Graefe's Arch Clin Exp Ophthalmol
(2002) 240:111–113

LABORATORY INVESTIGATION

DOI 10.1007/s00417-001-0407-1

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Outflow facilities through Descemet's membrane in rabbits

Received: 1 March 2001
Revised: 22 October 2001
Accepted: 5 November 2001
Published online: 11 January 2002

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Abstract *Background:* The outflow pathway in viscocanalostomy, a new procedure in glaucoma surgery, is unclear; however, outflow through Descemet's membrane has been postulated. This study evaluates outflow rates through Descemet's membrane at different IOP levels in rabbits. *Methods:* 51 Descemet's membranes without endothelium from enucleated rabbit eyes were installed in a double-ring system, the Minuth sheet. Different intraocular pressure levels (20, 25, 30, 40, 50 mmHg) were applied to one side of the system. The system was filled with balanced salt solution. The total amount of fluid percolating through Descemet's membrane was measured after 12 h. Based on this, flow rates were calculated. The area of Descemet's membrane was 6.9 mm^2. *Results:* At the pressure of 20 mmHg the flow rate was less than 0.003 µl/min. At pressures above 30 mmHg flow rates ranged from 0.04 µl/min to 0.15 µl/min with a mean of 0.09 µl/min. To achieve pressure control at high pressures, an area of at least 150 mm^2 of Descemet's membrane would be needed. *Conclusion:* Descemet's membrane provides good outflow resistance in rabbit eyes. Based on our results for pressure control by outflow through Descemet's membrane only, at least the whole corneal area is needed. If the same is true in humans, additional outflow sources are necessary in cases of viscocanalostomy.

Introduction

Recently new glaucoma procedures such as viscocanalostomy and deep sclerostomy, which are considered to be nonpenetrating filtration procedures, have been developed for glaucoma patients.

Various studies [1, 4, 11, 12] have shown a lowering effect on intraocular pressure (Fig. 1); however, the mechanism of action is still under debate. While in penetrating filtering glaucoma procedures a direct outflow path between the anterior chamber and the subconjunctival space is created, in nonpenetrating filtering procedures an outflow was postulated through the intact Descemet's membrane into a corneal / scleral cavity connected to Schlemm's canal.

This study evaluates the outflow rates through Descemet's membrane at different intraocular pressure (IOP) levels in rabbits.

Material and methods

Fifty-one Descemet's membranes were isolated from 9- to 12-month-old rabbits. The animals were professionally killed at the breeder's establishment. The eyes were enucleated as soon as possible, within 1 h, as a fresh organ extraction. The treatment of the animals was in line with ethical guidelines. Within 12 h after the death of the rabbits the anterior segment of the each eye was dissected and Descemet's membrane was denuded of endothelial cells. Then Descemet's membrane was dissected from the stroma using forceps. The prepared Descemet's membrane was installed in a double-ring system, the Minuth sheet (Fig. 2).

This device consisted of a double-chamber system with two independent ports. Descemet's membrane was the only relevant resistance between the two chambers. This enabled the measurement of the different outflow rates through Descemet's membrane at different levels of IOP.

The area of Descemet's membrane which was evaluated was 6.9 mm^2. The system was filled with balanced salt solution (BSS). The consistency of the system was guaranteed by silicone sealing

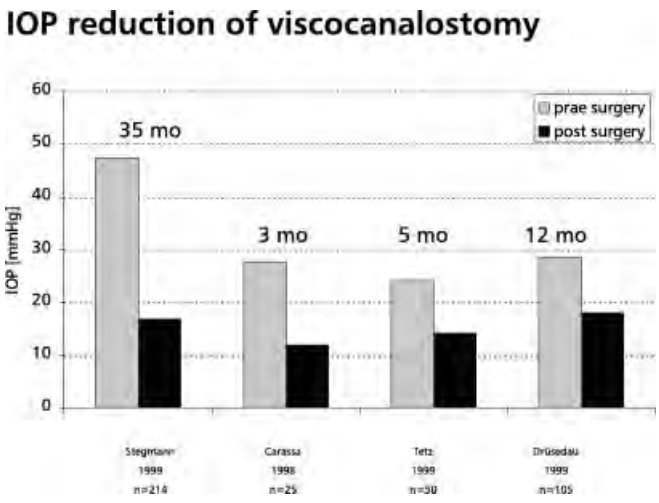

Fig. 1 The findings of different studies regarding the IOP-lowering effect over time

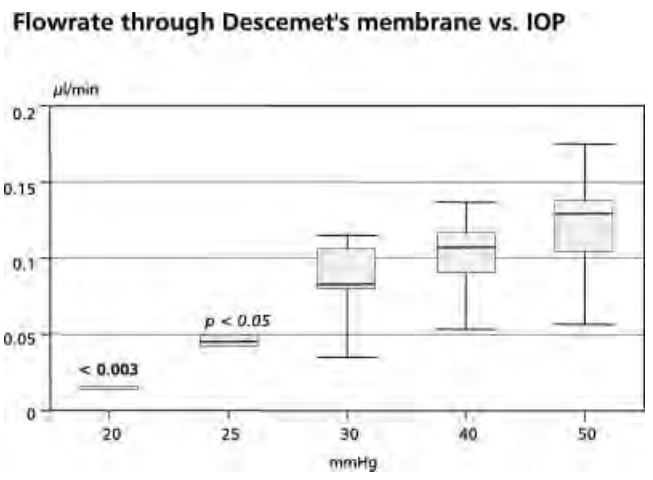

Fig. 3 Outflow facility of a 6.9-mm² area of Descemet's membrane in rabbits

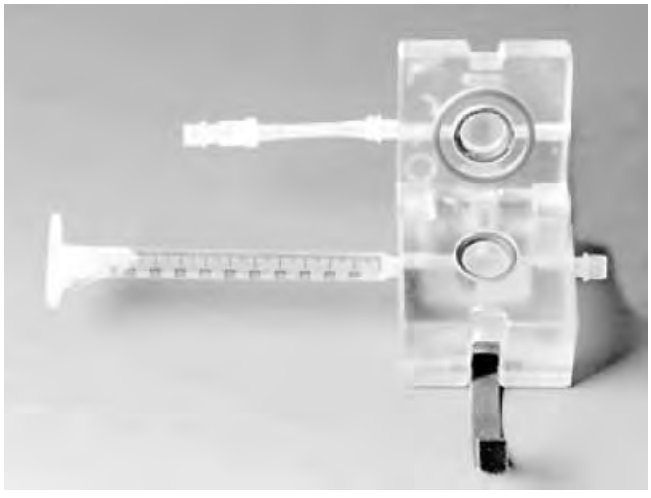

Fig. 2 The double-chamber unit and Minuth sheet (supplier: Minucells and Minutissue, Bad Abbach, Germany)

of the rings. To ensure the watertightness of the system, in a prestudy a 10-µm-thick PMMA plastic sheet was installed as a control. No percolation of BSS could be detected.

In the present study different IOP levels (20, 25, 30, 40, 50 mmHg) were applied to one side of the system. The total amount of fluid percolating through Descemet's membrane was measured after 12 h; based on the findings, flow rates were calculated.

Results

The results are summarized in Fig. 3.

At an IOP of 20 mmHg the flow rate was less than 0.003 µl/min. At 25 mmHg the rate increased to 0.06 µl/min. This was significantly lower than the flow rates at 30 mmHg (0.09 µl/min) 40 mmHg (0.11 µl/min) and 50 mmHg (0.13 µl/min). The range and mean of the flow rate increased with higher IOP.

Discussion

Based on the results of this study Descemet's membrane provides good outflow resistance in rabbits. Assuming a production of 2–3 µl/min of aqueous an area of 150 mm² would be needed to keep IOP at 30 mmHg. To achieve IOP control in this case the whole cornea of the rabbit might be dissected. These findings are consistent with the results of Kain and Bühl [8], who found that at IOP levels above 30 mmHg water began to flow through Descemet's membrane in rabbit eyes. They dissected the whole cornea down to Descemet's membrane and removed the endothelium in a whole-eye model.

Fatt [6] tried to calculate the permeability of Descemet's membrane from the swelling of the cornea. He found that the membrane had a substantial resistance to water pressure at physiologic IOP levels. Also Cogan and Kinsey [2] found a reduced flow rate through Descemet's membrane of 0.0003 µl/min at physiologic pressures.

The results of the findings of this present study in rabbits might be able to be extrapolated to humans. Examination of the anatomy of the cornea showed a similar structure of Descemet's membrane in rabbits and humans (Table 1) [5,7]. If the results of this study are used to answer the question of whether Descemet's membrane might plays a role in the outflow in the viscocanalostomy procedure, two further considerations may be of relevance. Descemet's membrane is thicker in the periphery

Table 1 Thickness of Descemet's membrane [5, 7, 10]

	Rabbit	Human
Center of cornea	7–8 µm	5–7 µm
Periphery	10 µm	10 µm
With aging	45 µm	30 µm

of the cornea and thicker in the elderly population (Table 1) [5, 7].

In this study the center of Descemet's membrane was tested and the rabbits were young. The corneal window in viscocanalostomy might be even less permeable in glaucoma patients, since they are older and the window in Descemet's membrane is created in the periphery of the cornea. For surgical procedures like the viscocanalostomy, this implies that the size of the Descemet's window would not substantially influence the IOP regulation and would play a smaller role in the outflow rate of the procedure.

Although in patients Descemet's membrane is covered by an intact corneal endothelial cell layer, in this study we used a preparation of Descemet's membrane which had been denuded of the endothelium, for the following reasons. The corneal endothelium represents an additional resistance to aqueous outflow. In whole perfused rabbit eyes, Kain and Bühl observed permeability of Descemet's membrane without endothelium at an IOP of 30 mmHg, whereas the outflow resistance increased to a pressure of 80 mmHg in eyes with Descemet's membrane and endothelium. Thus, an even smaller amount of aqueous outflow than demonstrated in our experiments can be expected in patients with a Descemet's window after viscocanalostomy, in which the endothelium is supposed to be untouched. Furthermore, according to Kain and Bühl, the influence of corneal endothelium on the aqueous outflow depends on the post-mortem time of the tissue [8]. Thus, preparation of Descemet's membrane without endothelium almost certainly excludes variation of results in our study due to different post-mortem times of the tissue.

Other studies support other outflow sources in viscocanalostomy. There are suggestions that the resistance to aqueous outflow is not reduced by the Descemet's window but by microperforations of Descemet's membrane or microlesions of the trabecular meshwork. This would be facilitated by the 50-µm thickness of the anterior trabecular meshwork [3]. In addition, Lim et al. [9] found that deroofing of Schlemm's canal in procedures such as deep sclerostomy caused a drop in IOP of a perfused human autopsy eye to 0.9 mmHg. This may also show that the procedure of deep sclerostomy may result in a mechanism similar to a "microtrabeculotomy".

In summary, if the findings of this study apply to humans, additional outflow sources in viscocanalostomy or deep sclerostomy are necessary to provide pressure control in glaucoma patients with elevated IOP. Preliminary results from other study groups suggest microlesions during preparation of Descemet's membrane or deroofing of Schlemm's canal, which will function as a "microtrabeculotomy". Further investigation is necessary to cast light on the mechanism of action of deep sclerostomy and/or viscocanalostomy.

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