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Short communication

Laughter elevates the levels of breast-milk melatonin

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Abstract

Objective: Patients with atopic eczema (AE) often complain of sleep disturbance. Melatonin is involved in sleep, and the levels of blood melatonin in patients with AE are decreased in comparison to healthy subjects. However, the levels of breast-milk melatonin had only been reported in healthy subjects. Laughter increased natural killer cell activity in blood and free radical-scavenging capacity in saliva in healthy subjects. Thus, the effect of laughter on the levels of breast-milk melatonin was studied in mothers with AE. Moreover, the effect of feeding with breast milk after laughter on allergic responses in infants was studied. **Methods:** Forty-eight infants aged 5–6 months were enrolled. All of the infants had AE and were allergic to latex and house dust mite (HDM). Half (n=24) of the mothers of these infants were patients with AE, while another 24 mothers were healthy subjects. The mothers viewed either an 87-min humorous DVD (*Modern Times*, featuring Charlie Chaplin) or an 87-min nonhumorous weather information DVD at 2000 h. After viewing, breast milk was collected sequentially from 2200, 2400, 0200, 0400 to 0600 h. The levels of breast-milk melatonin were measured. In addition, skin wheal responses to HDM and histamine were studied in infants. **Results:** Laughter caused by viewing a humorous DVD increased the levels of breast-milk melatonin in both mothers with AE and healthy mothers. In addition, allergic responses to latex and HDM of infants were reduced by feeding with breast milk after laughter of mothers with AE or of healthy mothers. **Conclusion:** Laughter increased the levels of breast-milk melatonin in both mothers with AE and healthy mothers, and feeding infants with increased levels of melatonin-containing milk reduced allergic responses in infants. Thus, laughter of mothers may be helpful in the treatment of infants with AE.
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Keywords: Laughter; Breast-milk melatonin; Atopic eczema; Skin prick test; Latex

Introduction

Laughter increases natural killer cell activity in blood and enhances free radical-scavenging capacity in saliva in healthy subjects [1,2]. On the other hand, laughter decreases the plasma levels of neuropeptides and neurotrophins and reduces allergic skin wheal responses in patients with atopic eczema (AE) [3,4]. Patients with AE often complain of sleep disturbance. Melatonin is involved in sleep and stress responses, and serum melatonin levels are decreased in AE patients [5,6]. AE patients are vulnerable to stress [7]. Moreover, melatonin activates Th1 lymphocytes by increasing IL-12 production, which in turn reduces allergic responses [8]. Thus, it would be tempting to speculate that elevation of melatonin levels could improve (1) sleep disturbance, (2) stress responses, or (3) allergic responses. We have previously reported that laughter elevates the levels of breast-milk leptin in mothers with AE [9]. Since the levels of breast-milk melatonin had only been reported in healthy mothers [10], the effect of laughter on the levels of breast-milk melatonin was studied in mothers with AE. In addition, we evaluated the modulation of allergic responses in infants, who were fed by breast milk before and after laughter of mothers.

Methods

Subjects

After obtaining informed consent, 48 infants with mild AE (SCORAD index: mean, 9; 95% CI, 7–11) (25 female

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Table 1
Effect of laughter on the levels of breast-milk melatonin

	Melatonin (pg/ml)					
	Healthy mothers			Mothers with AE		
		Viewing			Viewing	
	No viewing	Control DVD	Humorous DVD	No viewing	Control DVD	Humorous DVD
2200 h	5.2 (0.3)	5.3 (0.3)	7.1 (0.4)*	3.2 (0.2)	3.3 (0.2)	6.5 (0.3)**
2400 h	8.4 (0.4)	8.2 (0.4)	10.2 (0.6)*	5.9 (0.3)	5.7 (0.3)	9.3 (0.5)**
0200 h	15.6 (0.8)	15.9 (0.9)	20.8 (1.0)*	10.7 (0.5)	11.0 (0.6)	19.9 (0.9)**
0400 h	12.2 (0.7)	12.5 (0.8)	16.5 (0.8)*	8.5 (0.4)	8.8 (0.5)	15.4 (0.8)**
0600 h	5.5 (0.4)	5.1 (0.4)	6.3 (0.4)*	3.7 (0.2)	3.5 (0.2)	5.9 (0.3)**

Healthy mothers or mothers with AE either did not view DVD or viewed a control DVD or a humorous DVD at 2000 h, and the levels of breast-milk melatonin were sequentially measured after viewing from 2200 to 0600 h. Values are shown as mean (SE).

* Significant increase ($P<.05$) compared to viewing a control DVD or no viewing in each group.

** Significant increase ($P<.01$) compared to viewing a control DVD or no viewing in each group.

and 23 male; mean age, 5 months; range, 4–6 months) and 48 mothers of them were studied [11]. None of the mothers smoked a cigarette or drank alcohol or caffeine. Among 48 mothers, 24 mothers were healthy subjects (mean age, 26 years; range, 23–32 years), while another 24 mothers were patients with mild AE (SCORAD index: mean, 8; 95% CI; 6–10; mean age, 26 years; range, 22–33 years). Inclusion criteria were as follows: healthy mothers or their infants without any allergic or infectious diseases, and mothers and their infants with AE but not bronchial asthma or allergic rhinitis, or any infectious diseases. Exclusion criteria were as follows: subjects having sleep disorder, psychological or metabolic diseases, or taking any oral medication. All of the infants with AE were allergic to latex and house dust mite (HDM) as detected by skin prick test and serum IgE levels to these allergens. Serum IgE levels (mean±S.E.) in healthy mothers and AE patients were 68±5 and 1249±47 IU/ml, respectively. All of the AE patients including infants and mothers were treated with washing with povidone iodine and application of pinetar (Yoshida Pharmaceutical Co. Ltd., Tokyo, Japan) mixed with zinc oxide ointment, but without oral medication [4]. Participants were asked not to apply topical ointment for 6 h before the study. None of them had skin infection at the time of study.

Study design

The study was conducted by cross-over style. Randomly assigned 24 healthy mothers or 24 AE mothers first viewed an 87-min humorous DVD (*Modern Times*, featuring Charlie Chaplin) at 2000 h as reported previously [3]. After 2 weeks, they viewed an 87-min control nonhumorous DVD featuring weather information at 2000 h. Alternatively, another 24 healthy mothers or 24 AE mothers first viewed a control nonhumorous DVD (weather information) at 2000 h, and after 2 weeks they viewed a humorous DVD (*Modern Times*) at 2000 h. Laughter was assessed by the staff who watched whether subjects laughed or not. The staff confirmed that all of the subjects laughed while viewing the humorous DVD, while they did not laugh at all when viewing the control DVD. Moreover, the emotion of the subjects was assessed by questionnaire. All of the subjects were satisfied and relaxed while viewing the humorous DVD, but not when viewing the control DVD.

Table 2
Effect of laughter of mothers on allergic responses in infants

	Diameter of wheal (mm) in infants							
	Healthy mothers				Mothers with AE			
	Control DVD		Humorous DVD		Control DVD		Humorous DVD	
	1700 h	0300 h	1700 h	0300 h	1700 h	0300 h	1700 h	0300 h
Latex	4.1 (0.3)	4.3 (0.3)	4.2 (0.3)	3.7 (0.3)*	4.6 (0.4)	4.8 (0.4)	4.6 (0.4)	3.9 (0.3)**
HDM	5.5 (0.4)	5.3 (0.4)	5.8 (0.5)	5.1 (0.4)*	6.4 (0.6)	6.5 (0.6)	6.4 (0.6)	5.2 (0.4)**
Histamine	5.2 (0.4)	5.0 (0.4)	5.3 (0.4)	5.4 (0.5)	5.7 (0.5)	5.6 (0.5)	5.8 (0.5)	5.9 (0.5)
Control	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

Healthy mothers or mothers with AE viewed a control DVD or a humorous DVD at 2000 h, and they fed infants with breast milk at 1800, 2200, and 0200 h. At 1700 (before viewing) and 0300 h (after viewing), skin prick test for latex, HDM, histamine, or control solution was performed on infants. Values are shown as mean (S.E.).

* Significant reduction ($P<.05$) compared to 0300 h after viewing a control DVD or to 1700 h after viewing a humorous DVD in each group.

** Significant reduction ($P<.01$) compared to 0300 h after viewing a control DVD or to 1700 h after viewing a humorous DVD in each group.

All of the mothers and infants slept well in identical air-conditioned rooms when the lights went out, and they woke and fed infants in semidarkness; a flashlight was used to illuminate only the sampling area. After viewing, breast milk was collected sequentially from 2200, 2400, 0200, 0400 to 0600 h. After another 2 weeks of study with viewing, none of the mothers viewed any DVD anymore ("no viewing"), and breast milk was also obtained sequentially from 2200, 2400, 0200, 0400 to 0600 h. Milk was centrifuged at 4°C, and fat was discarded, and the levels of breast-milk melatonin were measured by ELISA (Immuno Biological Laboratories, Hamburg, Germany) [10].

On the other hand, infants were set to feed with breast milk at 1800, 2200, and 0200 h after milk samples were taken, and their allergic responses to latex (C-serum), HDM (Torii Pharm. Co., Ltd., Tokyo, Japan), histamine, and control solution were determined by skin prick test at 1700 and 0300 h, and wheal responses were measured [4]. This study was approved by the Ethical Committee of Moriguchi-Keijinkai Hospital. Statistical analysis was performed with Wilcoxon signed-rank test.

Results and discussion

We first confirmed by questionnaire whether sampling of breast milk every 2 h caused stress to mothers or their babies. All of the mothers answered that sampling caused no stress to them or to their infants. As shown in Table 1, the levels of breast-milk melatonin in healthy mothers were higher than those in mothers with AE during 2200 to 0600 h. Viewing a control DVD had no effect on the levels of breast-milk melatonin in healthy mothers or in mothers with AE. In contrast, viewing a humorous DVD elevated the levels of breast-milk melatonin in healthy mothers and in mothers with AE from 2200 to 0600 h, although elevation of the levels of breast-milk melatonin was more marked in mothers with AE.

As shown in Table 2, skin wheal responses to latex and HDM of infants were significantly reduced after feeding by healthy mothers or mothers with AE after viewing a humorous DVD, but not after viewing a control DVD. Again, reduction of wheal responses was more marked in infants fed by mothers with AE. This reduction was allergen specific, since wheal responses to histamine were not reduced.

The mechanisms of the elevation of melatonin levels and reduction of allergen-specific wheal responses by laughter remain to be elucidated. AE patients suffer from chronic stress, and melatonin levels were decreased in AE patients, while laughter counteracted stress-induced responses [4,5]. Laughter also elevated milk leptin levels which were reduced in AE patients [9]. On the other hand, melatonin was involved in stress responses [12]. Melatonin also reduced stress-induced mast cell degranulation in dermis in rats [13]. It is tempting to speculate that relaxation by laugher may elevate melatonin levels, which in turn may reduce allergic responses by inhibiting mast cell responses. In fact, melatonin interfered with differentiation and/or proliferation of mast cells induced by estradiol in frogs [14]. Thus, it is possible that melatonin may reduce allergen-induced mast cell degranulation in human. The possibility that factors other than melatonin in breast milk have some role in the reduced allergic responses should also be considered. Among those factors, transforming growth factor-beta and beta-defensin-2 are possible candidates, since both of them inhibit mast cell responses, and breast milk contains them [15–17]. In fact, our preliminary study revealed that laughter increased transforming growth factor-beta levels in breast milk (manuscript in preparation). A study is currently in progress to elucidate the exact mechanisms.

Collectively, these results suggest that laughter may elevate the levels of breast-milk melatonin, and feeding with breast milk containing higher levels of melatonin may reduce allergic responses in infants with AE. Laughter may be helpful in the study of melatonin production and treatment of AE.

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