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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INTRODUCTION

A wealth of experimental and clinical data support the role of nitric oxide (NO) in lowering intraocular pressure (IOP).[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 these effects by using bioengineered human 3D-HTM/HSC™ constructs.[5]

METHODS

In vivo pharmacological testing

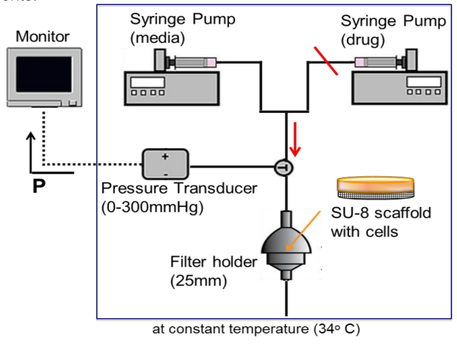
Ocular normotensive New Zealand white (NZW) rabbits and Beagle dogs as well as ocular hypertensive (hypertonic saline-induced) NZW rabbits or (laser-induced) Cynomolgus monkeys were used. All animals were treated with NCX 667 (30µL) at the indicated dose or vehicle (PBS with Cremophor EL 5%, DMSO 0.3%, BAC 0.02%). IOP was recorded prior to dosing and at different time points post dosing using a pneumatonometer (Model 30™ Reichert, Depew, NY, USA). One topical drop of the local anesthetic (Novesina® 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 discarded (post keratoplasty) donor tissue rings were used.[5] HTM cells were plated in IMEM containing 10% FBS, 0.1mg/mL 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 (100units/mL), streptomycin (0.1mg/mL) and L-glutamine (0.292mg/mL).

3D Co-culture of HTM and HSC cells on SU-8 scaffold. A previously described method was used.[5] 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 cultured. To create 3D-HTM/HSC™ constructs, the individual micro-fabricated scaffolds were seated 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 cells (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-IMEM) 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, Y-27632 (10µM), served as positive control. Pressure was continuously monitored and the "outflow facility" calculated mathematically after the treatments.[5]



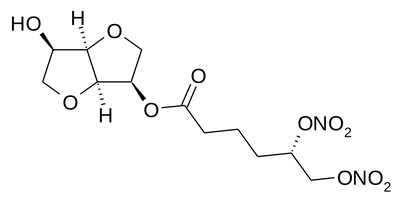
PURPOSE

To study the effects of NCX 667 on conventional outflow using bioengineered human trabecular meshwork/Schlemm's canal (3D-HTM/HSC™) constructs

RESULTS

NCX 667 lowers intraocular pressure (IOP) in animal models of ocular hypertension and glaucoma

Chemical structure

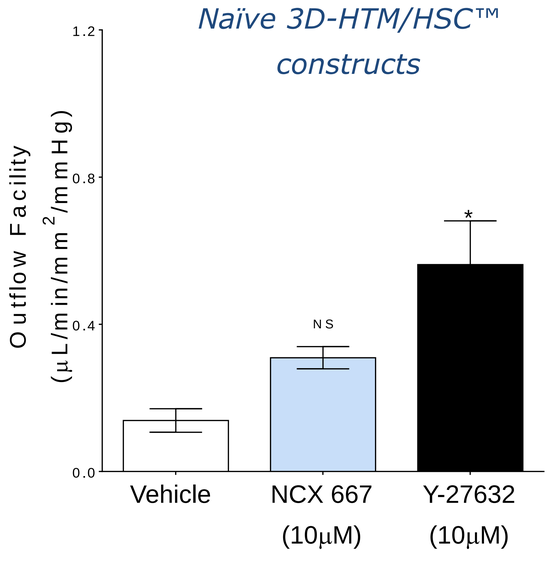


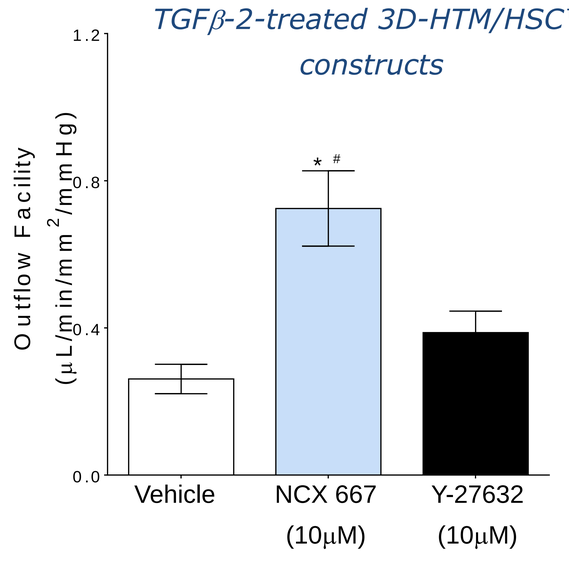
(S)-((3R,3aR,6R,6aR)-6-hydroxyhexahydofuro[3,2-b]furan-3-yl)5,6-bis(nitrooxy)hexanoate

Species	Model	Dose (%)	IOP change[a]	
			E_{max} ± SEM (mmHg)	T_{max} (min)
New Zeland White rabbit	Ocular normotensive	0.1 0.3 1	- 2.7 ± 0.4 - 4.6 ± 1.0* - 5.3 ± 0.8*	30-60
	Hypertonic (5%) saline-induced ocular hypertensive	0.1 0.3 1	- 0.4 ± 1.1 - 7.7 ± 0.5* - 11.8 ± 0.6*	--
Beagle dog	Ocular normotensive	0.1 1	- 2.4 ± 0.6 - 3.3 ± 0.5*	30-60
Cynomolgus monkey	Laser-induced ocular hypertensive	1	- 7.3 ± 2.3*	30-60

Data are reported as mean ± SEM of n=6-10. [a]IOP change was calculated as follows: (Drug IOP_{Tmax}- Drug IOP_{T0}) - (Veh IOP_{Tmax}- Veh IOP_{T0}). *p<0.05 vs. vehicle at the respective time point.

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





**p<0.05 vs. vehicle, #p<0.05 vs. Y-27632, Bonferroni's Multiple Comparison Test*

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

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