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# Anti-proliferative and anti-migratory effect of hyperforin on human dermal fibroblasts

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**Introduction:**
Hyperforin (HYP), a bicyclic polyprenylated phloroglucinol derivative, is one of the main active ingredients of Hypericum perforatum [1]. Apart from its known antidepressant activity, other effects such as anti-inflammatory, anti-microbial, anti-proliferative and differentiation-inducing action were identified after in vitro application of HYP on keratinocytes [2]. Therefore, HYP was suggested as an interesting candidate in topical therapy of skin diseases such as psoriasis, atopic dermatitis or fibrosis. In the present study HYP was used as stable hyperforin dicyclohexylammonium salt (HYP-DCHA). The influence of different HYP-DCHA concentrations on primary human dermal fibroblasts (HDF) isolated from neonatal foreskin was investigated with different assays in order to substantiate the potency of HYP and to establish an experimental in vitro setup for prospective investigations of HYP-DCHA formulations.

**Methods:**
HDF were cultivated in DMEM supplemented with 10 % fetal calf serum (FCS), 2 mM l-glutamine and antibiotics under standardized culture conditions. HYP-DCHA was dissolved in DMSO, subsequently diluted with medium to different concentrations with a final DMSO concentration of 0.1% (v/v). Viability of HDF was performed with an MTT assay. Additionally a cytotoxicity assay (CellTox™ Green cytotoxicity assay, Promega) was performed as well as a life-dead staining with fluorescent dyes, i.e., Hoechst 33342, calcein and propidium iodide. For all these assays the cells were incubated for 24 h along with the different HYP-DCHA concentrations.

Investigation of a potential anti-proliferative effect was done by determining the cell number of HYP-treated cells once a day over a time period of 4 days with a Z2 Coulter-Counter system (Beckman Coulter, Krefeld, Germany).

A scratch assay was performed in order to analyze the impact of HYP-DCHA on the migration behavior of treated cells. Micrographs of the scratch were taken with a confocal laser scanning microscope Nikon eclipse Pi (Nikon, Tokyo, Japan) half-hourly within at least 16.5 h.

**Results:**
The MTT test showed no significant loss of viability upon HYP-DCHA incubation at concentrations of 50 nM to 1 µM. At 5 and 10 µM HYP-DCHA, a slight decrease in viability was

detected, whereas the cytotoxicity assay did not display any cytotoxicity at any tested HYP-DCHA concentration. The latter was confirmed by life-dead staining which did not show any cytotoxicity of HYP-DCHA. The cell counting experiment clearly showed a dose-dependent anti-proliferative effect of HYP-DCHA on HDF cells. That means in the present study, the decrease in viability is not an indication of cytotoxycity but with regard to the reduce cell number a strong hint at an anti-proliferation effect. In Addition the scratch assay clearly showed an anti-migratory effect of HYP-DCHA-treated HDF cells at a 10 µM HYP-DCHA concentration.

**Conclusion:**
HYP-DCHA does not only affect human epidermal cells as previously described in literature but also inhibits proliferation and migration of HDF cells.

**References:**
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