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Original article

Influence of antlerogenic stem cells on the healing of lesions in the corneal epithelium and corneal stroma in rabbits

M. Kiełbowicz[1], P. Kuropka[2], M. Cegielski[3], Z. Kiełbowicz[4], P. Trębacz[5], M. Hebel[1], R. Aleksiewicz[1]

[1] Unit of Animal Surgery and Diagnostic Imaging, Faculty of Veterinary Medicine and Animal Science, Poznań University of Life Sciences, Wołyńska 33, 60-637 Poznań, Poland
[2] Department of Biostructure and Animal Physiology of Wrocław University of Environmental and Life Sciences, Norwida 25, 50-375 Wrocław, Poland
[3] Department and Division of Histology and Embryology of Wrocław Medical University, Chałubińskiego 6a, 50-368 Wrocław, Poland
[4] Department and Clinic of Surgery of Wrocław University of Environmental and Life Sciences, Grunwaldzki 51, 50-366 Wrocław, Poland
[5] Division of Small Animal Surgery Faculty of Veterinary Medicine, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland

Abstract

The aim of the study was to compare the effects of corneal healing in case of application of stem cells in various forms, in relation to the antibiotic-assisted procedures. Rabbits were divided into 4 groups in the first stage of the experiment. Group 0 (negative control group) was not subjected to any actions, which would cause damage to the cornea. The remaining three groups had their cornea damaged. Group 1 (positive control group) – no drugs were administered during the experiment. Rabbits in group 2 were administered with ointment containing stem cells to the lesion, while group 3 – with ofloxacinum. The stem cells were administered during the first five days, twice a day, onto the corneal surface. The further course of the experiment consisted of observing the rate of healing of the injured cornea and assessment of its transparency, size of lesion, hyperaemia, eyelid spasm and outflow from the conjunctival sac after 5, 10 and 20 days.

In the second stage the animals were euthanised after clinical examination on the twentieth day of the experiment, in order to analyse the corneal reparative processes on the same day. The studies revealed that the application of antlerogenic stem cells had a positive effect on the healing process of corneal defects. The application thereof not only shortened the healing time, but also weakened or arrested the development of side effects. The results have demonstrated that the epithelial proliferation in each group was different. The longest was maintained in the group with stem cells, the shortest – in the group with chemotherapeutics. The use of antlerogenic stem cells had a positive effect on the healing process of corneal lesions. The use of stem cells helped to maintain high transparency of the cornea.

Key words: eye, stem cells, cornea, rabbit, healing, lesions

Correspondence to: M. Kiełbowicz, e-mail: przychodnia.kielbowicz@gmail.com

Introduction

Stopping the advancement of eyesight destruction, preventing infection, inducing repair mechanisms, eliminating pain and discomfort, and shortening the recovery time to achieve total transparency of the cornea are some of the most important objectives of corneal lesions treatment.

Nowadays, there has been an increasing use of pluripotent cells in regenerative medicine (Cegielski et al. 2006, Cegielski and Kalisiak 2008, Cegielski et al. 2009, Cegielski et al. 2010). Pluripotent cells are the elements supporting and accelerating the healing process. They are particularly important in tissues where there are no substitute cells (or their number is low), such as cartilage tissue or cornea that do not contain blood vessels (Cegielski 2009).

Antlerogenic stem cells are characterised by the synthesis and secretion of numerous substances inducing regenerative processes (Cegielski 2009). They constitute an interesting study material as they can affect the proliferation and differentiation of corneal epithelial cells as well as connective tissue cells. In addition, they can induce an immune response. The response is usually weak in the cornea which contains no blood vessels and is associated with adverse processes of blood vessel penetration.

Stem cells play an important role in the initiation and continuation of the growth process of cervid antlers. These cells are the basis for antler growth, leading to periodical, seasonal tissue regeneration every year and they differentiate to form chondroblasts and osteoblasts (Bonewald and Mundy 1990, Amedee et al. 1994, Boskey 1995). The mesenchymal stem cells were obtained for this study by StemCells Spin Sp. z o. o. (limited liability company), a company from Wrocław, from red deer antlers (*Cervus elaphus*) which created a stable line of antlerogenic cells MIC-1 (Li et al. 2005, Cegielski et al. 2006, Cegielski et al. 2009). The studies confirmed the possibility of their continuous division, rapid growth and ease of culture maintenance (Cegielski and Kalisiak 2008, Cegielski 2009). Cells growing in culture are spindle-shaped, pointed sharply and are described as fibroblast-like. They have an ability of multipotent differentiation (Rolf 2008). The lack of class II histocompatibility antigens (MHC II) and inhibition of T and B lymphocyte activity prevent the rejection of stem-cell transplants (Javazon et al. 2004, Rayan et al. 2005).

Antlerogenic stem cells also produce and store numerous growth factors, such as insulinlike growth factor I and II (IGF-1, IGF-2), transforming growth factor (TGF), nerve growth factor (NGF), fibroblast growth factor (FGF), granulocyte growth factor (G-CSF), granulocyte and macrophage growth factor (GM-CSF), hepatocyte growth factor (HGF) and epidermal growth factor (EGF). This contributes to their regenerative capacity (Cegielski et al. 2010).

The aim of the study was to compare corneal healing supported with stem cells with physiological healing and healing supported with ofloxacinum.

Materials and Methods

The animals used in the study were New Zealand rabbits from a licensed laboratory animal provider. During the experiment, the animals were kept at the Department and Clinic of Surgery at the Faculty of Veterinary Medicine of Wrocław University of Environmental and Life Sciences in one-animal cages with constant access to drinking water and granulated food. Prior to the experiment, the rabbits underwent a two-week quarantine. This study was authorised by the II Local Ethics Committee for Experiments on Animals at the Wrocław University of Environmental and Life Sciences – permission no. 107/2010.

The mesenchymal stem cells were obtained for this study from red deer antlers (*Cervus elaphus*) which created a stable line of antlerogenic cells MIC-1 (Cegielski et al. 2006, Cegielski et al. 2009, Li 2005). The cells were grown at 37°C in a CO_2 incubator in DMEM, 10% foetal bovine serum (FBS) and antibiotics. All media, reagents, enzyme, serum, culture dishes and disposable plastics used in production of the cells possessed all necessary certificates. The cells were regularly examined for their viability, microscopically controlled to verify cell morphology, sterility, endotoxins and mycoplasma contents.

The cells growing in the culture were spindle shaped – referred to as fibroblasts, and their microscopic image is comparable to the image of human mesenchymal stem cells harvested from the bone marrow. The distinguishing features of the antler stem cells are their poor diversity and pluripotency. The cells were cultured in a clean-room of the pharmaceutical laboratory according to standard operational procedures. The antlerogenic stem cells were harvested from the culture and subjected to sonication in order to obtain the desired form. The cultured cells were characterised according to the requirements of the European Pharmacopoeia and frozen in liquid nitrogen in the mastercell bank. Prior to commencement of production, the banked cells were thawed.

Description of the active substance

The active factors were the antlerogenic stem cells. The cellular explants were harvested from culture

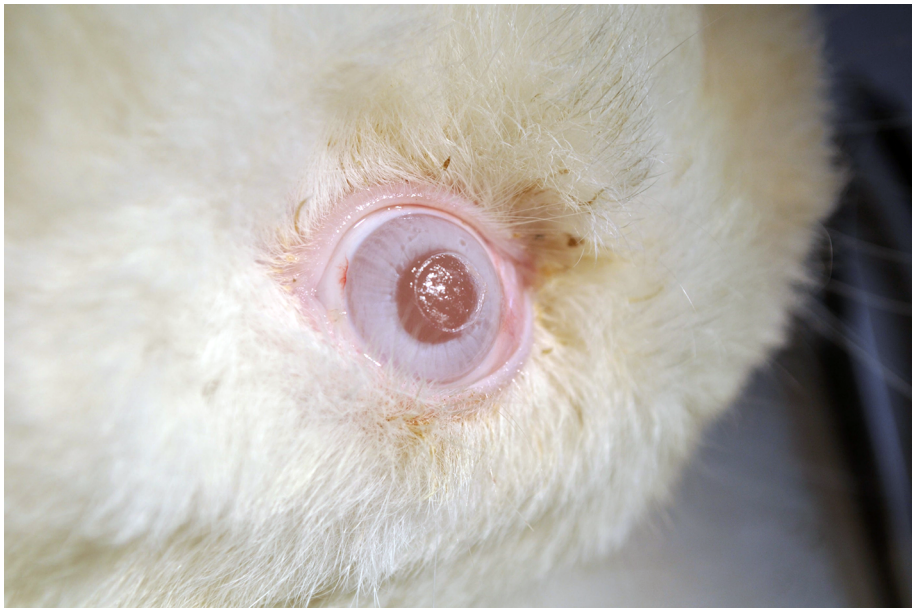

Fig. 1. The rabbit cornea with a corneal lesion made using a trephine.

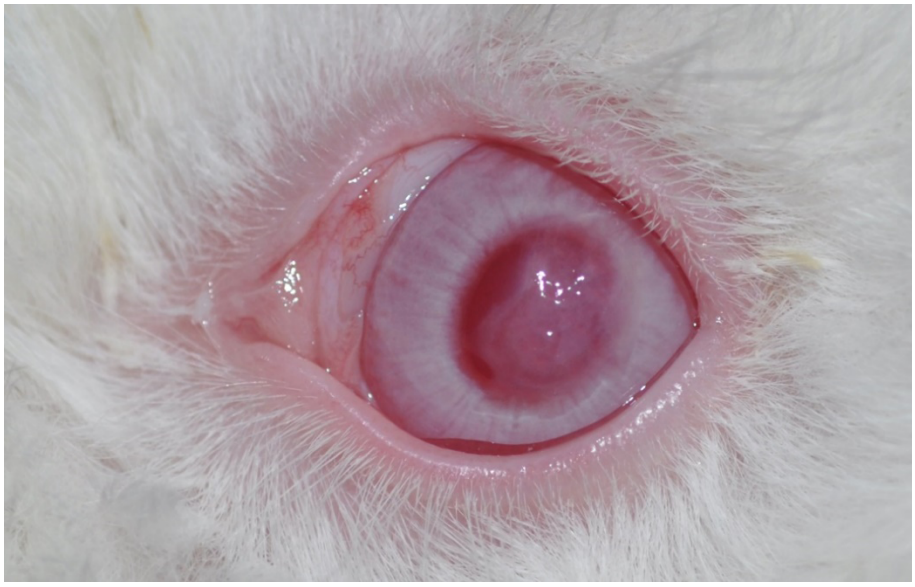

Fig. 2. The rabbit cornea in group 1 on the 20th day of the experiment.

dishes containing a population of antlerogenic stem cells (MIC-1 cell line) (Cegielski 2009).

In the present experiment, the rabbits were divided into 4 groups marked as 0, 1, 2 and 3. In group 0, no manipulations causing lesions to the cornea (group 0 negative) were performed. In groups 1, 2 and 3, consisting of 6 rabbits each, a corneal lesion 6 mm in diameter and reaching the corneal stroma was made with a trephine (Fig. 1). For this purpose, the animals were anesthetised using a mixture of Torbugesic® (butorphanol) at a dose of 0.2 mg/kg of body weight i.m. with Cepetor® (medetomidine) at a dose of 150 mcg/kg of body weight i.m. and Bioketan® (ketamine) at a dose of 35 mg/kg of body weight i.m. After the rabbits had been anesthetised, a catheter was placed in the auricular marginal vein and anesthesia was continued with a constant infusion of Propofol® (propofol) at a dose of 0.1 mg/kg of body weight i.v. Then, a circular fragment of the epithelium, 6 mm in diameter, was removed from the central part of the right cornea. During the procedure, analgesia was maintained with an intravenous supply of Fentanyl® (fentanyl) at a dose of 23 mcg/kg i.v. After the procedure, Vetergesic® (buprenorphine) was administered at a dose of 20 mcg/kg i.m. every 8 hours for two days and Metacam® (meloxicam) was given at a dose of 0.2 mg/kg of body weight s.c. for 3 days.

Group 1 was a positive control, where no drugs

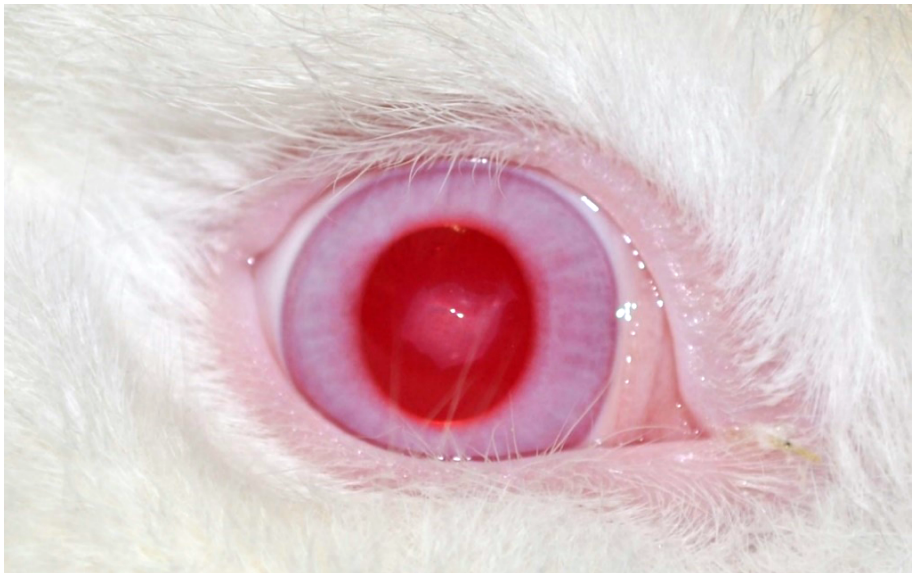

Fig. 3. The rabbit cornea in group 2 on the 20th day of the experiment.

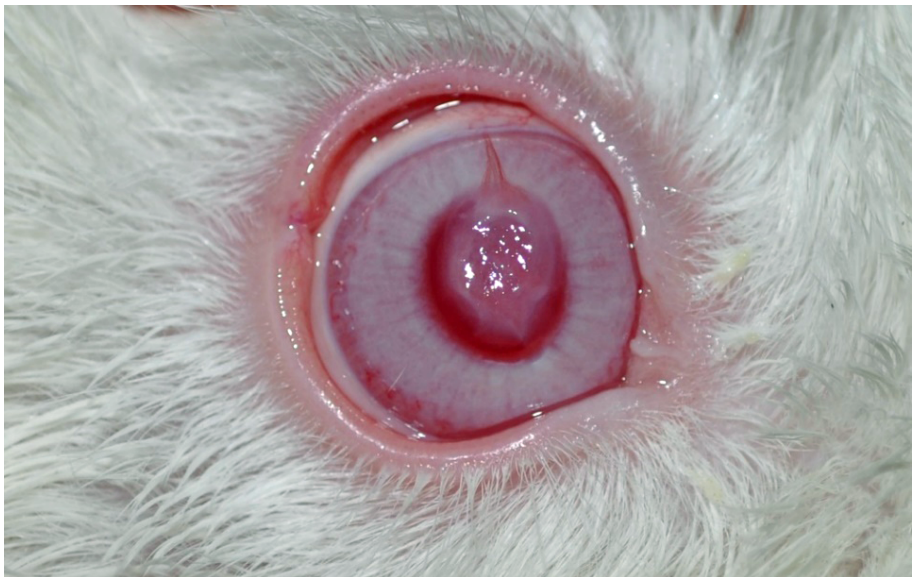

Fig. 4. The rabbit cornea in group 3 on the 20th day of the experiment.

were administered during the course of the experiment. In group 2, an ointment containing stem cells was administered to the lesion twice a day for the first 5 days, and in group 3 – an ointment with Floksal® (ofloxacinum) was administered twice a day for 20 days (Fig. 2-4). In the last stage of the experiment, accelerated corneal healing was observed. The transparency of the cornea was assessed on 5th, 10th, 15th and 20th day of the experiment. The progress of corneal healing was evaluated based on the following criteria:

Transparency of the cornea (where 1 – full transparency; 2 – opacity of the cornea with clearly visible iris; 3 – disrupted transparency with partially invisible iris; 4 disrupted transparency with visible iris contours; 5 – disrupted transparency with invisible structures of the eyeball anterior pole).

Conjunctival hyperaemia (where 1 – no conjunctival hyperaemia; 2 – blurring of blood vessel contours; 3 – invisible blood vessels with slight redness of conjunctiva; 4 invisible blood vessels with conjunctival swelling and redness; 5 – invisible blood vessels with distinctive conjunctival swelling, dark-red in colour).

Eyelid spasm (1 – no eyelid spasm, 2 – eyelid spasm, and 3 – strong eyelid spasm).

Size of the lesion (1 – no lesion, 2 – lesion of up to 1 mm, 3 – lesion of up to 3 mm, 4 lesion of up to 5 mm, and 5 – lesion with a diameter exceeding 5 mm).

Penetration of blood vessels (1 – no penetrating blood vessels, 2 – single penetrating peripheral blood vessels, 3 – numerous penetrating peripheral blood vessels, 4 numerous peripheral blood vessels and

Table 1. Clinical evaluation of corneal healing. The study was carried out on 5th day of the experiment.

	Positive control (group 1)	Stem cells (group 2)	Control – ofloxacinum (group 3)
Transparency	2, 2, 3, 2, 2, 2 Average: 2.17	3, 3, 2, 2, 3, 3 Average: 2.67	3, 4, 4, 3, 3, 3 Average: 3.33
Hyperaemia	1, 2, 5, 1, 1, 2 Average: 2	2, 3, 1, 1, 4, 1 Average: 2	4, 5, 5, 3, 3, 4 Average: 4
Eyelid spasm	1, 3, 3, 2, 2, 2 Average: 2.16	1, 2, 1, 1, 1, 1 Average: 1.16	3, 3, 3, 3, 1, 3 Average: 2.67
Size of the lesion	5, 5, 5, 5, 5, 5 Average: 5	4, 4, 4, 3, 5, 4 Average: 4	5, 5, 5, 5, 5, 5 Average: 5
Penetration by blood vessels	1, 2, 3, 1, 1, 2 Average: 1.67	1, 1, 1, 1, 1, 1 Average: 1	3, 5, 3, 1, 1, 2 Average: 2.5
Outflow from the conjunctival sac	5, 5, 5, 3, 2, 3 Average: 3.83	2, 4, 2, 3, 3, 3 Average: 2.83	4, 4, 3, 2, 2, 3 Average: 3

single blood vessels penetrating the entire cornea and 5 – significant penetration of the whole cornea by blood vessels).

Outflow from the conjunctival sac (1 – no outflow from the conjunctival sac, 2 moderate serous outflow from the conjunctival sac, 3 – strong serous outflow from the conjunctival sac, 4 – mucopurulent outflow and 5 – purulent outflow from the conjunctival sac).

The clinical evaluation was done together with the recording of the corneal healing stages with the use of a camera. The clinical evaluation was followed by an objective micromorphological examination of the corneas obtained from the rabbits after their euthanasia. All animals were euthanised after the clinical examination on the twentieth day of the experiment, and material for histological analysis was taken thereafter. Before being euthanised the animals were anesthetised using a mixture of Torbugesic® (butorphanol) at a dose of 0.2 mg/kg of body weight i.m. with Cepetor® (medetomidine) at a dose of 150 mcg/kg of body weight i.m. and Bioketan® (ketamine) at a dose of 35 mg/kg of body weight i.m., a catheter was placed in the auricular marginal vein and euthanasia was performed with infusion of Morbital® (pentobarbital sodium, pentobarbital) at a dose of 0.6 ml/kg of body weight i.v. The samples were fixed in a 4% solution of formalin, pH 7.0 - 7.2, buffered with calcium carbonate. The corneas were rinsed in running water, then dehydrated in alcohol and embedded in paraffin. Five µm-thick sections were stained with haematoxylin and eosin. A histological analysis was performed with the "Nikon Eclipse 80i" optical microscope – Nomarski contrast and non-polarised transmitted light. Micromorphometric tests were performed using the NisElements Ar software. At least 10 measurements of the corneal thickness without the epithelium were made at a distance of 100 µm from the central toward the peripheral part of the lesion. The sections that had been clearly damaged during the preparation were not taken into consideration in the course of the morphometric studies. Those were the sections that showed excessive stratification, clear cuts or tangential cuts.

Statistical analysis

Morphometric data were analysed in Statistica 13 PL software. This included: descriptive statistics (mean, standard deviation, minimum and maximum values), verification of normal distribution (data content) using the Shapiro-Wilk test; verification of homogeneity of variance using the Bartlett's test. All the hypotheses were verified at the significance level of p less than 0.05.

Results

Clinical examination

Negative control

In the negative control group, there was no loss of corneal transparency, no conjunctival hyperaemia, eyelid spasms or outflow from the conjunctival sacs throughout the experimental period.

Other groups

In groups 1 and 3, there was a clinically significant loss of corneal transparency. The shortest duration of inflammation was observed in those groups where nothing was administered during the course of the experiment. In group 2, where the stem cells were administered, conjunctival hyperaemia persisted until 5th day of the experiment (Table 1). Conjunctival hyperaemia, which lasted until day 20, was observed in group 3 (Table 4). Eyelid spasms were not noticeable

Table 2. Clinical evaluation of corneal healing. The study was carried out on 10th day of the experiment.

	Positive control (group 1)	Stem cells (group 2)	Control – ofloxacinum (group 3)
Transparency	2, 3, 4, 2, 3, 2 Average: 2.67	2, 3, 2, 2, 3, 2 Average: 2.33	3, 3, 4, 3, 3, 3 Average: 3.16
Hyperaemia	1, 5, 2, 1, 1, 2 Average: 2	2, 1, 1, 2, 1, 1 Average: 1.33	2, 2, 2, 2, 2, 1 Average: 1.83
Eyelid spasm	1, 3, 3, 2, 2, 2 Average: 2.17	1, 1, 1, 1, 1, 1 Average: 1	2, 2, 1, 2, 2, 2 Average: 1.83
Size of the lesion	5, 5, 4, 4, 5, 5 Average: 4.67	2, 1, 1, 1, 1, 1 Average: 1.17	5, 4, 4, 5, 5, 4 Average: 4.5
Penetration by blood vessels	1, 5, 3, 2, 2, 3 Average: 2.67	1, 1, 1, 1, 1, 1 Average: 1	2, 2, 2, 1, 2, 2 Average: 1.83
Outflow from the conjunctival sac	1, 5, 5, 2, 5. 4 Average: 3.67	2, 2, 2, 1, 2, 2 Average: 1.83	5, 4, 2, 3, 2, 3 Average: 3.17

Table 3. Clinical evaluation of corneal healing. The study was carried out on 15th day of the experiment.

	Positive control (group 1)	Stem cells (group 2)	Control – ofloxacinum (group 3)
Transparency	2, 2, 3, 3, 2, 2 Average: 2.33	3, 3, 2, 2, 2, 2 Average: 2.33	3, 4, 3, 4, 3, 4 Average: 2.5
Hyperaemia	1, 2, 5, 1, 1, 1 Average: 1.83	1, 1, 1, 1, 1, 1 Average: 1	1, 2, 2, 2, 1, 2 Average: 1.67
Eyelid spasm	1, 1, 2, 2, 2, 2 Average: 1.67	1, 1, 1, 1, 1, 1 Average: 1	2, 2, 1, 1, 2, 2 Average: 1.67
Size of the lesion	4, 4, 2, 3, 3, 3 Average: 1.17	1, 1, 1, 1, 1, 1 Average: 1	4, 1, 3, 2, 2, 2 Average: 2.33
Penetration by blood vessels	1, 2, 4, 2, 2, 2 Average: 2.17	1, 1, 2, 1, 1, 1 Average: 1.17	2, 2, 2, 2, 2, 2 Average: 2
Outflow from the conjunctival sac	1, 3, 3, 1, 1, 1 Average: 1.67	1, 1, 2, 1, 1, 1 Average: 1.17	4, 1, 2, 2, 2, 1 Average: 2

in the group of rabbits treated with stem cells. They had the longest duration in group 3. In group 2, which received stem cells, the lesions began to heal on day 10 of the experiment (Table 2). In the remaining groups, healing was observed after 20 days (Table 4). The penetration of blood vessels into the cornea was observed only in the group where no medications were administered and in the group administered with ofloxacinum. In the group which received stem cells, the outflow from conjunctival sacs was observed up to day 5 of the experiment (Table 1). In the control group, where no drugs were administered, the outflow persisted until 10th day of the experiment (Table 2), and in the group where the antibiotic was administered – until 15th day of the experiment (Table 3).

The analysis of clinical observations showed a slight disruption of corneal transparency during the experimental days in groups 2 and 3. Heperaemia decreased significantly on day 20 in group 1, and on 10th day in groups 2 and it decreased by half in group 3. Eyelid spasm was the lowest in group 1 on 20th day of the experiment, while in group 2 on day 10 and in group 3 on 15th day. Intensity of the lesion was significantly reduced on day 10 in group 2, and on 20th day in groups 1 and 3. Also, penetration of the blood vessels was absent in group 2, and remained relatively high in groups 1 and 3. Outflow from the conjunctival sac decreased slightly in all experimental groups reaching the lowest grade on day 20 of the experiment.

Histological examination

In order to compare the corneal status in individual groups of animals, a morphological analysis was conducted on 20th day after the performed procedure. Comparative studies showed variations in the degree of mobilisation of fibroblast and epithelial cells in the examined corneas. In group 1, there was a slight increase in the thickness of the epithelium and the corneal wall, especially in the area outside the lesion

Table 4. Clinical evaluation of corneal healing. The study was carried out on 20th day of the experiment.

	Positive control (group 1) (Photo. 2)	Stem cells (group 2) (Photo. 3)	Control – ofloxacinum (group 3) (Photo. 4)
Transparency	3, 3, 3, 3, 3, 3 Average: 3	2, 2, 3, 2, 3, 3 Average: 2.5	3, 3, 4, 3, 4, 2 Average: 3.17
Hyperaemia	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 1, 1, 1, 1 Average: 1	2, 2, 2, 2, 1, 2 Average: 1.83
Eyelid spasm	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 1, 1, 1, 1 Average: 1	1, 2, 2, 1, 2, 2 Average: 1.67
Size of the lesion	2, 2, 1, 1, 2, 1 Average: 1.5	1, 1, 1, 1, 1, 1 Average: 1	1, 2, 2, 1, 2, 2 Average: 1.67
Penetration by blood vessels	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 2, 1, 2, 1 Average: 1.33
Outflow from the conjunctival sac	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 1, 1, 1, 1 Average: 1	1, 1, 2, 1, 2, 1 Average: 1.33

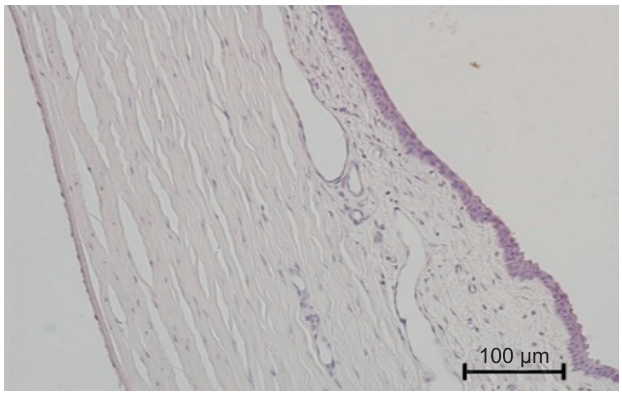

Fig. 5. The rabbit cornea in group 1 on 20th day of the experiment - histological image. x40 hematoxylin and eosin (HE) staining.

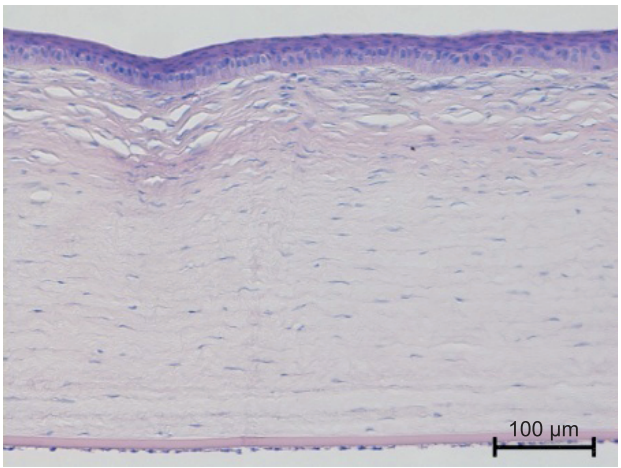

Fig. 6 Rabbit cornea in group 2 on 20th day of the experiment - histological image. HE x40.

(Fig. 5). In the corneal stroma, lymphocytic infiltration from local vessels was noted. In group 2, the epithelium cells decreased gradually from the peripheral zone towards the centre. That was accompanied by an increase in fibroblast activity (Fig. 6). That group was characterised by numerous irregularities of the epithelium and corrugations caused by disruption of the collagen fibre architecture just below the epithelium. In the area of cornea attachment to the eye bulb and corneal stroma, eosinophiles were noted. In group 3, the epithelium showed a slightly larger corrugation and the epithelium did not yet fully cover the lesion site (Fig. 7). The newly formed collagen fibres in the lesion were characterised by a regular arrangement, and there were fewer fibroblasts among them.

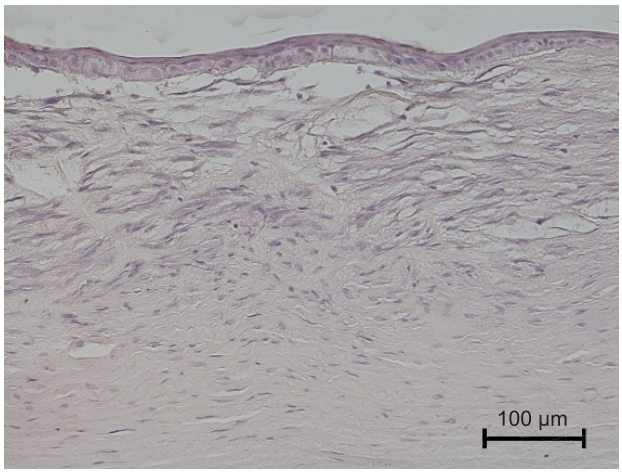

Fig. 7. The rabbit cornea in group 3 on 20th day of the experiment - histological image. HE x40.

Results of morphometric studies

The morphometric and statistical analysis of the corneal thickness on 20th day after the procedure showed that the highest corneal thickness was observed in group 1. That was due to the strong corrugation of collagen fibres of the connective tissue in the area of the lesion. The minimal corneal thickness was observed in group 3, which was caused by the highest degree of filling of the lesion with the connective tissue. The mean values of the corneal thickness 20 days after

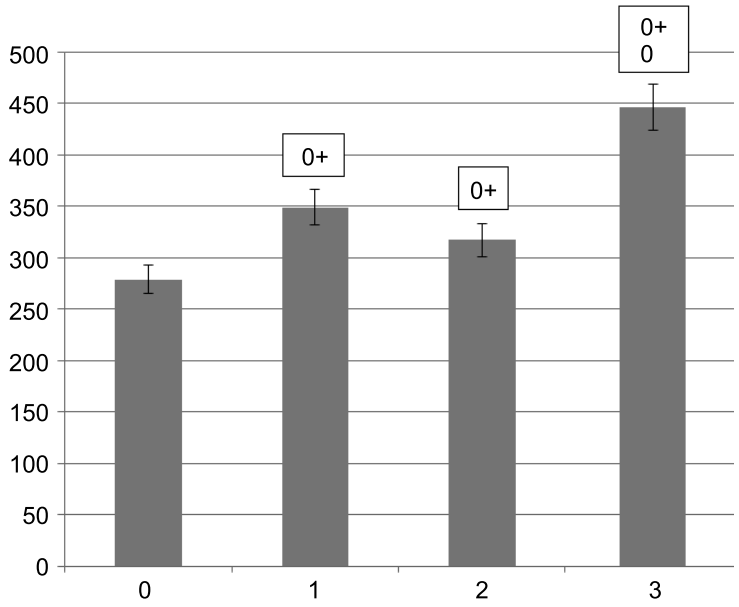

Fig. 8. Maximum thickness of the cornea in µm (vertical axis – thickness in µm, horizontal axis: group 0 – positive control group, 1 – no drugs administered during the experiment, 2 – stem cells ointment administered, 3 – ofloxacinum administered).

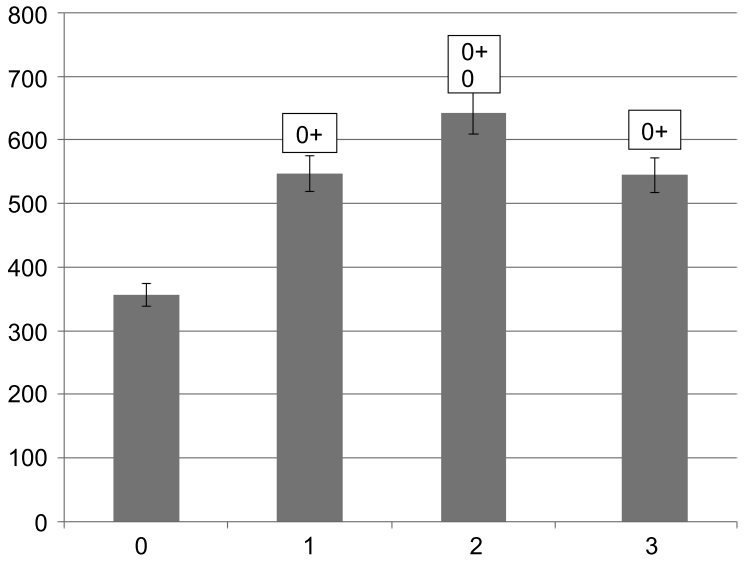

Fig. 9. Minimum thickness of the cornea in µm (vertical axis – thickness in µm, horizontal axis: group 0 – positive control group, 1 – no drugs administered during the experiment, 2 – stem cells ointment administered, 3 – ofloxacinum administered).

the procedure obtained from all the animals demonstrated that the cornea was the thickest in group 3 and the thinnest in group 2 (Fig. 8-10).

Discussion

Undoubtedly, shortening the time of lesion healing is associated with increased proliferation of epithelial cells and connective tissue. This may lead to undesirable effects, especially those that may affect subsequent eyesight. The excess of tissues in the regenerative process is undesirable because it determines the size of the scar and it limits the field of view.

The process described by Tuft et al. 1986 in rats and rabbits (Tuft et al. 1986, Tuft et al. 1989) is consistent with that observed in the present study in all groups of rabbits. In the present study, the initiation and development of the inflammatory process, the migration of cells towards the lesion site and the activation of fibroblasts and angioblasts as well as the silencing of the processes were visible very clearly. It is worth pointing out that while there are scientific definitions of inflammatory infiltrations, there are no data on the nature and type of such infiltrations. It is unclear whether they are of lymphocytic, granulocytic or of any other type. In the present study, the infiltration and migration

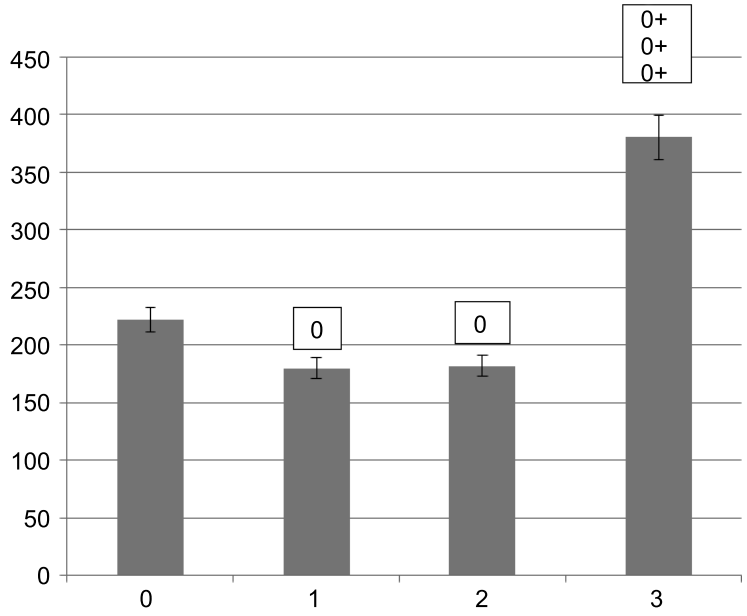

Fig. 10. Average thickness of the cornea in µm (vertical axis – thickness in µm, horizontal axis: group 0 – positive control group, 1 – no drugs administered during the experiment, 2 – stem cells ointment administered, 3 – ofloxacinum administered).

of eosinophil cells was observed at the level of the corneal stroma in group 2, while only a lymphocytic infiltration was found in group 1.

The size of the lesion and the manner in which it was induced play a significant role in the process of lesion healing (Ho et al. 1974, Tuft et al. 1986, Koch 1996). In our experiment, we found that the use of antlerogenic stem cells not only reduced the lesion, but it was beneficial considering the decrease of hyperaemia, eyelid spasm and progression of blood vessels into the cornea.

Interestingly, several short impulses with a laser can cause moderate inflammation without creating a lesion, while the use of twice as large impulses in addition to significant corneal damage also induce generalised inflammation of the cornea (Ho et al. 1974). This process, called keratoplasty, can be used to modify the degree of inflammation in the healing cornea. Similar studies were carried out by Zhang, who showed that the processes occurring in the cornea of animals are practically identical to those occurring in humans (Zhang et al. 2008).

Tuft, in his analysis, focused mainly on epithelial cells and the activation of fibroblasts, whose hyperplasia and increased deposition of collagen fibres are necessary for corneal regeneration (Tuft et al. 1986). He showed that the speed of epithelial covering of the lesion is crucial for the correct reconstruction of the shape and the microstructure of the cornea. In the present study, the oedema associated with this process and concurrent increased corneal thickness peaked on day 20 in study group 3. In turn, the lowest thickness of epithelium was obtained in 2nd group, where no drugs were administered.

Yu-li Pi et al. (2012) tried to transplant corneal epithelial cells after corneal injury with alkaline solutions, which led to one of the strongest inflammatory conditions together with increased angiogenesis and a number of complications (Pi et al. 2012). The method they used allowed an effective reduction of the level of unwanted changes, such as angiogenesis and the number of infiltrating cells. Hence, the method has led to the desired changes, including rapid regeneration of the corneal epithelium. A characteristic feature of the proliferative processes of epithelial cells was the appearance of characteristic invaginations of the epithelial reproductive layer into the connective tissue and a local elevation of surface cells above the remaining cells. In the present study, such processes were observed in the group treated with stem cells. Unfortunately, the authors of this study were not able to analyse the condition and arrangement of collagen fibres or their effect on the subsequent transparency of the cornea.

Zhang drew attention to the developing phenomenon of fibrosis around the scar, which was particularly important in the case of corneal stroma transplantation from patient to patient (Zhang et al. 2008). An abnormal and disturbed course of collagen fibres has a very significant impact on the subsequent transparency of the cornea.

In the present study, the relatively weakest fibroblast activity was observed in group 2 treated with stem cells. The new collagen fibres ran parallel to each other, as opposed to other groups where they had been

arranged in a random, undirected manner, often coiled in the form of clusters. The collagen fibres in the examined rabbits were of different thickness. In general, thin fibres are easier to adapt to the prevailing biomechanical loads than thick fibres. It is also easier to arrange them in accordance with the adjacent fibres.

Ho et al. (1974) applied EGF (epidermal growth factor) to induce an inflammatory process and then observed the speed at which the regenerative processes proceeded (Ho at al. 1974). He showed that the epithelial cell proliferation rate is linear and persists throughout the healing process.

In the present study, it was also observed that epithelial proliferation in each group was different. The longest was maintained in the group treated with stem cells, the shortest – in the group treated with chemotherapeutics. The conducted clinical study revealed that the use of antlerogenic stem cells has a positive effect on the healing process of corneal lesions. The use of stem cells helps to maintain high transparency of the cornea throughout the whole experiment. No unwanted effects, such as eyelid spasms or strong tearing, were observed. Vessel penetration was not found in any of the cases. The corneal lesion as well as the outflow from the conjunctival sacs was only noticeable until the fifth day of the experiment.

Therefore, the use of antlerogenic stem cells not only shortened the lesion healing time, but also weakened or stopped the development of side effects.

In summary, it has been shown that the use of stem cells influences the healing of corneal lesions. Their effect is characterised by shortening the healing time, maintaining transparency of the cornea and slowing or stopping the development of side effects.

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