10-Hydroxystearic Acid – A Bio-Derived Lipid to Counteract Aging Effects on Human Skin

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INTRODUCTION

Intrinsic aging is superimposed to photoaging, which is induced by UV light as major cause for premature aging [1,2]. In particular, aged skin shows prominent alteration in the extracellular matrix of dermal connective tissue [3,4].

It is well known that fatty acids play a key role as membrane components, metabolic fuel and gene regulators [5]. Some of the latter are ligands of peroxisome proliferator-activated receptors (PPARs), a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. Recent findings indicated that PPARα activation protects from UV-induced damage and intrinsic aging [6].

HYPOTHESIS

Fatty acids screening revealed 10-hydroxylstearic acid as PPAR agonist that might mitigate aging effects. The aim of this study was to sustainably produce *(R)*-10-hydroxystearic acid (10-HSA) and substantiate its biological effects against aging markers *in vitro* and *ex vivo*.

MATERIAL AND METHODS

Reporter Gene Assay for PPAR transactivation and EC_{50} determination

The applied mammalian one hybrid system is based on a co-transfection assay using the PPARα, PPARβ or PPARγ receptor construct, the luciferase reporter plasmid and a renilla-expressing plasmid (pRL-tk) for normalization.

Synthesis of *(R)*-10-Hydroxystearic Acid

The hydration of oleic acid (81%, KLK OLEO, Germany) was catalyzed by the oleate hydratase (EC:4.2.1.53) from a cell-free extract produced in *Escherichia coli* which yielded the enantiopure *(R)*-10-hydroxyoctadecanoic acid (content ≥99w/w%; INCI name: hydroxyl stearic acid) from representative pilot batches.

Measurement of Collagen type I and III in Fibroblasts

Biological evidence was obtained by High Content Analysis for collagen type 1 and type 3 on primary human dermal fibroblast cultures after treatment with test compounds for 48 h.

Ex vivo Skin Treatment

Human skin from abdominal plastic surgery was used after informed consent of the donors. Test samples and control (4 µl) were topically applied on skin biopsies (Ø 8 mm) 24 h and 1 h prior UVB irradiation (1 J/cm^2) for MMP-1, sunburn cells, and p53 analysis. For collagen type 3 the samples were applied daily for 6 days. We used immunohistochemistry (IHC) on human *ex vivo* skin for the analysis of collagen type 3 and p53 protein. Sunburn cells were counted after on haematoxylin/eosin-stained (H&E) skin sections. UVB-induced MMP-1 expression was determined by RT-qPCR from full thickness skin.

RESULTS AND DISCUSSION

10-Hydroxystearic acid is a PPARα agonist

We defined the half-maximal effective concentrations (EC_{50}) of 10-hydroxstearic acids for all known peroxisome proliferator-activated receptors (PPARs). 10-HSA preferably bound to PPARα, while EC_{50} values for PPARβ and PPARγ could not be detected (>15 µM). Interestingly, the enantiomer *(R)*-10-hydroxystearic acid (10-HSA) seemed to be more active than the racemic mixture of 10- or 9-hydroxstearic acids.

Table 1: EC_{50} concentrations for ligand binding to PPARα. GW 7647 is a selective agonist of human PPARα (Cayman Chemicals, USA) and was used as reference (EC50 = $1.21 \cdot 10^{-8}$). All concentrations were tested in biological triplicates.

Structure	EC_{50} [µM] PPARα
(R)-10-hydroxystearic acid (10-HSA)	5.54
9-hydroxystearic acid (racemate)	12.3
10-hydroxystearic acid (racemate)	11.9

Bioconversion from oleic acid resulted in enantiopure *(R)*-10-hydroxystearic acid

To consider the aspect of sustainability replacing classical chemical synthesis, we established a regio- and enantioselective bioconversion of vegetable-derived oleic acid to *(R)*-10-hydroxystearic acid by a highly active hydratase (yield 81 %, purity >99.5%, Fig. 1).

Figure 1. Production of 10-HSA on kilogram scale in the 200 L Reactor Mini Plant

RESULTS AND DISCUSSION

10-HSA stimulated Collagen type 1 and type 3 synthesis in human skin fibroblasts

10-HSA significantly and dose-dependently induced collagen on human dermal fibroblasts (Fig. 2A/B) after 48 hours of incubation. The amount of collagen type 1 doubled and collagen type 3 synthesis was stimulated by 244% compared to the medium control when 10-HSA was added at 5 µM concentration.

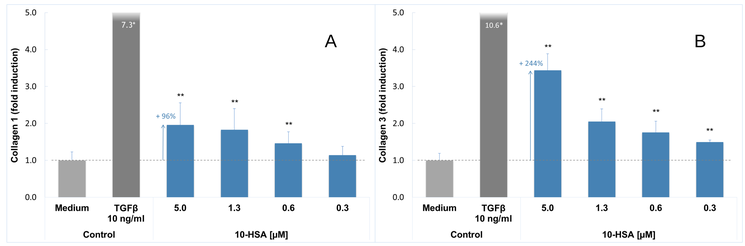

Figure 2. Collagen type 1 (**A**) and type 3 (**B**) stimulation in primary human dermal fibroblasts. Mean values ± SD, n=3, * p<0.01 relative to medium control.

10-HSA markedly induced *ex vivo* synthesis of collagen type 3

IHC staining of the skin sections topically treated with 10-HSA at 0.1% concentration showed a significant stimulation of collagen type 3 by 57% compared to the control at day 6 (Fig. 3).

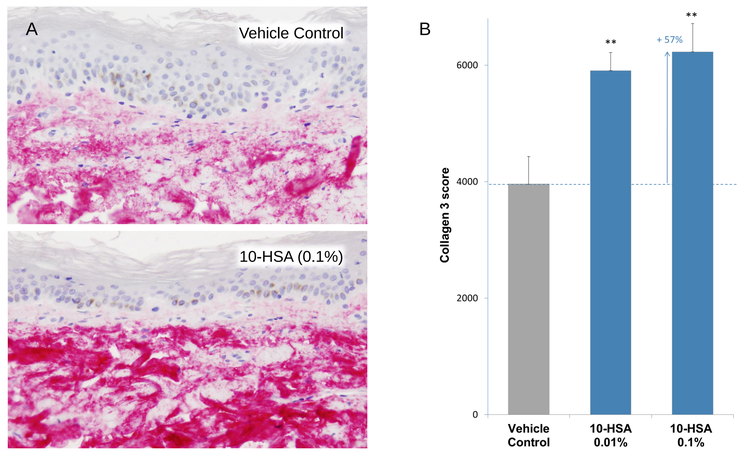

Figure 3. **A)** IHC staining for type 3 collagen in human *ex vivo* skin. Comparison of vehicle (DMSO) vs. 10-HSA treatment (0.1% = 3.3 mM) at day 6. **B)** Quantification of type 3 collagen of IHC stained sections. Mean values ± SEM, n=12, * p<0.01 significance relative to vehicle control.

UVB-induced MMP-1 expression, sunburn cells and p53 protein were reduced *ex vivo* by 10-HSA

Gene expression of **MMP-1** was upregulated by a factor of 3.4 one day after the UVB irradiation (1 J/cm^2; Fig. 4A). A significant decrease of 83% in MMP-1 expression was observed in skin explants topically treated with 0.1% 10-HSA compared the irradiated vehicle control.

As expected, UVB irradiation significantly increased the incidence of **sunburn cells** more than two-fold vs. non-irradiated control (Fig. 4B). Treatment with 0.1% 10-HSA reduced the formation of sunburn cells by 49% compared to the vehicle-treated, UVB-irradiated controls.

Interestingly, commercial sunscreens (SPF 50+) only partially protected the induction of both biomarkers, although a relatively high amount of 8 mg/cm^2 was applied onto the skin surface.

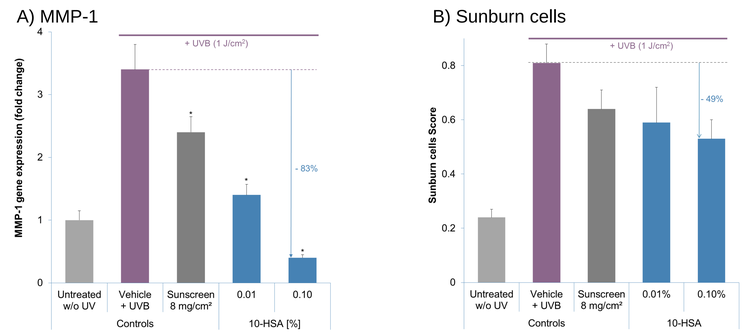

Figure 4. Comparison of *ex vivo* treatments vs. vehicle (DMSO) 24 h after UVB irradiation. **A**) UVB–induced MMP-1 gene expression from skin sections by RT-qPCR. Sunscreen: La Roche-Posay, Anthelios 50+. **B)** Sunburn cells quantification on H&E-stained skin sections. Sunscreen: Eucerin 50+ Kids. Mean values ± SEM, n = 12; * p<0.05 vs. vehicle control (DMSO) + UVB.

Stress marker **p53** protein was induced 80-fold on immunostained sections 24 hours after UVB irradiation (1 J/cm^2) of human skin biopsies. (Fig. 5). Our test compound 10-HSA inhibited UV-induced p53 formation by 46% vs. irradiated vehicle control skin.

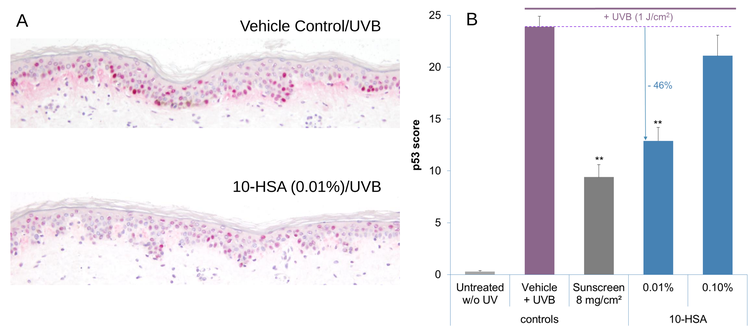

Figure 5. **A**) IHC stained skin section for p53 analysis. **B**) Image analysis of p53-stained skin sections. Mean values ± SEM , n = 12, ** p<0.01 significance vs. vehicle (DMSO) control.

CONCLUSIONS

- Naturally occurring 10-hydroxystearic acid was identified as PPARα agonist
- 10-HSA can be produced by a sustainable bioconversion with a regio- and entioselective hydratase
- 10-HSA acts as a potential anti-aging active by significantly modulating aging and photo-aging markers

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