Deferiprone Stimulates Aged Dermal Fibroblasts via HIF-1α Modulation

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Abstract

Background: Hypoxia-inducible factor 1α (HIF-1α), a transcription factor responsible for tissue homeostasis and regeneration, presents reduced functionality in advanced age. In addition to absence of oxygen, sequestration of iron also stimulates HIF-1α. Therefore, we analyzed the efficacy of the iron-chelator deferiprone (DFP) at stimulating dermal fibroblasts.

Objectives: The main objective of this study was to quantify the DFP concentrations capable of stimulating dermal fibroblasts in vitro and to correlate the effective DFP concentrations with the ability of DFP to penetrate the epidermis, reach the dermis, and activate HIF-1α in vivo.

Methods: We measured cell proliferation, metabolic activity, HIF-1α expression, and lactate dehydrogenase levels of both young and aged fibroblasts after a 24-hour in vitro preconditioning with DFP. In addition, we evaluated cell survival rates and morphology with different cellular stainings. Finally, we performed a transdermal permeation study with a 1% DFP topical formulation to quantify the concentration required to reach the dermis.

Results: In vitro administration of iron-chelation therapy (156-312.5 µg/mL DFP) on aged fibroblasts resulted in activation of various antiaging processes. The concentration required to reach the dermis within 24 hours was 1.5% (0.15 mg/mL), which corresponds well with the effective doses of our laboratory analyses.

Conclusions: The activation of HIF-1α by DFP enhances cell metabolism, proliferation, and survival of fibroblasts while reducing lactate dehydrogenase levels. Modulation of HIF-1α is linked to activation of key regeneration enzymes and proteins, and by proxy, antiaging. Therefore, the antiaging properties of DFP and its satisfactory dermal penetration make it a promising regenerative agent.

Editorial Decision date: May 22, 2020; online publish-ahead-of-print June 1, 2020.

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Aging is a phenomenon affecting all living beings. At a cellular and molecular level, aging is determined by, among other factors, environmental influences, genetic predisposition, and nutrition. Age-related skin changes are mainly triggered by the combination of intrinsic (e.g., DNA oxidation and damage accumulation) and extrinsic factors (e.g., UV exposure and smoking). Whereas intrinsic aging is a degenerative process representing the biological clock of the human body, external aging, also known as “photoaging,” is the result of skin exposure to external factors, most importantly UV radiation. The combination of these changes results in significant impairments of the regenerative capacity of the skin. To date, most cosmetic products only provide adequate skin hydration, rather than an agent capable of delivering long-lasting effects. No single approach is currently able to address all facets of age-related skin degeneration, namely, epidermal and dermal atrophy, loss of connective tissue, and diminished vascularization.

Recent evidence suggests that all these changes hinder the skin’s intrinsic healing capacity and thereby lead to chronic wounds as well as impaired tissue homeostasis in aged skin. The hypoxia-inducible factor 1 (HIF-1) pathway represents a key mechanism in both conditions. HIF-1 is a master transcription factor which regulates hypoxia response element (HRE) genes. This signaling pathway manifests itself in a hypoxic response which leads to enhanced proliferation, survival, and neovascularization. HIF-1 is essential for skin homeostasis and is mainly expressed in the basal layer of the epidermis. The intracellular presence of HIF-1α activates over 100 downstream genes, chiefly responsible for cell processes such as angiogenesis, cell proliferation, migration, and glucose metabolism.

Advanced age, similar to diabetes and other degenerative skin diseases, has been shown to correlate with attenuated HIF-1 function. HIF-1 consists of a highly regulated α subunit and a constitutively expressed β subunit. The oxygen-sensitive prolyl-hydroxylase (PHD) is the direct enzymatic regulator of HIF-1α. In aging, PHD is increased, resulting in excessive HIF degradation and subsequent impaired release of growth factors, reduced neovascularization, as well as inadequate tissue quality and regeneration. This enzyme therefore represents a target to rescue senescing dermis. Specifically, the active site of PHD consists of Fe, an iron moiety that can be targeted by iron chelators. In this way PHD can be sequestered and inactivated, inhibiting its function of HIF-1α degradation, and allowing dimerization of the HIF-1 molecule, kickstarting the HIF pathway (Figure 1).

In this study, we compared activation of the HIF-1 pathway by the iron chelator deferiprone (DFP; 3-hydroxy-1,2-dimethyl-4(1H)-pyridone; MW 139.154 g/mol) in young and aged fibroblasts. Aged as well as young fibroblasts were treated with various concentrations of DFP in order to identify the effective concentrations able to act as HIF stimulating factor (HSF), inducing a reactivation of aging-depleting mechanisms and positively affecting the skin. Considering the prospective dermatologic application of this molecule, we also performed a transdermal permeation study, in order to correlate laboratory observations with potential clinical translation.

**METHODS**

The penetration experiment was conducted in collaboration with the laboratory of Eurofins ADME BIOANALYSES (Vergeze, France) according to established protocols. We followed the Organisation for Economic Co-operation and Development guideline for testing of chemicals and skin permeation (guideline 428, April 2004). The human skin samples (mean donor age, 62 years; 400 μm thick) were obtained under institutional review board approval through Eurofins ADME BIOANALYSES.

**Cell Acquisition and DFP Preconditioning**

DFP was obtained from Donau Kanol (Ried im Traunkreis, Austria) and the 24-well plates were purchased from Greiner (#662160). Aged and young dermal fibroblasts from healthy donors were purchased from Promocell GmbH (Heidelberg, Germany) and tested between November 2017 and May 2018. The donors of the aged fibroblasts were 60 (male) and 65 (female) years old, and the female donor of the young fibroblasts was aged 29 years. All fibroblasts were cultured in Dulbecco’s modified Eagle’s medium (Biochrom Ltd, Cambridge, UK) supplemented with 10% fetal calf serum (heat inactivated) and 1% penicillin/streptomycin/amphotericin B (Capricorn Scientific, Ebdsdorfergrund, Germany). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO2. After 5 passages, various concentrations of DFP (10, 5, 2.5, 1.25, 0.625, 0.3125, 0.156, and 0.078 mg/mL) were initially evaluated to assess the effective dose, and analysis was performed after 24 hours of DFP exposure as described below. In order to examine the long-term effects of DFP treatment, medium was changed 24 hours after constant DFP administration and samples were analyzed 4 and 8 days after DFP treatment. All conditions were performed in triplicate.

**Cell Proliferation**

Cells were counted with a Casy Cell Counter (OLS, Bremen, Germany), an electric field, multichannel, cell-counting system. The number of viable cells was used to monitor cell proliferation.
Live/Dead Staining

Living/dead cells were detected with calcein AM (1 \(\mu\)g/mL) and propidium iodide (2 \(\mu\)g/mL). The live/dead staining kit (Thermo Fisher Scientific, Waltham, MA) was used according to the manufacturer’s instructions. Photographs were taken with an Axio Observer Z1 fluorescence microscope and analyzed with Axiovision software (Carl Zeiss AG, Oberkochen, Germany).
**HIF-1α Expression: Enzyme-Linked Immunosorbent Assay**

HIF-1α (92 kDa) subunit expression was determined in cell culture lysates after 24 hours of treatment with 156.25 µg/mL DFP (Duoset; R&D Systems, Minneapolis, MN). The assay was carried out as described in the manufacturer’s protocol. Plates were analyzed with a Mithras LB 940 microplate reader (Berthold Technologies GmbH &Co KG, Bad Wildbad, Germany).

**Metabolic Activity**

To determine metabolic activity, WST-1 assay (Roche Life Science, Penzberg, Germany) was performed as described in the manufacturer’s protocol. Absorbance at 450 nm was measured in a Mithras LB 940 microplate reader.

**Lactate Dehydrogenase (LDH) Release**

After incubation, 100 µL of supernatant was taken from each well and analyzed with a cytotoxicity detection kit (Roche Life Science, Penzberg, Germany) according to the manufacturer’s instructions. A Mithras LB 940 microplate reader was used for optical density measurements.

**β-Actin Staining**

For β-actin cytoskeleton staining, fibroblasts were fixed with 3.7% formaldehyde, permeabilized with 0.2% Triton X-100 in phosphate-buffered saline (PBS), and stained with Phalloidin-TRITC (0.5 µg/mL; Sigma-Aldrich, St Louis, MO) for 1 hour at 37°C. DAPI (0.75 ng/mL; Thermo Fisher Scientific, Waltham, MA) was used as counterstaining. Photographs were taken with an Axio Observer Z.1 fluorescence microscope and analyzed with Axiovision software.

**In Vitro Skin Permeation**

To translate our findings into a clinical setting, a drug permeation study was performed on February 17, 2019 in order to evaluate the in vitro dermal absorption of a DFP-carrying cream. A vertical Franz diffusion cell model was used as previously described.26 We manufactured a topical cream containing 1% (10 mg/mL) DFP. Briefly, dermatomized human skin samples (between 300 and 400 µm thick) from abdominalplasty samples were excised and cut into small pieces (2 cm × 2 cm). The dimension and thickness of these samples were ideal for testing molecule permeation, and the permeation rate was comparable to that of facial skin.

Skin samples were mounted between the 2 compartments of the diffusion cell with the stratum corneum facing the donor compartment (n = 3, 3 replicates). The DFP preparation was homogeneously applied (2.5 mg/cm²) on each skin sample in order to simulate the conditions of human exposure. Isotonic PBS solution agitated with a magnetic stirrer and maintained at 37°C by a circulating water jacket was used as a receptor phase. Following 24 hours of cream exposure the skin samples were removed, washed with PBS, and dried with an absorbent towel. The skin samples were frozen at –20°C and later analyzed for drug content via high-performance liquid chromatography.

**Statistical Analysis**

All assays were performed in triplicate in at least 3 independent experiments. Data were expressed as mean [standard deviation]. All statistical analysis was performed with Microsoft Excel version 16.0 and GraphPad Prism. We performed t tests for pairwise comparisons; P < 0.05 was considered statistically significant.

**RESULTS**

**Preconditioning with 156.25 µg/mL DFP Significantly Accelerates Cell Proliferation**

After incubation with various DFP concentrations we calculated the numbers of living cells at 1, 4, and 8 days after adding DFP. Treatment with 156.25 µg/mL DFP resulted in aged fibroblasts proliferating at a significantly higher rate (P < 0.01) than aged fibroblasts treated with other DFP concentrations (Figure 2). Therefore, this concentration was selected for the following experiments.

**DFP Administration Increases HIF-1α Protein Levels**

We next confirmed that our selected concentration was sufficient to stimulate HIF-1α expression on a cellular level. Following administration of 156.25 µg/mL DFP for 24 hours, we observed the stimulation of HIF-1α levels on both young and aged fibroblasts. HIF-1α levels after DFP treatment in aged fibroblasts were comparable to the levels in young cells (Figure 3).

**DFP Treatment Enhances Survival Rate of Aged Fibroblasts**

After a continuous treatment with 156.25 µg/mL DFP for 24 hours, viability and cell proliferation were significantly increased in both young and aged fibroblasts (P < 0.05). Remarkably, the proliferative capacity of aged fibroblasts after 24 hours of DFP treatment was comparable to that of untreated young fibroblasts (Figure 4).
DFP Upregulates Cell Metabolism and Simultaneously Reduces LDH Levels

After 24 hours of treatment with 156.25 µg/mL DFP, aged fibroblasts present a significantly higher metabolic activity ($P < 0.05$). The enhanced metabolic activity in aged fibroblasts exceeded the effects observed in young fibroblasts (Figure 5A). Interestingly, 24 hours of treatment with 156.25 µg/mL also led to a significant reduction of LDH release ($P < 0.05$), suggesting reduced cellular stress. The reduced cellular stress in aged fibroblasts shows no significant difference when compared with the reduction observed in young fibroblasts (Figure 5B).

Aged Fibroblasts Treated with DFP Display Normal Cell Morphology

Normal and healthy fibroblast morphology was confirmed after DFP treatment as an indicator of the health of a cell population. Preconditioned fibroblasts displayed a morphology comparable to that of control cells, which was verified by staining of the actin filaments in the cytoskeleton (Figure 6).

DFP Can Penetrate Human Skin in an Effective Dose

We used a 1% (10 mg/mL) DFP formulation homogeneously applied on each skin sample ($n = 3$, 3 replicates). The mean absorption of the skin was 1.50% [0.65%] of the applied dose, ie, 150 [65] µg/mL DFP. Considering our in vitro findings identifying 156.25 µg/mL DFP as an efficient dose to stimulate aged fibroblast, these data suggest that DFP can be effectively delivered transdermally via a topical formulation (Figure 7). The results of the DFP permeation study are summarized in Table 1.
Figure 4. Deferiprone (DFP) treatment enhances the survival rate of aged fibroblasts. Cell viability and proliferation are significantly increased in both young and aged fibroblasts \( (P < 0.05) \) after 24-hour continuous treatment with 156.25 µg/mL DFP. Both (A) young and (C) aged untreated dermal fibroblasts show no particular proliferative activity after 24 hours.
DISCUSSION

The physiologic activation of HIF-1α is significantly involved in tissue regeneration. Therefore, activation of this network could potentially have antiaging properties, through the action of new collagen strains, elastin, glycosaminoglycans, and nutritive blood vessels. Recent studies on human epidermal cells showed that the controlled upregulation of HIF-1α substantially increases the growth potential of keratinocytes and fibroblasts, improving the formation of viable and stratified epidermis. It has been further reported that various growth factors, such as fibroblast growth factor 9, transforming growth factor α, platelet-derived growth factor B, and insulin-like growth factor 1, are regulated by HIF in different cell lines. In addition to peptide growth factors, HIF further induces cell proliferation via activation of extracellular signal-related kinase (ERK) signaling. Suppression of dual-specificity protein phosphatase 2, an ERK-specific phosphatase, by HIF-1α prolongs ERK phosphorylation and thus may promote cell proliferation.

Our in vitro analysis of the viability and survival rate of fibroblasts showed significant effects of DFP preconditioning. This could be related to the fact that HIF-1α activates the transcription of different genes encoding BNIP3, a BH3-domain protein capable of inducing selective mitochondrial autophagy. Autophagy triggered by HIF-1-dependent BNIP3 expression is required for cell survival under conditions of prolonged hypoxia. The reduced cytoplasmic iron concentrations are potentially able to activate this network, allowing fibroblasts to achieve higher survival rates and higher metabolic activities, resulting in general activation of antiaging processes.

Interestingly, although the metabolic activity was increased in aged fibroblasts, younger fibroblasts were not significantly altered. Limiting iron in younger cells creates artificial anemia, which may affect the younger fibroblasts in a more profound way relative to the aged fibroblasts, which experience an enhanced activation of the HRE mechanism. Because fibroblasts of different ages respond differently to pharmacology, it is impossible to say whether DFP harms younger cells.

In addition, the trend of reduced activity was not significant, nor was the difference in activity without DFP. An explanation could be the fact that DFP treatment may affect

Regarding DFP-treated fibroblasts, both (B) young and (D) aged fibroblasts present a high proliferative activity. (B) Young DFP-treated fibroblasts present even a higher proliferation rate than (D) aged DFP-treated fibroblasts. As previously observed in our preliminary experimental studies, the proliferative capacity of aged fibroblasts after 24-hour treatment with DFP (D) is higher than the proliferative capacity of untreated young fibroblasts (A) (scale bars, 200 µm).
aged fibroblasts more profoundly than young fibroblasts, based on a selective improvement of HIF activity.

As previously mentioned, HIF-1 upregulation in aged fibroblasts via iron chelators has been described as exceeding hypoxic HIF-1α stabilization in young cells. This was corroborated by findings of global augmentation of HIF-1α. Systemic iron chelator therapy promotes neovascularization only in areas of ischemia and did not produce neovascularization in uninjured skin in a mouse model. This selective effect was confirmed by Duffy et al., who demonstrated no significant effect of DFP on human endothelial cells in the absence of ischemic coronary artery disease. Therefore, it can be argued with reasonable confidence that DFP treatment should not harm or alter younger cells without any HIF deficiencies.

In addition to HIF-1α, the PHD2-VHL pathway further regulates HIF-2α, which dimerizes with HIF-1β and activates target gene expression. Increased levels of both HIF-1α and HIF-2α are potentially able to synergistically strengthen cellular networks. Recent studies suggest that HIF-2α may regulate SOD2 and other genes encoding antioxidant proteins essential for cell survival. Lastly, the local reduction of cytoplasmic iron concentrations is potentially able to influence epigenetic factors, including

**Figure 6.** Aged fibroblasts treated with deferiprone (DFP) display normal cell morphology. Normal and healthy fibroblast morphology was confirmed after DFP treatment (B, D), an indicator of the health of the cell populations. In addition to acting as scaffold, the red cytoskeleton is key to cytoplasmic transport, cellular division, and signaling. The nuclei are colored blue. Preconditioned fibroblasts (B, D) displayed a morphology comparable to that of control cells (A, C), which was verified by staining of the actin filaments in the cytoskeleton.
Deferiprone (DFP) can penetrate human skin in an effective dose. In a Franz diffusion cell model the mean absorption of skin was 1.50% [0.65%] of the applied dose, ie, 150 [65] µg/mL DFP. Based on our in vitro findings that identified 156.25 µg/mL DFP as the most efficient dose to stimulate aged fibroblasts, these data suggest that DFP can be effectively delivered transdermally via a topical formulation.

DNA methylation and histone acetylation, in order to modulate hypoxia-responsive gene expression of aged fibroblasts. The lack of free iron profoundly affects expression of many noncoding RNAs classes that may have different implications in skin rejuvenation.

We report herein the impact of DFP towards HIF-1α expression and cell survival only after 24 hours of DFP preconditioning, in order to highlight its intrinsic ability to act in a very short timeframe. In our preliminary analyses, we observed that DFP significantly enhances cell survival after 1, 4, and 8 days. No further increase was observed at the 12th and 15th day after DFP administration. The most evident images are those at 24 hours, which we decided to report here. This behavior highlights the ability of the DFP to act as an effective HIF-1α upregulator, a cell survival inductor, and an ideal molecule for potential daily clinical use. This cellular behavior could moreover exclude potential DFP-mediated and uncontrolled proliferation during the subsequent days after treatment.

The possibilities of a therapeutic modulation of hypoxia-inducible signaling pathways by repurposing iron chelators are promising and we were able to demonstrate that an upregulation of HIF-1α stimulates aged fibroblasts effectively. However, in most cell types, and even in fibroblasts with DFP concentrations higher than 625 µg/mL, the reduction of cytoplasmic concentrations of iron leads to decreased cell proliferation. Interestingly, with a well-controlled DFP administration at specific concentrations we observed significant rejuvenation of aged fibroblasts. A low-dose DFP application of 156.25 µg/mL over a limited period of 24 hours achieved the highest efficacy. This concentration is potentially able to penetrate the epidermis, reach the dermis, and affect dermal fibroblasts in the most effective way. However, we obtained good results even with higher or lower concentrations (eg, 78.125, 312.5, and 625 µg/mL). In conclusion, despite the wide variability, both lower and higher DFP concentrations act positively by improving cell proliferation, survival, and viability through the different skin layers. Based on these data, the local impact of the different concentrations of DFP would not adversely affect the structure and/or the functionality of the skin layers.

The use of DFP in an intermittently applied topical formulation could lead to a significant improvement in skin quality. Many individuals apply skincare products for approximately 12 to 16 hours between cleansing. Hence, this time frame could be the most appropriate one for DFP preconditioning. However, the maximum reliability and the highest significance of employed cellular stainings is reached mainly at the 24-hour evaluation time point. Even the permeation test was performed after a 24-hour topical treatment. Therefore, we decided to analyze our samples after 24 hours, in order to obtain the most accurate laboratory tests and the closest approximation of clinical reality.

We have in this study compared the effective concentration of DFP able to generate downstream effects of the HIF-1 pathway on both young and aged fibroblasts. We further assessed DFP permeation to explore the possibility of DFP transdermal efficacy. The in vitro analyses determined the most effective concentration of DFP to be 156.25 µg/mL. By administering a 1% (10 mg/mL) DFP-carrying cream we were able to demonstrate that 1.5% (0.15 mg/mL) DFP topically applied reaches the dermal layer of the skin. This suggests that DFP has the ability to act dynamically across the dermis. This critical characteristic differentiates it from other agents currently used in cosmetic medicine, such as highly hydrophilic hyaluronic acids, which due to their nature and molecular size do not easily cross the stratum corneum. We acknowledge the limitations of our study, namely, the use of only female-derived fibroblasts, the small sample size of the ex vivo tissue group, and analysis of only the acute effect of DFP on cellular behavior. These limitations represent clear future research focuses, which would help promote DFP as a safe and effective skin care product.
Table 1. Individual Results Obtained After Deferiprone Application on Human Skin (1% w/w Deferiprone) in a Vertical Franz Diffusion Cell Model

<table>
<thead>
<tr>
<th>Test substance applied, ng</th>
<th>Skin, ng</th>
<th>Skin, µg/cm²</th>
<th>Skin, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>5.38</td>
<td>0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>B</td>
<td>4.43</td>
<td>0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>C</td>
<td>4.43</td>
<td>0.03</td>
<td>0.71</td>
</tr>
<tr>
<td>D</td>
<td>4.92</td>
<td>0.04</td>
<td>0.88</td>
</tr>
<tr>
<td>E</td>
<td>4.92</td>
<td>0.04</td>
<td>0.88</td>
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<tr>
<td>F</td>
<td>4.92</td>
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<tr>
<td>G</td>
<td>4.92</td>
<td>0.04</td>
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<tr>
<td>H</td>
<td>4.92</td>
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<tr>
<td>I</td>
<td>4.92</td>
<td>0.04</td>
<td>0.88</td>
</tr>
<tr>
<td>Mean</td>
<td>4.92</td>
<td>0.04</td>
<td>0.88</td>
</tr>
<tr>
<td>SD</td>
<td>0.19</td>
<td>0.01</td>
<td>0.05</td>
</tr>
<tr>
<td>CV (%)</td>
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<td>9.81</td>
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A topical 1% (10 mg/mL) deferiprone formulation was homogeneously applied on each skin sample (n = 3, 3 replicates). The mean absorption of the skin was 1.50% (0.65%) of the applied dose, ie, 0.35 (0.15) µg/cm². The association of our experimental analyses and this transdermal study enables our observations towards a translational approach. CV, coefficient of variation; SD, standard deviation.

CONCLUSIONS

The possibilities of a therapeutic modulation of hypoxia-inducible signaling pathways by repurposing iron chelators are promising. An upregulation of HIF-1α mediated by iron chelation stimulates aged fibroblasts effectively. We were able to demonstrate that the in vitro administration of DFP has the potential to activate the HIF-1 pathway in aged fibroblasts, resulting in enhanced cell proliferation, survival, and metabolism while reducing cellular stress and preserving physiologic cell morphology. Translation of this formula to human skin showed sufficient penetration, demonstrating its efficacy and applicability in clinical use. If it can be supported by solid clinical trial data, HIF-1 pathway modulation could prove a promising new paradigm in aesthetic medicine.

Disclosures

Drs Thor and Duscher are co-founders of, and have equity positions in, Tomorrowlabs GmbH, a commercial-stage biotech company that produces products based on HSF technology. The other authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Funding

Tomorrowlabs GmbH provided research grant support for the study.

REFERENCES


