ФОТО И СПЕКТРАЛЬНЫЙ ФЛУОРЕСЦЕНТНЫЙ АНАЛИЗ ОБЛАСТИ ТРАВМЫ СПИННОГО МОЗГА НА ЖИВОТНЫХ МОДЕЛЯХ

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Резюме

Цель работы - проследить динамику изменения флуоресцентных сигналов в приповерхностных слоях тканей травмированных участков спины лабораторных животных, что позволит, по косвенным признакам, оценить информативность флуоресцентной диагностики для последующего возможного диагностического мониторинга фотодинамической терапии спинного мозга. Модельными животными были крысы Вистар. Моделировалось два типа контузий: пневмоконтузия и контузия падающим грузом. Флуоресцентные измерения проводились фотографическим и спектрометрическим методом с препаратами метиленовый синий и индоцианин зеленый. Для фоторегистрации флуоресцентного ответа использовался стробоскопический флуоресцентный имиджер с длиной волны возбуждения 630 нм. Спектральные измерения проводились с помощью спектрометра ЛЕСА-01-БИОСПЕК, с возбуждением He-Ne лазером (632,8 нм). Показано, что оба метода позволяют оценивать величину флуоресценции метиленового синего и индоцианина зелёного в исследуемых тканях, а фотографический метод позволяет также получить пространственное распределение флуоресценции. Общая тенденция, обнаруженная в полученных данных – более интенсивная и равномерная флуоресценции дорсальной области крыс метиленовым синим, и менее интенсивное, но более контрастное распределение индоцианина зелёного. Представленные методы неинвазивны, что делает их привлекательными для диагностического использования. Однако из-за малой глубины приема сигнала состояние позвоночника можно определить лишь косвенно, по состоянию приповерхностных слоев тканей, накапливающих фотосенсибилизатор.

Ключевые слова: Флуоресцентная диагностика, спектральный анализ, метиленовый синий, индоцианин зелёный, область спинного мозга, спинальная травма.

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PHOTO AND SPECTRAL FLUORESCENCE ANALYSIS OF THE SPINAL CORD INJURY AREA IN ANIMAL MODELS

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Abstract

The purpose of the work is to follow the dynamics of changes in fluorescent signals in the near-surface layers of tissue of injured areas of the back of laboratory animals, which will allow, by indirect evidence, to evaluate the information content of fluorescence diagnosis for subsequent possible diagnostic monitoring of photodynamic therapy of the spinal cord. The model animals were Wistar rats. Two types of contusions were modeled: pneumocontusion and contusion by a falling load. Methylene blue and indocyanine green were used as photosensitizers. Fluorescence measurements were carried out by imaging and spectrometric methods. A stroboscopic fluorescence imager with an excitation wavelength of 630 nm was used to acquire fluorescence images. The LESA-01-BIOSPEC spectrometer with a He-Ne laser excitation allowed to obtain spectra. It was shown that both methods make it possible to estimate the fluorescence value of methylene blue and indocyanine green in the tissues under study. Moreover, the photographic method also allows to obtain the spatial distribution of fluorescence. The general trend found in the data is a more intense and uniform fluorescence of the dorsal region of rats with methylene blue and a less intense, but more contrasting distribution of indocyanine green. The presented methods are non-invasive, which makes them attractive for diagnostic use. However, due to the shallow depth of signal reception, the condition of the spine can be determined only indirectly, by the condition of the near-surface layers of tissue that accumulate the photosensitizer.

Key words: Fluorescence diagnosis, spectral analysis, methylene blue, indocyanine green, paravertebral area, spinal trauma.

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Introduction

Despite the significant efforts of clinicians around the world, spinal cord injury (SCI) remains one of the most pressing problems in modern neurosurgery. Thus, the social and economic consequences of this medical problem cannot be overstated [1]. Healthcare studies in developed countries indicate an incidence of SCI of 4–6 cases per 100,000 inhabitants per year, with severe long-term consequences for patients and, as a result, a huge impact on society.

Fluorescence diagnosis is based on the excitation of fluorescence of a photosensitizer accumulated in biological tissues and registration of the fluorescent signal from the tissue under study, followed by analysis. Classically, this procedure is used to identify foci of neoplasms of various localizations and their boundaries [2, 3]. In addition, the method is often used intraoperatively for navigation during surgery [4, 5]. Moreover, fluorescence diagnosis can be used, for example, to assess the effectiveness of photodynamic therapy (PDT) (measurements before/during/after a PDT session) [6, 7]. This possibility is considered in this work to analyze the prospects of fluorescence diagnosis when performing PDT for spinal cord injuries. Previously, fluorescence studies have already been used for the spinal cord to identify and influence tumor neoplasms [8-10] using various photosensitizers [11], as well as for invasive studies of a different nature [12-14].

The purpose of the present work is to follow the dynamics of changes in fluorescent signals in the near-surface layers of tissue of injured areas of the back of laboratory animals, which will allow, by indirect evidence, to evaluate the information content of fluorescence diagnosis for subsequent possible diagnostic monitoring of PDT of the spinal cord.

Materials and Methods

Model animals

The experimental animals were Wistar rats, 2.5-3 months old, females weighing 150–200 g, and males weighing up to 240 g. Modeling of contusion injury was carried out in 2 modifications - pneumo contusion and moderate contusion by a falling weight. Pneumo contusion was simulated by a blank shot at point-blank range from an IZH-53M spring pneumatic pistol. When modeling a moderate contusive spinal cord injury, a custom-made setup was used. The setup was in the form of a pipe 50 cm high and 20 mm in diameter, mounted on a tripod, dropping a cylindrical load weighing 350 g from a height of 50 cm, which is equivalent to 1.96 N/cm^2 in terms of force on the vertebrae. The animal's behavior was recorded using a Samsung A9 smartphone camera. Animals were removed from the experiment by immediate decapitation under chloral hydrate anesthesia. Imaging and spectral measurements of fluorescence were carried out once a day for 4 days, starting from the day of the simulated injury (1 hour after injury).

Photosensitizers

Fluorescence diagnosis was carried out using two photosensitizers - methylene blue (MB) and indocyanine green (ICG). Drug administration regimens are presented in Table 1.

Таблица 1
Режим введения фотосенсибилизаторов
Table 1
Modes of administration of photosensitizers

Фотосенсибилизатор Photosensitizer	Способ введения Administration way	Доза Dose	Экспозиция, мин. Exposition, min	Режимы введения Administration regimens
МС MB	внутрибрюшинно intraperitoneally	20 мг/кг 20 mg/kg	10	Раз в день, перед флуоресцентной диагностикиой, в течение 4 дней once a day before FD for 4 days
ИЗ ICG	внутрибрюшинно intraperitoneally	10 мг/кг 10 mg/kg	5	Раз в день, перед флуоресцентной диагностикиой, в течение 4 дней once a day before FD for 4 days

* МС – метиленовый синий, ICG – индоцианиновый зеленый.
* MB – methylene blue, ICG – indocyanine green.

For a better understanding of the working ranges of the technique under study, see the emission spectra of the photosensitizers used in the work [15] and the fluorescence spectra of endogenous fluorophores [16, 17].

Equipment

For photographic registration of the fluorescence response of photosensitizers and endogenous fluorophores, a stroboscopic fluorescence imager (SFI) was used. The SFI consisted of a red LED with a central wavelength of 630 nm and an optical power of 1 W to excite the fluorescence of light-sensitive components accumulated in biological tissues, two white LEDs (with an optical power of 200 mW each) to create uniform illumination of the surgical field, as well as one violet LED (not used in this study) (Fig 1a). The spectrum of the red LED was corrected by a bandpass filter with a central wavelength of 636 nm. A long pass filter (LPF) with a cut-on frequency of 660 nm was installed in front of the camera lens. SFI allowed to obtain pairs of frames: one frame with the fluorescence excitation LEDs and backlight LEDs turned on (respectively, with fluorescence) and the other with only backlight LEDs turned on (background frame). Subtracting the background frame helped to reduce the impact of background light.

Spectral measurements of fluorescence of tissues of laboratory animals were carried out using a LESA-01-BIOSPEC spectrometer Fig. 1b (BIOSPEC, Moscow, Russia) connected to a He-Ne laser with a radiation wavelength of 632.8 nm.

Fluorescence index

The fluorescence index (FI) was used to quantify fluorescence intensity when processing spectral data. It was calculated by dividing the area under the fluorescence spectrum curve by the area under the scattering spectrum curve of the excitation He-Ne laser.

Results

To distinguish the spectra of photosensitizers from the spectrum of endogenous fluorophores in Fig. 2 a spectrum taken on an intact animal is shown.

Fluorescence images

Below are examples of fluorescence images obtained in the area of spinal cord injury in laboratory animals obtained with SFI (Fig. 3).

Spectra

Below are the examples of obtained spectra from the region of spinal cord injury in laboratory animals, obtained using a spectrometer for the methylene blue (MB) (Fig. 4) and indocyanine green (ICG) (Fig. 5).

Fig. 4a shows spectra and diagrams of fluorescence signals obtained in an area away from the injury (healthy area). Fig. 4b shows spectra and diagrams of fluorescence signals taken in the area of injury (trauma area). Histograms express fluorescence indices (FI) (see description in the "Materials and Methods" section) for the corresponding rat on different days in chronological order (day 1 - day 4) and characterize the accumulation of the

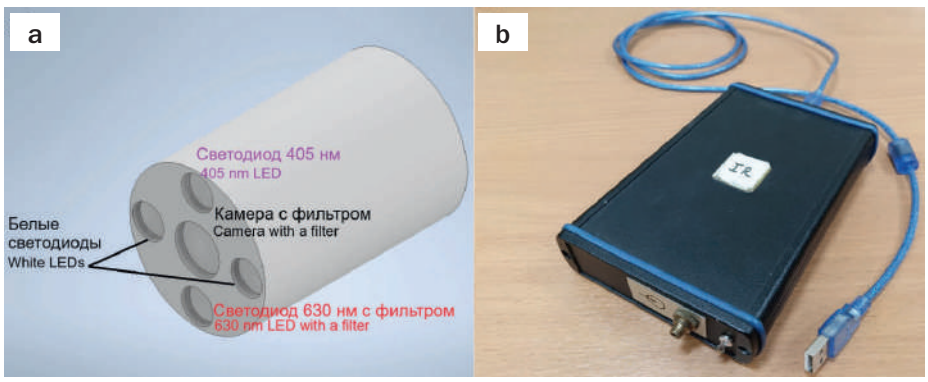

Рис. 1. Диагностическое оборудование: a – стробоскопический флуоресцентный имиджер (СФИ); b – спектрометр ЛЕСА-01 БИОСПЕК.
Fig. 1. Diagnostic equipment: a – stroboscopic fluorescence imager (SFI); b – spectrometer LESA-01 BIOSPEC.

photosensitizer in the study area. The histogram columns correspond in color to the presented spectra.

The "norm" was considered to be the area of the back located at a distance from the area of the animal's injury. The "trauma" was considered to be the directly injured area of the back.

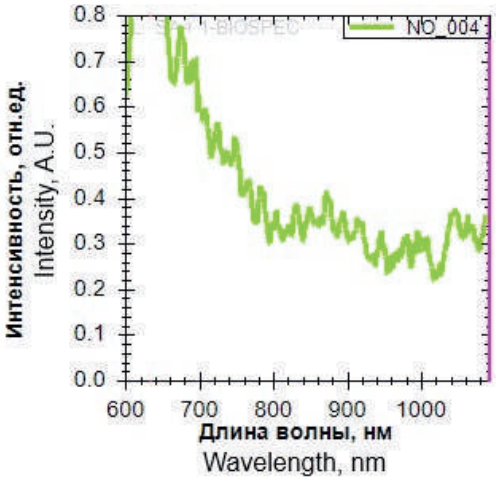

Рис. 2. Спектр флуоресценции спинной области интактного животного.
Fig. 2. Fluorescence spectrum of an intact animal dorsal area.

The distinctive fluorescence peak of indocyanine green was recorded around 880 nm (Fig. 5). In some spectra, this peak was nearly indistinguishable from the tissue autofluorescence spectral signal, which did not allow reliable analysis.

Discussion

The study showed that both methods under consideration can reliably detect the fluorescence signal from methylene blue, both in the area of injury and in normal conditions. The general trend, noticeable both in the spectra and in the images, is a more intense (in the case of spectra) and brighter and uniform (in the case of images) fluorescence of the dorsal region of rats with methylene blue than with indocyanine green. The relatively weak signal from indocyanine green is explained by the suboptimal wavelength of the exciting radiation (636 nm at SFI and 632.8 at spectral measurements), which in the wavelength range is located closer to the absorption band of methylene blue. However, it is worth noting that in the case of indocyanine green, a more contrasting fluorescence pattern is observed in the images. This stronger contrast in measurements can be explained by its accumulation in the main vessels and lymph flows.

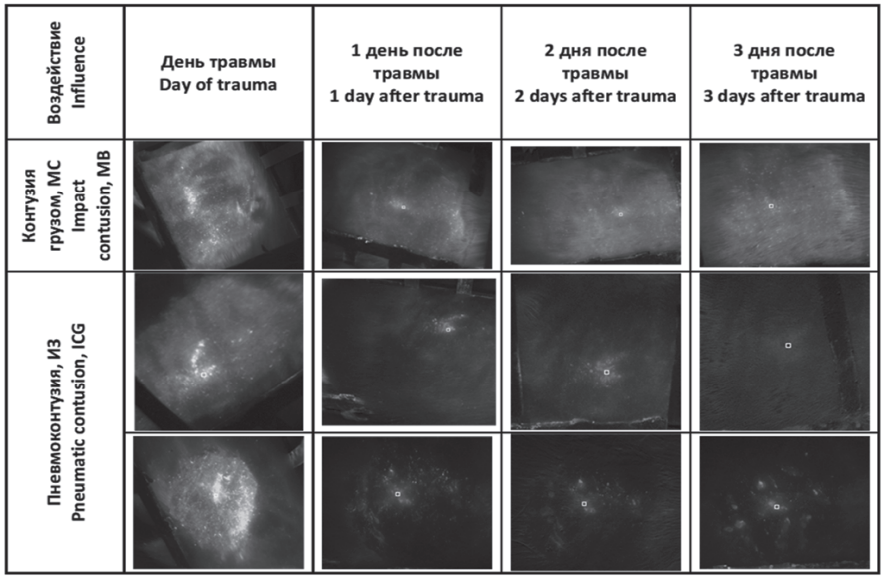

Рис. 3. Примеры СФИ изображений флуоресценции метиленового синего (МБ) и индоцианинового зеленого (ИЗ), полученных на лабораторных животных в ходе исследований на 1-4 сутки после моделируемой травмы спинного мозга (включая день травмы).
Fig. 3. Examples of SFI fluorescence images of methylene blue (MB) and indocyanine green (ICG) obtained on laboratory animals during studies on days 1-4 after simulated spinal cord injury (including the day of injury.

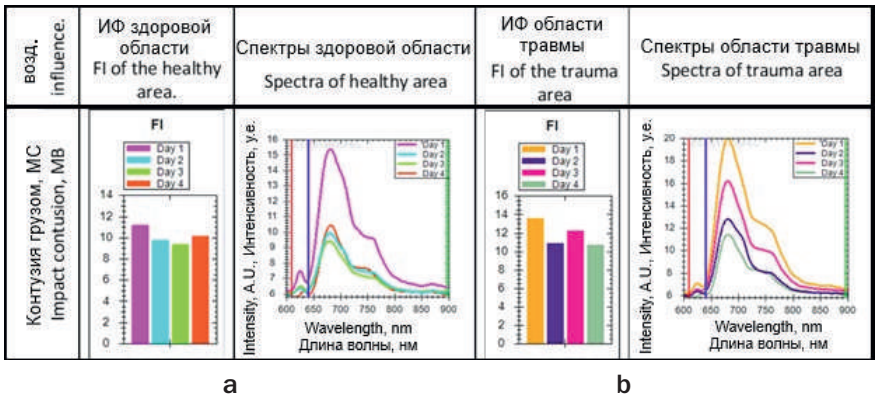

Рис. 4. Примеры спектров флуоресценции метиленового синего (МБ), полученных на лабораторных животных в ходе исследований на 1-4 сутки после моделирования травмы спинного мозга (ИФ – индекс флуофесценции): a – область за пределами травмы; b – зона повреждения спинного мозга.
Fig. 4. Examples of methylene blue (MB) fluorescence spectra obtained on laboratory animals during studies on days 1-4 after modeling spinal cord injury (FI – fluorescence index): a – area outside the injury; b – area of the spinal cord injury.

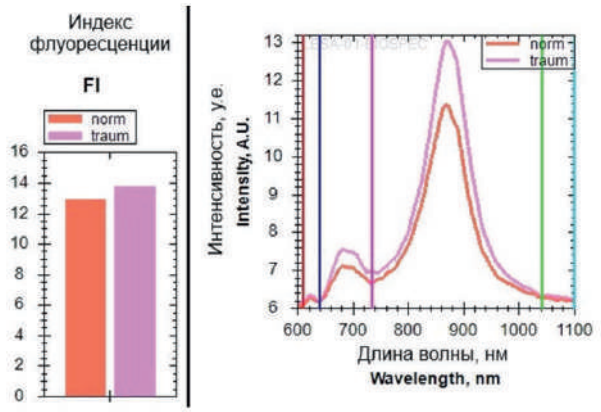

Рис. 5. Примеры индексов флуоресценции и спектров флуоресценции индоцианина зеленого (ИЗ), полученных в области нормы и травмы.

Fig. 5. Examples of indocyanine green (ICG) fluorescence indexes and fluorescence spectra obtained from normal area and the area of trauma.

Analysis of the averaged data results shows that on the first day of measurements (immediately after injury) the strongest MB and ICG fluorescence signal is visible in the injured area, which is explained by the fact that the increase in edema and the formation of hematomas occur gradually, therefore, there were fewer obstacles to detecting the signal in the injured area than in the following days. In subsequent days, the intensity of the fluorescence signal in the area of injury decreases. In the normal area, the signal decreases more slowly and almost imperceptibly, and the fluorescence intensity is lower than in the injured area. The results obtained in the form of images show a similar picture: the injury attenuation function is ahead of the normal attenuation function, due to which the contrast of the injury against the normal background in frames obtained with SFI is reduced.

Also, intense fluorescence of both drugs was observed both in hematomas and in areas of skin damage after shaving, which may be caused by the accumulation of the drug circulating in the bloodstream in hyperemia. Therefore, in future experiments, the rats should be depilated instead of shaving to avoid adding damage to the skin and thus introducing uncertainty into the experiment.

Conclusion

The presented methods are non-invasive, which makes them attractive and promising for diagnostic use. However, due to the shallow depth of signal reception, the condition of the injured spine can be determined only indirectly, by the fluorescence signals from the near-surface layers of the back accumulating photosensitizers. However, detecting the difference in the fluorescence signals from the "normal" area and the area of injury, as well as in the dynamics of the signal by day, makes it possible to detect and evaluate the degree of hematoma healing and reduction of hyperemia, which are often indistinguishable to the naked eye. This suggests that the method can be potentially used to control PDT in spinal cord injuries.

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