INVOLVEMENT OF PEROXISOME PROLIFERATOR ACTIVATOR RECEPTORS (PPARs) IN THE PHOTOPROTECTIVE ACTIVITY OF BIO201

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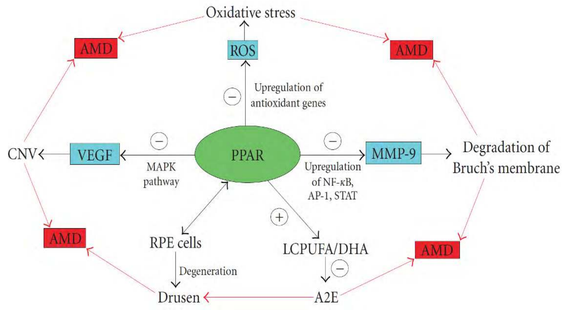
Introduction

According to Herzlich et al. (PPAR Research, 2008, article ID389507), PPARs play a key role in the protection of RPE cells against AMD.



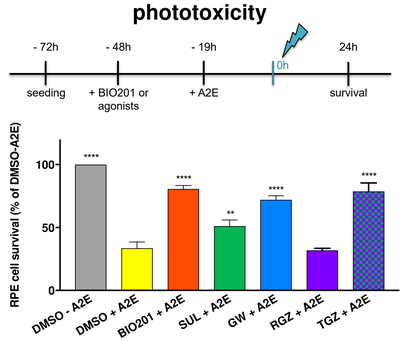
There is increasing evidence for the involvement of PPARs in the protection against the development of Age-Related Macular Degeneration (AMD). PPAR receptors comprise three sub-types of nuclear receptors (PPARα, PPARβ/δ and PPARγ) involved in the control of lipid and carbohydrate metabolism, as well as inflammatory processes. They are characterized by a large ligand-binding pocket and therefore accommodate diverse types of endogenous or exogenous ligands. BIO201 is a pharmaceutical grade preparation of the di-apo-carotenoid norbixin. It 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. The mechanism by which BIO201 exerts *in vitro* and *in vivo* protective effects on RPE was studied.

Materials and Methods

The photoprotective effect of BIO201 was tested in an *in vitro* model of phototoxicity of porcine primary RPE cells challenged with A2E then blue-light illuminated. Cell survival was measured 24h after illumination. BIO201 protective activity was then characterized by a pharmacological approach employing selective agonists and antagonists of the different PPAR subtypes.
In order to study the antioxidant and anti-apoptotic properties of BIO201, Arpe-19 cells were treated with antimycin A and PENAO, two pro-oxidant compounds, and ROS and PARP cleavage were measured by flow cytometry (DCFDA) and Western Blot, respectively, in the presence or the absence of BIO201 (30 µM).
To determine whether BIO201 was able to activate the 3 PPAR isoforms, Cv-1 cells were transfected with luciferase reporter plasmid (pG5-TK-pGL3) in the presence of pGal4hPPAR$_x$ (the vector expressed chimeric proteins containing the Gal4 DNA-binding domain fused to a human PPAR ligand-binding domain coding sequence) expression vector, and luciferase was measured after 16h contact with various concentrations of BIO201 (or other substances).
The expression level of the PPARs was assessed by Western Blot.
Statistical analyses were performed by one-way ANOVA followed by Dunnett's test.

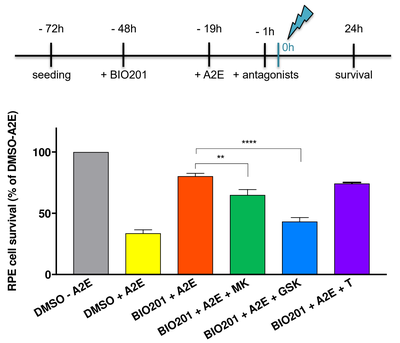
Results

BIO201, PPARα and PPARβ/δ agonists protect RPE cells against A2E-mediated phototoxicity



**p<0,01 ****p<0,0001 compared to DMSO + A2E

BIO201 protection is partially reversed by PPARβ/δ and PPARγ antagonists

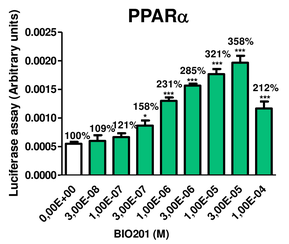


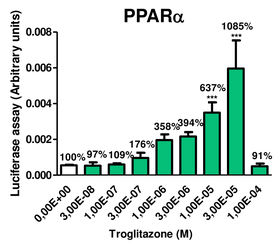
**p<0,01 ****p<0,0001 compared to BIO201 + A2E

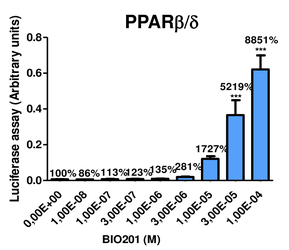
Molecule	Abbreviation	Function	Conc. (µM)
sulindac	SUL	PPARα agonist	200
GW0742	GW	PPARβ/δ agonist	30
rosiglitazone	RGZ	PPARγ agonist	20
troglitazone	TGZ	PPARα, γ dual agonist	20
MK886	MK	PPARα antagonist	5
GSK3787	GSK	PPARβ/δ antagonist	1
T0070907	T	PPARγ antagonist	10

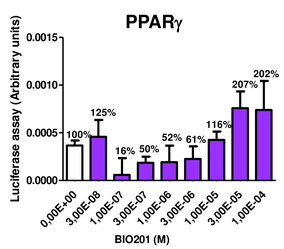
BIO201, as well as sulindac, GW0742 and troglitazone significantly protect RPE cells against A2E-indiced phototoxicity. Rosiglitazone, a specific PPARγ agonist, shows no photo-protective activity.
BIO201 protective activity is partially reversed by PPARα and PPARβ/δ antagonists.

BIO201 activates hPPARα and, even more efficiently, hPPARβ/δ









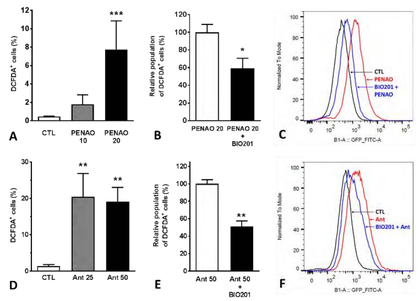
BIO201 is able to significantly trans-activate PPARα (C ≥ 0.3 µM) and PPARβ/δ (C ≥ 3 µM), but it does not trans-activate PPARγ.

Troglitazone, which is in fact a dual PPARα/γ agonist indeed activates PPARα (C ≥ 1 µM). This result suggests that the photoprotective effect of troglitazone on RPE cells is mediated by its interaction with PPARα.

These experiments however do not prove a direct binding of BIO201 to PPARs.

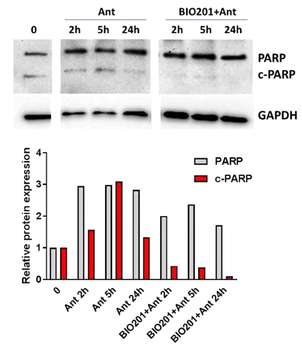
*p<0,05 **p<0,001 compared to not treated

BIO201 reduces ROS production and abolishes PARP cleavage in Arpe-19 cells



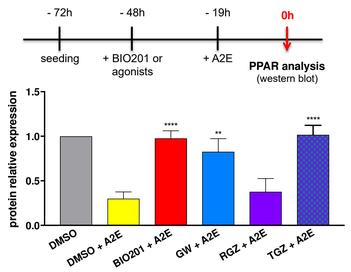
*p<0,05, **p<0,01 ***p<0,00, Mann-Whitney test

BIO201 (30 µM) significantly protects ARPE-19 cells against ROS induced by PENAO (A, B, C) or Antimycin A (D, E, F).



BIO201 (30 µM) decreases c-PARP expression induced by Antimycin A in ARPE-19 cells, suggesting the protection from ROS-induced apoptosis.

BIO201 counteracts PPARa protein degradation induced by A2E



**p<0,01 ****p<0,0001 compared to DMSO + A2E

We observe here for the first time a rapid and profound reduction of PPARα protein expression upon A2E treatment. This is abolished when RPE cells are treated with BIO201 before A2E exposure. GW0742 and troglitazone, two photoprotective PPAR agonists, display similar patterns. On the contrary, rosiglitazone, which is lacking cytoprotective activity, is unable to sustain PPARα protein expression. PPARα expression is not modified by BIO201 or PPAR agonists when used alone.
PPARβ/δ and γ protein expression were not affected by A2E treatment.

Conclusions

- Both photoprotection and transactivaction results indicate that BIO201's beneficial effect on RPE cells is linked with PPARα and/or PPARβ/δ activation.
- BIO201 inhibits ROS production and apoptosis.
- Prevention of PPARα protein degradation appears to be a key feature in BIO201 photoprotective activity.
- Further experiments are underway with BIO201 (i) to better understand the crosstalk between the PPARs and (ii) to elucidate the signaling pathways involved in RPE cell survival.

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