NCX 470, a nitric oxide (NO)-donating bimatoprost, preserves rabbit eyes from biochemical and functional changes associated with endothelin-1 (ET-1)-induced ischemia/reperfusion injury of optic nerve head and retina

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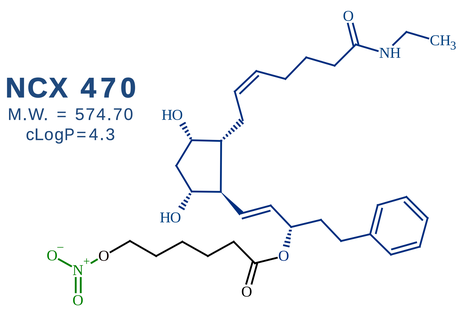
INTRODUCTION

- NCX 470 is a dual-acting New Molecular Entity (NME) with two pharmacologically active metabolites, namely: nitric oxide (NO) and the prostamide, bimatoprost.[1]



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 a pivotal ph3 clinical trial in patients with ocular hypertension or laucoma.[2]
- NCX 470 was shown to improve ocular hemodynamics and retinal cell physiology following ET-1-induced ischemia/reperfusion injury of the optic nerve head and retina.[3]

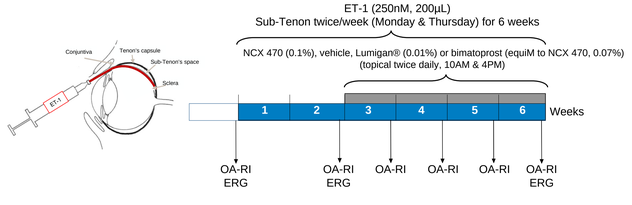
MATERIALS AND TEST SYSTEM

Animal model

Previously described[3] endothelin-1 (ET-1)-induced ischemia / reperfusion injury model was used (see scheme 1 below).

Vehicle (30 µL/eye), NCX 470 (0.1%, 30 µL/eye), Lumigan® (bimatoprost 0.01% ophthalmic solution, 30 µL/eye) or bimatoprost at equimolar dose as that released by NCX 470 (0.07%, 30 µL/eye) were administered as eye drops, bid starting on week 3 concomitantly to ET-1 until the end of the experiment (see scheme 1 below).

Scheme 1



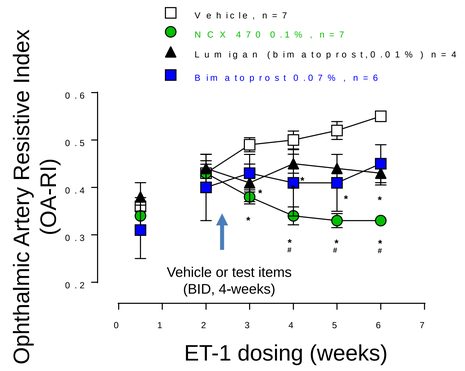
PURPOSE

Compare NCX 470 (0.1%) to Lumigan® (bimatoprost 0.01% ophthalmic solution) and bimatoprost (0.07% - equiM to that released by NCX 470 0.1%) on ocular hemodynamics and retinal cell physiology

RESULTS

Functional measurements

Ocular hemodynamics



*p<0.05 vs. Vehicle at the same time point, two tailed t-test
#p<0.05 vs. Lumigan or bimatoprost at the same time point, two tailed t-test

Photoreceptor function (ERG)

Dark adapted scotopic response 3.0 – Rod/Cone

Time after the 1st ET-1 dose	Week 0 Baseline	Week 2 ET-1	Week 6 ET-1 + Test items
Vehicle	139±18	110±12*	89±11
NCX 470 (0.1%)	130±9	90±9*	124±11#
Lumigan (0.01%)	165±3	114±9*	112±6
Bimat. (0.07%)	115±13	91±5	89±9

Similar trend was found following 'Dark adapted scotopic 0.01 response – Rod response' and 'Light adapted photopic 3.0 response – Cone response'.
Data are reported as mean ± S.E.M, n=4-6
*p<0.05 vs. baseline; #p<0.05 vs. week 2, two tailed t-test

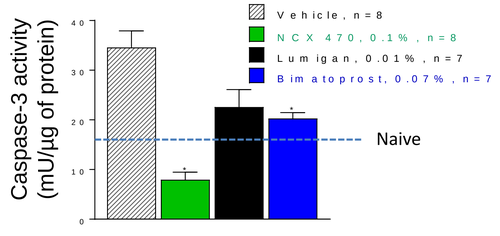
Biochemical measurements

Inflammatory cytokines

Groups	IL-1β	TNFα
	pg/mL	pg/mL
Vehicle	14.5±0.4	128.9±20.8
NCX 470 (0.1%)	10.4±0.7*	38.6±4.2*#
Lumigan (0.01%)	11.6±1.0*	67.6±12.2*
Bimat. (0.07%)	11.2±0.9*	58.7±2.5*

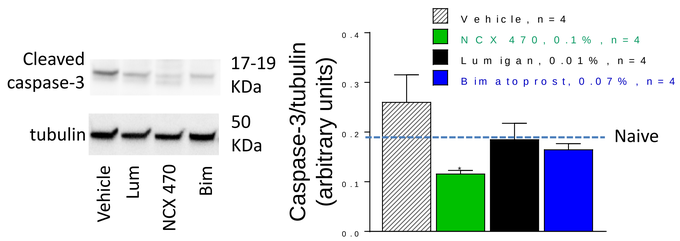
*p<0.05 vs. vehicle, #p<0.05 vs. Lumigan or Bimatoprost, two-tailed t-test

Caspase-3 activity



*p<0.05 vs. vehicle, two-tailed t-test

Cleaved caspase-3 protein expression



*p<0.05 vs. vehicle, two-tailed t-test

CONCLUSIONS

Repeated NCX 470 topical dosing improves ocular hemodynamics and retinal cell function after ischemia/reperfusion injury

Effects are only partially shared by bimatoprost pointing to NO as the major contributor

METHODS

Functional measurements

Electroretinogram (ERG).[3] ERGs recording took place under anaesthesia (ketamine 20mg/kg and xylazine 5mg/kg i.m.). The eyes were dilated with tropicamide 1% and adapted to darkness for at least 2h prior to ERGs recording. The ERGs were recorded using Retimax (CSO, Florence, Italy). Measurements were taken as indicated in scheme 1.

Ophthalmic Artery Resistive Index (OA-RI).[3] OA-RI was taken using an Ecocolor Doppler Philips Ultrasound HD7XE (Philips, Milan, Italy) as indicated in scheme 1. Pourcelot resistive index for ophthalmic artery (OA-RI) was calculated as follows: (PSV – EDV)/PSV where PSV and EDV refer to Peak Systolic Velocity and End Diastolic Velocity, respectively.[3]

Biochemical measurements

Retina was collected from all animals at the end of the study and tissues frozen.

Caspase-3 activity. Caspase-3 activity was determined using a fluorescent substrate following the methods described by Stennicke and Salvesen.[4]

Caspase-3 western blot analysis. The method described by Liu et al.[5] was generally followed. Briefly, 40µg of proteins were separated by SDS-PAGE, electro-transferred on polyvinylidene difluoride (PVDF) membranes and incubated with Caspase-3 antibody (2µg/mL, Invitrogen, Massachusetts, USA) followed HRP-conjugated secondary antibodies. Tubulin was used as house-keeping protein.

IL-1β and TNFα content. IL-1β and TNFα were quantitatively determined on aliquots (100µL) of retina homogenate supernatants, using the RayBio® Rabbit ELISA kit for IL-1β and TNFα (RayBiotech, Norcross, GA, USA). Briefly, 96-well plate coated with the antibodies specific to rabbit IL-1β or TNFα were incubated with the samples. Then, the biotinylated anti-rabbit cytokine antibody was added to the wells and reacted with HRP-conjugated streptavidin. The plate was then read at 450nm. IL-1β and TNFα quantification was performed using respective standard curves obtained with known amounts of each cytokine exposed to identical reaction conditions.

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4. Stennicke & Salvesen. Methods Enzymol. 2000; 322:91-100.
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Commercial Relationships Disclosure:
F. Impagnatiello, E. Bastia, S. Brambilla and C. Galli, Nicox Research Institute (E); D. Hubatsch, Nicox Ophthalmics, Inc. (E); S. Sgambellone, S. Marri, G. Provensi, L. Lucarini and E. Masini (F)

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