Molecular Pharmacology (USA) Limited

Influence of trace elements on the TNF-α suppression of an anti-arthritic cream

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INTRODUCTION

An extract of the anti-arthritic Thermalife Cream (AUSTR 27419) contains 13 trace elements (Table 1). Diffusion studies were undertaken to assess the permeability of human epidermis to the trace elements (exps.1 and 2, Fig. 1). Non-penetrating trace elements were discarded from the test formula (T2), and compared with the original formula (T1) for *in vitro* anti-inflammatory efficacy (TNF-α secretion in LPS-challenged human monocytes).

Table 1: Concentration (µg/g) of trace elements in T1.

Metallic species	Concentration in T1
Nickel sulphate heptahydrate	0.52 µg/g
Sodium fluoride	1.2 µg/g
Cupric sulphate	1.0 µg/g
Zinc Chloride	2.35 µg/g
Ammonium molybdate	0.35 µg/g
Cobaltous chloride hexahydrate	1.0 µg/g
Ferrous sulfate	10.0 µg/g
Manganese sulfate	1.82 µg/g
Magnesium sulfate heptahydrate	4.0 µg/g
Sodium gold chloride	1.75 µg/g
Ammonium vanadate	0.0012 µg/g
Potassium chromate	0.0105 µg/g
Boric acid	1.15 µg/g

METHODS

Exps.1 and 2: Human epidermis was mounted in vertical Franz type diffusion cells (stratum corneum facing up). T1 cream (n=4) or no cream (n=4) was applied to the donor compartment of diffusion cells, with PBS in the receptor compartment (3.0ml; stirred continuously at 37˚C). 240 Min after administration the receptor fluid was analysed for presence of metal ions by ICP-MS. A replication study (exp. 2) used a different skin donor.

Exp. 3: Human monocyte cultures (10% FCS, 5% CO^2) were either stimulated with 500ng/ml LPS (E.coli 0111:B4,) or not in the presence of 10% T1, 10% T2, or no treatment. 24 Hours after incubation, culture media were collected, centrifuged, and assayed (cytokine ELISA). Statistical analyses used a Treat by LPS ANOVA ($p < 0.05$).

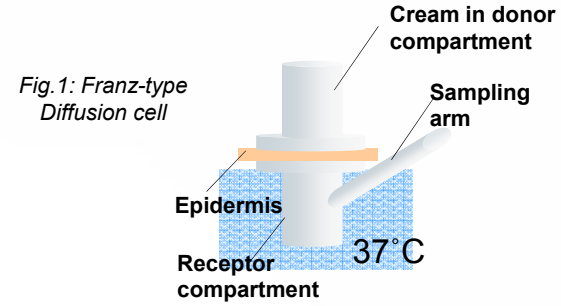

Fig.1: Franz-type Diffusion cell

RESULTS

Zinc was the only trace element to penetrate the human epidermis significantly (Exp.1: 0.59 [T1] vs 0.22 [control]; Exp.2: 0.039 [T1] vs 0.02 [control]). Both formulations strongly suppressed LPS-induced TNF-α secretion. T2 with zinc only was more effective than T1 (Treat: $F_{2,12}$=57.13, $p<0.0001$; LPS: $F_{1,12}$=245.47, $p<0.0001$; Treat by LPS: $F_{2,12}$=70.01, $p<0.0001$).

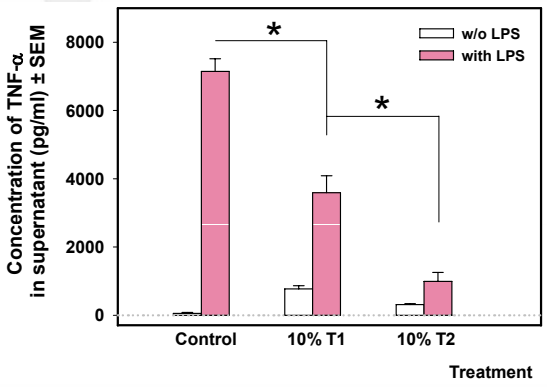

Fig. 2: Suppression of TNF-α secretion in LPS-challenged human monocytes, measured in triplicate 24hrs after treatment with 10% T1 (13 trace elements), 10% T2 (zinc chloride), or no treatment.

CONCLUSION

In the T1 cream base, zinc was the only trace element that could penetrate human epidermis *in vitro.* The efficacy of Thermalife extracts to reduce LPS-stimulated human monocyte TNF-α secretion *in vitro* was retained and enhanced in a formulation with only zinc chloride as trace element.