Original Paper

Skin Pharmacology and Physiology

Skin Pharmacol Physiol 2011;24:210–217
DOI: 10.1159/000324296

Received: April 13, 2010
Accepted after revision: January 11, 2011
Published online: March 11, 2011

Fifty-kDa Hyaluronic Acid Upregulates Some Epidermal Genes without Changing TNF-α Expression in Reconstituted Epidermis

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Key Words
Hyaluronic acid · Skin penetration · Anti-wrinkle effect · Skin inflammation

Abstract
Background: Due to its strong water binding potential, hyaluronic acid (HA) is a well-known active ingredient for cosmetic applications. However, based on its varying molecular size, skin penetration of HA may be limited. Recent studies have demonstrated that low-molecular-weight HA (LMW HA) may show a certain proinflammatory activity. We thus aimed to characterize an LMW-sized HA molecule that combines strong anti-aging abilities with efficient skin penetration but lacks potential proinflammatory effects. ***Methods:*** Total RNA and total protein were isolated from reconstituted human epidermis following incubation with HAs of various molecular weights (20, 50, 130, 300, 800 and 1,500 kDa). Tumor necrosis factor-α expression was determined using quantitative PCR. Genomic and proteomic expression of various junctional proteins was determined using Affymetrix and common Western blotting techniques. ***Results:*** LMW HA of approximately 50 kDa did not significantly alter tumor necrosis factor-α expression compared to 20-kDa HA, but revealed significantly higher skin penetration rates than larger sized HA associated with increased expression of genes and proteins known to be involved in tight junction formation and keratinocyte cohesion. ***Conclusion:*** LMW HA of approximately 50 kDa shows better penetration abilities than larger-sized HA. In addition, LMW HA influences the expression of various genes including those contributing to keratinocyte differentiation and formation of intercellular tight junction complexes without showing proinflammatory activity. These observations contribute to current knowledge on the effects of LMW HA on keratinocyte biology and cutaneous physiology.

Introduction

Hyaluronic acid (HA) represents a naturally occurring glycosaminoglycan biopolymer, located mostly in the intercellular matrix of the dermis but has also been found in the epidermal layer of the skin [1]. Its molecular structure

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is highly conserved between mammalian species [2, 3]. HA represents one of the major matrix substances in which cells and fibrous constituents of the dermal matrix including collagen and elastin are embedded [4]. Its invariant chemical structure reduces the likelihood of immunologic reactions, thus increasing its biocompatibility. HA molecules are also known to significantly contribute to the maintenance of the extracellular space. Due to their enormous water-binding capacity, HA molecules are highly relevant in controlling tissue hydration [5, 6]. Additionally, HA seems to play a pivotal role in tissue regeneration since recent studies suggest that the integrity and balance of matrix components themselves, which undergo degradation and reconstruction, assure normal tissue function and contribute to the regulation of wound healing [7, 8]. Besides being commonly utilized as an attractive therapeutic agent in medical fields such as ophthalmology and rheumatology, its extraordinary properties make HA a promising candidate for a variety of cosmetic applications, particularly in the context of skin regeneration, tissue augmentation and other anti-aging treatments [3]. However, this plethora of potential beneficial features is limited by the molecular size of HA, which can reach up to 2,000 kDa, thus interfering with efficient skin penetration.

This phenomenon may easily be addressed by fragmentation of high-molecular-weight HA. However, recent studies have shown that low-molecular-weight (LMW) HA or HA fragments may be involved in various biological processes including cell proliferation, angiogenesis, migration, maturation, activation of protein tyrosine cascades and proinflammatory activity [9–11]. In a study by Voelcker et al. [12], HA fragments were recognized by so-called Toll-like receptors (TLRs) 2 and 4 on human melanoma cells leading to activation of these cells and thus production of proinflammatory mediators.

In the present study, we thus aimed to identify an LMW HA molecule that combines strong anti-aging and moisturizing abilities with efficient skin penetration but lacks the proinflammatory effects mediated by TLRs.

Materials and Methods

Material

HA was produced using a novel, solvent-free methodology based on a new fermentation strain of *Bacillus subtilis*. This fermentation process results in a protein-free HA with constant molecular weight that appears as a very fine powder after the process of spray drying.

LMW HA is produced by controlled thermal hydrolysis of high-molecular-weight HA. Briefly, the HA powder containing residual humidity is heated up to 120–130°C for different periods of time, and hydrolysis is performed under controlled conditions until specifications of viscosity are achieved.

Methods

In vitro Culturing of Reconstituted Human Epidermis Constructs in the Presence of HA

Reconstituted human epidermis was purchased from SkinEthic (Nice, France) and incubated for 24 h in standard maintenance medium at 37°C and 5% CO_2 as previously described [13] before starting the experiments. In total, three different types of experiments employing reconstituted human epidermis constructs treated with various molecular weight forms of HA were conducted. In order to evaluate the possible proinflammatory effects of very LMW (VLMW) HA, the reconstituted human epidermis was incubated for 48 h with 50 μl phosphate-buffered saline (PBS) containing 0.5% 20-, 50-, 130- or 320-kDa HA, respectively. PBS-treated reconstituted human epidermis served as control. After incubation, RNA was isolated and served as template for cDNA generation. Using quantitative RT-PCR, the expression of the proinflammatory cytokine tumor necrosis factor-α (TNF-α) was determined.

Another set of experiments was performed to characterize the effects of HA on keratinocytes on a molecular level. In this context, reconstructed human epidermis was treated topically with 50 μl of a thin liquid O/W emulsion (composition: 3% glyceryl stearate-ceteth-20; 1% stearyl alcohol; 4% cetearyl ethylhexanoate; 4% caprylic/capric triglyceride; pH adjusted to 6 with NaOH; water: ad. 100%) containing 0.5% of 50-kDa HA and 800-kDa HA, 0.2% retinol as positive control or not active at all as vehicle control. The emulsion type vehicle was chosen because retinol is insoluble in water. Retinol was dissolved in the oil phase, whereas HA was dissolved in the water phase. Following 48 h application, RNA was extracted for transcriptome analysis using microarray technique and TaqMan® RT-PCR assays (see below).

A third set of experiments using reconstituted human epidermis constructs was conducted in order to confirm some of the observations from the microarray study on the protein level. Therefore, the models were topically treated with 0.05 and 0.5% 50-kDa HA (commercially available as HyaCare®50) dissolved in water for 72 h (n = 4). Afterwards, protein extracts were prepared and used for Western blotting analysis (see below).

In order to determine penetration properties of differentially sized HA molecules, diffusion of tritiated HA with a molecular weight of about 50, 300, 800 or 1,500 kDa through dermatomed porcine ear skin was assessed using Franz diffusion cells as previously described [14]. In brief, full-thickness skin samples of visually intact skin from the ear of 5-month-old female domestic pigs were dermatomed to a thickness of about 750 μm using an Acculan GA 643 (Aesculap, Tuttlingen, Germany). The samples were then mounted in modified Franz static dermal penetration cells with the external surface of the stratum corneum facing the donor chamber. After 5 and 22 h of HA application, the level of radioactivity in the receptor phase was determined and expressed as $ng \cdot cm^{-2} \cdot h^{-1}$, which served as a parameter for skin penetration.

RNA Isolation

Total RNA was extracted from cultured skin constructs using RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. RNA concentration was assessed spectroscopically using SmartSpec Plus (Biorad, München,

Germany). Purity and integrity of the RNA was determined by an Agilent 2100 bioanalyzer with a 6000 Nano LabChip reagent set (Agilent Technologies, Santa Clara, Calif., USA). RNA samples were stored at –80°C until analysis.

Quantitative RT-PCR

Reverse transcription was performed using the First-Strand cDNA Synthesis Kit (Super Script III, Invitrogen, Life Technologies, Carlsbad, Calif., USA) according to the guidelines of the manufacturer starting with 100 ng purified RNA from each sample. Quantitative PCR was carried out with an Opticon DNA engine (MJ Research, Waltham, Mass., USA) using SYBR Green PCR Master Mix (Applied Biosystems, Darmstadt, Germany) according to the manufacturer's instructions. The following gene-specific primers were used for TNF-α: forward 5′-CTG TGG CCC AGG CAG TCA GA-3′ and reverse 5′-GGC GTT TGG GAA GGT TGG AT-3′. Glycerin aldehyde phosphate dehydrogenase (GAPDH) served as a housekeeping gene with the following primer pair: forward 5′-ACC ACA GTC CAT GCC ATC AC-3′ and reverse 5′-TCC ACC ACC CTG TTG CTG TA-3′.

DNA Microarray and Data Analysis

Gene expression profiles were determined using Affymetrix HGU133 plus 2.0 GeneChips (Affymetrix, Santa Clara, Calif., USA) using 2 μg of total RNA pooled from three reconstituted human epidermis skin models. Gene chip assays and initial analysis were carried out as previously described [15].

TaqMan RT-PCR Assays

TaqMan first-strand cDNA synthesis was performed with the reverse transcription system from Promega (Madison, Wisc., USA) according to the manufacturer's instructions. Real-time RT-PCR analysis was performed with an ABI7900HT machine (Applied Biosystems). All reagents necessary for running TaqMan RT-PCR assays, including primers and probes were purchased from Applied Biosystems and used according to the manufacturer's instructions. TaqMan analysis of transcripts for CALB1 (Hs01077193_m1), CLDN4 (Hs00533616_s1), CLDN7 (Hs00600772_m1), CLDN17 (Hs00273276_s1), OCLN (Hs00170162_m1), TJP2 (Hs00178081_m1) and 18S-rRNA (Hs99999901_s1) as endogenous control, was performed with predesigned and optimized Assays on Demand (Applied Biosystems). The reaction parameters were as follows: 2 min 50°C hold, 10 min 95°C hold, followed by 40 cycles of 10 s 95°C melt and 1 min 60°C annealing and extension. Measurements were performed in triplicate for both donors. Results were analyzed with an ABI sequence detector software version 2.0 (Applied Biosystems). Relative quantification was performed using 18S-rRNA as reference as described earlier [16].

Western Blotting

In order to confirm the observed genomic changes on a protein level, reconstructed human epidermis models (SkinEthic) were topically treated with 0.05 and 0.5% 50-kDa HA for 72 h (n = 4). Then, proteins were extracted using standard protocols and 20 μg of the resulting supernatant was run out on a 4–20% SDS-polyacrylamide gel and subsequently electrotransferred to immune-blot PVDF membranes (Bio-Rad Laboratories, Hercules, Calif., USA). PVDF membrane sheets were then probed with primary antibody against tight junction protein-1 (TJP-1; BD Bioscience, Franklin Lake, N.J., USA), tight junction protein-2 (TJP-2; Santa Cruz Biotechnology, Calif., USA), E-cadherin (BD Bioscience), β-catenin (BD Bioscience), occludin (Santa Cruz Biotechnology), claudin-4 (Santa Cruz Biotechnology), claudin-17 (Abcam, Cambridge, Mass., USA), claudin-1 (Santa Cruz Biotechnology) and GAPDH (Abcam). The signals were then detected using horseradish peroxidase-conjugated secondary antibody, developed with chemiluminescent substrates (Pierce Biotechnology, Rockford, Ill., USA) and were quantified with a density scan (Lumi-Imager™ F1, Roche Diagnostics, Mannheim, Germany). After quantification, the intensities of the protein signals were normalized to the respective GAPDH signals.

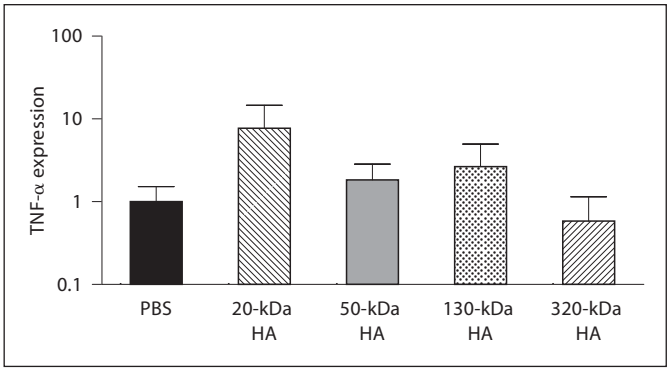

Fig. 1. VLMW HA is associated with an increased proinflammatory response. Total RNA was isolated from reconstituted human epidermis following incubation with PBS or HA of various molecular weights for 48 h. TNF-α expression was determined using quantitative RT-PCR with GAPDH serving as endogenous control. Mean value of the target was divided by the mean value of the endogenous control to obtain a normalized mean quantity per sample. Results shown represent n = 3 per group, as indicated in the main text. Bars represent means, error bars correspond to SEM.

Statistical Analysis

Results are presented as mean ± SEM (n = 3 per group, at each time point, if not stated differently). The data were analyzed using one-way ANOVA and Kruskal-Wallis test, and differences were considered significant at a p value of <0.05.

Results

VLMW HA Is Associated with an Increased Proinflammatory Response

VLMW HA with a molecular weight of 20 kDa revealed markedly increased expression of TNF-α (7.7 ± 6.9) in reconstructed epidermis models compared to models treated with PBS or HA molecules of higher molecular weight (1 ± 0.5; 1.8 ± 1.0; 2.7 ± 2.3 and 0.58 ± 0.6, HA 50, 130 and 320 kDa, respectively), indicating a proinflammatory effect of VLMW HA (fig. 1). In con-

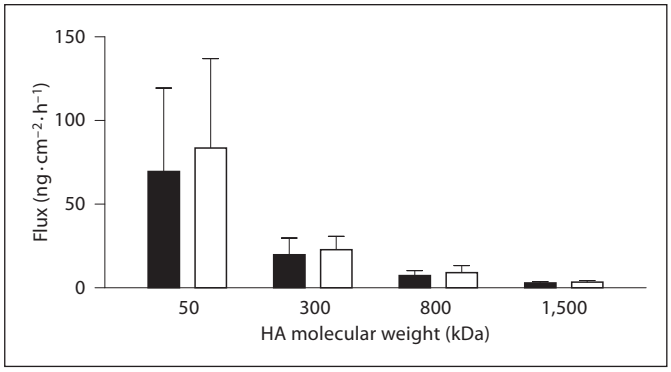

Fig. 2. LMW HA reveals improved penetrating properties compared to HA with higher molecular weights. HA molecules with different molecular weights, namely 50, 300, 800 and 1,500 kDa, were tested for their ability to penetrate pig ear skin (expressed in $ng \cdot cm^{-2} \cdot h^{-1}$) following 5 (■) and 22 h (□) of HA incubation. Results shown represent n = 3 per group, as indicated in the main text. Bars represent means, error bars correspond to SEM.

trast, HA molecules with a molecular weight >50 kDa did not induce marked upregulation of TNF-α expression compared to controls (fig. 1).

LMW HA Reveals Improved Penetration Properties Compared to HA with a Molecular Weight of >300 kDa

HA molecules with different molecular weight, namely 50, 300, 800 and 1,500 kDa, were tested for their ability to penetrate pig ear skin after 5 and 22 h of HA application. As shown in figure 2, only small amounts of HA with a molecular weight of 300 kDa and more (19.8 ± 10 and 22.8 ± 8; 7.3 ± 3 and 9.1 ± 4.2; 2.9 ± 0.8 and 3.4 ± 1 $ng \cdot cm^{-2} \cdot h^{-1}$; HA 300, 800 and 1,500 kDa, respectively) penetrated the skin, as seen at 5 and 22 h of incubation. In contrast, LMW HA with only 50 kDa revealed markedly higher skin penetration compared to the one with 300-kDa HA (69.5 ± 49.9 and 83.5 ± 53.4 $ng \cdot cm^{-2} \cdot h^{-1}$; fig. 2). Within duration (5 vs. 22 h) of incubation, the flux of HA remained constant over time.

LMW HA Is Associated with Increased Expression of Genes Known to Be Involved in Keratinocyte Differentiation and Cohesion

LMW HA with a molecular size of 50 kDa markedly affected up- or downregulation of about 120 genes (data not shown) including key genes involved in keratinocyte regulation as well as genes that play important roles for their cohesion. In contrast, HA with a molecular weight of about 800 kDa only led to differential expression of 40 genes (data not shown). A selection of up- or downregulated genes and their associated functions is presented in table 1.

Table 1. Effects of LMW HA with 50 kDa and HA with a molecular weight of 800 kDA on gene expression in reconstructed epidermis: microarray analysis

	Fold change vs. vehicle	
	HA 50 kDa	HA 800 kDa
Junctional control		
Occludin	3.4	2.0
Claudin-4	3.8	2.4
Claudin-7	7.5	2.8
Claudin-17	5.1	3.2
Aquaporin-3	2.3	2.0
Desmocollin-2	1.8	1.4
TJP-2	1.9	1.3
Striatin, calmodulin-binding protein	2.2	1.5
Kinesin family member C3	2.6	1.8
Differentiation/epidermis development		
Kallikrein-6	3.8	3.3
Kallikrein-14	2.0	2.1
Repetin	4.6	2.3
Keratin-34	3.0	1.4
Forkhead box Q1	4.0	3.2
ATPase, Ca^{2+} transporting, type 2C, member 1	–2.2	–1.6
Ceramide kinase	–2.5	–2.0
Small proline-rich protein-4	2.7	2.2
Biliverdin reductase A	–1.9	–1.3
Mitogen-activated protein kinase kinase-1	2.1	1.5
Calbindin-1	11.3	4.4
S100 calcium-binding protein A12 (calgranulin C)	2.0	1.6
Others		
Thrombospondin-1	3.0	2.6
Sirtuin	2.9	1.6
TIMP metallopeptidase inhibitor-3	2.2	1.8
Glutathione peroxidase-2	3.3	2.7
Heparin-binding EGF-like growth factor	3.4	2.2
Interleukin-10 receptor-β	–2.7	–2.0
Interleukin-18	–1.8	–1.9

In particular, incubation of reconstructed human epidermis with 50-kDa HA for 48 h resulted in marked upregulation of calbindin-1, occludin, claudin-4, -7, and -17, TJP-2 compared to vehicle treatment (table 1). Notably, upregulation of all these genes was less pronounced in the case of 800-kDa HA (table 1).

Due to the fact that RNA pooled from 3 biological replicates was used for microarray studies, the obtained results were judged as an indication that tight junction control is modulated by application of 50-kDa HA. For further verification, the results for selected targets were

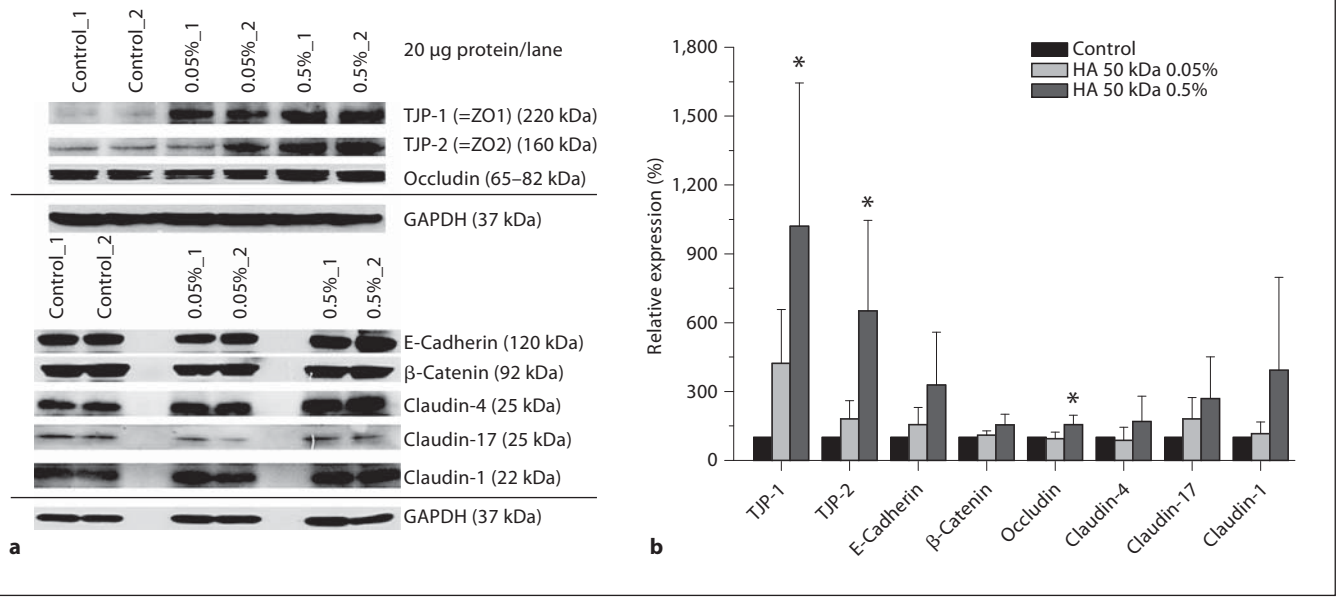

Fig. 3. a LMW HA is associated with increased expression of proteins known to be involved in keratinocyte regulation and cohesion. Accumulation of TJP-1, TJP-2, claudin-1, -4 and -17 as well as E-cadherin, β-catenin and occludin was determined in reconstituted human epidermis harvested after 72 h of topical treatment with either 0.05 or 0.5% 50-kDa HA. **b** Histograms depict intensities of the phosphorylated protein bands divided by the total form of the respective protein (n = 4). Bars represent means, error bars correspond to SEM. * $p < 0.05$, statistical significance for every comparison between groups.

Table 2. Effects of LMW HA with 50 kDa and HA with a molecular weight of 800 kDA on gene expression in reconstructed epidermis: qRT-PCR analysis

Target	HA 50 kDa		HA 800 kDa	
	fold change vs. vehicle	SD/p value	fold change vs. vehicle	SD/p value
Calbindin-1	5.1	0.28/0.00	1.2	1.41/0.64
Claudin-4	3.0	0.27/0.01	3.2	0.42/0.04
Claudin-7	6.1	0.41/0.01	2.1	0.44/0.06
Claudin-17	3.7	0.52/0.04	2.2	0.45/0.14
TJP-2	1.5	0.92/0.06	1.3	0.22/0.01
Occludin	1.1	0.19/0.01	0.8	0.25/0.10

reproduced independently by quantitative real-time PCR (qRT-PCR) experiments, in this case without pooling and including statistical analysis. Results are shown in table 2. In general, the qRT-PCR experiments confirmed the previous microarray results. With the exception of the occludin gene, all genes were upregulated by treatment with 50- or 800-kDa HA, and the effects were generally more pronounced in case of 50-kDa HA. All genes except the one encoding TJP-2 proved significantly ($p \leq 0.05$) upregulated by 50-kDa HA.

LMW HA Is Associated with Increased Expression of Proteins Known to Be Involved in Keratinocyte Differentiation and Cohesion

In order to confirm the superior stimulatory effects of 50-kDa HA on gene expression also on the protein level, we conducted a series of Western blotting experiments. Treatment of human epidermis models with 50-kDa HA resulted in increased protein levels of a number of TJPs in a dose-dependent manner. Specifically, TJP1 and TJP2 were found to be markedly increased after 72-hour posttreatment. The observed increase was accompanied by induction of claudin-1, -4 and -17 as well as E-cadherin, β-catenin and occludin (fig. 3). However, while incubation of human epidermis with 0.5% 50-kDa HA led to significant upregulation of TJP1, TJP2 and occludin, treatment with 0.05% 50-kDa HA did not significantly alter the amount of the respective proteins (fig. 3).

Discussion

HA, first discovered in the vitreous humor of the eye in 1934 [17] and subsequently synthesized in vitro in 1964 [18], consists of a basic unit of two sugars, glucuronic acid and N-acetylglucosamine, polymerized into large macromolecules of over 30,000 repeating units [3]. Due to its enormous water-binding capacity and its beneficial effects on cell proliferation, migration and thus tissue regeneration, HA has recently gained prominence in rejuvenative medicine as the injectable dermal filler of choice for the treatment of cutaneous lines and wrinkles [3]. However, applicability and potential beneficial activity features of HA have been shown to be limited by the molecular size of HA, which may reach up to 2,000 kDa and thus may interfere with efficient skin penetration. In contrast, VLMW HA or HA fragments may be recognized by so-called TLRs potentially leading to production of proinflammatory mediators [12]. At sites of inflammation, HA molecules undergo rapid degradation due to massive production of hyaluronidases by infiltrating inflammatory cells and bacterial invaders [19]. These HA fragments have been implicated in the process of injury and repair since they have been shown to activate inflammatory cells such as macrophages and dendritic cells to express proinflammatory mediators and enzymes degrading extracellular matrix [20–23]. Recent studies identified TLR-4 as the HA fragment binding receptor that links HA degradation and activation of inflammatory cells [24, 25]. In this study, we thus aimed to characterize an LMW-sized HA molecule that combines strong antiaging and moisturizing abilities with efficient skin penetration but misses the proinflammatory effects mediated by TRLs. By measuring TNF-α expression in reconstituted human epidermis after incubation with variedly sized LMW HA, we found that VLMW HA with a molecular weight of around 20 kDa led to marked upregulation of TNF-α expression in keratinocytes, indicating an inductive effect on the inflammatory response. In contrast, HA molecules with a molecular weight of 50 kDa or more did not alter the TNF-α expression profile in these cells to a large extent. However, differences in TNF-α expression may be due to penetration differences of the variedly sized HA molecules applied to the reconstituted human epidermis.

Having shown that HA with a molecular weight of 50 kDa and higher did not reveal proinflammatory activity in keratinocytes, we tested the penetrating properties of variedly sized LMW HA, namely 50, 300, 800 and 1,500 kDa. It was observed that a significantly higher skin penetration of 50-kDa HA molecules occurs compared to the ones with a molecular weight of 300-kDa HA and above. This indicates a relevant dependence of percutaneous transport on the HA molecular weight with a better permeation apparent for the lower-molecular-weight fractions. The route for this penetration is unknown, but as the molecular weight is much too high for percutaneous absorption, a follicular route is highly likely. Various skin delivery systems have been discussed [26]. Recent results on follicular penetration obtained at the Center for Experimental and Applied Cutaneous Physiology, Charité – Universitätsmedizin Berlin, Germany, emphasize that the hair follicles represent a highly relevant and efficient penetration pathway and reservoir for topically applied substances [27]. It has been demonstrated that the penetration depth of the particles can be influenced by their size resulting in the possibility of a differentiated targeting of specific follicular structures. In vitro measurements on pig ear skin appear to be equal or even superior for the analysis of follicular penetration, as compared to in vitro investigations on excised human skin indicating the suitability of this porcine tissue as a model for human skin [28, 29].

Having identified the smallest molecular size of HA that does not induce an inflammatory response but shows the best bioavailability within the range analyzed, we next aimed to characterize the molecular impact of a LMW HA of about 50 kDa on keratinocytes in comparison to a HA molecule with a molecular weight of 800 kDa, which is a representation of the commonly utilized HA used as active ingredient in cosmetic preparations for years. Using transcriptome analysis, we found that incubation of keratinocytes with LMW HA significantly affected overall up- or downregulation of about 120 genes, in particular genes known to be involved in keratinocyte regulation as well as genes that play important roles for their cohesion. HA with a molecular weight of about 800 kDa, in contrast, only induced differential expression of 40 genes, whereas topical application of 0.5% retinol under the same conditions changed the expression of about 160 genes (data not shown). These findings demonstrate the high gene regulatory potential of LMW HA. The treatment specifically led to marked upregulation of a broad variety of different TJPs on a genomic and proteomic level. TJPs comprise a novel group of integral membrane proteins necessary for cell-to-cell contacts and responsible for the barrier function in epithelial and endothelial cells in various tissues. The tight junction membrane domain contains at least three distinct proteins, named occludin, claudin and junctional adhesion

molecule, all of which have been discussed to contribute to keratinocyte differentiation and formation of intercellular tight junction complexes [30, 31].

The data obtained in our in vitro experiments suggest that the novel LMW HA may be associated with better anti-wrinkle properties in contrast to high-molecular-weight HA based on improved skin penetration. Clinical data elucidating the clinical effects of topically applied HA of differing molecular weight support our hypothesis (data not published yet). HA is a substance with various physiological functions in different cell and tissue types. Clearly, one of its major functions in skin is to constitute the structural backbone of the dermal extracellular matrix promoting proper tissue architecture. Secondly, it binds large amounts of water, thereby keeping the dermal skin layer properly moisturized. Apart from these well-characterized functions, a growing number of studies suggest that HA also participates in different cellular functions, including regulation of metabolite diffusion, cell migration, cell signaling, cell proliferation and differentiation, wound healing, cell adhesion, angiogenesis and inflammation [32]. Although the largest amount of skin HA is present in the dermis, significant amounts could also be detected in the epidermis, in particular in the upper spinous and granular layers [33–35], but also in the stratum corneum [36]. It is assumed that HA contributes to maintenance of epidermal skin hydration. Other functions of epidermal HA might include regulation of cell mitosis (basal layer) as well as recruitment of Langerhans cells via their CD44 receptors [37, 38] and wound healing/regeneration processes [34]. Notably, HA levels were found to be greatly reduced in aged epidermis, but not in aged dermis. This age-related epidermal HA deficiency might be compensated by topical delivery via cosmetic formulations containing HA.

Conclusions

Beneficial features of HA may be related to particular molecular sizes. We found that an LMW HA of approximately 50 kDa penetrates the skin better than larger-sized HA and, accordingly, influences the expression of many genes including those contributing to keratinocyte differentiation and formation of intercellular tight junction complexes which are reported to be reduced in aged and photodamaged skin. This can be utilized without running the risk of increased proinflammatory activity.

Acknowledgements

This study was funded by Evonik Goldschmidt, Essen, Germany, and by Novozymes Biopolymers, Copenhagen, Denmark.

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