The Effect of Steroids on the Viability of Endothelial Cells of Stored Cornea

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The change of endothelial cell viability due to corticosteroid treatment in stored rabbit corneas was investigated.

Hydrocortisone was injected into the anterior chamber of enucleated eyes which were stored in a moist chamber. After 24, 48, or 72 hours of storage, the cornea was removed and stained with trypan blue. The unstained endothelial cells were counted under the light microscope in order to determine the density of viable endothelial cells. The same procedures were done on the contralateral eye with normal saline injected into the anterior chamber instead of hydrocortisone as a control.

The density of viable endothelial cells in the corticosteroid-treated group was higher than that of the control group by 1.75%, 14.39%, and 27.40% in 24, 48, and 72 hour-stored corneas, respectively.

Key words: endothelial cell viability, corticosteroid, trypan blue, stored cornea.

INTRODUCTION

The degree of corneal endothelial damage in a human donor eye is proportional to the quantity of lysosomal hydrolytic enzymes present in the aqueous humor. Autolysis, which is brought about by the release of lysosomal hydrolytic enzymes, has been prevented in the cornea by the use of lysosomal membrane stabilizers such as corticosteroids.

It has been suggested that endothelial damage would be reduced if the donor eyes were treated with corticosteroids. The presented study was conducted in order to investigate the change in endothelial cell viability due to corticosteroid treatments in stored rabbit corneas.

MATERIALS AND METHODS

Fifteen healthy New Zealand white rabbits of either sex, weighing from 2.0 kg to 2.5 kg, were killed with an intravenous air injection. Both eyes of the freshly killed rabbits were enucleated, and one was used in the corticosteroid-treated group and the other in the control group.

In the corticosteroid-treated group, 40 μg of hydrocortisone sodium succinate mixed with 0.05 ml saline was injected into the anterior chamber with 26G needle. The osmolality of the mixed hydrocortisone was 280 to 290 mOsmol and the pH was 7.0 to 8.0. In the control group, 0.05 ml of saline was injected into the anterior chamber in the same...
manner.
The whole eye balls were washed with a 2% gentamicin solution and stored in a moist chamber at 4°C. Both corneas of each pair were stored for the same length of time (24, 48, or 72 hours). Central marking was done with fluorescein and a 26G needle, and the cornea was removed with a 3 mm scleral rim.
The corneal buttons were placed endothelial side up on a glass slide. The tissue was briefly rinsed in 0.9% sodium chloride followed by a 90 second stain with 0.25% trypan blue. The buttons were then carefully rinsed in 0.9% sodium chloride.
The corneal buttons were flattened on a slide with four radial incisions to enable light microscopic examination. A binocular microscope (AO Microstar 10 Series) with 0.025 mm² grid was used to count the unstained endothelial cells at a magnification of 400X. The counts were performed 5 times within 0.3 mm diameter of the central cornea. The mean number of viable endothelial cells in the corticosteroid-treated group was compared with that in the control group by paired t-test.

RESULTS

The mean number of viable endothelial cells was 3956 cells/mm² in the corticosteroid-treated group and 3888 cells/mm² in the control group after 24 hours of storage. There were 1.75% more cells in the corticosteroid-treated group than in the control group, but there was no significant difference between them by paired t-test (Fig. 1). Supravital staining revealed a similar figure in both groups. That is, endothelial cells appeared to be regularly arranged in a hexagonal mosaic, and cell boundary was distinct. Only a few endothelial cells were stained with trypan blue (Photo. 1, 2).
The mean number of viable endothelial cells was 3752 cells/mm² in the corticosteroid-treated group, and 3280 cells/mm² in the control group after 48 hours of storage. There were 14.39% more cells in the corticosteroid-treated group than in the control group, and there was a significant difference between the two groups by paired t-test (Fig. 2). After 48 hours of storage, endothelial cells maintained their hexagonal mosaic pattern and distinct cell boundaries, but several endothelial cells which were stained with trypan blue were scattered in the endothelium and these damaged cells had lost their cell boundaries (Photo. 3). The percentage of stained cells in the endothelium of the control group was somewhat higher than that of the corticosteroid-treated
group, and cell boundaries were somewhat irregular in the control group (Photo. 4).

After 72 hours of storage, the mean number of viable endothelial cell was 3236 cells/mm² in the corticosteroid-treated group, and 2540 cells/mm² in the control group. There were 27.40% more cells in the corticosteroid-treated group than in the control group, and there was a significant difference between them by paired t-test (Fig. 3). The endothelial mosaic was intact but exhibited many damaged cells surrounded by intact cells (Photo. 5) in the corticosteroid-treated group. In the control group, numerous endothelial cells were severely damaged and even unstained viable endothelial cells had lost their mosaic pattern (Photo. 6).

Graphic representation of the above results is presented in Fig. 4 and Fig. 5.

The difference in the mean viable endothelial cells between the corticosteroid-treated group and the control group increases as the duration of storage.

**DISCUSSION**

Corticosteroid, a lysosomal membrane stabilizer, reduces the release and activation of the lysosomal marker enzyme β-glucuronidase and acid phosphatase in stored cornea, and the degree of corneal endothelial damage in a human donor eye is proportional to the quantity of lysosomal hydrolytic enzymes. Furthermore, it has been demonstrated that the addition of $10^{-8}$ M hydrocortisone to M-K medium improved endothelial cell bicarbonated barrier function. When prompt enucleation is not possible after death, corticosteroid injection into the anterior chamber of the unenucleated eyes of a corneal donor has been recommended.

It seemed to us that the viability of the endothelial cells in the stored cornea would be increased if autolysis by lysosomal hydrolytic enzymes, were prevented by employing a lysosomal membrane stabilizer with corticosteroid treatment. Our results in this study support the hypothesis that corticosteroid could increase the viability of the stored cornea.
The effect of corticosteroid on the survival of corneal endothelial cells.

In order to determine the viability of corneal endothelium, trypan blue staining was used in this study. Specular microscope can evaluate the structural integrity but not the viability of corneal endothelium. Structural preservation of corneal endothelium does not necessarily reflect the ability of the cells to resume their normal function under physiologic conditions. Furthermore, the corneal epithelium and stroma become thick and hazy during storage, making viewing of the endothelium through specular microscope difficult. It is impossible for a transmission electron microscope to evaluate the panoramic state of the corneal endothelium and for a scanning electron microscope to evaluate the viability of the corneal endothelium exactly. Therefore, supravital staining with trypan blue, alizarin red S, or indocyanin green was thought to be a suitable method to evaluate the viability of corneal endothelial cells in a panoramic fashion. Trypan blue is normally excluded from viable cells, whereas in cell death the dye can permeate the cell membranes and stain the nucleus.

Corneal endothelium reveals an inhomogeneity between the central and peripheral regions with a variation of 10% in cell density. Therefore, we chose one small area in comparing the number of endothelial cells in the two groups. The central cornea of a 3 mm diameter was preferred in the present study because estimates by trypan blue is more precise in the central region than in the peripheral region, and peripheral corneal endothelium is more vulnerable to trauma than central during manipulation.

The density of viable endothelial cells in the corticosteroid-treated group was higher than that in the control group. It suggests that injection of corticosteroid into the anterior chamber of the enucleated eye ball in the moist chamber or addition of corticosteroid to the M-K media or K-sol may reduce endothelial cell loss during corneal storage.

The difference in the viability between the corticosteroid-treated group and the control-group was negligible in 24 hour storage. This indicates that the effect of corticosteroid begins to appear 24 hours or more after treatment with corticosteroid.

REFERENCES

Photo. 1. Endothelial cells from a 24 hour stored cornea in the steroid group.

Photo. 2. Endothelial cells from a 24 hour stored cornea in the control group.

Photo. 3. Endothelial cells from a 48 hour stored cornea in the steroid group.

Photo. 4. Endothelial cells from a 48 hour stored cornea in the control group.

Photo. 5. Endothelial cells from a 72 hour stored cornea in the steroid group.

Photo. 6. Endothelial cells from a 72 hour stored cornea in the control group.