NCX 470, a nitric oxide (NO)-donating prostaglandin analog, restores ocular hemodynamics and photoreceptor function after endothelin-1-induced ischemia/reperfusion injury in rabbits

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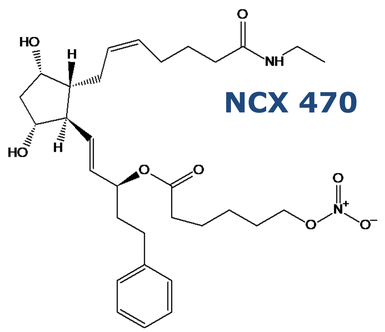
INTRODUCTION

NCX 470 is a nitric oxide (NO)-donating prostaglandin analog in clinical development for the reduction of intraocular pressure (IOP) in patients with open-angle glaucoma or ocular hypertension[1]. This work evaluated IOP changes, ocular hemodynamic (ophthalmic artery resistive index, OA-RI) effects and neuroprotective activity (electroretinogram, ERG) exerted by NCX 470 after endothelin-1 (ET-1)-induced ischemia/reperfusion injury in rabbits. Furthermore, we also addressed NCX 470-mediated changes in oxidative stress markers (e.g. manganese superoxide dismutase, MnSOD; reduced gluthatione, GSH) as well as changes in apoptotic cell markers (8-hydroxydeoxyguanosine, 8-OHdG) in retina and iris/ciliary body.

Data would argue that NCX 470 reduces oxidative stress and apoptosis in the retina after ET-1-induced ischemia/reperfusion and ameliorates ocular hemodynamics as well as photoreceptor function. Additional studies are needed to establish whether these effects are interdependent.

MATERIALS AND TEST SYSTEM

Nitric oxide (NO)-donating bimatoprost



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

Animal model

Endothelin-1 (ET-1)-induced ischemia/reperfusion in rabbits. The endothelin-1 (ET-1)-induced ischemia/reperfusion model was used.[2] Ischemia/reperfusion injury was induced in rabbits by sub-tenon injection (twice/week for 6 weeks) of 200 µL of 250 nM ET-1 (Fluka, Israel) dissolved in water using a lacrimal cannula under anaesthesia produced by ketamine and xylazine injected intramuscularly. After ET-1 injection, a drop of tobramycin (0.3% ophthalmic solution) was instilled in each eye. NCX 470 (0.1%, 30 µL/eye) or vehicle (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).

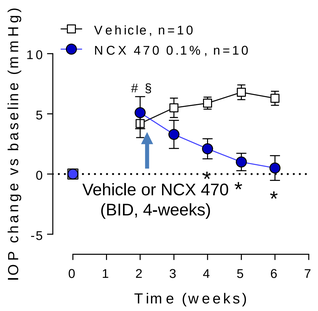
PURPOSE

Determine if NCX 470, a NO-donating bimatoprost with clinically proven IOP-lowering effects, improves ocular hemodynamic and retinal physiology

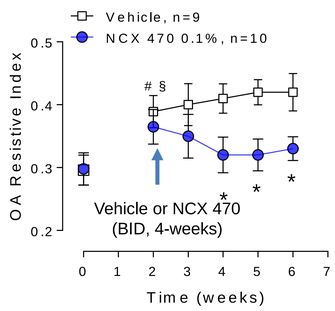
RESULTS

Functional measurements

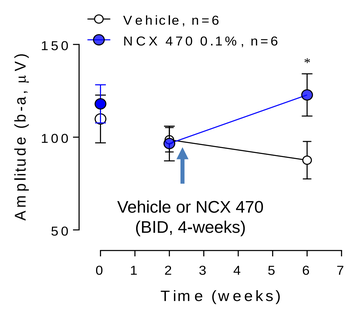
Intraocular pressure



Ocular hemodynamics



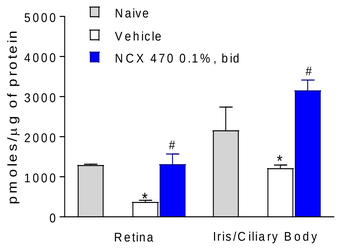
Photoreceptor function



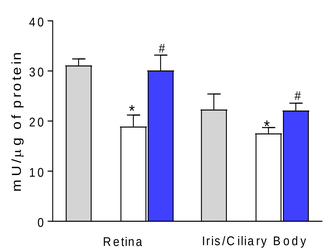
** p<0.05 vs. Vehicle at the same time point; #, § p<0.05 vs. basal (time 0), two tailed t-test*

Biochemical measurements

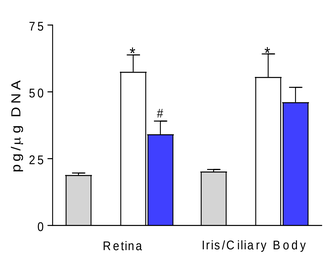
Reduced Glutathione (GSH)



Mn Superoxide Dismutase (MnSOD)



8-Hydroxy-2-deoxyguanosine (8-OHdG)



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

CONCLUSIONS

Repeated ocular dosing with NCX 470 (0.1%, bid) reverses ET-1-induced changes in IOP, OA-RI and ERG suggesting improved ocular hemodynamics and retinal cell physiology

METHODS

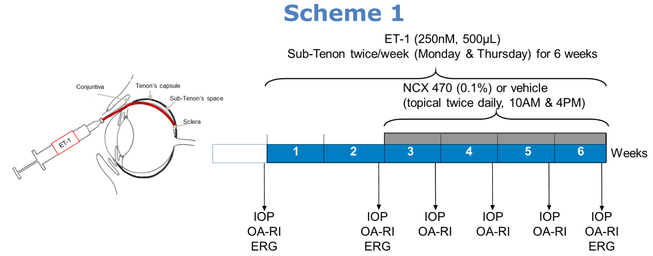
Functional measurements

Intraocular pressure (IOP). IOP was measured using a pneumatonometer (Model 30 Classic; Reichert, Depew, NY, USA) after animals were left for 36h free of treatment. One drop of oxybuprocaine hydrochloride (4 mg/ml) was instilled before each set of pressure measurements.

Electroretinogram (ERG). ERGs recording took place under anaesthesia (ketamine and xylazine 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). 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]

Scheme 1



Biochemical measurements

Retina and iris/ciliary body were collected from all animals at the end of the study and tissues frozen.

Glutathione (GSH). Tissues were homogenized in PBS, pH 7.4 200 µL of 40 mM N-ethylmalemide (Sigma-Aldrich Chemie, Steinheim, Germany) were added to 0.5 mL of sample, incubated for 30 min and diluted with 0.1 mL of NaOH 0.1 N and 1.8 mL phosphate-EDTA buffer; at the end 0.1 mL σ-phthaldialdehyde (Analyticals, Carlo Erba, Milan, Italy, 1 mg/mL in methanol, Uvasol) was added. After 20 min at RT the fluorescence was read at 420 nm emission & 350 nm excitation. The content of GSH was estimated against a standard curve and normalized for protein content.

Manganese Superoxide Dismutase (MnSOD). MnSOD activity was determined in a fixed volume of homogenate according to a previously described method.[2,4] A competitive inhibition assay that uses xanthine-xanthine oxidase-generated superoxide to reduce nitro blue tetrazolium (NBT, Sigma) to blue tetrazolium was used. In a spectrophotometer at a wavelength of 560 nm, 50 µL xanthine oxidase was added to samples and the reduction of NBT monitored for 30s.

8-Hydroxy-2-deoxyguanosine (8-OHdG). DNA isolation was performed as previously described[4] with minor modifications. Samples containing DNA were used for 8-hydroxy-2-deoxyguanosine (8-OHdG) determination using an ELISA kit (JalCA, Shizuoka, Japan), following the instructions provided by the manufacturer. The absorbance of the product was measured at 450 nm.

REFERENCES

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3. Galassi F. et al., Ophthalmologica. 2002; 216: 123-128.
4. Lucarini L. et al., JCMM. 2017; 21:324–335

Commercial Relationships Disclosure:
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