

Modulation of fecal polyamines by viewing humorous films in patients with atopic dermatitis

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Background/objective Alteration of intestinal flora was involved in the pathogenesis of atopic dermatitis. Patients with atopic dermatitis were less colonized with *Lactobacilli* or *Bifidobacterium*, whereas they were more colonized with *Staphylococcus aureus* or *Enterobacteria*. Consequently, fecal levels of bacterial metabolite (polyamines) were reduced. In contrast, stress also induced intestinal mucosal dysfunction against bacteria and impaired intestinal barrier function. We studied the effect of relaxation by viewing humorous films on fecal flora and fecal levels of polyamines.

Methods Twenty-four healthy individuals and 24 patients with atopic dermatitis either viewed seven control nonhumorous films or seven humorous films sequentially for 7 days. Before and after viewing, feces were obtained, and fecal flora and fecal levels of polyamines were assessed.

Results Neither viewing humorous films nor viewing control nonhumorous films had any effect on healthy individuals. In contrast, viewing humorous films

(i) increased colonization with lactobacilli and bifidobacterium, (ii) decreased colonization with *S. aureus* and *Enterobacteria*, and (iii) increased fecal levels of polyamines; whereas viewing control nonhumorous films failed to do so in patients with atopic dermatitis.

Conclusion Viewing humorous films may modulate fecal levels of polyamines by restoring intestinal flora in atopic dermatitis. *Eur J Gastroenterol Hepatol* 00:000–000 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Atopic dermatitis (AD) is a complex disease that impacts on the quality of life of patients and families to a significant degree. It was reported that alteration of intestinal flora was involved in the pathogenesis of AD. AD patients were less colonized with *Lactobacilli* or *Bifidobacteria*, whereas they were more colonized with *Staphylococcus aureus* or *Enterobacteria* [1–3]. Consequently, fecal levels of bacterial metabolite, such as polyamines (putrescine, spermidine, cadaverine) were reduced [4,5]. In contrast, administration of probiotics yogurt containing *Bifidobacterium animalis* subsp. *lactis* LKM512 increased fecal levels of polyamines [6]. AD patients were vulnerable to stress, and stress-enhanced allergic responses, whereas relaxation by viewing humorous films reduced allergic responses in AD patients [7–9]. Stress also induced intestinal mucosal dysfunction against bacteria and impaired intestinal barrier function [10,11]. We studied the effect of relaxation by viewing humorous films on fecal flora and fecal levels of polyamines in AD patients.

Materials and methods

Patients

Twenty-four control healthy individuals (12 female and 12 male, mean age 28 years, range 25–34) and 24 mild AD

patients (Scoring Atopic Dermatitis index: mean 10, range 6–13; 12 female and 12 male, mean age 28 years, range 25–34) were studied after obtaining informed consent [12]. All of the AD patients were treated with application of zinc oxide ointment, but without oral medication [13]. All of the healthy individuals and AD patients had normal bowel habit, and they were asked to avoid fermented milk, lactic acid bacterial drinks, and fermented soybean during the study. None of them took any drugs including antibiotics, or had any gastrointestinal diseases, or any psychological diseases. This study was approved by the Ethics Committee of Moriguchi-Keijinkai Hospital.

Study design

Randomly assigned 12 healthy individuals (six female and six male) or 12 AD patients (six female and six male) firstly viewed seven humorous films ‘Modern Times’ (United Artists), ‘The Best Bits of Mr. Bean’ (Tiger Television Production), ‘There is something about Mary’ (20th Century Fox), ‘Mr. Bean’s Holiday’ (Universal Pictures), ‘Tootsie’ (Columbia Pictures), ‘Animal House’ (Universal Pictures), and ‘Circus’ (United Artists) sequentially for 7 days. After 2 weeks, they viewed seven control nonhumorous films featuring different weather information sequentially for 7 days. Alternatively, other

12 healthy individuals (six female and six male) or 12 AD patients (six female and six male) firstly viewed the identical seven control nonhumorous films for 7 days, and after 2 weeks they viewed the identical seven humorous films for 7 days. Before viewing (day 1) or after viewing seven films (day 7), their feces were sampled. In a preliminary study, we studied about washout design. Ten AD patients viewed seven humorous films sequentially for 7 days, and feces were sampled after 1 week, 2 weeks, or 3 weeks. Colonization with *Lactobacilli* and *Bifidobacteria* was significantly increased, whereas colonization with *S. aureus* and *Enterobacteria* was significantly decreased in all feces obtained after 1 week of viewing seven humorous films as compared with those feces obtained before viewing. In contrast, colonization with *Lactobacilli*, *Bifidobacteria*, *S. aureus* or *Enterobacteria* was not altered in any feces obtained after 2 weeks or 3 weeks of viewing seven humorous films. These results indicated that a 2-week washout period was sufficient. Moreover, the mood of participants was assessed by completion of the Positive and Negative Scale (PANAS) questionnaire at baseline (before viewing; day 1) and after viewing seven films (day 7) [14].

Fecal flora analysis

Whole fecal samples were stored anaerobically (Anaero Pack Kenki; Mitubishi Gas Chemical Company Inc, Tokyo, Japan) at 4°C immediately after defecation and provided for fecal flora analysis within 24 h after defecation. Three nonselective media and nine selective media were used, and bacterial species were identified by colonization, Gram staining, morphology, lecithinase reaction, aerobic growth, and sporulation [15]. There was no modification in bacterial amounts during the period existing between the defecation and the measurements.

Measurement of fecal levels of polyamines

Frozen feces were diluted 10-fold with PBS (pH 7.2) and extracted twice by intense mixing for 1 min, and left to stand for 15 min at 25°C. After extraction, the precipitate was removed by centrifugation (10 000g for 20 min at 4°C), and the supernatant was filtered and used as fecal extract. Levels of polyamines (putrescine, spermidine, spermine, and cadaverine) were measured by high-performance liquid chromatography according to the method reported earlier [16]. In preliminary experiments, various amounts of putrescine, spermidine, spermine, and cadaverine have been added in the extracts to study whether one of these substances is absorbed on the precipitate. However, none of these substances are absorbed. In addition, there was no modification in polyamines contents during the period existing between the defecation and the measurements.

Statistical analysis

Statistical analysis was performed with Wilcoxon signed rank test.

Results

Effect of viewing humorous films on the mood

Viewing humorous films significantly ($P < 0.05$) improved the mood, whereas viewing control nonhumorous films failed to do so in healthy individuals. PANAS scale (positive affect vs. negative affect) (mean $\pm$ SEM) were as follows: 29.8 ± 1.9 vs. 15.3 ± 1.0 (baseline), 29.0 ± 2.1 vs. 15.4 ± 1.1 (after viewing control nonhumorous films), and 38.6 ± 3.0 vs. 12.3 ± 0.8 (after viewing humorous films) in healthy individuals. Similarly, but more markedly ($P < 0.01$), viewing humorous films improved the mood, whereas viewing control nonhumorous films failed to do so in AD patients. PANAS scale (positive affect vs. negative affect) (mean $\pm$ SEM) were as follows: 21.3 ± 1.4 vs. 20.4 ± 1.3 (baseline), 21.1 ± 1.5 vs. 20.8 ± 1.6 (after viewing control nonhumorous films), and 36.5 ± 2.1 vs. 13.5 ± 1.2 (after viewing humorous films) in AD patients.

Effect of viewing humorous films on fecal flora

As shown in Table 1, analysis of fecal flora showed that AD patients were less colonized with *Lactobacilli* and *Bifidobacteria*, whereas they were more colonized with *S. aureus* and *Enterobacteria* as compared with healthy individuals. Neither viewing control nonhumorous films nor viewing humorous films had any effect on fecal flora in healthy individuals. However, viewing humorous films (i) significantly increased colonization with *Lactobacilli* and *Bifidobacteria*, (ii) significantly decreased colonization with *S. aureus* and *Enterobacteria*, and (iii) increased fecal levels of polyamines; whereas it failed to do so in AD patients. There was no change of stool consistency or frequency after viewing control nonhumorous films or viewing humorous films in healthy individuals or in AD patients.

Effect of viewing humorous films on fecal levels of polyamines

As shown in Table 2, basal levels of fecal polyamines (putrescine, spermidine, spermine, and cadaverine) were significantly lower in AD patients than those in healthy individuals. Neither viewing control nonhumorous films nor viewing humorous films had any effects on fecal levels of polyamines in healthy individuals. In contrast, viewing humorous films significantly increased fecal levels of all of polyamines, whereas viewing control nonhumorous films failed to do so in AD patients.

Discussion

We showed that AD patients were less colonized with *Lactobacilli* and *Bifidobacteria*, whereas they were more colonized with *S. aureus* and *Enterobacteria* as compared with healthy individuals. This is in accordance with earlier results, and this imbalance may favor for T-helper type 2 cytokines production [1–4]. In contrast, LKM512 yogurt consumption improved intestinal flora, induced T-helper type 1 cytokine production, and improved

Table 1 Effect of viewing humorous films on fecal flora

	Healthy individuals				Atopic dermatitis			
	Before		After		Before		After	
	Count	Incidence	Count	Incidence	Count	Incidence	Count	Incidence
Total count	10.9±0.5	100	10.8±0.5	100	10.7±0.4	100	10.8±0.5	100
Total anaerobes	10.9±0.5	100	10.8±0.5	100	10.5±0.4	100	10.6±0.5	100
Total aerobes	8.1±0.2	100	8.1±0.2	100	8.3±0.2	100	8.2±0.3	100
<i>Lactobacilli</i>	5.8±1.2	100	5.9±1.1	100	4.9±1.2*	100	5.6±1.3**	100
<i>Bifidobacteria</i>	9.5±1.3	100	9.5±1.3	100	8.8±1.1*	100	9.6±1.4**	100
<i>Staphylococcus aureus</i>	3.4±1.0	60.2	3.3±0.9	60.0	4.8±1.4***	89.5	3.9±1.0 [†]	64.1
<i>Enterococci</i>	7.7±1.5	100	7.6±1.6	100	8.4±1.7***	100	7.9±1.5 [†]	100
<i>Clostridia</i> (+) ^a	4.5±1.1	7.6	4.4±1.1	7.2	4.7±1.2	24.3	4.6±1.2	22.4
<i>Clostridia</i> (–) ^b	7.2±1.3	100	7.1±1.4	100	7.9±1.7	100	7.8±1.6	100
Bacteroidaceae	10.1±0.6	100	10.2±0.6	100	9.6±0.5	100	9.8±0.4	100
<i>Eubacteria</i>	9.2±0.5	100	9.1±0.4	100	9.3±0.4	100	9.0±0.5	100
Yeast	3.5±0.7	61.4	3.3±0.7	62.1	3.6±0.5	64.2	3.5±0.6	62.8

Bacterial counts expressed as mean ± SD of log10 per gram of wet feces.

^a+ indicates lecithinase positive.

^b– indicates lecithinase negative.

*Significant decrease ($P<0.01$) compared with healthy individuals.

**Significant increase ($P<0.01$) compared with before.

***Significant increase ($P<0.01$) compared with healthy individuals.

[†]Significant decrease ($P<0.01$) compared with before.

Table 2 Effect of viewing humorous films on fecal levels of polyamines

	Polyamines (μmol/l)			
	Control nonhumorous films		Humorous films	
	Before	After	Before	After
Healthy individuals				
Putrescine	286.7±21.3	267.5±19.1	292.8±23.3	302.2±28.7
Spermidine	164.7±9.8	154.7±8.4	146.8±8.2	153.1±9.1
Spermine	41.9±2.7	42.6±2.9	45.5±3.1	43.4±3.0
Cadaverine	1649±87	1753±92	1740±91	1696±89
Atopic dermatitis				
Putrescine	152.3±10.5*	141.5±10.2	156.8±13.3*	232.7±16.1**
Spermidine	78.1±5.2*	69.5±4.9	72.5±5.0*	115.6±8.8**
Spermine	24.2±1.1*	21.6±1.0	22.8±1.3*	35.9±1.6**
Cadaverine	859±61*	814±53	831±57*	1251±82**

Twenty-four healthy individuals and 24 patients with atopic dermatitis viewed control nonhumorous films and humorous films for 7 days. Before and after viewing, fecal levels of polyamines were measured. Values are means ± SE.

*Significant decrease ($P<0.01$) compared with healthy individuals.

**Significant increase ($P<0.01$) compared with before.

itching by increase in colonization with *Bifidobacterium* in AD patients [4]. In contrast, stress also induced imbalance of intestinal flora in humans [17]. Thus, we hypothesized that relaxation by viewing humorous films would improve intestinal flora. Our results showed that relaxation by viewing humorous films increased in colonization with *Lactobacilli* and *Bifidobacteria*, whereas it decreased in colonization with *S. aureus* and *Enterobacteria* in AD patients. These results indicated that relaxation by viewing humorous films improved intestinal flora in AD patients.

We also showed a reduction of fecal levels of polyamines (putrescine, spermidine, spermine, and cadaverine) in AD patients. This is in accordance with earlier reports that fecal levels of polyamines (putrescine, spermidine,

cadaverine) were reduced, except, for fecal levels of spermine, these were elevated in AD patients [2–5]. However, fecal levels of spermine varied depending on healthy individuals (<5 – 89.5 μmol/l) [16]. It is possible that the different results may be because of the variation depending on participants. Moreover, spermine levels were measured in AD patients treated with steroid ointment for a long time in an earlier study [5]. In contrast, none of our AD patients were treated without steroid ointment as we have reported earlier [18]. The use of steroid ointment may affect the levels of spermine. A more detailed study is currently in progress to elucidate the different results.

We further showed that relaxation by viewing humorous films restored fecal levels of polyamines in AD patients. Fecal levels of polyamines are dependent on the balance of colonization with polyamines-producing and polyamines-absorbing bacteria in the intestine. It is speculated that *Lactobacilli* and *Bifidobacteria* are polyamines-producing bacteria, whereas *S. aureus* and *Enterobacteria* are polyamines-absorbing bacteria [2,5,6]. However, a fecal amount of polyamines does not only arise from bacteria, but also from food. Therefore, we asked all of the participants to avoid polyamines-rich foods, such as fermented soybean during the study. Consequently, the amount and the quality of the food ingested were almost equivalent in the case of AD patients and in the case of healthy individuals. These results excluded the effect of food-derived polyamines. Moreover, the amount of feces was almost equivalent in AD patients and in healthy individuals.

It was reported that spermine and spermidine induced intestinal barrier maturation in the suckling rat [19]. Levels of polyamines in human milk were high during

some period after delivery. These results suggested that polyamines stimulated gastrointestinal tract proliferation and maturation in newborns [20–22]. Moreover, LMK512 yogurt consumption improved intestinal mucosal barrier function by increase in fecal levels of polyamines in AD patients [5]. Similarly, administration of probiotics improved the imbalance of intestinal flora, which in turn restored fecal levels of polyamines [6]. Administration of probiotics also improved clinical symptoms of AD in mouse and man [23–26]. In contrast, stress disrupted intestinal flora and promoted bacterial translocation [27,28]. Psychological stress increased in the number of *Escherichia coli* and decreased relative proportion of Lactobacilli and *E. coli* in the small intestine in rats [29]. It was also reported that stress aggravated symptoms of AD, whereas psychological intervention was useful in the treatment of AD [7,30]. However, correlation between stress, AD, polyamines and bacterial flora in feces should be investigated in detailed study.

Collectively, these reports indicated that intestinal flora was modulated by stress, and imbalance of intestinal flora aggravated AD. It is tempting to speculate that chronic stress may induce an imbalance of intestinal flora, which in turn may reduce fecal levels of polyamines in AD patients. In contrast, relaxation by viewing humorous films may restore an imbalance of intestinal flora, which in turn may increase polyamines content of the intestinal lumen in AD patients. This is not surprising. Stress enhanced plasma neurotrophin levels and allergic responses, whereas viewing humorous films counteracted stress-induced enhancement of plasma neurotrophin levels and allergic responses in AD patients [9]. Moreover, relaxation has beneficial effect on irritable bowel syndrome [31]. However, more detailed study is necessary to elucidate the mechanisms. Particularly, it is important to study intestinal barrier function that can be measured with gut permeability probes. This point is currently under investigation.

These results indicate that there may be interest for the medical practitioner to know the fecal content of polyamines, as it may be a good parameter during the treatment of AD. In fact, the medical practitioner could observe the clinical and immunological condition in addition to the skin condition by measurement of fecal content of polyamines [4,5]. In conclusion, our results may be helpful for the study of the effect of emotion on fecal flora and fecal polyamines in AD.

Acknowledgement

Conflicts of interest: none declared.

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