June 2020

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Innovative active fights Zombie cells for well-ageing

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Senescence is a cellular response characterised by morphological changes, a stable growth arrest (in order to prevent tumoral proliferation) and a change in the cell's secretome. Indeed, senescent cells, called Zombie cells, produce a large amount of senescence-associated secretory phenotype (SASP) which is responsible for a pro-inflammatory response, for collagen degradation, for free radical production, and for the transmission of the senescent state.[1] As a result, senescent cells tend to accumulate with age and 20 to 60% of the skin cells are actually senescent.[2]

The senescence process may be induced by several causes. Ageing is the most common phenomenon (after a certain number of division, a cell may go into senescence), but another frequent cause is stress, especially oxidative stress that induces inflammation. The combination of ageing and moderated inflammation caused by stress is called inflamm'aging, also known as "secret killer". This progressive and insidious phenomenon has visible effects in the long term and prevents the skin from ageing well.

When a cell is exposed to stress, there are a few possible outcomes. It can be repaired, it can undergo apoptosis (the programme cell death) if it is too severely damaged, or it can undergo senescence.

Apoptosis will induce a high energy expenditure since the cell will have to be replaced and requires the division of a healthy cell. The same is true for necrosis, which consumes less energy but also requires the remaining healthy cells to replace the eliminated cell. These two processes cause a significant energy impact for the body, and therefore for the skin.

Senescent cells still maintain their production activities although in a degraded form. Senescence thus seems to be an acceptable compromise at a lower energy cost. However, senescence is a source of extensive and chronic inflammation as described before, the inflamm'aging.

Scutaline (INCI name: *Scutellaria baicalensis* root extract) is an active ingredient made from the dried roots of the *Scutellaria baicalensis*, a plant traditionally used in Chinese medicine and naturally rich in two polyphenols: Baicalein and Wogonin that have been described to reduce SASP.[3] Baicalein is an activator of the Nrf2 pathway. It thus provides strong anti-oxidative benefits by stimulating the production of anti-oxidants such as NADP(H): Quinone oxidoreductase 1 (NQO1) and glutathione.[4] Baicalein is also a COX-2 inhibitor.[5] It therefore inhibits prostaglandin (PGE_2) synthesis and participates in reducing inflammatory symptoms like redness and pain.

Wogonin is a NFkB inhibitor that provides potent anti-inflammatory benefits.[6] It is also responsible for degrading HIF-1α (hypoxia inducible factor-1α) that is key for regulating the angiogenesis process that may limit inflammation and redness.[7] Using a patented extraction method, the concentration of these two molecules was maximised for stronger benefits to the skin.

We focused on a strategy consisting of slowing the entry of cells into senescence during ageing and also decreasing the ability of senescent cells to affect nearby healthy cells and to cause a chronic low-grade inflammation leading to skin premature ageing.

In this paper, we will present how Scutaline (referred to as *'Scutellaria baicalensis* root extract') is able to meet our strategy to help the skin to age well.

Preventing stress-induced senescence

Senescent cells present several specific markers such as an increased senescence-associated-ß-galactosidase (SA-ß-Gal) activity and have a local impact in the tissue due to the secretion of pro-inflammatory mediators and to an overproduction of MMP-1 that will lead to collagen degradation.[2]

In order to assess *Scutellaria baicalensis* root extract's ability to prevent senescence, human dermal fibroblasts were exposed to H_2O_2 for 2 hours and cultivated for 3 days in the presence or in the absence of *Scutellaria baicalensis* root extract.

While the exposure to H_2O_2 led to a dramatic increase in the number of senescent cells as assessed by monitoring

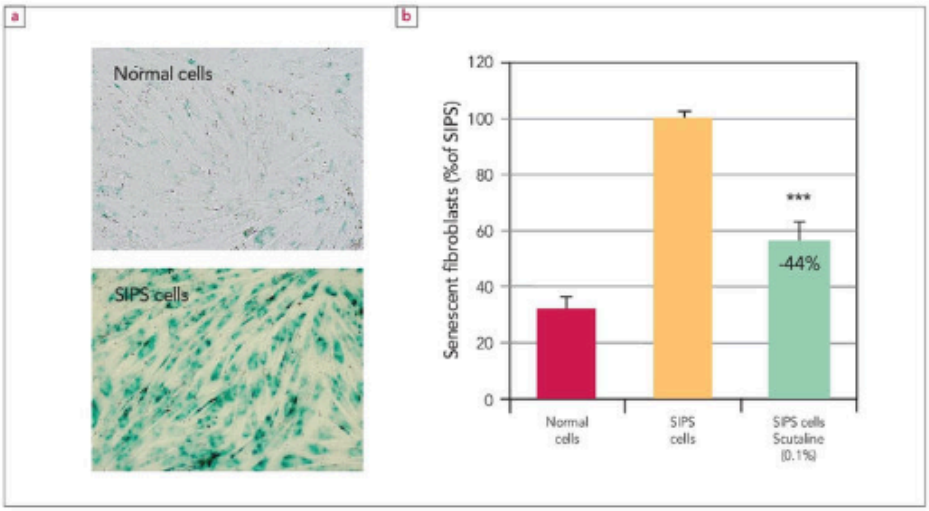

Figure 1: Scutaline prevents stress-induced premature senescence (SIPS).
NHDF were exposed to H_2O_2 for 2h, and for 3 days in the presence or in the absence of Scutaline.
Senescent fibroblasts were observed (a) and quantified (b).
Senescent cells appear in blue green. *** p-value<0.001 vs. SIPS cells, n=9-18.

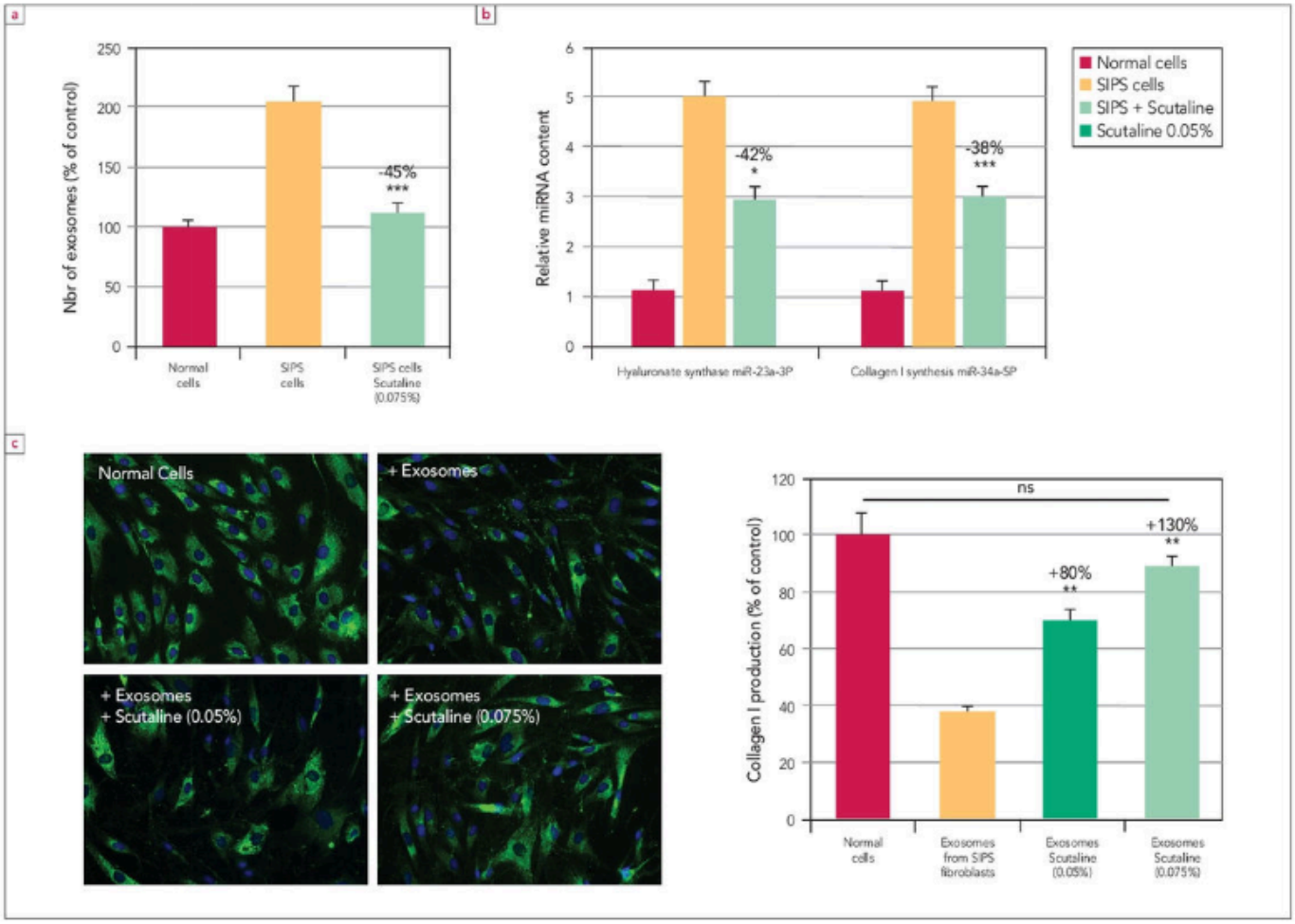

Figure 2: Scutaline prevents senescence spreading.NHDF were exposed to H_2O_2 for 2h and then left for 3-4 days. Cells were then cultivated in the presence or in the absence of Scutaline for 48h. The exosomes were then isolated and applied on normal fibroblasts for 72h. a) The number of exosomes produced was then quantified, b) the miRNA they contain were analysed by qRT-PCR, and c) the collagen I production of fibroblasts exposed to exosomes from SIPS fibroblasts was observed by immunofluorescence and quantified (collagen I appears in green and cell nuclei in blue (DAPI)).
*** p-value < 0.001, ** p-value < 0.01, * p-value < 0.05 vs SIPS, ns no significant vs normal cells.

SA-ß-Gal activity, treatment with *Scutellaria baicalensis* root extract (0.1%) decreased the number of senescent cells by 44%. *Scutellaria baicalensis* root extract thus provided a 65% protection against stress-induced premature senescence (SIPS) (Fig 1).

By preventing fibroblasts from entering into the senescence state, *Scutellaria baicalensis* root extract maintains their normal function and regulates their redox status, their energy production and their production of MMP-1 (data not shown). *Scutellaria baicalensis* root extract therefore acts in a preventive mode by limiting the natural process of senescence. This strategy allows skin that is still relatively young to defend itself against stress.

Decreasing the ability of senescent cells to promote senescence and to cause inflamm'aging

Senescent cells have the ability to spread senescence. This transmission process is mediated by exosomes which are small excreted vesicles of endoplasmic origin that contain microRNA (miRNA) specific to certain genes. Once these exosomes reach a nearby cell, they fuse to the membrane and release their content in this cell. miRNA will then inhibit the expression of specific genes in the target cell, and trigger the senescence phenotype.[8-10]

In order to assess the ability of *Scutellaria baicalensis* root extract to decrease the capacity of senescent cells to further promote senescence, fibroblasts under SIPS were cultivated for 48h in the presence or in the absence of *Scutellaria baicalensis* root extract. The number of exosomes they produce was quantified and the miRNA they contain was analysed using quantitative RT-PCR (Fig 2A, 2B).

Senescent cells secrete twice as many exosomes as normal cells and they contain high amount of miRNA that will lead to the inhibition of genes involved in key skin parameter such as hyaluronate synthase or collagen I synthesis.

Treatment with *Scutellaria baicalensis* root extract strongly decreases the number of exosomes secreted by fibroblasts under SIPS and the amount of miRNA they carry (Fig 2A, 2B). *Scutellaria baicalensis* root extract therefore reduces the inhibition of several key genes. As a result, target cells maintain an optimal activity such as their ability to produce collagen (Fig 2C).

By preventing healthy cells from being 'contaminated' by nearby senescent cells, *Scutellaria baicalensis* root extract maintains an optimal collagen production for a denser and firmer skin.

Among all the SASP produced by a senescent cell, many are pro-inflammatory such as IL-6 and are responsible for inflamm'aging, a low-grade chronic inflammation that can lead to premature ageing.

Macrophages are key actors of the innate immune response. Once activated by a danger message such as the presence of bacteria in the tissue, they secrete high levels of ROS (especially nitric oxide (NO)), pro-inflammatory cytokines, and lipid mediators such as prostaglandin (PGE_2) in order to obliterate it.

However, in the case of a chronic inflammation, macrophages are recruited even in the absence of pathogen. This creates a micro-environment rich in free

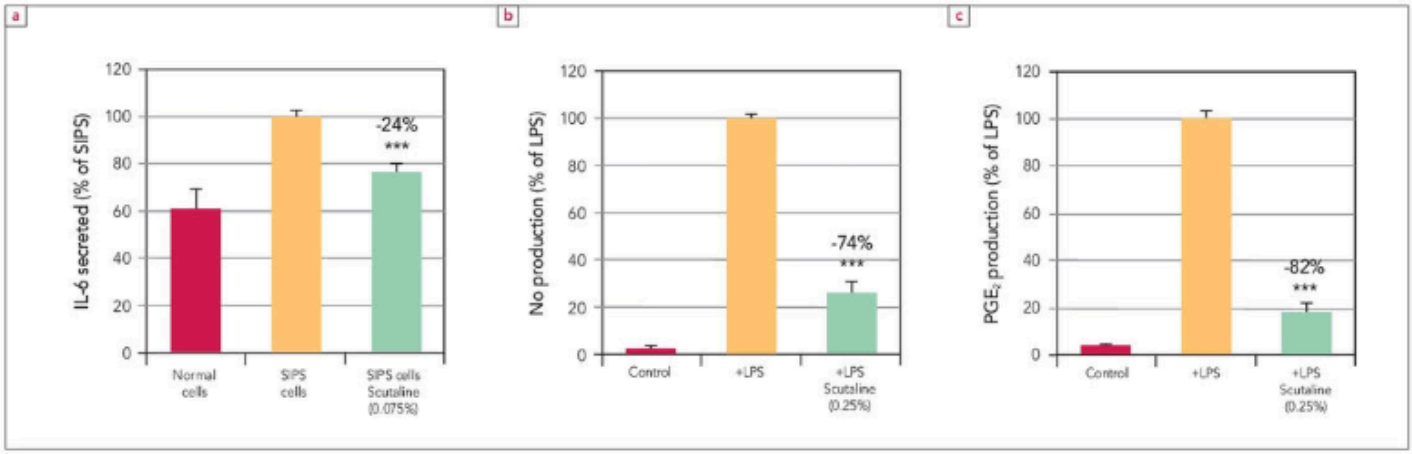

Figure 3: Scutaline prevents inflamm'aging. a) NHDF were exposed to H_2O_2 for 2h and then left for 3 days. Cells were then cultivated in the presence or in the absence of Scutaline. The amount of IL-6 secreted was measured using ELISA assay. b, c) LPS-activated macrophages were cultivated in the presence or in the absence of Scutaline for 24h. The productions of NO (b) and PGE_2 (c) were measured using spectrophotometry and ELISA assay respectively. ***p-value<0.001

radicals which maintains the inflammatory reaction and rich in PGE_2 that may cause pain, redness and/or oedema.

The treatment with *Scutellaria baicalensis* root extract (0.075%) leads to a 24% decreased in the amount of IL-6 produced by senescent fibroblasts. Furthermore, in the presence of *Scutellaria baicalensis* root extract (0.25%), activated macrophages produce 74% and 82% fewer NO and PGE_2 respectively.

Taken together these data suggest that *Scutellaria baicalensis* root extract is capable of decreasing the senescence-induced inflammation by reducing the number of recruited macrophages and by decreasing the severity of any unspecific response from activated macrophages. *Scutellaria baicalensis* root extract may therefore reduce local pain, redness and oedema.

This curative effect may thus limit inflamm'aging in more mature skins and support them in the well-ageing process.

Benefits for the skin

By ensuring an optimal activity to skin cells and by preventing chronic low-grade inflammation, *Scutellaria baicalensis* root extract may therefore be a good candidate for topic pre-ageing or anti-ageing treatments.

In order to assess *Scutellaria baicalensis* root extract's anti-ageing properties, 20 female volunteers received a treatment with Scutaline (2%) applied twice a day on the face for 28 days.

The volunteers were divided into two groups: "prevention group" (aged 40-50) in order to assess *Scutellaria baicalensis* root extract's ability to prevent ageing, and a "correcting group" (aged 51-68) in order to assess *Scutellaria baicalensis* root extract ability to correct the effects of ageing. Several key parameters of the skin were instrumentally measured and assessed by a dermatologist and by the volunteers themselves.

All the assessed skin parameters were improved in both groups.

In the Prevention Group, skin hydration was quickly increased, skin redness was steadily improved, and skin biomechanical properties were improved by the end of the treatment. This is consistent with the clinical evaluation. Indeed, the patients observed most of the benefits after only 14 days of treatment, especially for comfort parameters such as hydration, skin tone and softness. As for the skin firmness, skin elasticity and wrinkles were also improved but in a more time dependent way (Fig 4A).

In the Correction Group, skin hydration was quickly increased, skin redness reached a plateau after only 14 days and skin firmness and elasticity were steadily increased. The patients that received the treatment observed that the comfort parameters were strongly improved after only 14 days of treatment while the anti-ageing benefits (skin firmness, elasticity and wrinkles) were slightly delayed when compared to the Prevention Group (Fig 4B).

Treatment with *Scutellaria baicalensis* root extract thus globally improves the skin with quicker benefits for skin tone, hydration and softness. The processes that require a biological and/or biomechanical effect such as firmness, elasticity or wrinkle reduction are also improved at the end of the treatment but require a longer time (Fig 4).

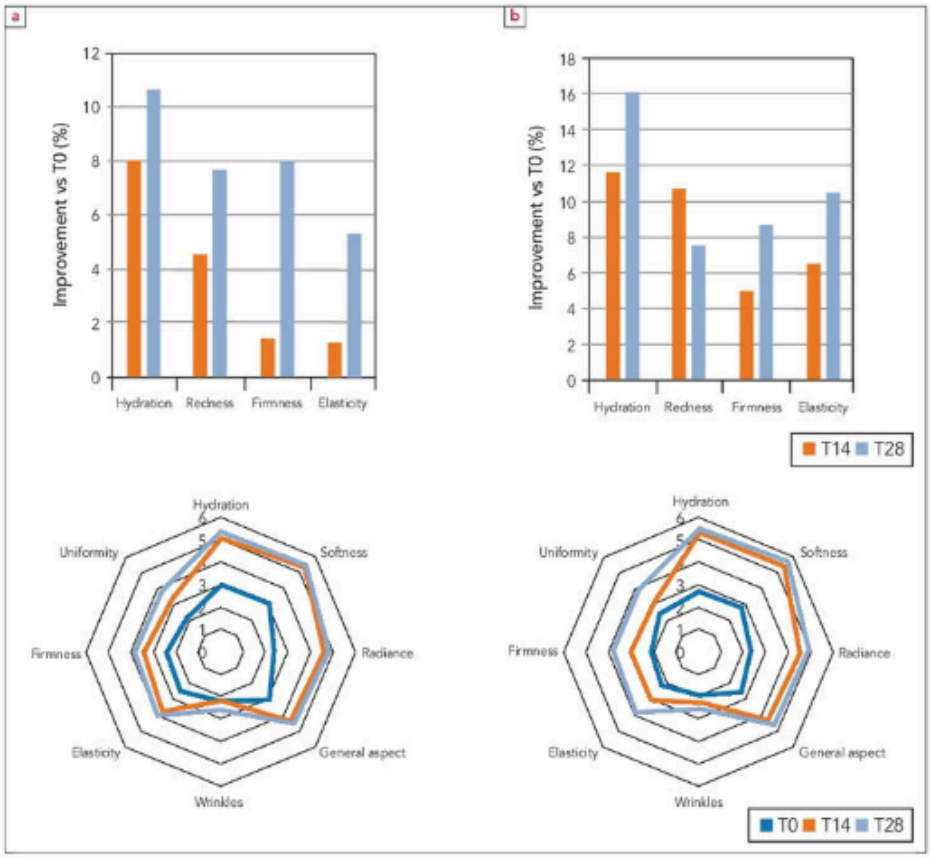

Figure 4: Scutaline improves skin parameters in human volunteers. Instrumental measure (top) of the improvement (expressed in % vs T0) of skin firmness, elasticity (cutometry), hydration (corneometry) and redness (VISIA), and clinical scoring (bottom) of several skin parameters by self-evaluation using a scoring method: 1 Very bad ; 2 Moderately bad ; 3 Slightly bad ; 4 Slightly good ; 5 Moderately good ; 6 very good a) Prevention group. b) Correction group.

Conclusion

Senescent cells are responsible for generating a local inflammation while also spreading senescence to other nearby or far away cells. This transmission process may lead to inflamm'aging, a chronic inflammation that causes skin premature ageing (dehydration, wrinkles, sagging of facial muscle, pigmentary disorders, loss of radiance, etc.).

Scutaline (INCI name: *Scutellaria baicalensis* root extract) is a cosmetic active capable of fighting senescence in a dual way. It is able to prevent healthy skin cells from becoming senescent after an exposure to a stress (oxidative stress, UV, pollution…), and it is able to reduce the negative impact of already senescent cells on their environment while preserving their beneficial effects (senescent cells still produce collagen (although in reduced quantity) and contribute to the extracellular matrix biomechanical features.

Scutaline thus allows for a quick reduction of inflammation that leads to a decrease of redness and pain and to an increase in skin hydration and comfort. Furthermore, Scutaline ensures the normal activity of skin cells (collagen production…) for a skin with optimal biomechanical properties such as firmness and elasticity.

Scutaline protects against senescence in young skin. Scutaline also limits the effects of inflamm'aging on mature skin. Therefore, by providing preventive and curative benefits, Scutaline may be a good candidate for supporting the skin during the 'well-ageing' process. PC

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