A SELENIUM-RICH THERMAL SPRING WATER PREVENTS UV- AND CHEMICALLY-INDUCED INFLAMMATION

A. RICHARD, A. ROUGIER

La Roche-Posay Dermatological Laboratories, Asnières, France.

INTRODUCTION

Due to their high level of reactivity, oxygenated species are liable to initiate chemical or structural modifications of proteins, nucleic acids or membrane lipids leading to changes in the morphology and function of the cells. By increasing glutathione peroxidase (GSH.Px) activity present in the skin, selenium regulates the production of reactive oxygen species and thus could play a significant role in skin aging and cancerization, as well as in inflammatory skin disorders such as eczema and psoriasis (1, 2, 3, 4).Because of its unique chemical composition (Table I), among which very high concentration in selenium, we focused our attention on the protective effect of the selenium-rich La Roche-Posay Thermal Water (LRP TW) against deleterious effects of UVA and UVB irradiations on fibroblasts and keratinocytes in culture.

MATERIAL AND METHODS

Protective effect of Se and Se-rich thermal water against UVA induced cytotoxicity

Culture conditions

Fibroblasts: Human fibroblasts were cultured for 10 days in:

Medium I = EMEM reconstituted with demineralized water (Millipore)

Medium II = Medium I supplemented with Sodium selenite (Na_2SeO_3), (56 µg/l; as in LRP TW)

Medium III = EMEM reconstituted with LRP TW (Table I).

All mediums contained 1.8 mM Ca^{++} and were then supplemented with 2% foetal calf serum (FCS).

Keratinocytes: Isolated Keratinocytes (5) were first sub-cultured with fibroblasts (3T3) (6) and grown (4 days) in MCDB 153 (Gibco, France). Before irradiation, they were then placed in the following medium for 24 hours:

Medium I = MCDB 153 reconstituted with demineralized water (Millipore)

Medium II = MCDB 153 reconstituted with LRP TW.

Irradiation procedures

UVA: Fibroblasts were exposed to UVA (365 nm, 36 J/cm²)(Vilbert Lourmat illuminating table, France). Lipoperoxidation and Se-GSH.Px activity were assessed just after exposure and cell viability 3 days after.

UVB: Keratinocytes were submitted to increasing doses (50 to 200 mJ/cm²) (Philips TL 20/12 lamp, 300-320 nm). Mediums were then replaced and the cultures incubated for 24 hours before measuring cell viability and IL1α release.

End points

Fibroblasts:

Cell viability was measured using the MTT conversion test (7).

Lipid peroxidation was assessed on the culture supernatant by the TBARS assay (8).

Se-GSH.Px activity was measured on sonicated cells by monitoring NADPH consumption (9).

Keratinocytes:

Cell viability was quantified using the neutral red uptake assay (NRU) (10)

Interleukin 1α release was measured on the cell culture supernatant by ELISA (RPA 528 Amersham) (11)

Protective effect of the Se-rich thermal water against chemically induced irritant dermatitis

4 mg/cm² of the following formulations were applied twice a day for 4 days on 2 cm² of the ventral forearm of 10 volunteers (5 males, 5 females, mean age 23).

Gel I = 0,5% carbomer 940 + demineralized water

Gel II = 0.8% carbomer 940 + LRP TW

On the 5th day, 65 µl/cm² of a 0.75% SLS water solution was applied onto the pre-treated areas under occlusive patch for 24 hours.

On the 6th day, changes in cutaneous blood flow were assessed by Laser Doppler Velocimetry (LDV).

Table 1:
La Roche-Posay thermal water physicochemical analysis

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

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

RESULTS

Protective effect of Se and Se-rich LRP TW against UVA induced cytotoxicity

Fibroblast survival is increased by respectively a factor of 1.6 and 1.8 ($p<0.005$) when cultured in the presence of Se (medium II) or LRP TW (medium III) compared to fibroblasts grown in Se-free medium (medium I) (fig. 1). Moreover, a decrease of respectively a factor of 1.8 (46%) and 1.7 (42%) ($p<0.005$) in lipoperoxides (TBARS) was observed when cells were cultured in mediums II or III (fig. 2a).This protective effect of Se or Se-rich LRP TW against UVA induced lipoperoxidation can be related to the activation by a factor of respectively 2.5 and 2.6 ($p<0.001$) of the Se-GSH.Px (fig. 2b).

Protective effect of the Se-rich LRP TW against UVB induced cytotoxicity

Keratinocytes cultured in medium III have a better resistance to increasing doses of UVB (fig. 3a). Thus the UVB dose required to kill 50% of the cells (IC 50) is twice when keratinocytes are cultured in medium III (respectively 80 ± 33 and 150 ± 33 mJ/cm²) (fig. 3b).Whatever the medium, IL1α release is increased up to a UVB dose of 150 mJ/cm² (fig. 4). However, IL1α release is decreased by a factor of about 2 when keratinocytes were cultured in medium III.

Protective effect of the Se-rich LRP TW against SLS induced irritant dermatitis

The increased blood flow observed in SLS induced inflammatory skin is reduced by 46% ($p<0.001$) when volunteers were pre-treated with the LRP TW gel compared to non treated volunteers, whereas, a decrease of only 15% was observed in volunteers pre-treated with demineralized water gel (fig. 5).

Figure 1:
Effect of Selenium and La Roche-Posay thermal water on survival of human fibroblasts following UVA irradiation

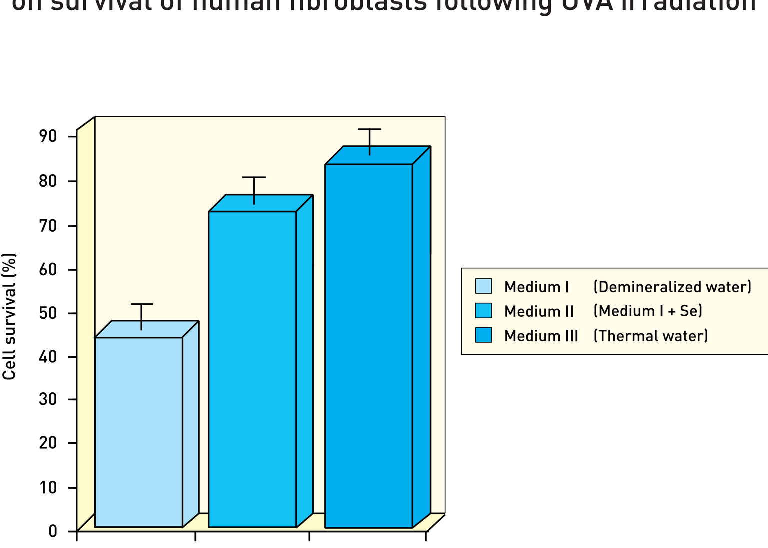


Figure 2:
Effect of Selenium and La Roche-Posay thermal water on lipid peroxidation and glutathione peroxidase (Se-GSH, Px) activity in human skin fibroblasts exposed to UVA

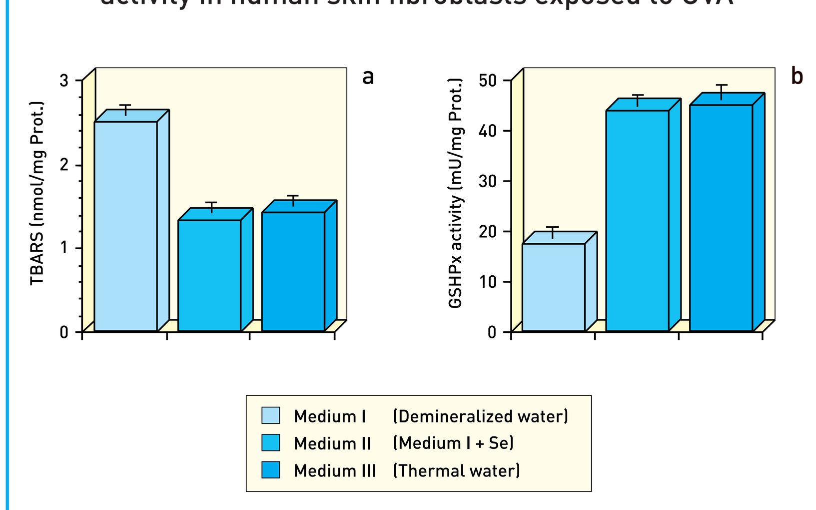


Figure 3:
Effect of La Roche-Posay thermal water on survival of human keratinocytes exposed to increasing doses of UVB

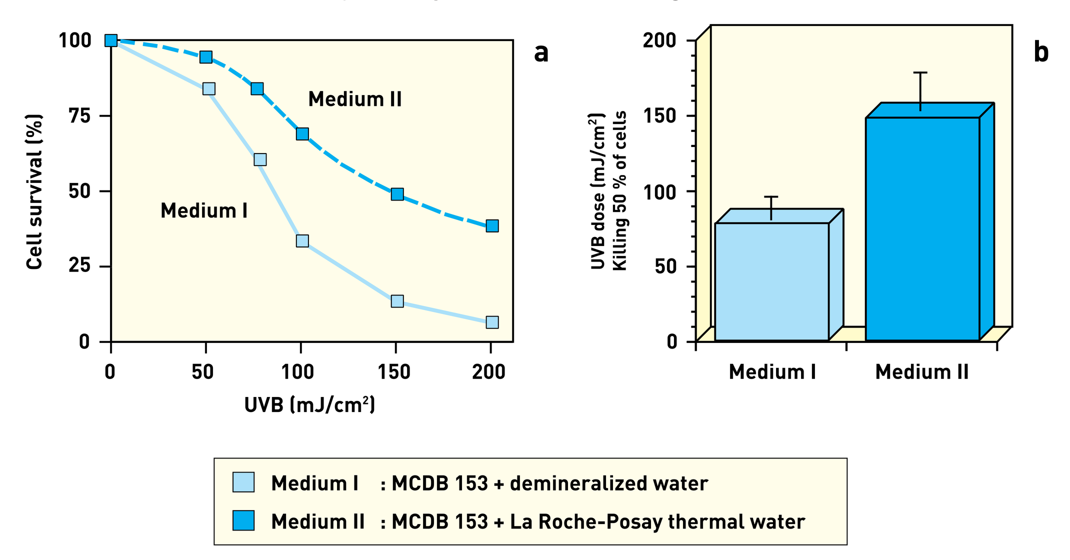


Figure 4:
Effect of La Roche-Posay thermal water on ILα released by human keratinocytes exposed to increasing doses of UVB

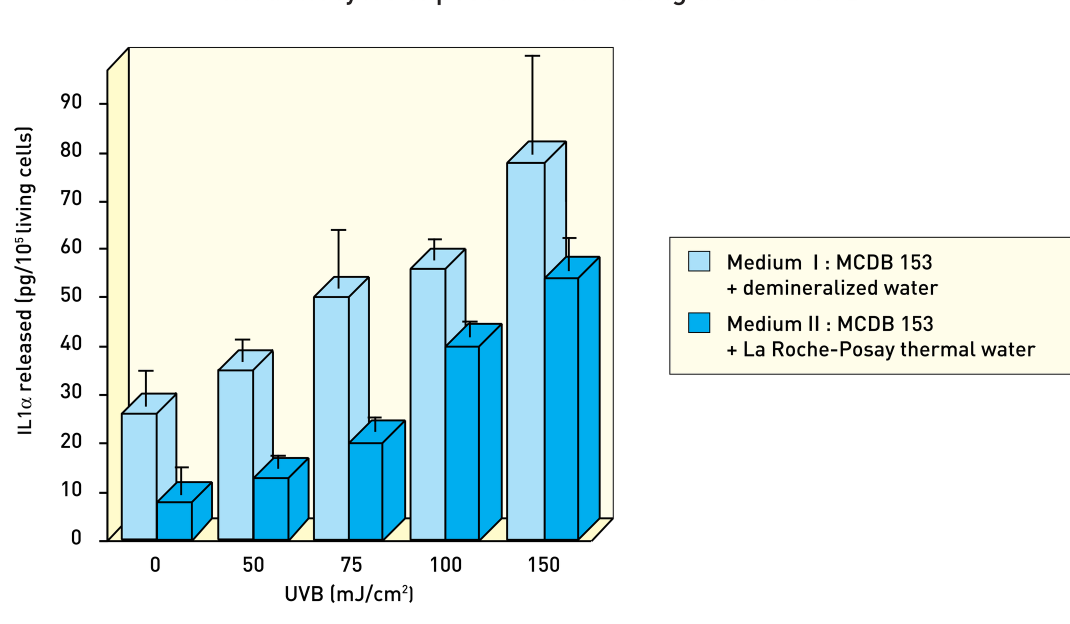
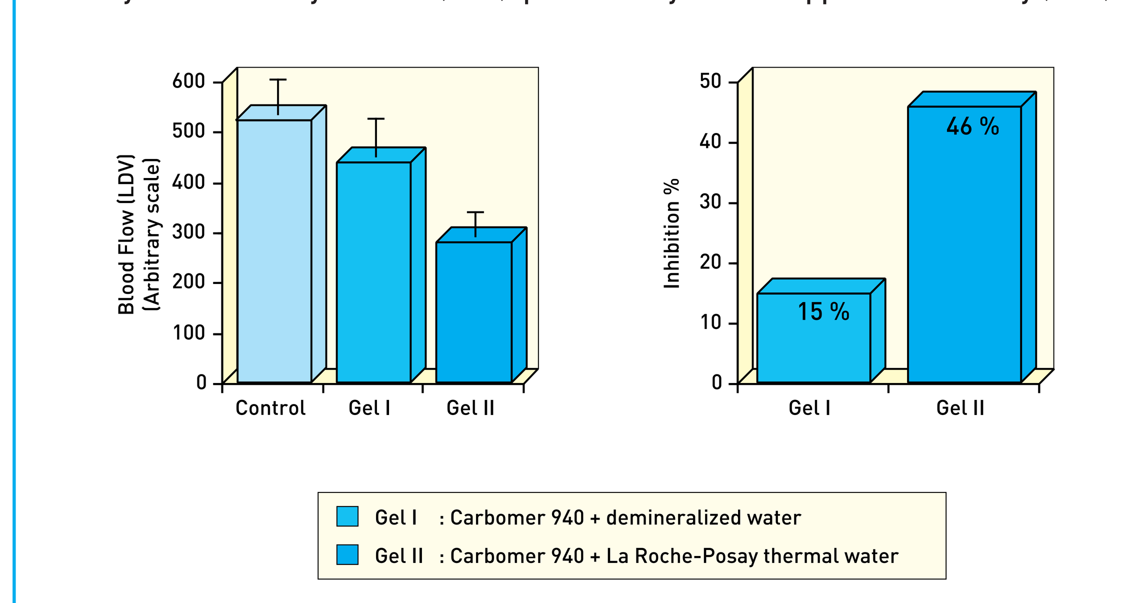


Figure 5:
Preventing effect of La Roche-Posay thermal water on inflammatory skin reaction induced by Sodium Lauryl Sulfate (SLS) quantified by Laser Doppler Velocimetry (LDV)



DISCUSSION

In UVA induced skin damages, free radicals or active oxygen species are directly involved in DNA strand breaks and cross links, protein modifications, cell membranes lipoperoxidation (12, 13). Moreover, a link between Se-GSH.Px activity and ageing has been previously described (14, 15). We have demonstrated that selenium can induce a protective effect on fibroblasts exposed to UVA. Thus, a decrease in UVA lethality as well as a decrease in UV induced lipid peroxidation probably due to an increase in Se-GSH.Px activity, were observed. Recently, it has been shown that daily topical applications of a cream containing LRP TW in hairless mice chronically exposed to UVB delay the appearance of spinocellular carcinomas and increase Se-GSH.Px activity (16), even if free radicals in UVB induced skin cancers are probably less predominant compared to direct damages to the DNA (17, 12). On keratinocytes, following UVB irradiation the NRU assay measures the disruption of lysosomial and/or cell membranes which can lead *in vivo* to sunburn cells formation (18, 19). However, beside cytotoxic effects, UVB irradiation can induce inflammatory reactions. Both *in vitro* and *in vivo*, these reactions are accompanied by inflammatory mediators release among which IL1α (20, 21). Our results showed that, following UVB irradiation LRP TW can greatly reduce the release of this mediator *in vitro*. Concerning chemically induced irritant dermatitis, LRP TW demonstrated a preventing effect against inflammatory reaction induced *in vivo* by a surfactant such as SLS.

CONCLUSION

Anti-oxidant properties of vitamins (A, E, C), trace-elements (Se, Cu, Zn, Mn,...) and reduced glutathion against deleterious cellular effects of oxygenated radicals have been widely documented (22). The high Se level of La Roche-Posay thermal water and the presence of this trace-element in the prosthetic group of the anti-oxidant enzyme (GSH.Px) suggest that the observed protective effects of La Roche-Posay thermal water against cell toxicity induced by UV light could be due to its natural anti-free radicals properties. Thus, beside its well known therapeutic benefits in curing inflammatory skin deseases such as eczema, psoriasis or in healing burns and scars, our results showed that La Roche-Posay thermal water, by its anti-free radical and anti-inflammatory activities can play an important role in the prevention of deleterious effects of UV light and/or chemically induced irritant dermatitis. Altogether, these results justify the use of this selenium-rich thermal water as « active excipient » in topical formulations. This could be of particular interest in patients with « sensitive skin ».

REFERENCES

1. Michaelsson G, B Berne, Carlmark B, Stand A. *Acta Derm Venereol*. 1989, **69**: 29-34.
2. Juhlin L. Blood glutathion peroxidase in skin disease. *Acta Derm Venereol*. 1986, **62**: 211-214.
3. Fairris GM, Perkins PJ, Lloyd B, Clayton BE. *Acta Derm Venereol*. 1989, **69**: 359-362.
4. Fairris GM, Perkins PJ, Lloyd B, Hinks L, Clayton BE. *Ann Clin Biochem*. 1989, **26**: 83-88.
5. Regnier M, Schweizer A, Michel S, Bailly C, Prunieras M. *Exp Cell Research*. 1986, **165**: 63-72.
6. Rheinwald JG, Green H. *Cell*. 1975, **6**: 331-344.
7. Mossman T. *J Immunol Methods*. 1983, **65**: 55-63.
8. Aust SD. Lipid peroxidation. In Greenwald R.A. (ed). *Handbook of Methods for Oxygen Radical Research.* CRC Press, Boca Raton, USA. 1986, 203-207.
9. Frohé L, Günzler WA. Assays for glutathione peroxidase. In Packer L. (ed). *Methods in Enzymology*. Academic Press, Orlando, USA. 1989, **105**: 114-121.
10. Borenfreund E, Puerner JA. *J of Tissue Culture Methods.* 1984, **9**: 7-9.
11. Cohen C, Dossou KG, Rougier A, Roguet R. *Toxicol*. In Vitro 1991, **5**: 407-410.
12. Tyrell RM, Keyse SM. *J of Photochem Photobiol*. 1990, **4**: 349-361.
13. Morlière P, Moysan A, Santus R, Hüppe G, Mazière JC, Dubertret L. *Biochem Biophy Acta*. 1991, **1084**: 261-268.
14. Zhang L, Maiorino M, Roveri A, Ursini F. *Biochem Biophys Acta*. 1989, **1006**: 140-143.
15. Pigeolet E, Remacle J. *Mech Ageing Dev*. 1991, **58**: 58-93.
16. Cadi R, Beani JC, Belanger S, Richard MJ, Richard A, Favier A, Amblard P. *Les Nouvelles Dermatologiques*. 1990, **10**: 1-7.
17. Coohil TP, Peak MJ, Peak JG. *J of Photochem Photobiol.* 1987, **46**: 1043-1050.
18. De Leo VA, Horlick H, Hanson D, Eisinger M, Harber LC. *J Invest Dermatol*. 1984, **83**: 323-326.
19. Gruenwedel DW, Cruikshank MK. *Toxicol Applied Pharmacol*. 1989, **50**: 1-7.
20. Murphy GM, Dowd PM, Hudspith BN, Brostoff J, Greaves MW. *Photodermatology*. 1989, **6**: 268-274.
21. Roguet R, Cohen C, Rougier A. In Rougier, A. Goldberg and H.I. Maibach (eds): *In Vitro Skin Toxicology*. Liebert Inc., New York, USA. 1994, 141-149.
22. Fuchs J, Packer L. Oxidative Stress in Dermatology. 1993, Marcel Dekker Inc., New York.

LA ROCHE-POSAY
LABORATOIRE DERMATOLOGIQUE