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APPLICATION OF CHITOSAN FILMS FOR THE TREATMENT OF AGE-RELATED MACULAR

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ABSTRACT

In the last decades ophthalmic drug delivery systems providing sustained and controlled drug release are of great interest. To improve ocular drug efficiency and bioavailability, there is a significant effort done towards the development of new drug delivery systems for ophthalmic administration. In the current research an attempt was made to prepare new drug delivery systems based on polymeric materials with prolonged and controlled therapeutic effect. Film casting method was used in order to obtain chitosan/mexidol composites. The influence of drug concentration on the drug release kinetics was investigated.

Key words: Mexidol, Chitosan, Ringer-Locke solution, Therapeutic effect, Drug delivery systems.

INTRODUCTION

Drug delivery directly into the eye is complicated due to removal mechanism of precorneal area, resulting in decrease of therapeutic response. Conventional ocular delivery systems like eye drops, suspensions and ointments show some disadvantages such as rapid corneal elimination, repeated instillation of drug and short duration of action. Moreover, regular drug installation is danger for eye and can result in toxicity, the risk of unwanted side effects[1,2]. However, successful therapy of many diseases is possible only if uniform concentration of the drug is ensured to an organism for prolonged period of time[3].

Various natural and synthetic polymers like cellulose derivatives, pectin, dextran, alginates, polyvinyl pyrrolidone, polyvinyl alcohol, etc. are used for the preparation of

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therapeutic films[4,5]. These polymers are physiologically inert, hydrophobic, soluble in water, available and cheap[6]. Perspective materials for this purpose are natural polysaccharides. Recently, new various applications using polysaharides are highlighted, such as artificial skin and surgical sutures that are absorbed naturally after surgery and ophthalmology, as corneal contact lenses in ophthalmology, etc.[7-9]

One of the most important issues in the ophthalmology is drug treatment of age-related macular (AMD), aimed to stimulate the functioning portions of the retina, rather than restoring the affected areas. Currently used pharmacological substances do not provide sufficiently stable effect on the treatment of AMD. Risk factor may be low levels of antioxidants in the body[10].

Mexidol is a succinic acid salt (succinate) belonging to the synthetic antioxidants group. In ophthalmology mexidol initially was used for the treatment of diabetic retinopathy and chronicopticneuropathy[11].

The main advantages of the polymer films are the possibility of programmed drug delivery by controlling the macromolecular structure and chemical nature of the polymeric matrix at the same time reducing the toxic side effects and the frequency of admission of commonly used drug dosage forms. First the films based on chitosan were developed in treating AMD. First the effectiveness of the use of polymer films based on chitosan in the treatment of AMD was substantiated experimentally and clinically confirmed.

EXPERIMENTAL

Materials

Chitosan with molecular weight of 250 kDa was purchased from Sigma-Aldrich (USA) and was used without further purification. Pharmaceutical grade mexidol - succinate, 2-ethyl-6-methyl-3-hydroxypyridine was applied from Pharmasoft (Russia). Sodium chloride, potassium chloride and calcium chloride was purchased from Biopharma (Ukraine) Hydrochloric acid was purchased from Reachem (Russia).

Preparation of chitosan/drug composites

To obtain the polymer matrix 1% chitosan solution in 0.1 N hydrochloric acid at 60°C under stirring for 4-5 hrs. Mexidole (50, 100, and 150 mg/g) was dissolved in the obtained polymer solution. Chitosan/mexidole solution was poured on the horizontal glass surface and dried for 2-3 days at 20-40°C until residual moisture was of 5-7%. Polymer composite film containing mexidol was carefully removed from the cup and placed in a

sterile plastic bag. Samples of size 2 × 4 mm were formed from the obtained chitosan/ mexidol films.

Thermal properties of the films

Thermal properties of the films were investigated by thermogravimetric analysis (TGA) on the instrument Mettler Toledo TGA/SDTA 851 (Switzerland). TGA was carried out in the temperature range from 50 to 500^{o}C at a heating rate 5^{o}C/min.

Evaluation of *in vitro* drug release

In order to determine the prolonged properties *in vitro* the release of mexidol from chitosan films was investigated. The drug release was determined using UV/VIS spectroscopy (Jasco UV/VIS 7850, Japan) at 297 nm in quartz cuvettes with thick of 10 mm at 25^{o}C. Visual acuity was assessed using a projector ACP 7EM" firm «Topcon» (Japan). As the lease medium was used Ringer-Locke solution. Ringer-Locke solution - a standard isotonic solution 6.5 g NaCl, 0.42 g KCl, 0.25 g $CaCl_2$ and 1 mole of sodium bicarbonate is dissolved in one Liter of distilled water.

Design of *in vivo* tests

Biomedical tests of obtained films were carried out jointly with the staff of the Kazakhstan Research Institute for Eye Diseases. *In vivo* experiments were studies in 20 chinchilla rabbits (range of weight from 3.0 to 3.5 Kg) at the age of 4-6 months. Implantation into the suprachoroidal space of polymer film was produced in the right eye of 10 rabbits, and polymer film loaded mexidol in another 10 rabbits. On the left eye was produced choroidalrevascularization surgery with implantation of episcleral flap. Surgery was performed on the operating microscope "LOMO".

Implants of size 2 × 4 mm were formed from polymer film. Anesthesia was conducted by the introduction of 5% kallipsola solution in marginal vein of the rabbit. Intrascleral tunnel was formed in the direction of posterior pole of the eyeball and suprachoroidal space was opened. In the formed tunnel was implanted polymeric chitosan film. On the conjunctival continuous seam was imposed, and then ointment with antibiotic and corticosteroid was laid into the conjunctival sac.

Clinical results

Clinical tests were conducted in accordance with the order of the Chairman of the Pharmaceutical Control of Health Ministry of RK from 12 September 2008 №219 "On approval of the list of recommended drugs to conduct clinical tests".

RESULTS AND DISCUSSION

Thermal properties of the films

TGA analysis graphs of chitosan films showed that the thermal degradation of the films began at 200^{o}C. In general, the process of degradation took place at the temperature range of 200-380^{o}C. Polymer films based on chitosan and films containing mexidol might be heat sterilized in an autoclave. The TGA diagram of chitosan films is shown in Figure 1.

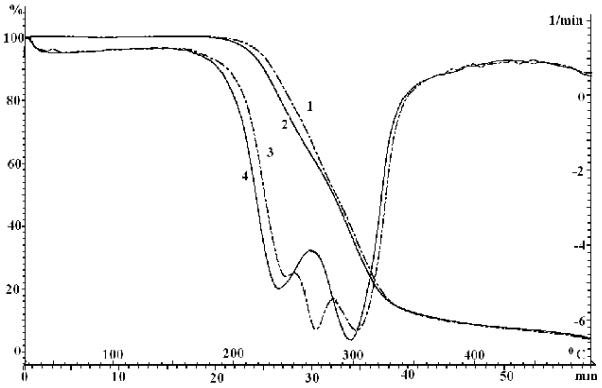

Fig. 1: TGA (1.2) and DTA (3,4) of the diagram of chitosan and chitosan films containing films mexidol

Evaluation of *in vitro* drug release

In vitro release kinetics of mexidol from the chitosan films was investigated to determine the drug release properties. It was established that the release consisted of three main stages: water sorption by a film and its swelling, the drug diffusion in a film at the phase interface "polymer system-environment" and the drug diffusion in the solvent volume. To determine the influence of drug loading on its release kinetics, chitosan films were loaded with 100 mg of mexidol, respectively with thickness of 15-20 microns. Obtained results showed that the drug was diffused practically completely into Ringer-Locke solution within 4-6 hrs (Figure 2).

Diffusion coefficients for the chitosan film, calculated at the initial stage of release was within $7.5 \cdot 10^{-6}$ and $8.3 \cdot 10^{-6}$ cm^2/sec. Increasing the film thickness by 3 times the diffusion coefficient decreased twice. Diffusion of the therapeutic agent in a matrix that was confirmed by inverse relationship between the release rate and film thickness played the limiting role in the release of mexidol from polymeric film system.

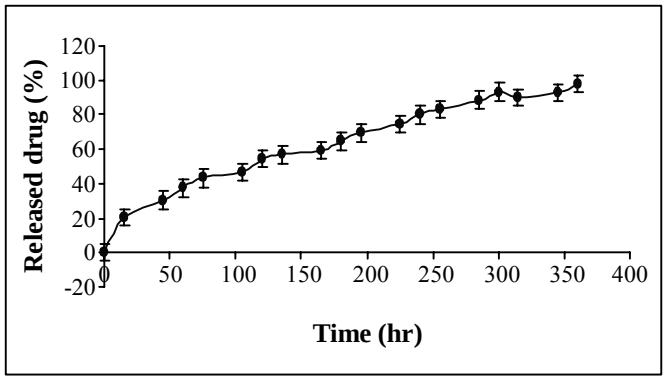

Fig. 2: Mexidol release from chitosan films

Design of *in vivo* tests

Implants of size 2 × 4 mm were formed from polymer film. Anesthesia was conducted by the introduction of 5% kallipsola solution in marginal vein of the rabbit. Intrascleraltunnel was formed in the direction of posterior pole of the eyeball and suprachoroidal space was opened. In the formed tunnel was implanted polymeric chitosan film. On the conjunctival continuous seam was imposed, and then ointment with antibiotic and corticosteroid was laid into the conjunctival sac.

On gross examination at all times of observation the enucleated eyes was correct eyeball round shape with a smooth surface without visible changes. After surgery in 7-10 days the local reaction was observed in the focal infiltration of the conjunctiva plasma cells, lymphocytes, leukocytes (Figure 3). Blood vessels were dilated, full-blooded.

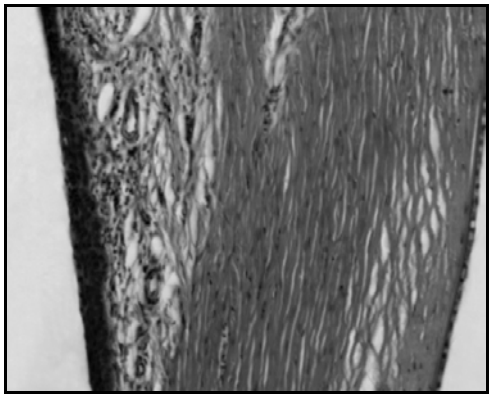

Fig. 3: Colouring with hematoxylin and eosin X 200

Histological tissue preparation of the rabbit conjunctiva in 7 days after implantation of chitosan film.

In 14 days after surgery a single cell cluster of lymphocytes, macrophages, soft fibrous tissue containing blood vessels with proliferation of fibroblasts was observed in interference of the sclera (Figure 4).

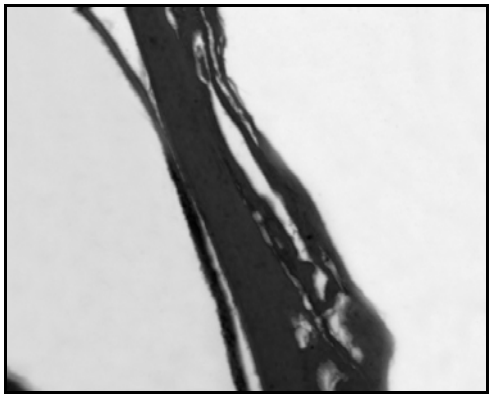

Fig. 4: Histological preparation of sclera in 14 days after implantation of chitosan film

By the 21th day the polymer is fully dissolved. Loose connective tissue containing isolated macrophages, fibroblasts, and thin-walled vessels was determined in its place.

By the 30th day tender fibrous scar submitted fibroblasts and collagen fibrils randomly-seated was defined.

Thus, it was experimentally proved that the introduced chitosan film into the suprachoroidal space did not cause toxic effects on the eye.

This method was shown high efficiency in the treatment of advanced stages of AMD, which was manifested in the improvement of visual function and stabilization process. A month later, there was an increase in visual acuity of 21.6% after 6 months the initial level was by 18.7%.

Clinical results

Effectiveness of using of chitosan film was studied on 10 patients (10 eyes) with AMD. There were 12 people in control group (12 eyes). Operation of suprachoroidal space was performed to 22 patients, 10 of them with implantation into the suprachoroidal space polymer films based on chitosan.

As the main criteria of state of eye bottom of both patients groups were assessed the presence of hemorrhages, hard exudates, ischemic "soft" exudative lesions, newly formed blood vessels, the presence of pigment and neuroepithelium detachments. Before the treatment ophthalmoscopic picture was comparable in both groups. Ophthalmoscopically in the eye bottom of 8 patients (8 eyes) were occurred exudative lesions localized in the macular and paramacular, soft cotton-like lesions with hard exudates were in 4 patients (4 eyes). Hemorrhages in the retina were found in 4 patients (4 eyes). In 12 patients (12 eyes) in the macular exudative detachment of the pigment epithelium in the macula and paramacular was occurred. In 3 (3 eyes) in addition to the pigment epithelium detachment neuroepithelium detachment was detected. In patients 2 (2 eyes) retinal cysts was revealed.

After treatment in the main group 70% of patients the amount and area of ischemic lesions decreased. Resorption of hemorrhages was in 3 months. In the control group after treatment in a month the resorption of hemorrhages was in 16% complete and in 50%. Soft cotton-like lesions decreased in 30%.

Thus, the clinical studies showed the effectiveness of the developed method for the treatment of "wet" form of AMD. Operation of suprachoroidal space with implantation of chitosan film helped to reduce macular edema by 29% and improved visual function and stabilization process. The data suggested that, after the combined operation there was the positive dynamics from the eye ground.

CONCLUSION

In the paper data, on using of polymeric composite materials in ophthalmology were generalized. The data indicated significant prospects of the biopolymer at the treatment of various eye defeats and the construction of new high-performance drugs with the prolonged and controlled action. Chitosan films containing mexidol were developed. The results of physico-chemical studies showed the high efficiency of using the natural polysaccharide chitosan as a matrix for the ophthalmic application. The prolonged drug release was 90-95% during 12-16 h. The profile of kinetic curves corresponded to kinetics of the first order and it was controlled by diffusion of the therapeutic agent in a matrix.

TGA analysis graphs of chitosan films showed that the thermal degradation of the films began at 200^{o}C. In general, the process of degradation took place at the temperature of 200-380^{o}C. Polymer films based on chitosan and chitosan films containing mexidol could be heat sterilized in an autoclave.

Morphological studies of the effect of polymer films based on chitosan were carried out on the experimental eyes of animals. *In vivo* tests were studies in 20 chinchilla rabbits at the age of 4-6 months. Implantation into the suprachoroidal space of polymer film was produced in the right eye of 10 rabbits, and polymer film loaded mexidol in another 10 rabbits. This method was shown high efficiency in the treatment of advanced stages of AMD, which was manifested in the improvement of visual function and stabilization process.

Effectiveness of chitosan film was studied on 10 patients (10 eyes) with "wet" AMD. Suprachoroidal space surgery was performed to 22 patients, 10 of them with the implantation of chitosan film.

Application of this method leaded to improvement of visual function and stabilization process. A month later, there was an increase in visual acuity of 21.6% after 6 months the initial level was by 18.7%.

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