The anti-inflammatory efficacy test of Cosmetic Ingredients Using Bioartificial Skin Model

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Abstract

In this study, an efficacy study of Portulaca Extract (PE) and β-glucan, candidates for cosmetic additives, was performed using artificial skin model (AS). The AS consists of collagen gel matrix populated by ATCC human skin fibroblast cell line that is overlaid with epidermal human skin keratinocyte cell line. Cytotoxicity and anti-inflammatory activity of PE and β-glucan were assessed using monolayer and AS models by measuring cell viability and the secretion of interleukin-1α.

Introduction

The use of animals for safety evaluation is increasingly being criticized from an ethical point of view so it has become necessary to develop alternative test methods which do not use animals. Since skin responses such as irritation are a complex process where several cell types interact to elicit the inflammatory response[1], fibroblast or keratinocyte monolayer model do not represent proper response of real skin and the extent of epidermal differentiation attained in artificial skin (AS) with the appearance of a stratum corneum, which allows for the topical application of test samples, suggested that the artificial skin may provide a realistic model[2].

Materials and methods

Cell

In this study we used two kinds of cell lines which were ATCC haman skin fibroblast and human skin keratinocyte cell line. Both of cell lines

were cultured with Dulbecco's modified Eagle medium (DMEM, Gibco BRL, Grand Islands, N.Y., USA) supplemented with 10% fetal bovine serum (FBS, Gibco Co.) in 37℃, 5% CO_2 incubator.

Artificial Skin (AS)

The dermal equivalent (DE) culture were prepared from Cellmatrix (Nitta-gelatin Co.) according to a modification of the method described by Bell et al. (1) and Yang et al. (2). 1×10^5 cells / ml of fibroblasts were mixed with collagen solution (the ratio of collagen, 5×DMEM and raft buffer is 7 : 2 : 1). After 7days 3×10^5 cells / ㎠ of keratinocytes were seeded onto DE and then submersion-cultured with DMEM containing 10% FBS + K-SFM supplemented with EGF (epidermal growth factor) and BPE (bovine pituitary extract) for 1week. the developing multilayered artificial skin was cultured at the air liquid interface for 2weeks.

Exposure of Test Substances and MTT assay

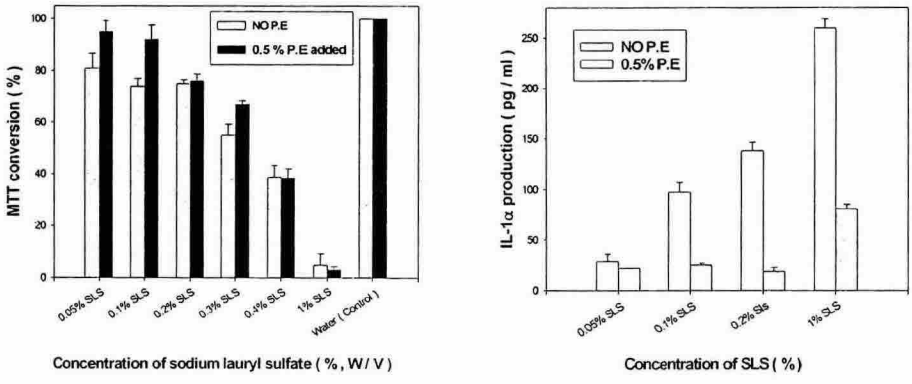
SLS (sodium lauryl sulfate, Fisher Scientific, Pittsburgh, Pa., USA) was used for the exposing substance. Before the test chemical exposure, medium was aspirated from the wells and then each concentration of SLS was dispensed into the inserts. After 4hours portulaca extract or β-glucan was dispensed in each insert and then cultures were incubated for 24h at 37℃, 5% CO_2 incubator. Medium was aspirated from cultures and 1.5ml PBS (phosphate-buffered saline) containing 0.33 mg MTT/ml Cultures were then incubated for 4h, then the PBS solution was aspirated and formazan blues were extracted in 3ml of isopropanol (Sigma Co.) acidified with 0.04N HCl (Junsei Chemical Co., Tokyo, Japan) for 2h at room temperature. The absorbance was read at 570nm in an ELISA

Assay for interleukin-1α production

IL-1α (interleukin-1α) determination were performed using the ENDOGEN human Interleukin-1α ELISA kit (Endogen Co., USA) which is an in vitro enzyme-linked immunosorbent assay the quantitative measurement of human IL-1α in serum. The absorbance was read at 450nm in an ELISA

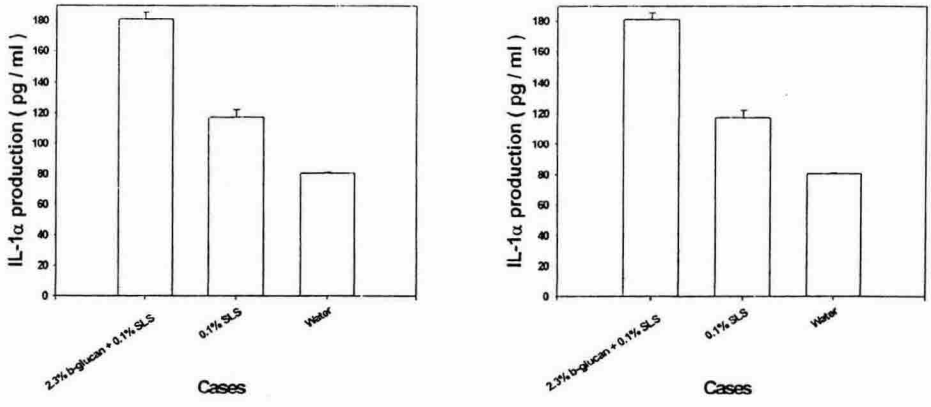
Results and Discussion

The results of this study demonstrated that an efficacy of Portulaca Extract (PE) and β-glucan, candidates for cosmetic additives, with artificial skin model (AS) which has studied in our laboratory. As shown in fig. 1 (a), the viability of samples added to portulaca extract is higher than not added. While the amount of interleukin-1α production of ASs added to 0.5% portulaca extract is much lower than just added to sodium lauryl sulfate (b). The results of this study demonstrate that portulaca extract is able to alleviate inflammation induced by sodium lauryl sulfate. Fig. 2. (a) is the comparison of MTT conversion in AS added 2.3% β-glucan and not added after sodium lauryl sulfate has been spreaded. As shown (a), MTT conversion of ASs added 2.3% β-glucan is much higher than not added and Inteleukin-1α production of ASs added 2.3% β-glucan is also higher than not added (b). As result of this study, the reason that pre-inflammatory mediator interleukin-1α increased rapidly might be not because of increasing the inflammatory response but because of cell proliferation.



(a) (b)

Fig. 1. Dose-response comparison of MTT conversion in AS(artificial skin) exposed to each concentration of sodium lauryl sulfate and then evaluate the efficacy of portulaca extract (a). The comparison of IL-1α production between the Artificial Skin added 0.5% portulaca extract and nothing added (b).



(a) (b)

Fig. 2. The comparison of MTT conversion in AS(artificial skin) exposed to 0.1% sodium lauryl sulfate and then evaluate the efficacy of β-glucan (a). The comparison of IL-1α production between artificial skin added sodium lauryl sulfate and then 2.3% β-glucan and sodium lauryl sulfate only (b). After 24h being added the β-glucan. the culture medium was sampled for interleukin-1α production assay. The absorbance read at 450nm in an ELISA.

Conclusion

Through this study with AS manufactured by our laboratory we could evaluate the efficacy of portulaca extract which has been used in cosmetic formula as an anti-inflammatory and moisturizing agent and 2.3% β-glucan which has known to has anti-tumor and immuno-stimulating properties in vitro. According the result our data from AS (artificial skin) was more similar to the data from in vivo test compared with monolayer culture (data not shown). Based on our culture system futher protocol for 3D AS model containing melanocyte is ongoing to whitening effects test of substances for cosmetic formula and phototoxicity test in vitro.

Reference

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