Supplementary Materials: Anti-Oxidant, Anti-Inflammatory and Anti-Angiogenic Properties of Resveratrol in Ocular Diseases

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1. Measurement of COX-1 and COX-2 Activity

Trans-resveratrol (Resv) was acquired from Sigma-Aldrich (St. Louis, MO, USA). It was tested using the COX fluorescent inhibitor screening assay kit (Cayman, Ann Arbor, MI, USA) according to the authors of Reference [1]. Scalar concentrations (specified in the graphs) of Resv were tested on purified, ovine COX-1 and human recombinant COX-2. The commercial inhibitors SC-560 and DuP-697 active on COX-1 and COX-2 isozymes, respectively, were used as controls.

The fluorescent product of the reaction (Resorufin; excitation wavelength 530–540 nm; emission wavelength 585–595 nm) was quantified using a fluorimeter (Fluostar Omega, BMG labtech, Ortemberg, Germany).

2. Estimation of *in Vitro* Anti-Inflammatory and Anti-Angiogenic Properties

Cultured human ARPE-19 cells (human retinal pigment epithelial cells) (American Type Culture Collection, Manassas, VA, USA) were grown, according to [2], in DMEM/F12 medium (Gibco-Invitrogen, Cergy-Pontoise France) containing 10% heat-inactivated fetal calf serum (56 °C for 30 min), antibiotics (100 IU/mL penicillin, 100 μg/mL streptomycin) (Gibco), and 1 mM sodium pyruvate (Gibco). The cells were seeded at 25–32 × 10^3/cm² in 75-cm² tissue cultured flasks (Falcon) containing 13 mL of culture medium. They were incubated at 37 °C in a humidified atmosphere of air containing 5% CO_2. The culture medium was changed every 2 days, and the cells were passaged once a week by trypsinization (0.05% trypsin-0.02% EDTA) (Gibco).

ARPE-19 cells were exposed to 20 μg/mL of LPS (0128:B12) and co-treated during 24 h with *trans*-resveratrol at 50, 30, 10 or 1 μM (see above for origin and purity) according to [3]. Cell supernatant were collected and the levels of 6 cytokines (IL-8, IL-1b, IL-6, IL-10, TNF α, and IL-12p70) were measured by flow cytometry using multiplex Cytometric Bead Array Kit (BD Bioscience). Finally, the level of the major angiogenic factor VEGF-A was measured in these media by ELISA (eBioscience, Paris, France).

References

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