

Effects of Chronic Ocular Hypertension and Hypotensive Drug Treatment on Ocular Physiology and Biotransport using Dynamic Gadolinium-enhanced MRI

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Target Audience: Researchers and clinicians with interest in basic and translational applications of dynamic gadolinium (Gd)-enhanced MRI to study ocular physiology and pharmacological interventions.

Purpose: Glaucoma is an irreversible neurodegenerative disease of the visual system and is the second leading cause of blindness in the world. Although there are a number of known risk factors for glaucoma, currently, the only clinically approved treatment approaches to glaucoma target at lowering elevated intraocular pressure (IOP). To date, the relationships between IOP and ocular physiology such as aqueous humor flow in the eye are not fully elucidated [1]. While elevated IOP in glaucoma is related to an increased resistance to aqueous outflow from the anterior chamber (AC), the relative contribution of impaired outflow to the pathogenesis of glaucoma remains incompletely understood. Gd-enhanced MRI has been reported to non-invasively visualize flow dynamics of aqueous humor in the eye because of the small molecular weight of the Gd contrast agent and its high permeability to blood-aqueous barrier, mimicking aqueous components in the AC of the eye [2]. In this study, dynamic Gd-enhanced MRI was employed to evaluate *in vivo* the ocular physiology and biotransport in a rat model of microbead-induced ocular hypertension and in healthy, normotensive rats after topical applications of 3 ophthalmic hypotensive eye drops that affected IOP through different processes, in order to better understand the mechanisms of aqueous humor dynamics and its relationships to IOP and glaucoma.

Methods: Animal Preparation: Twenty-six adult Sprague-Dawley rats were divided into 4 groups. Eight rats received unilateral microbead-induced chronic ocular hypertension to the right eye by injecting a mixture of 5 μ L of 10 μ m microbeads and 5 μ L of 15 μ m microbeads into the AC (MB group) to block aqueous outflow and induce sustained elevated IOP for about 2 weeks [3]. In addition, 18 healthy, normotensive rats were topically administered to the right eye with 0.005% latanoprost (Lat group; n=6), 0.5% timolol maleate (Tim group; n=6) and 0.2% brimonidine tartrate (BT group; n=6) ophthalmic hypotensive solutions at an hour and 30 min before MRI experiment, so as to reduce IOP by increasing aqueous outflow [4], by suppressing aqueous inflow [5] and by both increasing outflow and suppressing inflow [6] respectively for the 3 medications. IOP was measured using the Tonolab rebound tonometer immediately before MRI experiment. Rats were anesthetized with a mixture of air and isoflurane (3% for induction and 1.25% for maintenance) during MRI experiment. Gd-DTPA (Magnevist) was intraperitoneally (i.p.) injected at a dose of 0.3mmol/kg after one T1-weighted image (T1WI) at baseline was acquired. **MRI Protocols:** All scans were performed using a 9.4-Tesla/31-cm Varian/Agilent horizontal bore scanner with a transmit-receive volume coil. T1WI at 30s-temporal resolution was continuously acquired for 2 hours using a fast spin echo sequence. The imaging parameters were: TR/TE=600/8ms, ETL= 8, FOV=2.6x2.6cm², acquisition matrix=256x256, slice thickness=1mm. Slices were oriented to bisect the center of the eyeballs. **Data Analysis:** Manual regions of interest (ROI) were drawn on AC and vitreous body of both eyes in T1WI (Figure 1). The time course from each ROI measurement before and after Gd injection was fitted into a 6th-degree polynomial using MatLab. The peak % Gd signal enhancement and time-to-peak were calculated from the Gd characteristic curves and compared between eyes and across groups.

Results: Immediately before MRI experiments, the IOPs of MB, Lat, Tim and BT groups in the left, untreated eyes were 13 \pm 2, 16 \pm 2, 12 \pm 3, and 12 \pm 3 mmHg respectively, whereas those in the right, treated eyes were 26 \pm 4, 11 \pm 3, 8 \pm 1, and 7 \pm 1 mmHg respectively (p<0.05 between contralateral eyes for all groups). Figure 1 shows the basic rat eye anatomy on a T1WI at 1 hour after Gd i.p. injection. Hyperintensity was observed in the AC of both eyes because of Gd entrance and accumulation presumably via the blood-aqueous barrier. Figure 2 shows time courses of T1W signal enhancement at the AC and vitreous in both eyes. As shown in Figures 2 and 3, the right AC in MB group had a significantly higher peak intensity and shorter time to peak than left AC. On the contrary, the right AC in Lat group had a lower peak intensity and longer time to peak than left AC. Tim group showed similar but insignificant trends as Lat group (p=0.16 and p=0.20 respectively). For BT group, both left and right AC showed a significantly delayed time-to-peak relative to other 3 groups (post-hoc Tukey's tests, p<0.05). Among all vitreous body measurements, only the MB group showed significantly stronger Gd enhancement in right vitreous than left vitreous. No significant difference was observed in peak intensity in left, untreated AC among MB, Lat and Tim groups (post-hoc Tukey's tests, p>0.05).

Discussion and Conclusion: Our current results demonstrated that Gd signal enhancement in the eye may reflect the physiological states of the aqueous humor dynamics in the presence of altered IOP in an experimental model of glaucoma or during pharmacological manipulations. In microbead-induced ocular hypertension, the microbeads injected into the AC block the aqueous outflow in the eye. This might explain the increased Gd accumulation/peak enhancement and shorter peak time in the right AC in relation to reduced Gd clearance in MB group. Chronic ocular hypertension also appeared to disrupt aqueous-vitreous barrier integrity causing Gd to leak into vitreous body [2]. This might explain the significantly higher T1W signal enhancement in right vitreous body relative to left vitreous. Latanoprost is a prostaglandin analogue which reduces IOP by increasing drainage of aqueous humor [4]. Gd clearance might therefore increase causing longer time to peak with lower peak intensity in the right, treated AC in Lat group. Timolol is a non-selective beta-adrenergic receptor antagonist which reduces IOP by reducing aqueous humor formation [5]. This might explain the apparently reduced Gd signal increase rate in right AC in Tim group. While significant IOP reduction was observed in the right treated eye of BT group immediately before MRI experiment, the reason for the drastic differences in Gd enhancement compared to latanoprost and timolol in both treated and untreated AC is currently unclear. Brimonidine is known to act through a number of different mechanisms, many of which are still poorly understood. One possible explanation to current observations may be related to brimonidine as a selective alpha-2 adrenergic agonist which might cause systemic cardiovascular side effects such as bradycardia and reduced blood pressure, and lead to a systemic change in Gd dynamics in both eyes of BT group [7]. Further studies are currently ongoing to confirm this issue. Whether the lower peak time in left, untreated AC of Lat and Tim than MB group was related to systemic side effects of Lat and Tim and/or different baseline IOP during MRI scans remained to be elucidated [4], although IOP differences between contralateral eyes measured immediately after MRI experiments remained apparent across groups (data not shown). In conclusion, ocular Gd-enhanced MRI provided a non-invasive model system to visualize and investigate spatiotemporally the aqueous humor dynamics and its relation to IOP and glaucoma. It may also allow evaluation of efficacy of anti-glaucoma agents through longitudinal monitoring of aqueous flow *in vivo*. To date, there are only few methods enabling to assess the effect *in vivo* on aqueous humor production and uptake. Our current method may provide a new alternative tool for providing such information. Our results also echoed with previous reports [4,7] and drew attention to understanding the potential systemic side effects of topical ophthalmic hypotensive drug applications on the untreated eyes.

References: [1] Tim A.L.C., TVST, 2013; [2] Chan K.C., Exp Eye Res, 2008; [3] Crish S. D., PNAS, 2010; [4] Rajan M.S., Eye, 2003; [5] Yang Q., IOVS., 2012; [6] Bowman R.J.C., Eye, 2004; [7] Mungan N.K., J AAPOS, 2003;

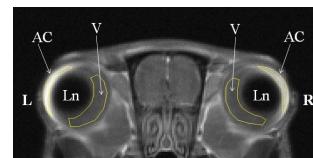


Figure 1. T1-weighted image (T1WI) at 1 hour post Gd i.p. injection, showing basic rat eye anatomy and ROIs of AC and V in yellow. AC: Anterior chamber, V: Vitreous body, Ln: lens.

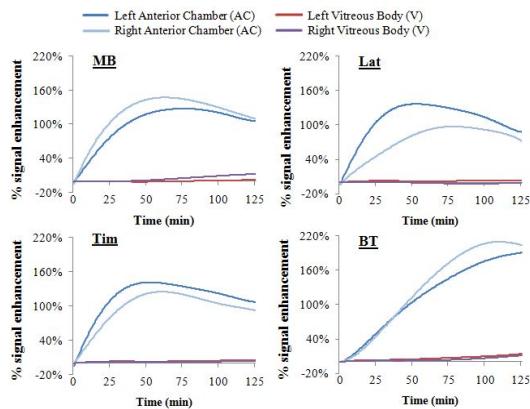


Figure 2. Fitted time courses of % Gd signal enhancement in anterior chamber and vitreous of both eyes of the chronic ocular hypertension (MB) and hypotensive drug treatment (Lat, Tim, BT) groups. All treatments were performed to right eyes only.

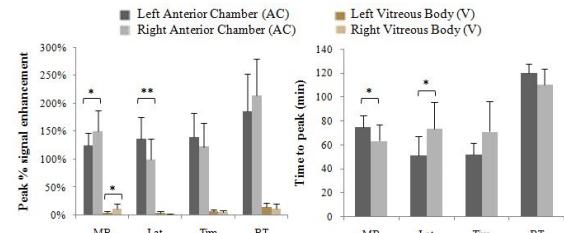


Figure 3. Quantitative comparisons of peak % Gd signal enhancement (AC and V) and time to peak (AC only) of all groups. (two-tailed paired t-tests between treated and untreated eyes: *p<0.05, **p<0.01)