IFSCC Mexico Conference 2021

OSM 欧诗漫

NT_91

Establishment and application of LPS-induced keratinocyte inflammation model

Sun, Fanghui[1]; Song, Xiaojie[2]; Huo, Gang[3];

[1,2,3] R&D Center, Osmun Biological Co., LTD., Zhejiang, China.

Introduction:

With the increasingly complex living environment, itching, tingling and other skin inflammation has become a common skin problem. Therefore, screening of anti-inflammatory cosmetic ingredients is a research hotspot in the daily chemical industry.

LPS-induced inflammatory model is a widely used method for screening anti-inflammatory agents, but LPS concentration, cell type and laboratory conditions are all important factors affecting the experimental results.

In this study, we developed a model of LPS-induced KC inflammation, and explored the application of this model in screening out active compounds with anti-inflammatory effects.

Materials & Methods:

Chemicals and Reagents

Human primary keratinocytes were extracted from skin tissue and used in all experiments. Related reagents and their manufacturers include: LPS (*E.coli* 0111:B4) (Sigma,USA) ; MTT(Biosharp Life Science, China) ; penicillin and streptomycin (Biological Industries, Israel) ; ELISA kits (Abcam, UK) ; KSFM(Gibco, USA) .

Cell culture

Keratinocytes were grown in complete KSFM and incubated at 37℃ in 5%CO_2 incubator. Cells were harvested by centrifugation at 1000 rpm for 5 minutes. Medium for cells was changed after every other day.

Cell viability assay

Cells were seeded in 96 well-plates at a density of 3×10^3 cells per well. The cells were treated with 0.01-100μg/mL LPS when the cell confluence reached about 90%. The plates were incubated for 24h, 0.5mg/mL MTT was added to each well. After 3h, DMSO was added and the absorbance was measure with plate-reader at 560nm.

ELISA assay

Cells were seeded in 24 well-plates at a density of 5×10^4 cells per well. The cells were treated with 0.01-100μg/mL LPS or 100μg/mL LPS combined with substance A (a mixture of magnolia, peony root bark and scutellaria extract) when the cell confluence reached about 90%. After incubation for 24h, the medium supernatant was collected and the concentration of IL-1α and IL-8 was detected by ELISA kits.

Results & Discussion:

1. Effects of LPS on cell viability of KC

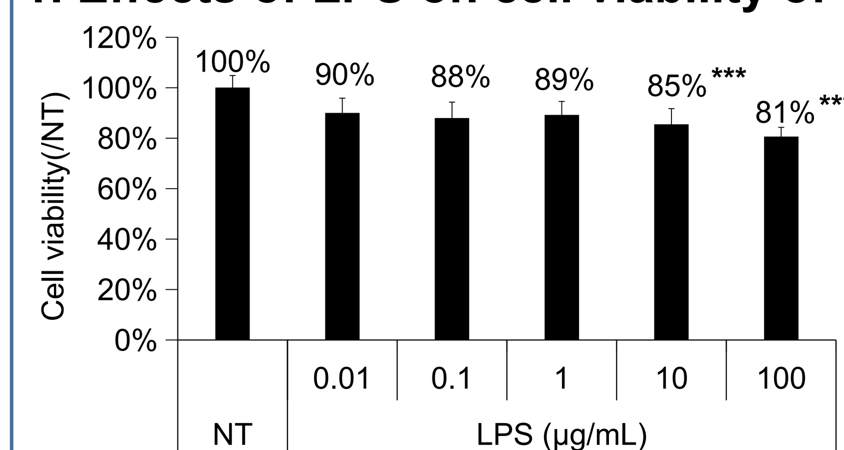

Fig. 1 Cytotoxicity of different concentrations of LPS on KC.

- After treatment with 0.01-100μg/mL LPS, the viability of KC was all higher than 80%, it showed that 0.01-100μg/mL LPS didn't have big influence on the cell viability of KC,and this concentration range could be used for subsequent experiments.

2. Effect of LPS on IL-1α expression in KC

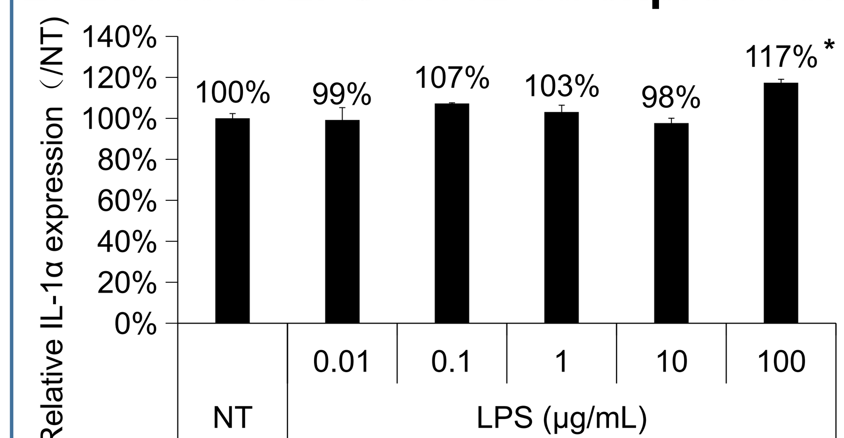

Fig. 2 Effect of LPS on the expression of IL-1α.

- The level of IL-1α in KC was not significantly changed when treated with 0.01-10μg/mL LPS, but increased by 17% with 100μg/mL LPS.Thus, KC can be induced to produce numerous IL-1α by 100μg/mL LPS.

3. Effect of LPS on IL-8 expression in KC

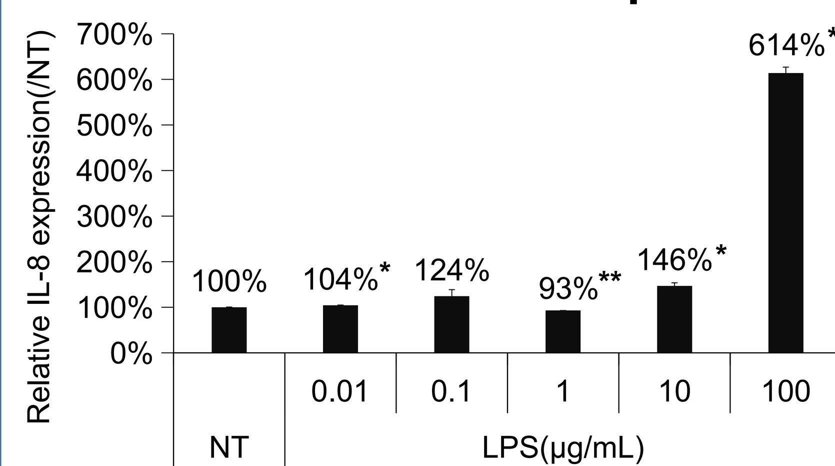

Fig. 3 Effect of LPS on the expression of IL-8.

- 0.01-1μg/mL LPS could cause slight fluctuation of IL-8 in KC, 10μg/mL and 100μg/mL LPS could up-regulate IL-8 expression in KC by 46% and 514% respectively. Therefore, both 10μg/mL and 100μg/mL LPS can be used to induce KC to produce plentiful IL-8.

4. Application of LPS-induced KC inflammation model

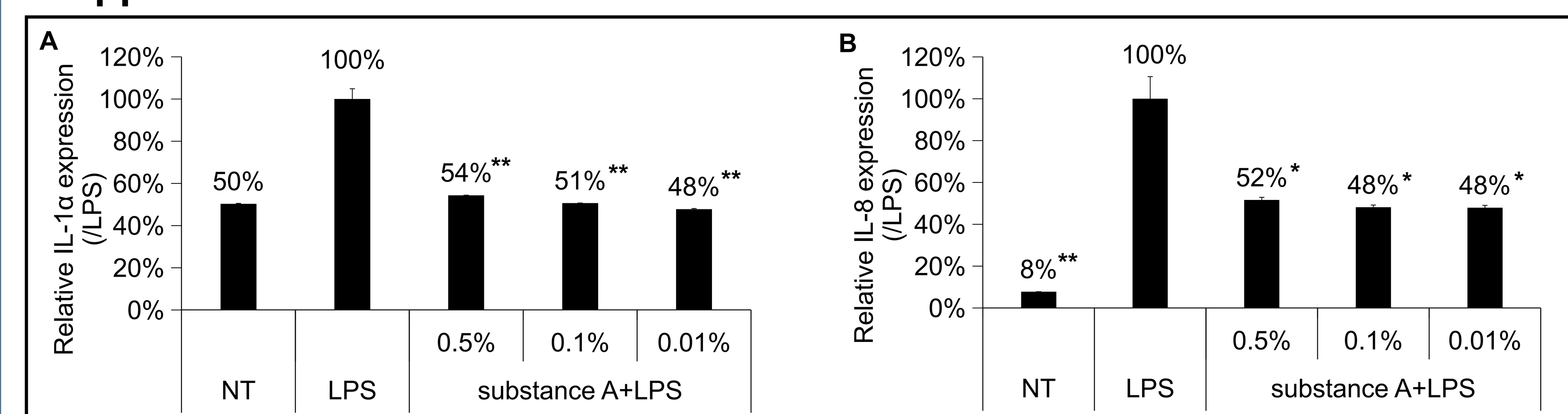

Fig. 4 Effect of substance A on LPS-induced changes in the expressions of IL-1α and IL-8.

- 100μg/mL LPS can evidently increase the levels of IL-1α and IL-8 in KC, while substance A can reduce the the level of IL-1α and IL-8 by about 50%. The 0.01% substance A had the strongest inhibitory rate. And the inhibitory rate of IL-1α and IL-8 were 52%. Since IL-1α and IL-8 are the markers of inflammatory response, it indicates that substance A has certain anti-inflammatory effect.

Discussion:

Besides KCs, other skin-resident cells (Langerhans cells, melanocytes and macrophages) also secrete cytokines that participate in local immune modulators. Different cells play different roles in skin inflammation and respond to inflammation to different degrees. In addition, after external stimulation, the expression of various types of cytokines are different, and their roles in the inflammatory response are also diverse. This experiment only studied the inflammatory response on KC and only two cytokines, which can't fully and comprehensively elaborate the problems related to skin inflammation.Therefore, inflammatory responses on other skin cells and the effects of LPS on other cytokines will be investigated in our follow-up plan,so as to explore the inflammatory reaction of skin from various perspectives to obtain more accurate experimental results.

In short, this model we developed fills in the lack of researches on the effect of LPS on KC, provides suggestions and guidance for other researchers to carry out related experiments, and provides a new reference for the study of inflammatory skin, and contributes to the uncovering of anti-inflammatory cosmetics.

Conclusions:

100μg/mL LPS is more suitable for inducing KC to produce inflammatory response compared with other concentrations. Therefore, a model of LPS-induced KC inflammation was developed, and the model could successfully screen out cosmetic ingredients with anti-inflammatory effects.

Aknowledgments:

This work was supported by the Osmun Biological Co., LTD.

References:

1. Nathan K. Archer, Jay-Hyun Jo, Steven K. Lee, *et.al.*(2019) Injury, dysbiosis and filaggrin deficiency drive skin inflammation via keratinocyte IL-1α release.J Allergy Clin Immunol. 143(4): 1426–1443.
2. Walana, W., Wang, J., Yabasin, I.B., *et.al.*(2018) IL-8 analogue CXCL8 (3-72) K11R/G31P, modulates LPS-induced inflammation via AKT1-NF-kβ and ERK1/2-AP-1 pathways in THP-1 monocytes, Human Immunology.79(11):809-816.
3. Nedoszytko, B., Sokołowska-Wojdyło, M., Ruckemann-Dziurdz´nska, K., et.al.(2014) Chemokines and cytokines network in the pathogenesis of the inflammatory skin diseases: Atopic dermatitis,psoriasis and skin mastocytosis. Postep. Dermatologii i Alergol. 31: 84–91.