THE ROLE OF HYALURONAN IN THE TREATMENT OF EPIDERMAL ATROPHY

Lenka Machková¹, Iva Dolečková¹

¹ Contipro a.s., Dolní Dobrouč, Czech Republic

INTRODUCTION

Senile skin atrophy is a common side effect of intrinsic aging and UV exposition and it affects both dermal and epidermal layers of the skin. While dermal atrophy is mostly associated with the loss of connective tissue, epidermal atrophy manifests as overall thinning of epidermis, reduction of keratinocyte size and flattening of the dermoepidermal junction. Compared to senile atrophy, steroid skin atrophy frequently arises during glucocorticoid therapy and causes serious skin damages, particularly when applied to fragile skin.

MATERIALS AND METHODS

In this study, we investigated the ability of 0,1 % high (1500 - 1800 kDa, HMW-SH), low (320 kDa, LMW-SH) and very low (15 kDa, VLMW-SH) molecular weight sodium hyaluronate (SH) to reverse the general signs of the skin atrophy (decreased cell viability, lower total protein level) and modulate the specific signs of epidermal atrophy (expression of matrix metalloproteinases) in the *in vitro* model of dexamethasone-treated HaCaT keratinocytes. We also performed *in vivo* study to evaluate the effect of VLMW-SH and LMW-SH topical application on thickness of living epidermis using the method of *in vivo* reflectance confocal microscopy.

RESULTS

The anti-atrophic potential of sodium hyaluronate (SH) has been studied previously and it was demonstrated on a group of patients affected by steroid atrophy¹.

Our results show that VLMW-SH significantly reverses all the signs of dexamethasone-induced atrophy in HaCaT keratinocytes investigated in this study and increases the average epidermal thickness of aged skin *in vivo*. HMW-SH demonstrated less pronounced effect and LMW-SH showed no significant anti-atrophic abilities neither *in vitro* nor *in vivo*.

CONCLUSION

In conclusion, we confirmed the importance of hyaluronan in senile and steroid-induced skin atrophy. However, therapeutic potentials of HMW, LMW and VLMW-SH show significant differences and should be investigated in detail. These results support the beneficial effects of SH and the possibility of its application in the therapy of atrophic epidermal skin.

IN VITRO ANTI-ATROPHIC EFFECT OF HMW, LMW and VLMW SODIUM HYALURONATE

Figure 1. *In vitro* model of dexamethasone-induced atrophy. HaCaT keratinocytes were treated with 1 µM dexamethasone for 72 hrs, then the cells were washed, cultivated in fresh medium with/without SH. Sodium hyaluronate used in this study was produced using a non-haematolytic microbial strain *Streptococcus equi*. After 24 hrs, the markers of atrophy were investigated.

Table 1. Markers of atrophy studied in dexamethasone-treated HaCaT keratinocytes.

MARKERS	+ DEXAMETHASONE
Cell viability	↓
Protein level in lysate	↓
MMPs' expression	↑MMP-7 (elastase) ↓MMP-13 (collagenase type I)

Figure 2. The viability of atrophic HaCaT keratinocytes (+/- SH). Untreated = non-atrophic; DXM = atrophic. The viability was determined using resazurin assay. The values represent means ± SEM of the HaCaT keratinocytes viability (* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 compared to DXM ctrl, n = 5).

Figure 3. Protein level in atrophic HaCaT keratinocytes (+/- SH). Untreated = non-atrophic; DXM = atrophic. The protein level was determined using BCA protein assay. The values represent means ± SEM of the protein level in the lysate of HaCaT keratinocytes (* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 compared to DXM ctrl, n = 5).

Figure 4. MMP-7 and MMP-13 gene expression level in atrophic HaCaT keratinocytes (+/- SH). Untreated = non-atrophic; DXM = atrophic. The gene expression was determined using real-time qRT-PCR. The values represent means ± SEM of the gene expression of MMP-7/MMP-13 in HaCaT keratinocytes (* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 compared to DXM ctrl, n = 5).

IN VIVO ANTI-ATROPHIC EFFECT OF VLMW SODIUM HYALURONATE

Figure 5. Epidermal thickness of the the skin after topical application of 0,2 % VLMW-SH (A) and 0,2 % LMW-SH (B). In contrast with LMW-SH, VLMW-SH significantly increased the thickness of living epidermis. It was determined using *in vivo* reflectance confocal microscopy. In both (A) and (B) double-blind, split-face and placebo-controlled studies, 28 healthy female volunteers with living epidermal thickness below 50 µm participated. The values represent means ± SEM of the epidermal thickness (* p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001 compared to placebo, n = 28).

REFERENCES

1. Kaya, G. et al. Hyaluronate Fragments Reverse Skin Atrophy by a CD44-Dependent Mechanism. PLOS Med. 3, e493 (2006).