INTRODUCTION

A wealth of experimental and clinical data support the role of nitric oxide (NO) in lowering intraocular pressure (IOP).<ref>1,2 NCX 667 is a novel NO donor known to decrease IOP in models of ocular hypertension and glaucoma following single or repeated daily dosing alone or combined with prostaglandin analogues.3,4 However, direct evidence of the cellular mechanism(s) involved remains elusive. Here we expanded previous data on the IOP-lowering activity of NCX 667 using various animal species and models and started to address the contribution of changes in conventional outflow to new outflow pathways by using bioengineered human 3D-HTM/HSC™ constructs.5,6</ref>

METHODS

In vivo pharmacological testing

Ocular normotensive New Zealand white (NZW) rabbits and Beagle dogs as well as ocular hypertensive (hypertonic saline-induced) NZW rabbits or laser-induced Cynomolgus monkeys were used. All animals were treated with NCX 667 (30 µL) at the indicated dose or vehicle (PBS with Cremophor EL 5%, DMSO 0.3%, BAC 0.02%). IOP was recorded prior to dosing and at different time points post dosing using a pneumotonometer (Model 30° Reichert, Depew, NY, USA). One topical drop of the local anaesthetic (Novesan® 0.4%, ophthalmic solution or 0.5% proparacaine hydrochloride) was applied to the eye prior to each IOP measurement.

3D-HTM/HSC™ Tissue Technology

Cell culture. Primary human trabecular meshwork (HTM) cells isolated from dissected (post keratoplasty) donor tissue rings were used. 7 HTM cells were plated in MEM containing 10% FBS, 0.1% gentamicin and maintained at 37°C in a humidified atmosphere with 5% carbon dioxide. Similarly, primary human Schlemm’s canal (HSC) cells were cultured in DMEM containing 10% FBS, penicillin (100 units/mL), streptomycin (0.1 mg/mL) and L-glutamine (0.292mg/mL).

3D culture of HTM and HSC cells on SU-8 scaffold. A previously described method was used.8 Briefly, epoxy-based photoresist SU-8 (MicroChem Corp., Westborough, MA) was used to develop free-standing biomimetic porous microstructures serving as the scaffold on which cells were outgrown. To create 3D-HTM/HSC™ constructs, individual micro-fabricated scaffolds were sealed on aluminum rings (15mm diameter) and placed in a 24-well plate followed by the seeding of 40,000–50,000 HTM cells. Once confluent, the HTM-containing constructs were inverted and HSC (40,000 cells/well) were cultured on the other side of the scaffold for 10 days. To mimic glaucomatous conditions, TGfβ-2 was applied to the newly formed 3D-HTM/HSC™ constructs for 6 consecutive days during which the media was changed every 3 days.

Perfusion Studies. Perfusion studies were performed as previously described.5 Ready to use 3D-HTM/HSC™ constructs were serum starved (1% FBS/DMEM) for 1 day and then perfused at various rates (2, 4, 8, and 16 µL/min) with vehicle (0.1% DMSO in culture media) or NCX 667 (10µM). The rho-associated protein kinase inhibitor, Y27632 (10µM), served as positive control. Pressure was continuously monitored and the “outflow facility” calculated mathematically after the treatment.<ref>5,6</ref>

RESULTS

NCX 667 increases outflow facility in naive and TGfβ-2-stimulated 3D-HTM/HSC™ constructs

CONCLUSION

NCX 667 lowers IOP in ocular normotensive and hypertensive animal models. These effects are likely due to an increase in outflow facility via TM/SC outflow pathway

NCX 667, a novel nitric oxide (NO) donor, lowers intraocular pressure (IOP) via stimulation of trabecular meshwork/Schlemm’s canal outflow facility

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