Repairing effect on skin barrier (clinical study).

Aim

If using monogalactosylceramide (CERAMIDES LS) repairs, strengthens the skin barrier function, its topical application in vivo on man will decrease TEWL and improve skin moisturization.

To do so, we have compared the barrier effect of a cream (dosed with 0.40% of CERAMIDES LS) with the one of a placebo cream by kinetic measurement of TEWL decrease, in an experimentation in double blind.

Protocol

- Study on 15 healthy or atopic female volunteers, being 65 years old on average, with a very dry and squamous skin on the inner side of forearm.
- Twice daily treatment, in the morning and in the evening, on the inner side of forearms during 3 weeks with randomization (2 mg/ml).
- Placebo cream on one forearm.
- Cream with 0.40% of CERAMIDES LS on the other forearm.

Before TEWL measurements, the subjects have been set in an air-conditioned room (20°C, 50% RH).

The comparative skin barrier effect has been evaluated before, then 45 minutes and 24 hours after a standardized topical application of each product, further to a treatment of controlled stripping, by a TEWL measurement at the beginning and at the end of the 3 weeks of treatment, the latest application dating back to 12 hours.

Results

- At the beginning of the treatment, cream with CERAMIDES LS has made it possible to decrease TEWL by 29.23% after 45 minutes and by 33.22% after 24 hours after the first application, in comparison with placebo cream.
- After 3 weeks of treatment, active cream has kept and increased its significant activity on the TEWL decrease by 34.93% after 45 minutes and by 35.26% 24 hours after the last standardized application, in comparison with placebo cream.

Conclusion

CERAMIDES LS at 0.40% in a cream, have clearly repaired and strengthened the skin barrier activity and the skin moisturization in comparison with a placebo cream.

A major and significant efficacy obtained after the first application (+33.22%) has been confirmed and even boosted after 3 weeks of application (+35.28% versus placebo).

Such an experiment has demonstrated that already at a low concentration (0.40%), lipids within CERAMIDES LS are bioavailable and physiologically functional. It has confirmed Eizani’s former works, showing the synergic efficacy of the combination: glycoconact with cholesterol + phospholipids.

Improvement of the skin barrier effect (%) by CERAMIDES LS at 0.40% vs placebo

Fig. 4. - Comparative study of the skin barrier effect at the beginning of the treatment (T1) and at the end of the treatment (T2). Topical application (2 mg/ml) on dry, squamous, stripped skin of placebo cream versus cream containing 0.40% CERAMIDES LS. TEWL measurements (10 ± 0.50% RH) at T0, T45 min and T24 h after application.

Fig. 6 - Co-operative study of the skin barrier effect at the beginning of the treatment (T1) and at the end of the treatment (T2). Topical application (2 mg/ml) on dry, squamous, stripped skin of placebo cream versus cream containing 0.40% CERAMIDES LS. TEWL measurements (10 ± 0.50% RH) at T0, T45 min and T24 h after application.

Fig. 6 – Comparative study of the skin barrier and permeability at the beginning of the treatment (T1) and at the end of the treatment (T2). Topical application (2 mg/ml) on dry, squamous, stripped skin of placebo cream versus cream containing 0.40% CERAMIDES LS. TEWL measurements (10 ± 0.50% RH) at T0, T45 min and T24 h after application.

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Sphingolipids, ceramides and skin

Sphingolipids are functionally important components of skin. They are present in transitional epidermal layers (S.G. and S.C.C.) containing glycolipids and ceramides whereas the superficial layers (S.C.D.) contain, non glycosylated ceramides.

Definition / Composition

CERAMIDES LS are concentrated and highly purified glyco-ceramides, analogous to 45% ceramides of human skin. They are combined to a lesser proportion of cholesterol and phospho-lipids.

CERAMIDES LS are of natural equine origin. They have a specific biological activity on the superficial epidermal layers. They act as glycolipids, as ceramides, as well as a ceramide precursor.

Composition:

- **Glycero-ceramide**: 65 - 100%
- **Phospholipids**: 25 - 38%

Skin benefits

1. **Repairing, strengthening the epidermal barrier**

   - CERAMIDES LS strengthen, keep and restore the lipidic and ceramides barriers being damaged or weakened.
   - They renew, repair and strengthen physiologically the substance cohesion of lipidic lamellar systems of intercellular spaces, being very sensitive to outer attacks.

2. **Strengthening the cohesion of horny layers**

   - The latter depends on integrity: corneocytes, corneodesmosomes and epidermal lipids/lamellar systems (ceramides, cholesterol, ...).
   - Modulators of the activity of enzymes being in charge of the integrity/degradation of corneodesmosomes.

3. **Epidermal lipido-protecting**

   - CERAMIDES LS avoid or repair the rough, scaly, dried, ery-thematous, irritated and uncomfortable features of the skin.
   - They strengthen its protection against damages induced by various attacks: anomic surfactants, retinoids, topical corticosteroids, solvents, strippings and against the effects of the environment to which the lipid barrier is very sensitive. Consequently, they strengthen/restore its hydoretaining capacity, its softness and in flexibility, decrease its sensitivity, reduce irritation and blotches, and its scaly feature.

Key advantages of CERAMIDES LS

- They combine with glycero-ceramides determined proportions of cholesterol and phospholipids (potentially effects of the activity of ceramide').
- They act as a ceramide precursor and as 45% ceramides, because it is activated by epidermal' idases (glycolipid conversion$\rightarrow$ceramide conversion).
- Homologous to human epidermal lipids.
- High purity, constant composition and quality. Well tolerated.
- A molecule of glycero-ceramide is fixed on each molecular level 45% ceramides; hence, a strengthened amphiphility of ceramide (extremely hydrophobic molecule), and consequently:
  - Easier incorporation into any type of cosmetic escipients (O/W and W/O emulsions) where it plays the cosurfactant part and, moreover, spontaneously forms in situ liposomal nanovesicles.
  - Efficiency at a low dosage. Excellent performance/price ratio.

Strengthening and structuring activity on the epidermal superficial layers

**Aim / Principle**

- We demonstrate that an emulsion containing CERAMIDES LS specifically strengthens SC by complex lipids and that this effect is located on SC. Skin of a healthy volunteer (33 years old) with a dry skin and a lipid deficiency. Biopsies of 3 areas corresponding to 3 experimen-
tal conditions: control area, placebo area, emulsion area with 0.30% CERAMIDES LS. Applications twice a day, during 2 days.
- After fixation of biopsies, sections with cryoncoteic (20 µm) and Baker’s histochemical staining for stain- ing sphingolipids, visible in blue-black. By histochemistry, we have quantified the intensity of the blue-black color being directly proportional to the quantified sphingolipids.
- An average of 15 measurements has been carried out and a statistical calculation according to Kruskall Wallis.

**Cosmetic use**

- Care of stressed, sensitive, scaly, rough and dry skin (prevention/repair). Care skin hand- cream.
- Anti-age care (aged skin often suffe-
ing from lipid deficiencies, especially ceramides).
- Sun Care

**Dose / Solubility / Mode of incorporation**

1. **Dose of use**: 0.20% up to 0.50%. 2. **Solubility**: CERAMIDES LS are soluble in fats and oils, dispersible in water.

**3. Mode of incorporation**

CERAMIDES LS must be dissolved in the oily phase of emulsions at 80°C or dispersed in water at 80°C.

**Analytical characteristics**

1. **Aspect**: beige powder of characteristic color.
2. **Specifications**: upon request.
3. **Tolerance**: Good.
4. **Efficacy**: Test summaries hereafter.

**Clinical study on volunteers with atopic dermatitis.**

**Aim**

- To evaluate the efficacy of a cream containing 0.5% CERAMIDES LS, so as to check whether the latter may reduce the use of topical steroids, and their side effects.

**Protocol**

- 17 patients have been selected (dermatological center in Tokyo). Topical treat-
ment by cream containing 0.5% CERAMIDES LS. Application twice or three times a day after washing, instead of applying steroids. Application on dermatotic lesions.
- Evaluation by means of the following tests: self-assessment, score, psychological. Evaluation by a dermatologist (before and after 2 weeks of treatment).

- **Efficacy**: lower use of steroids
- **Unchanged** application of steroidal treatments necessary.

**Results**

1. Effective: 12 cases; Excellent: 1 case; Unchanged: 4 cases; i.e. 76.5% of the subjects who, as a whole, have reacted towards the treatment in a good way and without any side effects.

**Conclusion**

CERAMIDES LS are prime components for the topical treatment of some affec-
tions of the skin, especially in case of dry skin linked to an atopic dermatitis. CERAMIDES LS are free from any side effect and are efficient.