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

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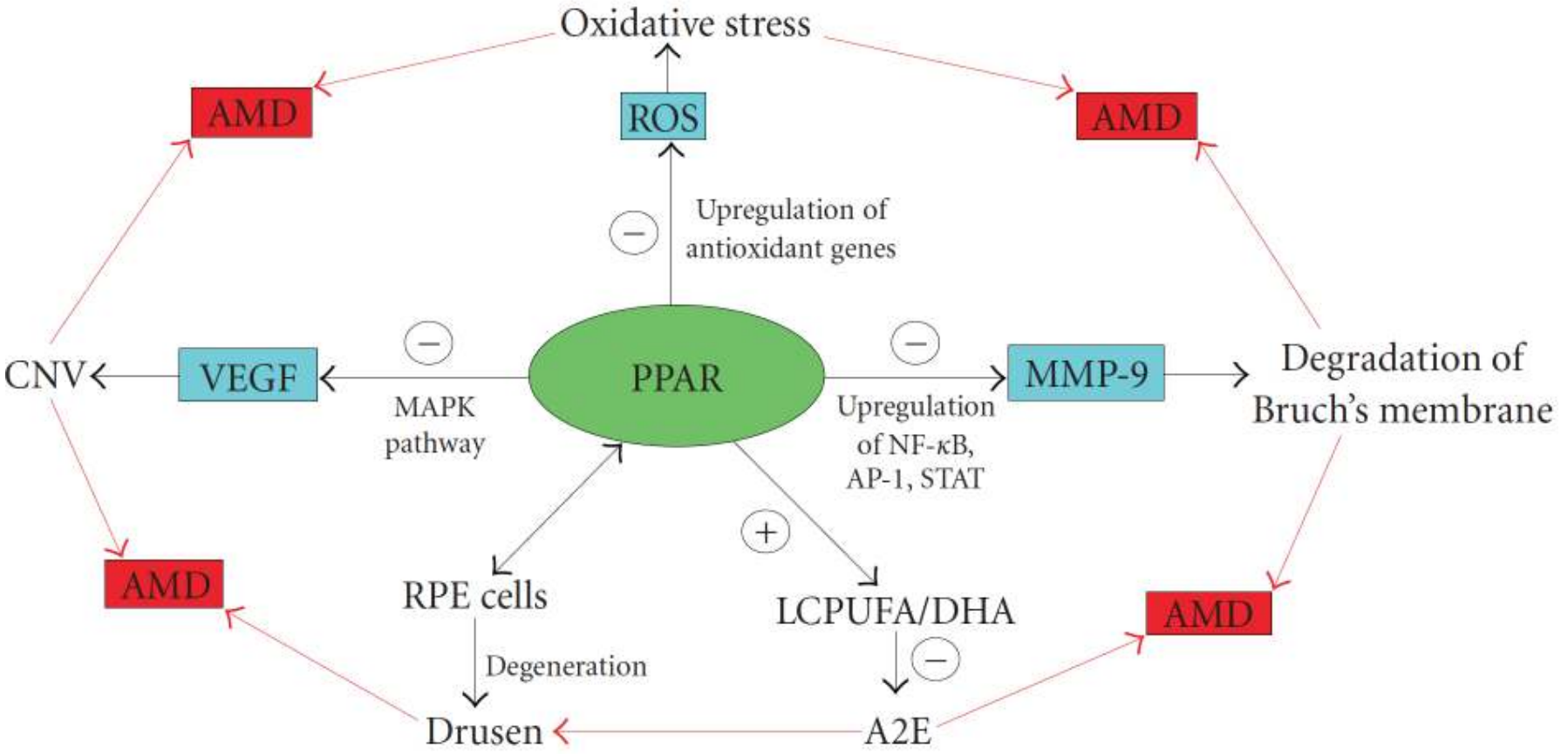
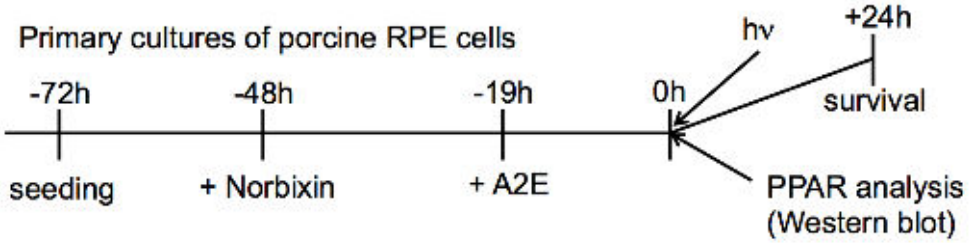
INTRODUCTION

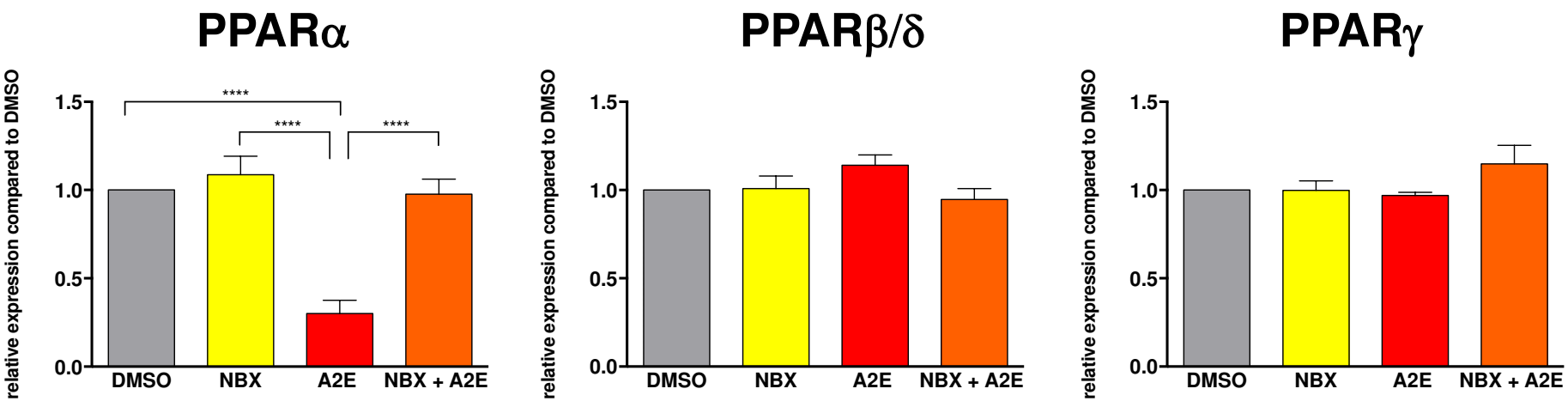
According to Herzlich (PPAR Res. 2008 Article ID 389507), PPARs play a key role in the protection of RPE cells against Age-Related Macular Degeneration



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

A2E sensitizes RPE cells to blue light damage and reduces PPARα protein levels. Both effects are blunted by BIO201, a preparation of the diapo-carotenoid norbixin developed by Biophytis[1].
No significant effect is observed on other PPARs levels.



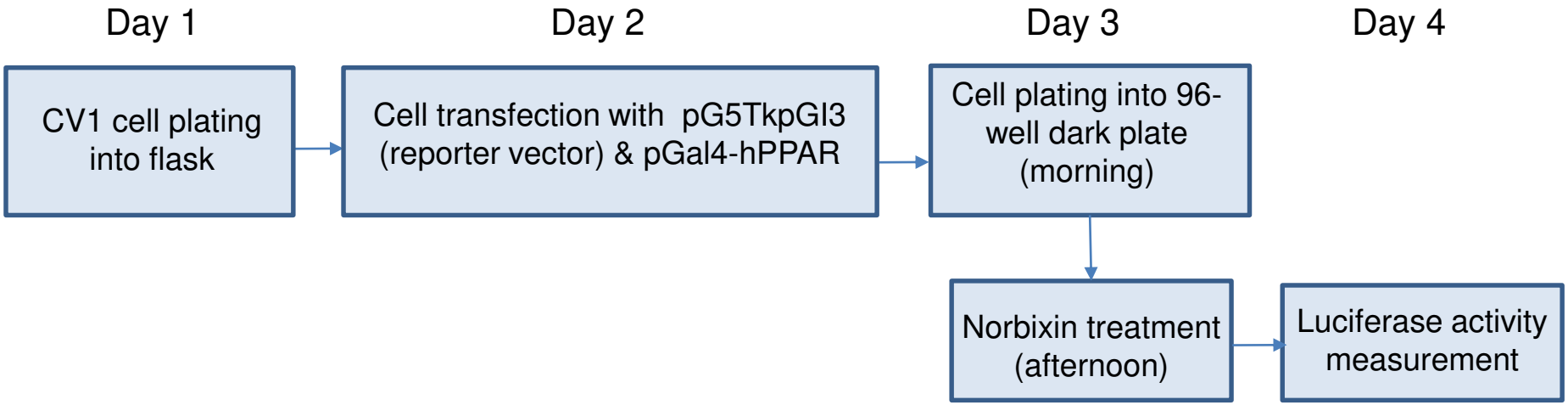


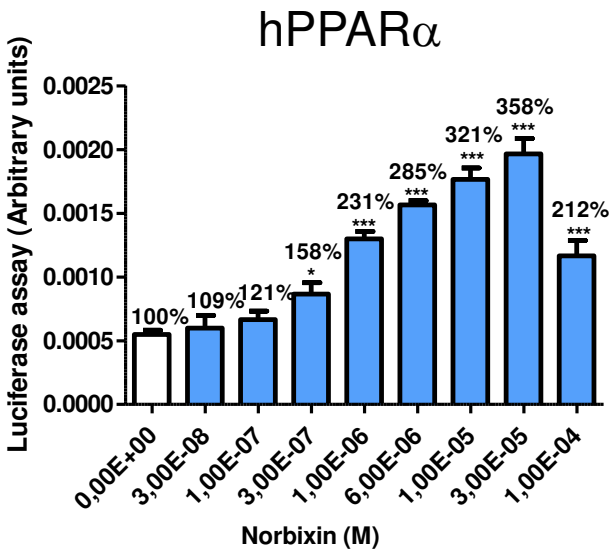
NBX: norbixin 20 µM
A2E: 30 µM

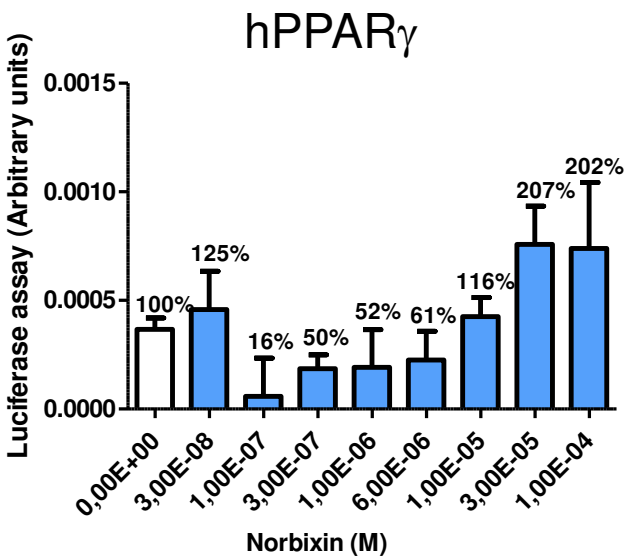
The effect on PPARα is post-translational and is also observed with inverted protocols (where treatment with A2E precedes that with norbixin)

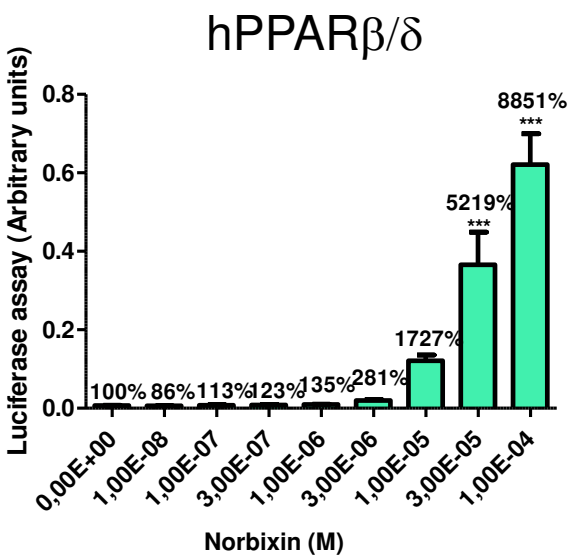
[1](Fontaine et al., PLoS ONE 2016, DOI:10.1371/journal.pone.0167793).

BIO201(norbixin) activates hPPARα and even more efficiently hPPARβ/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 PPARα with a K_i of 16.5 µM, and to PPARγ with a K_i of 1.15 µM

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 PPARβ/δ (K_a *ca.* 3.2 µM)

Effect of various pharmacological agents

Compound	Effect
PPARα agonist	**Sulindac** does not protect RPE cells and does not maintain PPARα levels
PPARα antagonist	**MK886** protects RPE cells and maintains PPARα levels
PPARβ/δ agonist	**GW0742** protects RPE cells and maintains PPARα levels
PPARβ/δ antagonist	**GSK3787** reduces protection by norbixin
PPARγ agonist	**Troglitazone** protects RPE cells and maintains PPARα levels
PPARγ 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 PPARα 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 PPARα levels. Thus the presence of PPARα appears required for photoprotection.

• Agonists of PPARγ and PPARβ/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.

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