Enhancement of Interleukin-1α Mediated Autocrine Growth of Cultured Human Keratinocytes by Sho-saiko-to

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ABSTRACT—We investigated the effects of Sho-saiko-to, the most commonly used herbal medicine in Japan, on the production of interleukin (IL)-1α by cultured human epidermal keratinocytes. IL-1α production was significantly promoted by treatment with 100 or 500 μg/ml Sho-saiko-to for 24 or 48 hr. Expression of IL-1α receptors was the most markedly upregulated after treatment with 500 μg/ml Sho-saiko-to for 24 hr and with 100 or 500 μg/ml for 48 hr; these cells showed the characteristics of multilayered differentiated keratinocytes. The presence of an anti-IL-1α antibody during the treatment with 500 μg/ml of Sho-saiko-to for 24 or 48 hr or with 100 μg/ml for 48 hr significantly down-regulated the synthesis by the keratinocytes and induced damages in them. Keratinocytes treated with Sho-saiko-to might produce IL-1α and express IL-1α receptors. IL-1α may regulate the proliferation and differentiation of keratinocytes after Sho-saiko-to treatment. These findings suggest that Sho-saiko-to enhances the autocrine growth mediated by IL-1α.

Keywords: Sho-saiko-to, Cultured keratinocyte, Interleukin-1α, Autocrine growth

The Japanese herbal medicine Sho-saiko-to consisting of a mixture of aqueous extracts from seven different herbs, has been reported to inhibit human immunodeficiency virus replication (1) and to suppress the proliferation of carcinoma cells in vitro by promoting their apoptotic death (2). In addition, it has an immunoregulatory function, displaying anti-inflammatory and anti-allergic actions (3), and it has been used in the treatment of various diseases.

Synthesis and secretion of interleukin (IL)-1α by human keratinocytes have been well-documented, and the rate of secretion can be modulated in response to a various noxious stimuli (4). IL-1 promotes keratinocyte growth and proliferation via an autocrine type of mechanism (5). IL-1 plays a major role in immune regulatory and inflammatory responses (6).

We investigated the effects of Sho-saiko-to on the production of IL-1α by cultured human epidermal keratinocyes.

MATERIALS AND METHODS

Sho-saiko-to

Sho-saiko-to powder (Lot No. 920009002PO), provided by Tsumura & Co., Tokyo, was purified by high-performance liquid chromatography. Sho-saiko-to was dissolved by stirring it in medium at the concentration of 1 mg/ml for 30 min at 37°C; then it was filter-sterilized. The solution was diluted with medium to 2 concentrations (100 and 500 μg/ml). The dye exclusion test revealed that at these concentrations, Sho-saiko-to had no influence on the viability of the keratinocytes.

Cell culture

Normal human keratinocytes were isolated from normal human skin tissues, obtained during plastic surgery with informed consent, and treated with 1% dispase (Godo Shusei, Tokyo) in Dulbecco’s modified Eagle medium (Gibco Laboratories, Grand Island, NY, USA) at 4°C overnight. Cells were cultured (7, 8) in serum-free K-GM (Kurabo, Osaka) at 37°C. Second to fourth passage cells were used for the experiments.

IL-1α assay

Cells were incubated in vitro at 37°C in KGM with or without the addition of Sho-saiko-to. After incubation, culture supernatants were collected and IL-1α assayed with an IL-1α ELISA kit (Otsuka Pharm., Tokyo). The
absorbance at 490 nm was measured with a microplate reader (MOR-A4i; Tosoh, Tokyo).

Expression of IL-1α receptor

The cell-surface expression of IL-1α receptors was assayed by a fluorescent antibody technique on a laser flow cytometer (EPICS Profile; Coulter, Inc., Hialeah, FL, USA). Cells treated with Sho-saiko-to were incubated with anti-IL-1α-receptor monoclonal antibody (Oncogene Science, Inc., Uniondale, NY, USA) for 30 min at 4°C, before staining with fluorescein isothiocyanate (FITC)-labeled anti-Ig antibody (1:40; Tago Inc., Burlingame, CA, USA). The fluorescence intensity was recorded for 5,000 cells in an appropriately gated area by both forward and side scatter, and each cell was plotted in a diagram with a log scale x-axis ($10^0$-$10^3$) for the fluorescence intensity of FITC and a log-scale y-axis ($10^0$-$10^3$) for the fluorescence intensity of phycoerythrin.

DNA synthesis assay

Analysis of DNA synthesis in keratinocytes was examined by the method described by Kato et al. (9, 10). Cells were pulsed with 0.5 μCi [3H]thymidine (Amersham-Japan, Ltd., Tokyo) for 4 hr and harvested onto filter paper. [3H]Thymidine incorporation was then measured by liquid scintillation counting.

Treatment with anti IL-1α antibody

Keratinocytes were cultured with 1.0 μg/ml anti IL-1α monoclonal antibody (Oncogene Science, Inc.) for 24 or 48 hr. This antibody neutralizes bioactivity when used at a ratio of 1 μg antibody to 250 pg IL-1α. The dye exclusion test revealed that at 1 μg/ml, the anti-IL-1α antibody had no influence on the cell viability of the keratinocytes.

RESULTS

Production of IL-1α in cultured keratinocytes

To detect IL-1α production by Sho-saiko-to, IL-1α in the supernatant of cultured keratinocytes was measured by ELISA. Figure 1 shows the kinetics of the Sho-saiko-to effect on IL-1α production by cultured keratinocytes. IL-1α production was significantly increased after treatment with either 100 or 500 μg/ml Sho-saiko-to for 24 or 48 hr.

Expression of IL-1α receptor

We analyzed the expression of IL-1α receptors by the fluorescent antibody technique. IL-1α receptors were expressed most markedly after treatment with 500 μg/ml Sho-saiko-to for 24 hr (Fig. 2) and with 100 or 500 μg/ml for 48 hr.

DNA synthesis after treatment with anti-IL-1α antibody

To examine the proliferation of keratinocytes and
Effect of anti-IL-1α antibody on the cells treated with Sho-saiko-to, the DNA synthesis was assayed. The DNA synthesis of keratinocytes was significantly upregulated after a 24-hr treatment with 100 pg/ml Sho-saiko-to. Constitutive DNA synthesis of keratinocytes was significantly down-regulated by the presence of anti-IL-1α antibody during the treatment with 500 pg/ml Sho-saiko-to for 24 or 48 hr or with 100 pg/ml for 48 hr (Fig. 3: A and B).

**Fig. 3.** Effect of Sho-saiko-to on constitutive DNA synthesis of cultured human keratinocytes after treatment with anti-IL-1α antibody for 24 hr (A) and 48 hr (B). Each column shows the mean ± S.D. of 6 cultures. Shown is a representative of 4 experiments. The values marked * are significantly different (P < 0.05) from the control values or the value of each control with no anti-IL-1α antibody by the paired Wilcoxon test.

Morphological observation

Phase-contrast microscopy revealed that cells showing upregulated expression of IL-1α receptor after treatment with Sho-saiko-to had the appearance of multilayered differentiated keratinocytes (Fig. 4: A and B). In the presence of an anti-IL-1α antibody, keratinocytes were damaged after treatment with 500 µg/ml Sho-saiko-to for 24 or 48 hr or with 100 µg/ml for 48 hr (Fig. 5), whereas keratinocytes after treatment with 100 µg/ml for 24 hr

**Fig. 4.** Phase-contrast micrographs (x100) of cultured human keratinocytes. A, Control; B, after a 24-hr treatment with 500 µg/ml of Sho-saiko-to, showing multilayered differentiated keratinocytes.
and keratinocytes cultured without Sho-saiko-to for 48 hr were not damaged.

DISCUSSION

This study showed that in cultured keratinocytes, treatment with Sho-saiko-to promoted IL-1α production, upregulated expression of the IL-1α receptor and caused proliferation and differentiation of keratinocytes. Enhancement of IL-1α production might have promoted the proliferation of keratinocytes after the 24-hr treatment with 100 μg/ml Sho-saiko-to, while enhancement of IL-1α production and its receptor expression might have promoted the differentiation of keratinocytes after the 24-hr treatment with 500 μg/ml or 48-hr treatment with 100 or 500 μg/ml sho-saiko-to. Since the anti-IL-1α antibody damaged the cells in the latter treatment, to die in apoptosis, but not those in the former treatment, IL-1α might act independently to differentiate keratinocytes, but acts cooperatively with other growth factors for their proliferation. The present findings suggest that Sho-saiko-to causes proliferation and differentiation of keratinocytes via enhancement of IL-1α-mediated autocrine growth.

Various effects of IL-1α on the proliferation of keratinocytes have been reported. Keratinocytes in culture produce IL-1α and proliferate in response to IL-1α (4–6). Blanton et al. (6) reported that the induction of surface IL-1 receptors was in parallel to the induction of squamous differentiation markers and that IL-1 may regulate proliferation and differentiation in the epidermis. Anti-IL-1α antibody can block the actions of IL-1α released from the keratinocytes (11). IL-1α has been shown to stimulate keratinocyte synthesis of IL-1α and transforming growth factor-α m-RNA (12). Our present findings are for the most part consistent with those reports.

IL-1α may regulate proliferation and differentiation of keratinocytes after Sho-saiko-to treatment. There may be an optimal dose and time for obtaining the best effects of IL-1α on keratinocytes by Sho-saiko-to. The herbal medicine Sho-saiko-to may have effects on the immune response and wound repair of keratinocytes.

REFERENCES