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Modulatory effects of selenium and strontium salts on keratinocyte-derived inflammatory cytokines

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Recently a change to a low-salt mineral water has been associated with an improvement in atopic dermatitis [2]. In a similar manner, balneotherapy using spa water has long been known as an effective approach to the management of inflammatory skin diseases such as psoriasis and atopic dermatitis. However, only a limited number of studies have addressed the mechanism(s) responsible for the overall satisfactory clinical results which so far have been only empirical in nature [11]. Trace elements such as selenium and strontium, which are sometimes found in high concentrations in spa waters, may play a significant role in the observed effects. Using a reconstituted skin model, we studied the in vitro modulatory effect of these two trace elements on the production of cutaneous inflammatory cytokines (IL-1α, TNFα, IL6) and compared the results with those obtained with a selenium- and strontium-rich spa water known to be particularly effective in the management of inflammatory skin diseases.

Samples of inflammatory skin and normal skin were obtained from biopsies of atopic dermatitis lesions and during plastic surgery, respectively. Skin was reconstituted according to the method of Prunieras [8] as modified by Basset-Seguin et al. [3]. After deepidermized dermis (DED) had been obtained, a 2-mm punch biopsy specimen of atopic dermatitis or normal skin was placed on top of each specimen of DED, which was then maintained at the air-liquid interphase with a metallic support in a 60-mm Petri dish. Under these conditions, one specimen from each of 14 atopic dermatitis skin and 14 normal skin were first incubated for one week (D0-D7) in a culture medium consisting of lyophilized minimal essential medium with Earle’s salts (EMEM; Gibco) reconstituted with Millipore water and supplemented with 10% fetal calf serum, 1-10 mmol/l choleratoxin (Sigma), 5 μg/ml insulin (Sigma), 10 ng/ml epidermal growth factor (Genzyme), 100 IU/ml penicillin (Gibco), 100 μg/ml streptomycin (Gibco) and 2.5 μg/ml Fungizone. Hydrocortisone was omitted. This medium is referred to as ‘control medium’. Then, after 7 days of culture (D7), reconstituted skin (RS) was incubated in duplicate with the same culture medium with or without strontium nitrate (SrNO₃, 260 μg/l), strontium chloride (SrCl₂, 260 μg/l), selenium chloride (SeCl₂, 60 μg/l), sodium selenate (SeNaO₃, 60 μg/l) or sodium selenite (SeNaO₄, 60 μg/l) (all Aldrich Chemical Co.). Alternatively on D7, Millipore water in the control medium was replaced by a selenium- and strontium-rich spa water (Source melusine, Roche Posay, France) without the addition of any other trace element. Media were changed twice weekly. The concentrations of strontium and selenium salts used above were similar to those of the spa water. On D14, the cultures were stopped and the RS frozen for immunohistochemical study.

Cryosections (4–6 μm) were treated for 30 min (20°C) with biotinylated rabbit anti-human cytokine antibodies (250 μg/ml IL-1α, 100 μg/ml IL-6, 100 μg/ml TNFα; Tebu). After washing in Tris-buffered saline (TBS), incubation was performed with a peroxidase-labelled avidin-biotin complex (Immunochem, Marseille, France) for 40 min (20°C). Specimens were incubated with 3-amino-9-ethylcarbazol (Immunochem) in acetate buffer (pH 5) and 0.014% hydrogen peroxide for 5 min, washed in water for 10 min, counterstained with haematoxylin for 90 s and washed in water for 10 min. Finally, the sections were mounted in buffered glycerin. For controls, specimens of normal and inflammatory RS were treated with cytokine antibody absorbed by preincubation for one hour at 20°C with cytokine (anti-IL-1 antibody with 200 U/ml purified human IL-1 (Tebu), anti-IL-6 antibody with 400 U/ml rIL-6...
(Tebu) or anti-TNFα antibody with rTNFα (Tebu) followed by the application of the biotin-avidin technique. Staining was quenched in all cases. Specific staining was also lost if either the primary antibody (anti-IL-1α, anti-IL-6 or anti-TNFα) or the secondary layer antibody was omitted, or if the primary antibody was replaced by an irrelevant IgG. Results are expressed according to the epidermal labelling intensity: – negative, + weak, ++ moderate, +++ strong.

In the same manner, the concentrations of inflammatory cytokines (IL-1α, IL-6 and TNFα) were determined in culture supernatants on days 10 and 14 using an immunoenzyme assay (ELISA; Immunotech). The detection limit of the assay was below 10 pg/ml of cytokine. No significant cross-reactivity was observed between the three cytokines. Interference of different salt concentrations on the ELISA results was allowed for by adding recombinant IL-1, IL-6 and TNFα together with the respective salt concentrations and without the salts. No difference was noted in the values. The results (the mean of two determinations for each medium) are expressed as an index of increase between days 10 and 14 according to the expression D14 – D10 x 100/D10.

In normal skin, epidermal labelling was weak (+) for IL-1α, and negative for IL-6 and TNFα with control medium. Subsequent incubation with the medium reconstituted with the spa water, or the control medium with exogenous strontium or selenium salts did not induce IL-6 or TNFα labelling, but IL-1α labelling disappeared in epidermis incubated with the three selenium salts.

In inflammatory skin, epidermal labelling was moderate (+++) for IL-1α and strong (++++) for both IL-6 (Fig. 1a) and TNFα with control medium. IL-1α labelling disappeared in epidermis incubated with SeNaO3 and was weak (+) with SeNaO3. No modifications were noted with SeCl2 (++), spa water (++) or strontium salts (++). IL-6 labelling was decreased with strontium, selenium salts and thermal water: no labelling with SeNaO3 (Fig. 1b), weak labeling (+) with SrNO3, SeNaO4, SeCl2 and thermal water, and moderate labelling (++) with SrCl2. TNFα labelling was slightly decreased (+++) with SrCl2, SeNaO4 and spa water and to a greater extent (+) with SrNO3, SeCl2 and SeNaO3.

The modulation of cytokine concentrations in culture supernatants is shown in Fig. 2. In both normal and inflammatory RS, the production, of all three inflammatory cytokines was lower after 10 days in medium containing strontium salts, selenium salts or spa water than in control medium. IL-6 was inhibited the most.

The RS model used in this study represents an in vitro model useful for simulating in vivo conditions. Indeed, with control medium, IL-1α labelling in normal and inflammatory RS was similar to that obtained by Kristensen et al. [6] in vivo in normal and psoriatic skin: it was predominant in basal cells in normal skin and spread throughout the spinous layer in inflammatory skin. TNFα and IL-6 labelling was not detectable in normal RS, although it has been found in vivo in normal epidermis [4, 7]. However, labelling in inflammatory RS was similar to that found in vivo in inflammatory diseases such as cutaneous psoriasis [4] or in allergic and irritant patch test reactions [5].

The addition of selenium or strontium salts to culture medium mediated the production of the three inflammatory cytokines in both normal and inflammatory RS. The combined immunohistochemical and ELISA techniques showed a selective inhibitory effect of selenium salts on IL-1α production. This effect was less evident with strontium salts, particularly in the immunohistochemical procedure. For TNFα, the inhibitory effect was greater with strontium salts. However, the most important modulating effect of both strontium and selenium salts was a decreased production of IL-6 both at the intra- and extracellular levels. Inhibition of IL-6 producing may have been due to a direct effect on this cytokine, or an indirect effect resulting from a decrease in IL-1α production which is known to stimulate IL-6 production [5], or a combination of these two mechanisms.

Furthermore, selenium- and strontium-rich spa water induced a moderate inhibitory effect on inflammatory cytokine production, particularly IL-6. This effect, which was intermediate between that of control medium and medium with added selenium or strontium salts, may have been due to the combined effect of both salts. Previous studies in animals have shown that a deficit in selenium salts in associated with an increased inflammatory reaction which disappears after selenium supplementation [1]. Two mechanisms have been suggested for this anti-in-

Fig. 1a, b IL-6 labelling of epidermis was strong (++++) in the supra basal layers with control medium (a) but disappeared after incubation in the presence of SeNaO3 (b)