In Vitro Characterization of Timolol Metabolism in Rabbit Ocular and Liver S9 Fractions

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Purpose
The number drugs on the market for topical ocular administration in treatment of ophthalmic diseases have been steadily increasing. Absorption and transport are well reported ocular delivery obstacles; however, the role of metabolism has been limited and inconsistent between human and preclinical species. The disposition of topically administered ophthalmic drugs is further complicated by the dependence on hepatic metabolism assessments in spite of low systemic exposure as well as the need for more sensitive analytical tools and ocular specific methodologies. The objective of this investigation was to understand whether eye has functional metabolic capacity toward timolol, a clinically used beta-adrenergic receptor antagonist. This investigation focuses on rabbits due to their use as an ophthalmological, pharmacodynamic model.

Methods
Metabolism of timolol was studied in rabbit ocular S9 fractions and compared with liver S9 fractions by data dependent high-resolution mass spectrometry (LC-MSⁿ). Metabolites were separated on an Atlantis T3 5μm 2.1x150 mm column (Waters, Milford, MA) over a 35 minute method at 0.25 mL/minute. Mobile phases A and B consisted of 10 mM ammonium formate in LC-MS grade water and LC-MS grade acetonitrile, respectively. Mobile phase B was initially set at 5% for 5 minutes and linearly increased to 95% over 23 minutes and held for 2 minutes before returning to starting conditions. Samples were analyzed in positive electrospray ionization with nine scan events. Full scans, collision induced dissociation (CID) product ion scans, and higher energy collision induced dissociation (HCD) product ion scans were obtained at 15,000 resolution or greater. Metabolites have a mass accuracy of less than 5 ppm and were manually interpreted using Xcalibur software version 2.0 (Thermo Fisher Scientific).

Results
Five metabolites of timolol, including oxidative and conjugative products were observed in rabbit liver S9 fractions, while only one metabolite was observed in the eye. Oxidative metabolites, including the CYP2D6 mediated hydroxylation on the morpholine ring, were liver specific. Most notably, a previously unreported N-acetyl conjugate of timolol was observed in ocular and liver S9 fractions.

Conclusion
Overall the results indicate that rabbit eyes have a metabolic capacity, including conjugative metabolism. Assessment of liver metabolism may be over-predictive and may serve as a poor surrogate to investigative ocular disposition for topically dosed therapies like timolol. From a functional activity perspective, the data suggests rabbits lack ocular CYP2D6 isoform. To the best of our knowledge, our investigation is the first investigation of timolol’s in vitro ocular metabolism and the first report of N-acetyl timolol conjugate.