#1119

BIO201: a new drug candidate for the treatment of dry AMD

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PURPOSE

Dry AMD is a major cause of visual impairment associated with aging, and no drug treatment is presently available. In collaboration with the Institut de la Vision, Biophytis develops a drug candidate (BIO201) based on norbixin, a di-*apo*-carotenoid, as API. We describe here the effects of norbixin on retinal pigmented epithelium (RPE) and retina photoprotection *in vitro* and *in vivo*.

MATERIALS AND METHODS

The photo-protective effect of norbixin, bixin, lutein, zeaxanthin and crocetin was evaluated on primary cultures of porcine RPE cells challenged with 30 µM A2E and illuminated with blue light (470 nm, 50 min) (N=16).

In vivo experiments measured the photo-protective effect of norbixin one week after blue light damage in the Abca4$^{-/-}$Rdh8$^{-/-}$ transgenic mouse model by intravitreal injections and in a standard rat blue light model of photodamage by repeated intraperitoneal injections. Twenty-eight 7-week-old mice were injected intra-vitreally in one eye with 120 µM norbixin (in 0.3% DMSO) or with DMSO alone, dark-adapted during 24 hours and exposed to blue light (4000 lux) for one hour. Ten mice were used as non-illuminated controls. Groups of 6-12 rats were injected intraperitoneally with either norbixin (10, 50, 100 mg/kg), α-phenyl-N-tert-butylnitrone (PBN, a potent free-radical trapping agent, 50 mg/kg), or an equivalent volume of saline 30 min prior to light damage and 2, 4 and 6 hours after the beginning of the exposure.

Full field scotopic electroretinogram was measured one week after light damage and eyes were removed for histology and photoreceptor quantification.

Bioavailability was tested in C57Bl/6 mice receiving bixin or norbixin, 50 mg/kg per os (in oil/DMSO 9:1) or 5 mg/kg intraperitoneally (in DMSO/tetraglycol/water 1:2:7) (N=4).

For statistical analyses, one-way ANOVA followed by Dunnett's tests were performed. All data are presented as mean+/- s.e.m. *p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001

CONCLUSIONS

1-Bixin and norbixin are more active than xanthophylls (lutein, zeaxanthin*) in vitro*

2-Norbixin has a much better oral bioavailability than bixin and was therefore selected for further studies

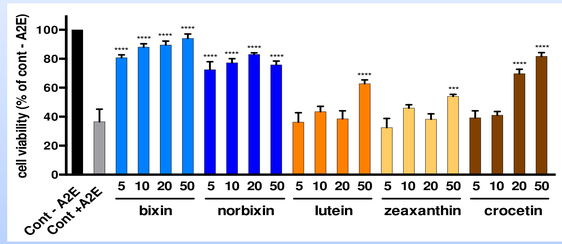
3-Norbixin provides an efficient photoprotection when injected intravitreally in Abca4$^{-/-}$Rdh8$^{-/-}$ mice eyes

4-Norbixin is at least as efficient as PBN by intraperitoneal injections in the blue light rat model

5-Chronic oral treatment of Abca4$^{-/-}$Rdh8$^{-/-}$ mice with norbixin is presently under investigation and appears to (1) reduce A2E accumulation and (2) prevent the degradation of ERG.

RESULTS

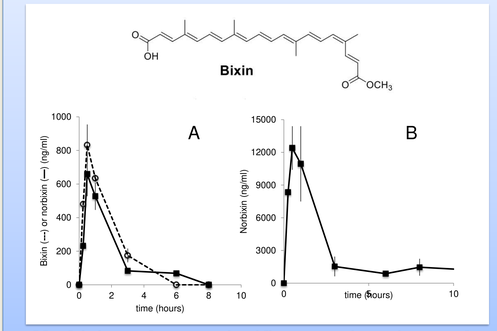
High protection of RPE cells by bixin and norbixin



Bixin and norbixin were highly protective even at 5 µM inducing 80.75 and 72.5 % RPE survival respectively whereas lutein, zeaxanthin and crocetin showed no protection at this concentration. At the different concentrations tested norbixin was always slightly less efficient than bixin.

Lutein, zeaxanthin and crocetin were also able to prevent RPE cell death at the highest concentrations. The concentrations of the substances are in µM. The positive control (cont – A2E) represents cells treated with DMSO alone. The negative control (cont + A2E) represents cells treated with A2E but not with substances.

Norbixin bioavailability is much better than that of bixin

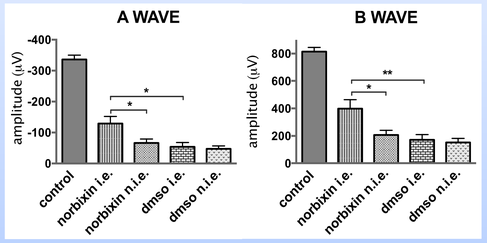


Blood was collected at various intervals and plasma levels were measured by HPLC-MS/MS.

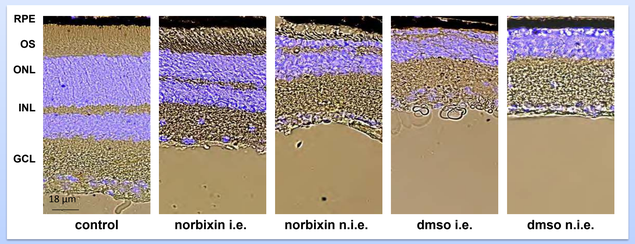
Ingested Bixin (A) bioavailability is quite low (*ca.* 2%) and this compound is rapidly converted into norbixin by esterases. Norbixin bioavailability (B) is much better and may become close to 100% with a suitable dosage form.

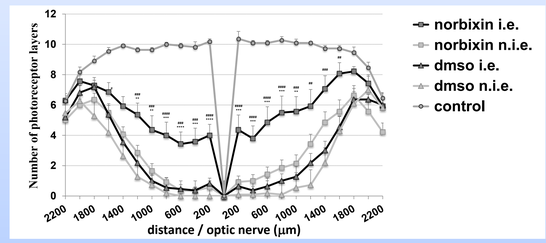
Norbixin undergoes significant conjugation as glucuronide(s) in mouse plasma.

Norbixin protects the retina of Abca4$^{-/-}$Rdh8$^{-/-}$ mouse against blue light induced phototoxicity



One week after BLD a- and b-wave amplitudes of ERG were significantly maintained in eyes injected with norbixin (i.e.) compared with non injected eyes (n.i.e.) or dmso injected eyes.

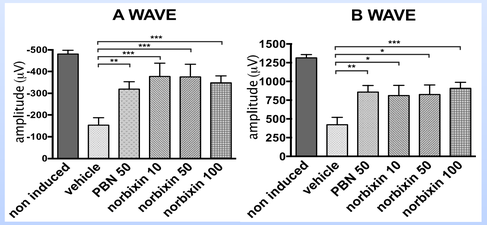




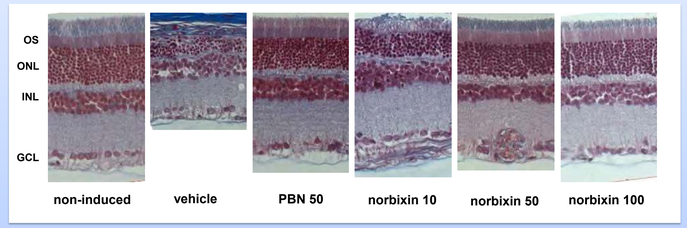
In the eyes injected with norbixin we observed a partial but clear protection of photoreceptor cells and outer segments compared to the contralateral eyes of the same mice or to the dmso-injected eyes. In the central retina, 4 to 6 rows of photoreceptors were still present one week after BLD compared to 1-2 rows for dmso or norbixin non-injected eyes .

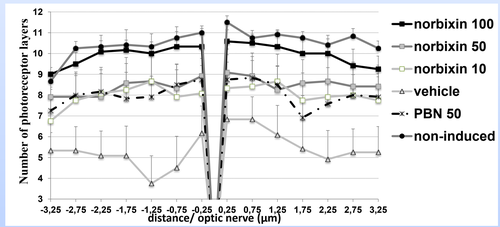
**: norbixin i.e. compared to norbixin n.i.e.; # : norbixin i.e. compared to dmso i.e.*

Norbixin protects the rat retina against blue light induced phototoxicity



One week after BLD a- and b-wave amplitudes of ERG were significantly maintained in eyes of norbixin and PBN injected rats compared to the vehicle injected group.





BLD induced a massive photoreceptor degeneration in vehicle-treated rats (4-6 rows remaining) compared to non-induced rats (10-11 rows). At 10 and 50 mg/kg norbixin induced a partial photoreceptor protection similar to that obtained with PBN (7-9 rows) whereas at 100 mg/kg the protection was very close to the non-induced control (9-10 rows compared to 10-11 rows).