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Screening of xerosis inhibitor from seaweed extracts using HaCaT keratinocyte

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Introduction

Xerosis(dry skin) and reduced barrier function occur as a result of reduced stratum corneum thickness and ceramide content (Imokawa et al., 1991), decreased with age (Rogers et al., 1996).

In this study, seaweed extracts have been tested for the increased production of ceramide contents from HaCaT keratinocyte as a preliminary screening.

Materials and Methods

Seaweed extracts Seaweed thalli were collected from the coast of Korea from October 2003 to January 2005. Methanol and water extracts were prepared according to Jin et al. (1997).

Cell culture The spontaneously immortalized human keratinocyte cell line HaCaT was cultured in Dulbecco's modified Eagle medium (DMEM) with 10% fetal bovine serum and 100 units/ml penicillin/streptomycin at 37℃ in an incubator containing 10% CO_2.

Lipid extraction from HaCaT HaCaT keratinocyte was seeded in 12-multiwell culture plate and grown up to approximately 50% confluence. Then the cells were treated with the seaweed extracts. After incubation for 24 h, the cells were washed twice with phosphate-buffered saline (PBS) and were harvested by scraping in 0.88% KCl. One mL of solution (chloroform/methanol/water 2:4:1.6) was used to extract the epidermal lipids from the harvested keratinocyte overnight at -20℃ (Bligh and Dyer., 1959). A mixture of chloroform and water (1:1) was added to the sample at room temperature. After shaking for 10 min, the mixture was centrifuged at 900G for 10 min, the chloroform phase was collected for lipid extraction.

TLC and lipid visualization Thin layer chromatogram was developed twice with chloroform: ethanol: acetic acid (190:9:1) to resolve lipids in the sample. After solvent development, the plate was air dried, sprayed with a solution (10% $CuSO_4$, 8% H_3PO_4) and charred at 180℃.

Results and Discussion

Of the 36 seaweed extracts tested, *Ishige sinicola* and *Helminthocladia australis* induced ceramide-like substance I from HaCaT keratinocyte. *Sargassum patens, Chondrus ocellatus* and *Gigartina tenella* induced less amounts of the ceramide.

Helminthocladia australis and *Pachymeniopsis elliptica* induced ceramide-like substance II from HaCaT keratinocyte.

Work is in progress to measure ceramide quantitatively and to confirm the main ceramide compounds responsible for the seaweed extracts.

References

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