MODULATORY EFFECTS OF SELENIUM AND STRONTIUM SALTS ON KERATINOCYTE-DERIVED INFLAMMATORY CYTOKINES

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INTRODUCTION

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. 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 reconstructed (RS) 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.

MATERIAL AND METHODS

Skin culture conditions

Samples of inflammatory skin and normal skin were obtained from biopsies of atopic dermatitis lesions and during plastic surgery, respectively. Skin was reconstructed according to the method of Prunieras (1) as modified by Basset-Seguin et al. (2). 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 g/ml streptomycin (Gibco) and 2.5 g/ml Fungizone. Hydrocortisone was omitted. This medium is referred to as «control medium». Then, after 7 days of culture (D7), reconstructed skin (RS) was incubated in duplicate with the same culture medium with or without strontium nitrate ($SrNO_3$, 260 μg/l), strontium chloride ($SrCl_2$, 260 μg/l), selenium chloride ($SeCl_2$, 60 μg/l), sodium selenate ($SeNaO_3$, 60 μg/l) or sodium selenite ($SeNaO_4$, 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 (Mélusine spring, La Roche-Posay, France), the composition of which is presented in Table 1. 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.

Pro-inflammatory cytokine release measurements

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 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.

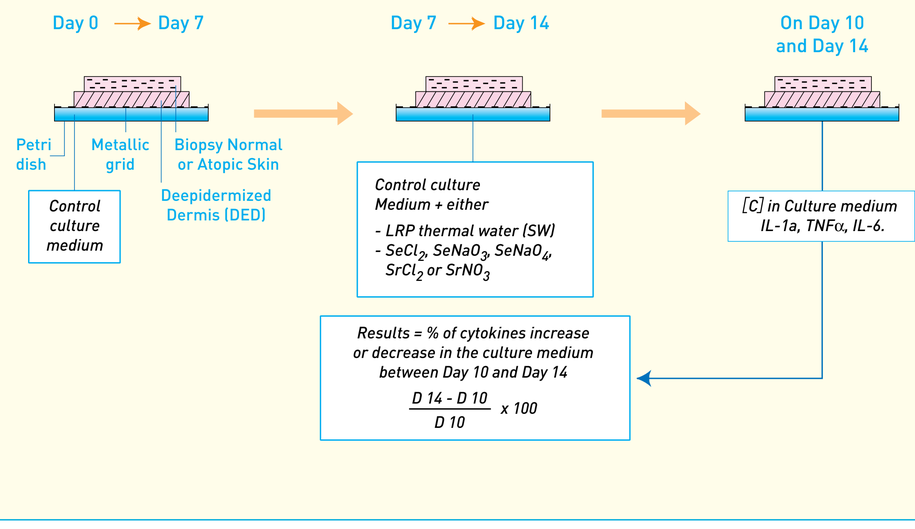
Table 1:
La Roche-Posay thermal water physicochemical analysis (Mélusine spring)

pH	7	Silica	31.6 mg/l
Temperature	13°C	Magnesium	4.4 mg/l
Resistance	1540 Ω	Strontium	0.3 mg/l
Dry residue	595 mg/l	Selenium	0.053 mg/l
Bicarbonates	387 mg/l	Zinc	< 0.005 mg/l
Calcium	149 mg/l	Copper	< 0.005 mg/l

Sodium Selenate 95%
Sodium Selenite 5%

Ref. : Bull. Acad. Natle. Med., 1996

Figure 1:
In vitro reconstructed (RS) skin model used to assess the effect of LRP thermal spring water and selenium salts on pro-inflammatory cytokines release



RESULTS

The modulation of cytokine concentrations in culture supernatants is shown in Figure 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 the most inhibited one. 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. (3) *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, 5). However, labelling in inflammatory RS was similar to that found *in vivo* in inflammatory diseases such as psoriasis (4) or allergic and irritant patch test reactions (6). 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. The inhibition of IL-6 production may be a direct effect or an indirect effect resulting from a decrease in IL-1α production which is known to stimulate IL-6 production (6), or a combination of these two mechanisms.The selenium- and strontium-rich spa water induced a moderate inhibitory effect on inflammatory cytokines production, particularly IL-6. This effect, which was intermediate between that of control medium and medium with added selenium or strontium salts, may be due to the combined effect of both salts. Two mechanisms have been suggested for this anti-inflammatory effect of selenium. First, it has been noted that *in vitro* incubation of granulocytes with selenium salts in the medium increases bactericidal and phagocytic activities (7). Secondly, as selenium is a component of glutathione peroxidase, it can catalyse the decomposition of peroxides and thus protect cells against inflammatory in situ reactions (8).

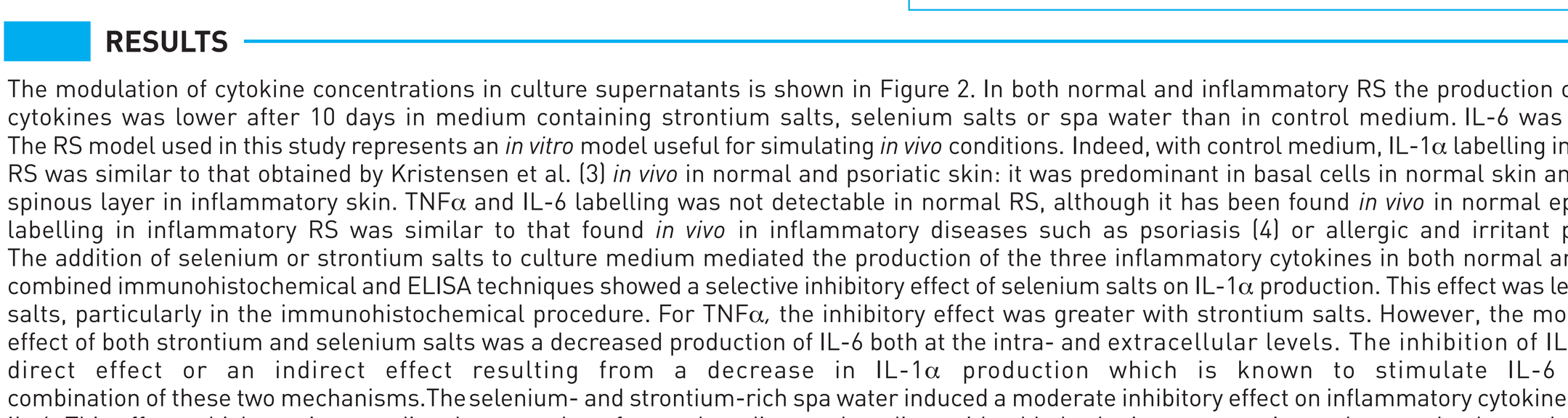


Figure 2:
Cytokine concentrations in culture media with different salts or spa water in control and inflammatory RS skin

Results are expressed as the percentage of increase according to the expression D14 - D10 x 100/D10 (D = day).
- Values for control normal skin (: pg/ml): IL-1α. 310 ± 10; TNFα. 30 ± 5; IL-6. 30 ± 8.
- Values for control inflammatory skin (: pg/ml): IL-1α. 205 ± 15; TNFα. 52 ± 3; IL-6. 8 ± 3 ***($SrNO_3$: strontium nitrate, $SrCl_2$: strontium chloride, $SeCl_2$: selenium chloride, $SeNaO_3$: sodium selenate, $SeNao_4$: sodium selenite, C: control medium, SW: spa water.***

CONCLUSION

The inhibitory effect of selenium and strontium salts on inflammatory cytokine production by keratinocytes may be another mechanism responsible for the anti-inflammatory effect of selenium salts. Moreover, these salts might play a role in the anti-inflammatory effect of locally applied selenium- and strontium-rich spa water reported in the treatment of psoriasis and eczema.

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