COMPARATIVE EXPERIMENTAL STUDY OF 5-ALA AND 5-ALA HEXYL ESTER SPECIFIC ACTIVITY

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Abstract

A comparative experimental study of the specific activity of drugs based on 5-aminolevulinic acid (5-ALA) and its hexyl ester (5-ALA HE) was carried out. Their ability to induce the synthesis of photoactive protoporphyrin IX in the healthy tissues of the rabbit bladder when instilling the drug solutions at various concentrations has been estimated. It was shown that 5-ALA HE results in the induction and accumulation of PPIX in the rabbit bladder epithelium at much lower concentrations than 5-ALA. Thus, a significant increase in the fluorescence intensity in comparison with the control was achieved by instillation of 5-ALA HE solution in the rabbit' bladder at a concentration of only 0.0001% (fluorescence intensity 2.20±0.60 a.u.), and for 5-ALA - only when using a solution at a concentration of 0.3% (fluorescence intensity 2.60±1.02 a.u.).

Keywords: fluorescence diagnosis, protoporphyrin IX, 5-aminolevulinic acid, hexyl ester of 5-aminolevulinic acid, bladder cancer.

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СРАВНИТЕЛЬНОЕ ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ СПЕЦИФИЧЕСКОЙ АКТИВНОСТИ 5-АЛК И ГЕКСИЛОВОГО ЭФИРА 5-АЛК

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Резюме

Проведено сравнительное экспериментальное исследование специфической активности препаратов на основе 5-аминолевулиновой кислоты (5-АЛК) и ее гексилового эфира (ГЭ 5-АЛК). Оценена их способность индуцировать синтез фотоактивного протопорфирина IX в здоровых тканях мочевого пузыря кролика при инстилляции растворов препаратов в различных концентрациях. Исследования показали, что ГЭ 5-АЛК вызывает индукцию и накопление ППIX в эпителии мочевого пузыря кролика в значительно меньших концентрациях, чем 5-АЛК. Так, достоверное увеличение интенсивности флуоресценции по сравнению с контролем удалось достичь при инстилляции в мочевой пузырь кролика раствора ГЭ 5-АЛК в концентрации всего 0,0001% (интенсивность флуоресценции 2,20±0,60 усл.ед.), а для 5-АЛК – только при использовании раствора в концентрации 0,3% (интенсивность флуоресценции 2,60±1,02 усл.ед.).

Ключевые слова: флуоресцентная диагностика, протопорфирин IX, 5-аминолевулиновая кислота, гексиловый эфир 5-аминолевулиновой кислоты, мочевой пузырь.

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Introduction

Among modern methods for early cancer diagnosis, fluorescence methods are currently considered the most promising, as they are based on the possibility of recognizing malignant tissue by the characteristic fluorescence induced by light radiation of exogenous or endogenous fluorochromes [1,2].

Fluorescence diagnostics (PD) of tumors is based on the selectivity of accumulation in tumor node tissues and the possibility of its detection by the spectra of exogenous fluorescence from the area illuminated by laser radiation. This method allows the detection of tumors, as well as determines their topography when scanning spots of exciting laser radiation on the surface of the tissue [3].

Fluorescence cancer diagnosis is the most promising for the detection of small tumors (up to 1 mm) localized in the surface layers (epidermis, mucosal epithelium), since the sensitivity of this method is significantly higher than of other modern early diagnosis methods. The effectiveness of the method depends on the level of accumulation and localization of the dye in individual structures of the tumor focus and surrounding tissue [4].

Photosensitizers of the first generation, which belong to hematoporphyrin derivatives group, have a number of disadvantages that reduce their diagnostic potential: low fluorescence intensity and fluorescence contrast (tumor vs. normal tissue). For this reason, the laboratories of many countries continue the search and synthesis of new photosensitizers with improved diagnostic properties.

One of the ways to create effective concentrations of the photosensitizer in the tumor tissue is to stimulate the body to produce porphyrins, which are endogenous photoactive compounds, in particular, the metabolite of heme protoporphyrin (PPIX) synthesis. One of the compounds that effectively induces the synthesis of endogenous PPIX is 5-aminolevulinic acid (5-ALA), an endogenous compound, one of the intermediate products of heme synthesis. Excessive administration of 5-ALA leads to an increased formation of PPIX, which is quickly utilized in healthy tissues, turning into heme under the action of the enzyme ferrochelatase [5,6]. In tumor cells, a deficiency of ferrochelase is observed, resulting in a temporary but significant increase in the level of PPIX, which remains in the tumor cells for several hours. The result is a high fluorescence contrast between the tumor and surrounding tissue, which in some tumors reaches 10-15 times [7]. The accumulation of 5-ALA-induced PPIX, which intensively fluoresces in the red region of the spectrum with a maximum at 635 nm, provides an opportunity to detect tumors and determine the precise boundaries of their spread [8].

Both in Russia and abroad, PD of bladder cancer is effected with 5-ALA-based preparations. Clinical studies have shown that the sensitivity of the PD method with 5-ALA-based preparations with fluorescence cystoscopy reaches more than 90%, which by far exceeds the maximum sensitivity of routine endoscopic examination of the bladder (up to 50%). At the same time, the high sensitivity of the method is accompanied by lower specificity (50–65%), which reduces its diagnostic accuracy [9].

Continuation of studies to improve fluorescence diagnostics led to the development of diagnostic tools based on 5-ALA methyl and hexyl esters, which undergo metabolic transformation in the body to 5-ALA [10]. Being more lipophilic compounds than 5-ALA itself, esters are better at penetrating biological membranes, so they accumulate in cells faster and to a greater extent, and become included in biosynthesis as PPIX precursors.

In this work, *in vivo* experiments were used to compare the ability of 5-ALA preparations and its hexyl ester (HE 5-ALA) at various concentrations to induce the synthesis and accumulation of photoactive PPIX in the tissues of the bladder.

Materials and methods

The study examined the specific activity of two drugs: 5-ALA and HE 5-ALA.

In experiments aimed at evaluation of the intensity of 5-ALA-induced fluorescence of protoporphyrin IX in the mucous membrane of the bladder with intravesical administration of 5-ALA, its solutions were used in concentrations of 3.0%, 0.3%, 0.03%, and 0.01%.

For preparation of solutions of the required concentration, 5-ALA powder is taken in an amount of 300 mg (to obtain a 3.0% solution), 30 mg (0.3% solution), 3 mg (0.03% solution) or 1 mg (0.01% solution) and dissolved in 10 ml of 5% sodium bicarbonate solution. The resulting solutions were clear, colorless. 10 ml of the solutions

was injected into the rabbit bladder immediately after preparation.

Another product used for the research was HE 5-ALA, a lyophilisate for the preparation of the solution used for instillations.

In the experiments, the specific activity of 0.2%, 0.01%, 0.002%, 0.001%, 0.0001% and 0.00005% solutions of HE 5-ALA preparation was investigated.

For preparation of solutions of the required concentration, HE 5-ALA powder is taken in an amount of 20 mg (to obtain a 0.2% solution), 1 mg (0.01% solution) and 0.2 mg (0.002% solution) and dissolved in 10 ml of 0.9% sodium chloride solution. To prepare 0.001%, 0.0001% and 0.00005% solutions, the 0.01% solution produved as described above was diluted with a 0.9% sodium chloride solution to 0.001%, 0.0001% and 0.00005% concentrations, respectively. The resulting solutions were clear, colorless. Solutions were injected, at a dose of 10 ml, into the rabbit bladder immediately after preparation.

Studies were performed on intact chinchilla rabbits, females weighing 2.5–3.5 kg. After preliminary sedation by intravenous administration of relanium (10–30 mg) or droperidol (5 mg), bladder immobilization was performed. Then, HE 5-ALA or 5-ALA solutions at concentrations listed above were injected into the bladder. The exposure time was 2 h, then the animal was killed by an overdose of anesthesia drugs, the bladder was removed and the specific fluorescence of PPIX was recorded.

Induced fluorescence in the bladder mucosa was evaluated by local fluorescence spectroscopy with excitation of radiation from a solid-state laser with a wavelength of 532 nm. During mathematical processing, the integrated fluorescence intensity in the range of 620–650 nm was normalized to the integrated tissue autofluorescence intensity in the range of 555–585 nm, the obtained value was designated as a diagnostic parameter (DP). Fluorescence was recorded by the contact method with the use of «Spektr-Claster» diagnostic unit (manufacturer: OOO «Klaster», Russia). The power density at the end of the fiber is 7 mW. In each tissue sample, from 5 to 10 spectra were measured to obtain a reliable result. The differences were considered significant at $p \leq 0.05$.

The control was the measured fluorescence spectra from the mucous membrane of the bladder of rabbits which were not administered the preparation.

Results and discussion

The dependence of 5-ALA induced PPIX fluorescence on the concentration of 5-ALA solution used for instillation is shown in Fig. 1. It can be seen that a 2-hour exposure to 3% and 0.3% solutions of 5-ALA preparation led to a significant induction of PPIX synthesis in rabbit bladder epithelium (DP – 2.69 ± 1.37 conventional units and 2.60 ± 1.02 conventional units, respectively). With a decrease in the concentration of the drug solution to 0.03%, a 2-fold decrease in the intensity of specific fluorescence of PPIX was observed (DP – 1.35 ± 0.32 conventional units) compared with the fluorescence observed with the use of 5-ALA in the form of 3% solution, and its uneven synthesis. The use of 5-ALA in the form of a 0.01% solution did not induce synthesis of endogenous PPIX in normal rabbit bladder epithelium (DP – 0.90 ± 0.03 conventional units), which was comparable with the control group.

Bladder exposure of an aqueous solution of HE 5-ALA for 2 hours at concentrations of 0.2%, 0.01%, 0.002% and 0.001% led to a significant induction of

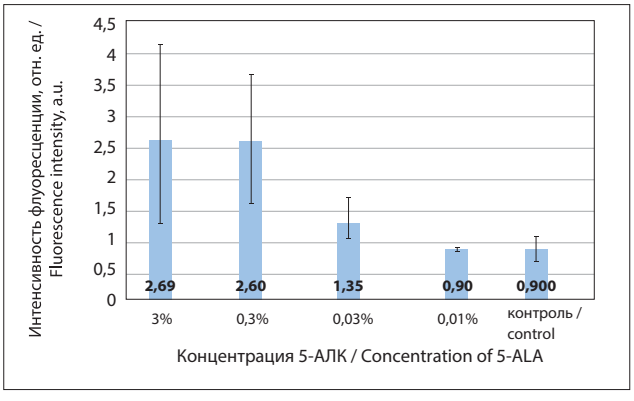

Рис. 1. Интенсивность нормированной флуоресценция ППIX в здоровом эпителии мочевого пузыря кролика после 2-часовой экспозиции растворов 5-АЛК
Fig. 1. PPIX normalized fluorescence intensity in the healthy epithelium of the rabbit bladder after a 2-hour exposure to 5-ALA solutions

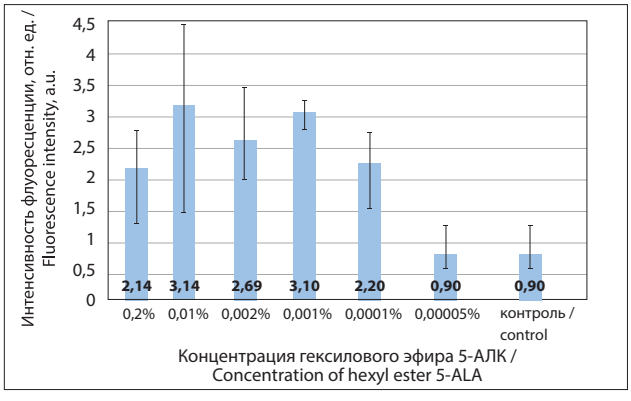

Рис. 2. Интенсивность нормированной флуоресценция ППIX в здоровом эпителии мочевого пузыря кролика после 2-часовой экспозиции растворов ГЭ 5-АЛК
Fig. 2. PPIX normalized fluorescence intensity in the healthy epithelium of the rabbit bladder after a 2-hour exposure to 5-ALA HE solutions

PPIX synthesis (DP – 2.14±0.59 conv. units, 3.14±1.64 conventional units, 2.69±0.78 conventional units and 3.10±0.16 conventional units, respectively). It should be noted that the fluorescence intensity of PPIX when HE 5-ALA solutions were used at concentrations of up to 0.001% was the same as when using a 0.3% 5-ALA solution. When the concentration of HE 5-ALA solution of the preparation was reduced to 0.0001%, a slight decrease (in the form of a trend, statistically unreliable) of the intensity of the specific fluorescence of fluorochrome to 2.20 ± 0.60 conventional units was observed. The introduction of a 0.00005% solution of HE 5-ALA did not induce endogenous PPIX synthesis in normal rabbit bladder epithelium (DP – 0.90 ± 0.03 conventional units), which was comparable with the control group.

Conclusion

A comparative analysis of the fluorescence level of PPIX after the introduction of 5-ALA and its hexyl ether, depending on the concentration of the injected solutions, showed a significantly higher efficiency of HE 5-ALA solutions. Upon instillation of a 0.0001% solution of HE 5-ALA solutions in a rabbit bladder, the fluorescence intensity (DP – 2.20 ± 0.60 conventional units) was comparable with the same indicator upon instillation of a 0.3% 5-ALA solution (DP – 2.60±1.02 conventional units) in a bladder. Thus, a comparison of the average fluorescence intensity data indicates that HE 5-ALA better induces and promotes the accumulation of PPIX in rabbit bladder epithelium at lower concentrations than 5-ALA.

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