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Effects of Magnesium Ascorbyl Phosphate on the Expression of Inflammatory Biomarkers after Treatment of Cultured Sebocytes with *Propionibacterium acnes* or Ultraviolet B Radiation

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Dear Editor:

Acne is a common skin disorder of the hair follicle. The proliferation of *Propionibacterium acnes* plays an important role in the pathophysiology of acne¹. In addition

to excessive sebum production, abnormal composition of the sebum and sebum peroxidation contribute to the formation of primary acne lesions². Inflammatory cytokines play a vital role in the formation and aggravation of acne

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lesions, and production of inflammatory cytokines in the sebaceous glands is increased by exposure to *P. acnes* and ultraviolet B (UVB) radiation³. Vitamin C is associated with several beneficial properties, including photoprotection from ultraviolet A and B radiation, improvement in a variety of inflammatory dermatoses, and antioxidant properties⁴. In

this study, we evaluated the effects of magnesium ascorbyl phosphate (MAP) on the expression of inflammatory biomarkers and sebum peroxidation after the treatment of cultured sebocytes with *P. acnes* or UVB radiation. Sebocytes obtained from the sebaceous glands of the occipital hair follicle were cultured in Dulbecco's modified

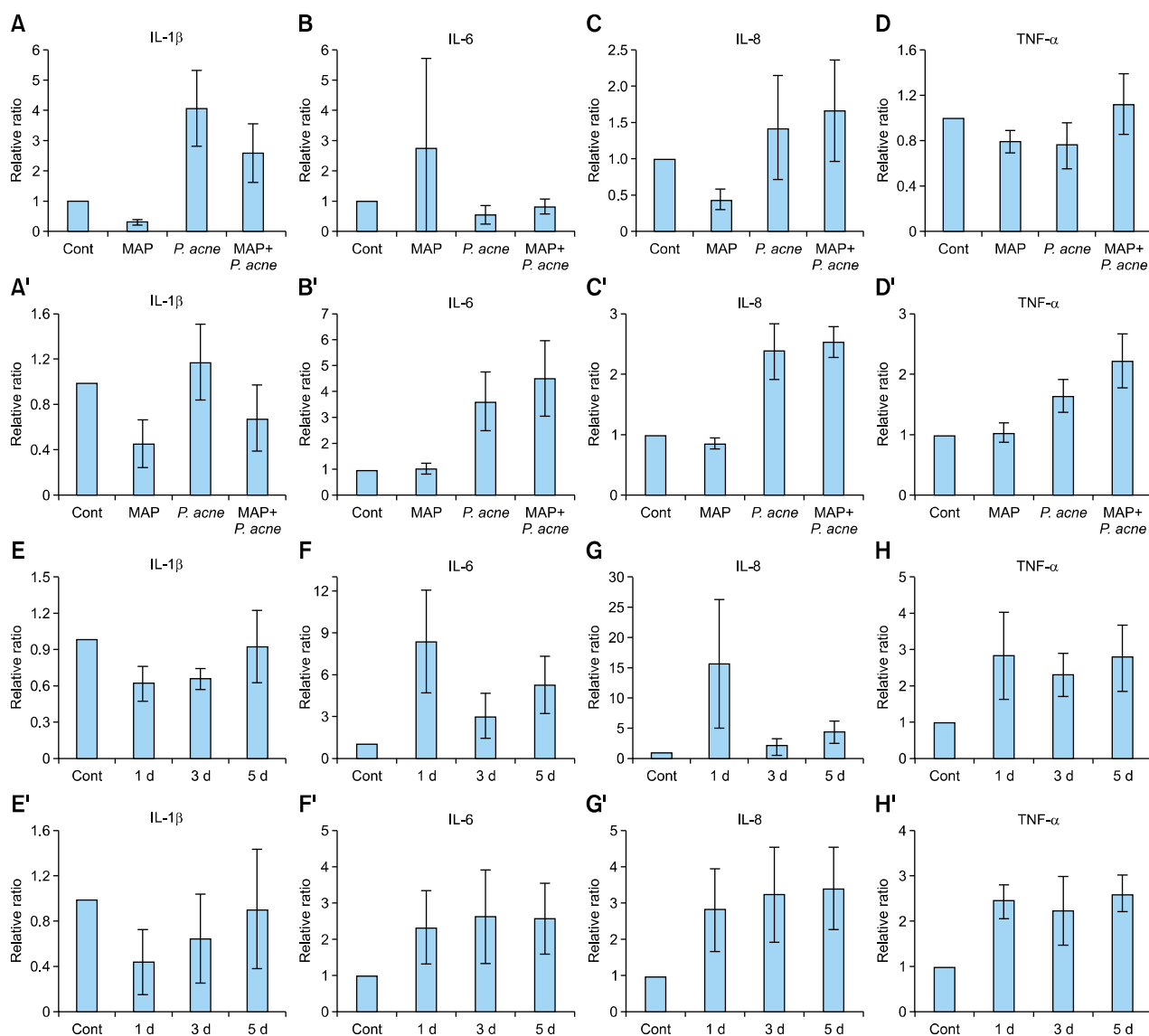


Fig. 1. (A~D) The gene expression of interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor α (TNF- α) after the treatment of cultured sebocytes with magnesium ascorbyl phosphate (MAP, 10^{-2} M), *Propionibacterium acnes* (10^{10} CFU/ μ l), or a combination of MAP (10^{-2} M) and *P. acnes* (10^{10} CFU/ μ l). (A'~D') The protein expression of IL-1 β , IL-6, IL-8 and TNF- α after the treatment of cultured sebocytes with MAP (10^{-2} M), *P. acnes* (10^{10} CFU/ μ l), or a combination of MAP (10^{-2} M) and *P. acnes* (10^{10} CFU/ μ l). MAP inhibited the upregulation of IL-1 β expression in cultured sebocytes induced by *P. acnes*. MAP did not inhibit the *P. acnes*-induced increase in the expression of IL-6, IL-8, and TNF- α after the treatment of cultured sebocytes with *P. acnes*. (E~H) The gene expression of IL-1 β , IL-6, IL-8 and TNF- α after the treatment of cultured sebocytes with MAP (10^{-2} M) and 40 mJ/cm² ultraviolet B (UVB) radiation. (E'~H') The protein expression of IL-1 β , IL-6, IL-8 and TNF- α after the treatment of cultured sebocytes with MAP (10^{-2} M) and 40 mJ/cm² UVB radiation. MAP decreased the expression of IL-1 β in cultured sebocytes 1, 3, and 5 days after exposure to 40 mJ/cm² UVB radiation. Cultured sebocytes treated with MAP did not show a decrease in the expression of IL-6, IL-8 and TNF- α after exposure to 40 mJ/cm² UVB radiation. Cont: control.

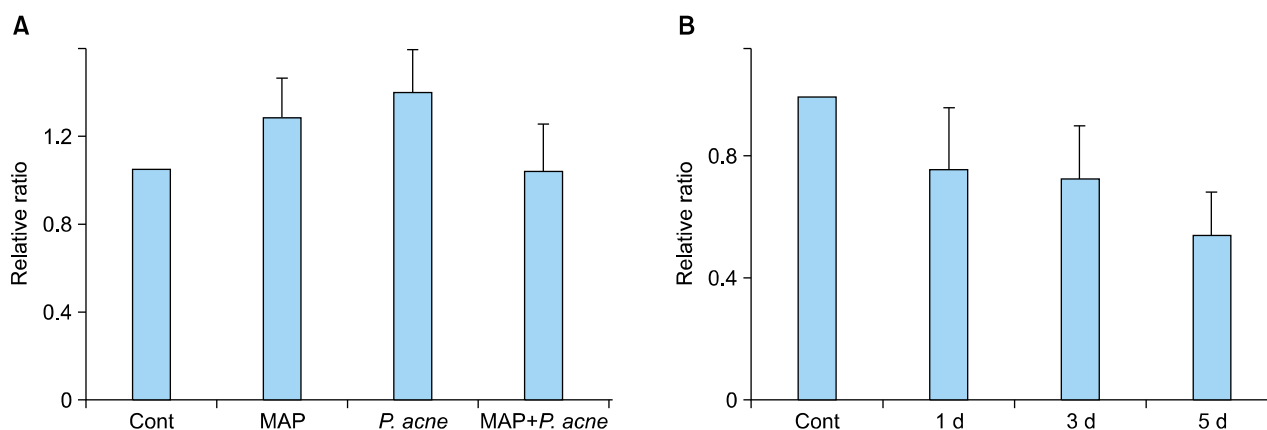


Fig. 2. Lipid peroxidation in cultured sebocytes. Magnesium ascorbyl phosphate (MAP, 10^{-2} M) decreased the levels of lipid peroxidation after the treatment of cultured sebocytes with *Propionibacterium acnes* (10^{10} CFU/ μ l) (A) or 40 mJ/cm² UVB radiation (B). Cont: control.

Eagle medium (DMEM; Hyclone Laboratories Inc., Logan, UT, USA) and Epilife (MEPI500CA; Gibco BRL, Grand Island, NY, USA). The sebocytes obtained after the second passage were used in this study after identification by hematoxylin and eosin (Muto Pure Chemicals Co., Ltd, Tokyo, Japan) and Oil Red O (Sigma-Aldrich, St. Louis, MO, USA) staining and immunocytofluorescence against cytokeratin 1 and 7 (Chemicon, Billerica, MA, USA).

MAP is a stable precursor of vitamin C that ensures constant delivery of vitamin C to the skin. The cultured sebocytes were treated for 24 h with MAP (10^{-2} M) (Sigma-Aldrich), *P. acnes* (10^{10} CFU/ μ l) or a combination of MAP (10^{-2} M) and *P. acnes* (10^{10} CFU/ μ l). In addition, the cultured sebocytes were treated with MAP (10^{-2} M) and 40 mJ/cm² UVB radiation using Dermopal (Daavin, Bryan, OH, USA). The cultured sebocytes were prepared for evaluation of gene and protein expression 1, 3, and 5 days after treatment with MAP and UVB radiation. The concentration of MAP was determined using an MTT assay.

The cultured sebocytes treated with MAP, *P. acnes*, or UVB were analyzed by real-time polymerase chain reaction (PCR) using the Maxima SYBR Green/Fluorescein qPCR Master Mix (2 \times) (Thermo Scientific, Vilnius, Lithuania) according to the manufacturer's protocol. Real-time PCR was performed in triplicate using the LightCycler (Roche Diagnostics, Indianapolis, IN, USA) under the following conditions: one cycle of 2 min at 50°C, followed by one cycle of 10 min at 95°C, 55 cycles of 10 sec at 95°C, and finally 30 sec at annealing temperature. The levels of interleukin (IL)-1 β , IL-6, IL-8, and tumor necrosis factor α (TNF- α) were analyzed using ELISA (R&D Systems, Shanghai, China) according to the manufacturer's instruction. A lipid peroxidation assay kit (Biovision, Milpitas, CA, USA),

which consists of 25 ml malondialdehyde (MDA) lysis buffer, 12.5 ml of phosphotungstic acid solution, 1 ml of 100 \times dibutyl hydroxy toluene (BHT), 4 bottles of thiobarbituric acid (TBA), and 100 μ l of 4.17M MDA standard, was used for the sensitive detection of the MDA.

MAP inhibited the upregulation of IL-1 β expression induced by *P. acnes* in cultured sebocytes (Fig. 1). However, MAP did not inhibit the *P. acnes*-induced increase in the expression of IL-6, IL-8, and TNF- α in the cultured sebocytes (Fig. 1). Further, MAP decreased the expression of IL-1 β in cultured sebocytes 1, 3, and 5 days after exposure to 40 mJ/cm² UVB radiation (Fig. 1). However, compared to untreated controls, cultured sebocytes treated with MAP did not show a decrease in the expression of IL-6, IL-8, and TNF- α after exposure to 40 mJ/cm² UVB radiation (Fig. 1). Lipid peroxidation increased after the treatment of cultured sebocytes with *P. acnes*. *P. acnes*-induced increase in the lipid peroxidation was inhibited after treatment of cultured sebocytes with MAP (Fig. 2). In addition, MAP decreased the lipid peroxidation in cultured sebocytes 1, 3, and 5 days after exposure to 40 mJ/cm² UVB radiation (Fig. 2).

P. acnes induces the expression of proinflammatory cytokines and chemokines not only in human keratinocytes but also in human sebocytes⁵. Inflammatory events induced by the production of proinflammatory cytokines and chemokines play an important role in the pathogenesis of inflammatory acne. Moreover, UV irradiation induces the expression inflammatory cytokines such as IL-1 β , IL-6, IL-8, and TNF- α in HaCaT cells and in human sebocytes^{6,7}. In addition, UV irradiation induces hyperplasia of sebaceous glands and sebocytes^{8,9}.

Pharmacologically active vitamin C has antioxidant and anti-inflammatory effects. MAP is one of the stable vitamin

C compounds that can attenuate the production of inflammatory mediators. However, whether MAP inhibits the expression of inflammatory mediators in cultured sebocytes treated with *P. acnes* or UVB radiation has not been reported thus far. Therefore, we assessed the effect of MAP on the responses of sebocytes treated with *P. acnes* and UVB radiation. Our results showed that MAP inhibited the increased expression of IL-1 β induced by *P. acnes* and UVB irradiation in cultured sebocytes. However, MAP did not inhibit the expression of IL-6, IL-8, and TNF- α induced by *P. acnes* and UVB irradiation in cultured sebocytes. Similar to lipopolysaccharide, *P. acnes* and UVB irradiation can facilitate the peroxidation of sebum lipids⁹. The singlet oxygen generated from coproporphyrin of *P. acnes* and from UV irradiation can promote the peroxidation of sebum lipids. Because of its antioxidant properties, MAP, similar to vitamin E, may be effective for preventing the peroxidation of sebum on the skin¹⁰. Our results showed that MAP decreased the sebum peroxidation in cultured sebocytes treated with *P. acnes* and UVB radiation.

In conclusion, our results showed that MAP has mild anti-inflammatory and antioxidative effects in cultured sebocytes. Thus, we propose that vitamin C should be considered a complementary therapy for the regulation of inflammatory acne.

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