Food and Chemical Toxicology 47 (2009) 669–673

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Safety studies of pseudo-ceramide SLE66: Acute and short-term toxicity

Osamu Morita [a,*], Ryosuke Ogura [a], Kazuhiko Hayashi [a], Minehiro Okuda [a], Koichi Yoshimura [b]

[a] Global R&D Safety Science, Kao Corporation, 2606 Akabane, Ichikai-machi, Haga-gun, Tochigi-ken 321-3497, Japan
[b] Global R&D, Kao Corporation, 2606 Akabane, Ichikai-machi, Haga-gun, Tochigi-ken 321-3497, Japan

ARTICLE INFO

Article history:
Received 11 June 2008
Accepted 11 November 2008

Keywords:
Pseudo-ceramide
Quasi-ceramide
Synthetic ceramide
Epidermal permeability barrier

ABSTRACT

Topical application of ceramides is reported to improve the structure and texture of the skin. Synthetic pseudo-cermaide, SLE66 has been shown to reduce dryness/scaling/itching of human skin. Although efficacy of topically applied ceramides and their analogs has been investigated to some extent, safety information is scarce. The objective of the present investigation was to evaluate potential adverse effects of SLE66. The oral LD_{50} of SLE66 in rats and mice was >5000 mg/kg, while dermal LD_{50} in rats was >2000 mg/kg. In animal and human studies, SLE66 did not cause skin irritation or sensitization. SLE66 does not possess phototoxicity or photosensitization potentials. Instillation of SLE66 into rabbit eye elicited transient conjunctival irritation. In 28 day repeat-dose studies, administration of SLE66 via gavage (daily) or by dermal application (five days/week) to Sprague Dawley rats at levels up to 1000 mg/kg/day did not cause mortality or morbidity. Compared to the controls, the clinical condition of the animals, body weights, feed consumption, hematology, clinical chemistry, organ weights, and gross necropsy findings were unaffected by oral or dermal administration of SLE66. The no-observed-adverse-effect level (NOAEL) for systemic toxicity following oral or dermal administration of SLE66 was 1000 mg/kg/day (the highest level tested).

1. Introduction

Ceramide is a family of lipid molecules composed of a sphingoid base and a fatty acid. The stratum corneum lipid domain is composed of approximately equimolar concentrations of cholesterol, free fatty acids, and ceramides (Coderch et al., 2003). Stratum corneum ceramides play a crucial role in structuring and maintaining the water permeability barrier function of the skin (Wartewig and Neubert, 2007). In several skin diseases, alterations of the content and/or molecular distributions of stratum corneum ceramides have been reported (Geilen et al., 1997). In most skin disorders that have a diminished barrier function, decrease in total ceramide content has been noted. In diseased skin, lipid composition is characterized by decreased levels of ceramide and altered ceramide profiles (Choi and Maibach, 2005). Topical application of formulations containing ceramides has been claimed to alleviate skin disorder caused by the diminished barrier function. Natural ceramides are found at trace levels in animals and plants, and their isolation for use in skin care products is tedious and expensive. Hence, several investigators have attempted to prepare synthetic pseudo-ceramides. As they do not contain a sphingol structure in their molecules, these compounds are defined as pseudo-ceramides. Using molecular designs, a highly functional synthetic pseudo-ceramide, SLE66 (CAS No.: 110483-07-3; Fig. 1), has been developed (Imokawa et al., 1986; Imokawa et al., 1989; Imokawa et al., 1991; Imokawa and Hattori, 1985; Wertz and Downing, 1983). In the studies by Imokawa and colleagues the effects of SLE66 were investigated following dermatological applications to the dry and scaly skin. The results of these studies show that the synthetic pseudo-ceramide exhibits water-retaining properties similar to those of the natural lipids. Subsequently, Mizutani et al. (2001) Nakamura et al. (1999) also reported that application of a cream containing 8% SLE66 to the skin of patients with atopic dermatitis resulted in the improvement of clinical symptoms of dryness, scaling, erythema, seropapules and itching. These studies suggest that SLE66 may improve the epidermal barrier function.

In recent years, ceramides have been implicated as important second messenger molecule, mediating a number of cellular responses to various exogenous stimuli and stress agents. Several factors such as heat, hypoxia, UV and ionizing radiation, anticancer drugs, cell stimulation with cytokines, antibodies, serum withdrawal, and antibodies have been shown to increase the intracellular concentration of ceramide (Testi, 1996; Hannun, 1996; Burek et al., 2001). In light of these findings, it is important to understand

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ICH, international conference on harmonization; 8-MOP, 8-metoxypsolaren; NOAEL, no-observed-adverse-effect level; ANOVA, analysis of variance.

* Corresponding author. Tel.: +81 285 68 7602; fax: +81 285 68 7452.
E-mail address: morita.osamu@kao.co.jp (O. Morita).

0278-6915/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2008.11.022

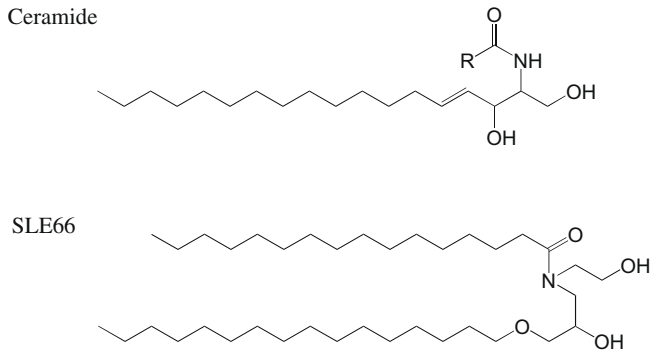

Fig. 1. Typical structure of ceramide and the structure of pseudo-ceramide (SLE66). *R* = alkyl chain.

any adverse effects of pseudo-ceramides such as SLE66. The objective of the present investigations was to study the potential toxicity of SLE66 following acute and short-term administration in rodents. Additionally, we also investigated the irritation and sensitization potentials of SLE66 in human subjects. The short-term repeat dose effects of SLE66 were investigated following oral and dermal administration of different doses.

2. Materials and methods

2.1. Test material

The test article (SLE66) used in the safety studies was white powder or lumps. The purity of SLE66 (>98%) was confirmed with InfraRed Liquid Film method and Gas Chromatography method. The test article used in the studies complied with the standard Product Specifications (melting point-69–77 °C; hydroxyl value-173–203; saponification value-80–110) including heavy metal analysis. The stability and homogeneity of test formulations was confirmed as per specific study protocol.

2.2. Acute studies

Oral and dermal LD_{50}: These studies were conducted according to Organization for Economic Co-operation and Development (OECD) guidelines (401 and 402; limit test). SLE66 in corn oil (also in water) was administered to Sprague Dawley rats at a dose of 5 g/kg body weight. The animals were observed daily for 15 days and were euthanized at the end of study for macroscopic examinations. In another set of similar experiments, ICR mice were administered a single oral dose of 5 g SLE66/kg and animals were observed. In dermal studies SLE66 61.5% (in distilled water) was applied to the dorso-lumbar region (hair removed) of Sprague Dawley rats, at a dose level of 2 g/kg and covered with gauze for 24 h. The animals were observed for mortality, clinical signs, and dermal responses to the treatment daily and on day 15 necropsy was performed.

2.3. Skin irritation studies

Rabbit studies: In a 4 h semi-occlusive skin irritation test, performed according to OECD guideline (404), SLE66 (0.5 g) was applied to skin of three New Zealand strain rabbits and the site was covered. Local dermal irritation was assessed for four days using a numerical system (0 = no reaction – 4 = severe reaction) for erythema and edema. In two separate, 24 h closed patch test following OECD guidelines, SLE66 (vehicle, 5%, 50%) in petrolatum and undiluted (neat, 0.5 g) was applied to normal and damaged back skin of Japanese white rabbits (6 females). The application sites were covered for 24 h and were observed at 3, 24 and 48 h for erythema and edema. In a cumulative skin irritation testing, SLE66 in petrolatum at a concentration of 0 (vehicle), 5 and 50% each was repeatedly applied (20 times over 5 weeks) to normal shaved clipped skin of Japanese white female rabbits (6 female) and the application sites were observed for irritation (erythema, edema) before each application.

Human studies: In a closed patch test in healthy volunteers (15/sex), a 0.02 ml of SLE66 (0, 5, 50%) in petrolatum was applied to the inside of upper arm of each subject for 48 hours using a fin chamber (closed patch). At 3, 24 and 48 h after patch removal the application sites were evaluated for erythema and edema. In another cumulative skin irritation testing in human subjects (15/sex), SLE66 in petrolatum (0, 5 and 50% each) was applied 20 times over 5 weeks, once daily, and the application sites were observed for irritation (erythema, edema) before each application.

2.4. Phototoxicity

SLE66 (0, 5 and 50%) in petrolatum, and 0.03% 8-metoxypsolaren (8-MOP) in ethanol (positive control) was applied to the back side of female Hartley guinea pig (20/group) and thirty minutes after the application UV light was irradiated only to the animals of UV exposure group. At 24, 48 and 72 h after the application, the sites were evaluated for erythema and edema.

2.5. Skin sensitization studies

In a guinea pig maximization test (GPMT), based on the results of initial experiments, the intradermal injection and topical application concentrations of 1% and 60% SLE66 in Alembicol D (triglyceride) were selected, respectively. For challenge application 30% and 60% of SLE66 in Alembicol D was used. The induction and challenge phases were conducted according to Magnuson and Kligman (1970) and following the EEC (Directive 84/449/EEC) guidelines. The GMPT experiment was repeated with some minor modifications. In place of Alembicol D, liquid paraffin was used as a vehicle. Seven days after intradermal injection, 30 min after application of 10% sodium lauryl sulfate in petrolatum, 0.1 ml of 50, 30 or 10% SLE66 in petrolatum was applied to the shaved application sites for 24 h by closed patch. At three weeks after the initiation of sensitization, 5% and 50% SLE66 in petrolatum (0.1 ml) were applied to the left abdomen for 24 h by closed patch and at 3, 24 and 48 h after patch removal sites were observed for reaction.

Photosensitization effects of SLE66 were evaluated according to the adjuvant-strip-AA method. Female Hartley white guinea pigs (10/group) were injected intradermally with 0.1 ml Freund's complete adjuvant to each of the four corners of the anterodorsal skin. Sites were tape-stripped and 0.1 ml of 50% SLE66 in petrolatum was applied and exposed to UV light. This procedure was repeated daily for five days and three weeks later 0, 5 and 50% SLE66 in petrolatum was applied and only one side was exposed to UV light. At 24 and 48 h after the challenge, the application sites were evaluated for erythema and edema.

2.6. Eye irritation study

In accordance with OECD guidelines (405), approximately 70 mg (0.1 ml by volume) of SLE66 was placed in the lower everted lid of one eye (other served as control) of each of New Zealand White strain rabbits ($n = 3$) and the eyes were examined at different time points for ocular irritation. In another ocular mucous membrane testing, undiluted SLE66 (100 mg) was instilled into eyes of group of female Japanese white rabbits and the eyes were washed 30 seconds later in one group. The cornea, iris, and conjunctiva were observed at different time points according to Draize's scoring system.

2.7. Repeat dose (28 day) studies

Animals and treatments: Both oral and dermal repeat dose studies were performed following a standard study protocol in accordance with OECD guidelines (407 and 410, respectively). For both the studies, Sprague-Dawley CD strain rats were used. For the oral study, 40 animals (20/sex) approximately 5 to 6 weeks old were housed in groups of five by sex in polypropylene grid-floor cages suspended over trays lined with absorbent paper. For the dermal study, 9 week old rats were individually housed in stainless steel wire-mesh cages throughout the study. The room temperature and relative humidity for both studies ranged between 18–27 °C and 41–93%, respectively. A 12 h light/dark cycle was maintained. The animals were allowed free access to food and water. For oral study, SLE66 formulation was prepared in peanut oil, while for the dermal study, it was ground to a powder and mixed with acetone to achieve a pasty consistency. For oral study, four groups, each of 10 rats (5/sex), were administered SLE66 via gavage at a dose of 0 (control), 150 (low), 400 (mid) and 1000 (high) mg/kg once daily for 28 consecutive days. For dermal study four groups (5/sex/group) were used and SLE66 was applied daily for 28 days at dose levels of 0 (vehicle; control), 100, 300, 1000 mg/kg/day to the dorsal clipped area of each rat. In the control and high dose group additional rats (five/sex) were included for 2 weeks post treatment recovery observations.

Clinical Observations, Survival, Body Weights and Feed Consumption: All animals were observed twice daily for mortality and moribundity. In addition, once daily cage side observations were performed to detect indications of toxic effects. Body weights and feed consumption were recorded weekly.

Clinical Pathology: Blood samples for clinical pathology evaluation (hematology and serum chemistry) were collected at necropsy. In oral study, blood for hematology and serum chemistry evaluations was collected from the orbital sinus puncture under halothane-anesthesia. Standard hematological parameters and plasma chemistry parameters (listed in Table 1) were determined.

Macroscopic and Microscopic Examination: A complete necropsy was conducted on all animals. At the time of necropsy, for oral study, over 35 tissues were collected and adrenals, brain, gonads, heart, kidney, liver, pituitary and spleen were weighed before fixation for histological observations. For dermal study, adrenals, liver, testis, kidneys and ovaries were weighed. In the dermal study, in addition to the above mentioned organs, treated and untreated skin was collected. For both oral and dermal study, the preserved tissues from control and high dose group were