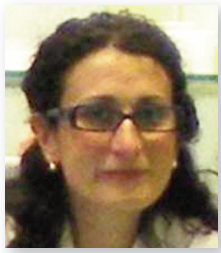
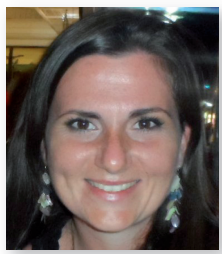
Small fragments sodium sulfated hyaluronate, more than hyaluronic acid, reduces LPS-induced cytokine/chemokine levels in HaCaT cells

Serena Lembo *
Maria Schiattarella *
Anna Balato
G. Caiazzo
Roberta Di Caprio
Giuseppe Monfrecola

Serena Lembo



Maria Schiattarella



Department of Clinical Medicine and Surgery Section of Dermatology, University of Napoli Federico II, Naples, Italy.

* Dr. Lembo and Dr. Schiattarella equally contributed to the production of this manuscript.

Summary

Small fragments sodium sulfated hyaluronate, more than hyaluronic acid, reduces LPS-induced cytokine/chemokine levels in HaCaT cells

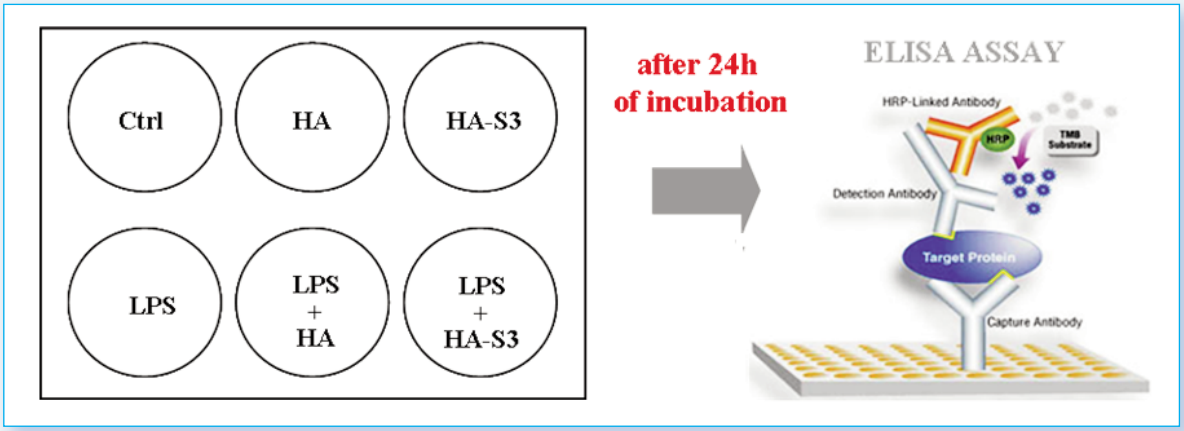
Hyaluronic acid (HA) is a linear non-sulphated glycosaminoglycan, used in dermatology as a biomaterial for bioengineering purposes, temporary dermal filler, stimulation of wound healing as well as drug vehicle in topical formulations. In addition to the well-characterized structural properties, extensive research on HA has revealed a range of vastly immune-modulatory effects, dependent on its size. In this in vitro study we investigated the ability of HA-S3, a small fragment HA (MW, molecular weight: 68 kDa) with degree of sulphatation of 3 and of HA fraction (MW:210 kDa) to reduce the bacterial induced inflammatory response in spontaneous immortalized keratinocytes. To this purpose, HaCaT cells were treated for 24 hours with 25 µg/ml of E. Coli derived bacterial lipopolysaccharide (LPS) in absence or presence of small fragment HA-S3 or HA. Cell viability was thereafter assessed using trypan blue stain and interleukin (IL)-8, IL-1β and tumor necrosis factor alpha (TNF-α) concentrations were determined in cell supernatants by single enzyme-linked immunoadsorbent assay (ELISA). Our results showed that cell viability was not affected either by HA-S3 or HA which in turn were able to reduce LPS-induced mortality. HA and especially HA-S3 were able to significantly reduce LPS-induced pro-inflammatory cytokines. Our observation might suggest new perspectives in the development of HA-S3 containing topical products able to modulate cutaneous inflammatory response.

Key words: *Hyaluronic acid, Anti-inflammatory effect.*

Hyaluronic acid (HA) is a linear non-sulphated glycosaminoglycan, composed of glucuronic acid and N-acetylglucosamine disaccharide units, abundant in the form of a *high-molecular-weight* (HMW) polymer ranging from 10^5 to 10^7 Da.[1] HA is able to bind water, building a pericellular coat, which, in turn, supports volume expansion and diffusion of metabolites. HA is nowadays used in dermatology as a biomaterial for bioengineering purposes, temporary dermal filler, stimulation of wound healing as well as drug vehicle in topical formulations[1]. In addition to the well-characterized structural properties, extensive research on HA has revealed a range of vastly immune-modulatory effects, focused on the regulatory influence exerted on the inflammatory pathway[2]. Further studies have deduced that the function of HA is dependent on its size, but the question remains controversial. Some authors state that HA fragments smaller than 500 kDa induces inflammatory responses, whereas larger MW HA mixtures are anti-inflammatory and anti-angiogenic[3]. Substantial evidence from other authors indicates that, in sites of cutaneous inflammation, HA, may be depolymerized in small fragments (MW < 200 kDa) that stimulates immunocompetent cells, including keratinocytes, to produce different soluble factors with antibacterial activity and to revert LPS-induced pro-inflammatory mediators[4]. *Fidia Farmaceutici S.p.A.* produced a small fragment HA (MW: 68 kDa)

Figure 1.

Study design. Schematic representation of the experimental setting with LPS (25 µg/ml) stimulation and HA or HA-S3 (1 mg/ml) treatment.

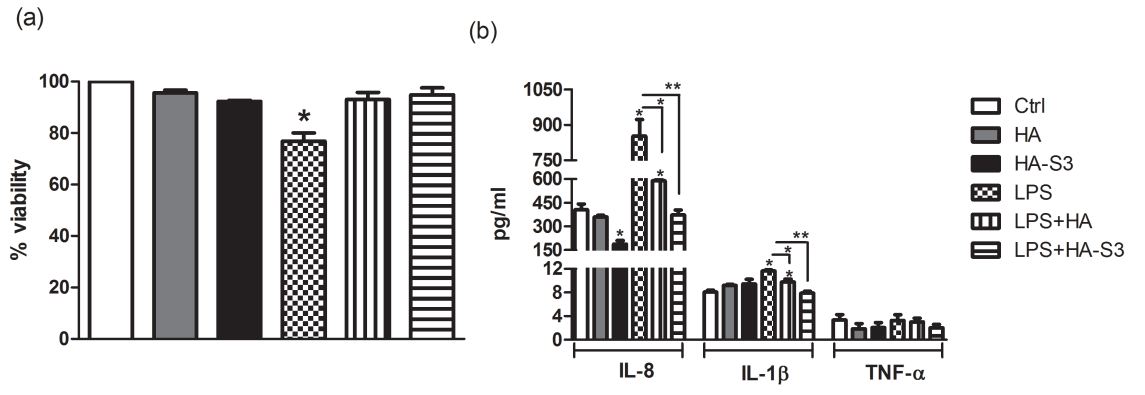


with degree of sulphatation of 3 (HA-S3) and a HA fraction (MW:210 kDa). In this in vitro study we investigated the ability of HA-S3 and HA to reduce the bacterial induced inflammatory response in spontaneous immortalized keratinocytes. To this purpose, HaCaT cells were treated for 24 hours with 25 µg/ml of E. Coli derived bacterial endotoxin-lipopolysaccharide (*LPS, Sigma-Aldrich, Oakville, ON, Canada*) in absence or presence of small fragment HA-S3 or HA (*1 mg/ml, Fidia Farmaceutici, Abano Terme, PD, Italy*) (Figure 1).

Cell viability was thereafter assessed using trypan blue stain and interleukin (IL)-8, IL-1β and tumor necrosis factor alpha (TNF-α) concentrations were determined in cell supernatants by single enzyme-linked immunoadsorbent assay (ELISA) (Figure 2) using commercially availa-

Figure 2.

Cell viability and protein assessment. (a) Evaluation of cell viability in HaCaT cells stimulated or not with LPS (25 µg/mL) and incubated with HA or HA-S3 (1 mg/ml). Cell viability was measured at 24 h. Statistical significance was determined with respect to the viability of non-stimulated cells. (b) IL-8, IL-1β and TNF-α protein levels in supernatants from HaCaT cells stimulated or not with LPS (25 µg /mL) and incubated with HA or HA-S3 (1 mg/ml) at 24h. All statistical analyses were performed using GraphPad Prism 4.0 (GraphPad Software Inc., La Jolla, CA). Data that passed the normality test were analysed with two tailed t-test. Values of $p < 0.05$ were considered significant. Data are expressed as means ± SD of three independent experiments, each performed in triplicate ($p < 0.05$, **$p < 0.01$).*



ble kit (*R&D Systems, Minneapolis, MN, USA*), according to the manufacturer's instructions. Our results demonstrated that HA-S3 or HA did not exert any toxic effect but both compounds were protective toward LPS-induce HaCaT cell damage. In particular, cell viability was not affected either by HA-S3 or HA which in turn were able to reduce LPS-induced mortality, increasing cell viability rate from 76% to 93 and 95% respectively (Figure 2a).

Concerning the inflammatory modulators assessed, LPS as expected [5], considerably enhanced protein levels of IL-8 and IL-1β in cell supernatants ($p < 0.05$). HA ($p < 0.05$) and especially HA-S3 ($p < 0.01$) were able to significantly reduce LPS-induced pro-inflammatory cytokines. A similar trend was found also for TNF-α (Figure 2b) which was slightly enhanced after LPS compared to control. Our data corroborates the findings of *Gariboldi et al.* who demonstrated that HA was able to revert to basal level, the increase of the same pro-inflammatory mediators induced by LPS in keratinocytes [4].

In our study a step forward has been made, resulting small fragment HA-S3 more effective than small HA. Our observation might suggest new perspectives in the development of HA-S3 containing topical products able to modulate cutaneous inflammatory response.

References

1. Anderegg U, Simon JC, Averback M. More than just a filler - the role of hyaluronan for skin homeostasis. Exp Dermatol. 2014; 23:295-03.

2. Hašová M1, Crhák T, Safránková B, et al. Hyaluronan minimizes effects of UV irradiation on human keratinocytes. Arch Dermatol Res. 2011; 303:277-84.

3. Jiang D, Liang J, Noble PW. Hyaluronan as an immune regulator in human diseases. Physiol Rev. 2011; 91:221-64.

4. Gariboldi S, Palazzo M, Zanobbio L, et al. Low molecular weight hyaluronic acid increases the self-defense of skin epithelium by induction of beta-defensin 2 via TLR2 and TLR4. J Immunol 2008; 181:2103-10.

5. Di Caprio R, Lembo S, Di Costanzo L, et al. Anti-inflammatory properties of low and high doxycycline doses: an in vitro study. Mediators Inflamm: 2015; 329418.