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Wool peptide derivatives for hand care

C. BARBA, S. MÉNDEZ, A. RODDICK-LANZILOTTA, R. KELLY, J. L. PARRA, and L. CODERCH, *IIQAB (CSIC), Jordi Girona 18–26, 08034 Barcelona, Spain (C.B., S.M., J.L.P., L.C., Canesis Network Ltd, Private Bag 4749, Christchurch, New Zealand (A. R.-L.), and Keratec limited, Springs Road, Lincoln, Canterbury, New Zealand (R.K.).*

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Synopsis

Hands experience much greater wear and tear during normal daily routines compared with most other parts of the body, and thereby demand specific needs from cosmetics targeted at hand care. Keratin proteins are the major structural component of the outer layers of the skin. In this work a novel keratin fraction from wool, which has high cystine content present in the S-sulphonated form, has been developed to target hand care applications. *In vivo* long-term studies were performed to evaluate the water-holding capacity and elasticity of hand skin following topical application of keratins. Moreover, protection of healthy skin against detergent-induced dermatitis was evaluated after topical application of the keratin-active formulation. Significant results in the measured biophysical parameters were found, which indicated an improvement in the skin's water-holding capacity, hydration, and elasticity for volunteers with dry skin as a result of the keratin peptide treatment. Results also indicated that the keratin peptide treatment can prevent some of the damaging effects associated with surfactant exposure.

INTRODUCTION

Keratin proteins are used extensively by the body as a major structural component of the outer layers of the skin. These keratin proteins, with a high content of the amino acid cystine, provide a greater structural stability to chemical agents and solvents than many other proteins types. Wool is predominantly composed of keratin proteins that combine to give it desirable properties such as strength, insolubility, and moisture regain. Different classes of keratin proteins are represented in the complex macromolecular structure, each of which has specific functions and characteristics.

The daily wear and tear experienced by the hands creates specific needs to be met by the formulators of hand care cosmetics. Issues such as moisturization and elasticity of hand skin are often more severe than issues experienced by other parts of the body.

In this study, a novel keratin protein fraction from wool has been developed to target hand care applications. The keratin peptide studied is a specific fraction of the wool fiber, which has been enzymatically hydrolyzed to give a peptide in the range of 6–8

amino acids (MW by SDS-PAGE<1000D), which has high cystine content present in the S-sulphonated form. This unique functionality maintains the antioxidant activity of the amino acid cysteine. As a hydrolysate, keratin peptide can penetrate into the skin and increase moisturization, protecting the hands.

The aim of this work was to study the efficacy of this new keratin active in a hand cream formulation. The effect of this keratin active when applied topically on a long-term basis to undisturbed hand skin on properties such as water-holding capacity and elasticity was determined. Finally, protection of healthy skin against detergent-induced dermatitis was evaluated after topical application of the keratin active formulation.

MATERIALS AND METHODS

CHEMICALS

Chemicals employed were keratin peptide (Keratec Limited, New Zealand); sodium lauryl sulphate, SLS (Merck, Darmstadt, Germany); and Cromadol GTCC, Polawax NF, stearic acid, Crodacol C90 EP, Propylparaben, Ultrez 21, acylate crosspolymer, and methylparaben (Croda, UK).

SAMPLE PREPARATION

Two hand creams were used; both were made from the formulation given below. The keratin cream was prepared by adding 3% keratin peptide (as supplied, i.e., 0.3% active) and the base cream by addition of 3% deionized water.

Base hand cream formulation. The formulation comprised Crodamol GTCC (10.0% w/w), Polawax NF (5.0% w/w), stearic acid (3.0% w/w), Crodacol C90 EP (2.0% w/w), Propylparaben (0.25% w/w), Ultrez 21 (8.0% w/w), acrylate crosspolymer 2%, Methylparaben (0.20% w/w), and deionized water (to 100.0% w/w).

SUBJECTS

Sixteen healthy volunteers (all females) phototype III-IV-V with a mean age of 33 ± 8 years (range 24–50 years) participated in both studies (Table I). All subjects were advised to avoid topical drugs or moisturizers on the tested zones for a week prior to the experiments. To obtain reliable measurements, the volunteers were acclimatized for 15 min in a conditioned room (20°C, 60% RH) before the experiments. Subjects were classified into group 1 (dry skin, mean initial capacitance < 45) and group 2 (hydrated skin, mean initial capacitance > 45) based on initial skin capacitance measurements, following the advice given by the instrument company (Courage & Khazaka).

BIOPHYSICAL MEASUREMENTS

Skin hydration was determined using a Corneometer CM 85 (Courage & Khazaka), which measures skin capacitance in arbitrary units (au). Transepidermal water loss (TEWL), which indicates the barrier function of skin, was measured using a Tewameter TM 210 (Courage and Khazaka). Elasticity was determined by a Cutometer SEM 575

Table I
Skin and Phototype of the Volunteers Who Participated in the Study

Volunteer	Age	Phototype	Initial skin capacitance	Kind of skin
1	24	IV	49.22	Hydrated
2	28	IV	32.33	Dry
3	45	III	34.57	Dry
4	30	III	47.22	Hydrated
5	29	V	59.55	Hydrated
6	26	III	51.00	Hydrated
7	38	IV	30.89	Dry
8	29	III	24.78	Dry
9	35	III	32.89	Dry
10	32	III	30.33	Dry
11	50	IV	29.56	Dry
12	28	IV	49.22	Hydrated
13	32	III	46.22	Hydrated
14	28	III	42.56	Dry
15	50	III	48.78	Hydrated
16	38	IV	42.22	Dry

(Courage and Khazaka) using Mode 1, where the measurements are performed with a constant negative pressure. Results are visualized in a curve that points out the viscoelastic qualities of the skin. The parameters that were considered in these studies are: R5, net elasticity, and R7, the portion of elasticity compared to the complete curve (the closer to 1 these parameters are, the more elastic the skin is). All parameters were recorded in accordance with established guidelines (1–4).

EFFECT OF KERATIN PEPTIDE HAND CREAM ON UNDISTURBED HAND SKIN

A long-term study was performed to test the effect of the formulations when applied repeatedly to undisturbed hand skin. Baseline measurements of TEWL, skin capacitance, and skin elasticity were taken on three marked zones of the dominant hand of the volunteers: two zones for topical treatment (base cream and cream containing 3% keratin peptide) and one untreated zone (control). Creams were randomly applied onto marked areas of 9 cm^2. After 24 h, skin capacitance and skin elasticity were evaluated, and then the formulations were applied again. The application of the formulations was repeated once a day during two and a half weeks (not including weekend days) with a total of 12 applications, and parameters were measured after 24 h following each application. TEWL was measured on the three zones at the end of the treatment. During all the treatment period, hands were left air exposed and no restrictions on washing were done.

EFFECT OF KERATIN PEPTIDE HAND CREAM ON SODIUM LAURYL SULPHATE (SLS) DISTURBED HAND SKIN

A test was performed to evaluate the protective effect of the keratin cream applied to undisturbed hand skin followed by SLS exposure. Following the long-term study on undisturbed hand skin, baseline measurements of TEWL, skin capacitance, and skin elasticity were taken in the three treated areas of the dominant hand of the volunteers in group 2. The three zones were then exposed to 2% SLS aqueous solution for 2 h (see

below), and the resultant irritation reaction was assessed after 2 h 30 min and 24 h after SLS exposure by measuring TEWL and skin capacitance (5–7).

SLS exposure. Fifty microliters of an aqueous solution of 2% SLS was pipetted onto a layer of filter paper placed in each of several aluminum chambers (d = 12 mm, large Finn Chambers, Epitest Oy, Finland). The chambers were fixed to the skin for 2 h with adhesive tape. Upon the removal of the patch, the skin was gently rinsed with water and allowed to dry.

DATA TREATMENT

The mean values and standard deviations (SD) were calculated. Results were presented doubly evaluated as a percentage of modification with respect to the basal (initial) value and subsequently as a percentage of modification with respect to the control zone at the same time. Dixon's test was used for detecting outliers, which were excluded from the data. ANOVA variance analyses were used to determine significant differences between values obtained from different treatments (significance level accepted $*p < 0.05$) using the Statgraphics® program. Statistics on the figures indicate the percentages (versus basal values) for the keratin peptide cream or the base cream treatments that are significantly different from the percentages (versus basal values) obtained for the non-treated control zone.

RESULTS AND DISCUSSION

EFFECT OF KERATIN PEPTIDE HAND CREAM ON UNDISTURBED HAND SKIN

A long-term study was performed to test the effect of the base hand cream formulation and the same formulation containing 3% keratin peptide. Evaluation of skin capacitance and elasticity was carried out 24 h after a daily application over two and a half weeks with a total of 12 applications, and TEWL was evaluated at the end of the treatment.

Initial tests on the skin capacitance of the volunteers clearly showed that they fell into two categories. Nine volunteers had dry skin (mean initial capacitance less than 40 a.u.), and seven had hydrated skin (mean capacitance greater than 45 a.u.). This is an important parameter in determining the efficacy of the keratin hand and nail cream, as will be discussed below.

Transepidermal water loss, TEWL, is a sensitive index of skin barrier integrity. A lower % change in TEWL (measured as water loss in g/m^2h) indicates the skin is acting more efficiently as a barrier to water loss and thus is better able to maintain its present moisture content. TEWL values before and after the treatment period for all 16 volunteers didn't show significant differences between the different treatments, indicating that for healthy skin in the absence of any disturbing treatment, variations in transepidermal water loss are too small to be taken into consideration.

The test population was separated into two distinct groups to allow differences in TEWL to be observed. Although no significant results were found, evaluation of TEWL indicated an influence of the keratin peptide cream for the group of volunteers with dry skin. A decrease in TEWL of 13% relative to the control zone was observed for the cream containing keratin peptide (Figure 1).

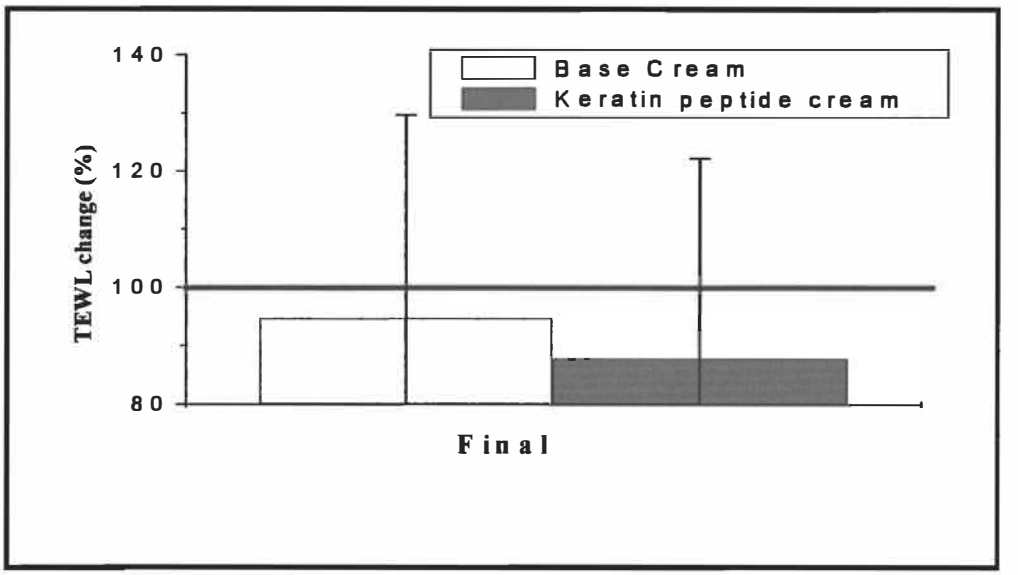

Figure 1. Final percentage of TEWL for the dry skin volunteers. Changes were doubly evaluated versus basal and control values.

Results of skin hydration tests by capacitance measurement indicated that there was a trend of increased hydration for the keratin peptide cream treatment. This increase was consistent for the dry skin volunteers (Figure 2), reaching an increase of 20% during the treatment period, even though it is not statistically significant.

For the elasticity parameters R5 and R7, the results showed a consistent trend of increasing elasticity in the zones treated with the keratin peptide cream, reaching a

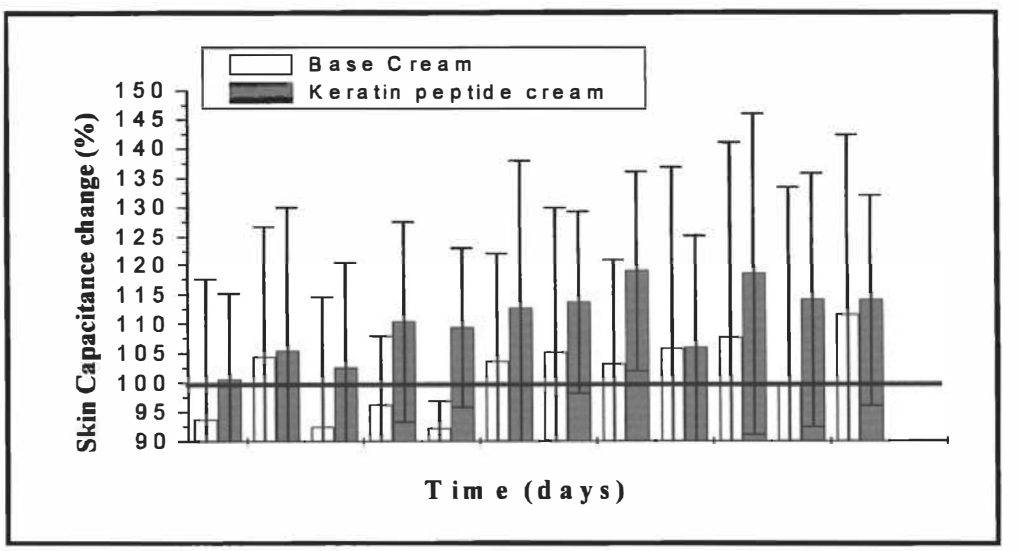

Figure 2. Variation of skin capacitance after topical applications for dry skin volunteers during the treatment period. Changes were doubly evaluated versus basal and control values.

significant increase in elasticity for almost all measurements during the treatment period (Figures 3 and 4).

It was predictable that only small differences in TEWL, hydration, and elasticity were obtained in the volunteers with already hydrated skin. However, treatment with keratin peptide cream on undisturbed hand skin showed beneficial effects on the volunteers with dry skin, significantly increasing the elasticity and showing trends of decreasing TEWL and increasing hydration.

EFFECT OF KERATIN PEPTIDE HAND CREAM ON SODIUM LAURYL SULPHATE (SLS) DISTURBED SKIN

It is well known that commonly used surfactants can irritate and damage the skin, decreasing its natural barrier function and thus its ability to retain moisture. To investigate the protective effect of the keratin peptide cream on hand skin, all skin zones (previously treated over a period of two weeks with keratin peptide cream, base cream, or control) received the same SLS application, and biophysical parameters were measured at 2 h 30 min and 24 h following SLS exposure.

The effect on SLS both on hydration and TEWL is always more marked for well-hydrated skin, and thus the differences obtained when the creams are applied are also more significant. The results obtained in this study showed that treatment of the skin with keratin peptide cream provided protection from SLS exposure evidenced by a smaller decrease in hydration and a smaller increase in TEWL values, particularly for volunteers with well-hydrated skin.

TEWL values show an initial increase in TEWL at 2 h 30 min, as a result of SLS exposure, followed by a recovery of the barrier function of the skin, demonstrated by a

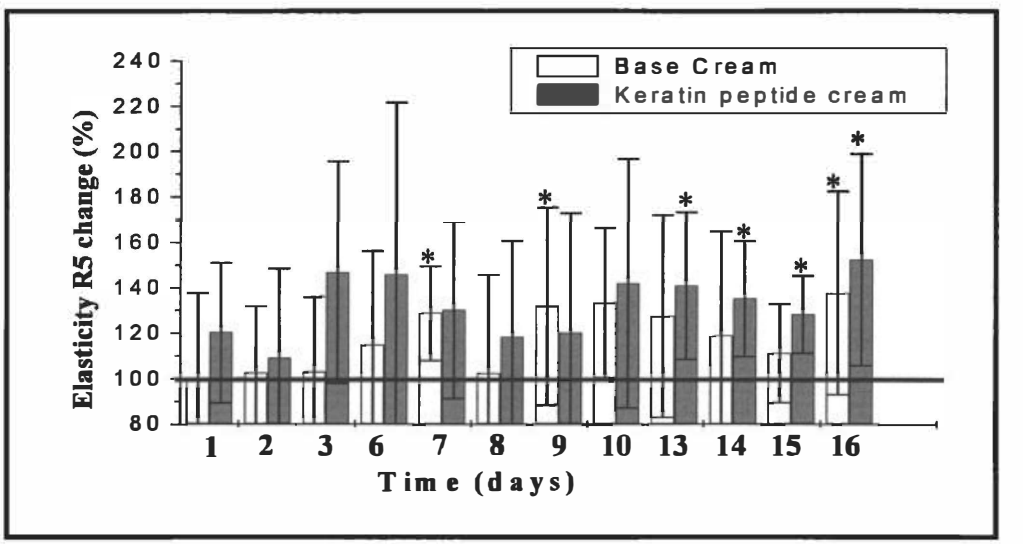

Figure 3. Variation for the elasticity parameter R5 for dry skin volunteers during the treatment period. Changes were doubly evaluated versus basal and control values. (*$p < 0.05$, significance is evaluated using ANOVA variance analysis between each percentage result and its corresponding control percentage result).

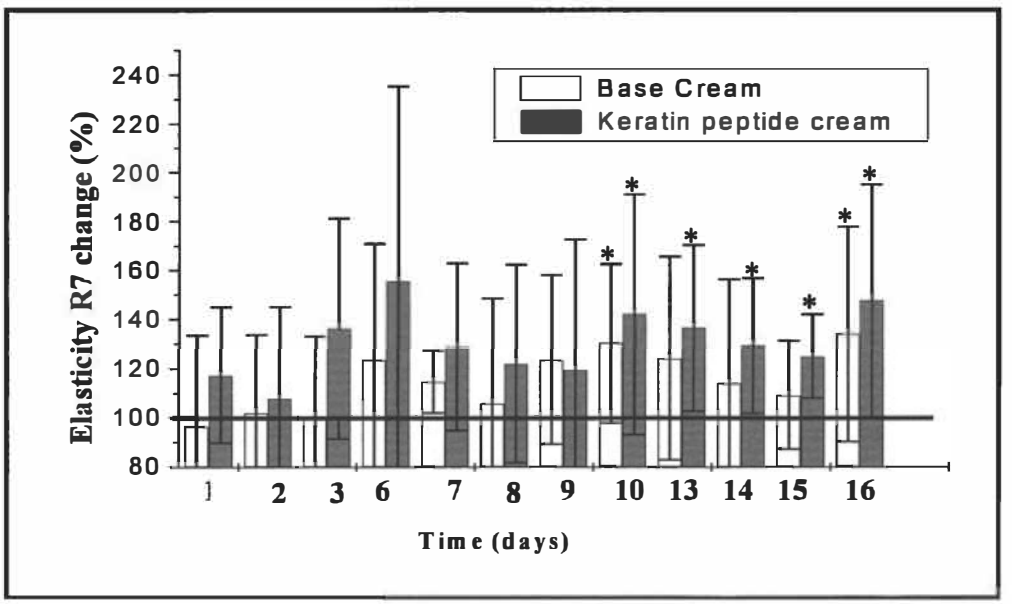

Figure 4. Variation for the elasticity parameter R7 for dry skin volunteers during the treatment period. Changes were doubly evaluated versus basal and control values. (*$p < 0.05$, significance is evaluated using ANOVA variance analysis between each percentage result and its corresponding control percentage result).

decrease in TEWL. It can be seen that in the zones treated with the keratin peptide cream the variation of TEWL after SLS exposure is less, with a decrease in TEWL of about 15% compared to the control zone (Figure 5).

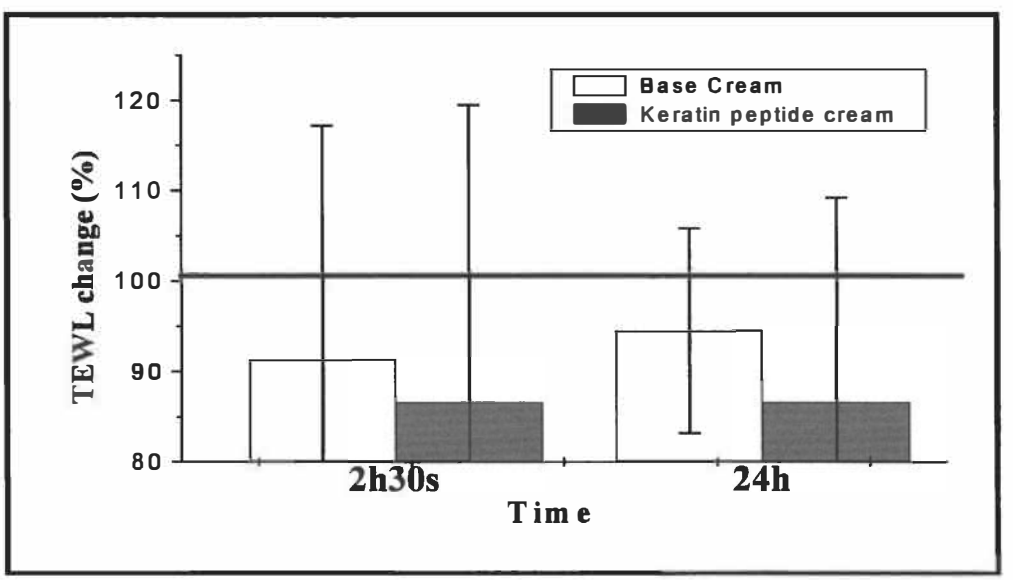

Figure 5. Variation of TEWL for the volunteers with well-hydrated skin at two different intervals following SLS exposure. Changes were doubly evaluated versus basal and control values.

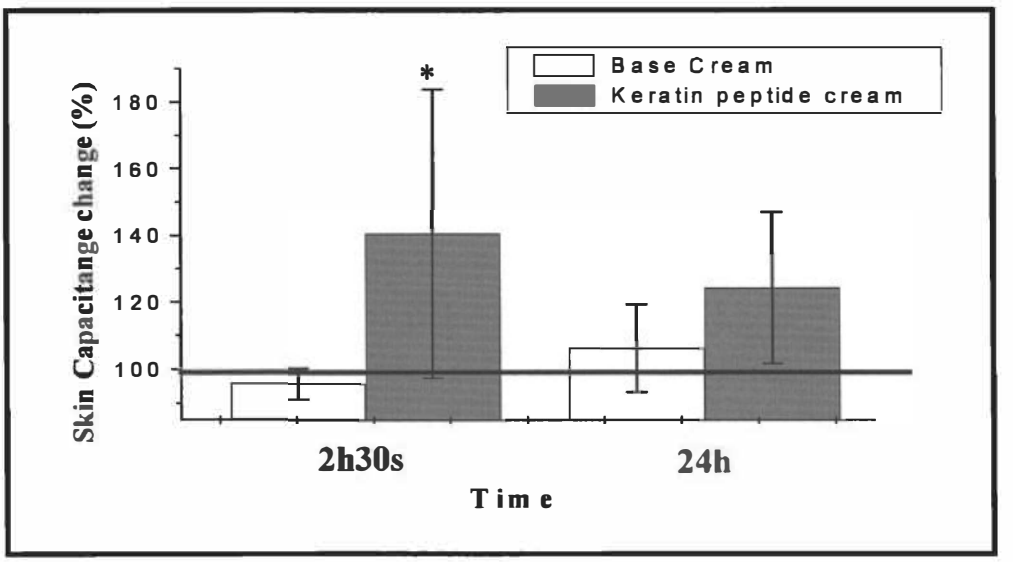

Figure 6. Variation of skin capacitance for well-hydrated skin at two different intervals following SLS exposure. Changes were doubly evaluated versus basal and control values (*$p < 0.05$, significance is evaluated using ANOVA variance analysis between each percentage result and its corresponding control percentage result).

Exposure to SLS has the effect of dehydrating skin. However, results showed that there was a significantly smaller decrease in hydration for skin zones treated with the keratin peptide cream (Figure 6).

CONCLUSIONS

Treatment with keratin peptide hand cream on undisturbed skin showed different effects depending on the initial hydration status of the skin. The keratin peptide cream treatment had beneficial effects on dry skin as evidenced by a decreased TEWL and increased hydration and elasticity. Skin treated with keratin peptide cream is more resistant to the damaging effects of SLS. While the applied peptides may not prevent the interference of SLS with the lipid layer of the stratum corneum, they do appear to assist the skin in retaining moisture following SLS exposure, thereby preventing some of the damaging effects of the surfactant insult.

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