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Review

The washout phenomenon in aqueous outflow – Why does it matter?

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ABSTRACT

The washout effect is a phenomenon in which the resistance to aqueous outflow diminishes with the volume of perfusate flowing through the outflow pathways, even if the perfusate is aqueous humor itself. One intriguing aspect of this phenomenon is that it appears to occur in the eyes of all species studied to date except humans. Even non-human primate eyes exhibit washout. Because washout does not occur in human eyes some have concluded that a greater understanding of this effect could not be relevant to the study of human primary open angle glaucoma. Those who have chosen to study this phenomenon realize that if a washout effect could be induced in the human eye, the result would be a reduction in outflow resistance and a drop in intraocular pressure – precisely the goal of all current therapy for open angle glaucoma. This article reviews the discovery of this phenomenon, the various lines of investigation aimed at unraveling its underlying mechanisms. It concludes with recent structural and functional comparisons that point to clear differences in the connectivity between the inner wall (IW) endothelial cells of Schlemm's canal and matrix or cells in the juxtacanalicular connective tissue (JCT) between human eyes that do not exhibit washout and non-human eyes that do exhibit washout. This enhanced connectivity consisted of a more complex array of elastic fiber connections between the IW and JCT in human eyes. This enhanced connectivity may withstand the hydrodynamic forces driving separation between the IW and JCT, which occurs in non-human eyes during washout. Strategies targeting JCT/IW or JCT/JCT connectivity in human eyes might be promising anti-glaucoma therapies to decrease outflow resistance, and thus IOP.

1. What is the "washout" effect?

The "washout effect" describes a phenomenon observed when an *in vivo* or enucleated non-human eye is experimentally perfused. As perfusion continues the outflow facility of the eye progressively increases (Kaufman et al., 1988; Erickson-Lamy et al., 1990), even if perfused with aqueous humor (Gaasterland et al., 1978). Washout was originally thought to be time-dependent (Erickson and Kaufman, 1981; Kaufman et al., 1988), but more recent studies have documented that the effect is actually perfusion volume-dependent (Johnson et al., 1991; Sit et al., 1997a).

Washout was first recognized by Barany and Scotchbrook (1954), who attributed the increase in outflow facility to a "washing away" of extracellular material (ECM). In their pioneering perfusion studies, they perfused hyaluronidase into enucleated bovine eyes and when "washout" was observed, they naturally concluded that the substrate for this enzyme, hyaluronic acid, was the material being washed out and pointed to the importance of hyaluronic acid as an element of resistance in the aqueous outflow pathway (Barany and Scotchbrook, 1954; Barany and Woodin, 1955; Barany, 1962, (Barany, 1964)). Other investigators have supported this notion (Peterson and Jocson, 1974). However, more recent work, described below, casts doubt on this conclusion.

2. Only one species, human, does not exhibit washout

Washout has been reported in all non-human mammalian eyes studied to date, including bovine (Fig. 1), pig, rabbit, dog, cat and guinea pig (Barany, 1962, (Barany, 1964); Epstein et al., 1982; Erickson-Lamy et al., 1988; Fourman and Fourman, 1989, Gaasterland et al., 1979; Hashimoto and Epstein, 1980; Melton and DeVille, 1960; Overby et al., 2002; Rao et al., 2001; Ruben et al., 1985; Van Buskirk and Brett, 1978; Yan et al., 1991) and even non-human primate eyes (Epstein et al., 1982; Erickson and Kaufman, 1981; Gaasterland et al., 1978, (Gaasterland et al., 1979); Kaufman et al., 1988; Peterson and Jocson, 1974). Some species have a greater washout effect than others (Melton and DeVille, 1960). Age does not

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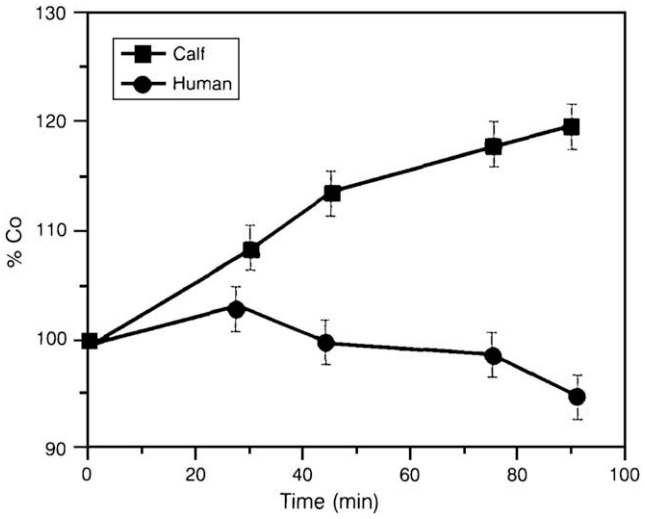

Fig. 1. "Washout" in bovine eyes but not in humans. Anterior segments from human eyes ($n = 13$) and neonatal calf eyes ($n = 9$) were perfused at 15<hsp sp="0.25"/>mmHg with Dulbecco's modified eagle medium (DMEM) in a 5% CO_2 environment at 37 °C. A progressive increase in facility of outflow over baseline facility (%C0) with time was noted in the calf eyes but not in the human eyes (from Erickson-Lamy et al., 1990).

affect washout in either rhesus or cynomolgus monkey eyes (Kiland et al., 2005). Possibly the most intriguing aspect of the washout effect, however, is that it does not occur in the human eye (Fig. 1) (Erickson-Lamy et al., 1990; Scott et al., 2007). The absence of washout in enucleated human eyes is unlikely to be the result of postmortem changes. Organ-cultured anterior segments, enucleated eyes, and *in vivo* monkey eyes undergo a similar magnitude of washout (Erickson-Lamy et al., 1990; Hu et al., 2006). Furthermore, since washout does not occur in the perfused human infant eye, the age of the donor cannot explain the difference in washout properties between human and other primate eyes (Erickson-Lamy et al., 1990). This being the case, some would argue that studies of washout are irrelevant to the human eye or to the pathogenesis of glaucoma in humans. But a thorough understanding of the mechanism of washout, and the reason for its absence in the human eye would likely provide important insight into the fundamental mechanisms that generate outflow resistance. Possibly most important is that by understanding washout we might be able to artificially induce a washout-like response in human eyes as a means of reducing intraocular pressure (IOP) in glaucoma.

The lack of washout in the human eye suggests that there is some unique aspect of outflow anatomy or physiology that distinguishes human eyes from most other species, including non-human primate eyes despite their anatomical similarity to humans.

3. Possible mechanism of washout

Several hypotheses have been put forward to explain the mechanisms governing the washout effect.

3.1. Washing out the ECM

Originally, washout was believed to be a "washing out" of glycosaminoglycans (GAGs), particularly hyaluronic acid, from the ECM in the outflow pathway (Barany and Scotchbrook, 1954; Barany and Woodin, 1955). However, washout has been shown to be a reversible process in both bovine and monkey eyes (Overby, 2002; Sabanay et al., 2004), and reversal occurred within 1–2 h. This timeline is less than would be necessary for secretion and organization of significant quantities of ECM (Hascall et al., 1991). This finding, combined with the findings of Knepper et al. (1984) and Johnson et al. (1993) that neither hyaluronate nor sulfated proteoglycans were depleted from the outflow pathway during washout, challenges the argument that washout results from a simple loss of hyaluronidase-sensitive GAGs from the ECM in the outflow pathway during perfusion. Additional evidence against this hypothesis is that neither a decrease in IOP nor an increase in outflow facility was found in living cynomolgus monkey eyes after removing hyaluronate or chondroitin sulfate proteoglycans with either single or multiple intracameral injections of the GAG-degrading enzymes, hyaluronidase or chondroitinase (Hubbard et al., 1997). In this regard, it should be noted that the purity of the hyaluonidase available in the late 1990s is far superior to that available to Barany in the middle 1950s.

3.2. Washing out plasma-derived protein

Another hypothesis has been that "washout" occurs due to a washing out of a depot of anterior segment protein located at the root of the iris and supplied by the ciliary body (Barsotti et al., 1992; Freddo et al., 1990; Johnson et al., 1993). The expected concentration of plasma-derived protein in an aliquot of aqueous humor obtained from the anterior chamber is about 1% of that in plasma. But it has been shown recently that the pathway by which plasma-derived proteins are added to the aqueous humor is via diffusion from the ciliary body stroma to the iris root (Fig. 2). From here,

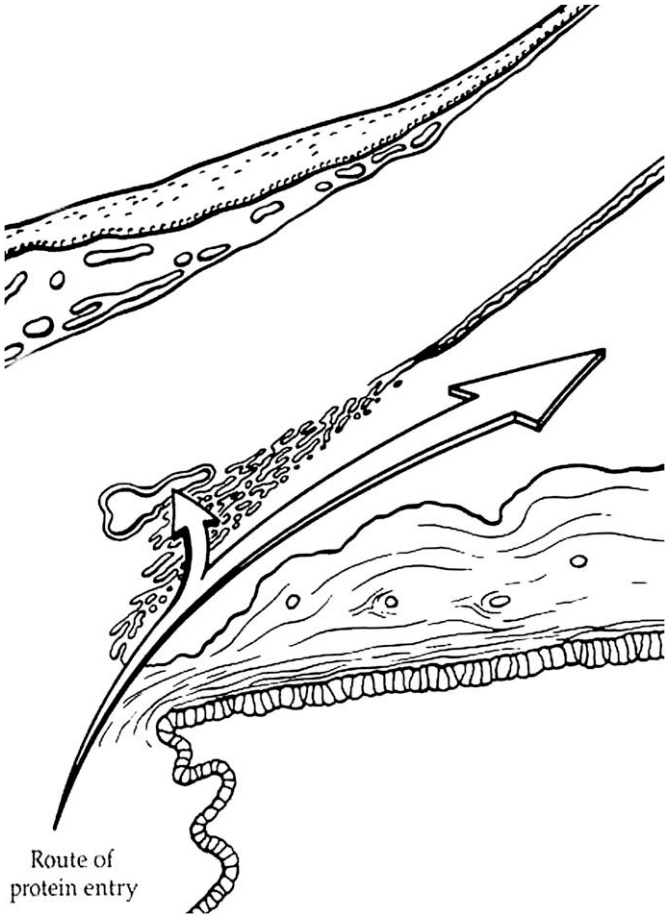

Fig. 2. The principal route of entry for plasma-derived proteins into the aqueous humor of the normal eye. Note the route of passage delivers protein in close proximity to the trabecular meshwork and aqueous outflow pathway (from Morrison and Freddo, 1996).