Effects of NCX 470, a nitric oxide (NO)-donating bimatoprost, in an *in vitro* 3D-human trabecular meshwork (TM)/Schlemm's canal (SC) tissue model

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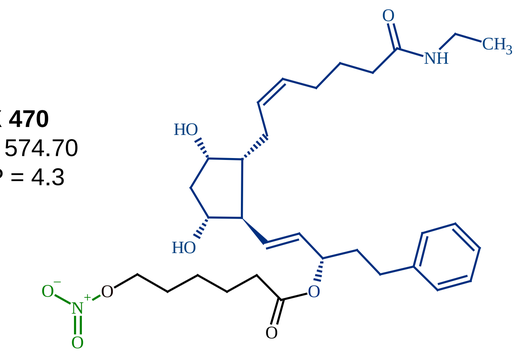
INTRODUCTION

- NCX 470 is a new dual-acting molecular entity with two pharmacologically active metabolites, nitric oxide (NO) and prostamide, bimatoprost.[1]

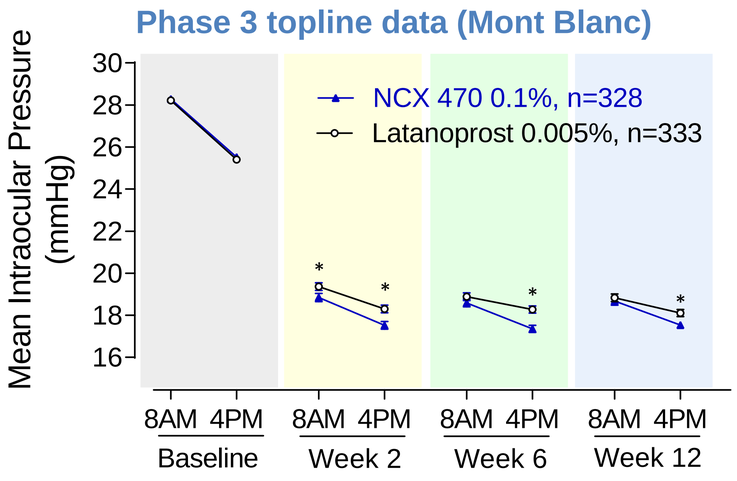
NCX 470
M.W. = 574.70
cLogP = 4.3



Hexanoic acid, 6-(nitrooxy)-(1S,2E)-3-[(1R,2R,3S,5R)-2-[(2Z)-7-(ethylamino)-7-oxo-2-hepten-1-yl]-3,5-dihydroxycyclopentyl]-1-(2-phenylethyl)-2-propen-1-yl ester

- NCX 470 demonstrated "non-inferiority" to latanoprost in decreasing intraocular pressure (IOP) in a pivotal phase 3 clinical trial (Mont Blanc, https://clinicaltrials.gov/ct2/show/NCT04445519) in patients with ocular hypertension or glaucoma (see graph below).[2]

Phase 3 topline data (Mont Blanc)



- In addition to its robust IOP-lowering activity and favorable safety profile, NCX 470 demonstrated retinal protective properties in previous studies performed in animal models of ischemia/reperfusion injury.[3]

METHODS

Trabecular meshwork (TM)/Schlemm's canal (SC) outflow in bioengineered human 3D-HTM/HSC™ constructs

Cell culture. Human trabecular meshwork (HTM) cells from 5 donors were isolated from discarded (post keratoplasty) donor tissue rings, plated in IMEM containing 10% FBS, 0.1mg/mL gentamicin and maintained at 37˚C in a humidified atmosphere with 5% carbon dioxide. Similarly, 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. SU-8 holder (MicroChem Corp., Westborough, MA) was used to develop free-standing biomimetic porous microstructures serving as a 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 seeding with 40,000–50,000 HTM cells. Once confluent, the HTM 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, TGF-β2 was applied for 6 consecutive days during which the media was changed every 3 days.

Perfusion Studies. Ready-to-use 3D-HTM/HSC™ constructs were perfused at various rates (2, 4, 8, and 16 µL/min) for 5h with vehicle (1% DMSO in culture media), NCX 470 (10µM in DMSO) or bimatoprost (10µM in DMSO). Pressure was continuously monitored and the outflow facility calculated as follows: CFl= (Δf/Δp)/A, where CFl=outflow facility, f=fluid flow, p=pressure and A=area of the cultured cells.

Aqueous humor (AH) dynamics in non-human primates (NHP)

Baseline IOP was measured (pneumatonometer Classic 30, Reichert) on day 1. Dosing of vehicle (Kolliphor® HS15, benzalkonium chloride, boric acid, EDTA, sorbitol, dibasic sodium phosphate, pH=6.0) and NCX 470 (0.1%) started immediately after baseline IOP for 3 consecutive days, bid. On day 4 IOPs were measured at 90, 180 and 300 min after AM dose. On day 4, animals were dosed with fluorescein (10%) and fluorophotometric scans (Fluorotron Master, OcuMetrics) of the cornea and anterior chamber were taken at 45min intervals to address changes in AH inflow (Fa), outflow facility (Cfl) and uveoscleral outflow (Fu). Central corneal thickness and anterior chamber depth were measured by ultrasound pachymetry (Sonomed). Cornea diameter was measured with calipers (Fine Science Tools). Cornea and anterior chamber volumes as well as Fa were calculated according to formulas reported elsewhere.[4,5] Outflow facility and uveoscleral outflow were calculated according to equations (1) and (2) respectively.

$$Cfl = \Delta Fa / \Delta IOP \quad (1)$$

$$Fu = Fa - Cfl\ (IOP - Pev) \text{ where Pev (episcleral venous pressure)} = 17mmHg \quad (2)$$

REFERENCES

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2. Fechtner R et al., American Glaucoma Society. 2023; Austin, TX, USA.
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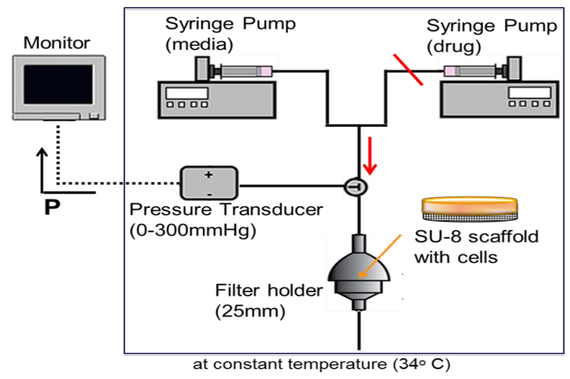
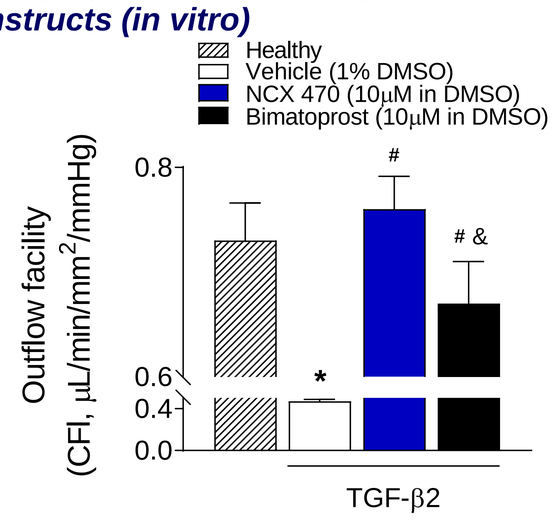
AIM

Explore NCX 470-mediated effects on aqueous humor (AH) dynamics using *in vitro* and *in vivo* models

RESULTS

Trabecular meshwork (TM)/Schlemm's canal (SC) outflow in bioengineered human 3D-HTM/HSC™ constructs (in vitro)

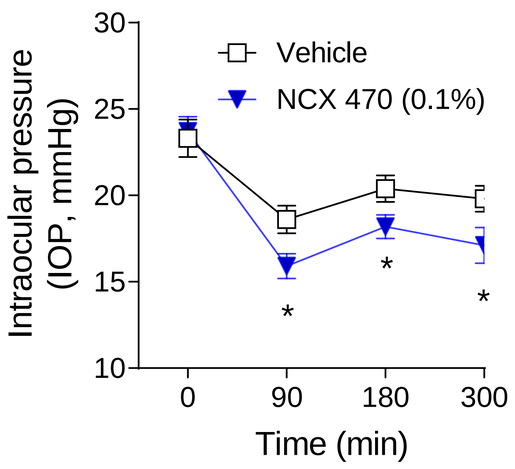




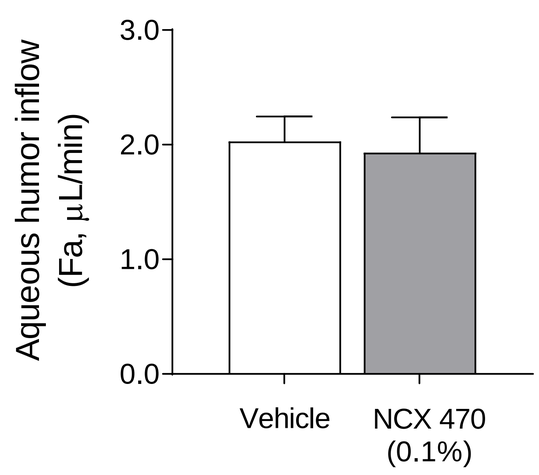
*Data are reported as mean ± SEM (n=4-5) *p<0.05 vs healthy; #p<0.05 vs vehicle; &p<0.05 vs NCX 470, Student's t-test (GraphPad Prism 7.05).*

Aqueous humor (AH) dynamics in non-human primates (in vivo)

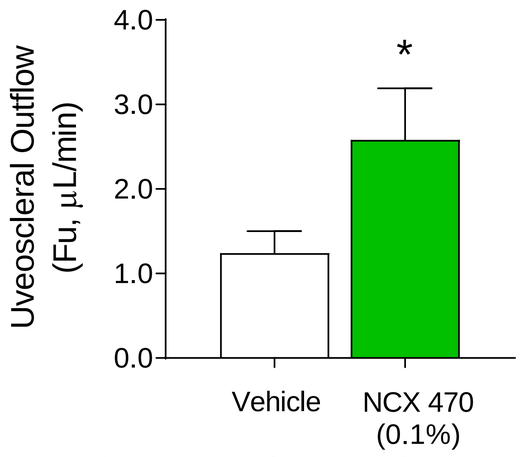
Intraocular pressure (IOP)



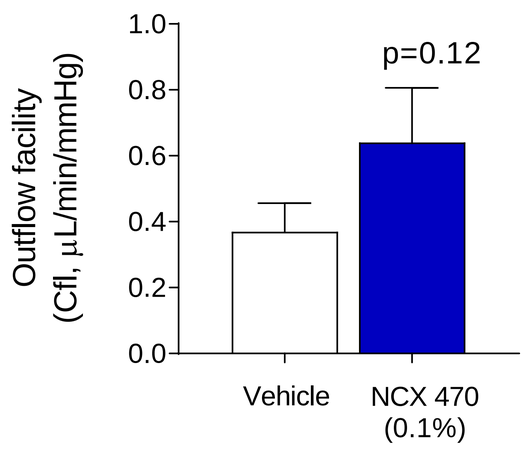
Aqueous humor inflow (Fa)



Uveoscleral outflow (Fu) (non-conventional pathway)



Outflow facility (Cfl) (conventional pathway)



*Data are reported as mean ± SEM of n=6-12 eyes. *p<0.05 vs vehicle using a multiple t-test (GraphPad Prism 7.05).*

SUMMARY

- NCX 470 improved outflow facility to greater extent compared to bimatoprost in 3D-HTM/HSC™ constructs.
- In non-human primates NCX 470 lowered IOP, increased uveoscleral outflow and, albeit not significantly, enhanced conventional outflow. No effects was observed on AH inflow.

CONCLUSION

NCX 470-mediated IOP lowering is a result of the concomitant contributions of conventional and uveoscleral outflow activated by nitric oxide and bimatoprost

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