STUDY OF PENETRATION ABILITY OF ADHESIVE SYSTEMS ON TEMPORARY TEETH BY CONFOCAL MICROSCOPY

Mamedov A.A.[1], **Loschenov V.B.**[2], **Morozova N.S.**[1], **Ryabova A.V.**[2], **Ivannikova K.O.**[1], **Orlova N.A.**[1]
[1]Sechenov First Moscow State Medical Univesity, Moscow, Russia
[2]Prokhorov General Physics Institute of the Russian Academy of Sciences, Moscow, Russia

Abstract

In this work, the authors evaluate the effectiveness of various adhesive systems (AS) in the temporary teeth restoration with composite materials. For this study, the authors used temporary teeth extracted for orthodontic reasons or lost due to a physiological change. Enamel samples with dentin were made from these teeth. Rhodamine B fluorochrome was added to each of the adhesive systems used. For the greater practical significance of research results, adhesive systems of several generations (IV, V, and VII) were chosen. In each sample, a cavity was prepared, and adhesive preparation was carried out according to the AS manufacturer instructions, followed by restoration with a composite material. Then, longitudinal slits were made so that the areas of enamel and dentin for which an adhesive protocol and restoration were performed could be observed in their entirety. The effectiveness of various generations was determined by the depth of penetration of AS components into the dentinal tubules. Fluorescent confocal microscopy was used to visualize the penetration ability of AS. Based on the results of the study, the authors concluded that IV and V generations of AS are the most effective.

Keywords: adhesive systems, dentin, dentistry, pediatric dentistry, confocal microscopy.

For citations: Mamedov A.A., Loschenov V.B., Morozova N.S., Ryabova A.V., Ivannikova K.O., Orlova N.A. Study of penetration ability of adhesive systems on temporary teeth by confocal microscopy, *Biomedical Photonics*, 2020, vol. 9, no. 2 , pp. 4–9. (in Russian) doi: 10.24931/2413–9432–2020–9–2–4–9.

Contacts: Morozova N.S., e-mail: kns74@bk.ru

ИССЛЕДОВАНИЕ ПЕНЕТРАЦИОННОЙ СПОСОБНОСТИ АДГЕЗИВНЫХ СИСТЕМ НА ВРЕМЕННЫХ ЗУБАХ МЕТОДОМ КОНФОКАЛЬНОЙ МИКРОСКОПИИ

А.А. Мамедов[1], **В.Б. Лощенов**[2], **Н.С. Морозова**[1], **А.В. Рябова**[2], **К.О. Иванникова**[1], **Н.А. Орлова**[1]
[1]Первый Московский государственный медицинский университет им. И.М. Сеченова, Москва, Россия
[2]Институт общей физики им. А.М. Прохорова РАН, Москва, Россия

Резюме

В работе представлены результаты исследования эффективности применения различных адгезивных систем (АС) при реставрации временных зубов композитными материалами. Для проведения исследования авторы использовали временные зубы, удаленные по ортодонтическим показаниям или утраченные вследствие физиологической смены. Из таких зубов были изготовлены образцы эмали с подлежащим дентином. В каждую из использованных АС был добавлен флуорохром (родамин В). Для большей практической значимости результатов были выбраны АС нескольких поколений: IV, V и VII. Далее в каждом образце отпрепарировали полость и провели адгезивную подготовку согласно инструкциям производителей соответствующих систем с последующей реставрацией композитным материалом. Затем для каждого образца изготавливали продольные шлифы с учетом полного попадания в область шлифа участков эмали и дентина, для которых проводили адгезивный протокол и реставрацию. Эффективность различных поколений определялась глубиной проникновения компонентов АС в дентинные трубочки. Для визуализации пенетрационной способности АС был использован метод флуоресцентной конфокальной микроскопии. По результатам исследования авторы делают вывод, что наибольшей эффективностью обладают АС IV и V поколения.

Ключевые слова: адгезивные системы, дентин, стоматология, детская стоматология, конфокальная микроскопия.

Для цитирования: Мамедов А.А., Лощенов В.Б., Морозова Н.С., Рябова А.В., Иванникова К.О., Орлова Н.А. Исследование пенетрационной способности адгезивных систем на временных зубах методом конфокальной микроскопии // Biomedical Photonics. – 2020. – Т. 9, № 2. – С. 4–9. doi: 10.24931/2413–9432–2020–9–2–4–9.

Контакты: Морозова Н.С., e-mail: kns74@bk.ru

Introduction

In modern restorative dentistry, composite materials are the materials of choice [1]. Their use has become widespread due to their strength and durability, as well as their user-friendly characteristics.

The success of the use of a specific composite material brand depends directly on the strength of its adhesion to hard dental tissues, enamel and dentin, and, therefore, only a well-chosen adhesive system (AS) can guarantee the stability of the restoration in the long term [2, 3].

Enamel and dentin processing performed before restoration can be divided into three stages: conditioning, priming, and bonding. At the first stage, orthophosphoric or organic acid is applied to the hard dental tissues, which decalcifies the intertubular and peritubular dentin, dissolves the smear layer formed during preparation, and opens the dentine tubules, which ensures the penetration of the components of the adhesive system into the thickness of the dentin. Then a primer is applied, which has hydrophilic groups that provide adhesion to dentine and hydrophobic groups that bind to the bond. The bond, also a component of the adhesive system, is applied after the primer, which provides adhesion to the composite material used for restoration [4].

Adhesive systems are classified into generations. 1st to 3rd generation systems are currently not in use. 4th generation AS are the most difficult to use: all three stages are separated, i. e., the conditioner, primer and bond are in separate vials. Further evolution of AS led to the integration of several processing stages into one. 5th generation AS have a separate enamel etching stage, but priming and bonding are combined. In the 6th generation systems, the conditioning stage is not separate, they are self-etching, and are supplied in two separate containers, requiring mixing before use. 7th generation is also self-etching, but with all stages combined into one [3]. Such adhesives are the easiest to use [5].

Full implementation of all the three stages of the adhesive protocol is difficult in the routine practice of a pediatric dentist [6]. Therefore, the most popular AS are self-etching ones and the systems where primer and bond are combined [7].

Over the past year, universal bonding systems have been gaining popularity. With them, the adhesive protocol is variable: depending on the clinical situation, the doctor decides whether to conduct the conditioning stage or not.

In this study, the authors compare the penetration ability of several kinds of the most popular AS using the method of fluorescent confocal microscopy.

Materials and methods

The study used intact primary masticatory teeth removed for orthodontic reasons or lost due to normal exfoliation. All parents of the patients signed informed voluntary consent to the use of primary teeth for the experimental study.

The primary teeth were used to make 63 samples of enamel with the subjacent dentin. Six AS most often used by pediatric dentists were selected for the study, two of which are universal. The adhesive protocol for each selected system was performed in accordance with the manufacturer's instructions.

The study used 4th generation AS (Bond A, 12 samples), 5th generation AS (Bond B, 10 samples), 5th generation AS (Bond C, 10 samples), 7th generation AS (Bond D, 11 samples), universal AS (Bond E, 10 samples) and universal AS (Bond F, 10 samples).

Additional characteristics of the studied AS are shown in Table 1.

Rhodamine B was selected as a fluorochrome, and its 0.01 wt-% solution was added to the bonding agents before their application [8].

The distribution of Rhodamine B fluorescence in tooth tissues was studied by laser scanning confocal microscopy with LSM-710 microscope (Carl Zeiss, Germany). To obtain images, a Plan-Apochromat lens with an X20 magnification (0.8 aperture) was used. For the study, tooth slices were placed on cover glasses with a thickness of 0.17 mm and observed in the plane of the cut. An argon laser with a wavelength of 458 nm (LASOS, Germany) was used to excite autofluorescence of dental tissues, and a DPSS laser with a wavelength of 561 nm (LASOS, Germany) was used to excite the fluorescence of Rhodamine B, in the ranges of 465 – 555 nm and 570 - 650 nm, respectively. Three-dimensional fluorescent images were obtained by registering a series of images with a step of 10 microns along the Z axis, followed by reconstruction of three-dimensional images with the use of ZEN software (Carl Zeiss, Germany).

The penetration capacity was determined by calculation of the average depth of penetration (*l*) and the number of filled dentine tubules in relation to all tubules in

the field of vision (n) as a percentage in images obtained by fluorescent confocal microscopy:

$$n = (n_{fill}/n_{total}) \times 100\%$$
$$I = I_{ave}/n_{fill},$$

where n is the percentage of filled dentinal tubules among the total number of dentinal tubules in the field of vision;

n_{total} is the total number of dentinal tubules in the field of vision;

n_{filled} is the total number of filled dentinal tubules in the field of vision;

I is the penetration capacity;

I_{ave} is the average depth of penetration of the adhesive system into the depth of dentin.

Statistical processing of the data.

The exact confidence bounds to the frequency were calculated based on the binomial distribution. Student's t-test was used to determine the significance of the differences.

The protocol of sample preparation for the study

1. Since dentine tubes are directed centrically to the pulp chamber, the most suitable area for examination is the gingival margin of primary teeth. Cylinders containing enamel with the subjacent dentin are cut out from the gingival margin of the crown of a primary tooth, and then divided into 4 parts.
2. A cylindrical drill is used to produce a cavity in the center of the block, pertaining to class V in Black's classification of cavities.
3. Adhesive preparation of the sample is performed according to the manufacturer's instructions.
4. The cavity is filled with a flowable compomer.
5. The sample is cut in half.
6. Confocal microscopy of the sample is performed.

Results and discussion

Results are shown in Fig. 1 and 2.

A comparison of the effectiveness of individual systems representing various AS types, performed with the use of confocal fluorescence microscopy, brought the authors to the conclusion that the best penetration ability is observed in 4th and 5th generation AS. There was no statistically significant difference between them in the number of filled tubules in the field of vision of the obtained image, or in the depth of penetration. However,

Таблица 1
Сравнительная характеристика адгезивных систем
Table 1
Comparison of the adhesive systems

Наименование Name	Поколение Generation	Схема использования Scheme of using the adhesive system
Bond A	IV	3 этапа: кондиционирование; прайминг; бондинг 3 steps: conditioning; priming; bonding
Bond B	V	2 этапа: кондиционирование; прайминг+бондинг 2 steps: conditioning; priming+bonding
Bond C	V	2 этапа: кондиционирование; прайминг+бондинг 2 steps: conditioning; priming+bonding
Bond D	VII	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding
Bond E	универсальная система universal system	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding
Bond F	универсальная система universal system	1 этап: кондиционирование+прайминг+бондинг 1 step: conditioning+priming+bonding

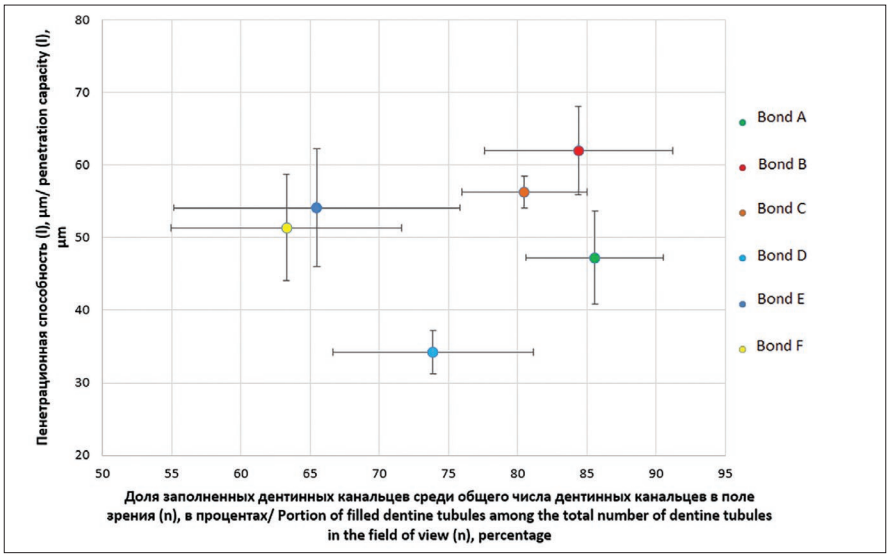

Рис. 1. Зависимость пенетрационной способности системы от доли заполненных дентинных канальцев
Fig. 1. Dependence of the penetration ability of the system on the fraction of filled dentinal tubules

significantly lower results were obtained for similar indicators in universal AS and 6th generation AS and universal bonding systems ($p<0.05$).

The results of this study can be explained as follows: during enamel and dentin preparation, a smear layer is inevitably formed on their surface, which acts as a barrier preventing AS components penetration into the thickness of the hard dental tissues [9]. However, etching with orthophosphoric acid at a concentration of 30-40% helps to remove the smear layer, providing microporosity of hard tissues and exposure of collagen fibrils [10]. Thus, the selected stage of conditioning contributes to the formation of a hybrid layer that is strongly integrated with dentin [11].

The results obtained by the authors are consistent with the data presented in the modern literature. T. Lemzi et al. [12] report in their literature review that AS with a separate conditioning stage show better results in comparison with self-etching AS. The authors made this conclusion based on an analysis of the results of *in vitro* studies, emphasizing that this fact could be confirmed by a larger number of such studies.

However, there are also data that do not confirm any significant superiority of AS with the separate stage of hard dental tissues etching. J. H. Jang et al. compared self-etching systems, where all three stages of adhesive preparation are combined into one, and total etching systems, where the stages of adhesive preparation are differentiated, in two ways: by examining the bursting micro-strength, noting the nature of the burst, and by comparing images of the adhesive layer obtained using a transmission electron microscope. In the first case, the authors noted the advantage of some kinds of total etching systems over self-etching adhesives, but noted that the break line always ran along the border of the adhesive interface. In the second case, there was no significant difference in the structure of the adhesive layer. The authors noted that in general, in terms of the results achieved, both self-etching systems and total etching systems are equally effective [13].

It is possible that statistically more reliable results can be obtained by using more samples of hard tissue of primary teeth and a greater variety of representative products of the selected AS generations.

The results of the study can be used by practicing pediatric dentists making a choice of an adhesive protocol for routine use, as well as by other researchers aiming at a more detailed study of adhesion of restorations to the hard tissues of primary teeth.

Conclusion

4th and 5th generation AS demonstrate high penetration ability, which allows us to recommend their use in the restoration of temporary teeth. The authors attribute the result to the fact that dentin conditioning with 37% orthophosphoric acid allows for more effective removal of the smear layer, enabling deeper penetration of the AS components into the dentine tubules. Thus, the exclusion of conditioning as a separate stage adversely affects the adhesion to the dentin of primary teeth. The authors want to emphasize the convenience and accura-

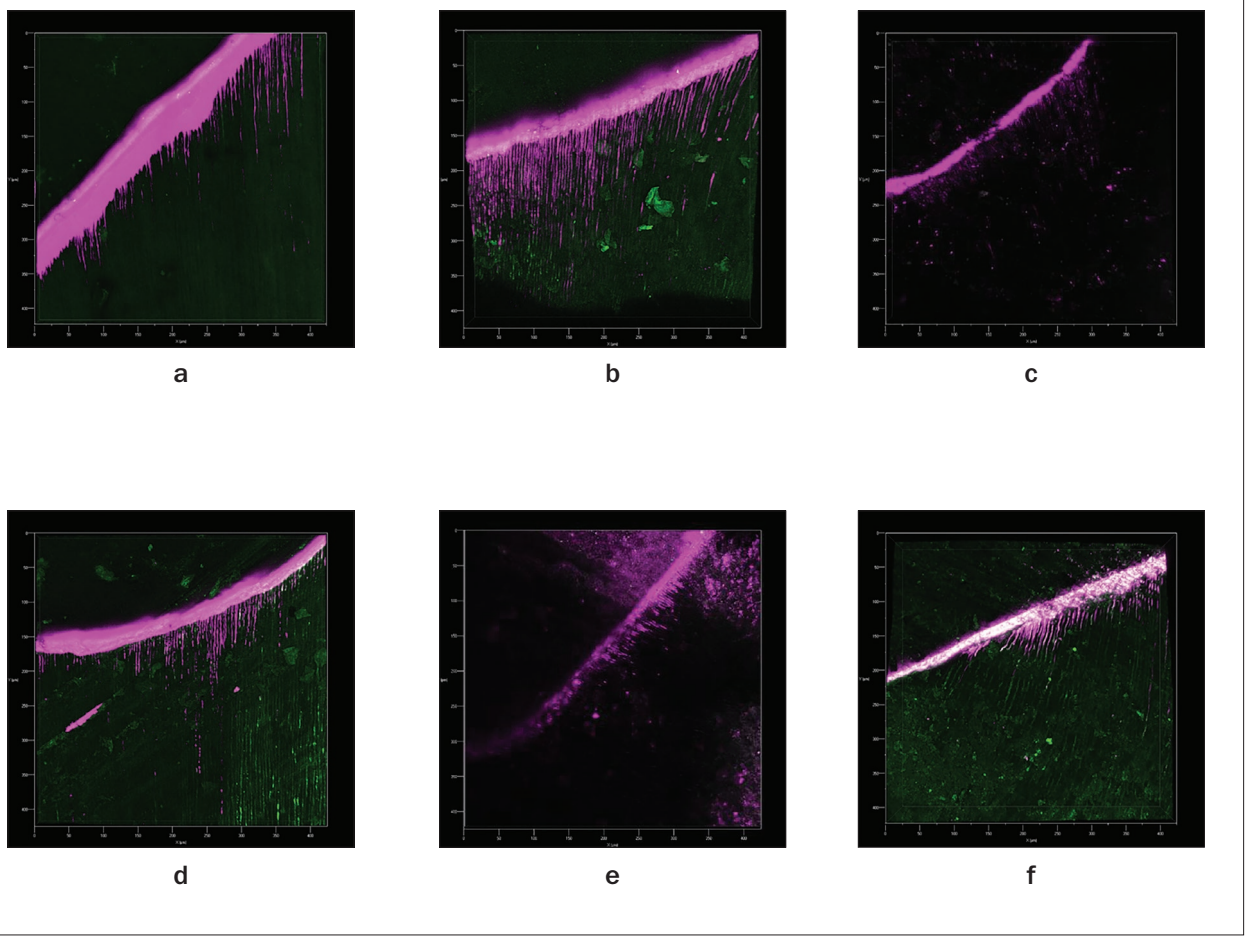

Рис. 2. 3D-реконструкция флуоресцентного изображения спила временного зуба при использовании различных адгезивных систем. Зеленым цветом подсвечены участки автофлуоресценции тканей зуба, розовым цветом – проникающая в дентин адгезивная система, меченная родамином В:
a – Bond A (IV поколение);
b – Bond B (V поколение);
c – Bond C (V поколение);
d – Bond D (VII поколение);
e – универсальная AC Bond E;
f – универсальная AC Bond F

Fig. 2. 3D reconstruction of a fluorescent image of temporary tooth sawn with various adhesive systems. Autofluorescence is shown in green, adhesive system labeled with rhodamine B – in pink:
a – Bond A (generation IV);
d – Bond B (generation V);
c – Bond C (generation V);
d – Bond D (generation VII);
e – universal Bond E;
f – universal Bond F

cy of their research method, fluorescent confocal microscopy, and hope for its further popularization in dentistry. This method can later be used to assess the penetration capacity of endodontic pastes and sealers, as well as to study colonies of microorganisms in the oral cavity and on hard dental tissues.

The performance of work was actively supported by the Core Facilities Center of the General Physics Institute of the RAS "Technological and Diagnostic Center for the Production, Research and Certification of Micro and Nanostructures."

REFERENCES

1. Welbure R.R., Duggal M.S., Hosey M.T. *Paediatric dentistry*. Oxford University Press, 2005. 445 p.
2. Ozer F., Blatz M.B. Self-Etch and Etch-and-Rinse Adhesive Systems in Clinical Dentistry, *Compend Contin Educ Dent*, 2013, vol. 34(1), pp. 12–18.
3. Aslanyan M., Eremin O., Trufanova Yu., Aslanyan Mel.A., Bykova O., Zavyalov A. Use of adhesive systems in dentistry: past and present, *Saratovskii nauchno-meditsinskii zhurnal*, 2018, no. 2, pp. 234–239.
4. Edward J.S. Bonding systems for restorative materials - a comprehensive review. *Pediatric Dentistry*, 1998, vol. 20(2), pp. 80–84.
5. Semikozov O., A clinical review on self-etch adhesives, *Problemy stomatologii*, 2010, vol. 4, pp. 12–14. (in Russ.)
6. Cameron A.C., Widmer R.P. *Handbook of pediatric dentistry*. Oxford, Elsevier Health Sciences, 2013.
7. Sofan E., Sofan A., Palaia G., Tenore G., Romeo U., Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type, *Ann Stomatol (Roma)*, 2017, vol. 8(1), pp. 1–17.
8. Choi A.N., Lee J.H., Son S., Jung K.H., Kwon Y.H., Park J.K. Effect of Dentin Wetness on the Bond Strength of Universal Adhesives. *Materials*, 2017, vol. 10(11), pp. 12–24.
9. Rosa W., Piva E., Silva A. Bond strength of universal adhesives: A systematic review and meta-analysis, *Journal of dentistry*, 2015, vol. 43(7), pp. 765–776.
10. Munoz M.A., Luque I., Hass V., Reis A., Loguercio A.D., Bombarda N.H. Immediate bonding properties of universal adhesives to dentine, *Journal of Dentistry*, 2013, vol. 41, pp. 404–415.
11. Munoz M.A., Sezinando A., Luque-Martinez I., Szesz A.L., Reis A., Loguercio A.D. Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives, *Journal of Dentistry*, 2014, vol. 42, pp. 595–602.
12. Lenzi T., Gimenez T., Tedesco T., Mendes F., Rocha R., Raggio D. Adhesive systems for restoring primary teeth: a systematic review and meta-analysis of in vitro studies, *International Journal of Paediatric Dentistry*, 2016, vol. 26(5), pp. 364–375.
13. Jang J.H., Lee M.G., Woo S.U., Lee C.O., Yi J.K., Kim D.S. Comparative study of the dentin bond strength of a new universal adhesive, *Dent Mater*, 2016, vol. 35(4), pp. 606–618.

ЛИТЕРАТУРА

1. Welbure R.R., Duggal M.S., Hosey M.T. Paediatric dentistry. – Oxford University Press, 2005. – 443 p.
2. Ozer F., Blatz M.B. Self-Etch and Etch-and-Rinse Adhesive Systems in Clinical Dentistry // Compend Contin Educ Dent. – 2013. – Vol. 34(1). – P. 12–18.
3. Асланян М.А., Еремин О.В., Труфанова Ю.Ю., Асланян Мел.А., Еремин А.О., Быкова О.А., Завьялов А.И. Применение адгезивных систем в стоматологии: прошлое и настоящее // Саратовский научно-медицинский журнал. – 2018. – № 2. – С. 234–239.
4. Edward J.S. Bonding systems for restorative materials - a comprehensive review // Pediatric Dentistry. – 1998. – Vol. 20(2). – P. 80–84.
5. Семикозов О.В. Клинический взгляд на самопротравливающие адгезивы // Проблемы стоматологии. – 2010. – № 4. – С. 83–87.
6. Cameron A.C., Widmer R.P. Handbook of pediatric dentistry. – Oxford: Elsevier Health Sciences, 2013.
7. Sofan E., Sofan A., Palaia G., Tenore G., Romeo U., Migliau G. Classification review of dental adhesive systems: from the IV generation to the universal type // Ann Stomatol (Roma). – 2017. – Vol. 8(1). – P. 1–17.
8. Choi A.N., Lee J.H., Son S., Jung K.H., Kwon Y.H., Park J.K. Effect of Dentin Wetness on the Bond Strength of Universal Adhesives // Materials. – 2017. – Vol. 10(11). – P. 12–24.
9. Rosa W., Piva E., Silva A. Bond strength of universal adhesives: A systematic review and meta-analysis // Journal of dentistry. – 2015. – Vol. 43(7). – P. 765–776.
10. Munoz M.A., Luque I., Hass V., Reis A., Loguercio A.D., Bombarda N.H. Immediate bonding properties of universal adhesives to dentine // Journal of Dentistry. – 2013. – Vol. 41. – P. 404–415.
11. Munoz M.A., Sezinando A., Luque-Martinez I., Szesz A.L., Reis A., Loguercio A.D., Influence of a hydrophobic resin coating on the bonding efficacy of three universal adhesives // Journal of Dentistry. – 2014. – Vol. 42. – P. 595–602.
12. Lenzi T., Gimenez T., Tedesco T., Mendes F., Rocha R., Raggio D. Adhesive systems for restoring primary teeth: a systematic review and meta-analysis of in vitro studies // International Journal of Paediatric Dentistry. – 2016. – Vol. 26(5). – P. 364–375.
13. Jang J.H., Lee M.G., Woo S.U., Lee C.O., Yi J.K., Kim D.S. Comparative study of the dentin bond strength of a new universal adhesive // Dent Mater. – 2016. – Vol. 35(4). – P. 606–618.

NARROW-BAND PHOTOTHERAPY AND SODIUM SALT OF THE SYNTHETIC DIAMIDE GAMMA-D-GLUTAMYL-D TRYPTOPHAN AS A COMBINED METHOD OF PSORIASIS TREATMENT: COMPREHENSIVE AND COMPARATIVE EVALUATION

Novikov Y.A.[1], **Okhlopkov V.A.**[2], **Troshina D.S.**[1], **Mozgovoy S.I.**[1], **Poleshchuk E.I.**[1], **Pravdina O.V.**[1]
[1]Omsk state medical University of Health of the Russian Federation, Omsk, Russia
[2]Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russia

Abstract

The paper presents the results of a comparative study of various immunosuppressive treatment methods of medium severity vulgar psoriasis (PASI from 10 to 20). The parameters used were PASI indices, p53 apoptosis marker expression indices, and expression indices of T-regulatory cell marker Foxp3. The study involved 96 patients, from 18 to 60 years old, divided by randomization into three groups. Patients in the 1st group (n = 32) received narrow-band phototherapy according to a 4-day regimen. We used the Waldmann UV-7002K instrument and F79/120W-TL01 lamps generating radiation with a maximum emission at a wavelength of 311 nm. We started with a dose of 0.05–0.1 J/cm^2, subsequently increased by 0.05–0.1 J/cm^2 for each procedure, in the absence of erythema. In total, patients received 12–15 procedures, with a course dose of 4.7–7.65 J/cm^2. In the 2nd group (n = 32), patients were treated with the sodium salt of the synthetic gamma-D-glutamyl-D tryptophan dipeptide. Patients of the 3rd group (n = 32) underwent combination therapy, which included both methods described above. As a result of treatment, the greatest decrease in the PASI index, as well as the level of expression of the marker of Foxp3 T-regulatory cells, was observed among patients who received the combined regimen. The data obtained indicate the high therapeutic efficacy of narrow-band phototherapy in combination with the sodium salt of the synthetic gamma-D glutamyl-D tryptophan synthetic dipeptide. It was also noted that the immunohistochemical marker p53, reflecting the cellular renewal of psoriatic papule keratinocytes, is an informative molecular-cellular indicator of the effectiveness of narrow-band phototherapy.

Keywords: psoriasis vulgaris, narrow-band phototherapy, UVB 311 nm, gamma-D-glutamyl-D sodium tryptophan, marker of apoptosis, p53, Foxp3+ T-regulatory cells.

For citations: Novikov Y.A., Okhlopkov V.A., Troshina D.S., Mozgovoy S.I., Poleschuk E.I., Pravdina O.V. Narrow-band phototherapy and sodium salt of the synthetic diamide gamma-D-glutamyl-D-tryptophan as a combined method of psoriasis treatment: comprehensive and comparative evaluation, *Biomedical Photonics*, 2020, vol. 9, no. 2, pp. 10–17 (in Russian). doi: 10.24931/2413–9432–2020–9–2–10–17.

Contacts: Troshina D.S., e-mail: kastaliya@mail.ru

УЗКОПОЛОСНАЯ ФОТОТЕРАПИЯ И ПРЕПАРАТ НАТРИЕВОЙ СОЛИ СИНТЕТИЧЕСКОГО ДИПЕПТИДА ГАММА-D-ГЛУТАМИЛ-D-ТРИПТОФАНА В КАЧЕСТВЕ КОМБИНИРОВАННОГО МЕТОДА ЛЕЧЕНИЯ ПСОРИАЗА: КОМПЛЕКСНАЯ И СРАВНИТЕЛЬНАЯ ОЦЕНКА

Ю.А. Новиков[1], **В.А. Охлопков**[2], **Д.С. Трошина**[1], **С.И. Мозговой**[1], **Е.И. Полещук**[1], **О.В. Правдина**[1]
[1]Омский государственный медицинский университет, Омск, Россия
[2]Федеральный научно-клинический центр реаниматологии и реабилитологии, Москва, Россия

Резюме

В работе представлены результаты сравнительного исследования различных иммуносупрессивных методов лечения вульгарного псориаза среднетяжёлой степени (PASI от 10 до 20). В качестве оцениваемых параметров использовали индекс PASI, уровни экспрессии

маркера апоптоза p53 и экспрессии маркера Т-регуляторных клеток Foxp3. В исследование были включены 96 пациентов в возрасте от 18 до 60 лет, разделённые методом рандомизации на три группы. Пациенты в 1-й группе (n=32) получали узкополосную фототерапию по 4-дневной схеме. Использовали прибор «Waldmann UV-7002K» и лампы F79/120W-TL01, генерирующие излучение с максимальной эмиссией на длине волны 311 нм. Начинали с дозы 0,05 – 0,1 Дж/см^2, повышая в последующем на 0,05 – 0,1 Дж/см^2 каждую процедуру, при отсутствии эритемы. Всего пациенты получали от 12 до 15 процедур с курсовой дозой облучения 4,7 –7,65 Дж/см^2. Во 2-й группе (n=32) пациентам проводили терапию натриевой солью синтетического дипептида гамма-D-глутамил-D-триптофана. Пациентам 3-й группы (n=32) проводили комбинированную терапию, включающую в себя сочетание двух вышеописанных методик. В результате лечения наибольшее снижение индекса PASI, а также уровня экспрессии маркера Т-регуляторных клеток Foxp3 наблюдали среди пациентов, получивших комбинированную схему. Полученные данные свидетельствуют о высокой терапевтической эффективности узкополосной фототерапии в комбинации с препаратом натриевой соли синтетического дипептида гамма-D-глутамил-D-триптофана. Также было отмечено, что иммуногистохимический маркер p53, отражающий клеточное обновление кератиноцитов псориатической папулы, является информативным молекулярно-клеточным показателем эффективности узкополосной фототерапии.

Ключевые слова: вульгарный псориаз, узкополосная фототерапия, UVB 311 нм, гамма-D-глутамил-D-триптофан натрия, маркер апоптоза, p53, Foxp3+ Т-регуляторные клетки.

Для цитирования: Новиков Ю.А., Охлопков В.А., Трошина Д.С., Мозговой С.И., Полещук Е.И., Правдина О.В. Узкополосная фототерапия и препарат натриевой соли синтетического дипептида гамма-D-глутамил-D-триптофана в качестве комбинированного метода лечения псориаза: комплексная и сравнительная оценка // Biomedical Photonics. – 2020. – Т. 9, № 2. – С 10–17. doi: 10.24931/2413–9432–2020–9–2–10–17.

Контакты: Трошина Д.С., e-mail: kastaliya@mail.ru

Introduction

Psoriasis is a chronic, multisystem inflammatory disease which most commonly manifests itself on the skin and in the joints. In addition to the physical aspects of the disease, psoriasis has a strong emotional and psychosocial impact on patients: it leads to stigmatization, poor self-esteem and stress, and affects interpersonal relationships [1].

The onset of the disease is induced and supported by dermal infiltrating lymphocytes with secondary increased keratinocyte proliferation and epidermal hyperplasia [2]. A normal balance between apoptosis and cell proliferation supports homeostasis of the epidermal compartment [3]. Psoriasis is a disease associated with decreased apoptosis activity [4]. More and more evidence has been emerging recently showing that two unique subsets of CD4+ T cells can play a role in the pathogenesis of psoriasis: T-helper cells-17 (Th17) and CD4+ CD25+ regulatory T cells (Treg) [5, 6]. The normal function of T-regulatory cells is aimed at suppressing inflammation and autoimmune reactions. A specific marker of Treg is the transcription factor Foxp3, through which their suppressor activity is implemented [7]. It is suggested that a change in the number or functional imbalance of Treg cells in the blood or tissue may cause a decrease in the regulation and subsequent hyperproliferation of T cells, which are pathogenic for psoriasis (Th17) [8].

Despite the significant impact of the disease on the quality of life, its diagnosis and treatment still involves difficulties [9, 10]. Therefore, a better understanding of the pathogenesis of this disease and the available treatment options is needed to ensure optimal therapy [1].

It is known that the basic means of treating mild and moderate psoriasis are such external remedies as topical corticosteroids and vitamin D3 analogues [11]. However, if local drugs do not cause an adequate response or their administration is not practical due to the extensive affected area of the body surface, a more appropriate treatment is systemic therapy [1].

Phototherapy is the main method for treating moderate to severe psoriasis, especially in the form that does not respond to local treatment. The currently available types of phototherapy are PUVA, broadband UVB, and narrowband UVB (NB-UVB). NB-UVB therapy is often used as a first-line treatment [12]. The effect of this method is due to the fact that ultraviolet light causes DNA damage, resulting in overexpression of p53 and, according to some data, stimulation of apoptosis of keratinocytes and T cells in the epidermis and dermis [13]; it changes the cytokine and T-cell profiles towards the predominance of Th2-link, which suppresses the differentiation of Th1/Th17-link lymphocytes and the synthesis of their cytokines (IL-12, IL-22, TNF, IFN-γ) [14].

Another method of systemic influence on the course of psoriasis is therapy with preparation of sodium salt of gamma-D-glutamyl-D-tryptophan, a synthetic dipeptide [15]. Its advantages include non-toxicity, effectiveness even in low concentrations, as well as a wide range of therapeutic doses. The drug has an immunosuppressive effect aimed at humoral and cellular immunity reaction, and it proportionally reduces the levels of T-helpers and T-suppressors, as well as the number of activation markers on lymphocytes, and suppresses T-cells proliferation.

In this study, our goal was to evaluate the effective-

ness of a combination of narrow-band phototherapy and therapy with gamma-D-glutamyl-D-tryptophan (a synthetic dipeptide) sodium salt based on clinical and immunohistochemical data.

The aim of the study was to draw conclusions about the effectiveness of each of the schemes based on comparative data on the expression of the p53 apoptosis marker and the Foxp3 T-regulatory cell marker in psoriatic plaque biopsies of patients receiving various immunosuppressive treatment regimens.

Materials and methods

As part of a cohort open prospective randomized controlled trial, in accordance with the inclusion and exclusion criteria, 96 patients with an advanced stage of vulgar psoriasis were involved in the study while undergoing inpatient treatment in the Budgetary Health Care Insitution "KKVD" of the Omsk Region. The research protocol was approved by the local Research Ethics Committee of Omsk State Medical University.

The criteria for inclusion in the study included age from 18 years, the presence of voluntary informed consent to participate in the study, the duration of the disease at the time of the study for at least 6 months, and the advanced stage of medium severity vulgar psoriasis, which corresponds to the PASI index from 10 to 20.

The exclusion criteria were the presence of decompensated chronic somatic pathology, complicated forms of psoriasis, previous hospitalization for psoriasis less than six months ago, the development of side effects to the therapy administered, a complicated allergic history, patient non-compliance, alcoholism, drug addiction, simultaneous administration of other medications that may affect the course of the primary disease.

All patients were randomly divided into three study groups. Group 1 patients (n=32) received narrow-band phototherapy on a 4-day schedule performed with Waldmann UV-7002K device and F79/120W-TL01 lamps (manufacturer: Herbert Waldmann GmbH & Co. KG, Germany), generating radiation in the wave range of 310-315 nm with a maximum emission at a wavelength of 311 nm. The initial radiation dose was 0.05-0.1 J/cm^2, followed by an increase of 0.05-0.1 J/cm^2 at each procedure subject to the absence of erythema. During hospitalization, patients received the treatment 12-15 times, achieving a course radiation dose of 4.7–7.65 J/cm^2.

In group 2 (n=32), the patients observed were receiving treatment with gamma-D-glutamyl-D-tryptophan sodium salt (OOO Pharma Bio, Russia, registration certificate PN-000022/02 of 10.08.2009). The treatment regimen consisted of two courses of intramuscular injections of 0.1% solution at a dose of 2.0 ml and 1.0 ml daily for 7 days in the first and second courses, respectively, with a 2-day break between courses.

Patients in group 3 (n=32) received combined therapy that included both methods described above.

In all the study groups, patients were also prescribed Retinol palmitate 55 mg + alpha-tocopherol acetate 100 mg, 1 capsule 3 times a day for 14 days, and a 30% solution of sodium thiosulfate, 10 ml, intravenously for 10 days. Externally, 3% sulfur-salicylic ointment and basic care products were prescribed, for the purpose of moisturizing the skin in the stationary stage of the treatment.

The severity and dynamics of the psoriatic process were assessed with the PASI dermatologic index, which was also used as a tool for evaluating the effectiveness of therapy. PASI was calculated with an application developed for this purpose.

In all the studied patients, biopsies of the affected skin areas were performed before treatment and on day 21 of therapy. Immunohistochemical study of p53 and Foxp3 expression in biopsies was performed at the Department of Pathological Anatomy of Omsk State Medical University.

Biomaterial was collected with a DERMO PUNCH skin biopsy needle with a diameter of 4 mm (manufacturer: STERYLAB s. r. l., Italy). Under local incisional anesthesia with 2% lidocaine solution, a biopsy was performed (within the dermis) of fresh morphoelements at the border with healthy skin before treatment, and residual lesions on day 21 of the therapy.

The resulting skin fragments were fixed in 10% neutral buffered formalin (pH 7.2 – 7.4) for 12 – 24 hours, and were fixed in paraffin. Slices were made from the paraffin blocks perpendicular to the skin surface, longitudinally through all layers, with a thickness of 4-5 microns, and were mounted on slides with poly-L-lysine adhesive coating (OOO Bio-Vitrum, Russia, registration certificate no. P3H 2015/2954 of 14.08.2015). The antigens were unmasked in a citrate buffer (pH 6.0) when boiled in water bath for 1 hour. As primary antibodies, ready-to-use (RTU) rabbit monoclonal antibodies were used (Spring Bioscience, Corp., USA, registration certificate No. ФС3 2010/07436 of 20.06.2010): Fohr3, PCH 101 clone, and P53, SPM 514 clone. To visualize the results of the immunohistochemical reaction, the detection kit REVEAL-Biotin-Free Polyvalent DAB (Spring Bioscience, Corp., USA, registration certificate No. ФС3 2010/07437 of 20.06.2010) was used. 3,3-diaminobenzidine tetrachloride, which is part of the commercial detection kit, was used as a chromogen. Cell nuclei were stained with Mayer's Hematoxylin for a period ranging from 30 seconds to 2 minutes.

The expression index of T-regulatory lymphocytes (nuclear label Foxp3) was calculated by counting the number of positively colored cells among the total number of mononuclears (≥1000 cells) in 10 fields of vision (X400), with the results expressed as a percentage. The apoptosis index was evaluated in a similar way.

Microparticles were viewed and photographed with an Axioskop 40 microscope using the AxioCam 503 color

camera (manufacturer: Carl Zeiss Microscopy GmbH, Germany) and ZEN software package.

Statistical processing of the research results was performed with Statistica V. 6 software package and Microsoft Office Excel 2016. The descriptive statistics of quantitative characteristics are represented by the median and standard deviations (in the Me±σ format), and the minimum and maximum indicators were also used. We used nonparametric methods (the Wilcoxon test to check the differences between two samples of paired measurements, the Kraskel-Wallis method to compare three independent groups), and the Spearman correlation coefficient to study the correlation between two variables measured in metric scales on the same sample (where $r \leq 0.3$ is a weak correlation, $0.3 < r < 0.7$ is a moderate correlation, and $r > 0.7$ is a strong correlation). The results were considered statistically significant at $p < 0.05$.

Results

Group 1 included patients aged 19 to 60 years. The duration of the disease varied from one year to 29 years, the median being 14.0±7.29 years. Individual values of the PASI index before treatment ranged from 10.2 to 20.0, median 16.25, and after a course of narrow-band phototherapy, the index decreased by values from 8.0 to 1.2, the median being 2.25.

The 2nd group included patients aged 18 to 60, with the duration of the disease within the range from one year to 44 years (8.0±14.04 years). The values of the PASI index before treatment ranged from 10.0 to 19.5, median 13.9, whereas after treatment they were from 6.0 to 1.3, median 3.25.

Group 3 included patients aged 18 to 60. The duration of the disease varied from one year to 35 years, with an average value of 6.5±8.89 years. The values of the PASI index before treatment ranged from 10.0 to 20.0, median 15.6, whereas after the latest treatment they were from 6.0 to 1.3, median 3.25.

The values of the PASI index before treatment did not differ significantly between the comparison groups ($H=5.83$; $p=0.55$) (see Table 1). After the completion of treatment, each group demonstrated a significant decrease in the index, but it was most significant in group 3, i. e., in patients who received combined therapy ($H=19.65$; $p=0.000$).

Prior to treatment, p53 protein was detected in 2.31-3.82% of epidermal cells, and no statistically significant differences were found among the three study groups ($H=9.2$; $p=0.1$). Positively expressing cells were located in the basal and suprabasal layers of the epidermis (Fig. 1).

After a course of narrow-band phototherapy that is able to induce apoptosis, the expression of the p53

Таблица 1
Динамика значений индекса PASI, экспрессии p53 и Foxp3 до и на 21 день лечения
Table 1
Dynamics of the PASI index, p53 and Foxp3 expression before and on 21 day of treatment

Показатель Indicator	1-я группа 1st group (n=32)	2-я группа 2nd group (n=32)	3-я группа 3rd group (n=32)	Критерий Краскела-Уоллиса, H Kruskal-Wallis test, H	p
PASI до лечения, M PASI before treatment, M	16,25	13,9	15,6	5,83	0,55
PASI на 21 день лечения, M PASI on 21 day of treatment, M	2,25	3,25	1,75	19,65	0,000
Экспрессия p53 до лечения, M p53 expression before treatment, M	2,31	3,82	3,14	9,2	0,1
Экспрессия p53 на 21 день лечения, M p53 expression on 21 day of treatment, M	5,32	1,85	4,86	18,89	0,000
Экспрессия Foxp3 до лечения, M Foxp3 expression before treatment, M	12,1	11,86	11,8	0,47	0,79
Экспрессия Foxp3 на 21 день лечения, M Foxp3 expression on 21 day of treatment, M	5,33	6,5	2,18	36,84	0,000

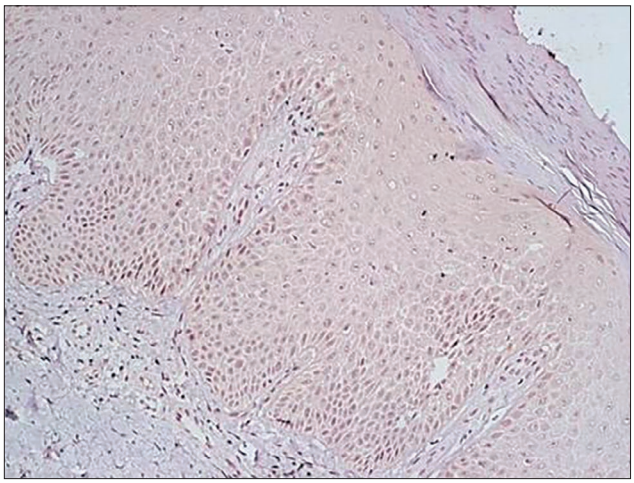

Рис. 1. Позитивно p53-экспрессирующие клетки в псориатической бляшке до начала лечения, Иммуногистохимия. Ув. 200
Fig. 1. Positively p53-expressing cells in psoriatic plaque before treatment, Immunohistochemistry. x200 magnification

marker in group 1 became significantly higher (see Table 2, Fig. 2a), which may indicate the protective function of the protein in relation to skin homeostasis, which is implemented by influencing the glycolysis process and by regulating cell growth. On the contrary, the number of epidermal cells expressing p53 in the skin biopsies of group 2 patients treated with the synthetic dipeptide gamma-D-glutamyl-D-tryptophan sodium salt agent significantly decreased on day 21 of treatment (see Table 2, Fig. 2b), which is consistent with the data of previous studies [15] and indicates the normalization of cellular cooperation and inhibition of hypertrophied functions of cellular immunity. In group 3, an increase in p53 expression was registered in 22 cases, and a decrease was noted in 10. Apparently, based on the research data, it is possible to evaluate the dominant role of a particular therapy method in the regression of the disease in a particular patient. As can be seen from Table 3, an increase in p53 expression was observed mainly in patients who previously received narrow-band phototherapy, while a decrease in the level of expression was observed mainly in those who had previously had only general treatment without immunosuppressive therapy.

Prior to the treatment, the level of positively expressing Foxp3 cells among mononuclear cells of dermal infiltrate ranged from 11.8 to 12.1%. The cells were located in the papillary layer of the dermis (Table 1, Fig. 3a). There were no statistically significant differences among the study groups (N=0.47; p=0.79) (Table 1). At day 21 of the treatment, a significant decrease in the expression of Foxp3-positive cells was observed in each group of patients (T=0.00; p=0.000) (table 2). The most pronounced decrease was in group 3, with the average value corresponding to 2.18% (N=36.84; p=0.000) (Table 1, Fig. 3b).

Таблица 2
Индексы экспрессии p53 и Foxp3+ клеток до начала лечения и на 21 день терапии
Table 2
p53 and Foxp3+ cells expression indices before and on 21 day of therapy

p53	Группы Groups						Foxp3	Группы Groups					
	1-я (n=32) 1st (n=32)		2-я (n=32) 2nd (n=32)		3-я (n=32) 3rd (n=32)			1-я (n=32) 1st (n=32)		2-я (n=32) 2nd (n=32)		3-я (n=32) 3rd (n=32)	
	До Before	21 день 21 day	До Before	21 день 21 day	До Before	21 день 21 day		До Before	21 день 21 day	До Before	21 день 21 day	До Before	21 день 21 day
Медиана Median	2,31	5,32	3,82	1,85	3,14	4,86		12,1	5,33	11,86	6,5	11,8	2,18
Минимум Minimum	0,58	0,87	0,54	0,0	1,11	0,0		10,1	0,3	2,78	0,63	5,78	0,0
Максимум Maximum	8,68	13,75	59,9	45,27	12,43	20,0		15,46	10,77	29,33	25,45	23,3	6,2
Стандартное отклонение Standard deviation	2,1	3,29	10,27	7,81	3,21	4,49		1,43	2,48	6,55	5,3	3,94	1,65
Критерий Уилкоксона, T Wilcoxon test, T	0,00		0,00		167,0			0,00		0,00		0,00	
p	0,000		0,000		0,07			0,000		0,000		0,000	

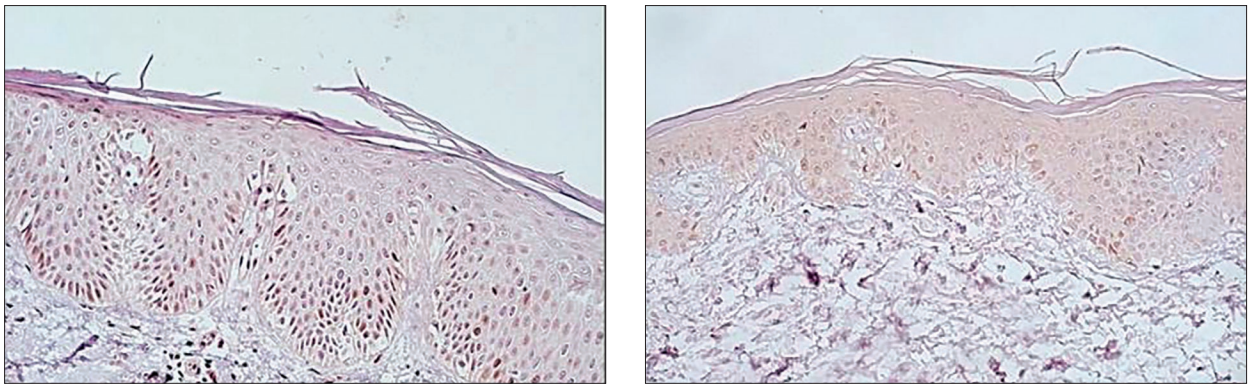

Рис. 2. Позитивно p53-экспрессирующие клетки в псориатической бляшке на 21 день лечения. Иммуногистохимия. Ув. 200
a – 1-я исследовательская группа;
b – 2-я исследовательская группа
Fig. 2. Positively p53-expressing cells in psoriatic plaque on 21 day of treatment. Immunohistochemistry. x200 magnification.
a – 1st research group;
b – 2nd research group

Таблица 3
Изменение экспрессии p53 в биоптатах кожи пациентов, получающих комбинированное лечение, в зависимости от вида терапии в анамнезе
Table 3
Change in p53 expression in skin biopsies of patients receiving combination treatment depending on the type of therapy in anamnesis

Результат комбинированного лечения Result of the combined therapy	Абсолютное число больных Absolute number of patients	Виды лечения в анамнезе Type of therapy in anamnesis			
		Терапия без применения иммуносупрессии Therapy without immunosuppression	Узкополосная фототерапия Narrow-band phototherapy	ПУВА-терапия PUVA-therapy	Метотрексат Methotrexate
Увеличение индекса экспрессии p53,% Increase in p53 expression index,%	22	3	15	2	2
Снижение индекса экспрессии p53,% Decrease in p53 expression index,%	10	8	–	–	2

There were a statistically significant moderate positive correlation and a strong correlation between the PASI index and the levels of Foxp3 + cells: the higher the PASI index in psoriasis, the greater the number of Foxp3+ cells found in the immunohistochemical analysis of the biopsy. There was also a weak negative correlation between the PASI value and p53 expression in group 1: the lower the initial PASI index in patients who received narrow-band phototherapy, the greater the number of p53+ cells detected in immunohistochemical studies (Table 4).

Discussion

This study included a comparative assessment of the dynamics of the PASI index, the expression levels of the p53 apoptosis marker, and the Foxp3 T-regulatory cell marker in patients with moderate psoriasis after treatment with different therapeutic regimens.

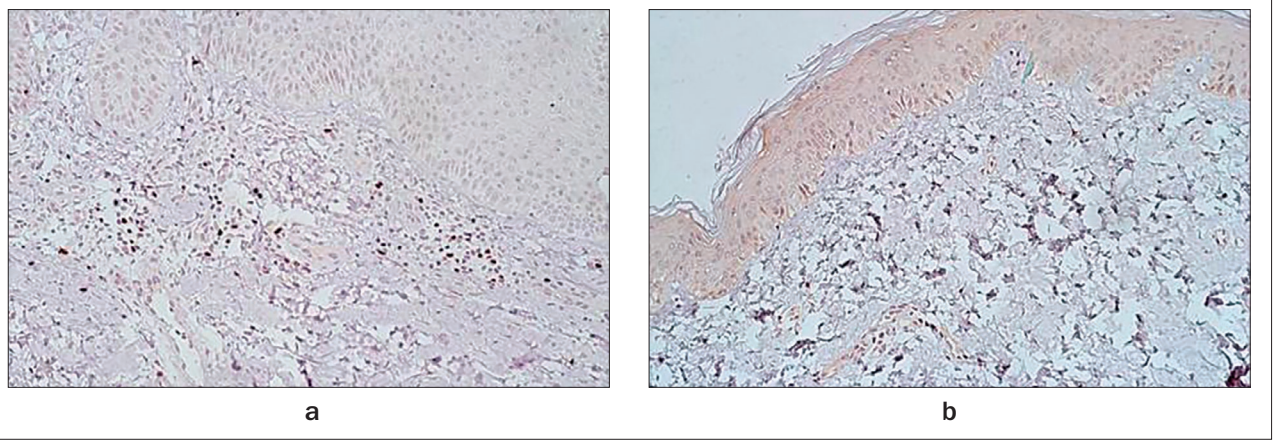

Рис. 3. Foxp3+ клетки в псориатической бляшке. Иммуногистохимия. Ув. 200
a – до лечения;
b – после лечения
Fig. 3. Foxp3+ cells in psoriatic plaque. Immunohistochemistry. x200 magnification
a – before treatment;
b – after treatment

As a result of treatment, the PASI index went down significantly in all study groups, but the greatest decrease was among patients who received combination therapy which included narrow-band phototherapy (UVB 311 nm) and the use of the gamma-D-glutamyl-D-tryptophan sodium salt preparation (group 3).

A study of the level of p53 expression showed a significant increase in group 1, in which patients received only narrow-band phototherapy. In the 2nd group of patients treated with gamma-D-glutamyl-D-tryptophan sodium salt agent, on the contrary, the number of epidermal cells expressing p53 significantly decreased, which indicates the normalization of cellular cooperation phenomena. In group 3, p53 expression levels were different among patients receiving combination therapy. An increase in p53 expression was observed mainly among patients who had previously received narrow – band phototherapy, and a decrease was observed among patients who earlier had been administered only general treatment.

Immunohistochemical staining with Foxp3+ monoclonal antibodies showed that the Foxp3+ cell fraction was significantly reduced in skin biopsies on day 21 of treatment in patients of all the three study groups. The decrease in Foxp3 expression was most pronounced in patients in study group 3.

A positive correlation was found between the PASI index and the level of Foxp3+expression.

Conclusion

As a result of the research, a statistically significant difference in the PASI diagnostic index before and after treatment was found in each study group. However, in

Таблица 4
Корреляция между индексом PASI, экспрессией p53 и Foxp3 в исследуемых группах
Table 4
Correlation between PASI index, p53 and Foxp3 expression within the studied groups

	1-я группа (n=32) 1st group (n=32)		2-я группа (n=32) 2nd group (n=32)		3-я группа (n=32) 3rd group (n=32)	
Показатели Indicators	r	p	r	p	r	p
PASI и Foxp3 PASI and Foxp3	0,81	0,000	0,35	0,000	0,78	0,000
PASI и p53 PASI and p53	–0,3	0,02	0,14	0,26	–0,2	0,12
Foxp3 и p53 Foxp3 and p53	–0,03	0,82	0,09	0,5	–0,08	0,5

group 3, the decrease in the PASI index was significantly more dramatic than in other groups. The obtained data indicate high therapeutic effectiveness of narrow-band phototherapy in combination with the gamma-D-glutamyl-D-tryptophan sodium salt agent. It is interesting that this combination results in a potentiation of the therapeutic effect, but, depending on the previous treatment, one of the methods plays a leading role.

REFERENCES

1. Kim B., Jerome D., Yeung J. Diagnosis and management of psoriasis, *Can. Fam. Physician,* 2017, vol. 63, no. 4, pp. 278–285.
2. Kalyan S., Shirazi N., Jindal R., et al. Effect of Methotrexate Therapy on p53 and Bcl2 Expression in Patients with Psoriasis: A Prospective Hospital-Based Cohort Study, *Ann. Med. Health. Sci. Res.*, 2018, vol. 8, no. 2, pp. 84–89.
3. Raj D., Brash D., Grossman D. Keratinocyte apoptosis in epidermal development and disease, *J. Invest. Dermatol,* 2006, vol. 126, no. 2, pp. 243–57.
4. Moorchung N., Vasudevan B., Kumar D., et al. Expression of apoptosis regulating proteins p53 and bcl-2 in psoriasis, *Indian Journal of Pathology and Microbiology,* 2015, vol. 58, no. 4, pp. 423–426.
5. Yoo I., Lee J., Song S., et al. T-helper 17 cells: the driving force of psoriasis and psoriatic arthritis, *Int. J. Rheum. Dis,* 2012, vol. 15, no. 6, pp. 531–537.
6. Mattozzi C., Salvi M., D'Epiro S. et al. Importance of regulatory T cells in the pathogenesis of psoriasis: review of the literature, *Dermatology,* 2013, vol. 227, no. 2, pp. 134–145.
7. Tang Q., Bluestone J. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation, *Nat. Immunol.,* 2008, vol. 9, no. 3, pp. 239–244.
8. Zhang L., Yang l., Wei J., et al. Characterization of Th17 and FoxP3+ Treg Cells in Paediatric Psoriasis Patients, *Scandinavian Journal of Immunology,* 2016, vol. 83, no. 3., pp. 174–180.
9. Feldman S., Fleischer A., Cooper J. New topical treatments change the pattern of treatment of psoriasis: dermatologists remain the primary providers of this care, *Int. J. Dermatol.,* 2000, vol. 39, no. 1, pp. 41–44.
10. Horn E., Fox K., Patel V. et al. Are patients with psoriasis undertreated? Results of National Psoriasis Foundation survey, *J. Am. Acad. Dermatol.,* 2007, vol. 57, no. 6, pp. 957–62.
11. Mason A., Mason J., Cork M., et al. Topical treatments for chronic plaque psoriasis: An abridged Cochrane Systematic Review, *J. Am. Acad. Dermatol.,* 2013, vol. 69, no. 5, pp. 799–807.
12. Menter A., Korman N., Elmets C., et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy, *J. Am. Acad. Dermatol.*, 2010, vol. 62, no. 1, pp. 114–35.
13. Weatherhead S., Farr P., Jamieson D, et al. Keratinocyte apoptosis in epidermal remodeling and clearance of psoriasis induced by UV radiation, *J. Invest. Dermatol.*, 2011., vol. 131, no. 9, pp. 1916–26.
14. Wong T., Hsu L., Liao W. Phototherapy in Psoriasis: A review of mechanisms of action, *J. Cutan. Med. Surg.*, 2013, vol. 17, no 1, pp. 6–12.
15. Korotkiy N.G., Udzhkhu V.Yu., Abdullaeva A.E. The therapeutic possibilities of Thymodepressin in patients with psoriasis and the mechanisms of its therapeutic effect, *Poliklinika*, 2013, vol. 1, no. 1, pp. 105–10. (in Russ.)

ЛИТЕРАТУРА

1. Kim B., Jerome D., Yeung J. Diagnosis and management of psoriasis // Can. Fam. Physician. – 2017. – Vol. 63, No. 4. – P. 278–285.
2. Kalyan S., Shirazi N., Jindal R., et al. Effect of Methotrexate Therapy on p53 and Bcl2 Expression in Patients with Psoriasis: A Prospective Hospital-Based Cohort Study // Ann. Med. Health. Sci. Res. – 2018. – Vol. 8, No. 1. – P. 84–89.
3. Raj D., Brash D., Grossman D. Keratinocyte apoptosis in epidermal development and disease // J. Invest. Dermatol. – 2006. – Vol. 126, No. 2. – P. 243–57
4. Moorchung N., Vasudevan B., Kumar D., et al. Expression of apoptosis regulating proteins p53 and bcl-2 in psoriasis // Indian Journal of Pathology and Microbiology. – 2015. – Vol. 58, No. 4. – P. 423–426.
5. Yoo I., Lee J., Song S., et al. T-helper 17 cells: the driving force of psoriasis and psoriatic arthritis // Int. J. Rheum. Dis. – 2012. – Vol. 15, No. 6. – P. 531–537.
6. Mattozzi C., Salvi M., D'Epiro S. et al. Importance of regulatory T cells in the pathogenesis of psoriasis: review of the literature // Dermatology. – 2013. – Vol. 227, No. 2. – P. 134–145.
7. Tang Q., Bluestone J. The Foxp3+ regulatory T cell: a jack of all trades, master of regulation // Nat. Immunol. – 2008. – Vol. 9, No. 3. – P. 239–244.
8. Zhang L., Yang l., Wei J., et al. Characterization of Th17 and FoxP3+ Treg Cells in Paediatric Psoriasis Patients // Scandinavian Journal of Immunology. – 2016. – Vol. 83, No. 3. – P. 174–180.
9. Feldman S., Fleischer A., Cooper J. New topical treatments change the pattern of treatment of psoriasis: dermatologists remain the primary providers of this care // Int. J. Dermatol. – 2000. – Vol. 39, No. 1. – P. 41–44.
10. Horn E., Fox K., Patel V. et al. Are patients with psoriasis undertreated? Results of National Psoriasis Foundation survey // J. Am. Acad. Dermatol. – 2007. – Vol. 57, No. 6. – P. 957–62.
11. Mason A., Mason J., Cork M., et al. Topical treatments for chronic plaque psoriasis: An abridged Cochrane Systematic Review // J. Am. Acad. Dermatol. – 2013. – Vol. 69, No. 5. – P. 799–807.
12. Menter A., Korman N., Elmets C., et al. Guidelines of care for the management of psoriasis and psoriatic arthritis: section 5. Guidelines of care for the treatment of psoriasis with phototherapy and photochemotherapy // J. Am. Acad. Dermatol. – 2010. – Vol. 62, No. 1. – P. 114–35.
13. Weatherhead S., Farr P., Jamieson D, et al. Keratinocyte apoptosis in epidermal remodeling and clearance of psoriasis induced by UV radiation // J. Invest. Dermatol. – 2011. – Vol. 131, No. 9. – P. 1916–26.
14. Wong T., Hsu L., Liao W. Phototherapy in Psoriasis: A review of mechanisms of action // J. Cutan. Med. Surg. – 2013. – Vol. 17, No. 1. – P. 6–12.
15. Н.Г. Короткий, В.Ю. Уджуху, А.Э. Абдуллаева. Терапевтические возможности тимодепрессина у больных псориазом и механизмы его лечебного действия // Поликлиника. – 2013. – Т. 1, №1. – С. 105–107.

ORIGINAL ARTICLES

PHOTODYNAMIC THERAPY OF CANCER OF LARGE DUODENAL PAPILLA AND EXTRAHEPATIC BILE DUCTS

Stranadko E.F.[1], Baranov A.V.[1], Duvansky V.A.[1], Lobakov A.I.[2], Morokhotov V.A.[2], Riabov M.V.[1]
[1]Skobelkin State Scientific Center of Laser Medicine FMBA, Moscow, Russia
[2]Moscow Regional Research and Clinical Institute ("MONIKI"), Moscow, Russia

Abstract

The problem of treating patients with malignant neoplasms of the extrahepatic bile ducts and the large duodenal papilla remains relevant due to the growing incidence, high mortality, and a pronounced decrease in the quality of life of patients, despite the radical surgery. The purpose of this study was to evaluate the effectiveness of photodynamic therapy (PDT) in inoperable patients with malignant tumors of these localizations.
The study is based on the treatment of 79 patients with adenocarcinoma of large duodenal papilla and extrahepatic bile ducts. 29 patients received palliative bile drainage operations with PDT. 50 patients in the control group only had palliative bile drainage operations. Patients in the study group received from 1 to 3 PDT courses in a year. In total, 29 patients received 52 PDT courses. The tolerance to the method and the life expectancy of patients were evaluated.
The median survival of patients who underwent PDT was 18 months (11–60 months); in the control group – 11.5 months.
PDT, in combination with bile drainage operation, is an effective method for the treatment of inoperable patients with malignant neoplasms of the extrahepatic bile ducts and the large duodenal papilla in the absence of severe complications and with easy tolerance to therapy by patients. For the treatment and prolongation of life of patients whose radical surgical treatment is associated with a high risk of death, PDT seems to be the best treatment option. Results of PDT treatment for cancer of this localization are comparable with the results of radical surgeries and exceed those for palliative surgeries.

Keywords: cancer of large duodenal papilla, cancer of extrahepatic bile ducts, photodynamic therapy, photosensitizer, laser.

For citations: Stranadko E.F., Baranov A.V., Duvansky V.A., Lobakov A.I., Morokhotov V.A., Riabov M.V. Photodynamic therapy of cancer of large duodenal papilla and extrahepatic bile ducts, *Biomedical Photonics*, 2020, vol. 9, no. 2, pp. 18–28 (in Russian). doi: 10.24931/2413–9432–2020–9–2–18–28.

Contacts: Stranadko E.F., e-mail: seph04@yandex.ru

ФОТОДИНАМИЧЕСКАЯ ТЕРАПИЯ РАКА БОЛЬШОГО ДУОДЕНАЛЬНОГО СОСОЧКА И ВНЕПЕЧЁНОЧНЫХ ЖЕЛЧНЫХ ПРОТОКОВ

Е.Ф. Странадко[1], А.В. Баранов[1], В.А. Дуванский[1], А.И. Лобаков[2], В.А. Морохотов[2], М.В. Рябов[1]
[1]Государственный научный центр лазерной медицины им. О.К. Скобелкина ФМБА, Москва, Россия
[2]Московский областной научно-исследовательский клинический институт им. М.Ф. Владимирского, Москва, Россия

Резюме

Проблема лечения пациентов с злокачественными новообразованиями (ЗНО) внепечёночных желчных протоков (ВЖП) и большого дуоденального сосочка (БДС) остается актуальной в связи с растущей заболеваемостью, высокой летальностью и выраженным снижением качества жизни пациентов, несмотря на проведение радикальной операции. Цель данного исследования заключалась в оценке эффективности применения фотодинамической терапии (ФДТ) у неоперабельных больных с злокачественной опухолью данной локализации.
В исследование вошли 79 больных аденокарциномой БДС и ВЖП. Для лечения 29 больных применяли паллиативные желчеотводящие операции и ФДТ, 50 больным, составившим группу контроля, выполняли только желчеотводящие операции. Количество курсов ФДТ в основной группе составило от 1 до 3 в течение года. В общей сложности 29 больным проведено 52 курса ФДТ. Оценивали переносимость метода и продолжительность жизни больных.
Медиана выживаемости больных, которым выполнялась ФДТ, составила 18 мес (11–60 мес), в группе контроля – 11,5 мес.
ФДТ в комбинации с желчеотводящими операциями является эффективным методом лечения неоперабельных больных с ЗНО БДС и ВЖП при отсутствии тяжелых осложнений и с легкой переносимостью терапии пациентами. Для лечения и продления жизни больных,

радикальное хирургическое лечение которых сопряжено с высоким риском развития летального исхода, ФДТ представляется оптимальным вариантом терапии. Результаты ФДТ по продолжительности жизни сравнимы с радикальными операциями и превышают таковые для паллиативных операций.

Ключевые слова: рак большого дуоденального сосочка, рак внепеченочных желчных протоков, фотодинамическая терапия, фотосенсибилизатор, лазер.

Для цитирования: Странадко Е.Ф., Баранов А.В., Дуванский В.А., Лобаков А.И., Морохотов В.А., Рябов М.В. Фотодинамическая терапия рака большого дуоденального сосочка и внепечёночных желчных протоков // Biomedical Photonics. – 2020. – Т. 9, № 2. – С. 18–28. doi: 10.24931/2413–9432–2020–9–2–18–28.

Контакты: Странадко Е.Ф., e-mail: seph04@yandex.ru

Introduction

The use of photodynamic therapy (PDT) for the treatment of patients with malignant neoplasms over the past 20 years has become so wide-spread that it is now used for tumors that are the most aggressive and difficult to access with other treatment methods [1-5]. One of these localizations is malignant tumors of the biliary tract. A group of malignant tumors that develop from the epithelium of the bile ducts is referred to by a general term of cholangiocarcinoma (ChC), which includes intrahepatic, portal, and distal forms. 80% of ChCs are extrahepatic: portal vein ChC and distal ChC [6-10]. The latter includes duodenal papilla carcinoma (DPC). ChC develops against the background of long-term chronic inflammation of the bile ducts and is very pernicious. It is radical surgery that gives hope to the patients. However, in 80-90% of patients, ChC is diagnosed only upon the observation of the first clinical sign of the disease, which is mechanical jaundice, and at that stage surgery is impossible due to the extent of the process. By the time when mechanical jaundice develops, the tumor often invades the large vessels of the hepatoduodenal ligament, the portal vein, or passes to the head of the pancreas, which reduces the possibility of performing radical surgery to 23-50% [11-15]. Performing this extensive and traumatic operation is accompanied by high rates of postoperative mortality (up to 40%), and 50-75% of patients have relapses of the disease [11, 16, 17]. Chemotherapy and radiation therapy are less than effective, and adjuvant chemotherapy does not reduce the frequency of postoperative relapses [8, 13-15]. The average life expectancy of these patients is 3 to 6 months after the diagnosis [8-10]. As early diagnosis remains difficult, and treatment options for ChC are limited, the prognosis of 5-year survival of the patients remains low and is on average 5-15% [9-11, 17].

Being a less invasive and relatively safe method of treatment, local excision of the tumor is used, but it is accompanied by a high frequency of relapses. As for radical surgery in connection with malignant tumors of the bile ducts, the authors of publications most often refer to a high frequency of relapses, reaching 80% of cases [18, 19]. The average life expectancy after radical surgery in the form of gastropancreatoduodenal resection (GPDR) is 1.5-2 years, and 5-year survival rate does not exceed 10-20% [4, 5, 11].

Patients with obturation jaundice and contraindications to GPDR usually undergo palliative interventions: draining endoscopic procedures with the introduction of endoprostheses or the creation of bypass anastomoses. However, the tumor quickly grows into an endoprosthesis, which is accompanied by restenosis and jaundice. The average life expectancy after palliative surgery is 4 to 12 months.

Radiation therapy of the malignant tumors of this zone, both external and endoluminal irradiation (brachytherapy), is technically difficult to perform, ineffective, and, therefore, rarely used. For the treatment of this pathology, chemotherapy is also ineffective.

This situation makes us look for less traumatic and, at the same time, more effective methods of treating patients with malignant tumors of duodenal papilla and extrahepatic bile ducts. A method that meets these requirements is photodynamic therapy (PDT).

PDT is a well-known method of treating various diseases and, above all, malignant tumors, in which a photosensitizer (FS), which selectively accumulates in tissues with increased metabolism, is activated by local irradiation of pathologically altered tissues with light ath a wavelength corresponding to the peak of absorption of the PS. The photodynamic reaction that occurs in the presence of oxygen causes the generation of singlet and other reactive oxygen intermediates that have a detrimental effect on tumor tissues, which subsequently leads to tumor resorption [5, 20]. The action of reactive oxygen intermediates is manifested either by direct cytotoxic damage to the tumor [5, 16], or by the destruction of the blood vessels feeding the tumor [20, 21]. At the same time, depending on the photosensitizer used, the vascular mechanism accounts for up to 60% of the antitumor effect of PDT, while the direct cytotoxic effect

is about 30%. Along with that, PDT stimulates the immune responses of the body both due to the direct inflow of lymphocytes, neutrophils, and macrophages into the PDT-exposed tumor tissues (which in itself leads to a slowdown in tumor growth due to the release of tumor necrosis factor by macrophages), and due to the action of cytokines (interleukin 6 and interleukin 10) responsible for the antitumor effect of PDT [17, 22-28].

PDT opens up wide opportunities for various therapeutic effects of radical and palliative nature when other methods of treatment have already been exhausted or are not applicable at all.

The first publication on the use of PDT for inoperable common bile duct cancer in a female patient who had 7 PDT courses for 4 years appeared in 1991 [29, 30]. It was only much later that publications appeared on the few series of clinical observations on the use of PDT for bile duct cancer [21, 31, 32].

The analysis of literature sources on the use of various methods of elimination of obturation jaundice in ChC, i. e., surgical, combined and complex methods of ChC treatment, including literature reviews covering more than 2,200 patients undergoing percutaneous transhepatic biliary drainage and 8,100 patients undergoing endoscopic biliary drainage [27], indicates the unanimous opinion of researchers that the use of PDT for the treatment of DPC and extrahepatic bile duct cancer is possible in the cases when surgical intervention involves significant risk, in patients with severe comorbidities, in the presence of a disseminated, technically non-removable tumor (palliative PDT intended to relieve the bothersome symptom of mechanical jaundice due to the biliary tract obturation by a tumor), the presence of locoregional and remote metastases in patients (palliative PDT), and the patients' refusal of surgical treatment.

Materials and methods

The work performs a comparative analysis of the treatment effectiveness of two groups of patients with biliary tract cancer who had counterindications for radical surgical treatment.

We observed 79 patients with cancer of the major duodenal papilla and extrahepatic bile duct cancer. The main group included 29 patients who underwent percutaneous transhepatic drainage, endoscopic stent placement, or T-shaped drainage device installation during trial laparotomy in 2001-2015; at the second stage, after elimination or reduction of jaundice, PDT was applied. The control group included 50 patients who had only surgery to evacuate bile in the period from 1991 to 2001. Histological verification of the diagnosis confirmed that all patients had adenocarcinoma of various degrees of differentiation. In the main group, the tumor focus was localized in the porta hepatic in 2 (6.9%), in the choledochus, in 3 (10.3%), in the gall bladder and cystic duct, in 4 (13.8%), and in duodenal papilla, in 20 (69.0%) patients. In the control group, liver cancer was diagnosed in 21 (42.0%), choledochal cancer in 9 (18.0%), gallbladder and cystic duct cancer in 6 (12.0%), and duodenal papilla cancer in 14 (28.0%) patients.

The main reason for refusal to perform radical surgical treatment in the main group was the presence of severe somatic pathology and, therefore, a high anesthetic risk.

The clinical picture of patients was dominated by mechanical jaundice phenomena, and in this connection, the majority of patients underwent various palliative surgical interventions, depending on the location of the tumor. In the main group of 3 patients with DPC, the phenomena of mechanical jaundice were eliminated by conservative therapy and PDT, which resulted in recanalization of tumor stenosis, leading to an improvement in the outflow of bile to the duodenum. Later, those patients had stents installed in the duodenal papilla duct and terminal choledochus 6, 9, and 11 months after PDT due to the renewed growth of the tumor. On average, the jaundice-free period after PDT in mono mode was 7.8 months.

Depending on the dissemination of the tumor process and the conditions created by previous medical interventions, we used various methods of light delivery to the therapy site:

- endoscopic surface irradiation of the DPC from the side of the duodenal lumen with a light guide equipped with a straight diffuser;
- endoscopic endoluminal irradiation of the duodenal papilla duct and the terminal part of the common bile duct with a light guide with a 2 - 4 cm long cylindrical diffuser inserted through the mouth of the duodenal papilla or papillosphincterotomic opening;
- irradiation of the tumor with a light guide with a cylindrical diffuser delivered to the tumor side through the lumen;
- transfistulous endoluminal irradiation of the common bile duct, cystic and hepatic ducts with light guides with a 2-5 cm long cylindrical diffuser with radiopaque metal markers in the presence of a bile fistula created earlier for biliary tract decompression;
- combined methods of light guidance using surface irradiation of duodenal papilla from the duodenal lumen and the terminal choledochus, with the introduction of a light guide with a cylindrical diffuser from the duodenum or terminal choledochus with the introduction of a light guide through a drainage catheter or through a transhepatic drainage.

In the case of endoscopic irradiation with a light guide with a cylindrical diffuser, the latter was introduced through the endoscope's biopsy channel under visual

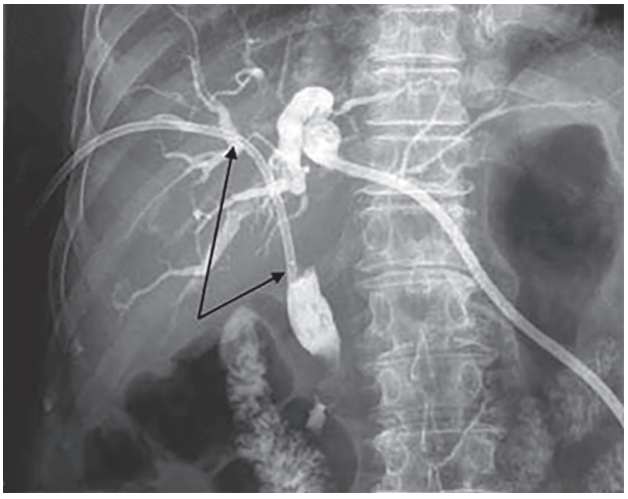

Рис. 1. Вариант чрезфистульного облучения у больной раком ворот печени Bismuth-IV с метастазами в печени и легких (продолжительность жизни 23 мес) после раздельного транспеченочного дренирования. Рентгеноконтрастные метки указаны стрелками
Fig. 1. Variation of transfistular irradiation in a patient with cancer of liver port Bismuth-IV with metastases in liver and lungs (survival time 23 months) after transherpatic draining. Radiopaque marks are indicated by arrows

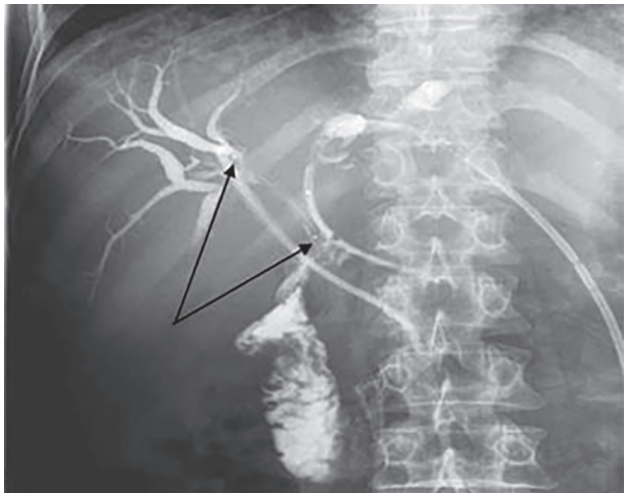

Рис. 2. Вариант чрезфистульного облучения у больной раком ворот печени Bismuth-IV после раздельной чрескожной холангиостомии. Рентгеноконтрастные метки указаны стрелками
Fig. 2. Variation of transfistular irradiation in a patient with cancer of liver port Bismuth-IV after transcutaneous cholangiostomy. Radiopaque marks are indicated by arrows

control into the choledochal lumen through the duodenal papilla mouth or papillosphincterotomic opening.

When using a light guide with a polished flat end or with a microlens at the end, the latter was also introduced through the endoscope biopsy channel, and the light irradiation was performed on the duodenal papilla tumor under visual control.

Transfistulous irradiation was performed in patients who had previously undergone surgical interventions in the form of external drainage of the bile ducts in order to address the issue of mechanical jaundice. Fistulography was performed, in which the extent of the tumor stricture and the adequacy of drainage standing were evaluated. Then a light guide with a cylindrical diffuser and radiopaque marks located on the borders of the diffuser was introduced through the drainage tube. The X-ray contrast markers being used for orientation, the diffuser was placed inside the stenosed bile duct with an overlap of the stenosis zone by 0.5 - 1 cm at each edge of the tumor (Fig. 1, 2). The length of the diffuser on the light guide was selected on the basis of the size of the tumor as determined by the length of the tumor stenosis.

After the placement of the light guide in the appropriate position, the drainage tube was pulled out, in part of in full, in order to avoid partial absorption of laser radiation by the walls of the drainage tube. With a stenosis length of more than 4-5 cm, light exposure was performed from several positions of the diffuser, moving the light guide under X-ray TV control. After the completion of the PDT procedure, the light guide was removed and the drainage tube was installed in the same position under x-ray control.

Transfistulous radiation was performed on 13 (46.4%) patients. In 4 of them, it was found impossible to re-install drainage in its initial position. The passage of bile is provided due to the expansion of the tumor-stenosed section of the bile duct as a result of PDT exposure.

The duration of the light exposure session in the PDT process was calculated based on the size of the tumor, the length of the diffuser of the light guide used, and the required specified energy density of the supplied laser radiation. This procedure was performed in an endoscopic or X-ray room, depending on the chosen method of laser radiation delivery. No pain relief was required during the procedure.

In case of the endoscopic method of irradiation, protease inhibitors and spasmolytics were prescribed in normal dosages in order to prevent the possible development of acute pancreatitis. Prevention measures were taken, depending on the situation, within 2 to 3 days after PDT.

Despite the satisfactory tolerability of the procedure by all patients, in the postoperative period 25 patients (89.3%) had a fever reaction and pain syndrome of varying severity, depending on the intensity of the photodynamic reaction of the tumor. Pain appeared, as a rule, 3 to 5 hours after the PDT session due to developing tissue edema, localized in the right hypochondrium and epigastric region, and completely stopped in 2 to 4 days. In 6 patients, it was necessary to administer narcotic an-

algesics to relieve the pain syndrome. Fever response, as an inflammatory component of PDT, was observed in 22 patients (75.8%). Body temperature normalized in 3 to 4 days after a PDT session. The remaining 4 patients had no adverse reactions associated with PDT.

The PS used was Photosens, sulfated aluminum phthalocyanin (FSUE «SSC NIOPIK», Russia, registration certificate PN000199/02 dated 04.03.2010) and Photoditazine, a derivative of e6 chlorin (OOO "VETA-GRAND", Russia, registration certificate no. ЛС 001246 dated 18.05.2012). Photosens accumulates in the tumor in a concentration that is 1.5 - 2 times higher than that in healthy tissues. Photoditazine is characterized by rapid accumulation in the tumor in maximum concentration after 2 to 3 hours, followed by a gradual decrease in concentration after 4 to 5 hours. After 24 hours, only trace quantities of the drug are detected in the blood, which makes it possible to avoid the long-term skin photosensitivity typical for first-generation PS products. Photoditazine has a high tropism to tumor tissues. According to various authors [35], the maximum contrast ratio varies from 5 to 20. Fotosens was administered in doses of 0.8-1.0 mg per kilo of body weight, Photoditazine was most often administered in doses of 0.6-0.7 mg per kilo of body weight, less often in 0.8-0.9 mg per kilo of body weight.

The PDT session was performed after a certain period of time corresponding to the achievement of maximum PS accumulation in the tumor. With Photosense used as PS, laser light exposure was performed after 24 hours from the moment of the drug administration, and with Photoditazine, after 2.5-3 hours.

The sources of laser radiation used were "Latus" semiconductor device (OOO "Atcus", Russia) with a maximum output optical power of 2 W and a wavelength of 662 nm, and LFT-02-Biospec laser system for photodynamic therapy (OOO "Biospec", Russia) with an output optical power of up to 2.5 W and a wavelength of 672 nm.

Laser irradiation was performed using flexible quartz monofilament light guides with a cylindrical diffuser and X-ray contrast markers, with an end diffuser or a microlens at the end (ZAO "Poluprovodnikovyie Pribory", OOO "Polironik", OOO "Elomed", Russia).

The output power at light exposure was 0.5 and 1.0 W, the power density was 200-500 mW/cm^2 or 200-500 mW/cm of the diffuser length. The energy density for endoluminal irradiation using light guides with a cylindrical diffuser ranged from 50 to 100 J/cm of the length of the diffuser to 250-300 J/cm.

Results

We managed to track the life expectancy of all patients in the main and control groups. The direct results of PDT application were evaluated based on clinical and instrumental data (X-ray computed tomography, magnetic resonance imaging and cholangiopancreatography, ultrasound, duodenoscopy with biopsy for duodenal papilla tumors). The number of PDT courses varied from 1 to 3 in a year. Follow-up examination of patients was performed in 3, 6, and 12 months after the treatment.

Despite the relatively satisfactory tolerability of the procedure by patients, after 12 (23%) PDT courses out of the 52 conducted, adverse reactions and complications were observed. The greatest number of complications was observed in patients with duodenal papilla carcinoma who had laser radiation brought to the affected area with the endoscopic method. The nature of adverse reactions and complications is shown in Table

The greatest number of adverse reactions and complications observed were in the form of cholangitis, which occurred 12-24 hours after the PDT session and was manifested as fever, the yellowing of the skin and sclera, increased blood bilirubin levels due to the direct fraction. Cholangitis was stopped with conservative treatment in 3 to 4 days. We do not consider the cholangitis symptoms as a specific complication of PDT. Cholangitis can occur as a result of any X-ray examination with contrast enhancement or an endoscopic intervention on the extrahepatic bile duct. Despite the preventive measures taken for all patients, one patient had pancreatitis sympthoms. They were also stopped by conservative measures in 3 days. Photosensitivity phenomena in the form of erythema of open skin areas were recorded in 1 patient, and are associated with prolonged excretion of Photosens and the patient's failure to observe the light regime.

The most severe complication was fibroulcerative duodenitis, which occurred in 3 cases, one of them with partial duodenal obstruction, which required longer-term conservative therapy. The phenomena of fibrinous-ulcerative duodenitis were observed in cases when Photosens was used, and are associated, in our opinion, with its lower tropicity to the tumor tissues compared to the preparations of the chlorin group.

The total number of complications was also higher when Photosens was used (58.3%) as PS, compared to Photoditazine (41.7%). There were no lethal outcomes.

The median survival time of patients who underwent PDT was 18 months (the minimum time being 12.5 months and the maximum 60 months). In the group where only palliative surgery was performed, the median survival was 11.5 months (minimum time: 4 months, maximum time: 14 months), $p < 0.0001$ (Fig. 3).

Discussion

In 1978, T. Dogherty published the first paper on the successful clinical use of PDT at the US National Cancer Institute. This article triggered the use of PDT for the treatment of cancer of superficial localizations and some visceral ones. After 7 years, J. S. McCaughan used PDT for a bile duct cancer patient [29]. It was a remarkable

Таблица
Побочные реакции и осложнения при ФДТ рака большого дуоденального сосочка и внепеченочных желчных протоков в зависимости от использованного фотосенсибилизатора, абс. ч.
Table
Adverse reactions and complications in PDT of cancer of the large duodenal papilla and extrahepatic bile ducts, depending on the photosensitizer used

Характер осложнения Type of complication	Фотосенс Photosens	Фотодитазин Fotoditazin	Всего Total
Панкреатит Pancreatitis	1		1
Холангит Cholangitis	1	5	6
Фибринозно-язвенный дуоденит Fibrinous-ulcerative duodenitis	3		3
Фибринозно-язвенный дуоденит с явлениями стеноза двенадцатиперстной кишки Fibrinous-ulcerative duodenitis with duodenal stenosis	1		1
Эритема кожи Skin erythema	1		1
Всего (%) Total (%)	7 (58,3%)	5 (41,7%)	12 (100%)

story of a persistent struggle for the life of a seemingly doomed person.

In 1985, a 50-year-old woman was diagnosed with a common bile duct adenocarcinoma during a cholecystectomy. A T-shaped drainage was left in place, and after 2 months, the first course of PDT with a hematoporphyrin derivative at a dose of 2 mg per kilo of body weight was performed during laparotomy. A light guide with a cylindrical diffuser was introduced with the use of a choledochoscope. After PDT, a U-shaped tube was left in the bile duct to prevent the development of jaundice. In the following 4 years, 6 more PDT courses were administered to the patient. The choledochoscope was inserted through a U-shaped drain tube. 2 years after the start of treatment for common bile duct cancer, the patient was diagnosed with endometrial cancer, for which she underwent surgery and radiation therapy. The patient died 4.5 years later [29].

In Russia, PDT was successfully used for the first time in 1998, for stage I duodenal papilla cancer (T1N0M0) in a 67-year-old patient who was admitted with mechanical jaundice and who had counterindications against surgical treatment because of a high degree of surgical and anesthetic risk due to severe concomitant diseases: coronary heart disease, bilateral nephrolithiasis, and pyelonephritis with partial renal failure. On 14.02.98, the pa-

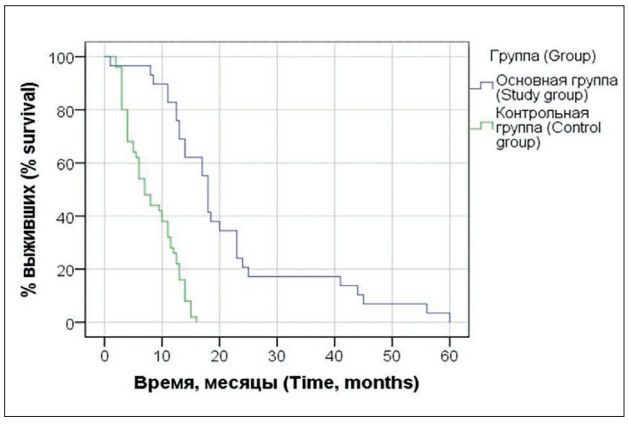

Рис. 3. Общая выживаемость пациентов основной и контрольной групп (по Каплан-Мейеру)
Fig. 3. Overal survival of patients of the study and control groups (Kaplan–Meier estimator)

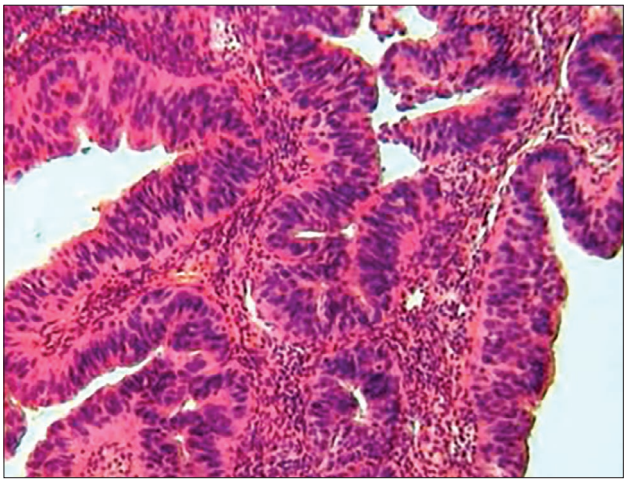

Рис. 4. Гистология. Высокодифференцированная темноклеточная сосочковая аденокарцинома. Окраска гематоксилин-эозином. Ув. х20. [25]
Fig. 4. Histology. Highly differentiated dark cell papillary adenocarcinoma (staining with hematoxylin-eosin, x20). [25]

tient underwent endoscopic papillosphincterotomy in Moscow City Clinical Hospital No. 1 (by Yu. V. Vasilenko). Histological examination revealed a tumor in the biopsy material that had the structure of a highly differentiated dark-cell papillary adenocarcinoma. Expressed cellular polymorphism with violation of the rows and polarity of tumor cells, with the presence of atypical mitoses in them. Tumor cells form glandular and glandular-like structures (Fig. 4) [25].

On 21.04.98, the patient had an intravenous injection of Photosens, a second-generation PS produced in Russia (FSUE «SSC NIOPIK», Russia, registration certificate PN000199/02 dated 04.03.2010) the dose being 0.8 mg per kilo of body weight, and after 24 hours a session of endoluminal laser irradiation was performed using a light guide with a 2 cm long cylindrical diffuser, and then, in addition, the duodenal papilla was superficially irradiated from the side of the duodenum lumen with a light guide with a polished end that creates direct exposure of the surface to the light beam. Irradiation was performed with ALT PDT-670/500 "Alpha-Photosens", a medical device manufactured by NPO "Polyus" based on a laser on yttrium aluminate with neodymium, generating light with a wavelength of 670 nm. Control gastroduodenoscopy performed 3 weeks after the course of PDT, revealed residual phenomena in the form of edema of the descending duodenum mucosa and the smoothness of the relief. The papilla tissues are edematous, whitish in color, dense. An influx of transparent bile from the choledochoduodenal anastomosis is observed, there are no signs of the violation of the patency of the terminal department of the choledoch. Histological conclusion on a biopsy from the papilla and the surrounding mucosa: there are signs of malignancy in the form of occasional glands, which have the epithelium characterized by polymorphism, hyperchromic nuclei, and single atypical mitoses. The surrounding tissues are observed to have the phenomena of sclerosis, edema, moderate inflammatory infiltration, dystrophic changes, which should be considered as manifestations of therapeutic pathomorphosis of the tumor [25] (Fig. 5).

The patient's condition was quite satisfactory, and the biochemical parameters normalized. Dynamic follow-up in 1, 5, 10, 16, 22, 24 and 30 months after PDT with a set of diagnostic tests (gastroduodenoscopy with multiple biopsies, computed tomography, ultrasound of the abdominal cavity) did not reveal a recurrence of the duodenal papilla tumor or its metastases. The results of a control histological examination 22 months after PDT: the mucous membrane with the phenomena of stroma edema, hyperplasia of part of the glands, and moderate signs of inflammatory changes. In some areas, the phenomena of fibrosis, uneven edema of the stroma with inflammatory infiltration consisting mainly of lymphocytes, are observed. No signs of malignant cells were found [25] (Fig. 6). The patient's body mass increased by 7 kg. The patient was observed for 3.5 years after the PDT course and died of concomitant pathology without signs of major duodenal papilla cancer recurrence [27].

Similar descriptions on the use of PDT of inoperable ChC can be found in the literature starting from the mid-1990s. [21, 31, 32]. For instance, M. Ortner et al. reported the effective use of PDT of unresectable ChC in 9 patients who earlier had had ineffective bile duct drainage by stenting [31].

Patients were intravenously administered Photophrin at a dose of 2 mg per kilo of body weight, and after 2 days, a session of endoluminal light exposure was performed with cholangioscope that had wavelength of 630 nm and energy density of 180 J/cm^2. Patients had a decrease in serum bilirubin for at least 2 months, and an improvement in the quality of life when assessed on the Karnofsky Scale from 32% before treatment to 70% after PDT. Over the 30-day period, there were no lethal outcomes.

At the 8th International Photodynamic Association World Congress (Vancouver, Canada, 2001), 2 reports were made on the use of PDT in ChC. A group of authors from Germany reported on PDT to 9 patients with ChC. 48 hours after intravenous administration of Photophrin at a dose of 2 mg per kilo of body weight, a PDT session was performed with a light source wavelength of 630 nm, light guides with a 3-7 cm long cylindrical diffuser, the effective fields overlapping the proximal and distal edges of the tumor stenosis. The luminous density was 180 J/cm^2 of the diffuser length. The light guide was introduced by retrograde cholangiopancreatography

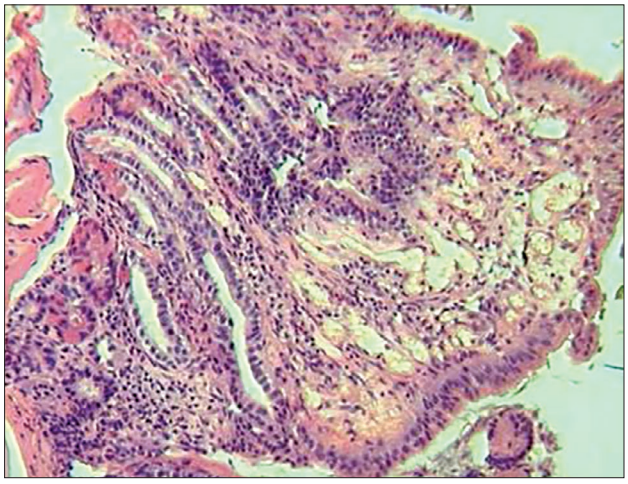

Рис. 5. Гистология. Аденокарцинома большого сосочка двенадцатиперстной кишки. Проявления терапевтического патоморфоза опухоли через 3 недели после фотодинамической терапии. Окраска гематоксилин-эозином. Ув. х20. [25]
Fig. 5. Histology. Adenocarcinoma of the major duodenal papilla. Manifestation of therapeutic pathomorphosis 3 weeks after PDT (staining with hematoxylin-eosin, x20). [25]

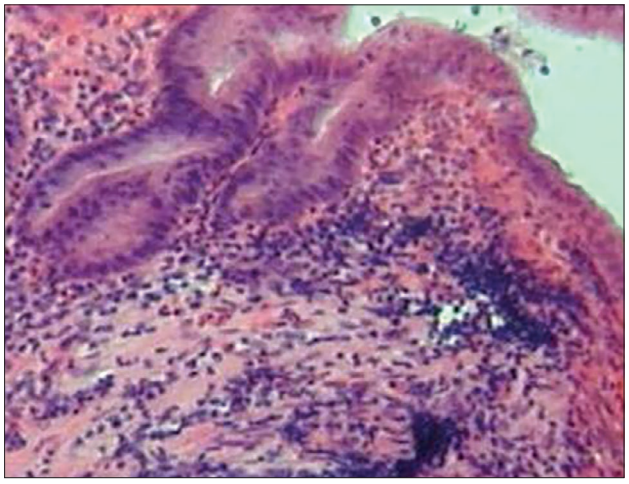

Рис. 6. Гистология. Большой дуоденальный сосочек. слизистая оболочка с явлениями отека стромы, гиперплазии части желез и умеренными признаками воспалительных изменений без признаков злокачественного роста. Окраска гематоксилин-эозином. Ув. х20. [25]
Fig. 6. Histology. Large duodenal papilla, mucosa with signs of edema, hyperplasia of some glands and moderate signs of inflammatory changes but no malignant growth (staining with hematoxylin-eosin, x20). [25]

method or through percutaneous drainage. For 3 patients who had only partial effect or none at all, PDT was repeated in 3 months. At the end of the PDT session, all patients were fitted with 1 or 2 plastic stents or had a percutaneous biliary drainage. Of the 9 patients, 3 were alive in the period from 51 to 225 days.

The median survival was 78 days, with variations from 12 to 371 days. The survival rate of patients depended on the localization of the tumor in the bile ducts according to the Bismuth classification. With multiple foci and lesions of the right and left hepatic ducts (Bismuth IV), the 90-day mortality rate was 80%. In all cases, the death was caused by the dissemination of the process. The authors conclude that PDT of disseminated ChC is a new promising local method of treatment without severe complications that increases the life expectancy of this category of patients [28].

Another report from the California Pancreatic and Biliary Diseases Institute (Los Angeles, USA) discusses the effectiveness of a combination of treatment methods, including endoscopic metal stent placement, high-dose brachytherapy, and PDT, in 13 patients with disseminated inoperable ChC Bismuth type III and IV. Stents were placed in the right and left hepatic ducts. Brachytherapy was performed in 3 patients at a dose of 30 Gy, coarsely fractionated, while 10 patients received 60 Gy in 2 separate courses.

PDT was performed 2 days after intravenous administration of hematoporphyrin at a dose of 2 mg per kilo of body weight by endoscopic intraluminal irradiation. As a result, the bilirubin level in the patients' blood serum decreased, and the quality of life and survival increased. The average life expectancy (17 months) was slightly different from that after brachytherapy administered as a single therapy. However, it significantly exceeded the quality of life and life expectancy of patients who were treated only with drainage and chemotherapy [33].

In the Russian Federation, only a few works on the use of PDT in the treatment of ChC were published [1-3]. A group of authors from the St. Petersburg I. I. Mechnikov State Medical Academy reported the use of PDT in 6 patients with common hepatic duct cancer. At the first stage, drainage of the bile ducts was performed, which was followed, after the elimination of the cholestasis phenomena, by a course of PDT with Radachlorine, with the light energy density of 200 J/cm^2. The average life expectancy was 360 days. All patients had progressive tumor growth along the duct. The authors consider PDT as a method of choice for palliative therapy in inoperable hepaticocholedochus tumors.

Analytical reviews of the literature on the issue published in the last 10 years confirm that PDT is useful in the treatment of inoperable ChC [5, 9, 11, 12, 16, 27].

Our data on the analysis of PDT effectiveness for duodenal papilla and extrahepatic bile duct cancer in inoperable patients in comparison with the results of drainage and other palliative operations are comparable with the results obtained by other authors.

Conclusion

Our research has shown that PDT in combination with bile-evacuating surgery is an effective method of treating inoperable patients with DPC and extrahepatic bile duct cancer; it is not accompanied by severe complications and is easily tolerated by patients. PDT is the optimal method for treating and prolonging the life of patients with somatically burdened history who have counterindications to radical surgical treatment, or in cases when such treatment involves a high risk of lethal outcome.

The methods developed for bringing laser radiation to the site of exposure (endoscopic surface, endoscopic intraluminal, transfistulous intraluminal) provide adequate access to all departments of the extrahepatic bile ducts for PDT.

Fotoditazine and Photosens used for PDT have comparable clinical effectiveness, but the use of Fotoditazine is preferable due to fewer adverse reactions and complications.

The results of PDT in terms of life expectancy are quite comparable to radical surgery and exceed those for palliative surgery.

REFERENCES

1. Dolgushin B.I., Sergeeva O.N., Frantsev D.Yu., Kukushkin A.V., Panov V.O., Virshke E.R., Kosyrev V.Yu., Cherkasov V.A., Trofimov I.A., Chistyakova O.V., Moroz E.A., Pogrebnyakov I.V., Shishkina N.A. Intraductal photodynamic therapy for portal cholangiocarcinoma in inoperable patients, *Annaly khirurgicheskoi gepatologii*, 2016, vol. 21, no. 3, pp. 106–118. (in Russ.)
2. Lazarev S.M., Savinov I.P., Ivanov A.S., Drach L.L., Muradov G.G. Ways to increase the effectiveness of photodynamic therapy of Klatskin tumors, *Lazernaya meditsina*, 2011, vol. 15, no. 2, pp. 66. (in Russ.)
3. Nechushkin M.I., Patyutko Yu.I., Dolgushin B.I. i dr. Sovremennaya strategiya lecheniya raka vnepechenochnykh zhelchnykh protokov in *Vozmozhnosti sovremennoi onkologii v diagnostike i lechenii zlokachestvennykh zabolevanii* [The possibilities of modern oncology in the diagnosis and treatment of malignant diseases]. Moscow, 2003. pp. 96–99.
4. Stranadko E.F. The main stages of development and the current state of photodynamic therapy in Russia, *Lazernaya meditsina*, 2012, vol. 16, no. 2, pp. 4–14. (in Russ.)
5. Agostinis P., Berg K., Cengel K.A., Foster T.H., Girotti A.W., Gollnick S.O., Hahn S.M. Photodynamic therapy of cancer: an update, *Cancer J. Clin.*, 2011, vol. 61(4), pp. 250–281.
6. Talreja J.P., Kahaleh M. Photodynamic therapy for cholangiocarcinoma, *Gut and Liver*, 2010, vol. 4, suppl. 1, pp. 62–66.
7. Cai Y., Cheng N., Ye H., Li F., Song P., Tang W. The current management of cholangiocarcinoma: A comparison of current guidelines, *BioScience Trends*, 2016, vol. 10(2), pp. 92–102.
8. Lewis H.L., Rahnemai-Azar A.A., Dillhoff M., Schmidt C.R., Pawlik T.M. Current management of perihilar cholangiocarcinoma and future perspectives, *Chirurgia*, 2017, vol. 112(3), pp. 193–207.
9. Rizvi S., Khan S.A., Hallemeier C.L., Kelley R.K., Gores G.J. Cholangiocarcinoma – evolving concepts and therapeutic strategies, *Clinical oncology*, 2018, vol. 15(2), pp. 95–111.
10. Wang Y., Pang Q., Jin H., Zhou L., Hu X., Qian Z., Man Z., Yang S., Liu H. Albumin-bilirubin grade as a novel predictor of survival in advanced extrahepatic cholangiocarcinoma, *Gastroenterology research and practice*, 2018, 8902146. doi: 10.1155/2018/8902146
11. Gao F., Bai Y., Ma S.R., Liu F., Li Z.S. Systematic review: photodynamic therapy for unresectable cholangiocarcinoma, *J Hepatobiliary Pancreat Science*, 2010, vol. 17(2), pp. 125–131.
12. Squadroni M., Tondulli L., Gatta G., Mosconi S., Beretta G., Labianca R. Cholangiocarcinoma, *Critical reviews in oncology/hematology*, 2017, vol. 116, pp. 11–31.
13. Patyutko Yu.I., Kotel'nikov A.G., Kosyrev V.Yu. Mikhailov M.M., Sokolova I.N., Sagaidak I.V., Akhmetov M.Sh. Current data on the possibilities of surgical treatment of patients with pancreatic and

ЛИТЕРАТУРА

1. Долгушин Б.И., Сергеева О.Н., Францев Д.Ю. и др. Внутрипротоковая фотодинамическая терапия при воротной холангиокарциноме у неоперабельных больных // Анналы хирургической гепатологии. – 2016. – Т. 21, № 3. – С. 106–118.
2. Лазарев С.М., Савинов И.П., Иванов А.С. и др. Пути повышения эффективности фотодинамической терапии при опухолях Клатскина // Лазерная медицина. – 2011. – Т. 15, № 2. – С. 66.
3. Нечушкин М.И., Патютко Ю.И., Долгушин Б.И. и др. Современная стратегия лечения рака внепеченочных желчных протоков. В кн.: Возможности современной онкологии в диагностике и лечении злокачественных заболеваний. – Москва, 2003. – С. 96–99.
4. Странадко Е.Ф. Основные этапы развития и современное состояние фотодинамической терапии в России // Лазерная медицина. – 2012. – Т. 16, № 2. – С.4–14.
5. Agostinis P., Berg K., Cengel K.A., et al. Photodynamic therapy of cancer: an update // Cancer J. Clin. – 2011. – Vol. 61(4). – P. 250–281.
6. Talreja J.P., Kahaleh M. Photodynamic therapy for cholangiocarcinoma // Gut and Liver. – 2010. – Vol. 4, Suppl. 1. – P. 62–66.
7. Cai Y., Cheng N., Ye H., et al. The current management of cholangiocarcinoma: A comparison of current guidelines // BioScience Trends. – 2016. – Vol. 10(2). – P. 92–102.
8. Lewis H.L., Rahnemai-Azar A.A., Dillhoff M., et al. Current management of perihilar cholangiocarcinoma and future perspectives // Chirurgia. – 2017. – Vol. 112(3). – P. 193–207.
9. Rizvi S., Khan S.A., Hallemeier C.L., et al. Cholangiocarcinoma – evolving concepts and therapeutic strategies // Clinical oncology. – 2018. – Vol. 15(2). – P. 95–111.
10. Wang Y., Pang Q., Jin H., et al. Albumin-bilirubin grade as a novel predictor of survival in advanced extrahepatic cholangiocarcinoma // Gastroenterology research and practice. – 2018. – 8902146. doi: 10.1155/2018/8902146
11. Gao F., Bai Y., Ma S.R., et al. Systematic review: photodynamic therapy for unresectable cholangiocarcinoma // J Hepatobiliary Pancreat Science. – 2010. – Vol. 17(2). – P. 125–131.
12. Squadroni M., Tondulli L., Gatta G., et al. Cholangiocarcinoma // Critical reviews in oncology/hematology. – 2017. – Vol. 116. – P. 11–31.
13. Патютко Ю.И., Котельников А.Г., Косырев В.Ю. и др. Современные данные о возможностях хирургического лечения больных раком поджелудочной железы и периампулярной зоны // Современная онкология. – 2000. – Т. 2, № 1. – С. 12–15.
14. Witzigmann H., Berr F., Ringel U., et al. Surgical and palliative management and outcome in 184 patients with hilar cholangiocarcinoma: palliative photodynamic therapy plus stenting is comparable to r1/r2 resection // Ann Surg. – 2006. – Vol. 244. – P. 230–239.
15. Wiedmann M., Caca K., Berr F., et al. Neoadjuvant photodynamic

periampullary cancers, *Sovremennaya onkologiya*, 2000, vol. 2, no. 1, pp. 12–15. (in Russ.)
14. Witzigmann H., Berr F., Ringel U., Caca K., Uhlmann D., Schoppmeyer K., Tannapfel A., Wittekind C., Mossner J., Hauss J., Wiedmann M. Surgical and palliative management and outcome in 184 patients with hilar cholangiocarcinoma: palliative photodynamic therapy plus stenting is comparable to r1/r2 resection, *Ann Surg*, 2006, vol. 244, pp. 230–239.
15. Wiedmann M., Caca K., Berr F., Schiefke I., Tannapfel A., Wittekind C., Mössner J., Hauss J., Witzigmann H. Neoadjuvant photodynamic therapy as a new approach to treating hilar cholangiocarcinoma, *American cancer society*, 2003, vol. 97(11), pp. 2783–90.
16. Fayter D., Corbett M., Heirs M., Fox D., Eastwood A. A systematic review of photodynamic therapy in the treatment of precancerous skin conditions, Barretts oesophagus and cancers of the biliary tract, brain, head and neck, lung, oesophagus and skin, *Health Technol Assess*, 2010, vol. 14(37), pp. 1–288.
17. Gollnick S.O., Liu X., Owczarczak B., Musser D.A., Henderson B.W. Altered expression of interleukin 6 and interleukin 10 as a result of the photodynamic therapy in vivo, *Cancer Res*, 1997, vol. 57(18), pp. 3904–3909.
18. Ortner M.A., Dorta G. Technology insight: photodynamic therapy for cholangiocarcinoma, *Nat Clin Pract Gastroenterol Hepatol*, 2006, vol. 8, no. 3, pp. 459–467.
19. Ortner M.A. Photodynamic therapy for cholangiocarcinoma, *Lasers Surg Med*, 2011, vol. 43(7), pp. 776–780.
20. Stranadko E.F. Mechanisms of photodynamic therapy, *Rossiiskii onkologicheskii zhurnal*, 2000, no. 4, pp. 52–56. (in Russ.)
21. Abulafi A.M., Allardice J.T., Williams N.S., van Somern N., Swain C.P., Ainley C. Photodynamic therapy for malignant tumors of the ampulla of Vater, *Gut*, 1995, vol. 36(6), pp. 853–856.
22. Gollnick S.O., Owczarczak B., Maier P. Photodynamic therapy and anti-tumor immunity, *Lasers Surg Med*, 2006, vol. 38, pp. 509–515.
23. Korbelik M., Stott B., Sun J. Photodynamic therapy-generated vaccines; relevance of tumor cell death expression, *Br. J. Cancer*, 2007, vol. 97, pp. 1381–1387.
24. Korbelik M., Cecic I., Merchant S., Sun J. Acute phase response induction by cancer treatment with photodynamic therapy, *Int. J. Cancer*, 2008, vol. 122, pp. 1411–1417.
25. Stranadko E.F., Meshkov V.M., Vasilenko Yu.V., Ryabov M.V., Makhinya V.A., Volkova N.N. Photodynamic therapy of major duodenal papilla cancer, *Lazernaya meditsina*, 2002, vol. 6, no. 1, pp. 9–13. (in Russ.)
26. Stranadko E.F., Vasilenko Yu.V., Lobakov A.I., Meshkov V.M. i dr. Possibilities of palliative photodynamic therapy in endoscopic practice, *Annaly khirurgii*, 2003, no. 2, pp. 20–24. (in Russ.)
27. Duan F., Cui L., Bai Y., Li X., Yan J., Liu X. Comparison of efficacy and complications of endoscopic and percutaneous biliary drainage in malignant obstructive jaundice: a systematic review and meta-analysis, *Cancer imaging*, 2017, vol. 17(1), pp. 27–32.
28. Nesbigall T., Huttenberger D., Albert F.W. Outcome of Patients with Cholangiocarcinoma after Local Treatment with Photodynamic Therapy. *IPA 8th World Congress of Photodynamic Medicine June 5 – 9. Clinical and Basic Applications of Photodynamic Medicine*. Vancouver, 2001. pp. 27–28.
29. McCaughan J.S., Mertens B.F., Cho C., Barabash R.D., Payton H.W. Photodynamic therapy to treat tumors of the extrahepatic biliary ducts. A case report, *Arch. Surg*, 1991, vol. 126, pp. 111–113.
30. *A clinical manual: Photodynamic Therapy of Malignancy* by McCaughan J.S. as edt. Austin: R.G. Landes Company, 1992. 248 p.
31. Ortner M.A., Liebetruth J., Schrejber S., Hanft M., Wruck U., Fusco V., Müller J.M., Hörtnagl H., Lochs H. Photodynamic therapy of nonresectable cholangiocarcinoma, *Gastroenterology*, 1998, vol. 114(3), pp. 536–542.
32. Ortner M.A., Caca K., Berr F., Liebetruth J., Mansmann U., Huster D., Voderholzer W., Schachschal G., Mössner J., Lochs H. Succesful photodynamic therapy for nonresectable cholangiocarcinoma: a therapy as a new approach to treating hilar cholangiocarcinoma // American cancer society. – 2003. – Vol. 97(11). – P. 2783–90.
16. Fayter D., Corbett M., Heirs M., et al. A systematic review of photodynamic therapy in the treatment of precancerous skin conditions, Barretts oesophagus and cancers of the biliary tract, brain, head and neck, lung, oesophagus and skin // Health Technol Assess. – 2010. – Vol. 14(37). – P. 1–288.
17. Gollnick S.O., Liu X., Owczarczak B., et al. Altered expression of interleukin 6 and interleukin 10 as a result of the photodynamic therapy in vivo // Cancer Res. – 1997. – Vol. 57(18). – P. 3904–3909.
18. Ortner M.A., Dorta G. Technology insight: photodynamic therapy for cholangiocarcinoma // Nat Clin Pract Gastroenterol Hepatol. – 2006. – Vol. 8, No. 3. – P. 459–467.
19. Ortner M.A. Photodynamic therapy for cholangiocarcinoma // Lasers Surg Med. – 2011. – Vol. 43(7). – P. 776–780.
20. Странадко Е.Ф. Механизмы действия фотодинамической терапии // Российский онкологический журнал. – 2000. – № 4. – С. 52–56.
21. Abulafi A.M., Allardice J.T., Williams N.S., et al. Photodynamic therapy for malignant tumors of the ampulla of Vater // Gut. – 1995. – Vol. 36(6). – P. 853–856.
22. Gollnick S.O., Owczarczak B., Maier P. Photodynamic therapy and anti-tumor immunity // Lasers Surg Med. – 2006. – Vol. 38. – P. 509–515.
23. Korbelik M., Stott B., Sun J. Photodynamic therapy-generated vaccines; relevance of tumor cell death expression // Br. J. Cancer. – 2007. – Vol. 97. – P. 1381–1387.
24. Korbelik M., Cecic I., Merchant S., Sun J. Acute phase response induction by cancer treatment with photodynamic therapy // Int. J. Cancer. – 2008. – Vol. 122. – P. 1411–1417.
25. Странадко Е.Ф., Мешков В.М., Василенко Ю.В., Рябов М.В., Махиня В.А., Волкова Н.Н. Фотодинамическая терапия рака Фатерова соска // Лазерная медицина. – 2002. – Т. 6, № 1. – С. 9–13.
26. Странадко Е.Ф., Василенко Ю.В., Лобаков А.И., и др. Возможности паллиативной фотодинамической терапии в эндоскопической практике // Анналы хирургии. – 2003. – № 2. – С. 20–24.
27. Duan F., Cui L., Bai Y., et al. Comparison of efficacy and complications of endoscopic and percutaneous biliary drainage in malignant obstructive jaundice: a systematic review and meta-analysis // Cancer imaging. – 2017. – Vol. 17(1). – P. 27–32.
28. Nesbigall T., Huttenberger D., Albert F.W. Outcome of Patients with Cholangiocarcinoma after Local Treatment with Photodynamic Therapy // IPA 8th World Congress of Photodynamic Medicine June 5 – 9. Clinical and Basic Applications of Photodynamic Medicine. – Vancouver, 2001. – P. 27–28.
29. McCaughan J.S., Mertens B.F., Cho C., et al. Photodynamic therapy to treat tumors of the extrahepatic biliary ducts. A case report // Arch. Surg. – 1991. – Vol. 126. – P. 111–113.
30. A clinical manual: Photodynamic Therapy of Malignancy / McCaughan J.S. – Austin: R.G. Landes Company, 1992. – 248 p.
31. Ortner M.A., Liebetruth J., Schrejber S., et al. Photodynamic therapy of nonresectable cholangiocarcinoma // Gastroenterology. – 1998. – Vol. 114(3). – P. 536–542.
32. Ortner M.A., Caca K., Berr F., et al. Succesful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study // Gastroenterology. – 2003. – Vol. 125. – P. 1355–1366.
33. Renner I.G. Photodynamic Therapy for Cholangiocarcinoma // IPA 8th World Congress of Photodynamic Medicine June 5 – 9. Clinical and Basic Applications of Photodynamic Medicine. – Vancouver, 2001. – P. 28.

randomized prospective study, *Gastroenterology*, 2003, vol. 125, pp. 1355–1366.

33. Renner I.G. Photodynamic Therapy for Cholangiocarcinoma. *IPA 8th World Congress of Photodynamic Medicine June 5 – 9. Clinical and Basic Applications of Photodynamic Medicine*, Vancouver, 2001, pp. 28.

TRANSPUPILLARY THERMOTHERAPY OF CHOROIDAL MELANOMA

Naumenko L.V.
N.N. Alexandrov National Cancer Center, Lesnoy, Belarus

Abstract

A retrospective analysis of the immediate and long-term effectiveness of the treatment of the choroidal melanoma using transpupillary thermotherapy (TTT) was carried out. The study included 84 patients with choroidal melanoma (C69.3) who received treatment between 2007 and 2018. Patients were sampled from the Belarusian Cancer Register. The average values of the thickness of the tumor were 2.6±1.3 mm, the diameter of the base - 7.2±3.3 mm. TTT was carried out using a diode laser with a wavelength of 860 nm and radiation power of 200 - 800 mW, the exposure time was 60 s, and the diameter of the laser spot was varied between 1 and 3 mm. The entire surface of the tumor was thermally treated with overlapping fields from the periphery to the top. 75 patients underwent a single session of TTT, while 9 - two sessions with an interval of 3–4 weeks. As a result of the treatment, 51 (60.7%) patients showed complete tumor resorption, 28 (33.3%) patients had stabilization of the tumor process, and 5 (6.0%) patients had no effect. In the group of patients with stabilization of the tumor process, continued growth was recorded in 16 (19.1%) patients with follow-up periods of 3 months to 4 years. In 19 (37.3%) patients from the group with complete tumor regression, relapse was observed 1 to 8 years after TTT. Metastatic disease (disease progression) developed in 5 (5.9%) patients, of which in 1 patient during the first 12 months, in 1 patient - after 4 years, and in 3 patients more than after 5 years of the follow-up observation. Analysis of the effectiveness of TTT of choroidal melanoma showed that an increase in the thickness and diameter of the base of the tumor focus results in the decrease of immediate effectiveness, and the rise of the likelihood of continued tumor growth.

Keywords: choroidal melanoma, transpupillary thermotherapy, photodynamic therapy, brachytherapy.

For citations: Naumenko L.V. Transpupillary thermotherapy of choroidal melanoma, *Biomedical Photonics*, 2020, vol. 9, no. 2, pp. 29–35. (in Russian) doi: 10.24931/2413–9432–2020–9–2–29–35.

Contacts: Naumenko L.V., e-mail: larisanau@mail.ru

ТРАНСПУПИЛЛЯРНАЯ ТЕРМОТЕРАПИЯ МЕЛАНОМЫ СОСУДИСТОЙ ОБОЛОЧКИ ГЛАЗА

Л.В. Науменко
Республиканский научно-практический центр онкологии и медицинской радиологии им. Н.Н. Александрова, Лесной, Республика Беларусь

Резюме

Проведен ретроспективный анализ непосредственной и отдаленной эффективности лечения меланомы сосудистой оболочки глаза методом транспупиллярной термотерапии (ТТТ). Исследование включало 84 пациента с меланомой сосудистой оболочки глаза (С69.3), получивших лечение в период 2007–2018 гг. Выборка больных проведена из базы Белорусского канцер-регистра. Средние значения толщины опухоли составили 2,6±1,3 мм, диаметра основания – 7,2±3,3 мм. ТТТ проводили с использованием диодного лазера, длина волны 860 нм, мощность излучения 200 – 800 мВт, экспозиция 60 с, диаметр лазерного пятна от 1 до 3 мм. Термическому воздействию подвергали всю поверхность опухоли, поля располагали с перекрытием, от периферии к вершине опухоли. У 75 пациентов проведен один, у 9 – два сеанса ТТТ с интервалом 3 – 4 нед. В результате проведенного лечения у 51 (60,7%) пациента зарегистрирована полная резорбция опухоли, у 28 (33,3%) – стабилизация опухолевого процесса, у 5 (6,0%) – отсутствие эффекта от лечения. В группе пациентов со стабилизацией опухолевого процесса продолженный рост зафиксирован у 16 (19,1%) при сроках наблюдения от 3 мес до 4 лет. У 19 (37,3%) больных из группы с полной регрессией опухоли зарегистрирован рецидив заболевания на сроках от 1 до 8 лет наблюдения после проведения ТТТ. Метастатическая болезнь (прогрессирование заболевания) развилась у 5 (5,9%) пациентов, из них в течение первых 12 мес – у 1 больного, через 4 года – у 1, в сроки наблюдения более 5 лет – у 3. Анализ эффективности ТТТ в зависимости от размеров меланомы хориоидеи показал, что с увеличением толщины и диаметра основания опухолевого очага непосредственная эффективность снижается, а вероятность продолженного роста опухоли возрастает.

Ключевые слова: меланома хориоидеи, транспупиллярная термотерапия, фотодинамическая терапия, брахитерапия.

Для цитирования: Науменко Л.В. Транспупиллярная термотерапия меланомы сосудистой оболочки глаза // Biomedical Photonics. – 2020. – Т. 9, № 2. – С. 29–35. doi: 10.24931/2413–9432–2020–9–2–29–35.

Контакты: Науменко Л.В., e-mail: larisanau@mail.ru

Introduction

Melanoma of the choroid of the eye is the most common tumor in the membranes of the eye among adult population. According to the Belarusian Cancer Registry, the gross intensive incidence rate per 100,000 population was 1.1 in 2011, and 1.2 in 2016 [1]. Over a 5–year period, the detection rate of stage I of the disease increased from 5.4% in 2011 to 16.4% in 2016 [2].

Despite a large range of treatment possibilities, choroidal melanoma is prone to hematogenous metastasis, which leads to a fatal outcome. Improving patient survival is possible only when the tumor is diagnosed at the early stages of the disease. Small tumors are now treated with laser technologies, one of which is transpupillary thermotherapy (TTT). The method is non-invasive. The infrared spectrum of a diode laser is delivered with a wavelength of 810 nm to the surface of choroidal melanoma (CM) through transparent optical media of the eye at the height of the cycloplegia [3, 4]. When exposed to the laser, the temperature of the tumor increases to 45-60° C, which leads to obliteration of the tumor vessels with the development of necrosis. According to various authors, lasers can achieve therapeutic effect if the tumor is no more than 2 mm thick [5].

C.L. Shields, J.A. Shields et al. believe that the maximum depth of penetration of TTT is 4 mm, which allows treatment of small CM tumors [3]. Absorption of diode laser radiation is higher in more pigmented tumors. The advantages of TTT in comparison with radiation therapy include precise localization of the laser beam, rapid tumor necrosis, and the ability to treat patients on an outpatient basis with minimal damage to the surrounding intact vascular membrane. According to various authors, tumor relapses after TTT in CM range from 9 to 28% [3]. Due to the energy of the laser beam, complications may include tractional retinal detachment (44%), retinal vein occlusion (26–41%), retinal artery occlusion (12%), cystic macular edema (9–23%), epiretinal membrane development (23%), vitreous hemorrhage (10%), retinal neovascularization (6%), foveal traction (4%), chorioretinal scarring (4%), optic disc atrophy (2%), regmatogenic (1%) and serous retinal detachment (1%), optic disc edema (<1%), and cataract (<1%) [3, 5].

A direct correlation was found between the development of disease recurrence and the number of prognostic risk factors (RF) for tumor growth. The latter include tumor thickness of > 2 mm (according to ultrasonography), the presence of subretinal fluid found at optical coherence tomography, clumped orange pigment, the localization of tumor edge near the optic nerve disc, hypoechoic character of the tumor with hyperreflexive contour, absence of drusen, the base diameter being > 5 mm [5]. According to A. Mashayekhi et al., the relapse rate after 10 years of follow-up was 18% in patients with one to two RFs, 35% in those with three to five RFs, and 55% in people with more than six RFs [6]. TTT is less preferable in cases where the number of RFs exceeds three. S. Turcotte et al. presented the results of treatment of 8 patients with CM who received an average of three sessions of TTT. The average thickness of the tumor before treatment was 2±0.8 mm. In 3 (38%) patients, continued growth of the tumor was registered. No deaths due to metastases were registered. The authors conclude that patients with continued tumor growth have a high risk of relapse when the number of RFs is more than one. Loss of visual acuity directly related to TTT treatment was observed in 25% of the patients [7].

In a prospective non-randomized study, M. M. Chojniak et al. [8] evaluated the results of treatment of 27 patients with an average tumor thickness of 2.7 mm and a base diameter of 8.52 mm. After 3 sessions of treatment with a follow-up period of 45 months, the average thickness of the tumor decreased to 1.34 mm ($p<0.001$), and the diameter of the base of the tumor, to 5.48 mm ($p<0.001$). Complications were observed in 12 (44%) patients and included retinal vascular occlusion, optic disc atrophy, vitreous hemorrhage, regmatogenic retinal detachment, and maculopathy. The rate of optic disc atrophy after treatment was high, being observed in 60% of patients. Visual acuity remained unchanged in 9 (33%) patients, improved in 5 (19%) and decreased during the first 6 months after treatment in 13 (48%). Marginal recurrence of the tumor was detected in 2 (7%) patients. The eyes were preserved in all patients. In 1 case, the development of metastases was observed. The authors conclude that TTT is an effective treatment for small-sized CM. Reduced visual acuity occurred in the early stages after treatment and was mainly associated with the treatment of subfoveal and perifoveal tumors. According to the authors, it is necessary to conduct long-term randomized studies in order to achieve a better insight into the effectiveness of the treatment method.

B. M. Stoffelns et al. evaluated 10-year TTT results in 26 patients with small-sized CMs. The tumors were located behind the equator and had a base diameter of ≤12 mm and a thickness of ≤4.5 mm. The patients had an average of 1.4 treatment sessions. Ten years after treatment, tumor regression was achieved in 16 patients, initial regression with subsequent tumor recurrence in 6, and insufficient tumor regression in 4. Two patients died due to metastatic liver damage. The authors conclude that MCs treated with TTT require careful monitoring, as local relapses and complications later develop in the treatment area. Complications were observed in 14 patients, including maculopathy in 8 cases, macular edema in 6, choroidal neovascularization in 4, and posterior synechiae with iris atrophy in 1 case [9].

In the study of A. A. Yarovoy et al. ("MNTK "Eye Microsurgery"", Russia), 78 CM patients with a tumor thickness of up to 3.6 mm were treated; the follow-up period

ranged from 2.5 to 108 months. The criteria for evaluating the treatment were the status localis, the number of enucleations, tumor relapses, complications, and visual acuity. In 51 patients, the tumor regressed completely, and in 20 patients, partially. The absence of results from treatment was registered in 7 cases: 2 of them were followed with ophthalmectomy, and 5 with brachytherapy with ^{106}Ru. Tumor recurrence was detected in 10 patients within 7 to 54 months after the end of the treatment. It should be noted that in 8 patients the tumor regression was partial. Additional TTT was used to successfully treat 6 patients with relapsed disease, and 4 were administered brachytherapy. No metastases were registered. The authors concluded that the main prognostic adverse factors of TTT are the thickness of the tumor of more than 3 mm, basal diameter of more than 10.2 mm, non-pigmented tumor, maximum systolic blood flow rate of more than 11.7 cm/s, the presence of subretinal fluid and incomplete regression after treatment. The TTT method is an experimental method for treating CM. Patient preparation for TTT has to be based on careful selection of patients with due consideration of prognostic factors and functional perspectives [10].

Data from a retrospective review by K. Gündüz et al. [11] on the use of TTT in 20 patients with CM and 4 with choroidal nevi, showed that patients with vascular nevi were found to have risk factors for the development of melanoma, or secondary neovascularization of the vascular membrane was observed. The average initial initial diameter of the tumor was 6.6 mm, and its thickness was 3 mm. The average number of TTT sessions was 2.5 (from 1 to 6), and the average resorption of the tumor thickness was 1.2 mm. In 9% of patients, a relapse developed after 12 months, in 27%, 5 years after the treatment. Due to neovascular glaucoma, the eye was enucleated in 2 (8.3%) patients, and one (4.1%) patient underwent exenteration due to extraocular spread of the tumor. A positive result was achieved in 21 (87.5%) patients. In one (4.1%) patient with relapse and extracranial spread of the tumor, liver metastasis was detected. The authors believe that TTT can be used in the treatment of small-sized CMs, but the high incidence of complications and relapses requires careful monitoring of patients even upon the achievement of a flat chorioretinal scar.

J.M. Caminal et al. [12] presented the treatment results of 13 patients with CM (average height 2.02±0.54 mm, diameter 7.60±1.98 mm). In 11 (84.6%) patients with an average follow-up period of 42.46±26.29 months, the foveal subretinal fluid was completely absent. The average number of TTT sessions was 1.38±0.77. 69.3% of the patients had their visual acuity preserved or improved after treatment. Continued growth of the tumor was registered in 5 patients, regardless of the presence of subretinal fluid. In those cases, brachytherapy was performed. The authors concluded that TTT in most cases can be effective in achieving good visual acuity in the presence of foveal subretinal fluid with small CMs.

The data of academician A. F. Brovkina et al., obtained by analyzing the results of treatment of 30 patients with CM, show that the effectiveness of the technique is confirmed only in tumors with thickness less than 2 mm [13]. With a larger tumor thickness, TTT can only be used in combination with brachytherapy. The risk factors that impair the effectiveness of TTT were identified: tumors with a thickness of more than 2 mm, amelanotic or slightly pigmented, as well as having subretinal exudate. The development of complications and their nature depend on the basic size of the CM, the number of sessions, and previous treatment (local destruction of the tumor). A comparison of the data available in the scientific literature and the experience of the authors allows us to determine clear indications for TTT in the treatment of choroidal melanoma and identify factors that contribute to the prevention of possible complications.

In the study by V. M. Stoffelns et al. [14], TTT was administered to 26 patients with MH with a tumor prominence of up to 3 mm. During the follow-up period of 20 weeks, complete tumor resorption was achieved in 89% of patients. In CM, the probability of complete resorption of the tumor while preserving the eye declines with the increasing prominence of the tumor. In small CM tumors with a posterior location, tumor resorption was achieved much faster after TTT treatment compared to brachytherapy.

Although the penetration capacity of TTT according to literature data reaches 4 mm, the best effect from TTT is obtained in the treatment of tumors with prominence of up to 2-3 mm and a base diameter of less than 10 mm, well pigmented, with no subretinal fluid and low blood flow rate. The prognosis for maintaining residual visual acuity after TTT (especially when the number of sessions increases) is debatable and depends on the localization of the tumor process; it is often impossible to maintain visual acuity and visual field. Complications that develop after TTT are associated with the area of thermal exposure, the proximity to the macular zone and the optic disc.

Materials and methods

The study included 84 patients with choroidal melanoma (ICD-10: C69.3) who received treatment in the period from 2007 to 2018. A sample of patients was taken from the database of the Belarusian Cancer Registry.

In 75 (89.3%) of the patients, a melanoma of the vasculature of the eye was the only tumor. Synchronous colon cancer was registered in 1 (1.2%) patient. 8 (9.5%) patients were diagnosed with metachronous cancer, including one case of basal cell skin cancer, ductal breast carcinoma, pancreatic cancer, prostate cancer, lung cancer, skin melanoma, and 2 cases of kidney cancer. The

sample included 29 (34.5%) men, and 55 (65.5%) women, the minimum age being 20 years, and the maximum 84, with the average age of patients being 59±13 years. The average value of the tumor thickness was 2.6±1.3 mm, and that of the base diameter was 7.2±3.3 mm. The average systolic blood flow rate was 7.7±3.4 cm/s. The level of blood flow in the tumor was assessed by a Doppler ultrasound scan.

TTT was performed with a diode laser with the wavelength of 860 nm, the radiation power from 200 to 800 mW, the exposure of 60 seconds, and the diameter of the laser spot from 1 to 3 mm. The entire surface of the tumor was subjected to thermal exposure, the exposure fields were placed with overlapping, from the periphery to the top of the tumor. 75 patients had one, and 9 had two sessions of TTT with an interval of 3 or 4 weeks.

The immediate result of treatment was evaluated according to the WHO recommendation for solid tumors. Complete resorption of the tumor was characterized by the formation of a full-fledged focus of atrophy in the area of the former location of the tumor, but a possible dispersion or a slight accumulation of pigment were allowed. The criteria for the stabilization of the neoplastic process were a decreased size of the tumor or, in severe pigmentation, no size changes and the absence of blood circulation. The absence of effect from the treatment was understood as no changes on the part of the tumor or an increase in its size with the preservation or reinforcement of the blood flow in it. The positive result of treatment was understood as complete resorption or stabilization of the tumor process.

In the dynamic monitoring of patients with tumor process stabilization, a continued growth of the tumor in the eye membranes was understood as a condition when, against the background of stabilization, an increase in the size and appearance of the vascular network in the tumor were registered. A relapse was understood as a condition when the growth of the tumor was registered against the background of an atrophic chorioretinal focus (complete regression). The progression of the disease was understood as the emergence of distant metastases in other organs.

To calculate the survival rate, we used the adjusted cumulative 5-year survival rate with the actuarial method.

Results and discussion

In 79 of 84 patients (94.0%), the immediate effect of treatment was assessed as stabilization or complete resorption of the tumor. Complete resorption of the tumor was registered in 51 (60.7%) patients, and stabilization of the tumor process in 28 (33.3%) patients. 5 (6.0%) patients were observed to have no effect from the treatment. Of 28 patients with tumor process stabilization achieved, continued growth was recorded in 16 (19.1%), with follow-up periods of 3 months to 4 years.

Of the 51 patients with complete tumor regression, 19 (37.3%) had a relapse in the observation period, one to eight years of follow-up after TTT. In all cases, TTT or brachytherapy was performed to preserve the eye. In 3 patients, after the relapse treatment with brachytherapy, the tumor process progressed. Enucleation due to continued growth, relapse, and complications of TTT was performed in 12 (14.3%) patients. 5 (5.9%) patients developed distant metastases, including one case during the first 12 months and one in 4 years, and 3 cases during the follow-up period of more than 5 years. Complications registered in connection with TTT included post-radiation opticoretinopathy in 6 (7.1%) patients, local retinal hemorrhage in 3 (3.6%) patients, and partial hemophthalmia in 1 (1.2%) patient with diabetic retinopathy.

For a more accurate assessment of the treatment outcomes with due consideration for the size of the tumor, patients were divided into five subgroups.

Table 1 shows the height and diameter of the tumor base before treatment.

Tables 2 and 3 provide summary data on immediate and long-term TTT results in patients with CM, depending on the size of the tumor.

In the first subgroup, 8 (57.2%) patients had complete tumor resorption, 6 (42.8%) had stabilization, and 2 (14.3%) had relapse. In a patient with juxtapapillary localization of the tumor, relapse was detected in the fourth year of follow-up after registering the effect of TTT. In connection with a relapse, 2 additional courses of TTT were conducted. The eyeball was removed after 9 months due to the development of secondary painful glaucoma against the background of a complete resorption of the tumor. In the second patient, a local relapse was detected in the fifth year of the follow-up after the registration of atrophy; the eye was removed, and a year later liver metastasis was registered. A continued growth against the background of stabilization of the tumor process was recorded in a female patient four years after the registration of the effect of treatment. Brachytherapy was performed, the process was stabilized, but a year later, a distant metastasis process was diagnosed.

In the second subgroup of patients, 9 (52.9%) cases showed complete tumor resorption, and stabilization in 8 (47.1%) patients. A relapse was diagnosed in 2 (17.7%) patients, one of them had two relapses registered one after another: 3 years and then 4 years after the treatment. In the second case, the relapse developed a year later. Both patients were administered brachytherapy as additional organ-preserving treatment. Continued growth against the background of stabilization was observed in 1 (5.9%) patient, ophthalmectomy was performed, but a year later metastatic disease was detected.

Таблица 1
Характеристика высоты и диаметра основания меланомы хориоидеи (M ± m)
Table 1
Characterization of the height and diameter of the base of the choroidal melanoma (M ± m)

Показатели Parameters	Подгруппы Subgroups				
	1 (n=14)	2 (n=17)	3 (n=21)	4 (n=13)	5 (n=19)
Диаметр основания, мм Base diameter, mm	2,4±1,2	5,0±0,5	7,0±0,6	8,8±0,5	11,5±1,5
Высота, мм Height, mm	1,5±1,0	2,1±0,9	2,7±1,2	2,5±1,3	3,8±1,1

Таблица 2
Непосредственные результаты транспупиллярной термотерапии в зависимости от размеров меланомы хориоидеи (абс. ч./%)
Table 2
Immediate results of transpupillary thermotherapy depending on the size of the choroidal melanoma (No. patients / %)

Эффект лечения Treatment effect	Подгруппы Subgroups				
	1 (n=14)	2 (n=17)	3 (n=21)	4 (n=13)	5 (n=19)
Полная резорбция Complete resorption	8/57,2	9/52,9	15/71,4	9/69,2	10/52,6
Стабилизация Stabilization	6/42,8	8/47,1	6/28,6	3/23,1	5/26,3
Отсутствие эффекта No effect	–	–	–	1/7,7	4/21,1

Таблица 3
Отдаленные результаты транспупиллярной термотерапии в зависимости от размеров меланомы хориоидеи (абс. ч./%)
Table 3
Long-term results of transpupillary thermotherapy depending on the size of the choroidal melanoma (No. patients / %)

Отдаленные результаты ТТТ Long-term results of TTT	Подгруппы Subgroups				
	1 (n=14)	2 (n=17)	3 (n=21)	4 (n=13)	5 (n=19)
Рецидив Reccurence	2/14,3	2/17,7	7/33,3	3/23,1	5/26,3
Продолженный рост Continued growth	1/7,1	1/5,9	2/9,5	2/15,4	4/21,1
Энуклеация Enucleation	2/14,3	1/5,9	3/14,3	1/7,7	5/26,3
Прогрессирование Progression	2/14,3	1/5,9	2/9,5	1/7,7	–

In the third subgroup, complete resorption of the tumor was observed in 15 (71.4%), and stabilization in 6 (28.6%) patients. Relapse occurred in 7 (33.3%) patients in follow-up periods of one year (2 cases), 2 years (3 cases), 3 years (1 case), and 4 years (1 case). Enucleation was performed on 3 patients, including, in one case, due to the relapse and painful glaucoma in the fourth year of the follow-up, and on 2 patients due to the relapse in the follow-up periods of 4 and 5 years. Brachytherapy was performed in 4 patients in connection with local relapse. Metastases in the liver and bones were detected in 2 patients with recurrent CM in a year and in 5 years. In 2 observations, during the year, continued growth was recorded against the background of stabilization. Pa-

tients were successfully treated with brachytherapy and 2 courses of TTT.

Complete resorption of the tumor in the fourth subgroup was registered in 9 (69.2%) patients, stabilization in 3 (23.1%); in one case (7.7%), the effect of treatment was absent. Relapse occurred in 3 patients in the second, fourth and seventh years of follow-up. Continued growth was recorded in 2 cases. All patients were administered TTT and brachytherapy. Enucleation was performed in one case under the observation due to the lack of effect from treatment. A patient with continued growth was diagnosed with metastatic liver damage 4 years after the treatment.

In the fifth subgroup, tumor resorption was recorded in 10 (52.6%), stabilization in 5 (26.3%), and no effect from treatment in 4 (21.1%) patients. Relapse was registered in 5 patients: in one, it occurred in 2 years after the registration of complete resorption of the tumor, in 3, in 5 years, and in one case, 8 years after the registration of complete resorption of the tumor. Five enucleations were performed in the periods from 2 months to 2 years, two of them due to the continued tumor growth, and three due to the lack of effect from treatment. No cases of metastatic disease were registered in this subgroup.

The survival rate of patients with a single eye tumor (n=75) was analyzed, without including patients with multiple primary diseases. One-year overall survival was 100%, 5-year and 10-year survival was 95.4±2.1% and 79.8±6.9%, respectively.

Conclusion

As a result of TTT, 51 (60.7%) patients were registered to have complete resorption of the tumor, 28 (33.3%) had the tumor process stabilized, and 5 (6.0%) had no effect from the treatment. Of 28 patients with tumor process stabilization, continued growth was recorded in 16 (19.1%) cases with follow-up periods of 3 months to 4 years. Of the 51 patients with complete tumor regression, 19 (37.3%) had a relapse in the observation period, one to eight years of follow-up after TTT. 5 (5.9%) patients developed distant metastases, including one case during the first 12 months and one in 4 years after the completion of TTT, and 3 cases during the follow-up period of more than 5 years. The analysis of the TTT effectiveness in relation to the size of the focus of choroid melanoma showed that the method's direct efficiency is reduced in proportion to the increase in the thickness and diameter of the base of the tumor, and the probability of tumor growth after TTT administration increases. One-year overall survival in patients who had only one tumor, that of the eye, was 100%, 5-year and 10-year survival was 95.4±2.1% and 79.8±6.9%, respectively.

REFERENCES

1. Okeanov A.E., Moiseyev P.I., Levin L.F. et al. *Statistika onkologicheskih zabolevaniy v Resbublike Belarus (2008–2018): Belarusian kantser-registr* [Statistics of cancer diseases in the Republic of Belarus (2008–2017): Belarusian Cancer Registry]. Ed. O.G. Sukonko. Minsk, N.N. Alexandrov National Cancer Centre of Belarus, 2018. 286 p.
2. Naumenko L.V., Zhilyaeva E.P. *Algoritmi diagnostiki i lecheniya zlokachestvennyh novoobrazovaniy: klinicheskiy protocol* [Algorithms for the diagnosis and treatment of malignant neoplasms: a clinical protocol]. Ed. O.G. Sukonko. S.A. Krasny. Minsk, Professional editions, ch. 63, 2019. pp. 493–509.
3. Shields C.L., Shields J.A., Perez N., Singh A.D., Cater J. Primary transpupillary thermotherapy for choroidal melanoma in 256 consecutive cases. Outcomes and limitations. *Ophthalmology*, 2002, vol. 109 (2), pp. 225–234.
4. Houston S.K., Wykoff C.C., Berrocal A.M., Hess D.J., Murray T.G. Lasers for the treatment of intraocular tumors. *Lasers Med Sci*, 2013, vol. 28 (3), pp. 1025–1034.
5. Rishi P., Koundanya V.V., Shields C.L. Using risk factors for detection and prognostication of uveal melanoma. *Indian J Ophthalmol*, 2015, vol. 63 (2), pp. 110-116. doi:10.4103/0301–4738.154373
6. Mashayekhi A., Shields C.L., Rishi P., Atalay H.T., Pellegrini M., McLaughlin J.P., Patrick K.A., Morton S.J., Remmer M.H., Parendo A., Schlitt M.A., Shields J.A. Primary transpupillary thermotherapy for choroidal melanoma in 391 cases: importance of risk factors in tumor control. *Ophthalmology*, 2015, vol. 122 (3), pp. 600–609.
7. Turcotte S., Bergeron D., Rousseau A.P., Mouriaux F. Primary transpupillary thermotherapy for choroidal in determinate melanocytic lesions. *Can J Ophthalmol*, 2014, vol. 49 (5), pp. 464–467.
8. Chojniak M.M., Chojniak R., Nishimoto I.N., Allemann N., Erwenne C.M. Primary transpupillary thermotherapy for small

ЛИТЕРАТУРА

1. Океанов А.Е., Моисеев П.И., Левин Л.Ф. и соавт. Статистика онкологических заболеваний в Республике Беларусь (2008–2018): Белорусский канцер-регистр/под ред. О.Г. Суконко.– Минск: Республиканский научно-практический центр онкологии и медицинской радиологии им. Н.Н. Александрова, 2018.– 286 с.
2. Науменко Л.В., Жиляева Е.П. Алгоритмы диагностики и лечения злокачественных новообразований: клинический протокол/под ред. О.Г. Суконко, С.А. Красного.– Минск: Профессиональные издания, ч. 63, 2019.– С. 493–509.
3. Shields C.L., Shields J.A., Perez N. et al. Primary transpupillary thermotherapy for choroidal melanoma in 256 consecutive cases. Outcomes and limitations//Ophthalmology.– 2002.– Vol. 109 (2).– P. 225–234.
4. Houston S.K., Wykoff C.C., Berrocal A.M. et al. Lasers for the treatment of intraocular tumors//Lasers Med Sci.– 2013.– Vol. 28 (3).– P. 1025–1034.
5. Rishi P., Koundanya V.V., Shields C.L. Using risk factors for detection and prognostication of uveal melanoma//Indian J Ophthalmol.– 2015.– Vol. 63 (2).– P. 110-116. doi:10.4103/0301–4738.154373
6. Mashayekhi A., Shields C.L., Rishi P. et al. Primary transpupillary thermotherapy for choroidal melanoma in 391 cases: importance of risk factors in tumor control//Ophthalmology.– 2015. –Vol. 122 (3).– P. 600–609.
7. Turcotte S., Bergeron D., Rousseau A.P. et al. Primary transpupillary thermotherapy for choroidalindeterminatemelanocyticlesions//CanJOphthalmol.–2014.–Vol.49 (5).– P. 464–467.
8. Chojniak M.M., Chojniak R., Nishimoto I.N. et al. Primary transpupillary thermotherapy for small choroidal melanoma//Graefes Arch Clin Exp Ophthalmol.– 2011. –Vol. 249 (12).– P. 1859–1865.

choroidal. *Graefes Arch Clin Exp Ophthalmol*, 2011, vol. 249 (12), pp. 1859–1865.
9. Stoffelns B. M., Schoepfer K., Vetter J., Mirshahi A., Elflein H. Long-term follow-up 10 years after transpupillary thermotherapy (TTT) for small, posterior located malignant melanomas of the choroid. *Klin Monbl Augenheilkd*, 2011, vol. 228 (4), pp. 277–283.
10. Yarovoy A. A., Magaramov D. A., Bulgakova E. S. Which choroidal melanoma should be treated with primary transpupillary thermotherapy? Our experience from 78 patients. *Eur J Ophthalmol*, 2010, vol. 20 (1), pp. 186–193.
11. Gündüz K., Karslioğlu M. Z., Köse K. Primary transpupillary thermotherapy of choroidal melanocytic lesions. *Middle East Afr J Ophthalmol*, 2011, vol. 18 (2), pp. 183–188.
12. Caminal J. M., Mejia-Castillo K. A., Arias L., Catala J., Rubio M., Garcia P., Pujol O., Arruga J. Subthreshold transpupillary thermotherapy in management of foveal subretinal fluid in small pigmented choroidal lesions. *Retina*, 2013, vol. 33 (1), pp. 194–199.
13. Brovkina A. F., Borisova Z. L. Optimization of indications for transpupillary thermotherapy in choroidal melanomas. *Vestnik Oftal'mologii*, 2010, vol. 126 (4), pp. 48–52. (in Russian)
14. Stoffelns B. M., Schoepfer K., Jochem T., Faldum A. Tumor regression in malignant choroidal melanomas after transpupillary thermotherapy (TTT) versus ruthenium brachytherapy and sandwich therapy – acomparativeanalysis. *Klin Monbl Augenheilkd*, 2010, vol. 227 (4), pp. 262–268. (in German)

9. Stoffelns B. M., Schoepfer K., Vetter J. et al. Long-term follow-up 10 years after transpupillary thermotherapy (TTT) for small, posterior located malignant melanomas of the choroid//Klin Monbl Augenheilkd. – 2011. – Vol. 228 (4). – P. 277–283.
10. Yarovoy A. A., Magaramov D. A., Bulgakova E. S. Which choroidal melanoma should be treated with primary transpupillary thermotherapy? Ourexperience from 78 patients//Eur J Ophthalmol.– 2010.– Vol. 20 (1).– P. 186–193.
11. Gündüz K., Karslioğlu M. Z., Köse K. Primary transpupillary thermotherapy of choroidal melanocytic lesions//Middle East Afr J Ophthalmol.– 2011. – Vol. 18 (2). – P. 183–188.
12. Caminal J. M., Mejia-Castillo K. A., Arias L. et al. Subthreshold transpupillary thermotherapy in management of foveal subretinal fluid in small pigmented choroidal lesions//Retina. – 2013. – Vol. 33 (1). – P. 194–199.
13. Бровкина А. Ф., Борисова З. Л. Оптимизация показателей транспупиллярной термотерапии меланомы хориоидеи//Вестник Офтальмологии. – 2010. – Т. 126, № 4. – С. 48–52 (in Russian).
14. Stoffelns B. M., Schoepfer K., Jochem T., et al. Tumor regression in malignant choroidal melanomas after transpupillary thermotherapy (TTT) versus ruthenium brachytherapy and sandwich therapy – a comparative analysis//Klin Monbl Augenheilkd.– 2010. – Vol. 227 (4). – P. 262–268.

STATISTICS ON THE WORK OF GASTROENTEROLOGISTS IN THE RUSSIAN FEDERATION IN 2013–2017

Guryanova N.E., Sachek O.I., Ivanova M.A.
Central Research Institute of Organization and Informatization of Health Care, Ministry of Health of Russia, Moscow, Russia

Abstract

In recent decades, increasing importance in modern diagnostic algorithms is given to techniques that use optical systems, including those with the ability to examine organs in various spectral ranges and in fluorescence modes. The rapid development of modern technologies and their implementation in the field of healthcare requires constant improvement of the organization of medical care to the population. Endoscopic services in Russia began to emerge in the 70s of the 20th century. In the early stages, it was represented by independent diagnostic rooms based on large medical clinics and research centers. Over the past decades, endoscopic methods of diagnosis and treatment have moved far forward. For the successful use of endoscopic technologies in clinical practice, qualified medical personnel are needed, including oncologists, gastroenterologists, and other specialists proficient in endoscopic techniques. The human potential of endoscopy was initially formed by part-timers, most often from surgeons and therapists. In this regard, it is necessary to analyze the activities of specialist doctors who provide medical care in outpatient conditions. The aim of the study was to analyze the activities of gastroenterologists providing outpatient medical care at the federal level and in the pilot regions of the Russian Federation. The multidirectionality of trends regarding the level of burden on gastroenterologists in the subjects of the Russian Federation is established. In all federal districts, a decrease in the number of visits per gastroenterologist and a decrease in the part-time coefficient was observed.

Keywords: endoscopic methods of treatment and diagnostics, gastroenterologists, photodynamic therapy (PDT), subjects of Russian Federation.

For citations: Guryanova N.E., Sachek O.I., Ivanova M.A. Statistics on the work of gastroenterologists in the Russian Federation in 2013–2017, *Biomedical Photonics*, 2020, vol. 9, no 2, pp. 36–44. (in Russian) doi: 10.24931/2413–9432–2020–9–2–36–44.

Contacts: Guryanova N.E., e-mail: guryan8@yandex.ru

СТАТИСТИЧЕСКИЕ ПОКАЗАТЕЛИ РАБОТЫ ВРАЧЕЙ-ГАСТРОЭНТЕРОЛОГОВ В РФ В 2013–2017 гг.

Н.Е. Гурьянова, О.И. Сачек, М.А. Иванова
ФГБУ «Центральный научно-исследовательский институт организации и информатизации здравоохранения» Минздрава России, Москва, Россия

Резюме

В последние десятилетия все большее значение в современных диагностических алгоритмах отводится методикам, использующим оптические системы, в том числе с возможностью осмотра органов в различных спектральных диапазонах и во флуоресцентных режимах. Стремительное развитие современных технологий и их внедрение в практическое здравоохранение требуют постоянного совершенствования организации медицинской помощи населению. Эндоскопическая служба в России начала зарождаться в 70-е годы XX столетия. На первых этапах становления служба была представлена разрозненными диагностическими кабинетами на базе крупных медицинских клиник и научно-исследовательских центров. За последние десятилетия эндоскопические методы диагностики и лечения шагнули далеко вперед. Для успешного использования эндоскопических технологий в клинической практике необходимы квалифицированные медицинские кадры, в том числе врачи-онкологи, гастроэнтерологи, другие специалисты, владеющие эндоскопическими методиками. Кадровый потенциал эндоскопии изначально формировался из числа совместителей, чаще из врачей-хирургов и терапевтов. В этой связи необходим анализ деятельности врачей–специалистов, оказывающих медицинскую помощь в амбулаторных условиях. Целью исследования явился анализ деятельности врачей-гастроэнтерологов, оказывающих медицинскую помощь амбулаторно, на федеральном уровне и в пилотных регионах Российской Федерации. Установлена разнонаправленность тенденций относительно уровня нагрузки на гастроэнтерологов в субъектах страны, вошедших в исследование. Во всех федеральных округах отмечено уменьшение числа посещений по заболеванию на 1 врачебную должность и снижение коэффициента совместительства.

Ключевые слова: эндоскопические методы лечения и диагностики, врачи-гастроэнтерологи, фотодинамическая терапия (ФДТ), субъекты Российской Федерации.

Для цитирования: Гурьянова Н.Е., Сачек О.И., Иванова М.А. Статистические показатели работы врачей-гастроэнтерологов в РФ в 2013–2017 гг. // Biomedical Photonics. – 2020. – Т. 9, № 2. – С. 36–44. doi: 10.24931/2413–9432–2020–9–2–36–44.

Контакты: Н.Е. Гурьянова, e-mail: guryan8@yandex.ru

Introduction

In recent decades, more and more importance in modern diagnostic algorithms is given to methods that use optical systems, including those which allow for examining organs in various spectral ranges and in fluorescent modes. Modern endoscopic technologies for diagnosis and treatment brought dramatic changes to the fight against gastrointestinal (GI) diseases [1-3].

Endoscopy has significantly enhanced its capacity recently due to the development and improvement of medical equipment and the appearance of innovative methods. The use of minimally invasive technologies allows for thorough endoscopic diagnostics of early gastrointestinal cancers [4]. One of the methods of treatment of GI diseases, including esophagus diseases, is photodynamic therapy (PDT) [5]. PDT has been approved for use in the United States since December 1998. The widespread use of PDT is due to its ability to have a therapeutic effect on large surfaces of the mucous membrane without the need for their full visualization. The therapeutic impact can be provided through a fiber that conducts light perpendicularly to the axis of the endoscope, which makes this technology ideal for use in gastroenterology [6, 7]. Diseases of the digestive system, among which the predominant part is liver disease [8], are one of the causes of mortality among the working-age population [9]. Due to the high incidence of digestive system diseases and related mortality, the analysis of the activities of gastroenterologists is highly relevant.

The issues of personnel security and the activities of medical specialists, as well as the organization of medical care for patients, are very important both for practical health care and for scientific research [10-15].

The purpose of the study was to analyze the activities of gastroenterologists providing medical care in outpatient settings.

Materials and methods

The method of descriptive statistics presents the results of calculating extensive and intensive indicators that characterize the activities of gastroenterologists. A comparative analysis of the dynamics of indicators for the period from 2013 to 2017 in the constituent entities of the Russian Federation was based on the Federal Statistical Reporting form No. 30 "Information about the medical organization".

Results and discussion

The results of the analysis of gastroenterologists' activity showed a predictable decrease in the dual job holding coefficient from 1.32 in 2013 to 1.2 in 2017, the Annual Growth Rate (AGR) being equal to - 9.1% (Fig. 1).

Due to the fact that the provision of medical organizations with gastroenterologists increased during the study period, it is natural to expect the workload per specialist doctor to have decreased. In the Russian Federation as a whole, the number of visits to gastroenterologists during the analyzed period decreased by 19.5% per 1 position (from 4750,9 to 3823,0). The workload indicators for gastroenterologists practicing in outpatient settings are observed to decrease every year. The maximum decrease in the annual growth indicators occurred in 2014. (AGR = -10.9%) (Fig. 2).

However, during the analyzed period, the share of visits in connection with diseases increased slightly from 94.3% in 2013 to 94.8 in 2017 (AGR = +0.6%). Nevertheless, it should be noted that the entire period of the study is characterized by fairly high values of the indicators. On average, the percentage of visits n connection with diseases was 94.4%. The minimum value of 93.6% was recorded in 2015, and the maximum value, 95.1%, was in 2016. As shown in Fig. 3, during the analyzed period, the percentage of gastroenterologist appointments in connection with diseases in the Russian Federation as a whole decreased every year until 2015. An unprecedented increase in the number of visits to specialist doctors in 2016 (95.1%) was followed by a decline to 94.8% in 2017.

We conducted an analysis of the dual job holding indicator for gastroenterologists, which revealed that in all Federal Districts (FOs) the AGR values are negative. The leading position is held by the North Caucasus Federal District (-20.2%), and the second by the Southern Federal District (-16.5%). In the Far Eastern Federal District, slight fluctuations in the indicator were observed, with similar values at the endpoints over the entire period of the study (Fig. 4).

A comparative analysis of the dual job holding coefficient for gastroenterologists providing medical care in outpatient settings was conducted for the pilot entities of the Russian Federation in respect of the period from 2013 to 2017. The results of the study allowed us to identify 5 regions with the highest and lowest values of the dual job holding indicator (Table 1).

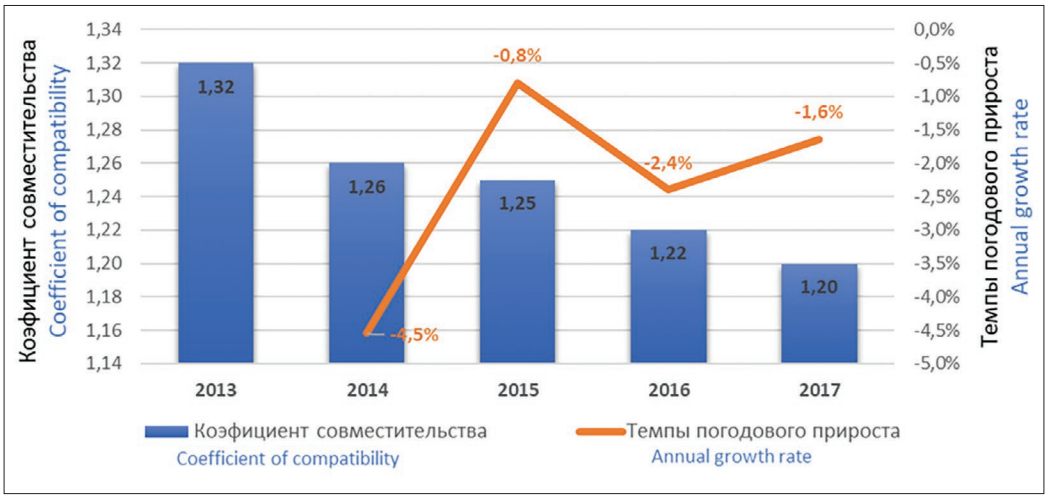

Рис. 1. Динамика коэффициента совместительства врачей-гастроэнтерологов и темпов погодового прироста в Российской Федерации за период 2013–2017 гг.
Fig. 1. Dynamics of the coefficient of gastroenterologists part-timing of in the Russian Federation and the rate of annual growth of indicators in 2013–2017

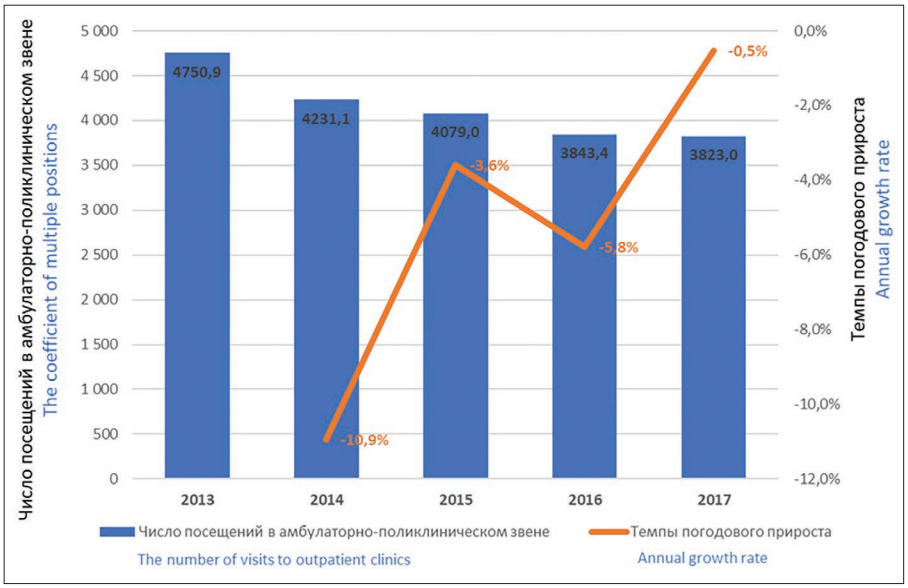

Рис. 2. Динамика числа посещений на 1 должность врача-гастроэнтеролога в амбулаторных условиях и темпы погодового прироста в Российской Федерации за период 2013–2017 гг.
Fig. 2. Dynamics of the number of visits of outpatients per gastroenterologist in the Russian Federation and the rate of annual growth of indicators in 2013–2017

It should be noted that in 2017, in such pilot entities as the Republic of Tatarstan, the Udmurt Republic and the Moscow Region, along with high dual job holding coefficients (1.92, 1.48 and 1.45, respectively), maximum AGRs were also observed (23.1%, 15.6%, and 10.7%). The minimum value of this indicator in 2017 was found in the Tomsk Region (0.93). At the same time, the Sverdlovsk Region was found to have the largest negative value of the indicator (-21.6%).

By 2017, a decrease in the number of visits per 1 gastroenterologist position was observed in most Federal Districts. A positive annual growth of the indicator (2.7%) was registered only in the far Eastern Federal district. The leading position in the number of visits to a gastroenterologist in 2013 was held by the Southern and Central Federal Districts, where the values of the indicator exceeded the national level by 15.1 and 13.8% (5466.9 and 5405.8 against 4750.9, respectively). By 2017, the Southern Federal district continued its lead, with the value exceeding the all-Russian average indicator by 13.0%. At the same time, the Central Federal district had the highest negative AGR of the indicator (-32.1%) and was one of the

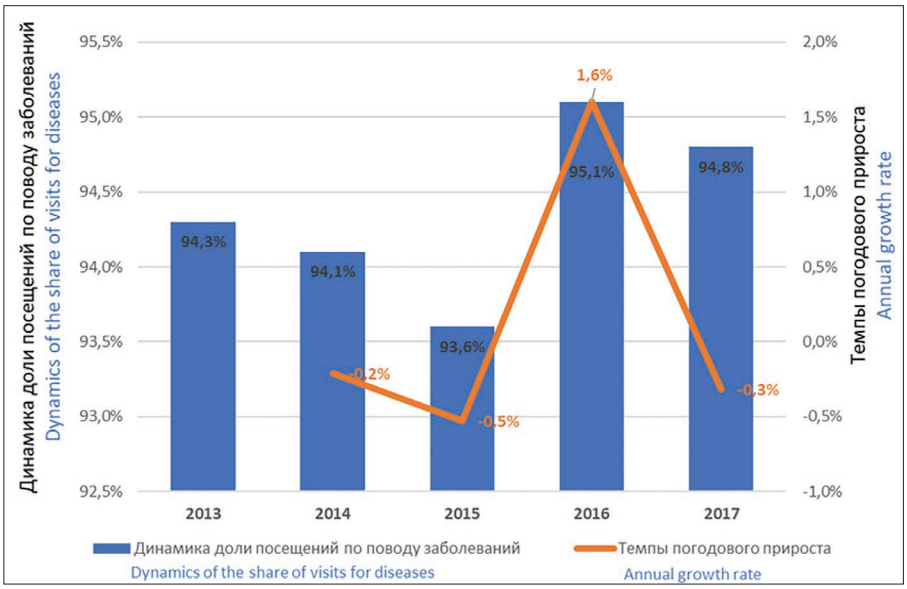

Рис. 3. Динамика доли посещений врачей-гастроэнтерологов и темпов погодового прироста в Российской Федерации за период 2013–2017 гг.
Fig. 3. Dynamics of the share of visits of gastroenterologists regarding diseases in the Russian Federation and the rate of annual growth of indicators in 2013–2017

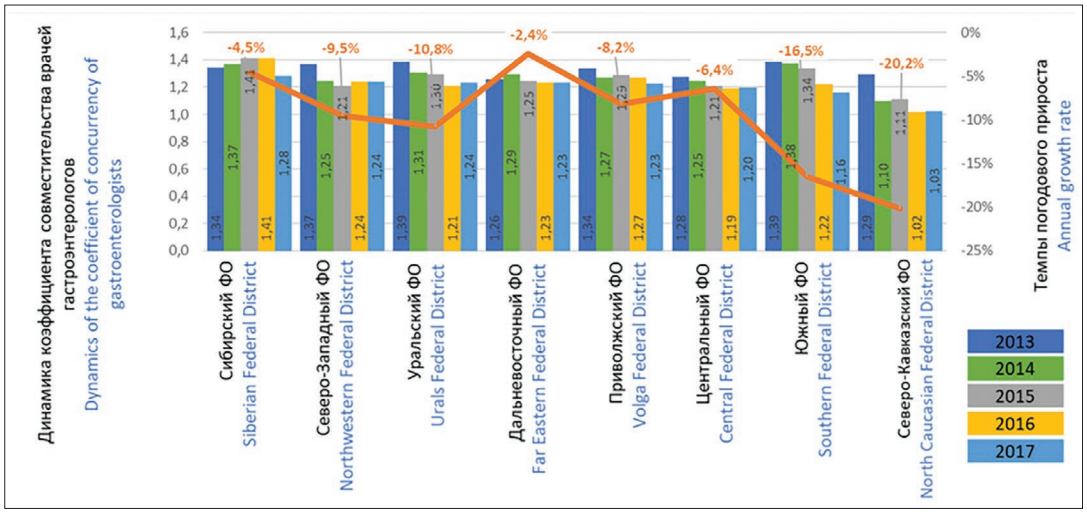

Рис. 4. Динамика коэффициента совместительства врачей-гастроэнтерологов и темпы погодового прироста показателя в федеральных округах Российский Федерации за период 2013–2017 гг.
Fig. 4. Dynamics of the coefficient of gastroenterologists part-timing in the Federal districts of the Russian Federation and the rate of annual growth of indicators in 2013–2017

Federal Districts with the lowest number of visits per 1 gastroenterologist position (Fig. 5).

Table 2 shows the subjects of the Russian Federation with the minimum and maximum values of the number of visits to gastroenterologists. The distribution of pilot entities by the lowest and highest values of the number of visits is based on 2017 data.

The study showed that individual entities where the indicators initially exceeded the average national level or were close to it, subsequently had a sharp decrease in the number of visits to gastroenterologists working on an outpatient basis. This was reflected in the AGR: the Ivanovo Region: 7367.3 in 2013 and 3581.5 in 2017, AGR = -51.4%; the Irkutsk Region: 6959.2 in 2013 and 3991.9 in 2017, AGR = -42.6%; Moscow: 6095.2 in 2013 and 3089.6 in 2017, AGR = - 49.3%. In contrast, the highest growth rates were in the Republic of Tatarstan (39.7%) and the Khabarovsk Territory (19.2%).

In 4 Federal District (Northwestern, Volga, North Caucasian, Far Eastern), a decrease in the specific weight of visits in connection with diseases was found (within the range from -0.6% to -1.7%). Despite that, the percent-

Таблица 1
Динамика коэффициента совместительства врачей-гастроэнтерологов, оказывающих медицинскую помощь в амбулаторных условиях, за период 2013–2017 гг. (данные пилотного исследования)
Table 1
Dynamics of the part-timing coefficient of gastroenterologists providing medical care in outpatient conditions in 2013–2017 (pilot study)

Субъекты РФ Subjects of the Russian Federation	2013	2014	2015	2016	2017	2013/2017 ТПП, % 2013/2017 AGR, %
Минимальные значения Minimum value						
Томская область Tomsk Oblast	1,11	1,21	1,13	0,93	0,93	–16,2
г. Москва Moscow	1,18	1,12	1,06	1,0	1,02	–13,6
Пермский край Perm Krai	1,23	1,09	1,23	1,1	1,06	–13,8
Новосибирская область Novosibirsk Oblast	1,19	1,13	1,27	1,49	1,07	–10,1
Свердловская область Sverdlovsk Oblast	1,39	1,29	1,15	1,06	1,09	–21,6
Максимальные значения Maximum value						
Московская область Moscow Oblast	1,31	1,38	1,35	1,35	1,45	10,7
Удмуртская Республика Udmurt Republic	1,28	1,39	1,8	1,48	1,48	15,6
Астраханская область Astrakhan Oblast	1,47	1,44	1,5	1,61	1,5	2,0
Иркутская область Irkutsk Oblast	2	1,97	1,64	1,46	1,68	–16,0
Республика Татарстан Republic Of Tatarstan	1,56	1,75	1,57	1,46	1,92	23,1

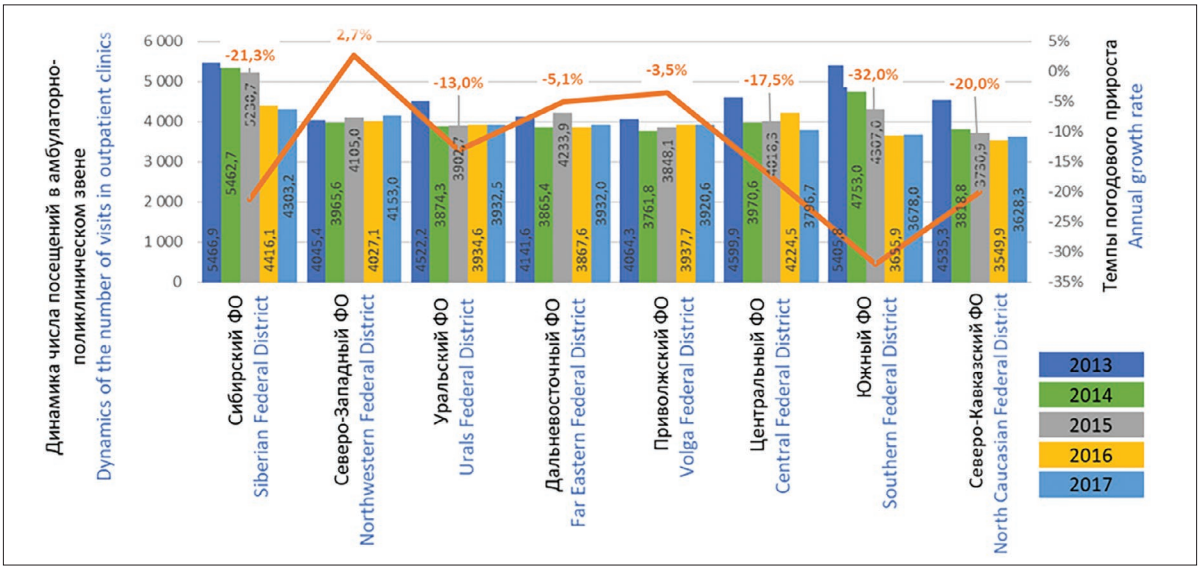

Рис. 5. Динамика числа посещений на 1 должность врача-гастроэнтеролога в амбулаторных условиях и темпы погодового прироста в федеральных округах Российской Федерации за период 2013–2017 гг.
Fig. 5. Dynamics of the number of visits of outpatients per gastroenterologist in the Federal districts of the Russian Federation and the rate of annual growth in 2013–2017

Таблица 2
Динамика числа посещений врачей-гастроэнтерологов, оказывающих медицинскую помощь в амбулаторных условиях, за период 2013–2017 гг., абс. ч. (данные пилотного исследования)
Table 2
Dynamics of the number of visits of gastroenterologists providing medical care in outpatient conditions in 2013–2017 (pilot study), number of patients

Субъекты РФ Subjects of the Russian Federation	2013	2014	2015	2016	2017	2017/2013 ТПП, % 2013/2017 AGR, %
Минимальные значения Minimum value						
Удмуртская Республика Udmurt Republic	3285,4	3150,6	3568,4	2446,1	2762,4	–15,9
Красноярский край Krasnoyarsk Krai	4779,1	3630,4	3400,8	3118,1	2943,8	–38,4
г. Москва Moscow	6095,2	5015,6	4020,9	3191,4	3089,6	–49,3
Новосибирская область Novosibirsk Oblast	3004	2828,6	3068,4	4052,5	3222,5	7,3
Пермский край Perm Krai	4371,7	3656,4	4061,3	3595,5	3309,3	–24,3
Максимальные значения Maximum value						
Хабаровский край Khabarovsk Krai	3998,1	4222,2	4690,8	4526	4764,2	19,2
Алтайский край Altai Krai	6316,4	5348,1	5561,7	5318	4783,8	–24,3
Астраханская область Astrakhan Oblast	6798,3	6130,8	5410	5603,9	5404,7	–20,5
Ставропольский край Stavropol Krai	4860,6	3698,6	4843,1	4418,5	5429,6	11,7
Республика Татарстан Republic Of Tatarstan	4312,8	5457,2	4675,6	4463,5	6024,3	39,7

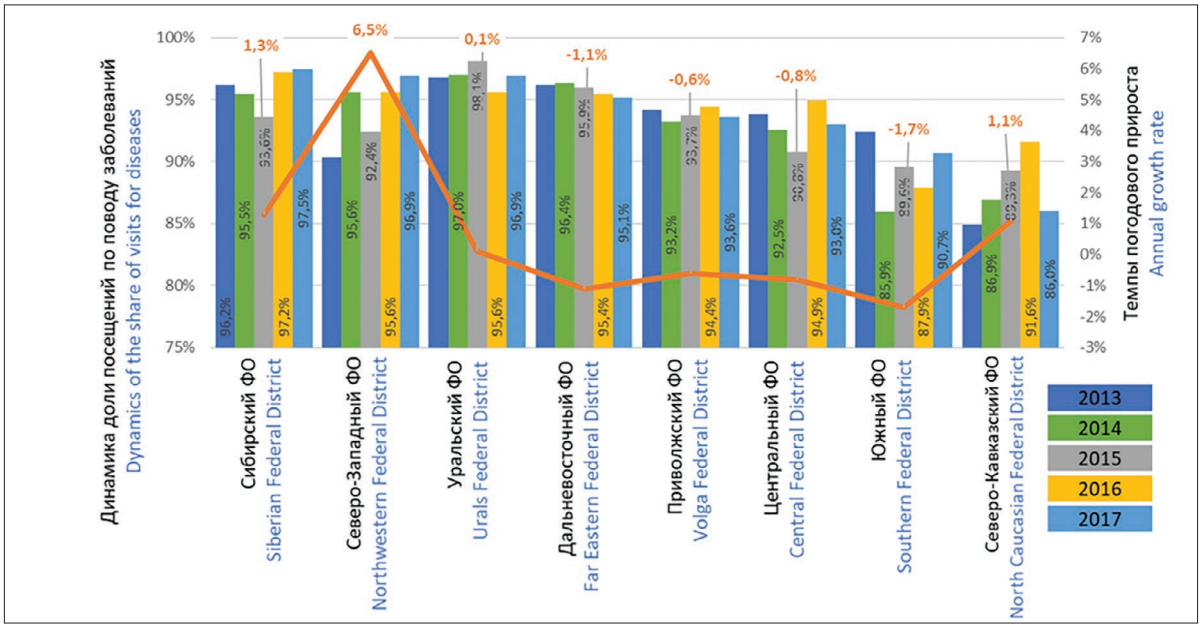

Рис. 6. Динамика доли посещений по поводу заболеваний гастроэнтерологов и темпы погодового прироста в федеральных округах Российской Федерации за период 2013–2017 гг.
Fig. 6. Dynamics of the share of visits regarding diseases in the Federal districts of the Russian Federation and the rate of annual growth in 2013–2017

Таблица 3
Динамика доли посещений по заболеванию врачей-гастроэнтерологов в 2013–2017 гг., % (данные пилотного исследования)
Table 3
Dynamics of the share of visits to gastroenterologists regarding diseases in 2013–2017 (pilot study), %

Субъекты РФ Subjects of the Russian Federation	2013	2014	2015	2016	2017	2017/2013 ТПП 2013/2017 AGR,%
Минимальные значения Minimum value						
Алтайский край Altai Krai	64,7	73,9	70,4	76,7	58,1	–6,5
Хабаровский край Khabarovsk Krai	94,5	84,6	87,9	80,3	82,3	–12,2
Республика Татарстан Republic Of Tatarstan	95,3	85,3	89,1	90,4	89,9	–5,4
Томская область Tomsk Oblast	94,8	86,4	86,3	98,3	90,7	–4,1
Ставропольский край Stavropol Krai	92,6	93,9	92,7	93,3	91,2	–1,4
Максимальные значения Maximum value						
г. Москва Moscow	98,4	98,6	99,4	99,8	99,9	1,6
Удмуртская Республика Udmurt Republic	99,9	99,8	99,3	99,9	100,0	0,1
Пермский край Perm Krai	99,8	99,4	98,6	99,9	100,0	0,1
Новосибирская область Novosibirsk Oblast	92,6	98,9	100,0	100,0	100,0	7,4
Московская область Moscow Oblast	94,0	95,5	81,9	100,0	100,0	6,0

age of visits to gastroenterologists in connection with diseases in these districts remained high (90.7 to 95.1%). The highest rates were registered in the Central (97.5%), Southern (96.9%), and Ural (96.9%) Federal Districts. In the Southern Federal District, the largest increase in the proportion of visits in connection with diseases was registered: from 90.4% in 2013 to 96.9% in 2017 (AGR=6.5%). The lowest indicator during the study period was observed in the Siberian Federal district: 84.9% in 2013 and 86.0% in 2017 (Fig. 6).

The analysis of the data provided in the federal statistical observation forms showed that during the study period, there was an increase in the share of gastroenterologist attendance in connection with diseases. The Astrakhan region had the most significant growth of 15.4%. A decrease in the medical appointments was observed in the 9 pilot subjects, especially significant in the Khabarovsk Territory (-12.2%).

In 2017, in 4 pilot subjects, the share of gastroenterologist attendance in connection with diseases was 100%. During the 5-year observation period in the Ivanovo region, the Udmurt Republic, the Perm Region and Moscow, this indicator exceeded 99.0%.

The distribution of entities by maximum and minimum indicators as of 2017 is shown in table 3.

Conclusion

The results of a study of gastroenterologists' activities for the period 2013-2017 revealed a natural decrease in the workload per doctor. During the analyzed period, in the Russian Federation as a whole, the number of visits to gastroenterologists per 1 position decreased by 19.5%. The dual job holding coefficients tend to decrease in all FOs of the country, especially in the North Caucasian (-20.2%) and Southern (-16.5%) FOs. At the same time, the share of visits to gastroenterologists in connection with diseases increased insignificantly: from 94.3% in 2013 to 94.8 in 2017 (AGR = 0.6%).

The number of visits per 1 medical position tends to decrease, with the exception of the Far Eastern Federal District. At the same time, the share of visits to gastroenterologists in connection with diseases in the North-

Western, Volga, North Caucasus, and Far Eastern Federal districts remains at a high level (from 90.7% to 95.1%). The highest rates were registered in the Central (97.5%), Southern (96.9%), and Ural (96.9%) Federal Districts. Among the country's regions, the highest growth rates were registered in the Republic of Tatarstan (39.7%) and in the Khabarovsk territory (19.2%).

The percentage of visits in connection with diseases during the 5-year observation period in the Ivanovo Region, the Udmurt Republic, the Perm Region and Moscow exceeded 99.0%. The results of the study of gastroenterologist activities in the Russian Federation in 2013-2017 showed the need for managerial decisions aimed at optimization of the specialists' workload.

REFERENCES

1. Levchenko N.V., Khrachkov V.V., Shavaliev R.R., Kislitsyn D.P. Antegrade papillotomy using YAG:HO laser for stenosis of the large duodenal papilla. *Biomedical Photonics.* 2018, vol. 7(1), pp. 21–27. (in Russian)
2. Tumanina A.N., Polezhaev A.A., Apanasevich V.A., Gurina L.I., Volkov M.V., Tarasenko A.Yu., Filonenko E.V. Experience of using photodynamic therapy in the treatment of esophageal cancer. *Biomedical Photonics*. 2019, vol. 8(2), pp. 19–24. (in Russ.)
3. Kundukhova E.R., Remizov O.V., Butaev T.M., Dzugaeva Z.I., Alagova A.R. main directions in solving the problems of endoscopy development. *Modern problems of science and education*, 2017, no 2. (in Russian)
4. Filonenko E.V. The history of development of fluorescence diagnosis and photodynamic therapy and their capabilities in oncology. *Russian Journal of General Chemistry*, 2015, vol. 85(1), pp. 211–216. (in Russian)
5. Sokolov V.V., Chissov V.I., Filonenko E.V., Sukhin G.M., Yakubovskaya R.I., Belous T.A., Zharkova N.N., Kozlov D.N., Smirnov V.V. Photodynamic therapy of cancer with the photosensitizer PHOTOGEM. *Proceedings of SPIE - The International Society for Optical Engineering*, 1995, vol. 2325, pp. 375–380.
6. Sokolov V.V., Chissov V.I., Filonenko E.V. *Sposob lazernogo oblucheniya pri endoskopicheskoi fotodinamicheskoi terapii nachal'nogo raka polykh organov. Metodicheskie rekomendatsii* [Method of laser irradiation in endoscopic photodynamic therapy of initial cancer of the hollow organs. Methodological recommendations]. Moscow, 2002, p. 8.
7. Chissov V.I., Sokolov V.V., Filonenko E.V. et al. Clinical fluorescence diagnostics in photodynamic carcinoma treatment with the photosensitizer Photogem. *Khirurgiya*, 1995, vol. 71 (5), pp. 37–41.
8. Korochanskaya N.V., Durleshter V.M., Kovalevskaya O.V., Serikova S.N., Popandopulo K.I. The morbidity and mortality rates from diseases of the digestive system in the Krasnodar Territory and the quality of medical care for gastroenterological patients. *Rossiiskii zhurnal gastroenterologii, gepatologii, koloproktologii*, 2019, vol. 29(4), pp. 30–37. (in Russ.)
9. Nolte E., McKee M. Measuring the health of the nations: analysis of mortality amenable to health care, *BMJ*, 2003, vol. 327, pp. 1129. DOI:10.1136/bmj.327.7424/1129//
10. Son I.M., Shipova V.M., Ivanova M.A. i dr. Rationing of outpatient care during primary care. *Zdravookhranenie*, 2014, vol. 7(7), pp. 6–8. (in Russ.)
11. Ivanova M.A. Rationing of the labor of the endocrinologist. *Zdravookhranenie*, 2016, vol. 2, pp. 52–55. (in Russ.)
12. Ivanova M.A. Rationing of labor is one of the ways to optimize the quality of medical care for patients]. *Sotsial'nye aspekty zdorov'ya naseleniya*, 2007, vol. 4(4), p. 6. (in Russ.)
13. Ivanova M.A. Rationing of labor - as the main tool for the formation of the staffing of outpatient facilities. *Sovremennye problemy zdravookhraneniya i meditsinskoi statistiki,* 2014, vol. 1, pp. 2–14. (in Russ.)
14. Kalashnikova I.A., Achkasov S.I. Peristomal skin complications and quality of life in patients with intestinal stoma. *Sbornik tez-*

ЛИТЕРАТУРА

1. Левченко Н.В., Хрячков В.В., Шавалиев Р.Р., Кислицин Д.П. Антеградная папиллотомия с использованием YAG:HO лазера при стенозе большого сосочка двенадцатиперстной кишки // Biomedical Photonics. – 2018. – Т. 7, № 1. – С. 21–27. https://doi.org/10.24931/2413–9432–2018–7-1–21–27
2. Туманина А.Н., Полежаев А.А., Апанасевич В.А., Гурина Л.И., Волков М.В., Тарасенко А.Ю., Филоненко Е.В. Опыт применения фотодинамической терапии в лечении рака пищевода // Biomedical Photonics. – 2019. – Т. 8, № 2. – С. 19–24. https://doi.org/10.24931/2413–9432–2019–8-2–19–24
3. Филоненко Е.В. История развития флуоресцентной диагностики и фотодинамической терапии и их возможности в онкологии // Российский химический журнал. – 2015. – Т. 85. – С. 211 – 216.
4. Кундухова Э.Р., Ремизов О.В., Бутаев Т.М., Дзугаева З.И., Алагова А.Р. Основные направления в решении проблем развития эндоскопии // Современные проблемы науки и образования. – 2017. – № 2. URL: http://scienceeducation.ru/ru/article/view?id=26388
5. Sokolov V.V., Chissov V.I., Filonenko E.V., Sukhin G.M., Yakubovskaya R.I., Belous T.A., Zharkova N.N., Kozlov D.N., Smirnov V.V. Photodynamic therapy of cancer with the photosensitizer PHOTOGEM // Proceedings of SPIE - The International Society for Optical Engineering. – 1995. – Т. 2325. – С. 375–380. https://doi.org/10.1117/12.199169
6. Соколов В.В., Чиссов В.И., Филоненко Е.В. Способ лазерного облучения при эндоскопической фотодинамической терапии начального рака полых органов. Методические рекомендации. – М., 2002. – С. 8.
7. Chissov V.I., Sokolov V.V., Filonenko E.V. et al. Clinical fluorescence diagnostics in photodynamic carcinoma treatment with the photosensitizer Photogem // Khirurgiya. – 1995. – Vol. 71. – №5. – pp. 37–41.
8. Корочанская Н.В., Дурлештер В.М., Ковалевская О.В., Серикова С.Н., Попандопуло К.И. Показатели заболеваемости и смертности от болезней органов пищеварения в Краснодарском крае и качество оказания медицинской помощи гастроэнтерологическим пациентам // Российский журнал гастроэнтерологии, гепатологии, колопроктологии. – 2019. – Т. 29(4). – С. 30–37. https://doi.org/10.22416/1382–4376–2019–29–4-30–37
9. Nolte E., McKee M. Measuring the health of the nations: analysis of mortality amenable to health care // BMJ. – 2003. – Vol. 32. – P. 1129. DOI:10.1136/bmj.327.7424/1129//
10. Сон И.М., Шипова В.М., Иванова М.А. и др., Нормирование труда амбулаторного приема при оказании первичной медицинской помощи // Здравоохранение. – 2014. – Т. 7. – С. 76–85.
11. Иванова М.А. Нормирование труда врача-эндокринолога // Здравоохранение. – 2016. – Т. 2. – С. 52–55.
12. Иванова М.А. Нормирование труда – один из путей оптимизации качества оказания медицинской помощи больным // Социальные аспекты здоровья населения. – 2007. – Т. 4(4). – С. 6.

isov. Materialy II s"ezda koloproktologov stran SNG, III s"ezda koloproktologov Ukrainy s uchastiem stran tsentral'noi i vostochnoi Evropy. Odessa. [Collection of abstracts. Materials of the II Congress of Coloproctologists of the CIS countries, IIIrd Congress of Coloproctologists of Ukraine with the participation of countries of central and eastern Europe. Odessa city], 2010, pp. 261–262.

15. Vorob`ev G.I., Achkasov S.I., Kalashnikova I.A. Organization of rehabilitation services for ostomy patients. *Menedzhment kachestva v sfere zdravookhraneniya i sotsial'nogo razvitiya,* 2007, vol. 2, pp. 55–56. (in Russ.)

13. Иванова М.А. Нормирование труда - как главный инструмент формирования штатного расписания амбулаторно-поликлинических учреждений // Современные проблемы здравоохранения и медицинской статистики. – 2014. – Т. 1. – С. 2–14.
14. Калашникова И.А., Ачкасов С.И. Перистомальные кожные осложнения и качество жизни у пациентов с кишечной стомой / Сборник тезисов // Материалы II съезда колопроктологов стран СНГ, III съезда колопроктологов Украины с участием стран центральной и восточной Европы. г. Одесса. – 2010. – С. 261–262.
15. Воробьев Г.И., Ачкасов С.И., Калашникова И.А. Организация служб реабилитации стомированных больных // Менеджмент качества в сфере здравоохранения и социального развития. – 2007. – Т. 2 – С. 55–56.

THERAPEUTIC PATHOMORPHOSIS IN MALIGNANT GLIOMA TISSUES AFTER PHOTODYNAMIC THERAPY WITH CHLORIN e6 (REPORTS OF TWO CLINICAL CASES)

Rynda A.Yu., Rostovtsev D.M., Olyushin V.E., Zabrodskaya Yu.M.
Russian Neurosurgical Institute named after Professor A.L. Polenov – a branch of «The National Medical Research Center named after V.A. Almazov», Saint-Petersburg, Russia

Abstract

In recent years, photodynamic therapy (PDT) has been increasingly introduced into the surgical practice of treating malignant neoplasms. In this publication, the authors show the appearance of therapeutic pathomorphosis *in vivo* in human malignant glioma cells after intraoperative photodynamic therapy. Tissue samples obtained 10–14 days after PDT revealed nuclear and cytoplasmic signs indicating apoptosis, necrosis, and autophagy. A decrease in the proliferative activity of glial tumor cells and their higher death count were detected.. Immunohistochemical analysis shows decreases expression of Ki-67 cell proliferation marker and decreased amount of transcription factor protein p53.

Keywords: photodynamic therapy, malignant gliomas, chlorin e6, therapeutic pathomorphosis, brain.

For citations: Rynda A.Yu., Rostovtsev D.M., Olyushin V.E., Zabrodskaya Yu.M. Therapeutic pathomorphosis in malignant glioma tissues after photodynamic therapy with chlorin e6 (reports of two clinical cases), *Biomedical Photonics*, 2020, vol. 9, no. 2, pp. 45–54 (in Russian). doi: 10.24931/2413–9432–2020–9–2–45–54.

Contacts: Rynda A.Yu., e-mail: artemii.rynda@mail.ru.

ЛЕЧЕБНЫЙ ПАТОМОРФОЗ В ТКАНЯХ ЗЛОКАЧЕСТВЕННОЙ ГЛИОМЫ ПОСЛЕ ФОТОДИНАМИЧЕСКОЙ ТЕРАПИИ С ХЛОРИНОМ e6 (СООБЩЕНИЕ О ДВУХ КЛИНИЧЕСКИХ СЛУЧАЯХ)

А.Ю. Рында, Д.М. Ростовцев, В.Е. Олюшин, Ю.М. Забродская
Российский нейрохирургический институт имени профессора А.Л. Поленова – филиал ФГБУ «Национальный медицинский исследовательский центр имени В.А. Алмазова», Санкт-Петербург, Россия

Резюме

Фотодинамическая терапия (ФДТ) в последние годы все более внедряется в хирургическую практику лечения злокачественных новообразований. В данной публикации авторами показано появление лечебного патоморфоза *in vivo* в клетках злокачественной глиомы человека после интраоперационной ФДТ. В образцах тканей, полученных через 10–14 дней после ФДТ, выявлены ядерные и цитоплазматические признаки, указывающие на апоптоз, некроз и аутофагию. Обнаружено снижение пролиферативной активности глиальных опухолевых клеток, увеличение числа случаев их гибели. По данным иммуногистохимии отмечено уменьшение экспрессии маркера клеточной пролиферации Ki-67 и снижение содержания белка транскрипционного фактора p53.

Ключевые слова: фотодинамическая терапия, злокачественные глиомы, хлорин е6, лечебный патоморфоз, головной мозг.

Для цитирования: Рында А.Ю., Ростовцев Д.М., Олюшин В.Е., Забродская Ю.М. Лечебный патоморфоз в тканях злокачественной глиомы после фотодинамической терапии с хлорином е6 (сообщение о двух клинических случаях) // Biomedical Photonics. – 2020. – Т. 9, № 2 – С. 45–54. doi: 10.24931/2413–9432–2020–9–2–45–54.

Контакты: Рында А.Ю., e-mail: artemii.rynda@mail.ru.

Introduction

Currently, photodynamic therapy (PDT) is actively used to treat tumors of various locations. Its use in the treatment of brain tumors was first suggested by I. Diamond in 1972 [1], and as early as in 1980, C. Perria described the results of treatment of malignant gliomas (MG) with PDT [2]. Later, scientists performed a significant number of small-scale clinical studies to assess the safety and effectiveness of this technique in glioma therapy [3, 4].

The PDT mechanism is aimed at the destruction of tumor cells and vessels that have accumulated the introduced photosensitizer (FS) and are located in the bed of the resected tumor, which is done with laser irradiation at a certain wavelength. Upon being administered in blood, a PS product is accumulated in the metabolically active tumor tissue.

The effect on the tumor tissue induces a number of intracellular and tissue mechanisms, as a result of which the cell dies through apoptosis, necrosis, or autophagy. The response to photodynamic effects depends on the type of tumor cell, its genetic or metabolic potential, as well as on the total amount of energy delivered by irradiation, the types of PS used, and their intracellular localization. The initial site affected by PDT can determine which pathway of cell death is activated. It is assumed that the autophagy process is triggered when cells damaged due to PDT try to retain damaged proteins and then remove them from the cell. The mechanism of apoptosis is triggered in the case of sufficiently severe damage in the cell that cannot be repaired. The use of PDT at maximum radiation doses leads to necrosis, since proteins involved in autophagy and apoptosis can be quickly destroyed, and cell integrity can be lost. In addition, the closure of tumor-feeding vessels can lead to local depletion of nutrients and oxygen and cause secondary necrosis associated with PDT [5-7].

A number of authors suggest that the method of cell death observed in PDT depends on the location of the greatest accumulation of PS inside the cell. With PS is localized in mitochondria, PDT will result in the loss of membrane permeability and the release of proapoptotic mediators, while the damage to the endoplasmic reticulum releases cellular calcium deposits. Damage to the lysosomes in which PS has accumulated leads to activation of proteolytic enzymes during laser radiation. Lysosomes can also merge with autophagosomes, leading to activation of hydrolysis of damaged organelles and their recirculation by autophagy. When PS is localized in more than one organelle, several pathways can participate in cell death simultaneously [7].

Additional mechanisms of the innate immune system trigger antiblastomic immunity in areas that are outside the PDT exposure zone. In some cases, PDT stimulates the immune system through several mechanisms, for example, by damaging cells through a combination of cytostatic mechanisms [7] and endogenous intracellular molecules known as Damage Associated Molecular Patterns (DAMPs) [8]. DAMPs that increase the immune system's sensitivity to tumor cells include Calreticulin, phosphatidylserine, adenosine triphosphate, peroxyredoxin 1, HMGB1, BCL-2, and annexin A1 [5, 9].

Among the many cytokines that can be activated by PDT, special attention is given to interleukin-1 (IL-1) and interleukin 6 (IL-6), serving as chemoattractants for various forms of immunocytes, including neutrophils, phagocytes, and lymphocytes [10]. Neutrophil penetration into cells that accumulated PS and were irradiated occurs within a few minutes after the PDT session, which increases the level of IL-1 and IL-6 in addition to the appearance of E-selectin in the cells of the perifocal tumoral inflammation zone. Some authors describe the significance of neutrophils in mediating PDT-induced cytotoxicity. In addition, activation of granulocyte-macrophage colony-stimulating factor (GM-CSF) leads to an increase in the number of neutrophils in tissues, potentiating the effectiveness of PDT. Cecic I. et al. showed the importance of the presence of anaphylatoxin C3a together with PDT-induced neutrophilic leukocytosis [11]. An inhibition of substances occurs in the cellular microenvironment, namely, of vasoendothelial growth factor, cyclooxygenase type 2, metalloproteinases of type 2 and type 9, Survivin apoptosis inhibitor, heat shock protein HSP-90, etc. In addition, PDT-induced release of other heat shock family proteins, such as HSP-47, 60, and 90, leads to increased sensitivity of the antigen-presenting complex to tumor cells [8]. The inhibition stops pathological angiogenesis and tumor cell proliferation. HSP70 binds to antigen-presenting cells (APCs) and facilitates antigen presentation, leading to dendritic cell maturation and activation of CD8 and cytostatic T-lymphocytes [12].

A PS fixed on the cell and exposed to laser radiation also activates non-immune mechanisms of antiblastoma resistance, such as carcinolytic cells (phagocytes, natural killers, cytotoxic T-lymphocytes), tumor necrosis factor-alpha, allogeneic inhibition factor and destruction of xenogenous cells (contact inhibition factors that inhibit the taxis and proliferation of tumor cells), alpha-lipoproteins of the surrounding tissue.

The outcome of these reactions is hypoxia, inflammation, and oxidative stress, leading to apoptotic and aseptic tumor necrosis. Secondary decrease in the synthesis of macroergic compounds in secondary tumor tissue hypoxia caused by the reduction of cytochrome C3, a lower ATP/ADP, and a reduction of NADN and NAD^+, causing a sharp decline in the respiratory capacity of mitochondria [13].

Activation of the lysosomal enzymes of the tumor cell causes intracellular catabolism of proteins and lipids, re-

sulting in the accumulation of underoxidized metabolic products, such as beta-oxybutyric acid and acetoacetic acid. There is a decrease in the activity of antioxidant systems of the glial tumor cell mainly due to the level of activity of superoxide dismutase and glutathione peroxidase, which triggers the activation of free radical oxidation and leads to the destruction of the cytoplasmic membranes of the tumor cell, mitochondria, lysosomes, as well as endoplasmic reticulum membranes, and the disruption of the cell's transport systems.

Activation of intracellular processes triggers a caspase cascade of complement activation with the formation of apoptosomal intracellular bodies. Caspases, through a series of biochemical reactions, activate the p53 protein, which suppresses the growth of tumor cells in the G1 phase. The point of application of PDT is also the endothelium of blood vessels and the system of macrophage cells, after irradiation of which the production of inflammatory mediators and cytokines (lymphokines, thromboxanes, prostoglandins, etc.) occurs, creating the vascular component for tumor stroma destruction [14].

It is extremely difficult to assess therapeutic pathomorphosis *in vivo* after PDT performed 7-14 days after surgery in patients with malignant glial tumors due to the intracranial location of the neoplasm. For its assessment, repeated surgical intervention is required in order to collect biopsy material, which is only possible subject to clinical indications. At the moment, there is almost no information in the literature concerning these data. The available scientific publications and reports are mainly based on the results of study of histological material obtained on models of glial tumors in animals, or on the analysis of glial tumor tissues *in vitro*, without blood flow and the microenvironment, with impaired biochemical and biophysical characteristics of the tissue, 6-72 hours after surgery [6, 15-20]. As a result, the data obtained are far from the changes that occur in real practice conditions.

The above-described results of studies conducted on various experimental models of glial neoplasms demonstrate that changes in tumor cells caused by PDT show signs of their death along the pathway of apoptosis, and less often by necrosis and autophagy [15-23]. There is evidence of the possibility of activation of PS in tumor cells located at a distance from the main focus or lying in the perifocal zone, since the penetration of radiated light into the brain tissue and tumors is limited by their physical and chemical properties. However, this is sufficient for a significant volumetric effect on the tissue achieved with the use of various diffusers to irradiate the entire cavity [4, 24-27].

Materials and methods

97 patients with supratentorial glial tumors were treated at Polenov Russian Neurosurgical Institute from 2004 to 2016.

According to the WHO classification, Grade IV was diagnosed in 49 (50.5%) patients, Grade III in 30 (31%), and Grade II in 18 (18.5%). Among patients with a Grade IV tumor, 48 had a morphological diagnosis of glioblastoma, and one of gliosarcoma. In the group of patients with Grade III tumors, anaplastic astrocytomas prevailed, while oligoastrocytomas and oligodendrogliomas were less common. The most common astrocytic tumors of Grade II were fibrillar-protoplasmic astrocytomas.

The patients underwent a complex treatment that included surgical removal of the tumor, intraoperative fluorescence diagnostics and PDT with Photoditazin, a second-generation chlorin e6 preparation (OOO VETA-GRAND, Russia, registration certificate No. ЛС 001246 dated 18.05.2012) [24, 25].

The degree of the totality of tumor removal was assessed with brain MRI (CT) data in the first 72 hours after surgery. In the vast majority of patients, a total (52 patients, 53.63%) or subtotal (33 patients, 34.0%) tumor removal was achieved. In 12 (12.37%) patients, the tumor was removed partially.

The choice and prescription of further adjuvant radiation or chemotherapy depended on the histological structure of the tumor.

An analysis of the effectiveness of PDT based on the results of morphological examination of biopsies obtained intraoperatively in 2 (4.1%) patients with Grade IV glial brain tumors of supratentorial localization was performed.

The methods of intraoperative photodynamic therapy

During the patient's stay on the operating table, after introductory anesthesia and 1.5 – 2 hours before the intended removal of the tumor, Photoditazine, a drug with chloride e6 as the active substance, diluted in 200 ml of saline solution in the dose of 1 mg per 1 kilo of body mass, was administered intravenously. The vial with the diluted agent was enclosed in a light-proof material. Photoditazine selectively accumulated in glioma tissue, while its concentration in normal brain tissue remained minimal, which allowed for determination of the tumor sites by the characteristic red fluorescence of chlorines (Fig. 1).

To perform fluorescence diagnostics in blue light, a fluorescent extension (LOMO, Saint Petersburg, Russia) was connected to the surgical microscope (LEICA OHS-1, Leica Microsystems, Germany). The fluorescent pattern with a high color contrast allowed to differentiate the tumor tissue that had accumulated Photoditazine from the intact tissue. At the same time, unaffected normal brain matter was also visible. The tissue with red fluorescence was gradually removed with due account for the functional and anatomical and physiological features of the tumor localization.

After removal, the neoplasms achieved thorough he-

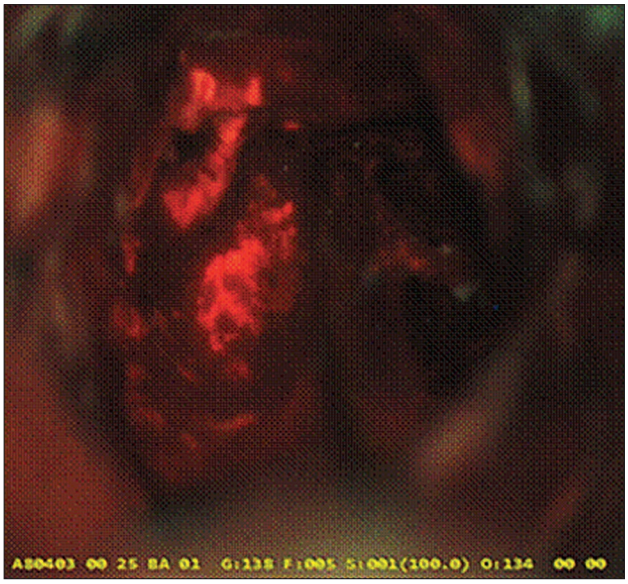

Рис. 1. Флуоресценция фотодитазина в глиобластоме
Fig. 1. Fluorescence of Fotoditazin in glioblastoma

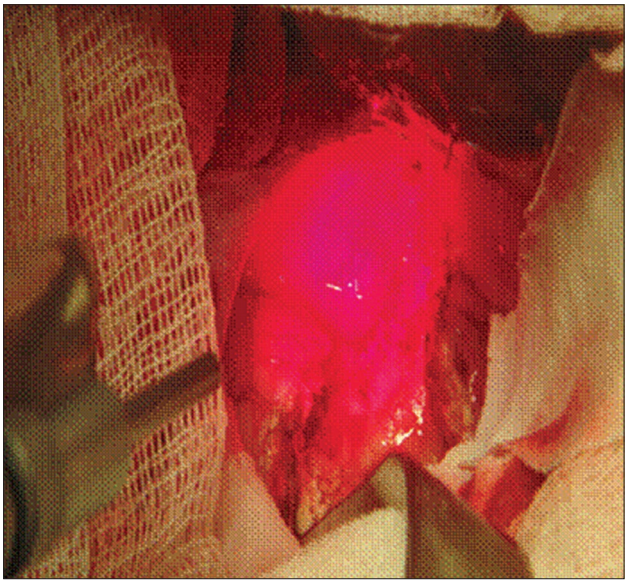

Рис. 2. Внешний вид операционного поля при проведении сеанса интраоперационной фотодинамической терапии
Fig. 2. A session of photodynamic therapy

mostasis in the perifocal zone. Then the distal end of a fiber instrument connected to a laser radiation source (Latus 2.5, Atkus, Russia) with a wavelength of 662 nm and a maximum power of 2.5 W was entered to reach the bed of the removed tumor, and laser irradiation was performed (Fig. 2). The duration of irradiation did not exceed 15-20 minutes. To prevent the risk of thermal damage to tissues during irradiation, the tumor bed was continuously irrigated with saline solution. The light dose was on average 180 J/cm^2. In the following 24 hours, the patient wore dark glasses to avoid direct sunlight on the retina to prevent its photodamage due to the presence of residual Photoditazine on it.

In all patients, the diagnosis was confirmed by histological examination of the surgical material in accordance with the WHO classification of CNS tumors. The material was fixed in 10% formalin, dehydrated in the standard way, and covered with paraffin. Sections with a thickness of 3-5 microns were made (Leica SM 2000R microtome, Leica Microsystems, Germany), stained with hematoxylin and eosin, and, if necessary, other types of staining were performed: van Gieson pycrofuxin, silver impregnation. A Leica 4000B laboratory microscope (Leica Microsystems, Germany) and a Leica DM 2500 laboratory microscope (Leica Microsystems, Germany) equipped with a digital camera and an adapted Adobe Photoshop CS 3 program were used for visualization and microphotography. The expression of p53 and Ki-67 (MIB-1) markers was determined by the immunohistochemical method.

Results

Two patients underwent repeated operations for the developed complications.

In the first observation, patient K., a 58-year-old male with glioblastoma of the right frontal lobe, was re-operated 10 days after PDT surgery in connection with the formation of an acute epidural hematoma in the area of surgery due to an injury sustained when he felt severe dizziness and fell down. In the postoperative period, the neurological status of the patient did not change.

During the first operation, photoditazine was administered intravenously at a dose of 1 mg per kilo of body mass 1.5 hours before the tumor was removed, to induce photodynamic effects. The PDT session was performed with a 1 cm long diffuser in continuous mode, an optical fiber diameter of 200 microns, an output optical power of 0.5 W, and an energy density of 180 J/cm^2. The duration of irradiation was 15 minutes.

In another observation, patient M., a 45-year-old male with glioblastoma of the left frontal lobe, was re-operated for osteomyelitis of the bone flap 2 weeks after PDT surgery. In the postoperative period, the patient's neurological status did not deteriorate.

To induce photodynamic effects during the first operation, Photoditazine was also administered intravenously at a dose of 1 mg per kilo of body mass 1.5 hours before the tumor removal. The duration of irradiation was 15 min. The irradiation was performed with a 1 cm long cone-shaped diffuser with a ball at the end, in continuous mode, with an optical fiber diameter of 200 microns,

and an output optical power of 0.5 W. The energy density was 180 J/cm^2.

In both cases, biopsies were taken from the bed of the removed tumor at the PDT site. The PDT-induced medical pathomorphosis was subject to pathomorphological and immunohistological evaluation. The tissue samples obtained after PDT were found to have nuclear and cytoplasmic signs indicating apoptosis, necrosis, and autophagy. Chromatin marginality along the intact nuclear membrane, chromatin condensation, swollen mitochondria with fragmentation of mitochondrial ridges, an increase in the number of cytoplasmic vacuoles, and membrane erosions were observed (Fig. 3, 4). In glial tumor biopsies, homogenization, loss of cell boundaries, the formation of ghost cells with pycnotic nuclei, perinuclear vacuolization and cytoplasm shrinkage, and pronounced vacuolar degeneration were observed. Individual cells in tumor nodules, mainly located at the borders, showed morphological changes characteristic of apoptosis: chromatin condensation, karyopyknozis, eosinophilic cytoplasm and increased nuclear-cytoplasmic ratio.

In the first case, against the background of PDT, pronounced fields of gliosis and necrosis, intracellular signs of chromatin condensation, cell fragmentation, apoptotic corpuscles, and the presence of phagosomes were observed. There were signs of typical dystrophic and alterative changes against the background of pronounced vascular disorders: stasis, sharp hyperemia, sludge phenomenon, microthrombosis, plasmarrhage and hemorrhage, and inflammatory infiltration.

In the second case, there was a marked increase in the number of cytoplasmic vacuoles, membrane erosion, fragmentation of mitochondrial ridges, karyopycnosis, the signs of autophagy, and the presence of "monster cells". The blood vessels were slit-shaped with partially formed thin walls and deformed lumen.

The foci of necrosis were surrounded by connective tissue (substitutive gliosis). The formation of lymphoplasmocytic infiltrates, as well as lymphocytic perivascular couplings, and the appearance of giant multinucleated and xanthomous cells, was observed around tumor cells groups. It should be particularly noted that the alterative changes in the tumor tissues had a gradient character, with the severity gradually decreasing from the center to the periphery.

The presence of therapeutic pathomorphosis after PDT was also observed in the immunohistochemical study of drugs, which revealed a decrease in the expression of Ki-67 cell proliferation marker from 31 to 7% in the first case and from 29 to 6% in the second case. There was a decrease in the protein content of transcription factor p53 after PDT from "+++" to "+" in both patients (Fig. 5, 6).

The catamnesis in the first patient after complex treatment with intraoperative PDT and subsequent radiation therapy (total boost dose: 95 Gy) and chemotherapy with Temozolomide (6 courses) was 19 months. In the second patient, after complex treatment with intraoperative PDT and subsequent radiation therapy (total boost dose = 60 Gy) and chemotherapy with Temozolomide (4 courses), the catamnesis was 17.5 months.

Discussion

High risks of continued tumor growth and low median survival in MG are the main reasons that adversely affect the outcome of treatment. Despite the high probability of tumor recurrence, many literature sources report that the delaying time to relapse and the median life expectancy of patients with MG depends on the degree of radicality of the surgical intervention. This pattern also remains true with repeated surgical interventions. However, it should be taken into account that glial tumors are characterized by invasive growth with the spread of tumor cells across perivascular spaces at a significant distance from the main tumor focus. The nature of glioma growth and the limited possibility of resection in functionally significant areas of the brain do not allow for the total removal of the tumor [3, 4, 24-26]. Therefore, the search for the latest treatment methods that achieve the maximum possible removal of tumor cells in the perifocal zone of the tumor and at a distance from the main focus remains an urgent problem. One of these methods is intraoperative PDT. The safety and effectiveness of this technique in the treatment of oncological diseases of different localities have been shown by a number of authors. In recent years, publications on the results of PDT use in patients with MG have become more frequent. However, reseach on the effectiveness of PDT in patients with MG based on the results of morphological studies is extremely rare. Those are mainly experimental models of cell strains and biopsy materials in animals with implanted human gliomas subjected to PDT.

The response of tumor tissues to intraoperative PDT is a complex of induced alterative/destructive changes in the glioma. Its assessment is of particular importance for determining the effectiveness of PDT, which occupies a crucial place in the treatment of MG.

In our study, the evaluation of the effectiveness of PDT was based not only on catamnesis data but also on the study of therapeutic pathomorphosis in the histological examination of tumor preparations before and after PDT.

It was found that the tissue and cellular targets of photoditazine PS are the vascular wall, plasma membrane of neoplastic tissue, and intracellular structures and mechanisms responsible for proliferation and biosynthesis processes. The intracellular arrangement of FS in various organoids (mitochondria, lysosomes, endoplasmic reticulum, cytoplasmic membrane, etc.) played an important role in the cell death mechanism. After PDT, the proliferative activity of tumor cells decreased. According to the immunohistochemical study, there was a decrease in the

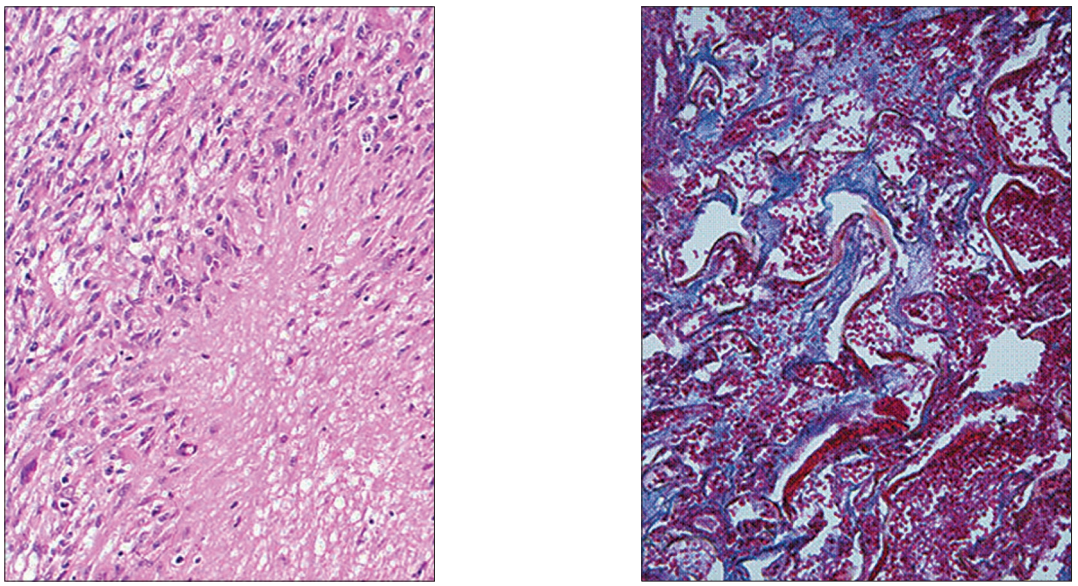

Рис. 3. Микрофотография препарата глиобластомы пациента К.:
a – до проведения фотодинамической терапии;
b – лечебный патоморфоз через 10 дней после проведения фотодинамической терапии. Окраска гематоксилином-эозином. Ув. х200.
Fig. 3. Micrograph of glioblastoma of patient K. (magnification x200, hematoxylin-eosin staining):
a – before photodynamic therapy;
b – therapeutic pathomorphosis 10 days after photodynamic therapy

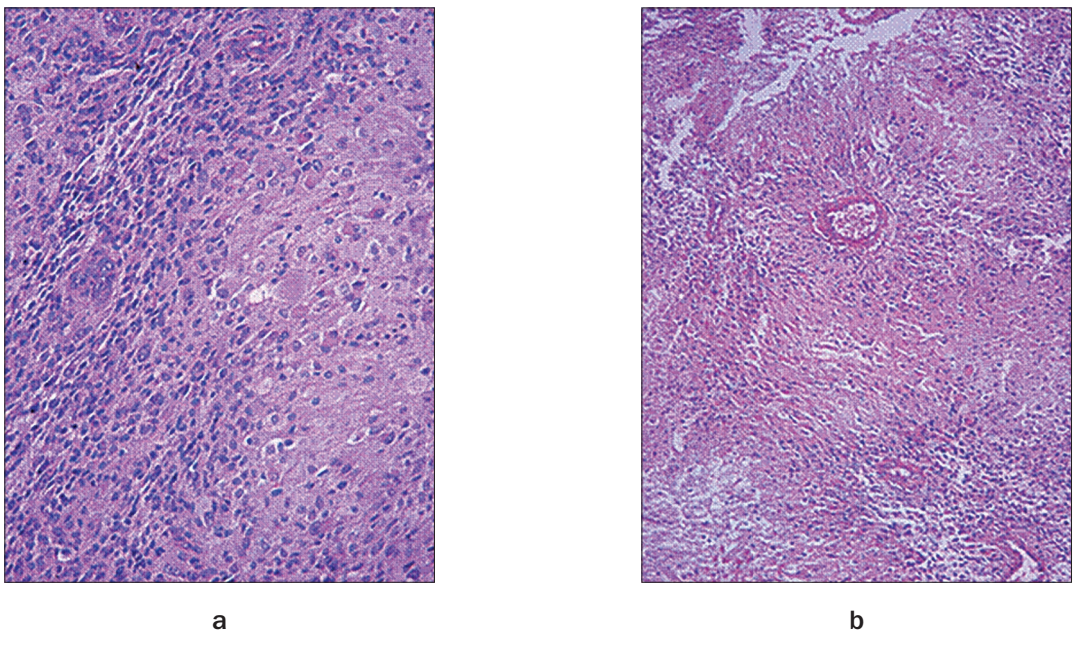

Рис. 4. Микрофотография препарата глиобластомы пациента М.:
a – до проведения фотодинамической терапии;
b – лечебный патоморфоз через 14 дней после фотодинамической терапии. Окраска гематоксилином-эозином. Ув. х200.
Fig. 4. Micrograph of glioblastoma of patient M. (Magnification x200, stained with hematoxylin-eosin):
a – before photodynamic therapy;
b – therapeutic pathomorphosis 14 days after photodynamic therapy

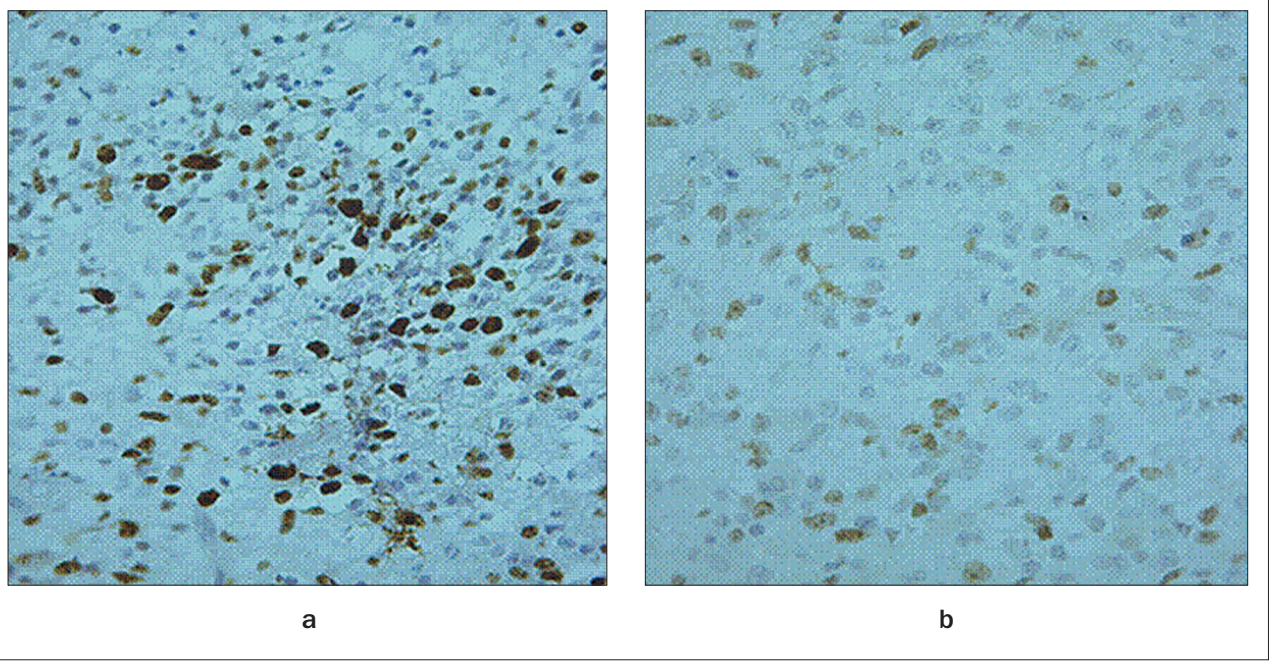

Рис. 5. Иммуногистохимия. Экспрессия Ki-67 в препарате глиобластомы пациента М.:
a – до проведения фотодинамической терапии;
b – лечебный патоморфоз через 14 дней после фотодинамической терапии. Ув. х400
Fig. 5. Immunohistochemistry. Expression of Ki-67 in glioblastoma of patient M. (magnification x400):
a – before photodynamic therapy;
b – therapeutic pathomorphosis 14 days after photodynamic therapy

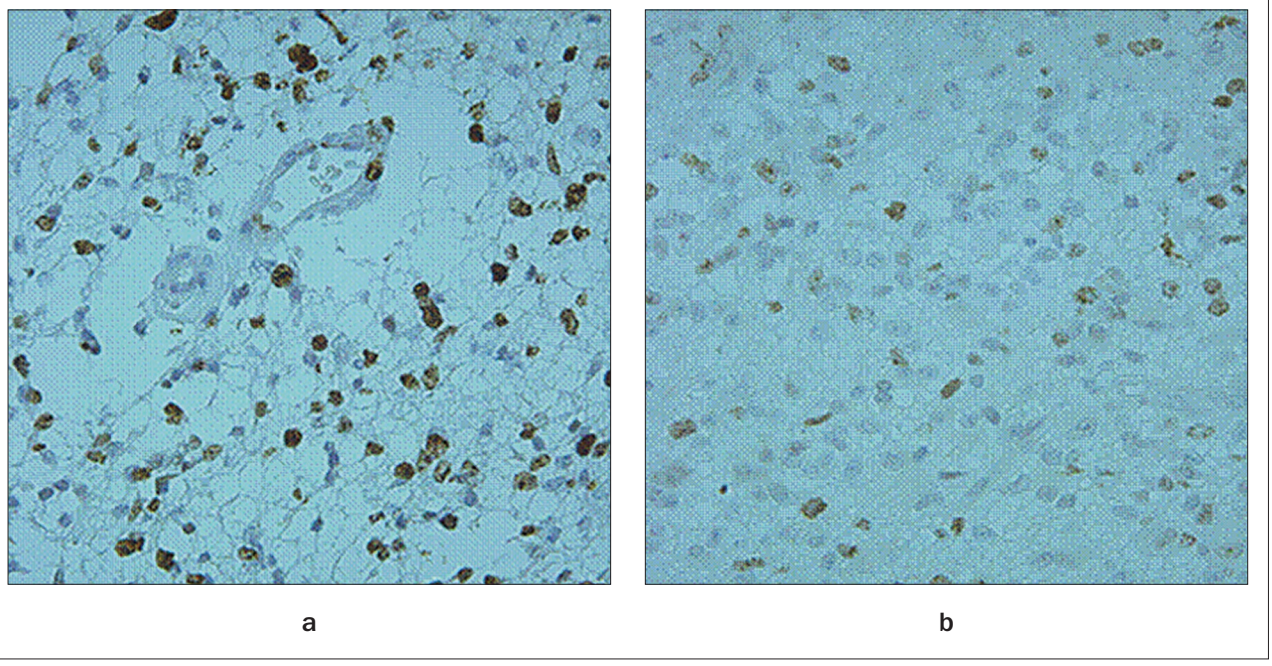

Рис. 6. Иммуногистохимия. Экспрессия p53 в препарате глиобластомы пациента М.:
a – до проведения фотодинамической терапии;
b – лечебный патоморфоз через 14 дней после фотодинамической терапии. Ув. х400.
Fig. 6. Immunohistochemistry. Expression of p53 in glioblastoma of patient M. (magnification x400):
a – before photodynamic therapy;
b – therapeutic pathomorphosis 14 days after photodynamic therapy

expression of the cell proliferation marker Ki-67 and the level of p53 transcription factor protein. Thus, a decrease in Ki-67 and p53 expression after PDT can be considered as a favorable predictor of therapy effectiveness.

The data obtained as a result of our study indicate morphological changes in glial tumor cells after PDT. However, the small scale of our clinical study does not allow us to draw unambiguous and reliable conclusions, so a further study of the issue is required.

Conclusion

The results of our work can testify to the effectiveness of PDT in the structure of complex treatment of patients with MG, as evidenced by the results of the application of the technique by many authors [3, 4, 7, 24-29].

The revealed morphological changes in glioblastoma tissues after PDT should be regarded as a manifestation of therapeutic pathomorphosis.

In the near future, PDT will definitely become the standard of treatment for patients with MG on a par with such methods as radiation and chemotherapy. Moreover, PDT has no systemic side effects on healthy tissues, such as those that occur after chemotherapy and radiation therapy, and belongs to the methods based on superselective action on tumor cells.

PDT is a promising and safe method that makes it possible to intraoperatively affect disseminated tumor cells lying in the perifocal zone and cause structural changes in those cells (therapeutic pathomorphosis), which determines the best long-term results of MG patients treatment. This technique should be used as part of comprehensive treatment in the surgery of glial brain tumors of supratentorial localization of varying degrees of malignancy. However, further clinical data is necessary to study the effectiveness and usefulness of PDT in MG patients.

REFERENCES

1. Perria C., Capuzzo T., Cavagnaro G., Datti R., Francaviglia N., Rivano C., Tercero V.E. First attempts at the photodynamic treatment of human gliomas, *J neurosurg sci*, 1980, vol. 24, pp. 119–129.
2. Diamond I., Granelli S.G., McDonagh A.F., Nielsen S., Wilson C.B., Jaenicke R. Photodynamic therapy of malignant tumours, *Lancet*, 1972, vol. 2, pp.1175–1177.
3. Kurzhupov M.I., Filonenko E.V., Loshakov V.A., Zaytsev A.M. Photodynamic therapy in neurooncology, *Ros. onkol. zhurn.*, 2010, no .4, pp. 45–48. (in Russ.)
4. Tzerkovsky D.A., Maslakov E.A., Bagrintsev D.A., Semak I.A., Protopovich Y.L., Chizh A.G., Tatur A.A., Fomenkov I.S., Stupak D.S. The role of photodynamic therapy in the treatment of primary, recurrent and metastatic malignant brain tumors, *Biomedical Photonics*, 2018, vol. 7, no. 2, pp. 37–49. (in Russ.)
5. Korbelik M., Sun J., Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response, *Cancer res*, 2005, vol. 65, pp. 1018–1026.
6. Yakubovskaya R.I., Morozova N.B., Pankratov A.A., et al. Experimental photodynamic therapy: 15 years of development, *Russian Journal of General Chemistry*, 2015, vol. 85(1), pp. 217–239.
7. Mroz P., Yaroslavsky A., Kharkwal G.B., Hamblin M.R. Cell Death Pathways in Photodynamic Therapy of Cancer, *Cancer*, 2011, vol. 3, pp.2516–2539.
8. Tesniere A., Panaretakis T., Kepp O., Apetoh L., Ghiringhelli F., Zitvogel L., Kroemer G. Molecular characteristics of immunogenic cancer cell death, *Cell seath siffer*, 2008, vol. 15, pp. 3–12.
9. Garg A.D., Agostinis P. ER stress, autophagy and immunogenic cell death in photodynamic therapy-induced anti-cancer immune responses, *Photochem photobiol sci*, 2014. vol. 13,pp. 474–487.
10. Kaplanski G., Marin V., Montero-Julian F., Mantovani A., Farnarier C. IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation, *Trends Immunol*, 2003, vol. 24, pp. 25–29.
11. Cecic I., Stott B., Korbelik M. Acute phase response-associated systemic neutrophil mobilization in mice bearing tumors treated by photodynamic therapy, *Int immunopharmacol*, 2006, vol. 6, pp. 1259–1266.
12. Todryk S., Melcher A.A., Hardwick N., Linardakis E., Bateman A., Colombo M.P., Stoppacciaro A., Vile R.G. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets

ЛИТЕРАТУРА

1. Perria C., Capuzzo T., Cavagnaro G. et al. First attempts at the photodynamic treatment of human gliomas // J neurosurg sci. – 1980. – Vol. 24. – P. 119–129.
2. Diamond I., Granelli S.G., McDonagh A.F. et al. Photodynamic therapy of malignant tumours // Lancet. – 1972. – Vol. 2. – P.1175–1177.
3. Куржупов М.И., Филоненко Е.В., Лошаков В.А., Зайцев А.М. Фотодинамическая терапия в нейроонкологии // Рос. онкол. журн. – 2010. – № 4. – С. 45–48.
4. Церковский Д.А., Маслаков Е.А., Багринцев Д.А. и соавт. Роль фотодинамической терапии в лечении первичных, рецидивных и метастатических злокачественных опухолей головного мозга // Biomedical Photonics. – 2018. – Т. 7, № 2. – С. 37–49.
5. Korbelik M., Sun J., Cecic I. Photodynamic therapy-induced cell surface expression and release of heat shock proteins: relevance for tumor response // Cancer res. – 2005. – Vol. 65. – P. 1018–1026.
6. Yakubovskaya R.I., Morozova N.B., Pankratov A.A., et al. Experimental photodynamic therapy: 15 years of development // Russian Journal of General Chemistry. – 2015. – Vol. 85(1). – P. 217–239.
7. Mroz P., Yaroslavsky A., Kharkwal G.B. et al. Cell Death Pathways in Photodynamic Therapy of Cancer // Cancer. – 2011. – Vol. 3. – P. 2516–2539.
8. Tesniere A., Panaretakis T., Kepp O. et al. Molecular characteristics of immunogenic cancer cell death // Cell seath siffer. – 2008. – Vol. 15. – P. 3–12.
9. Garg A.D., Agostinis P. ER stress, autophagy and immunogenic cell death in photodynamic therapy-induced anti-cancer immune responses // Photochem photobiol sci. – 2014. – Vol. 13. – P. 474–487.
10. Kaplanski G., Marin V., Montero-Julian F. et al. IL-6: a regulator of the transition from neutrophil to monocyte recruitment during inflammation // Trends immunol. – 2003. – Vol. 24. – P. 25–29.
11. Cecic I., Stott B., Korbelik M. Acute phase response-associated systemic neutrophil mobilization in mice bearing tumors treated by photodynamic therapy // Int immunopharmacol. – 2006. – Vol. 6. – P. 1259–1266.
12. Todryk S., Melcher A.A., Hardwick N. et al. Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake // J immunol. – 1999. – Vol. 163. – P. 1398–1408.

immature dendritic cell precursors to enhance antigen uptake, *J Immunol*, 1999, vol. 163, pp. 1398–1408.
13. Huang H.C., Mallidi S., Liu J., Chiang C.T., Mai Z., Goldschmidt R., Ebrahim-Zadeh N., Rizvi I., Hasan T. Photodynamic Therapy Synergizes with Irinotecan to Overcome Compensatory Mechanisms and Improve Treatment Outcomes in Pancreatic Cancer, *Cancer res*, 2016, vol. 76, p.1066–1077.
14. Chen B., Pogue B.W., Hoopes P.J. Vascular and cellular targeting for photodynamic therapy, *Crit rev. Eukaryot gene expr*, 2006, vol. 16, pp. 279–306.
15. Hambsch P., Istomin Y.P., Tzerkovsky D.A., Patties I., Neuhaus J., Kortmann R.D., Schastak S., Glasow A. Efficient cell death induction in human glioblastoma cells by photodynamic treatment with Tetrahydroporphyrin-Tetratosylat (THPTS) and ionizing irradiation, *Oncotarget*, 2017, vol. 8, no. 42, pp. 72411–72423.
16. Tirapelli L.F., Morgueti M., da Cunha Tirapelli D.P., Bagnato V.S., Ferreira J., Neto F.S., Peria F.M., Oliveira H.F., Junior C.G. Apoptosis in glioma cells treated with PDT, *Photomed Laser Surg*, 2011, vol. 29, no. 5, pp. 305–309.
17. Miki Y., Akimoto J., Yokoyama S., Homma T., Tsutsumi M., Haraoka J., Hirano K., Beppu M. Photodynamic Therapy in Combination with Talaporfin Sodium Induces Mitochondrial Apoptotic Cell Death Accompanied with Necrosis in Glioma Cells, *Biological and Pharmaceutical Bulletin*, 2013, vol. 36, is. 2, pp. 215–221.
18. Yuan S.X., Li J.L., Xu X.K. Underlying mechanism of the photodynamic activity of hematoporphyrin-induced apoptosis in U87 glioma cells, *International Journal of Molecular Medicine*, 2018, vol. 41, is. 4, pp. 2288–2296.
19. Fisher C.J., Niu C., Foltz W., Chen Y., Sidorova-Darmos E., Eubanks J.H., Lilge L. ALA-PpIX mediated photodynamic therapy of malignant gliomas augmented by hypothermia, *PLoS ONE*, 2017, vol. 12, no. 7, e0181654.
20. Boeuf-Murailleab G., Rigauxa G., Callewaert M., Callewaert M., Zambrano N., Van Gulick L., Roullin V.G., Terryn C., Andry M.C., Chuburu F., Dukic S., Molinari M. Evaluation of mTHPC-loaded PLGA nanoparticles for in vitro photodynamic therapy on C6 glioma cell line, *Photodiagnosis and Photodynamic Therapy*, 2019, vol. 25, pp. 448–455.
21. Rynda A., Rostovtsev D., Olyushin V, Zabrodskaya Yu.M. Fluorescence-Guided Resection of glial brain tumors with Fotoditazin, *Journal of Surgery*, 2018, vol. 6, is. 5, pp. 116–122.
22. Rynda A.Y., Olyushin V.E., Rostovtsev D.M. Photodynamic therapy of cerebral glioma – long term survival, *Vestnik Rossiiskoi Voenno-Meditsinskoi Academii*, 2017, vol. 2, no. 58, pp. 68–72. (in Russ.)
23. Abrahamse H., Hamblin M.R. New photosensitizers for photodynamic therapy, *Biochem j*, 2016, vol. 473, no. 4, pp. 347–364.
24. Shimizu K., Nitta M., Komori T., et al. Intraoperative Photodynamic Diagnosis Using Talaporfin Sodium Simultaneously Applied for Photodynamic Therapy against Malignant Glioma: A Prospective Clinical Study, *Front neurol*, 2018, vol. 9, pp. 1–9.
25. Osman H., Elsahy D., Saadatzadeh M.R., Maruyama T., Yasuda T., Fujii Y., Masamune K., Kawamata T., Maehara T., Muragaki Y. Acridine Orange as a Novel Photosensitizer for Photodynamic Therapy in Glioblastoma, *World Neurosurgery*, 2018, vol. 114, e1310–e1315.
26. Nitta M., Muragaki Y., Maruyama T., Iseki H., Komori T., Ikuta S., Saito T., Yasuda T., Hosono J., Okamoto S., Koriyama S., Kawamata T. Role of photodynamic therapy (PDT) using talaporfin sodium and semiconductor laser on prognosis of patients with newly diagnosed glioblastoma, *Neuro-oncol*, 2017, vol. 19, suppl. 6, no. 6, p. 20.
27. Kaneko S., Okura S.I., Tanaka T. Photodynamic applications (PDD, PDT) using aminolevulinic acid in neurosurgery in *Aminolevulinic Acid, Science, technology and application*. Okura I., Tanaka T.R. Eds. SBI ALA Promo Co., Ltd, 2015. pp. 119–140.
28. Singh K., Kouli O., Kanodia A., Goodman C., Eadie E., Ibbotson S.H., Hossain-Ibrahim K. Comparing Outcomes in Glioblastoma Multiforme patients undergoing Photodynamic Therapy with a
13. Huang H.C., Mallidi S., Liu J. et al. Photodynamic Therapy Synergizes with Irinotecan to Overcome Compensatory Mechanisms and Improve Treatment Outcomes in Pancreatic Cancer // Cancer res. – 2016. – Vol. 76. – P. 1066–1077.
14. Chen B., Pogue B.W., Hoopes P.J. Vascular and cellular targeting for photodynamic therapy // Crit rev. Eukaryot gene expr. – 2006. – Vol. 16. – P. 279–306.
15. Hambsch P., Istomin Y.P., Tzerkovsky D.A., et al. Efficient cell death induction in human glioblastoma cells by photodynamic treatment with Tetrahydroporphyrin-Tetratosylat (THPTS) and ionizing irradiation // Oncotarget. – 2017. – Vol. 8, No. 42. – P. 72411–72423.
16. Tirapelli L.F., Morgueti M., da Cunha Tirapelli D.P., et al. Apoptosis in glioma cells treated with PDT // Photomed Laser Surg. – 2011. – Vol. 29, No. 5. – P. 305–309.
17. Miki Y., Akimoto J., Yokoyama S., et al. Photodynamic Therapy in Combination with Talaporfin Sodium Induces Mitochondrial Apoptotic Cell Death Accompanied with Necrosis in Glioma Cells // Biological and Pharmaceutical Bulletin. – 2013. – Vol. 36, Iss. 2. – P. 215–221.
18. Yuan S.X., Li J.L., Xu X.K. Underlying mechanism of the photodynamic activity of hematoporphyrin-induced apoptosis in U87 glioma cells // International Journal of Molecular Medicine. – 2018. – Vol. 41, Iss.4. – P.2288–2296.
19. Fisher C.J., Niu C., Foltz W., et al. ALA-PpIX mediated photodynamic therapy of malignant gliomas augmented by hypothermia // PLoS ONE. – 2017. – Vol. 12, No. 7. – e0181654.
20. Boeuf-Murailleab G., Rigauxa G., Callewaert M., et al. Evaluation of mTHPC-loaded PLGA nanoparticles for in vitro photodynamic therapy on C6 glioma cell line // Photodiagnosis and Photodynamic Therapy. – 2019. –Vol. 25. – P. 448–455.
21. Rynda A., Rostovtsev D., Olyushin V. et al. Fluorescence-Guided Resection of glial brain tumors with Fotoditazin // Journal of Surgery. – 2018. – Vol. 6, Iss. 5. – P. 116–122.
22. Рында, А.Ю., Олюшин В.Е., Ростовцев Д.М. Фотодинамическая терапия глиом головного мозга – отдаленные результаты // Вестник Российской военно-медицинской академии. – 2017. – № 2 (58). – С. 68–72.
23. Abrahamse H., Hamblin M.R. New photosensitizers for photodynamic therapy // Biochem j. –2016. – Vol. 473, № 4. – P.347–364.
24. Shimizu K., Nitta M., Komori T., et al. Intraoperative Photodynamic Diagnosis Using Talaporfin Sodium Simultaneously Applied for Photodynamic Therapy against Malignant Glioma: A Prospective Clinical Study // Front neurol. – 2018. – Vol. 9. – P. 1–9.
25. Osman H., Elsahy D., Saadatzadeh M.R., et al. Acridine Orange as a Novel Photosensitizer for Photodynamic Therapy in Glioblastoma // World Neurosurgery. – 2018. – Vol. 114. – P. e1310–e1315.
26. Nitta M., Muragaki Y., Maruyama T., et al. Role of photodynamic therapy (PDT) using talaporfin sodium and semiconductor laser on prognosis of patients with newly diagnosed glioblastoma // Neuro-oncol. – 2017. – Vol. 19, Suppl. 6, No. 6. – P. 20.
27. Kaneko S., Okura S.I., Tanaka T. Photodynamic applications (PDD, PDT) using aminolevulinic acid in neurosurgery / Aminolevulinic acid. Science, technology and application by Okura I., Tanaka T.R. as eds. – SBI ALA Promo Co., Ltd., 2015. – P. 119–140.
28. Singh K., Kouli O., Kanodia A., et al. Comparing Outcomes in Glioblastoma Multiforme patients undergoing Photodynamic Therapy with a Second-Generation Photosensitiser vs 5-Aminolevulinic Acid – A Single Site Retrospective Analysis // Neuro-Oncology. – 2018. – Vol. 20, Suppl.3. – P. 265.
29. Dupont C., Reyns N., Deleporte P., et al. Intraoperative photodynamic treatment for high-grade gliomas // SPIE Proceedings Photodynamic Therapy VI. – 2017. – Vol. 10047.

Second-Generation Photosensitiser vs 5-Aminolevulinic Acid – A Single Site Retrospective Analysis, *Neuro-Oncology*, 2018, vol. 20, suppl. 3, pp. 265.

29. Dupont C., Reyns N., Deleporte P., et al. Intraoperative photodynamic treatment for high-grade gliomas, *SPIE Proceedings Photodynamic Therapy VI*, 2017, vol. 10047.