Effects of sphingomyelin and hyaluronic acid on prostaglandin E2 secretion in canine keratinocytes.

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Introduction

Keratinocytes are highly active immunological cells, with major control over the acute and the chronic phases of allergic reaction by means of inflammatory mediators' production[1]. Prostaglandin E2 (PGE_2) abundantly synthesized prostaglandin, especially at inflammation sites, has long been regarded as an important potentiator of acute inflammation[2].

Objective

The aim of this study was to asses the inhibitory effects of sphingomyelin (SM) and hyaluronic acid (HA) on PGE_2 secretion in canine keratinocytes cultures.

Material and methods

Cell cultures

Canine keratinocytes were freshly isolated from skin biopsies as previously described[3].

Cells ($6x10^4$) were plated onto collagen coated six-well plates and cultured during 24h in the presence of either sphingomyelin (SM) (Avanti Polar Lipids Inc., Alabster, AL, USA), hyaluronic acid (HA) (Bioiberica SA, Barcelona, Spain) or SM plus HA at increasing concentrations (0.001 mg/mL, 0.003 mg/mL and 0.01 mg/mL).

Cell stimulation

After 24h , cells were stimulated with poly-(I:C) at 10 µg/mL (InvivoGen, San Diego, CA, USA) during 4h in order to induce PGE_2 release. Supernatants were collected and placed at -80 ºC until measured.

PGE_2 release determination

To study PGE_2 synthesis and release an enzymatic immunoassay test with proven efficacy in canine cells was used[4] (Cayman Chemical Company, Ann Arbor, MI, USA).

Statistical analysis

Results of PGE_2 release (n=3) were expressed as mean ± standard error of the mean. Difference between means of different compounds concentrations and control poly-(I:C) treatment were tested for significance by analysis of variance (ANOVA) and Dunett's multiple comparison test.

References

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2. Morimoto K, Shirata N, Taketomi K *et al.* J Immunol. 2014: 192: 1130-1137.
3. Cerrato S, Ramio-Lluch L, Fondevila D *et al*. J Vet Med 2013: 2013: 231526.
4. Gueck T, Aschenbach JR, Fuhrmann H. Vet Dermatol 2002: 13: 301-305.

Conflict of interest

S. Cerrato: none declared, L. Ramió-Lluch: none declared, P. Brazís: none declared, S. Segarra is employed by Bioiberica SA, Barcelona, Spain. Bioiberica SA provided the compounds used in the study, A. Puigdemont: none declared.

Results

The results showed a significant ($p<0.001$) inhibition of PGE_2 secretion in the presence of SM at 0.001 mg/mL (54.7 ± 5.1%), 0.003 mg/mL (76.5 ± 2.3%) and 0.01 mg/mL (84.9 ± 2.6%).

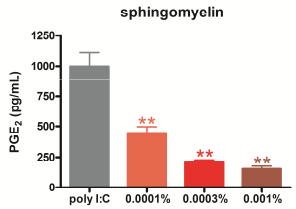

Figure 1. PGE_2 release inhibition induced by increasing SM concentrations in keratinocytes after poly-(I:C) stimulation. **P < 0.01 by comparison with poly-(I:C) control.

A significant 63.9 ± 11.9% inhibition was also seen with 0.01 mg/mL SM plus HA ($p<0.001$).

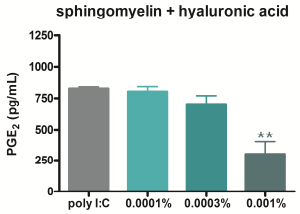

Figure 2. PGE_2 release inhibition induced by increasing SM+HA concentrations in keratinocytes after poly-(I:C) stimulation. **P < 0.01 by comparison with poly-(I:C) control.

Although a significant inhibitory effect was not observed with HA alone, a 24.6 ± 6.7% decrease in PGE_2 secretion was observed at 0.01 mg/mL.

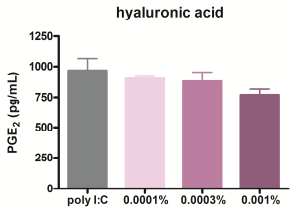

Figure 3. PGE_2 release inhibition induced by increasing HA concentrations in keratinocytes after poly-(I:C) stimulation.

Conclusion

Sphingomyelin may have a therapeutic potential in the management of inflammatory skin diseases due to its ability to down-modulate PGE_2 secretion in canine keratinocytes.

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