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The Influence of Oligosaccharides on Skin Aging: An Alternative to Retinoids

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Key words

anti-aging, oligosaccharides, retinoids, galactomannans, epidermal metabolism, extracellular matrix protection

Abstract

Retinol and its derivatives are recognized anti-aging molecules but they sometimes have irritating side effects. The authors investigate oligosaccharides obtained after enzymatic hydrolysis of Medicago sativa *as a non-irritating alternative to retinol.*

Multiple in vivo studies have shown that retinol and retinoids applied topically can stimulate the natural process of photoaging repair. They induce collagen synthesis, limit the fragmentation of elastic fibers or decrease the metalloproteinase activity. However, based on the amount and area of application, retinol and its derivatives can present irritating side effects in local applications. As a result, some cosmetic formulators look for molecules presenting biological properties identical to those of retinol but without the side effects, such as cutaneous irritation.

After screening several raw materials, we describe the biological properties of oligosaccharides obtained by enzymatic hydrolysis of *Medicago sativa*. Tested in vitro, the oligosaccharides support the synthesis of the major components of the skin, which are collagens type I, and limit the extracellular matrix degradation by decreasing the metalloproteinase-1 activity.

Biological properties comparable with those of the retinol could also be observed on epidermal cells. The oligosaccharides significantly increased the synthesis of the cytokeratines 4 and 19, which are specific markers of retinoid activity. Moreover, tested on young and old epidermal cells, the oligosaccharides improve cell proliferation and stimulated synthesis of Heat Shock Protein 27 (HSP 27), a marker of keratinocyte differentiation.

The molecules described are likely to limit the harmful effects of photoaging and represent an innovative alternative to retinol and its derivatives. The action of the active ingredient was determined by isolating several fractions of the product. These fractions were then tested on models of collagen I and cytokeratin 4 synthesis. We found that one fraction among those isolated behaved comparably to *Medicago sativa* extract in terms of the synthesis of collagen I and cytokeratin 4. The chromatographic analysis of this fraction showed that the active fraction is composed of oligosccharides primarily of galactomannans (Figure 1).

When applied to human skin, the efficacy of a preparation containing oligosaccharides, used against fine lines and wrinkles, could be confirmed using profilometry.

Retinoids such as retinol (vitamin A), retinaldehyde and retinoic acid (Figure 2) play an essential role in embryogenesis, reproduction and vision as well as in the control of cell growth and the differentiation of many adult tissues.[1,2]

In the beginning of the 20th century, vitamin A was identified as an essential nutrient whose principal source is the diet (animal fat, eggs, milk, vegetables).[3] The study of retinoids in dermatology then showed the value of topical application of retinoids to treat certain skin disorders such as acne or keratinization disorders such as psoriasis.[4]

There is a considerable body of published data on the effects of retinol and its derivatives in vitro on skin cells and, no less important, on the effects of topical applications of retinoids. Retinoids have been extensively investigated and have rapidly become reference cosmetic ingredients as a result of their elevated repair capacities.

All-trans retinoic acid (tretinoin or vitaminA acid) is the active metabolite of retinol (natural vitaminA). The use of retinol or its analogues in the form of topical application improves the characteristics of older skin with lines and wrinkles, as well as its texture and color. A significant improvement in the general appearance, as well as a dose-dependent effect that increased with treatment duration, have been reported.[5] These changes on the surface of the skin are accompanied by histological modifications in both the dermis and the epidermis.[6]

In spite of its benefits, retinoic acid can cause highly irritating side effects when applied locally, which is why it can no longer be used in cosmetic composi-

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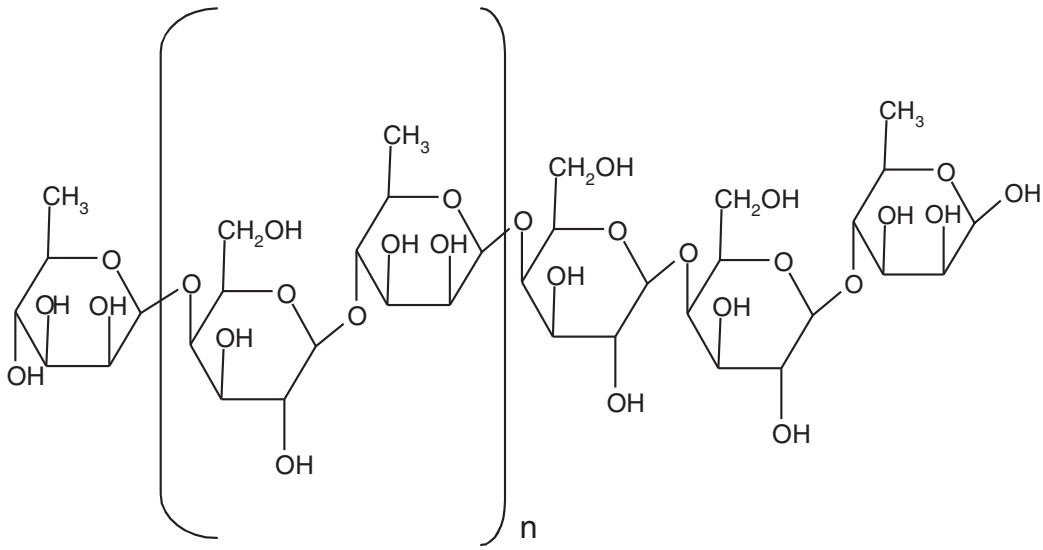

Figure 1. Structure of the galactomannan

tions; it was in fact banned from cosmetic applications in 1988 (10th adaptation of European Directive 88/233/EC). Cosmetics laboratories then became interested in other members of the family of retinoids: retinol, retinaldehyde and esters. Nevertheless, the use of retinol in formulations remains difficult because of its excessive instability to light, oxygen and heat. In addition, retinol also causes irritation when used above the 0.1% threshold. And although esters are more stable, they penetrate the skin with more difficulty.[7]

Therefore, we began to develop an active ingredient whose behavior is similar to retinol and that can, as the latter, participate in the regulation of dermal and epidermal metabolism in the absence of side effects caused by retinol and its derivatives. *Medicago sativa* (alfalfa) actives were obtained after screening several raw materials by a process involving several steps of controlled enzymatic hydrolysis leading to a purified active ingredient rich in galactomannans.

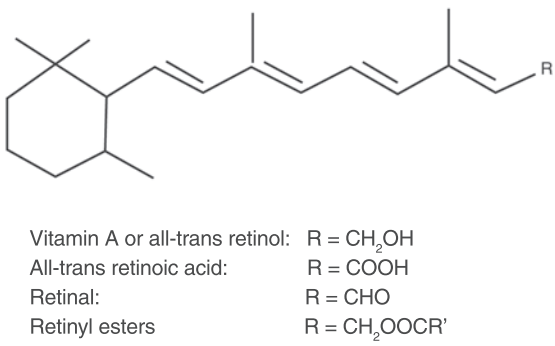

Figure 2. The family of retinoids

Study of Retinol-like Behavior

Stimulation of retinoid-specific markers: In addition to histology, one of the means of evaluating the epidermal response to retinoids is the assay of markers such as cellular retinoic acid-binding protein 2 (CRABP2) and the tazarotene-induced genes TIG1 and TIG2. These genes are rapidly upregulated in normal human epidermis after the application of retinoic acid under an occlusive bandage. Other markers are also of considerable value for detecting modifications specifically induced by retinoids in the

epidermis. According to the literature,[8,9] cytokeratins 4 (CK 4) and cytokeratins 19 (CK 19) are specific markers of retinoids that are more reliable and easier to study than CRABP2 or TIG.

The expression of CK 4 is easily followed by the use of commercial antibodies, while no ready-to-use tool of this type exists for the markers CRABP2 and TIG. Furthermore, the increased expression of CK 4 after 2 days of treatment with retinoic acid is 3 times higher than expression of the protein CRABP2.

We thus first determined the effect of the *Medicago sativa* extract (MSE) on these two specific markers of the activity of retinoids with Western blot. Keratinocytes were previously treated with MSE at different concentrations (1%, 2% and 3%) or with 10^{-5}M retinol and incubated for 6 days in a 37°C incubator in a moist atmosphere containing 5% CO_2.

Tested at 3% on cultures of human keratinocytes, MSE significantly increased the synthesis of CK 4 and CK 19 by 29% and 34%, respectively (Figure 3).

These results are comparable to those obtained with retinol, favoring the synthesis of CK 4 and CK 19 by 25% and 41%, respectively.

Epidermal metabolism: Retinoids, retinol in particular, play a key role in the maintenance and preservation of epidermal homeostasis. Retinoids affect keratinocyte differentiation and regulate the expression of a large number of epidermal proteins.[8] The protein HSP 27 was chosen to evaluate the influence of MSE on epidermal metabolism. This 27 kDa heat shock protein is localized in the upper layers of the epidermis and is involved in terminal keratinocyte differentiation by, among other things, participating in the formation of cornified cell envelopes.[10,11] According to work by Kindas-Mügge and Trautinger,[11] HSP 27 is a protein that can be considered as a marker of the differentiation of normal human keratinocytes.

MSE or retinol were added to the keratinocyte culture medium. The cells were then incubated for 6 days in a 37°C incubator in a humid atmosphere. HSP 27 levels were assayed with the Western blot technique.

When MSE at different concentrations was tested on normal human keratinocytes, the increases in the levels of HSP 27 were comparable to the increases achieved with retinol (Figure 4). This study showed that MSE favors keratinocyte differentiation.

Dermal metabolism: Retinoids not only play a role in epidermal metabolism, but also affect metabolism of the dermis. They are known to attenuate damage to the dermal matrix that occurs with increasing age.[4,12,13]

Among other things, retinoids stimulate the proliferation of fibroblasts, and favor the production of collagen and of several other key molecules such as decorin.[12] This can explain the reduction of lines and wrinkles following the topical application

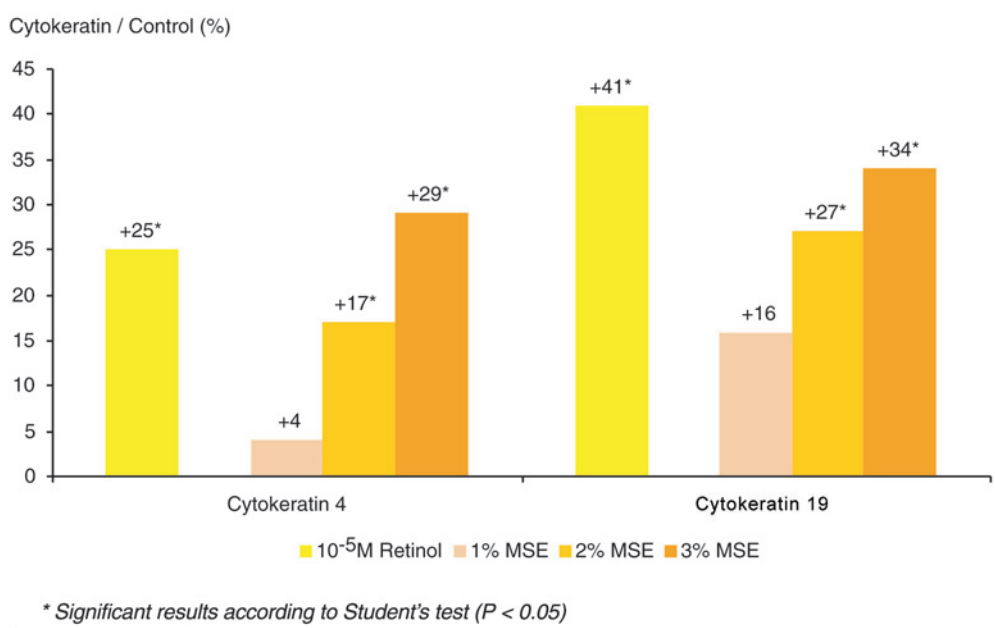

Figure 3. Influence of Medicago sativa extract on the expression of specific markers of retinoids (CK 4 and CK 19); comparison to retinol

of retinoids.[4,13] Retinoids also participate in the protection of the extracellular matrix in the course of extrinsic and intrinsic aging by blocking matrix metalloproteinases (MMPs), a family of enzymes responsible for the rupture of collagen fibers.[4,14]

The influence of MSE on the synthesis of collagen I was investigated using cultures of human fibroblasts and compared to that of retinol. Cells were treated with MSE or with 10^{-5} M retinol. After 48 hours of incubation, cell-free supernatants were recovered and collagen I was assayed with ELISA (enzyme-linked immunosorbent assay). Tested at 0.5%, MSE increased

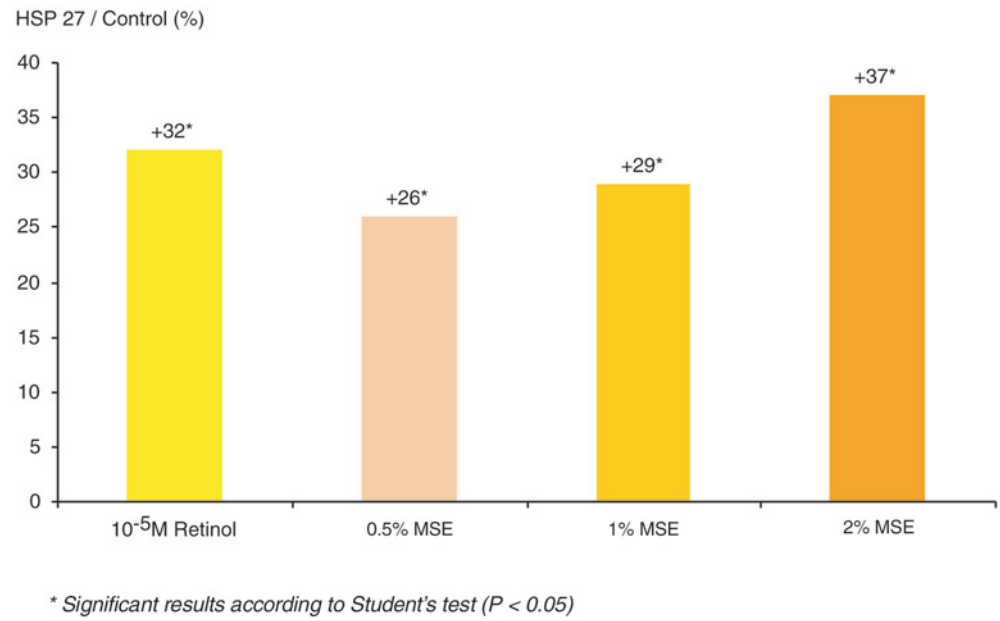

Figure 4. Effect of MSE on the synthesis of HSP 27; comparison to retinol

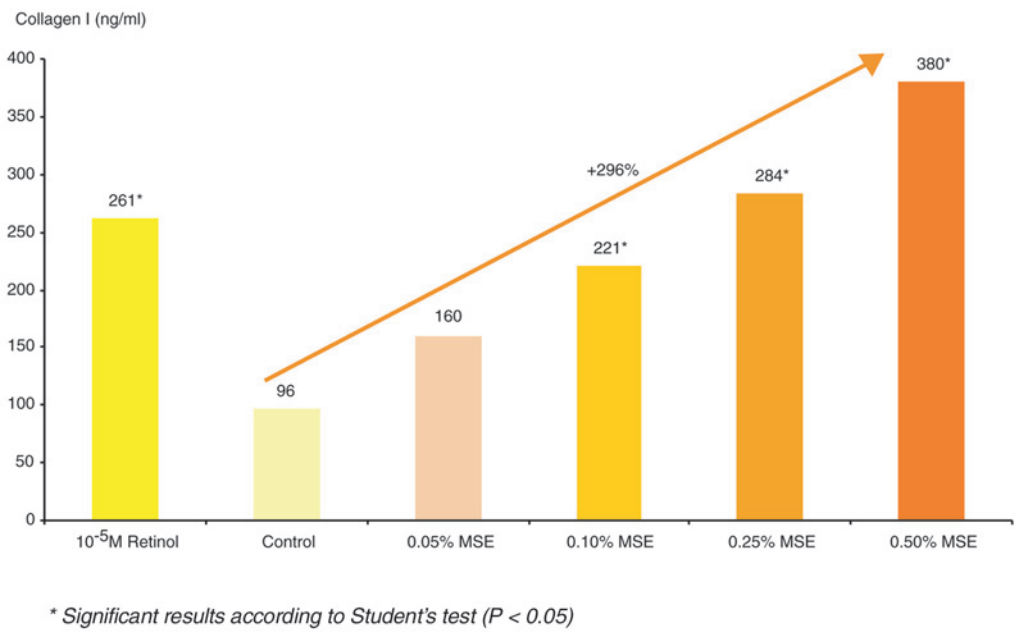

Figure 5. Effect of MSE on the synthesis of collagen I; comparison to retinol

the synthesis of collagen I by 296%, thereby protecting the extracellular matrix (Figure 5).

Inhibition of MMP-1, an enzyme responsible for the degradation of collagen I and III fibers, by MSE was determined using cultures of human fibroblasts subjected to UVA irradiation (20 J/cm^2) followed by ELISA. UVA irradiation of cells causes a 103% increase in the expression of MMP-1. The addition of retinol or MSE to the culture medium limited the expression of UVA-induced MMP-1. This effect was dose-dependent and comparable to that of 10^{-5} M retinol that reduced MMP-1 levels by 62% (Figure 6).

By reestablishing the balance between collagen synthesis and the degradation of its fibers, MSE acts like retinol to maintain a functional extracellular matrix.

Clinical Study

A comparative study was administered on 42 volunteers divided into two groups of 21 each. One group tested MSE formulated at 4% in an emulsion and the other tested the formula containing 0.15% of stabilized retinol. Each group applied the test product to one side of the face and the placebo to the other side, twice daily for 28 days. The results were analyzed by profilometry. The parameters selected to determine the anti-wrinkle effect were the total length of wrinkles and their depth. Figure 7 shows the results obtained in this study.

After 28 days of twice daily application, MSE significantly reduced the characteristic parameters of wrinkles. The total wrinkled surface was reduced by 17%; the total length of wrinkles decreased by 11%; and the depth of wrinkles decreased by 5%. This effect was comparable to that obtained with retinol formulated at 0.15%. Nevertheless, MSE caused no irritation reaction, while retinol at this dose resulted in irritation dermatitis reactions in 24% of the volunteers, causing their premature dropout from the study. In addition, the formula containing retinol caused sensations of paresthesia, the first stage of irritation dermatitis, in 47% of the volunteers.

Conclusion

Retinol and its derivatives are recognized anti-aging molecules but are responsible for intolerance reactions when used. In order to circumvent this problem, we have developed an active ingredient whose behavior towards cells is similar to that of retinol. *Medicago sativa* extract (MSE) was investigated in terms of both epidermal and dermal metabolism. Just as for retinol, MSE regulated the differentiation process. Tested at 2%, it led to a 29% stimulation of the synthesis of HSP 27, a marker protein of this process. In addition, this active ingredient favored the protection of the extracellular matrix by stimulating the synthesis of collagen I (+296%) and also by limiting the expression of MMP-1 induced by UVA irradiation (92% inhibition of UV-induced MMP-1 activity).

Finally, when tested directly in vivo, both MSE and retinol improved the general appearance of mature skin by causing a statistically significant reduction (p<0.05) of the total wrinkled

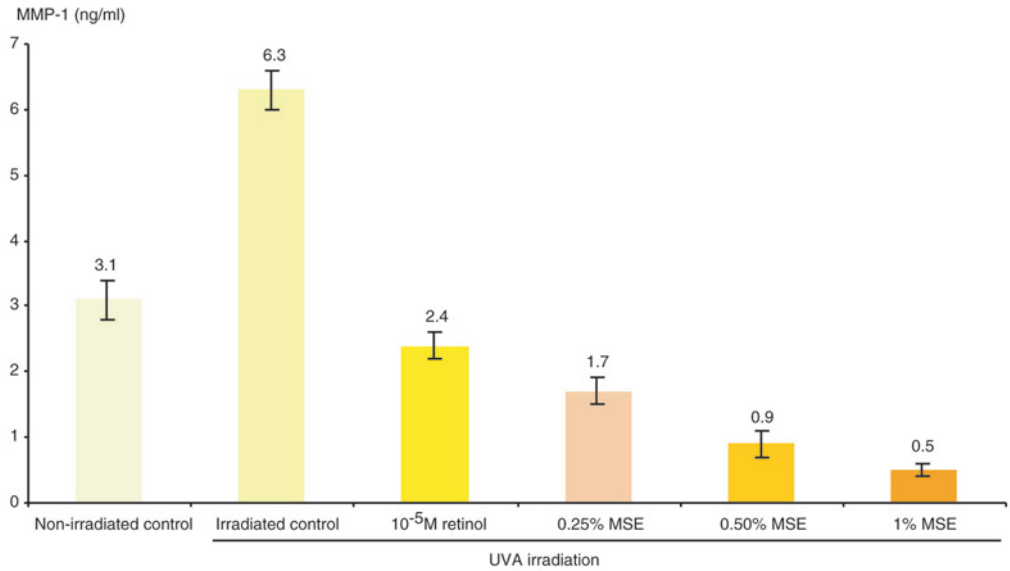

Figure 6. Effect of MSE on the synthesis of MMP-1 by UVA-irradiated human fibroblasts; comparison to retinol

surface by 17% and 26%, respectively. Several studies were conducted to understand the mechanism of action of the active ingredient. The composition of the active ingredient was investigated and four active fractions were isolated and tested on models of collagen I and cytokeratin 4 synthesis. One fraction among those isolated behaved comparably to MSE in terms of the synthesis of collagen I and cytokeratin 4. The gas chromatography analysis of this fraction showed that the active fraction is an oligosaccharide fraction rich in galactomannans.

MSE's action is comparable to that of retinol on skin metabolism. MSE also stimulated the synthesis of 2 molecules: cytokeratins 4 and 19, specific markers of the activity of retinoids.

Another study using the cDNA macroarray method provided further understanding of the mechanism of action of MSE. The gene expression profile of MSE was compared to that obtained with retinoic acid. It was found that the large number of genes modulated by retinoic acid was also regulated by treatment with MSE. Nevertheless and in contrast to retinoic acid, MSE did not modulate the expression of the genes for CRBP1 (cellular retinol-binding protein 1), RARγ1 (retinoic acid receptor γ 1) and CRABP2 (cellular retinoic acid-binding protein 2) that mediate the effects of retinoids. This suggests that the two treatments have a different mechanism of action that could be investigated further in order to

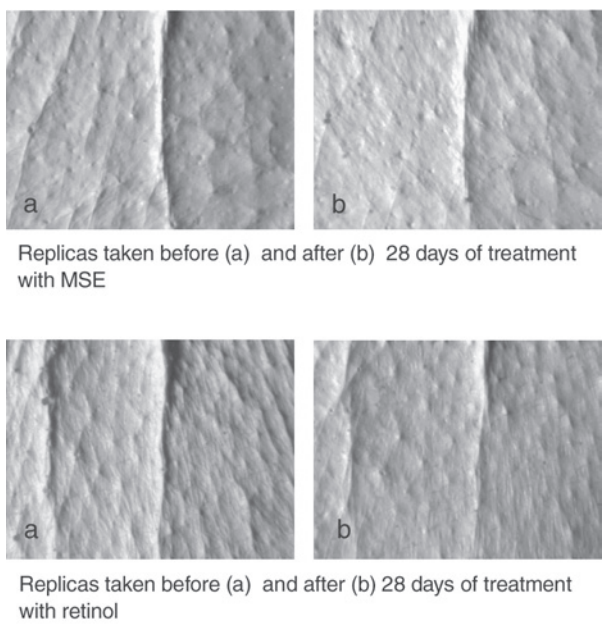

Figure 7. Anti-wrinkle effect of MSE; comparison to retinol

more precisely define the receptors affected by MSE.

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