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Ceramides in Skin Stress: Ultraviolet, Tape Stripping and Crowding

Stress induces changes in the ceramide content of the skin, and may lead to compromised barrier function or skin diseases. Skin has ways of responding to these stresses, as skin stressed by tape stripping, organic solvents, overcrowding and ultraviolet (UV) irradiation is reviewed.

These results indicate that physical stress decreases the CER content early, after which CER content increases to restore the damaged barrier.

The Role of Ceramides in the Skin

Nine subclasses of ceramide (CER) have been described in human stratum corneum (SC); these differ from each other by the sphingoid base groups (sphingosine, phytosphingosine, 6-hydroxysphingosine) and hydrocarbon chain length.[1] Ceramides are the main polar lipids of the SC intercellular lipids, playing an important role in skin barrier function and accounting for half of the SC lipids by weight.[2]

In addition to preponderance of CER, blockades of either CER synthesis or CER generation results in abnormal skin barrier function.[3,4] CER content and changes of composition are involved in skin diseases such as atopic dermatitis,[5-7] psoriasis,[8] contact dermatitis[9,10] and skin irritation.[11] Moreover CER must be present in mixtures of physiologic lipids for skin barrier recovery to occur at normal or accelerated rates after acute abrogation.[12] From these results, it can be seen that CERs play a crucial role in skin barrier function.

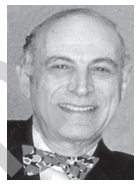
Stress adversely affects skin barrier function by CER change. Stress increases CER either by (1) acceleration of sphingomyelin (SM) hydrolysis resulting from activation of sphingomyelinase (SMase) or (2) increased de novo CER synthesis, through activation of either CER synthase or serine palmitoyltransferase (SPT). Stress-induced generation of CERs restores skin barrier function and induces apoptosis in cultured human keratinocytes (CHKs) to eliminate stress-induced cell damage.

Ceramides and Stripping

When the barrier function is damaged by organic solvents or tape stripping, a series of homeostatic systems accelerate and barrier function recovers. Denda et al.[13] investigated the relation between tape stripping and CER content, reporting that the total amount of CER in tape stripping-induced scaly skin did not differ significantly from that in control skin.

Schmuth et al.[14] assessed changes in SMase activity in epidermal homogenates obtained before and after acute disruption of permeability barrier with acetone or tape stripping. Enzyme activity increased significantly (1.4-fold) 2 hours (h) following barrier abrogation, whereas no differences in activity were noted at an earlier (0.25 h) and later (8 h) times.

Stachowitz et al.[15] investigated the effect of tape stripping on mRNA and protein involved in CER synthesis. SPT and CER synthase are the key enzymes for de novo CER synthesis. Stachowitz et al. measured mRNA and protein expression of SPT2 after tape stripping: expression of SPT2 differs depending on the time after tape stripping. The values of SPT2 mRNA expression 30 min and 2 h after tape stripping were lower compared to control levels in all cases. But 4 h after barrier disruption, expression had increased to its maximum level, and 8 h after barrier disruption no further increase in SPT2 mRNA was detected. In a rodent model, an increase in SPT mRNA showed a similar time curve of mRNA expression after barrier disruption. SPT2 mRNA expression was increased at 4 h after tape stripping; SPT activity increased 5–7 h after barrier disruption, returning to normal after 12 h.[28] These results indicate that physical stress decreases the CER content early,



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SOME ABBREVIATIONS USED IN THIS ARTICLE

CER	ceramide
CHKs	cultured human keratinocytes
mRNA	messenger RNA that encodes and carries information from DNA to sites of protein synthesis
RNA	ribonucleic acid
SM	sphingomyelin
SMase	sphingomyelinase
SPT	serine palmitoyltransferase
SPT2	serine-palmitoyl transferase 2

after which CER content increases to restore the damaged barrier.

Ceramides and Overcrowding

Stress from overcrowding (too many people living too close together) markedly influenced physiological conditions, water barrier function and barrier recovery rate.[16]

Ishida et al.[17] investigated the effect of overcrowding on change of skin surface lipids and recovery of skin barrier function after tape stripping in mice. Skin surface lipids were decreased markedly in the overcrowded mice, with significance compared with the controls.

To estimate the relationship of stress and CER composition, Aioi et al.[16] measured change of CER composition in overcrowding stress and normal groups. The amount of CERs in the stress group decreased significantly to 4.9±0.2 μg/cm^2 compared to 6.0±0.3 μg/cm^2 in the control group ($P < 0.001$). Thus, overcrowding stress induced impairment of barrier function and water retention properties with a decrease in CER. This decrease of CER contents in the SC perturbed the skin's barrier function.

Ceramides and Ultraviolet Irradiation

Investigators reported the correlation between the production of intracellular ceramides and ultraviolet (UV) irradiation in various cell types. The effect of UV irradiation on the CER content was different depending on dose and UV wavelength.[18-22]

Types of cellular damage induced by UVA exposure are related to the generation of reactive oxygen such as singlet oxygen and oxygen radicals. Singlet oxygen might mediate the nonenzymatic hydrolysis of SM leading to CER generation.[18] Grether-Beck et al.[18] and Maziere et al.[19] reported the relationship between singlet oxygen and CER generation upon UVA irradiation in keratinocytes. The production of CER was proportional to the intracellular content of reactive oxygen species; the increase of CER was prevented by the antioxidant vitamin E and enhanced by the prooxidant buthionine-sulfoximine.

The effect of UV irradiation on the CER content was different depending on dose and UV wavelength.

Grether-Beck et al.[18] reported that exposure to UVA radiation at a dose of 30 J/cm^2 resulted in a 3-fold increase in the level of CER in human keratinocytes, detectable as early as 15 min post-irradiation with a maximum after 30 min.

Maziere et al.[19] also reported the production of CER reached a maximum 2 h after UVA irradiation and an increase of approximately twofold was found for

9 J/cm^2. Beyond 9 J/cm^2, a decrease in CER was noted and became significant at 18 J/cm^2 UVA dose. This result was inconsistent with Grether-Beck et al.[18] Table 1 summarizes the effect of UVA radiation on the CER formation. The effect of UVA on the kinetics of CER generation differs depending on the experiment conditions.

Grether-Beck et al.[18] and Maziere et al.[19] demonstrated that singlet oxygen triggers a nonenzymatic mechanism of CER formation. The question of the pathways involved in CER generation in cells upon UVA exposure remains unresolved, if SMase or CER synthase is not activated. However, CER might arise from pathways other than SM degradation. Recently, investigators reported that SM deacylase and glucosylceramide deacylase were important modulators of CER generation.[7,23,24] Thus in addition to nonenzymatic pathway, the possibility that other enzymes are involved in the CER generation with UVA irradiation cannot be excluded.

Content of intracellular CER was increased after UVB irradiation in CHKs.[20-22] However, Shimizu et al.[25] reported that no significant production of CER was observed in UVB-irradiated HaCaT cells within 24 h after exposure to UVB (0.25 J/cm^2). In addition to HaCaT cells, Meguro et al.[26] reported that there was no change in the total amount of major SC lipids, cholesterol, free fatty acids and CER in UVB-irradiated hairless rats skin (40 mJ/cm^2/day for 15 days).

To clarify the CER production in human keratinocytes, Uchida et al.[20] assayed changes in the activities of two key enzymes of CER synthesis, CER synthase and SPT, in high dose UVB-irradiated CHKs. CER synthase activity is increased in CHKs treated with UVB (60 mJ/cm^2), resulting in elevated cellular CER levels leading to apoptosis. CER synthase activity increased at 4 h (1.2-fold) and 8 h (1.7-fold) following UVB exposure. In contrast, SPT activity did not change following irradiation. In contrast to higher UVB doses, the lower dose (23 mJ/cm^2) induced increased SPT activity in CHKs at later time points. Table 2 shows the effect of low dose and high dose UV irradiation on the CHK physiology. As shown in Table 2, the content of SM in CHKs did not change significantly after UVB exposure.

These data indicate that CER increase is due to de novo synthesis and not due to increased SM degradation. However, Magnoni et al.[22] reported that exposure to UVB (75 mJ/cm^2) irradiation resulted in rapid generation of CER in CHKs and SMase activity was involved in production of intracellular CER. Therefore,

Table 1. Effect of UVA irradiation on the generation of ceramides in human keratinocytes

	UVA dose 9 J/cm^2 (Reference 19)	UVA dose 30 J/cm^2 (Reference 18)
Ceramides increase (fold)	2.0	3.5
Time for maximum level	2 h	30 min
SMase activity	Decrease[a]	No change
Ceramide synthase	-	No change
SM content	No significant	Increase[b]
Singlet oxygen	Increase	Increase
Type of cells	NCTC 2544 human keratinocytes	Long-term cultured, normal human keratinocytes
Cultivation condition	Serum-containing media	Serum-free media

[a] Beyond 9 J/cm^2, SMase activity is decreased at higher dose of UVA (18 J/cm^2).

[b] SM increases up to 2.9-fold in keratinocytes upon exposure to $NDPO_2$, a generator of singlet oxygen. $NDPO_2$ is the disodium salt 3,3'-(1,4-naphthalidene) dipropionate.

Table 2. Comparing the effect of UVB and UVC irradiation on ceramide generation and enzyme activity in skin

	UVB dose 23 mJ/cm^2 (Ref 21)	UVB dose 60 mJ/cm^2 (Ref 20)	UVB dose 75 mJ/cm^2 (Ref 22)	UVB dose 250 mJ/cm^2 (Ref 25)	UVC dose 60 mJ/cm^2 (Ref 27)
Ceramide increase	1.25-1.35	1.3-1.9	2.0-5.0	No change	3.0-3.5
Time for maximum level	48 h	48 h	24 h	-	2-5 min
SPT activity	1.3-1.5	-	-	-	-
Ceramide synthase	-	1.2-1.7	-	-	-
SMase	-	-	2.5-3.5	No change	-
Apoptosis	ND	40%	41%	60%	10%
Cell types	CHKs	CHKs	CHKs	HaCaT	Melanoma

keratinocyte CER synthesis responds differently to doses of UVB irradiation. Taken together, CER might be a second messenger in the UVB-induced apoptosis of keratinocytes.

However, Deng et al.[27] reported that ceramide did not act as a general second messenger for melanoma cells induced by UVC (wavelength less than 280 nm). Exposure to UVC (60 mJ/cm^2) irradiation resulted in rapid generation of CER in melanoma cells and CER content increased significantly with time after UVC irradiation (3.5-3.0 fold from 2 to 60 min, respectively). Unexpectedly they also observed that UVC-induced melanoma apoptosis was low level (approximately 5–10%) in spite of high production of CER. The magnitude of relative ceramide increase after UVC (60 mJ/cm^2) irradiation was greater in melanoma cells than in keratinocyte cells after UVB (60 mJ/cm^2) irradiation at short irradiation time.

Regarding the effects of UVC irradiation on apoptosis and CER generation, one can say that those effects differ depending on cell types and doses of UV and that those effects remain unsolved.

Conclusion

CERs play a crucial role in formation of SC structure and in regulating skin barrier function. Stress-induced change of CER content may impair these properties and result in skin diseases. Stress-induced CER generation involves various enzymes and nonenzymatic pathways. Depending on the stress types, CER generation has different mechanisms.

Tape stripping increases SMase and SPT activities to restore barrier function. Singlet oxygen is involved in generation of CER upon UVA irradiation, while enzymatic pathway is involved in production of CER upon irradiation from UVB and UVC.

The effect of UVB irradiation on the CER generation varies with the dose of UV irradiation. The effects of UV irradiation on the apoptosis and CER generation differ depending on cell types, doses and wavelength of UV. However, stress-induced CER generation might play a crucial role in restoring barrier function and preventing skin disease.

Taken together, the commercial availability of cost-effective ceramides might lead to enhanced cutaneous biologic insights and possibly prophylactic and therapeutic interventions.

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