Diffusion of *Cortexin* and *Retinalamin* in eye sclera

Elina A. Genina[*1], Elena A. Zubkova[1], Anastasiya A. Korobko[1], Irina Yu. Yanina[1], Alexey N. Bashkatov[1], Tatiana G. Kamenskikh[2], Vladimir A. Galanzha[2], Valery V. Tuchin[1]
[1]Saratov State University, Saratov, Russia
[2]Saratov State Medical University, Saratov, Russia

ABSTRACT

Spectral measurements of sclera reflectance during its interaction with aqueous solutions of both *Cortexin* and *Retinalamin* have been carried out. Twenty three samples included in the study were human sclera and rabbit sclera *in vitro*. The results of the experiments have shown that penetration of *Cortexin* as well as *Retinalamin* into sclera tissue leads to the decrease of sclera reflection due to optical immersion. Estimation of diffusion coefficients of studied solutions was made on the basis of analysis of reflectance change dynamics of the sclera samples. Diffusion coefficients of *Cortexin* and *Retinalamin* in sclera tissue were estimated as $(4.4 \pm 2.7) \times 10^{-6}$ and $(1.82 \pm 0.14) \times 10^{-6}$ cm^2/sec, respectively. Obtained values of diffusion coefficient allowed estimating time needed for total penetration of both agents through scleral membrane at subtenon's injection of studied agents. The results are important for treatment of partial optic atrophy observed at primary open-angle glaucoma and others eye diseases.

1. INTRODUCTION

The problem of metabolic disorder correction in optic nerve at its partial atrophy development as a result of inflammation, intoxication, blood circulation disorder, glaucomatous optic neuropathy and others is an actual one.

Eye tissues, especially those, which are responsible for visual functions, are well isolated from systemic blood circulation by a multitude of barriers. The barriers provide for a high level of soluble selection. At the system application of drugs only 0.01 – 0.07 % of injected dose reach the eye tissues.[1]

A number of authors carried out comparative studies of effectiveness of medicine action on eye at different methods of introduction.[2] Analysis of drug concentration in eye tissues after oral, intramuscular and intravenous introduction has shown that the most effective method is intravenous introduction. Comparison of drug concentration in eye tissues at both intravenous and subconjuctival methods of introduction has revealed the concentration of the drugs is higher at the subconjuctival introduction than at the intravenous one. Research has shown that the drug introduction into the space between sclera and Tenon's capsule is the most expedient.[2]

Now in clinical practice different kinds of drug topical application are used. At the treatment of partial optic atrophy parabulbar or subtenon methods of introduction of suitable drugs are used.[3,4]

Many years' experience of the use of cytomedines (such as *Retinalamin* and *Cortexin*) in clinical practice has shown high effectiveness of the drugs in different areas of medicine, including ophthalmology. *Retinalamin* consists of calf's or pig's retina polypeptides and glycin. It regulates metabolic processes in retina, stimulates functions of cell elements, promotes to improvement of functional interaction of pigment epithelium and external segments of photoreceptors, increases retinal macrophage activity. *Cortexin* has tropy action on the nerve tissue, regulates metabolic processes of neuromediators and peroxidation in a cerebral cortex, optic nerve and retinal neurons.[5]

The goal of the study is *in vitro* research of sclera permeability as the most significant tissue barrier at the topical application of the both *Retinalamin* and *Cortexin*.

* eagenina@optics.sgu.ru

Saratov Fall Meeting 2006: Optical Technologies in Biophysics and Medicine VIII, edited by Valery V. Tuchin, Proc. of SPIE Vol. 6535, 65351Y, (2007) · 1605-7422/07/$18 · doi: 10.1117/12.741082

2. MATERIALS AND METHODS

2.1. Materials

Twenty three samples used in the study were human sclera obtained from 7 patients. The samples were obtained from enucleated eyes during elective surgery – enucleation of blind eye with forming functioning stump for artificial eye. Age of the patients was 38 – 45, all of them were males. The reasons of the enucleation were the followings: congenital absolute glaucoma, preathrophy owing to penetrating wound. Necessity of enucleation has arised because of unsatisfactory appearance of the blind eye at will of the patients. Rabbit sclera samples were also used. Time interval from *post mortem* to enucleating does not exceed 24 hours. After enucleating all samples were kept in a normal saline solution (0.9% NaCl) at the temperature 4 - 5°C, where they were stored up for spectroscopic measurements. All measurements were performed at room temperature about 20°C. Before the measurement pigment layer (lamina fuska) was removed from sclera samples. Thickness of the samples was measured by micrometer before the interaction with studied solutions and after that. Precision of the measurements was ±50 μm. The obtained values were averaged.

As immersion liquids aqueous solution of both *Cortexin* and *Retinalamin* were used. Concentration of *Cortexin* was 20 mg/ml, refractive index was measured by Abbe refractometer as 1,342 at λ = 589 nm. Concentration of *Retinalamin* was 25 mg/ml and refractive index was 1.346 at λ = 589 nm.

2.2. Experimental setup

Estimation of diffusion coefficients of studied solutions was made on the basis of analysis of reflectance change dynamics of the tissue samples. The measurements of sclera reflectance have been performed in the spectral range 450-900 nm using a commercially available optical multichannel spectrometer LESA-5 (BioSpec, Russia) with fiber-optical probe at room temperature about 20°C. The scheme of the experimental setup is shown in Fig. 1.

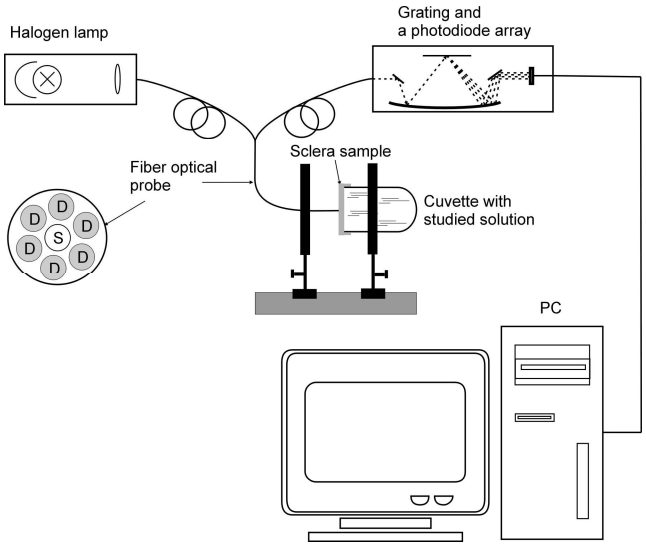

Fig. 1. Experimental setup for measurements of sclera reflectance spectra.
S and D mean source and detector fibers, respectively.

As a light source a 250 W xenon arc lamp with filtering of the radiation in the spectral range from 450 to 900 nm has been used in the measurements. Light was delivered to the sample and collected from the tissue using the originally designed optical probe. The fiber-optical probe consisted of seven optical fibers. All fibers had 200 μm core diameter and a numerical aperture of 0.22. The central fiber (S) delivered incident light to the tissue surface and the six fibers (D) placed around the central fiber collected reflected light. Distance between the delivering and receiving fibers was 290 μm. Time of signal accumulation was 100 ms. As a reference a white slab $BaSO_4$ with a smooth surface was used. For the spectrometric measurements each sample was fixed on the special cuvette with solution of *Cortexin* or *Retinalamin*.

2.3 Method for estimation of diffusion coefficient

The transport of drugs within tissues can be described in the framework of free diffusion model.[6-9] We assume that the following approximations are valid for the transport process: 1) only concentration diffusion takes place; i.e., the flux of the agent into the tissue at a certain point within the tissue sample is proportional to the agent concentration at this point; 2) the diffusion coefficient is constant over the entire sample volume.

Geometrically the tissue sample is presented as a plane-parallel slab with a finite thickness. Since lateral sides of the experimental samples were fixed, the one-dimensional diffusion problem has been solved. Diffusion equation of the drug transport has the form:

$$\frac{\partial C(x,t)}{\partial t} = D\frac{\partial^2 C(x,t)}{\partial x^2}, \tag{1}$$

where $C(x,t)$ is the concentration of agent, g/ml; D is the diffusion coefficient, cm^2/s; t is time, s; and x is the spatial coordinate, cm.

We also suppose that penetration of drug into the tissue does not change the concentration of the drug in the external volume. Besides, due to geometry of the measurements, penetration of *Cortexin* or *Retinalamin* into the sclera sample takes place from top surface of the sample only. The corresponding boundary conditions are

$$C(0,t) = C_0 \quad \text{and} \quad \frac{\partial C(l,t)}{\partial x} = 0, \tag{2}$$

where C_0 is drug concentration in external solution, g/ml, and l is sclera sample thickness, cm.

The initial condition corresponds to the absence of drug inside skin before the measurements,

$$C(x,0) = 0 \tag{3}$$

for all inner points of the sample.

Solution of Eq. 1 for a slab with a thickness l at the moment t with boundary (Eq. 2) end initial (Eq. 3) conditions has the form

$$C(t) = C_0\left(1 - \frac{8}{\pi^2}\sum_{i=0}^{\infty}\frac{1}{(2i+1)^2}\exp\left(-(2i+1)^2\, t\,\frac{\pi^2}{4}D\Big/l^2\right)\right), \tag{4}$$

where $C(t)$ is the volume-averaged concentration of agent within skin sample.

In a first-order approximation Eq. 4 is reduced to the form

$$C(t) \approx C_0\left(1 - \exp\left(-t\pi D/l^2\right)\right). \tag{5}$$

3. RESULTS AND DISCUSSION

Figs. 2 and 3 show spectral dependence of sclera reflectance during its interaction with studied solutions of *Cortexin* and *Retinalamin*, respectively. Change dynamics of sclera reflectance under action of *Cortexin* and *Retinalamin* at some wavelengths is presented in Figs. 3 and 5, respectively.

In initial time the form of reflectance spectra (see, Fig. 2, 4) is determined by spectral dependence of sclera scattering coefficient. Since absorption coefficient of scleral collagen as well as water and proteins of interstitial matrix is insignificant in studied spectral range, then we can neglect light absorbance in sclera.[10]

Spectral dependence of reflectance is determined wavelength of incident radiation as well as relation of refractive indices of sclera scatterers and surrounding interstitial liquid.[11] Refractive index of sclera interstitial liquid (after 24 hour's

keeping in saline) could be considered as matching to water refractive index, i.e. 1.332. Refractive index of collagen fibers is 1.411.[12]

Penetrating into sclera the drugs with refractive index larger than that of interstitial liquid causes matching of refractive indices of collagen fibers and surrouding medium. That leads to the decrease of light scattering (optical immersion) and as a consequence to the decrease of sclera reflectance[13,14] (see, Figs. 3 and 5).

It is important that the use of such high-polymer substances as *Cortexin* and *Retinalamin* as immersion agents leads to osmotic dehydration of tissue. The thickness of the samples decreases to about 20%, for example, before measurements the thickness of sclera sample was 0.5±0.08 mm, after that it was 0.38±0.05 mm. Dehydration of the studied samples causes significant increasing of scatterer volume fraction in tissue, that induces the rise of scattering coefficient and compensate the immersion effect.

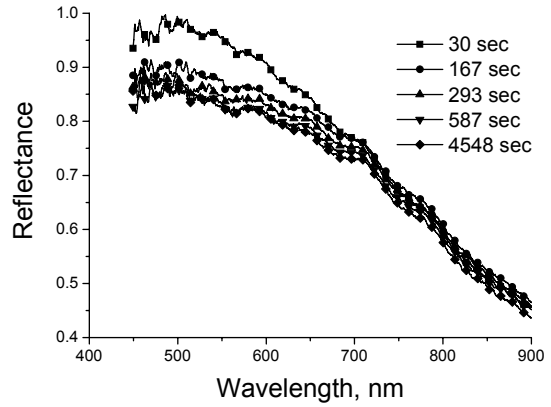

Fig. 2. Reflectance spectra of human eye sclera measured in different moments of interaction of tissue with *Cortexin* solution.

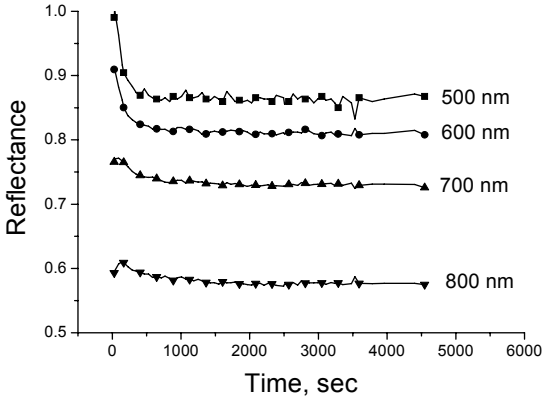

Fig. 3. Reflectance change dynamics of human eye sclera measured at different wavelengths during interaction of tissue with *Cortexin* solution.

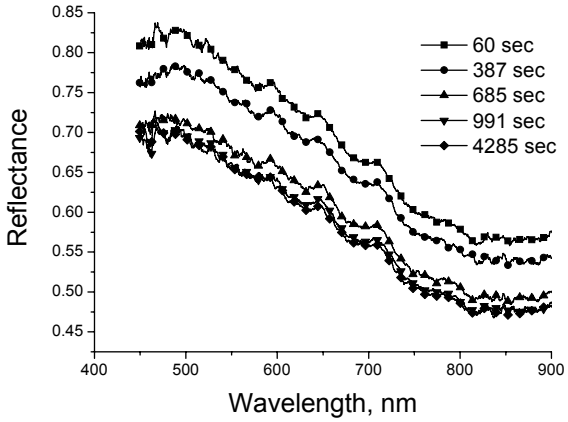

Fig. 4. Reflectance spectra of rabbit eye sclera measured in different moments of interaction of tissue with *Retinalamin* solution.

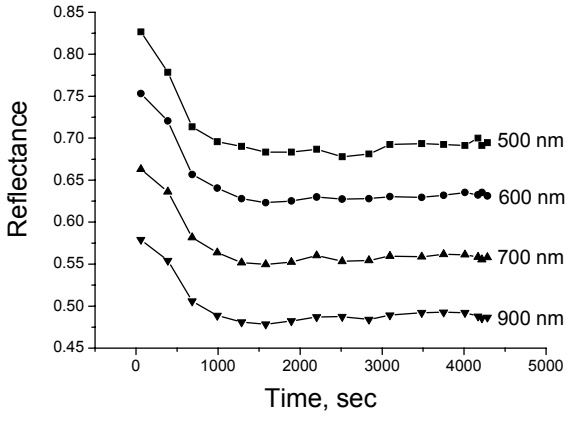

Fig. 5. Reflectance change dynamics of rabbit eye sclera measured at different wavelengths during interaction of tissue with *Retinalamin* solution.

Influence of water penetration on the change of sclera reflectance was researched by analogical method. Fig. 6 presents the change dynamics of human eye reflectance measured at different wavelengths during interaction of sclera sample with saline. It is well seen that water diffusion into the sclera sample practically did not change sclera reflectance in all studied spectral range. Thus, it can be concluded that the changes in the sclera reflectance spectra observed in Figs. 3 and 5 were connected with diffusion of *Cortexin* and *Retinalamin*, respectively, in the interstitial liquid of the sclera samples.

Analysis of reflectance change dynamics of the sclera samples allows us to estimate timing diffusion constant $\tau = \frac{l^2}{\pi D}$ of *Cortexin* and *Retinalamin* as 484.1±314.1 and 439.01±34.46 sec, respectively. Average diffusion coefficients of *Cortexin* and *Retinalamin* are $(4.4 \pm 2.7) \times 10^{-6}$ and $(1.82 \pm 0.14) \times 10^{-6}$ cm^2/sec, respectively. The values are in a good agreement with the known values of diffusion coefficients of different immersion agents in both tissues and interstitial liquid.[9,14,15]

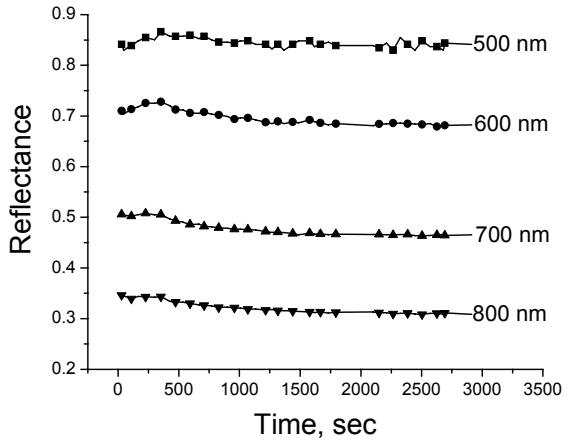

Fig. 6. Reflectance change dynamics of rabbit eye sclera measured at different wavelengths during interaction of tissue with saline.

Obtained value of diffusion coefficient allows estimating a time needed for total penetration of both *Cortexin* and *Retinalamin* through scleral membrane at subtenon's injection of studied agents. It is well known that at transmembrane diffusion of agent from small volume with nonzero concentration of the agent (injection area) into large volume with zero concentration of the agent concentration of the agent in small volume can be described by the following equation:[8]

$$C(t) \approx C_0 \exp\left(-Dt/l^2\right). \tag{6}$$

Substituting obtained data into the Eq. 6 it can be estimated that total penetration of both *Cortexin* and *Retinalamin* through eye sclera is in about 3 hours after the injection.

4. CONCLUSION

The results of the experiments have shown that penetration of *Cortexin* as well as *Retinalamin* into sclera tissue leads to the decrease of sclera reflection due to optical immersion. Analysis of reflectance change dynamics of the sclera samples allowed us estimating diffusion coefficient of both *Cortexin* and *Retinalamin* in sclera as $(4.4 \pm 2.7) \times 10^{-6}$ and $(1.82 \pm 0.14) \times 10^{-6}$ cm^2/sec, respectively. Total penetration of both agents through eye sclera is in about 3 hours after the injection.

The results are important for treatment of partial optic atrophy observed at primary open-angle glaucoma and others eye diseases.

ACKNOWLEDGEMENTS

The research described in this publication has been made possible, in part, by grants PG05-006-2 and REC-006 of U.S. Civilian Research and Development Foundation for the Independent States of the Former Soviet Union (CRDF) and the Russian Ministry of Science and Education, grants RUB1-570-SA-04 of CRDF, grant of the Russian Federal Agency of Education Russian Federation 1.4.06, and grant of RFBR No. 06-02-16740. The authors thank Dr. S.V. Eremina (Department of English and Intercultural Communication of Saratov State University) for the help in manuscript translation to English.

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