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Comparative studies between species that do and do not exhibit the washout effect

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Abstract

Ocular perfusion studies from all non-human species performed to date consistently demonstrate a perfusion-volume-dependent increase in aqueous outflow facility known as the ''washout'' effect. However, this ''washout'' effect does not occur in human eyes. We have recently documented that, in bovine eyes, the washout associated increase in facility correlates with the extent of physical separation between the juxtacanalicular connective tissue (JCT) and the inner wall endothelium lining the aqueous plexus (the bovine equivalent of Schlemm's canal). We hypothesize that if washout truly correlates with inner wall/JCT separation then this separation should not occur in human eyes that do not exhibit the washout effect, even after prolonged perfusion. Eight enucleated human and eight bovine eyes were used in this study. Aqueous humor outflow facility was measured at 15 mmHg for long-duration (3 h) or short-duration (30 min to 1 h) perfusion ($n = 4$ for each group). All eyes were perfusion-fixed at 15 mmHg, and examined morphologically with both light and electron microscopy. In bovine eyes, outflow facility increased 81% ($p = 0.049$) from 1.06 ± 0.06 μl/min per mmHg (mean ± SEM) at baseline to 1.92 ± 0.30 μl/min per mmHg after 3 h due to washout. The pre-fixation outflow facility in long-duration eyes (1.92 ± 0.30 μl/min per mmHg) was 2-fold greater than pre-fixation facility in short-duration eyes (0.92 ± 0.11 μl/min per mmHg; $p = 0.0387$). In human eyes, washout was not observed; baseline outflow facility was similar between both groups (0.18 ± 0.02 vs. 0.25 ± 0.08 μl/min per mmHg; $p = 0.518$); however, pre-fixation outflow facility in long-duration eyes showed a 40% *decrease* compared to baseline outflow facility in those same eyes ($p = 0.017$, paired Student's *t*-test). In bovine eyes, significant expansion and rarefaction of the JCT and inner wall/JCT separation was much more prevalent in long-duration eyes, and data from all bovine eyes revealed a correlation between the extent of inner wall/JCT separation and the absolute value of outflow facility measured immediately prior to fixation ($p = 0.0024$) as well as the washout-induced increase in outflow facility ($p = 0.0006$). In human eyes, no significant morphologic differences were observed between long- and short-duration perfusion, with no observed change in inner wall/JCT separation or expansion between the two groups. Morphologic analysis revealed that the previously described ''cribriform plexus'' of elastic-like fibers was far more extensive in the JCT of human eyes, appearing to form numerous connections to the inner wall endothelium. The cribriform plexus appears to function as a mechanical tether that maintains inner wall/JCT connectivity in human eyes by opposing hydrodynamic forces generated during perfusion, potentially explaining the lack of washout in humans.

Keywords: aqueous humor outflow facility; inner wall; JCT; washout; human eye; bovine eye; trabecular meshwork; light microscopy; electron microscopy

1. Introduction

The ''washout effect'' is a phenomenon in which perfusion of an eye at physiological pressure results in a volume-dependent increase in the measured facility of aqueous humor

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outflow. Washout was originally believed to result from a ''washing out'' of extracellular glycosaminoglyans (Bárány and Scotchbrook, 1954; Bárány and Woodin, 1955; Bárány, 1962, 1964) from the outflow pathway, but biochemical studies have failed to detect an appreciable change in either sulfated proteoglycans (Johnson et al., 1993) or hyaluronic acid (Knepper et al., 1984) from the outflow pathway following prolonged perfusion. While the rate of facility increase during washout can be slowed by perfusion with either serum (Kee et al., 1996; Johnson et al., 1993) or serum proteins (Epstein et al., 1978; Sit et al., 1997) or by perfusion with either pooled homologous aqueous humor (Bárány and Woodin, 1955; Gaasterland et al., 1978) or mock aqueous humor with similar biochemical constituents (Erickson and Kaufman, 1981; Gaasterland et al., 1979), the washout effect cannot entirely be eliminated (Bárány and Scotchbrook, 1954; Van Buskirk and Brett, 1978).

Possibly the most intriguing aspect of the washout effect, however, is that it does not occur in the human eye (Erickson-Lamy et al., 1990). This suggests that there is some unique aspect of outflow anatomy or physiology that distinguishes human eyes from most other species, including from non-human primate eyes that exhibit washout during perfusion both in vivo (Bárány, 1962, 1964; Erickson and Kaufman, 1981; Gaasterland et al., 1978, 1979; Kaufman et al., 1988) and in vitro (Epstein et al., 1982; Hashimoto and Epstein, 1980; Peterson and Joscon, 1974), despite their anatomical similarity to humans. A thorough understanding of the mechanism of washout, and the reason for its absence in humans would likely provide important insight into the fundamental mechanisms that generate outflow resistance. Such understanding might also permit us to artificially induce a washout-like response in human eyes as a means of reducing intraocular pressure in glaucoma.

Two recent studies (Overby et al., 2002; Sabanay et al., 2004) have documented that washout is a reversible process in both bovine and monkey eyes. A structural correlate to the facility increase during washout in bovine eyes appeared to be the degree of separation of the JCT from the inner wall of the aqueous plexus—the bovine equivalent to Schlemm's canal (Tripathi, 1971). This separation was proposed to increase outflow facility by disrupting a hydrodynamic interaction between the inner wall and JCT known as ''funneling'' (Johnson et al., 1992). The funneling theory states that the patterns of outflow through the JCT are confined to those regions nearest the pores in the inner wall, and this flow confinement reduces the filtration area through the JCT, thereby increasing its effective hydrodynamic resistance. Based upon our prior work in bovine eyes, we hypothesized that washout resulted from a disruption in the connectivity between inner wall and JCT that decreased outflow resistance by eliminating the funneling effect (Overby et al., 2002). The washout hypothesis and funneling theory emphasize the role of cellular and extracellular matrix adhesions that maintain the connectivity between the inner wall and JCT in the face of an opposing pressure gradient and thereby influence outflow resistance by controlling the local hydrodynamic patterns of outflow.

In this study, we hypothesize that if the structural correlate for washout is separation between the inner wall and JCT, then these morphological changes should not be found in human eyes subject to prolonged perfusion. Light and electron microscopy were used to compare the baseline morphological differences in the outflow pathway and morphological changes following prolonged perfusion in bovine eyes that exhibit washout and human eyes that do not exhibit washout. Our goal was to determine whether the absence of washout in human eyes relates to morphological differences unique to the human outflow pathway. A corollary of our hypothesis is that the absence of washout in the human eye may result from an enhanced connectivity between the inner wall and JCT, which would have important consequences for the regulation of outflow resistance in this species.

2. Materials and methods

Enucleated eyes from both bovine and human donors were used in this study. Eight bovine eyes were obtained from a local abattoir (Arena and Sons, Hopkinton, MA) and delivered on ice within 6 h post-mortem. Eyes with any discernible damage or accumulated blood in the angle of the anterior chamber were excluded. Eight human eyes from anonymous donors with no known history of eye disease (ranging from 33 to 89 years of age) were obtained from National Disease Research Interchange (Philadelphia, PA) within 24 h postmortem. Each eye was confirmed to be grossly normal by examination under a dissecting microscope.

2.1. Experimental ocular perfusion

All eyes were perfused with Dulbecco's phosphate-buffered saline containing 5.5 mM glucose (collectively referred to as DPBS) using a previously described constant-pressure ocular perfusion system (Erickson-Lamy et al., 1990). Briefly, this system maintains a constant IOP by a fixed height perfusion reservoir placed at 20.4 cm (15 mmHg) above the eye, while the changing weight of the reservoir is recorded as a measure of the perfusion flow rate. Outflow facility is calculated as a function of time as the ratio of the time-varying flow rate to IOP.

2.2. Experimental perfusion of bovine eyes

Bovine eyes were perfused at 15 mmHg for either a short-duration (30 min) or a long-duration (3 h) prior to fixation. Previous studies have shown that 3 h of perfusion at 15 mmHg is sufficient to induce appreciable washout in bovine eyes (Bárány and Woodin, 1955; Erickson-Lamy et al., 1988, 1990; Overby et al., 2002). For the short-duration perfusion, eyes were perfused for 30 min to allow for stabilization of outflow facility and to achieve a baseline facility measurement. For the long-duration perfusion, eyes were perfused for 30 min to achieve a stable baseline facility measurement, followed by 3 h of prolonged perfusion at the same pressure. All eyes were perfusion-fixed with 2%