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Expression of Antimicrobial Peptides and Proteins in Epidermis Equivalents Exposed to Salt Water and Narrowband Ultraviolet B Radiation

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Dear Editor:

The influence of salt water and ultraviolet (UV) radiation on the expression of antimicrobial peptides and proteins (AMPs) has not yet been established. AMPs play an important role in the pathogenesis of inflammatory skin conditions such as psoriasis and atopic dermatitis. Patients with these conditions have been shown to benefit from combined therapy using salt-water baths and UVB exposure. Nevertheless, the mechanisms of action of this combination therapy are still unclear, and standardized treatment regimens using specific salt concentrations and solutions with specific salt composition are lacking[1-3]. In the present pilot study, we investigated the effect of salt water soaks and narrowband UVB (NB-UVB) radiation on the expression of AMPs in human epidermis equivalents.

EpiDerm-200 tissue models (one lot of medium: EPI-100-NMM-AFAB) were purchased from MatTek Corporation (Ashland, MA, USA). The EpiDerm-200 model has recently been described in detail[3]. Epidermis equivalents were cultured under sterile conditions at the air-liquid interface using antibiotic-free, anti-fungal-free, and hydrocortisone-free EPI-100-NMM Medium from the same supplier. Following a 6-day period in culture medium, including a 3-day treatment period, the epidermis equivalents (n = 9 per group) were detached from the inserts with a 15-min incubation with 1 U/ml dispase (Worthington, Lakewood, NJ, USA) at 37°C. The upper side (horny layer) of the epidermis equivalents was treated for 20 min with 3% NaCl and 30% NaCl solutions. NB-UVB irradiations were performed using a Waldmann (Villingen-Schwenningen, Germany) UV 236 B therapy system. After soaking, the epidermis equivalents were immediately exposed to radiations at 130 mJ/cm^2 (irradiance: 7 mW/cm^2). Following the aforementioned treatments, relative gene expression analyses of epidermis equivalents were performed, as previously described in detail for human β-defensin-2

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(hBD2), psoriasin (S100A7), and LL37[3]. Relative quantities of all analyzed genes were normalized to that of the homogenously expressed housekeeping gene *RPL38*. mRNA levels of the analyzed genes were determined using the median log-transformation of gene expression. Analysis of data was performed using the statistical package MedCalc (MedCalc Software, Mariakerke, Belgium). The distribution of data was confirmed using the D'Agostino-Pearson test. To analyze AMP expression, we used the Kruskal-Wallis test, including the Conover post-hoc test, and the Mann-Whitney test. Since this was an exploratory pilot study, we refrained from correction for multiple testing (n=18). A $p<0.05$ was considered significant.

As demonstrated in Table 1, AMP mRNA expression in salt water-exposed epidermis equivalents was not different from that in untreated controls ($p>0.05$). The combination of salt-water soaks and NB-UVB irradiation, however, affected AMP expression. hBD2 mRNA level was significantly ($p=0.037$) lower in the salt-water−soaked and NB-UVB-exposed epidermis equivalents than in the controls exposed to only NB-UVB. Similarly, psoriasin mRNA level was significantly ($p=0.043$) lower in the soaked and irradiated epidermis equivalents than in the irradiated controls. With regard to AMP expression, there was no significant difference between 3% NaCl- and 30% NaCl-treated samples ($p>0.05$). Compared to untreated controls, NB-UVB-irradiated epidermis equivalents showed significantly higher LL37 mRNA expression ($p=0.04$). Combined treatment with 3% or 30% NaCl soak followed by NB-UVB exposure resulted in significantly higher LL37 expression than that observed in samples that were only soaked ($p=0.04$).

In a recent study, we systematically investigated how salt water soaks affected UV transmission in a psoriatic epidermis model. When UV transmission was measured in 5-nm steps, significantly increased transmission within the UVA as well as UVB wavelength ranges (305～360 nm) was observed after 30% NaCl soaks[3]. Whether these effects are due to altered hydration, changes in the scattering properties of the skin, and/or alterations in the chemical environment following exposure to different concentrations of salt water solutions remains unclear. Recently, Gläser et al.[2] reported a dose-dependent increase in hBD2 and psoriasin expression in keratinocytes exposed to broadband UVB radiation. Similar results were also reported by Kim et al[4,5]. In the aforementioned studies, however, higher UV doses than those used in the present study and/or broadband UVB devices have been applied. On the other hand, Felton et al.[6] observed increased post-NB-UVB expression for psoriasin and RNase7, but

Table 1. Median (range) antimicrobial peptide and protein mRNA* expression in epidermis equivalents following soaking in salt-water of different concentrations and irradiation with narrowband ultraviolet B (NB-UVB) over a 3-day period

Group	A) Untreated controls, B) only NB-UVB	C) 3% NaCl, D) plus NB-UVB	E) 30% NaCl, F) plus NB-UVB	Differences between groups (Kruskal-Wallis test, *p*-value)	Significant inter-group differences (Conover post-hoc test, $p<0.05$)
hBD2	0.07 (0.05～0.08)	0.03 (0.03～0.06)	0.04 (0.03～0.05)	0.348	
	0.1 (0.09～0.13)	0.01 (0.01～0.03)	0.05 (0.03～0.09)	*0.037*	*B vs. D and F*
p-value	A vs. B: 0.1	C vs. D: 0.12	E vs. F: 0.51		
Psoriasin	0.3 (0.08～0.31)	0.09 (0.07～0.13)	0.09 (0.08～0.1)	0.501	
	0.27 (0.25～0.34)	0.07 (0.05～0.07)	0.1 (0.05～0.1)	*0.043*	*B vs. D and F*
p-value	A vs. B: 1	C vs. D: 0.12	E vs. F: 0.83		
LL37	0.02 (0.01～0.025)	0.02 (0.01～0.04)	0.08 (0.05～0.11)	0.07	
p-value	0.49 (0.21～0.59)	0.18 (0.14～0.23)	0.28 (0.26～1.68)	0.08	
	A vs. B: 0.04	*C vs. D: 0.04*	*E vs. F: 0.04*		

*mRNA levels of the analyzed genes were determined using the median log-transformation of the gene expression. Relative mRNA expression levels were calculated using the comparative Δ-ΔC_t method. Gene expression was normalized to that of the homogenously expressed housekeeping gene *RPL38*. Statistically significant differences have been highlighted in italics.

not for hBD2. In the present study, low NB-UVB doses, which are comparable to starting doses for phototherapy for patients with fair skin-types, have been used. Nevertheless, we observed a significant decrease in hBD2 and psoriasin expression in NB-UVB-exposed epidermis equivalents, which were pretreated with salt-water soaks. The antimicrobial activity of hBD2 was shown to be sensitive to the concentration of NaCl in an assay. For example, an eightfold reduction in the growth of *Escherichia coli* was observed when salt concentration was increased from a 20 mM to 150 mM[7]. We speculate that saltwater soaks in combination with UV radiation not only reduce of hBD2 activity, but also its mRNA expression. In the present study, salt-water soaks alone led only to a tentative and insignificant decrease in hBD2 expression, likely due to the small sample size. Nevertheless, the reduction in hBD2 and psoriasin expression due to salt-water soaking led to decreased AMP mRNA expression in response to NB-UVB. Hence, in the presented experimental setting, NB-UVB appears to regulate the observed changes in AMP expression. In accordance with previous reports, we observed significantly higher LL37 expression in irradiated epidermis equivalents than in non-irradiated controls. This finding suggests that LL37 could be induced by inflammatory stimuli such as UV. UVB exposure was reported to trigger vitamin D synthesis that stimulates production of LL37, which was also supported by the increase in vitamin D receptor expression[5,6]. The limitations of the present experimental pilot study include the absence of AMP protein expression data, which was unfortunately impossible to obtain because of the limited material and funding.

In conclusion, we have shown in this pilot study that the combination of NB-UVB with salt-water soaks has differential effects on epidermal AMP mRNA expression in human epidermis equivalents. Future studies on the combined treatment with salt-water soaks and phototherapy using psoriatic epidermis equivalents are warranted in order to establish the mode of action of balneophototherapy.

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