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Pretreatment of skin with a *Ginkgo biloba* extract/sodium carboxymethyl-β-1,3-glucan formulation appears to inhibit the elicitation of allergic contact dermatitis in man

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The clinical efficiency of mitigating contact dermatitis with a *Ginkgo biloba* extract and carboxymethyl-β-1,3-glucan formulation was investigated in a double-blind versus placebo study using 22 subjects (Caucasian women aged 22–55 years) with allergic contact dermatitis from various substances in the European standard series. The formulation was applied to intact skin 2× a day for 2 weeks ("in use" application) prior to a single application of a selected contact allergen under a Finn Chamber for 24 h. Readings were carried out in a blind study by a dermatologist 2 and 3 days after patch removal. Representative photographs were taken of treated, placebo and untreated test areas. 68.2% of the panelists showed significantly reduced skin reactivity ($p=0.037$*) on the treated site 2 days after patch removal, versus untreated and/or placebo sites. This finding indicates that the *Ginkgo biloba*/carboxymethyl-β-1,3-glucan formulation can mitigate against allergic contact dermatitis.

Key words: allergic contact dermatitis; inflammation; *Ginkgo biloba*; carboxymethyl-β-1,3-glucan; patch testing; prevention. © Munksgaard, 1998.

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Allergic contact dermatitis is induced by a vast spectrum of agents, ranging from metals to drugs and certain cosmetics. In cosmetology, adverse reactions are not acceptable when evaluated against the potential benefit of the product. Few cases of contact dermatitis caused by cosmetics are diagnosed, and most of these are allergic contact dermatitis (1–4). Because there was a growing need for cosmetics specifically designed for truly allergic people, the "hypoallergenic" concept was introduced about 40 years ago, and took into account the dictionary definitions of "hypo" meaning "below" or "less than normal", and "allergenic" describing a substance that induces allergy. Cosmetic manufacturers have focused mainly on high degrees of purity for their ingredients, rigid manufacturing quality controls, simplicity of formulations, and, particularly, elimination of well-known sensitizing ingredients. Concerned that consumers might be misled by "hypoallergenic" claims, the Food and Drug Administration in 1974 attempted to define the term and set standards. Cosmetics labelled "hypoallergenic" must show, by means of scientific studies conducted on human volunteers, that significantly fewer adverse reactions occur compared to appropriate reference products.

Today, while " hypoallergenic" products comply with the above standards, an increasing frequency of allergic contact reactions to topical products is being reported (1), which indicates that other approaches for well-tolerated products must be identified. In this context, a formulation containing a *Ginkgo biloba* extract and carboxymethyl-β-1,3-glucan, which have, respectively, anti-free-radical properties and immunomodulatory effects, was investigated in a clinical study to evaluate the rôle of the mixture in mitigating allergic contact dermatitis, using a patch test technique on volunteers selected for their susceptibility to various allergens.

Materials and Methods

Subjects

The study was carried out on 22 preselected volunteers (Caucasian women aged 22–55 years), who reacted in a preliminary study to at least 1 of 8

known allergens in the European standard series. 3 were atopics, but they did not present any dermatological lesions. After giving informed consent, the subjects were requested not to apply any detergents or cosmetics to their forearms for 1 week prior to, and throughout the duration of the experiment. The study was approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Lyon A (France).

Allergens

4 allergens often implicated in allergic reaction to cosmetics were selected from the European standard series: nickel sulfate 5% in mineral oil (for 13 volunteers); balsam of Peru 25% in mineral oil (for 4 volunteers); fragrance mix 8% in mineral oil (for 4 volunteers); methyl-isothiazolinone 0.01% in water (1 volunteer). 20 μl of the allergen solution were applied under occlusive patches (Finn Chambers on Scanpor-Promedica) for 24 h.

Products

(a) 0.1% standardized *Ginkgo biloba* extract (Beaufour-Ipsen) containing low levels of terpenes, <0.5% (known to be involved in irritant processes and in contact allergy), and 32±3% of flavones and heterosides (Quercetin, Isorhamnetin and Kaempferol), obtained from *Ginkgo biloba* leaves, was mixed with 0.5% sodium carboxymethyl-β-1,3-glucan obtained by carboxymethylation of a polysaccharide produced by *Saccharomyces cerevisiae* (Mibelle-AG Biochemistry). The mixture was then incorporated into a Carbopol™ (Goodrich) gel. This formulation was tested for its irritant potential using a patch test technique, and was well tolerated. (b) A placebo (vehicle alone).

Protocol

2 μl/cm^2 of the formulation was applied over a 35 cm^2 area 2× a day for 2 weeks ("in use" test) on the volar forearm (right or left) of the 22 volunteers (the contralateral forearm received the placebo), in a double-blind test. The areas were randomized. On the 16th day, 20 μl of one of the contact allergens (specific for every subject) was applied on treated, placebo, and untreated sites (control) 15 min after the last application.

Visual scoring

Clinical observations were performed by a dermatologist 2 and 3 days after patch removal. The observations were scored for erythema, oedema and vesicles on a 5-point scale (0 to 4) according to criteria of the International Contact Dermatitis Research Group, as follows: 0=no reaction; 1=very slight erythema; 2=erythema with slight oedema, and maybe some papules (no vesicles, bullae); 3=erythema with oedema, papules, vesicles; 4=marked erythema with oedema, vesicles, bullae.

Pruritus was scored as follows: 0=none; 1=slight; 2=moderate; 3=marked; 4=severe.

"+" or "−" were added to visual scoring by the dermatologist when the reaction was obviously different from other sites, but not enough to be classified below or above.

Macrophotography

Photos representative of the reactions observed in the volunteers were taken in order to illustrate the difference between sites, 2 days after patch removal.

Statistics

Mean visual scorings (cutaneous reactions and pruritus) of active, placebo, and control sites were compared using the non-parametric Wilcoxon signed rank test (significant for $p<0.05$).

Results

Visual scoring

Results analysis shows that on the *Ginkgo biloba* extract/carboxymethyl-β-1,3-glucan formulation treated site, cutaneous allergic reactions were reduced compared to placebo and control sites. The reduction is significant between active and control sites 2 days after patch removal ($p=0.037$*), and at each time of evaluation the physician noted im-

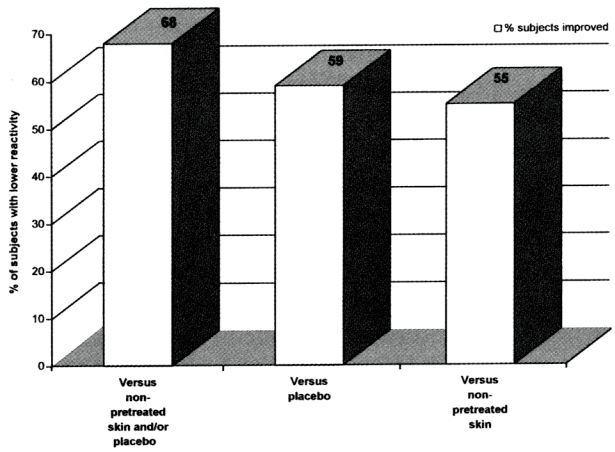
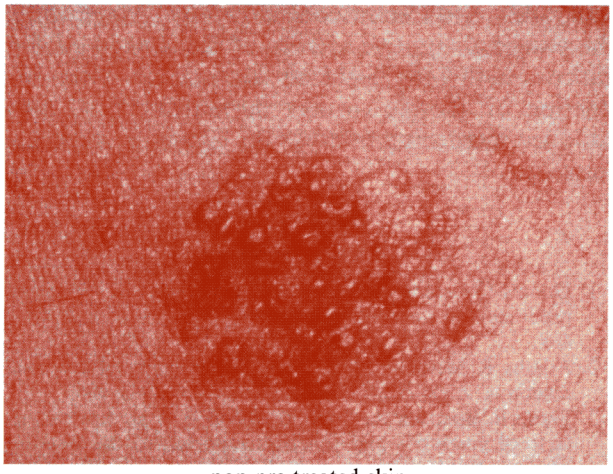
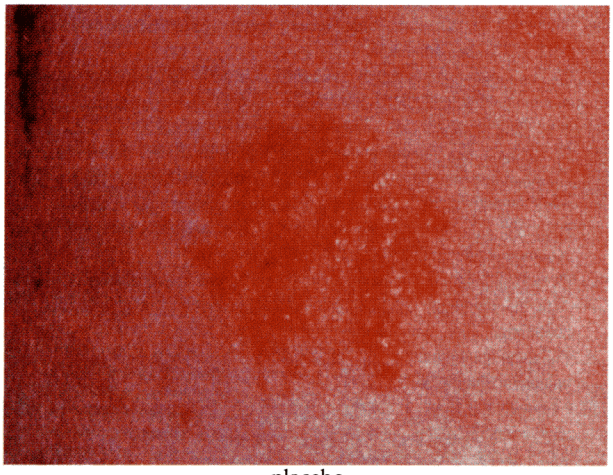


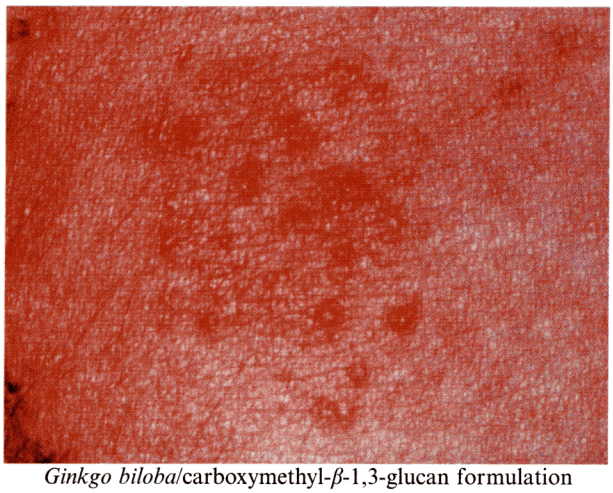
Fig. 1. Ratio of panellists improved regarding sensitization reactions at D2. 68% of the subjects showed a significant decrease in cutaneous reactions versus untreated skin and/or placebo.



non pre-treated skin



placebo



Ginkgo biloba/carboxymethyl-β-1,3-glucan formulation

Fig. 2. Cutaneous reactions 2 days after patch removal. Vesicles were more scattered and erythema was slightly reduced on the active site compared to control or placebo sites.

provement of the severity and nature of cutaneous reaction, such as no oedema or no vesicles, or vesicles more scattered and/or less erythema.

On 15 volunteers (68.2% of the whole panel), it was evident that the site that received the *Ginkgo biloba* extract/carboxymethyl-β-1,3-glucan formulation had a lower cutaneous reactivity compared to the control and/or the placebo sites for the cutaneous reactions (Fig. 1). Pruritus also decreased, though these changes were not statistically significant (data not shown).

Macrophotography

Photos of each tested site for the same volunteer, 2 days after patch removal, are shown in Fig. 2. Cutaneous reactions were decreased for the *Ginkgo biloba* extract/carboxymethyl-β-1,3-glucan formulation treated area as compared to control and placebo sites. Vesicles were more scattered and erythema was slightly reduced.

Discussion

Contact allergy is a complex phenomenon in which the skin immune system is involved, particularly immunocompetent Langerhans cells that trap antigens and initiate an immune response by stimulation of specific T-cells and keratinocytes that produce cytokines. Usually, allergic contact dermatitis is expressed as erythema, oedema (inflammatory processes), and pruritus. The formulation containing *Ginkgo biloba* extract and carboxymethyl-β-1,3-glucan was shown to be effective on the elicitation of allergic contact dermatitis induced by preselected allergens, especially for those typically contained in cosmetics, such as fragrance mix or methyl-isothiazolinone. *Ginkgo biloba* extract contains dimeric flavonoids (biflavones) and several publications have reported that these active ingredients can inhibit certain processes involved in the inflammatory response (main manifestation of allergic contact dermatitis): Della Logia et al. (5), investigating topical anti-inflammatory activity of *Ginkgo biloba* extract and some of its components (ginkgolides, bilobalide, biflavonic fraction and some pure biflavones) in the croton oil test on mice ears, demonstrated an anti-inflammatory action with a potency comparable to that of indomethacin for the extract (significant diminution of oedema of 16%, and, for bilobalide alone, inhibition rose to 64%). They showed that the ability of the ginkgolides to restrain the inflammatory processes mediated by platelet activating factor (PAF), the modulating effect of biflavones on histamine release from mast cells, and the reducing action of *Ginkgo biloba* extract on the amount of oxygen species produced by activated neutrophils, all contribute to balancing the inflammatory cascade. Moreover, Kurihara et al. (6) found that 1 *Ginkgo biloba* derivative is a specific PAF antagon-

ist and has an inhibiting effect on PAF-induced human eosinophil and neutrophil chemotaxis. These 2 types of cells are implicated in inflammation. Bombardelli et al. (7) showed that *Ginkgo biloba* dimeric flavonoids contained in *Ginkgo* extract had vasomotor activity and actions on microcirculation that ameliorate skin trophism. These activities could contribute to mitigating contact dermatitis elicited by the topically applied allergens used in this study.

In 1941, Pillmer & Echer (8) discovered zymosan, the first defined pharmaceutical yeast product. This β-(1-3) glucan isolated from the inner cell wall of *Saccharomyces cerevisiae* has been identified as a very potent stimulator of the immune system, with the ability to activate macrophages, neutrophils, and other cells that carry specific β-glucan receptors on their surface (9). Sandula et al. (10) compared the action of various β-glucan derivatives of different solubilities, degrees of substitution and molecular weight distributions on rat thymocytes. They found that carboxymethyl glucan with a degree of substitution of 0.75 exhibited the highest immunomodulatory activity. The derivative used in our study had this degree of substitution.

The findings from this double-blind versus placebo clinical study indicate that the *Ginkgo biloba*/carboxymethyl-β-1,3-glucan formulation applied in a preventive way on preselected allergic skin, can reduce cutaneous reactions induced by various allergens (metals, e.g., nickel, or cosmetic ingredients, e.g., methyl-isothiazolinone). While the exact mechanism is not clearly elucidated, it could be speculated that it is related to the combined effects of inhibition of release of inflammatory compounds and immunomodulatory activity of each ingredient. In order to elucidate the contribution of each ingredient, further clinical investigations will be conducted by testing each active ingredient separately at different concentrations. Meanwhile, the potential curative activity of the *Ginkgo biloba*/carboxymethyl-β-1,3-glucan association after induction of allergic contact dermatitis could also produce interesting findings on its action mechanisms.

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