



EVIDENCE FOR THE INVOLVEMENT OF PPARs IN THE PHOTOPROTECTIVE ACTIVITY OF BIO201 (NORBIXIN) ON RETINAL PIGMENT EPITHELIUM.

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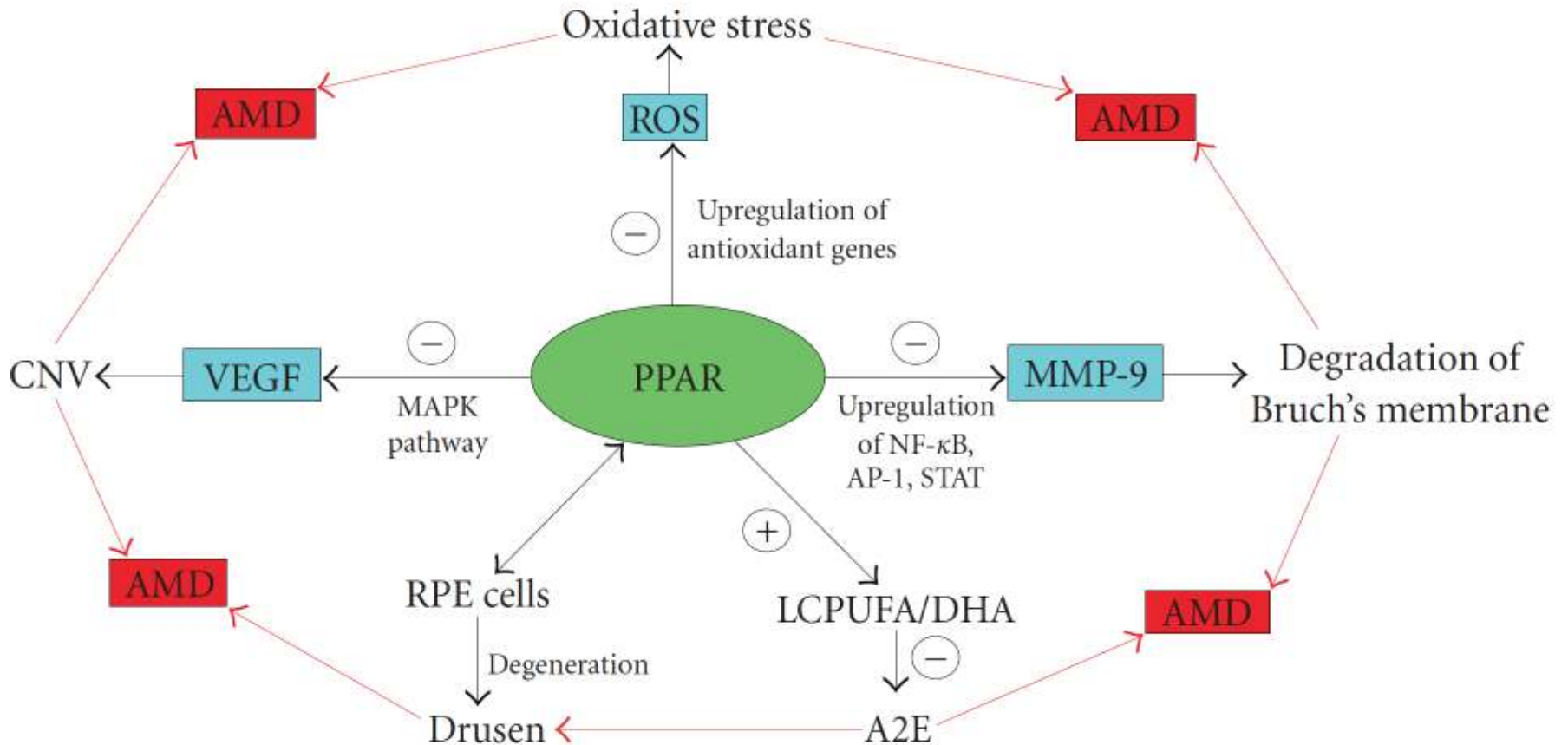
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Financial disclosure: Financial support came from Biophytis.

INTRODUCTION

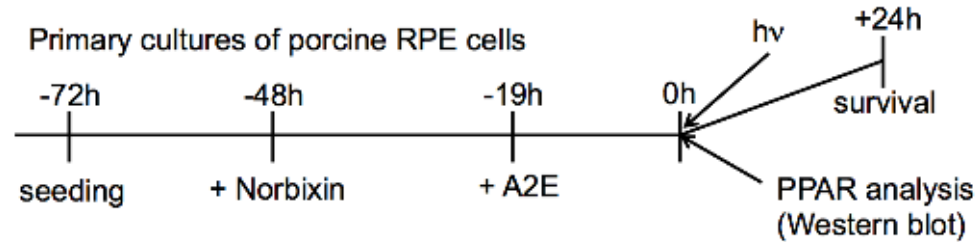
According to Herzlich (PPAR Res. 2008 Article ID 389507), PPARs play a key role in the protection of RPE cells against Age-Related Macular Degeneration



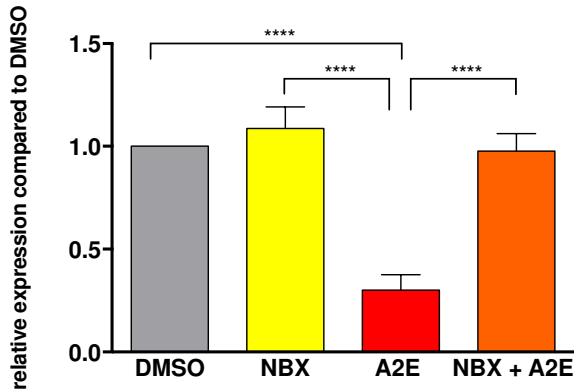
PPARs comprise three subtypes that can accommodate various types of ligands. The aim of the present work was to investigate the respective roles of PPAR α , PPAR β/δ and PPAR γ in response to BIO201 (norbixin) on the protection against the deleterious effects of A2E.

A2E sensitizes RPE cells to blue light damage and reduces PPAR α protein levels. Both effects are blunted by BIO201, a preparation of the diapo-carotenoid norbixin developed by Biophytis¹.

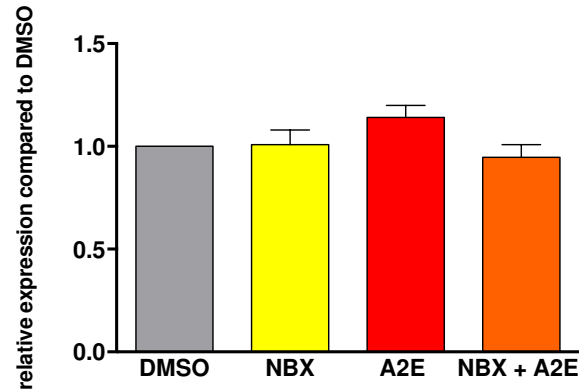
No significant effect is observed on other PPARs levels.



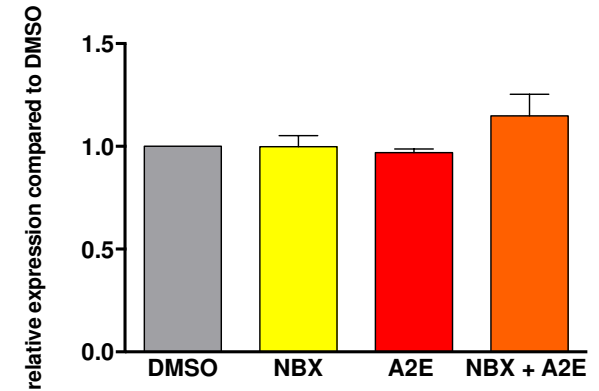
PPAR α



PPAR β/δ



PPAR γ



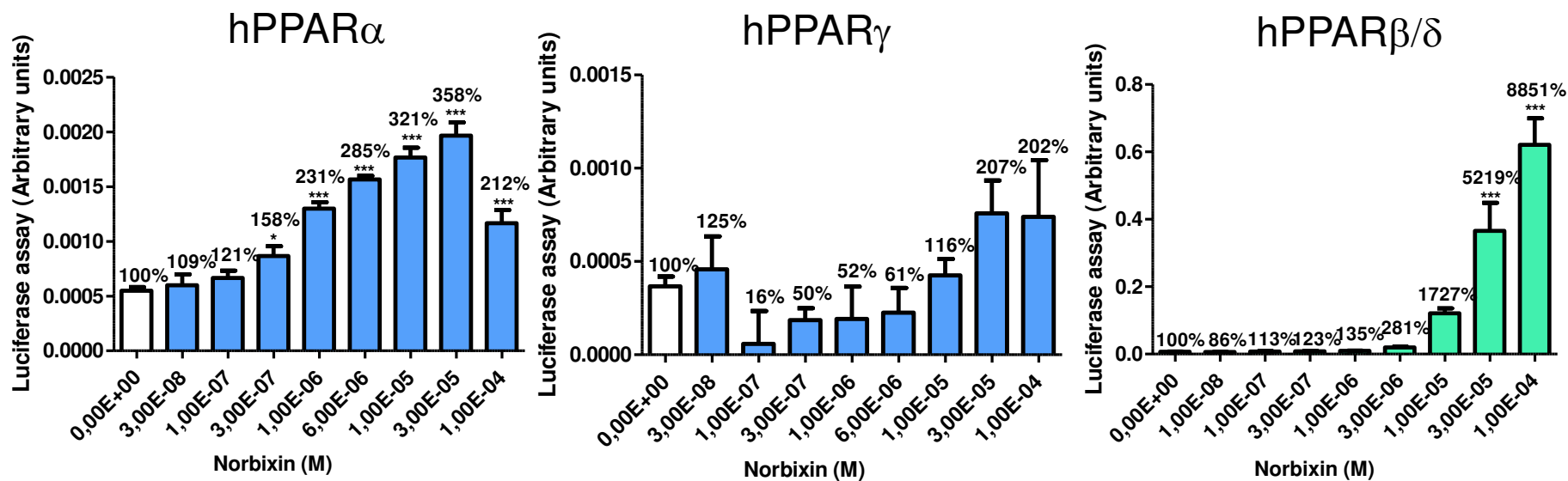
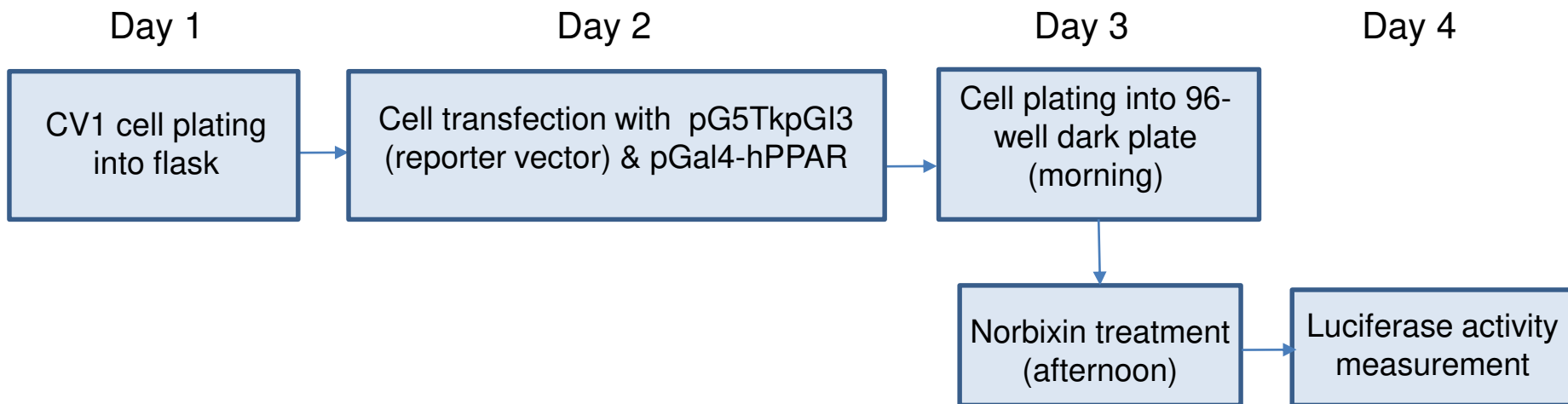
NBX: norbixin 20 μ M

A2E: 30 μ M

The effect on PPAR α is post-translational and is also observed with inverted protocols (where treatment with A2E precedes that with norbixin)

¹(Fontaine et al., PLoS ONE 2016, DOI:10.1371/journal.pone.0167793).

BIO201 (norbixin) activates hPPAR α and even more efficiently hPPAR β/δ



Such experiments do not tell us whether these effects are direct or indirect

DIRECT BINDING TESTS (competition with radioactive ligands):

BIO201 (norbixin) binds to PPAR α with a K_i of 16.5 μ M, and to PPAR γ with a K_i of 1.15 μ M

FRET ACTIVITY TESTS (induction of PPAR coactivator or corepressor recruitment):

BIO201 (norbixin) shows no agonist effect on any of the three PPARs, but has an antagonist effect on PPAR β/δ (K_a ca. 3.2 μ M)

Effect of various pharmacological agents

Compound	Effect
PPAR α agonist	Sulindac does not protect RPE cells and does not maintain PPAR α levels
PPAR α antagonist	MK886 protects RPE cells and maintains PPAR α levels
PPAR β/δ agonist	GW0742 protects RPE cells and maintains PPAR α levels
PPAR β/δ antagonist	GSK3787 reduces protection by norbixin
PPAR γ agonist	Troglitazone protects RPE cells and maintains PPAR α levels
PPAR γ antagonist	T0070907 reduces protection by norbixin and troglitazone

APOPTOSIS: BIO201 (norbixin) cytoprotective activity correlates with a significant reduction of both ROS levels ($p < 0.01$) and apoptosis induction upon treatment with antimycin A.

CONCLUSIONS

- These experiments have revealed the difficulty of interpreting experimental data, arising from the limited specificity of current PPAR agonists and antagonists, a potential source of multiple cross-reactions (e.g. troglitazone).
- There is some discrepancy between activation studies and binding experiments, which suggests that PPAR activation is mostly indirect.
- A2E induces PPAR α degradation, and all the tested compounds showing a protection of RPE cells against blue light damage in the presence of A2E are able to sustain PPAR α levels. Thus the presence of PPAR α appears required for photoprotection.
- Agonists of PPAR γ and PPAR β/δ protect RPE cells, whereas their antagonists reduce the protective effect of BIO201 (norbixin). Thus, both PPARs seem involved in BIO201 action.
- Additional strategies (siRNA, shRNA, ...) are under way in order to relieve remaining ambiguities and assess definitively the role of the three PPARs in BIO201 (norbixin) protective action.
- **Norbixin** is the active principle of **BIO201**, a 97% pure 9'-cis isomer prepared from the seeds of *Bixa orellana*. BIO201 has been formulated for improving its stability and *per os* bioavailability as **Macuneos**. Biophytis will start a phase 1 clinical trial in 2017 with Macuneos as an oral treatment for dry AMD.