Clearing senescent cells for rejuvenated skin

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Abstract

Cellular senescence is one of the hallmarks of ageing. Senescent cells that reside in the dermis as a result of the ageing process and oxidative stress, secrete pro-inflammatory factors that further contribute to ageing. Therefore, eliminating senescent cells has emerged as a promising anti-ageing therapy in the medical field in the past few years. This novel concept known as 'senolytics' helps to clear tissues of senescent cells without affecting healthy cells in order to reduce inflammation and rejuvenate the tissue. For the first time, this concept has been adapted for cosmetics. An extract from organic alpine rose leaves demonstrated a clear senolytic activity on senescent fibroblasts. In placebo-controlled clinical studies, the alpine rose extract prevented the formation of protein carbonyls, one of the most harmful irreversible modifications of proteins, upon UVA irradiation. In addition, treatment with alpine rose extract significantly reduced skin redness and increased elasticity.

Fibroblasts in the dermis are responsible for the production of collagen to form the connective tissue, as well as for assisting in the wound healing of the skin. When these fibroblasts either age by telomere shortening through too many cell divisions or encounter too much oxidative stresses, such as UV light and pollution, which cause damage beyond repair, they face a choice. They can either continue to divide despite the accumulated damage that would be propagated to their daughter cells, which potentiates the harmfulness. A second option is to undergo apoptosis, programmed cell death, to stop themselves from spreading the damage. However, there is a third option, which permanently blocks the cells from undergoing further cell divisions: the cells become senescent. Senescent cells are also called 'zombie cells'[1] for the following reason: whilst they no longer divide, they are also far from being dead. Senescent cells block an intracellular pathway that promotes their apoptosis in order to prevent their own elimination. Moreover, they continue to secrete signalling molecules such as cytokines that promote inflammation and can influence surrounding cells into also becoming senescent (Fig 1).[2] In younger tissue, senescent cells are usually cleared by the immune system. In aged skin and skin that has been exposed to consistent stress, the large number of senescent cells can no longer be cleared by the immune system without help and they therefore accumulate. The resulting chronic inflammation exacerbates the ageing process by promoting collagen degradation, which leads to a lack of skin elasticity. Not surprisingly, the formation and accumulation of senescent cells is one of the hallmarks of ageing.[3] As a result, eliminating senescent cells has emerged as a promising anti-ageing therapy in the medical field in the past few years.

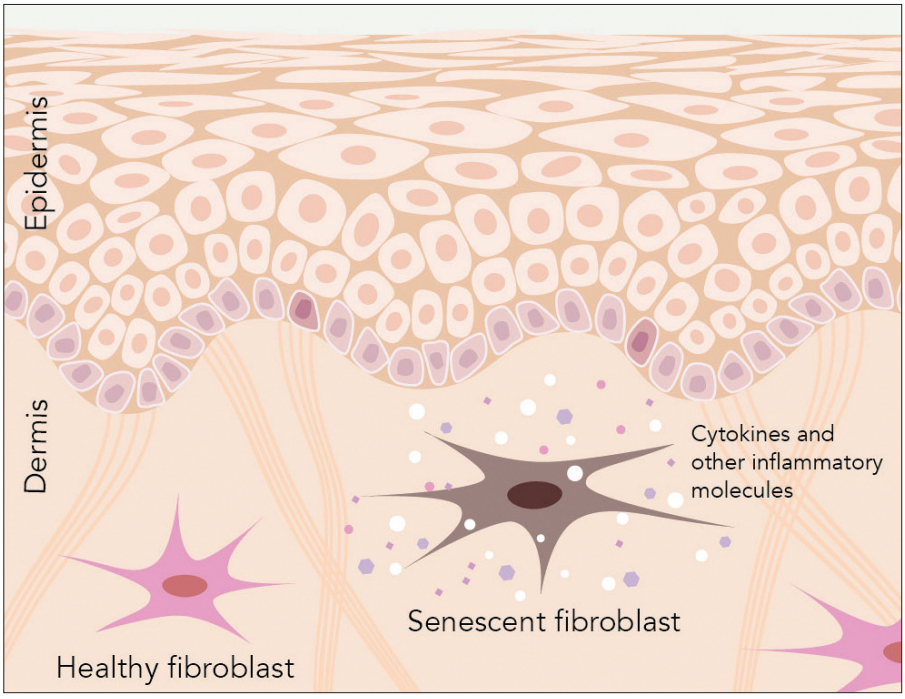

Figure 1: Schematic representation of a senescent fibroblast in the dermis. Secretion of cytokines by these senescent cells leads to constant inflammation in the skin, which results in collagen degradation and accelerated skin ageing.

Senolytic drugs to promote longevity

A novel concept known as 'senolytics' helps to clear tissues of senescent cells in order to reduce inflammation and rejuvenate the tissue. The term senolytics was coined in 2015 by researchers from the Mayo Clinic and the Scripps research institute in the US, who described a mechanism through which senescent cells are selectively eliminated without harming healthy dividing cells.[4] The targeted senescent cells are going into apoptosis and are subsequently cleared from the tissue. Clearing senescent cells both reduces negative effects of ageing pathologies and extends median lifespan.[5] Despite being a brand-new life science topic, there have already been more than 200 scientific publications that investigate the senolytic activity of numerous compounds. So far, the concept of

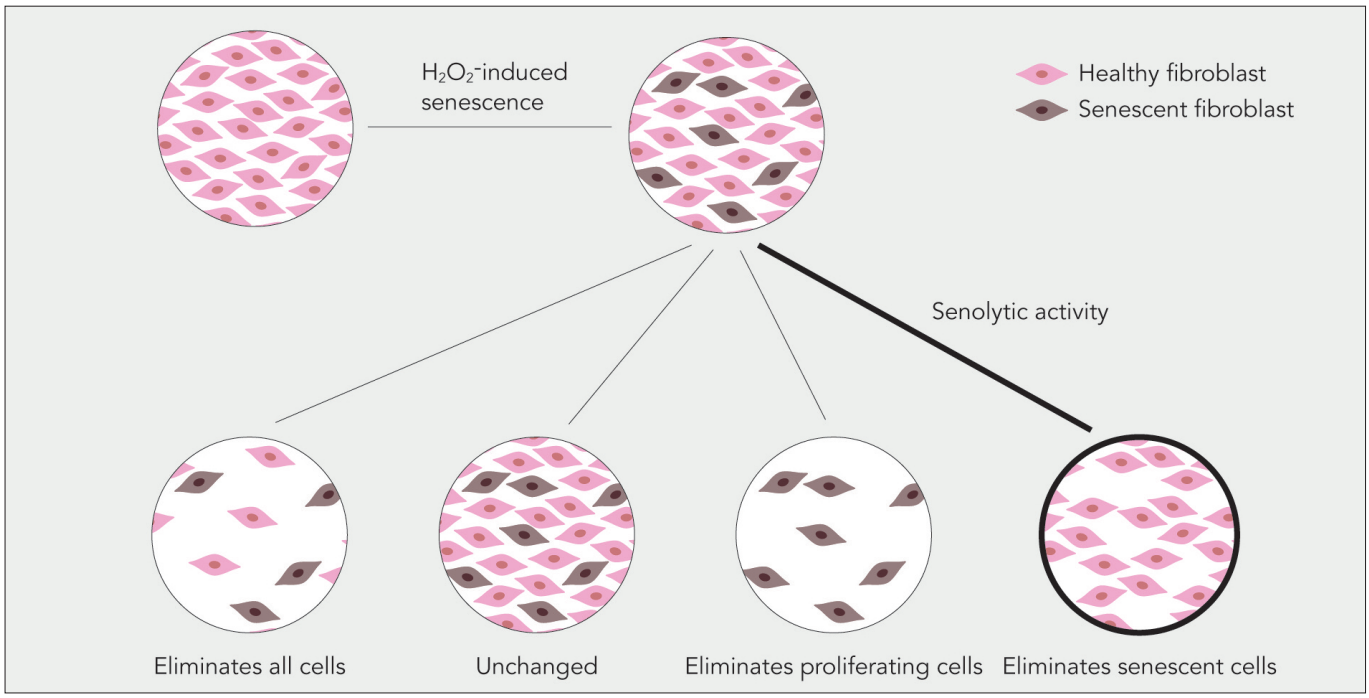

Figure 2: Schematic representation of the senolytic study concept. The desired senolytic activity depicted on the right results in the depletion of senescent cells while not affecting the healthy cell number.

senolytics has not yet been applied in the cosmetic field despite its great potential for skin rejuvenation.

Protein carbonylation

Environmental stress factors, such as UV light, infrared radiation, tobacco smoke, and pollution, generate reactive oxygen species (ROS). ROS oxidise proteins and lipids, which are the main components of cell membranes. This leads to carbonylation, which is one of the most harmful irreversible modifications of protein structure. Under normal conditions, the resulting carbonylated proteins are recycled by the proteasome. However, age and an increase in oxidative stress factors impair the proteasome activity, which leads to a further addition of lipid aldehydes to these carbonylated proteins resulting in their crosslinking. These high-molecular-weight superstructures resist degradation and accumulate over time, and they become cytotoxic and accelerate ageing by promoting cellular senescence. Therefore, the content of carbonylated proteins is the major indicator of oxidative damage and a hallmark of ageing.

An extract from organic Swiss alpine rose leaves

Rhododendron ferrugineum, which is also known as alpine rose, is one of the most typical and iconic plants of the Swiss Alps. To produce an active ingredient for cosmetics, the leaves of alpine roses were handpicked by organic farmers in the Swiss Alps through controlled wildcrafting. The extract from these leaves (INCI: Rhododendron Ferrugineum Extract (and) Glycerin (and) Aqua / Water, from here on alpine rose extract) inhibits the formation of carbonylated proteins and therefore protects the skin against the formation of premature senescent cells. Where cellular senescence has already occurred, the alpine rose extract can help to clear these cells from the tissue thanks to its senolytic activity.

Materials and methods

Senolytic assay

In order to distinguish between the prevention of senescence and true senolytic activity, Normal Human Dermal Fibroblasts were first stressed with 500 µM H_2O_2 for 2 hours to induce premature senescence through oxidative stress. The medium was then exchanged, and the cells were grown for 3 days to fully establish the senescent phenotype in a subpopulation of the cells. This mixture of senescent and healthy fibroblasts was then treated for 48 hours with either 1% alpine rose extract or Navitoclax (Cayman Chemical, Ann Arbor, USA), a known senolytic drug, or not treated (control). Following fixation with 2% formaldehyde and 0.2% glutaraldehyde, cells were stained with DAPI and the relative total cell number was determined by fluorescence measurement. Senescence-Associated β-galactosidase activity assay was performed according to (6) and a total of 400 cells were counted. Counting the β-gal-positive cells as a marker for senescence and calculating the percentage compared to the total cell number revealed the treatment efficacy.

In vivo protein carbonylation assay

A double-blind, placebo-contolled clinical study was performed with 12 volunteers (9 female/3 male, 40 - 54 years) who applied a placebo cream and 2% alpine rose extract in

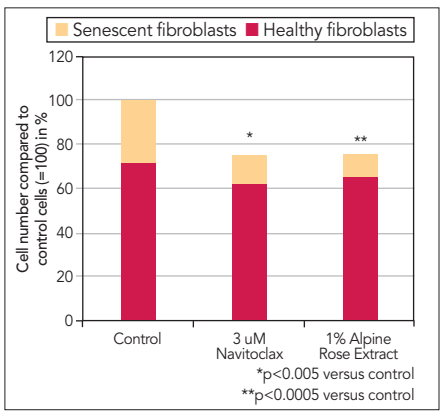

Figure 3: The alpine rose extract exhibits senolytic activity. Cell numbers of senescent and non-senescent cells are shown normalised to control cells in which senescence was induced with hydrogen peroxide.

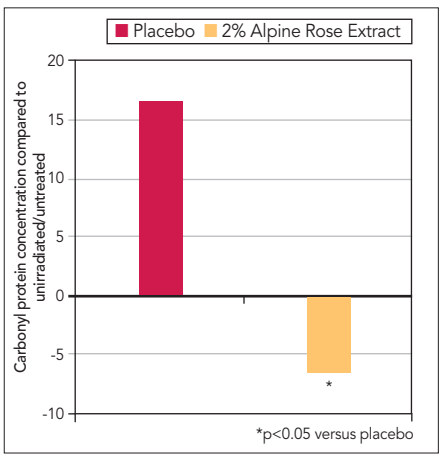

Figure 4: Prevention of protein carbonyl formation by the alpine rose extract upon UVA stress *in vivo*.

the same cream formulation, twice daily over a period of 2 weeks on each of their forearms. After 2 weeks of product application, the treated test sites were irradiated with 10.0 J/cm² UVA-light (solar-simulator SOL 500, Dr. Hönle, Munich, Germany), equipped with an H1 UV-filter blocking the UVB-portion of the solar spectrum. An untreated and unirradiated test site served as a control. Immediately after the irradiation, suction blister fluid samples (diameter 7.0 mm) were taken from the test sites and subsequently analysed with an ELISA-kit (Immundiagnostik AG, Bensheim, Germany) to measure the content of protein carbonyls in these fluids.

Clinical anti-ageing study

In a double-blind, placebo-contolled clinical study, forty-four Caucasian women aged between 40 and 65 years (mean age: 55 years) with redness on the cheeks were split into two groups. One group applied a cream with 2% alpine rose extract and the other group applied the corresponding placebo cream on the entire face and neck twice daily for 28 days. Skin colour was measured using a Spectrocolorimeter CM700-d (Konica Minolta, Japan) and skin elasticity was determined with a Cutometer MPA 580 (Courage + Khazaka, Germany). In addition, macrophotographs were taken before and after treatment with the Visia skin analysis system (Canfield Scientific, Germany).

Results and discussion

Alpine rose extract has senolytic activity

A challenge when screening for a senolytic effect is to distinguish between senescence delaying actives, such as antioxidants that minimise the damage that could lead to senescence, and true senolytic activity. To assess the latter, senescence was induced in fibroblasts by treatment with H_2O_2 for 2 h first and cells were then cultured for three more days. Only afterwards when the senescent phenotype is established, incubation with the potential senolytic active takes place. The number of senescent cells was quantified and compared to total cell number. Several different outcomes of the experiment are shown in Figure 2. Only actives that eliminate senescent cells while not affecting healthy fibroblasts are considered to possess senolytic activity (right option in Figure 2).

Treatment with 1% alpine rose extract significantly reduced the number of senescent cells while not affecting the number of healthy fibroblasts. Compared to control cells, which had 28.1% senescent cells in comparison to total cell number, treatment with alpine rose extract reduced that number to 10.1% senescent cells compared to the total cell number. The effect was similar to a treatment with the known senolytic drug Navitoclax, which reduced the percentage of senescent cells to 12.3% (Fig 3). The alpine rose extract therefore exhibits senolytic activity.

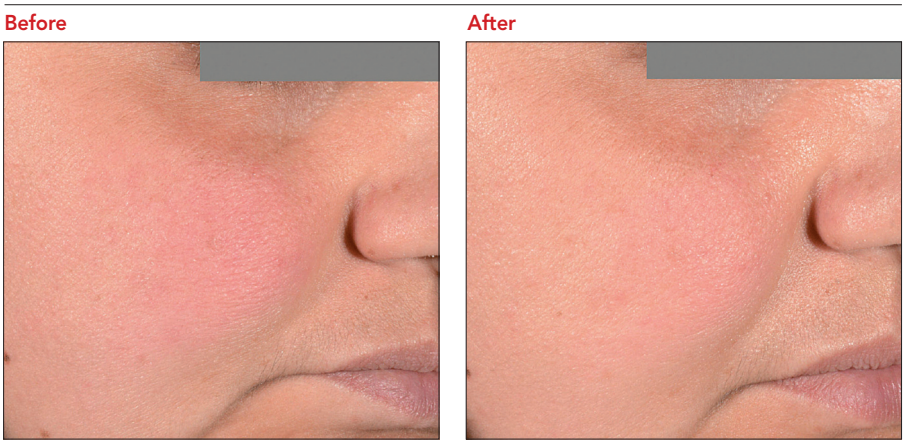

Figure 5: Before and after picture taken of a volunteer who applied 2% alpine rose extract twice daily for 14 days.

Alpine rose extract prevents protein carbonylation

To assess the ability of the alpine rose extract to protect against protein carbonylation *in vivo*, 12 female and male volunteers aged 40 to 54 years applied a cream with 2% alpine rose extract and a placebo cream twice daily for 14 days on the inner side of the forearms. After the final product application, the test sites were irradiated with 10 J/cm² UVA-light. Subsequently, suction blisters were induced, the suction blister fluids were collected, and their content of protein carbonyls was analysed as a marker of oxidative stress. The carbonyl protein content was significantly reduced in the suction blister fluid of the test site that was previously treated with alpine rose extract compared to the placebo-treated skin area (Fig 4). This indicated a protective effect *in vivo* against the damage caused by oxidative stress induced by UVA.

Increase in skin elasticity and decrease in redness

In another double-blind, placebo-controlled clinical study, twice daily application of 2% alpine rose extract for 14 days resulted in a reduction of the redness parameter a* by 8.4 %, which was significant compared to initial conditions as well as the placebo. The effect was also visible in macrophotographs taken of the volunteers (Fig 5). Furthermore, an increase in skin lightness by 2.1% was measured in volunteers who applied 2% alpine rose extract, which was significant compared to initial conditions and the placebo (data not shown). After 28 days of treatment, skin elasticity increased by 16.1%, which was significant compared to initial conditions and the placebo (Fig 6).

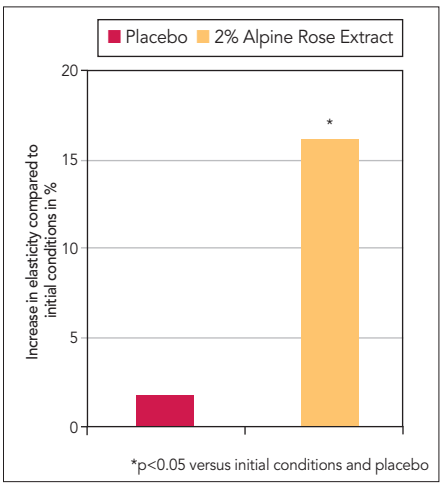

Figure 6: Increase in skin elasticity after 28 days treatment with 2% alpine rose extract.

Conclusion

An extract from organic alpine rose leaves inhibits the carbonylation of cutaneous proteins and therefore protect skin proteins against oxidative damage, a known cause of cellular senescence. When senescence has already occurred, the alpine rose extract eliminates senescent cells while not affecting healthy cells through its senolytic activity. In this way, skin redness is reduced, and skin elasticity is increased. PC

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