

## LETTER TO THE EDITOR

**A NOVEL TREATMENT OF CONTACT DERMATITIS BY TOPICAL APPLICATION OF PHOSPHOLIPASE A<sub>2</sub> INHIBITOR: A DOUBLE-BLIND PLACEBO-CONTROLLED PILOT STUDY**A. INGBER<sup>1</sup>, Y. COHEN<sup>2</sup>, M. KRIMSKY<sup>3</sup> and S. YEDGAR<sup>3</sup><sup>1</sup>*Department of Dermatology, Hadassah University Hospital, Jerusalem;*<sup>2</sup>*Morria Biopharmaceuticals, London, UK;* <sup>3</sup>*Department of Biochemistry, Hebrew University Medical School, Jerusalem, Israel**Received July 17, 2006 – Accepted November 24, 2006*

Phospholipase A<sub>2</sub> hydrolyzes membrane phospholipids releasing arachidonic acid and lysophospholipids. These are key precursors of inflammatory mediators, such as prostaglandins, leukotrienes, thromboxanes and PAF, in numerous inflammatory/allergic diseases, including skin inflammation. Accordingly, inhibition of PLA<sub>2</sub> has long been postulated as a potentially potent anti-inflammatory therapy. In the present study we tested the effect of a novel PLA<sub>2</sub> inhibitor on contact dermatitis in human subjects. A double-blind, placebo-controlled pilot study was conducted on contact dermatitis patients (n = 11) treated with the inhibitor-containing topical preparation (1% cream). Disease severity was assessed by physician's assessment before treatment (day 0) as well as after 14-days and 30-days. Patients treated with 1% PLA<sub>2</sub> inhibitor-containing cream showed a 69.9% reduction in disease score while placebo-treated patients showed a reduction of 36.5% with p = 0.0024. The clear improvement in the disease score of inhibitor-treated patients supports the involvement of PLA<sub>2</sub> activity in skin inflammation and the therapeutic prospective of its inhibition.

Phospholipase A<sub>2</sub> (PLA<sub>2</sub>) hydrolyzes membrane phospholipids to release arachidonic acid (AA) and lysophospholipids, both important precursors of inflammatory lipid mediators (1). AA is metabolized into the eicosanoid families, mainly via the lipoxygenase pathways (5, 12, 15-LOX), producing the leukotrienes, and the cyclooxygenase pathways (COX-1, COX-2) producing prostaglandins and thromboxanes. Products of both pathways are involved in inflammatory pathophysiology (2). LysoPL induce inflammatory cell activation and extravasation (3). Lyso-phosphatidylcholine is also the precursor of platelet-activating factor (PAF), a major inflammatory stimulant (4). Extracellular

PLA<sub>2</sub> also signal cells to produce other pro-inflammatory agents, including cytokines, nitric oxide and adhesion molecules (1). Several PLA<sub>2</sub> isoforms in human skin have been implicated in skin function and pathophysiology (5-7).

On these grounds, inhibitors of PLA<sub>2</sub> activity at the cell-membrane have been sought for the treatment of lipid mediator-related diseases (8). However, inhibitors that invade the cell impair its metabolism and viability. Therefore, it has been proposed that cell impermeable PLA<sub>2</sub> inhibitors would provide the desired treatment (9). Important players in cell protection from exogenous PLA<sub>2</sub> and other inflammatory injuries are the cell surface

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glycosaminoglycans (GAG), and GAG enrichment has been proposed for cell protection from inflammatory injury (10-11).

To address both needs (PLA<sub>2</sub> inhibition and GAG-enrichment), we have designed and synthesized a class of extra-cellular PLA<sub>2</sub> inhibitors (ExPLIs), composed of a lipidic PLA<sub>2</sub>-inhibiting moiety conjugated with GAG; while the lipid inhibits cell membrane phospholipids hydrolysis, the conjugated polymer prevents its internalization (10). Simultaneously, the lipid anchors the GAG to the membrane, thus elevating GAG levels on the cell surface. The ExPLIs have been previously found effective in suppressing diverse inflammatory and allergic processes *in vitro* and *in vivo* (12-14). In this paper we report the results from a double blind placebo-controlled pilot study in contact dermatitis patients using a 1% topical preparation of an ExPLI.

## MATERIALS AND METHODS

### *ExPLI preparation*

The ExPLI used in this study was HyPE, composed of phosphatidyl-ethanolamine (PE) conjugated to truncated hyaluronic acid (HA) forming phosphatidyl-ethanolamine-hyaluronyl amide (average MW 50 kDa) (15). The topical preparation of the 1% HyPE was formulated according to Hadassah Hospital Pharmacy formulation, using the following w/w % ratios: Water 70.0, Sodium Dodecyl Sulfate 0.6, Propylene Glycol 7.2, Cetyl Alcohol 10.6, Paraffin, White soft 10.6, HyPE 1.0.

### *Study design and experimental procedures*

Hand eczema was chosen in the present study due to its symmetrical phenotype, which enables the comparison between the two hands of each patient. The study group comprised a total of 11 female patients aged 19-50 (mean: 34.6). All the patients were positive for three selection criteria: clinical diagnosis of allergic contact dermatitis (ACD), positive patch test (to a minimum of 1 single allergen) and clinical relevancy. Patients in which ACD was not unequivocally diagnosed were not included in this study. All patients exhibited contact dermatitis on their hands, with some patients also exhibiting the disease on their forearms. The disease distribution was symmetrical in all patients at the study sites. One hand was treated with the ExPLI-containing cream, while the other was treated with the vehicle alone (Placebo). This set-up provided each patient with his own, internal control (placebo-treated hand), and made

the group statistically sufficient despite its small size, as clearly shown in the results below.

All patients were tested with Standard European Series patch testing kit (Chemotechnique Diagnostics Products, Malmö, Sweden). Patch tests were applied to the upper part of the patients' backs for 2 days and were read on days 2 and 4. Only ++ reactions were considered positive for the present study. All positive reactions were considered relevant. As shown in Table I, the most common allergens were potassium dichromate, fragrance, Balsam of Peru and Paraben mix. Patients refrained from any systemic treatment a month before the study and from topical treatment two weeks before the study.

Each patient received two color-coded tubes of cream, one tube with the active pharmaceutical ingredient (API), and the other containing just the vehicle (placebo). Except for their label color, the tubes were identical in size, color and odor. Both physician and patient were unaware of which tube contained the API. The patients were instructed to avoid their respective allergen triggers and to consistently apply cream from the blue marked tube to their right hand and forearm, and cream from the pink marked tube to their left hand and forearm, twice a day. The patients were strictly instructed to apply the same amount of cream, and to be careful not to mix the tubes, in order to avoid contra-lateral contamination. To avoid cross-application between the hands, the patients were instructed to use disposable gloves on the applying hand. Disease severity was assessed before treatment (day 0) as well as after 14 days and one month later, by the conventional Physician's Assessment (16) of Dryness, Scaling, Redness, Pruritis and Fissures, scoring from 0 (none) to 3 (severe), thus making the maximum score 15. The same physician evaluated the patients before and after treatment.

### *Statistical Analysis*

For each individual patient, the change in disease score in the drug-treated hand was compared to that in the placebo-treated hand, thus making the latter an internal control for each patient. Statistical significance was determined by the Paired Two-tailed Student t-test.

### *Ethical Permission*

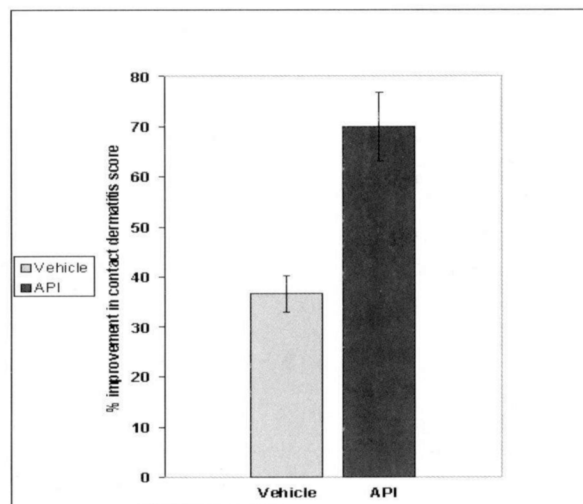
The study was approved by the Hadassah Hospital Ethical Committee and re-confirmed by The Israel Ministry of Health, based on animal safety data that showed no toxicity.

## RESULTS

All 11 enrolled patients completed the one month

**Table I.** Allergen patch test reactions and clinical distribution.

| Patient No. | Allergen                               | Clinical distribution          |
|-------------|----------------------------------------|--------------------------------|
| 1           | Potassium dichromate                   | Hand eczema                    |
| 2           | Nickel, Potassium dichromate           | Hand eczema                    |
| 3           | Fragrance, Paraben mix                 | Hand eczema                    |
| 4           | Fragrance                              | Hand eczema                    |
| 5           | Potassium dichromate                   | Hand eczema,<br>forearm eczema |
| 6           | Balsam of Peru                         | Hand eczema                    |
| 7           | Fragrance, Potassium dichromate        | Hand eczema                    |
| 8           | Balsam of Peru, Paraben mix            | Hand eczema,<br>forearm eczema |
| 9           | Paraben mix, fragrance                 | Hand eczema                    |
| 10          | Potassium dichromate, cobalt, nickel   | Hand eczema                    |
| 11          | Fragrance, Balsam of Peru, Paraben mix | Hand eczema,<br>forearm eczema |

**Fig. 1.** Improvement of contact dermatitis by HyPE (see Methods for details).

study. As shown in Fig. 1, after one month of treatment, the average visual score on the ExPLI-treated side was reduced by 69.9%, while on the vehicle-treated side the average visual score was reduced by 36.5% ( $p = 0.0024$ ), thus clearly showing the efficacy of the treatment. It should be noted that since the vehicle is used in our department as a "cooling cream" in cases of sub-acute dermatitis, its therapeutic effect is not unexpected.

## DISCUSSION

The marked improvement by the drug-containing cream over the vehicle, clearly demonstrates that the topical PLA<sub>2</sub>-inhibitor is effective in treating contact dermatitis.

Although molecules of the size of HyPE (about 50 kDa) are not normally expected to permeate the skin, HA is known to bind to epidermal receptors (CD44, RHAMM, ICAM-1). In inflamed/damaged skin, epidermal CD44 is enriched and exogenous HA is accessible to its binding (17). Accordingly, HA, mixed with pharmaceutically active ingredients, has long been used as a vehicle for drug delivery in topical preparations (17-18).

The results of the present study clearly demonstrate the therapeutic potential of the HA-lipid conjugates in treating skin diseases. In addition to lipid mediator production, PLA<sub>2</sub> activity is also linked to the production or action of other agents involved in skin inflammation, such as IL-1 in murine DTH (19), IL-8 in stimulated dermal fibroblasts (20), iNOS in psoriasis (21) and contact dermatitis (22), the T-cell adhesion molecules (23) and TNF $\alpha$  in psoriasis (24). Concordant with this, the ExPLIs were found effective in suppression of these mediators and amelioration of related inflammatory conditions in a number of *in-vitro* and *in-vivo* systems, including the suppression of endotoxin-induced production of cytokines and adhesion molecules in human lung microvascular endothelial cells (13), as well as in rats with endotoxin-induced sepsis (12), rats and mice with experimental allergic encephalomyelitis (EAE) (25), and rats with ovalbumin-induced allergic bronchitis (14).

The present study provides the first documentation in humans of the beneficial effects of treatment with ExPLIs. It further supports the notion that inclusive control of lipid mediator

production is a promising therapeutic approach to the treatment of inflammatory/allergic diseases in general, and skin inflammation in particular.

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