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Cortexin diffusion in human eye sclera

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Abstract. **Investigation of the diffusion of cytamines, a typical representative of which is cortexin, is important for evaluating the drug dose, necessary to provide sufficient concentration of the preparation in the inner tissues of the eye. In the present paper, the cortexin diffusion rate in the eye sclera is measured using the methods of optical coherence tomography (OCT) and reflectance spectroscopy. The technique for determining the diffusion coefficient is based on the registration of temporal dependence of the eye sclera scattering parameters caused by partial replacement of interstitial fluid with the aqueous cortexin solution, which reduces the level of the OCT signal and decreases the reflectance of the sclera. The values of the cortexin diffusion coefficient obtained using two independent optical methods are in good agreement.**

Keywords: *drug delivery, diffusion coefficient, reflectance spectroscopy, optical coherence tomography*.

1. Introduction

The use of cytamines in clinical practice has demonstrated high efficiency of the substances of this class in different fields of medicine, including ophthalmology [1–6]. The problems of increasing the efficiency of treatment in patients with retinal pathologies and correcting metabolic abnormalities in the visual nerve at its partial atrophy, developed as a result of inflammation, intoxication, blood circulation abnormality, or glaucoma optical neuropathy, are basic problems in modern ophthalmology [4, 5]. Biologically regulating therapy, based on the use of polypeptides (such as cortexin and retinalamin), is notable for a number of essential advantages and, first of all, its pathogenetic foundation. Any pathological process is accompanied with the abnormality of regulation of the informative molecules transport between the cells; therefore, the enhancement of the synthesis of regulatory peptides in the organism itself or their external administration may favour the suppression of a pathological process and the restoration of the functions lost [3, 5].

Cortexin is a complex of L-amino acids and low-molecular-weight active polypeptides having the molecular mass from 1 to 10 kDa, extracted from the cerebral cortex of calves or piglets. The basic amino acids are glutamine, asparagine, isoleucine, alanine, glycine, etc. Cortexin possesses a tissue-specific polyfunctional effect on the brain. The preparation increases the efficiency of the energy metabolism of the brain cells, improves the intracellular protein synthesis, and also regulates the processes of lipid peroxidation in the brain cells, visual nerve and retinal neurons, reduces the formation of free radicals, blocks the processes of free-radical oxidation [2, 4–7].

The delivery of a drug to the inner tissues of the eye under its topical administration is associated with certain difficulties. When using the preparation in the form of eye drops the main barrier on its way to the internal structures of the eye is the cornea. The cornea epithelium obstructs the penetration of lipophobic compounds, while the stroma of the cornea is poorly penetrable for lipophilic compounds and for large-size molecules (having the mass more than 500 kDa) [8]. Because of that, the parabulbar or subtenon approach [4] appears preferable to introduce hydrophilic preparations, to which cortexin belongs.

The investigation of the cortexin diffusion through sclera is an important problem in relation with evaluating the drug dose, necessary to provide sufficient concentration of the preparation in the inner tissues of the eye. However, despite numerous studies of sclera permeability for different preparations [9–12], the problem of quantitative estimation of penetration rate of cytamines at their transscleral delivery remains urgent.

The permeability coefficient of a fibrous tissue and the coefficients of diffusion of drugs in it may be estimated using the methods, based on measuring the time dependence of the change in the tissue optical properties as a result of the change in the refractive index of the interstitial liquid [11–15]. It is well known that the sclera is mainly composed of collagen fibres, packed in planar bundles, merged into the amorphous base substance [16, 17]. The inhomogeneities of the structure and the difference in refractive indices of hydrated collagen fibres and interstitial fluid cause multiple scattering of light in the sclera, which makes it opaque in the visible region of wavelengths [13]. Since the refractive index of the immersion liquid (drug) differs from that of the

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interstitial liquid of sclera, a partial replacement of the base substance of the sclera with the cortexin solution will cause an optical response of the tissue, i.e., the change in its transparency with respect to the probing radiation. The analysis of temporary dynamics of this process allows the estimation of the diffusion rate of the drug in the eye tissue.

In the present paper the results of *in vitro* experiments on measuring the diffusion rate of the drug 'Cortexin' through the human eye sclera are presented. The method is based on the analysis of the temporal dynamics of the signal of optical coherence tomography (OCT) and the reflectance spectra of the sclera under the conditions of its interaction with an aqueous solution of cortexin. The aim of the work is to investigate the permeability of sclera as the main barrier on the way of delivering high-molecular-weight preparations (represented by cortexin) to the internal eye tissues.

2. Materials and methods

2.1 Preparation of materials

The material for the *in vitro* study using the reflectance spectroscopy included 10 samples of the human eye sclera. The samples were obtained from enucleated human eyes in the course of a planned surgery, namely, the enucleation of a blind eye with formation of functioning stump for prosthesis. The OCT measurements were carried out using 5 samples of the rabbit eye sclera. The time dependence of the sclera thickness was also measured in 5 samples of the rabbit eye sclera.

Immediately after the enucleation, the samples were placed into saline (0.9 % aqueous solution of NaCl) and were kept in it at the temperature 4 – 5 °C until the beginning of the measurements. The experiments were performed no later than 24 hours after the tissue enucleation. Before the measurements the layer of retinal pigment epithelium was removed from the sclera samples. The thickness of the tissue was measured with a micrometer. For this purpose the samples were placed between two cover glasses; the measurements were carried out at several points of the sample. The error of each measurement was ±50 μm, the obtained thickness values were averaged.

The aqueous solution of 'Cortexin' (LLC 'Gerofarm', Russia) preparation (concentration 20 mg mL^{-1}) was investigated. The refractive index of the solution (1.342) was measured using the Abbe refractometer ($\lambda = 589$ nm) straight before the experiment.

All measurements were carried out at room temperature (~ 20 °C).

2.2 Method of reflectance spectroscopy

The coefficient of diffusion of cortexin in the sclera was estimated using the modification of the time dependence of the reflectance change in the studied tissue samples.

To perform spectrometric measurements each sample was fixed on a special cuvette with the studied solution in such a way, that the solution was in contact only with the external surface of the tissue sample. From the opposite side a fibreoptic probe consisting of seven fibres was applied to the sample. The central fibre (source) served to introduce radiation into the tissue, while six other fibres, symmetrically surrounding the source fibre, were aimed to collect the backscattered radiation. The diameter of each fibre was 200 μm, the numerical aperture of the fibres was 0.2, the separation between the centres of the source and detector fibres was 290 μm. A halogen lamp served as a light source in the spectral region from 450 to 1000 nm. The radiation, backscattered from the sclera sample, was registered with the LESA-5 fibreoptic spectrometer with the diffraction grating and multichannel detector (CJSC 'Biospec', Russia). The signal acquisition interval was 100 ms, the spectrometer was calibrated using a $BaSO_4$ plate.

In the studies of the interaction of the cortexin aqueous solution with the sclera samples it was assumed that this interaction affects only the refractive index of the interstitial liquid of the sclera, which leads to the reduction of the scattering coefficient of the tissue due to refractive index matching between the scatterers and the interstitial fluid. The analysis of the time dependence of this process allows estimation of the diffusion coefficient as a measure of the mean rate of the exchange flow of the drug into the tissue and of the water from the tissue [12].

The process of a drug transport in a tissue may be described within the framework of the free diffusion model. The use of this model may be considered as valid because the size of the cortexin molecule (the hydrodynamic radius of molecules having similar molecular weight is 2 – 3 nm [9]) is much smaller than the interfibrillar spacing in the sclera. According to the data of Ref. [13], the mean spacing between fibrils is ~ 285 nm, which allows cortexin molecules to diffuse freely in the interfibrillar space. At the same time, it is obvious that the rate of the cortexin diffusion in the interstitial liquid of the sclera will differ from the mean rate of diffusion of this agent, calculated for the whole sclera, because in this case the porosity of the tissue and, therefore, the tortuosity of the diffusion path, are not taken into account. In the present work we estimate the mean rate of a drug transport (i.e., the permeability coefficient) through the sclera, considered as a whole membrane, into the internal space of the eye. The solution of this problem is valuable for estimating the dose of the drug, delivered, e.g., to the eye retina.

The free diffusion model is widely used in the studies of the transport of molecules, having large molecular weight, through tissues [9, 10, 18, 19]. Thus, in [18] the axial propagation of protein microdose, injected into muscular fibrils, is analysed, and it is shown that the diffusion of large molecules (e.g., myoglobin) is well described by this model. In Refs [9, 10, 19] the free-diffusion model was also used to describe the diffusion of molecules having the molecular weight from 0.023 to 150 kDa.

In the present work we use two assumptions, related to the diffusion process: (i) only the concentration diffusion takes place, i.e., the flow of substance at a given point in the tissue is proportional to the substance concentration gradient; (ii) the diffusion coefficient is constant at all points inside the studied sample of the tissue.

Geometrically the sclera sample is represented by a plane-parallel plate of finite thickness. Since the area of the upper and lower surfaces of this plate is much larger than that of the side faces, it is possible to neglect the periphery effects and to solve a one-dimensional diffusion problem, i.e., the equation

$$\frac{\partial C(x,t)}{\partial t} = D\,\frac{\partial^2 C(x,t)}{\partial x^2},$$

where $C(x,t)$ is the concentration of cortexin in the sclera (g mL^{-1}); D is the diffusion coefficient ($cm^2\ s^{-1}$); t is the

time, during which the diffusion occurs (s); x is the spatial coordinate with respect to the depth of the tissue sample (cm). The boundary conditions, accounting for the geometry and the conditions of the measurements, are as follows: $C(0,t) = C_0$, $C(l,t) = 0$, where C_0 is the cortexin concentration in the solution; l is the thickness of the tissue sample (cm). The initial conditions indicate the absence of cortexin inside the sclera sample at the initial moment of time, i.e. $C(x,0) = 0$.

The solution of the diffusion equation with the initial and boundary conditions taken into account allows estimation of the mean concentration of cortexin solution inside the sclera sample at any instant of time [20–23]:

$$C(t) = \frac{C_0}{2}\left\{1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2} \times \exp\left[-\frac{(2i+1)^2 t\pi^2 D}{l^2}\right]\right\}. \quad (1)$$

The time dependence of the refractive index of the interstitial liquid can be obtained from the Gladstone–Dale law, according to which the refractive index of a solution $n_s(t)$ is composed of the mean refraction indices of its components, weighted by their volume fractions [24]:

$$n_s(t) = [1 - C(t)]n_{base} + C(t)n_{osm}, \quad (2)$$

where n_{base} is the refractive index of the interstitial fluid at the initial moment of time; n_{osm} is the refractive index of the cortexin solution. Since the sclera samples were kept in saline during ~ 24 hours before the beginning of the experiments, we assumed that at the initial instant of time the interstitial liquid in the sclera samples was replaced with the NaCl solution, whose refractive index is practically the same as that of water. The spectral dependence of the refractive index of water has the following form [25]:

$$n_w(\lambda) = 1.3199 + \frac{6.878 \times 10^3}{\lambda^2} - \frac{1.132 \times 10^9}{\lambda^4} + \frac{1.11 \times 10^{14}}{\lambda^6}, \quad (3)$$

where λ is the wavelength (nm).

The tissue optical model may be considered as a plate with the thickness l, containing the scatterers (collagen fibrils) in the form of thin dielectric cylinders with the mean diameter 100 nm [13], which is much smaller than the length of the cylinders. The fibrils lie in the planes, parallel to the sample surfaces, but within each plane layer they are oriented in a random way [13, 17]. The spectral dependence of the refractive index of the eye sclera scatterers is given by the expression [26]

$$n_c(\lambda) = 1.4389 + 1.588 \times 10^4\lambda^{-2} - 1.4806 \times 10^9\lambda^{-4} + 4.3917 \times 10^{13}\lambda^{-6}. \quad (4)$$

As the first approximation it was assumed that in the process of interaction between the tissue and cortexin the size of the scatterers is not changed. In this case all changes in the scattering of light by the tissue are due to the variation in the refraction index of the interstitial liquid.

In the visible range of wavelengths (except the absorption bands of blood) the absorption coefficient of a tissue is much smaller than the scattering coefficient. Since cortexin also has no strong absorption bands in the studied spectral range, the variation in the sclera reflectance is determined solely by the behaviour of the scattering coefficient.

The reflectance spectra of the eye sclera were modelled using the Monte Carlo (MC) method based on the algorithm presented in Ref. [27]. In the present work we modified the subroutine of photon registration for the backscattered photons, taking into account the geometry of the fibreoptic probe, used in the experiments. The MC modelling of the trajectories of photon packets included successive modelling of elementary events, namely, the generation of the photon mean free path, the events of scattering, absorption, reflection and/or refraction at the medium interfaces. The initial and the final state of photons were completely determined by the geometry of the radiation source and detector. The incident light was a narrow beam, directed along the normal to the tissue surface. In the modelling of the reflectance spectra all backscattered photons, appearing in the area of the receiving fibres of the fibreoptic detector, were detected taking into account the aperture of the receiving fibres. The specular reflection of photons from the boundary between the air and the tissue was separately taken into account. In each scattering event the new photon direction was chosen according to the Henyey–Greenstein phase function

$$f_{HG}(\theta) = \frac{1}{4\pi}\frac{1 - g^2}{(1 + g^2 - 2g\cos\theta)^{3/2}},$$

where g is the anisotropy factor; θ is the polar angle of scattering. The distribution over the azimuthal angles was taken to be uniform. The scattering coefficient and the anisotropy factor were calculated using the Mie theory [28]. Detailed description of this algorithm is presented in [12].

The system of equations, describing the time dependence of the cortexin concentration, formulates the direct problem. The inverse problem in this case is the restoration of the diffusion coefficient value from the variation in sclera reflectance. This problem was solved by minimising the target functional

$$F(D) = \sum_{i=1}^{N_t}[R(D,t_i) - R^*(t_i)]^2, \quad (5)$$

where $R(D,t)$ and $R^*(t)$ are the calculated and the measured values of time-dependent reflectance, respectively; N_t is the number of points, acquired during the registration of the time dependence of the reflectance. The 'complex' method [29] was used to minimise the target function (5). The iterative procedure was repeated until the agreement between the experimental and the calculated data was achieved. The calculation was performed for ten wavelengths (500, 550, 600, 650, 700, 750, 800, 850, 900, and 950 nm); the obtained values of the diffusion coefficients were averaged.

2.3 Method of optical coherence tomography

The measurements were carried out by means of the OCT system with the low-coherence broad-band near-IR source having the wavelength 1310 ± 15 nm and the output power 3 mW (LLC 'Superluminescent Diodes', Russia). The light beam, scattered by the sample, and the light beam, reflected from a mirror in the reference arm, formed an interfero-

gram that was registered with a photodiode. Two-dimensional images were obtained by scanning the incident beam over the surface of the sample in the lateral direction, combined with depth scanning (along the z axis) by moving the reference mirror of the interferometer. These images consisted of 450×450 pixels (2.2×2.4 mm). The data acquisition time for the whole image was about 3 s. The two-dimensional images were averaged in the lateral direction (~ 1 mm, which is enough for speckle noise suppression); finally, a single curve of the averaged OCT signal was plotted presenting a one-dimensional distribution of the reflected light intensity over the depth in logarithmic scale.

Before the experiment the sclera samples were placed into a special cuvette with saline having the room temperature. In the course of experiment with a whole eyeball its lower hemisphere was immersed in saline to prevent the dehydration of the sample. The OCT signal was registered before the application of the agent during ~ 7 min. Then 0.1 mL of cortexin was applied on the surface of the studied area. The total time of continuous monitoring was about 30 min.

The sclera permeability coefficient P was calculated by division of the thickness of the studied part (with the maximal change in the OCT signal) by the diffusion time of the agent, $P = z_{\mathrm{reg}}/t_{\mathrm{reg}}$. Since the cortexin diffusion manifests itself in the change in the OCT signal slope (OCTS) [30], which is relatively constant both before the agent application and after the attainment of saturation, the value of t_{reg} was calculated as the saturation time minus the time of the beginning of the OCTS variation (i.e., the time of the agent application).

3. Results and discussion

Figures 1 and 2 present the reflectance spectra of the human eye sclera, measured at different instants of time, and the time dependences of the sclera reflectance, measured at several wavelengths. These spectra characterise the variation in the optical properties of the sclera in the process of its interaction with the cortexin solution.

It follows from Fig. 1 that at the initial instant of time the sclera is a strongly scattering tissue with high enough reflectance. The shape of the spectrum is mainly determined

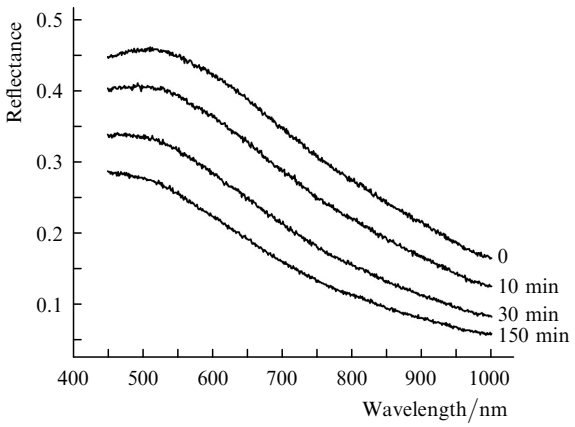

Figure 1. Typical reflectance spectra of the human eye sclera, measured at different instants of time in the process of interaction of the tissue with the cortexin solution.

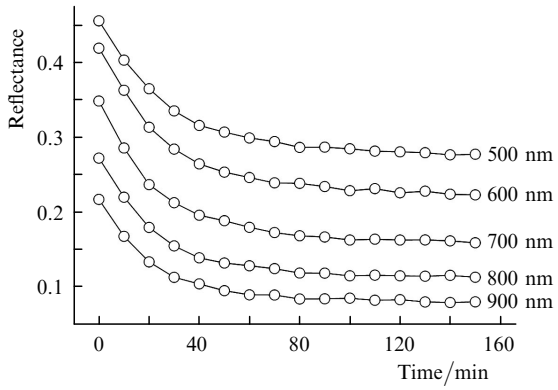

Figure 2. Time dependences of human eye sclera reflectance, measured at different wavelengths in the process of interaction of the tissue with the cortexin solution. The points represent experimental data; the curves are plots of the approximate dependences.

by the spectral dependence of the sclera scattering coefficient. The essentially lower reflectance in the long-wave spectral region is due to the outcome of the backscattered radiation from the area of detection. The absorption coefficients of collagen, water and proteins that are a part of the tissue are insignificant in the spectral region under study; therefore, the absorption of light in the sclera may be neglected [31, 32]. Since the sclera is characterised by a low content of blood, the absorption by haemoglobin also exerts almost no influence on the shape of the spectrum in the *in vitro* studies [32].

As shown earlier [11 – 14, 30, 32], the optical properties of fibrous tissues change under the action of immersion agents, to which some drugs can belong. In this case the dominant mechanism of the optical 'clearing' of the tissue is the matching between the refractive indices of the scatterers (mainly collagen fibres) and the interstitial fluid due to both the osmotic dehydration of the tissue and the diffusion of the immersion liquid into it. The dehydration of the tissue, as well as the modification of the structural fibres, leads to a denser and, correspondingly, more regular packing of the scatterers, which also increases the transparency of the tissue for the waves in the optical region [32]. Thus the scattering in the tissue appears to be reduced and, therefore, the reflectance decreases (Figs 1 and 2). On average, for the samples studied in the considered spectral region the reflectance decreased approximately by 22 ± 6 % during the first 10 minutes and by 47 ± 14 % during 20 minutes from the beginning of the cortexin action. It is important to note, that the use of such a high-molecular-weight compound as cortexin in the role of an immersion agent causes significant osmotic dehydration of the tissue.

In the course of the experiment the thickness of the sclera samples reduced, on average, by nearly 35 % (from 0.63 ± 0.02 to 0.41 ± 0.01 mm), which indicates the high osmotic activity of cortexin. Figure 3 illustrates the time variation in the eye sclera thickness as a result of the tissue dehydration under the action of cortexin. It is seen that the decrease in the tissue thickness continues for nearly 2 hours and can be sufficiently well described by the complicated power function

$$l(t) = \frac{9542.27 + 0.395t^{1.428}}{15404.5 + t^{1.428}}, \qquad (6)$$

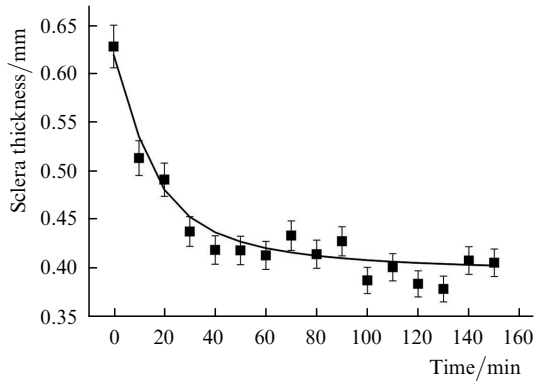

Figure 3. Time dependence of the human eye sclera thickness under the action of cortexin. The points represent experimental data; the solid curve is a plot of the approximate dependence (6).

where t is the time of cortexin action (s) and l is the thickness of the sclera (mm). The data, presented in Fig. 3, were used in MC modelling of the sclera optical clearing.

Since the solvent transport may strongly influence the structural and optical properties of a tissue, we studied the effect of water diffusion on the change in sclera reflectance. Figure 4 shows the typical dynamics of the human eye sclera reflectance, measured at several wavelengths in the sample under the action of saline. It is clearly seen, that when water, which is the base substance of saline, diffuses into the sclera, its reflectance only slightly changes in the entire spectral region under study. On average, the change in reflectance at the chosen wavelengths during the same time did not exceed 5 %. It follows that the changes in reflectance, observed in Figs 1 and 2, are caused mainly by the effect of cortexin, not water. One can see from Fig. 2 that the experimental data (points) are in good agreement with the approximating curves, obtained within the framework of the proposed model.

The solution of the inverse problem by means of numerical MC modelling of the process of the optical clearing in the human eye sclera, performed within the framework of the developed model and using the measured time dependence of the reflectance, allowed estimation of the diffusion coefficient of cortexin in the sclera. The calculations were performed at ten wavelengths and then averaged. The resulting data are presented in Table 1.

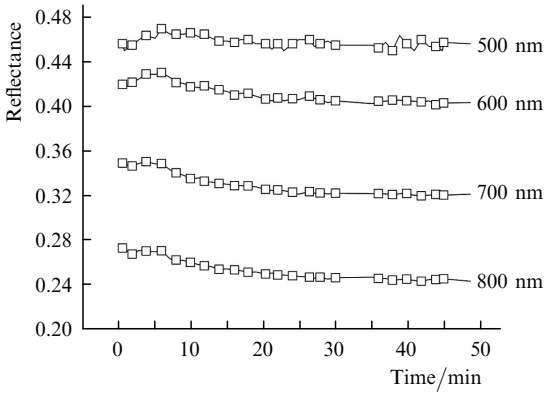

Figure 4. Time dependence of the human eye sclera reflectance at different wavelengths in the process of interaction with saline.

Table 1. Permeability coefficients of the eye sclera and diffusion coefficients of cortexin in the sclera, obtained using the methods of reflectance spectroscopy (RS) and OCT and their mean values.

Method of measurement	Permeability coefficient/cm s^{-1}	Diffusion coefficient/cm^2 s^{-1}
RS	$(1.57 \pm 0.95) \times 10^{-5}$	$(0.98 \pm 0.67) \times 10^{-6}$
OCT	$(2.40 \pm 0.32) \times 10^{-5}$	$(1.49 \pm 0.25) \times 10^{-6}$
Mean value	$(1.99 \pm 0.59) \times 10^{-5}$	$(1.24 \pm 0.36) \times 10^{-6}$

Figure 5a shows typical OCTS signals from the rabbit eye sclera in the process of cortexin diffusion at the probing depth 125 and 50 μm. The increase in the local concentration of cortexin deep in the tissue facilitates the decrease in its scattering coefficient and, therefore, the reduction of the OCTS signal. Approximately 12 min after application, the drop of cortexin completely impregnated the studied part of the sclera. When the preparation reached the internal space of the eye (aqueous humour), the inverse process began, associated with washing cortexin out of the tissue in the area of measurement, and the OCTS signal increased.

Table 1 summarises the values of the sclera permeability coefficient and the coefficient of cortexin diffusion in the sclera, obtained using different methods. By means of the OCTS method the permeability coefficient P was calculated from the slope of the experimental curve, and the diffusion coefficient was estimated using the following expression [33]:

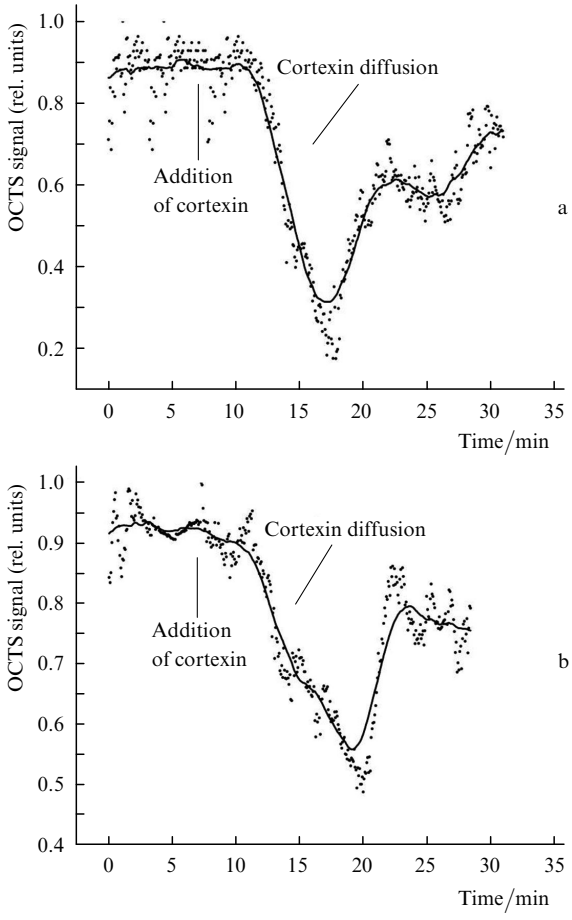

Figure 5. Time dependence of the OCTS signal in the process of interaction of the tissue with the cortexin solution. The signal is measured at the depth 125 μm (a) and 50 μm (b).

$$D = Pl_d, \quad (7)$$

where l_d is the depth of the tissue sample probing. At the probing depth 125 μm the diffusion coefficient appeared to be $(3 \pm 0.4) \times 10^{-7}$ cm^2 s^{-1}, and at $l_d = 50$ μm $D = (1.2 \pm 0.2) \times 10^{-7}$ cm^2 s^{-1}. The diffusion coefficient, averaged over all measurements, was $(1.49 \pm 0.25) \times 10^{-6}$ cm^2 s^{-1}.

When the method of reflectance spectroscopy was used, on the contrary, the diffusion coefficient was determined from the analysis of experimentally measured time dependence of reflectance, while the permeability coefficient was estimated using Eqn (7) and assuming the diffusion coefficient to be constant in all the volume of the tissue.

It follows from Table 1 that the permeability coefficients of the sclera and the coefficients of diffusion of cortexin, obtained using the methods of reflectance spectroscopy and OCT, are in sufficiently good agreement.

In vitro diffusion experiments with proteins, having a high (up to 150 kDa) molecular weight and diffusing through the samples of a fibrous tissue, carried out with the help of fluorescent methods, showed that sclera is permeable for large molecules [9, 12, 18, 19]. According to the data of Ref. [9], the permeability of sclera for fluorescein isothiocyanate – dextran with the molecular weight 4.4 kDa at 25 °C is $\sim 2.52 \times 10^{-5}$ cm s^{-1}. Then the diffusion coefficient is $D = Pl \approx 1.01 \times 10^{-6}$ cm^2 s^{-1} (here $l \approx 0.5$ mm is the mean thickness of the sclera sample). From the data, presented in [19], it can be found that the diffusion coefficient of dextran (10 kDa) equals 0.4×10^{-6} cm^2 s^{-1}. The diffusion coefficient of cytochrome (12.4 kDa) in the muscular fibrils at the temperature 22 °C is $\sim 0.16 \times 10^{-6}$ cm^2 s^{-1} [18], and the diffusion coefficient of retinalamine (10 kDa) in the human sclera equals 1.82×10^{-6} cm^2 s^{-1} [12]. The permeability coefficient of the rabbit sclera for water, determined by means of the OCTS method, is 6.6×10^{-5} cm s^{-1} [30], which approximately corresponds to $D = 0.18 \times 10^{-6}$ cm^2 s^{-1}.

The comparison of our results with the data from the literature yields that the values of the permeability coefficient of the sclera (and, correspondingly, the diffusion coefficients) for cortexin are close enough to the data, obtained for other agents. The difference between the coefficients, found by us and known from the literature, can be explained by the difference in structure and properties of the studied agents and tissues, as well as by the difference in applied experimental and computational methods.

4. Conclusions

The results of the experiments have shown that the penetration of cortexin into the eye sclera leads to the reduction of the reflectance and the OCT signal due to the optical immersion. The analysis of the time dependence of the reflectance of sclera samples allowed estimation of the diffusion coefficient of cortexin in the sclera, which appeared to be $(0.98 \pm 0.67) \times 10^{-6}$ cm^2 s^{-1}. The mean value of the diffusion coefficient, found using the OCTS method, was equal to $(1.49 \pm 0.25) \times 10^{-6}$ cm^2 s^{-1}. The results, obtained using two independent optical methods, are in good agreement. It should be taken into account, that the OCT method principally gives a local estimate of the diffusion coefficient, which is in our case at the depth of 50 or 125 μm, while the spectrophotometric method allows the determination only of the value, averaged over the whole sample thickness. Therefore, one should not expect full coincidence of the results, obtained using different methods.

These results are important for determining a number of parameters, such as the dose of the administered drug, sufficient for reaching the internal parts of the eye, the time, necessary for the efficient action of the preparation, etc., which is of great importance for the treatment of the partial atrophy of the visual nerve and a number of other ophthalmological diseases.

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