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Successful management of mild atopic dermatitis in adults with probiotics and emollients

Research Article

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Abstract: Atopic dermatitis is characterized by impaired skin and mucous membrane barrier function. Measures improving barrier integrity decrease the influence of environmental factors that might exacerbate inflammation. Ten adult patients with mild-to-moderate atopic dermatitis consumed for three months fermented with potent antioxidative probiotic, *L. fermentum* ME-3 (DSM 14241) goat milk 200 mg/day. A control group consisted of six patients, not supplemented by probiotic. All patients used emollients regularly. Skin iron levels, glutathione redox ratios (GSSG/GSH), diene conjugate (DC) amounts, blood glutathione status, oxidized low-density lipoprotein (oxLDL), and total antioxidativity was measured at the baseline and after three months. A significant decrease in skin iron levels, DC amounts, and glutathione redox ratio occurred in the probiotic-supplemented group compared to the control group ($P < 0.05$ for all indices). In the same group, blood levels of oxLDL decreased ($p < 0.05$), and GSH levels increased ($P < 0.001$) with concomitant improvement in the GSSG/GSH ratio. Blood antioxidativity markers also showed an improvement. The results of our study demonstrate that regular use of probiotics with antioxidative properties coupled with the use of lipid-containing emollients considerably decreases inflammation and concomitant oxidative stress in adult patients with mild-to-moderate atopic dermatitis. This effect was observed both in the skin and in the blood.

Keywords: *Atopic dermatitis • Oxidative stress • Glutathione redox ratio • OxLDL • L. fermentum ME-3*

Abbreviations

AD – atopic dermatitis,
DC – diene conjugates,
GSH – reduced glutathione,
GSSG – oxidized glutathione,
GSSG/GSH – glutathione redox ratio,
OxS – oxidative stress,
TAA – total antioxidative activity,
TAC – total antioxidant capacity.

1. Introduction

Atopic dermatitis (AD) is a common chronic relapsing inflammatory skin disorder characterized by defective skin barrier function [1]. The peak incidence of AD is in early childhood, however, 1- 3% of adults still experience relapses [2]. Allergic sensitization is not a uniform cause of relapses, and the exacerbation of symptoms can often be explained by genetically impaired skin as well as mucous membrane barrier function [1,3]. Lipid-based creams and ointments improving skin barrier function and decreasing transepidermal water loss are usually enough to control mild AD most of the time [1,4].

Probiotics are increasingly discussed as well as

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applied in the treatment and prevention of AD in small children [5-7]. In addition to being essential to normal immune development, probiotics have been shown to reduce systemic penetration of allergens [8]. Recently a significant antioxidative effect of some probiotics has been reported [9-11].

We hypothesized that regular use of emollients combined with the ingestion of antioxidative probiotics will decrease the influence of environmental irritants and allergens that exacerbate inflammation and enhance concomitant oxidative stress (OxS) in a patient with atopic dermatitis. To assess the effectiveness of these measures, several markers of OxS such as skin iron level, glutathione status, the amount of diene conjugates (DC), blood glutathione status, oxLDL level and total antioxidativity were measured before and after a three-month-period of regular use of emollients and probiotics.

2. Material and Methods

2.1. Patients

Sixteen volunteers (4 men, 12 women, with a mean age of 26±6.4 years, range 20-42) suffering from mild-to-moderate AD participated in the study. The disease severity was expressed using SCORAD index [12]. Patients were randomly distributed into two groups with no significant differences in age or SCORAD indexes at baseline. For three months, ten-patient groups consumed goat milk fermented with antioxidative probiotic, strain *L. fermentum* ME-3, 200g/day containing 3 x 10^9 CFU/ml [11]. A control group consisted of six patients who did not receive probiotic. All patients were encouraged to use bath oils and were regularly supplied with different emollient creams for everyday use (Eucerin®, Baierstorf, Hamburg; Decubal®, Dumex-Alpharma; Linola® fett N and Wolff Basis crème, Dr. August Wolff GmbH & Co, Arzneimittel, Germany; Aqualan® Orion Pharma). No systemic or local treatments were prescribed during the course of the study. Volunteers were asked to avoid any antioxidant supplements, over the counter medications, and instructed not to consume yogurts or any other fermented milk products. Normal biochemistry and hematology values, particularly albumin, fibrinogen, bilirubin, ferritin, and urea, were required at baseline. All subjects gave their written informed consent and the Tartu University Ethics Committee approved the investigation.

Blood for biochemical analyses was collected at the baseline and upon completion of the study. Skin punch biopsies of 4 mm were obtained before and after the study from patients' back. Epidermis and dermis were frozen together and stored at –80°C until used. Biopsies were kept in liquid nitrogen for five days, where they were carefully homogenized in 1.15% KCl solution with a homogenizer and centrifuged at 10,000 g for 10 min. The supernatants were kept on ice and used for antioxidant assays.

2.2. Biochemical analysis

For the assessment of skin iron levels, a special kit (Sigma Diagnostics St. Louis, USA) was used. The level of diene conjugates (DC) was measured as previously described [13]. Total glutathione and oxidized glutathione (GSSG) both in skin homogenates and blood were measured by the Griffith method [14]. To measure total antioxidant capacity (TAC) of the serum, a commercially available kit (Randox Laboratories Ltd. Ardmore, UK) was used. Total antioxidative activity (TAA) was assessed by using the lipid peroxidation test described elsewhere [10]. OxLDL level was measured by using the Mercodia Oxidized LDL Enzyme-Linked Immunosorbent Assay (ELISA) kit, manufactured by Mercodia AB, Uppsala Sweden; Cat No 10-1143-01.

2.3. Statistical analysis

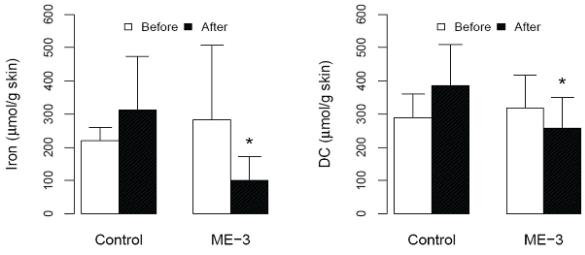
For statistical analysis, one-way ANOVA and Wilcoxon exact tests were used. Statistical analysis was implemented in the software package SAS/STAT. Free software R was used for studying correlations and their significance. Statistical significance was defined as $P < 0.05$.

3. Results

3.1. Patients characteristics

All patients suffered from AD in their early childhood. Two of them reported that AD disappeared when they went to school, but returned at puberty. One patient had concomitant asthma and two other patients reported family history of atopy. Examination revealed that all patients had dry skin. At the initial assessment, the median SCORAD index for ME-3 group and control group was 4.8±3.9 and 4.8±2.8, respectively. During the 3-month-study period, patients experienced improvement in their skin condition that resulted in SCORAD indexes of 1.9 ± 1.8 in probiotic group, and 2.3 ± 0.9 in the control group.

Figure 1. Levels of iron and diene conjugates in the skin homogenates were significantly decreased only in those AD patients who regularly used probiotic.



3.2. Decrease in OxS-related parameters in the skin

All patients participating in the study had mild-to-moderate symptoms of AD at baseline and therefore, SCORAD indexes did not alter significantly. Despite this fact, the markers of OxS significantly decreased, especially in the skin.

The decrease in OxS in the skin was evidenced by a significant decrease in the amount of free iron (Figure 1). Free ionic iron can directly catalyze the generation of free radicals; therefore, the amount of it in the cells is highly regulated; the excess of free iron is stored in iron-binding proteins such as transferrin and ferritin [15,16].

Increased free iron levels lead to increased lipid peroxidation (LP). Therefore, we determined the levels of DC, which is an intermediate product of LP. In patients who regularly used emollients and probiotic, the decrease in free iron levels was accompanied by the decrease in DC amount (P<0.05). On the contrary, in the skin homogenates from patients belonging to the control group, iron and DC levels did not change significantly during the study period (Figure 1).

Reduced glutathione (GSH) is an important intracellular antioxidant. While removing free radicals, GSH oxidizes to GSSG (oxidized glutathione). Therefore, the ratio of oxidized to reduced glutathione (GSSG/GSH) is a very reliable parameter of cellular OxS. In patients supplemented by probiotic bacteria *L. ferm*entum ME-3 skin redox ratio decreased (P<0.05) because of the decrease in the level of GSSG (P<0.001). The level of GSH did not change significantly (Table 1).

3.3. Decrease in OxS-related parameters in the blood

We also measured several OxS-related parameters at blood level. In patients' blood, another marker of LP, namely oxidatively modified LDL (oxLDL) was measured. The level of oxLDL decreased significantly in the ME-3 group compared to the control group at the end of the study (P<0.05) (Table 2). The serum levels of GSH enhanced significantly after consumption of *L. fermentum* ME-3 compared to control group (P<0.001). This resulted in improvement of the blood's glutathione redox ratio (P<0.05 compared to control group, Table 1).

Finally, plasma TAA and TAC levels were measured in probiotic groups, and the results were compared to the control group. There was an improvement in both, TAA and TAC in the group of patients, supplemented by L. fermentum ME-3 (P < 0.001, and P < 0.01 as compared to the control group, respectively; Table 2).

Further analysis showed that in ME-3 group, oxLDL level correlated negatively with blood total antioxidant capacity (TAC, r = 0.49; P = 0.05) and total antioxidant activity (TAA, r = 0.54; P = 0.05). TAC values, at the same time, were positively correlated with the level of GSH in blood (r=0.76, P<0.001). Both these correlations indicated that in probiotic supplemented group OxS had decreased during the 3-month period.

Table 1. The glutathione status and redox ratios of the skin and blood.

Skin						
	GSSG (µg/g skin)		GSH (µg/g skin)		GSSG/GSH	
	Before	After	Before	After	Before	After
ME-3 (n=10)	31±8	13±2	184.8±26.1	149.2±58.4	0.18±0.04	0.13±0.03*
		P=0.001				P=0.05
Control (n=6)	38±11	29±8	124±94	166±44	0.16±0.03	0.16±0.03
Blood						
	GSSG (µM/L)		GSH (µM/L)		GSSG/GSH	
	Before	After	Before	After	Before	After
ME-3	38±5	38±8	1075±73	1313±81*	0.032±0.003	0.025±0.006
				P=0.001		P<0.05
Control	40±12	35±11	1105.0±59.3	1153±89	0.036±0.010	0.030±0.007

**P<0.05 as compared to control group*

Table 2. Blood OxLDL levels and plasma total antioxidativity.

	OxLDL U/L		Total antioxidative activity		Total antioxidant capacity	
	Before	After	Before	After	Before	After
ME-3	90±20	72±16*	36±2	45±2*	0.90±0.04	0.098±0.05*
		P<0.05		P<0.001		P<0.01
Control	103±28	109±19	37±1	35±4	0.97±0.07	0.81±0.09

**P<0.05 as compared to control group*

4. Discussion

AD is characterized by skin barrier dysfunctions leading to increased antigen absorption [2] and enhanced reactivity to irritants. Barrier impairment holds also true of mucosal surfaces [3]. Previous studies suggest that probiotic therapy would be beneficial in the preservation of gut barrier integrity [17]. Therefore, we can assume that effects seen in our patients could be based on the improvement of barrier function both of the skin and gut.

Alongside with the creams that reveal their effects due to occlusion and protection, several mechanisms of probiotic action are suggested. These include, for example, inhibitory effect on the development of allergic type Th2 cells [18], enhancement of IgA and reduction in IgE production [19]. A significant anti-inflammatory and antioxidative effect of some probiotics has been reported [9-11]. Resent evidence also suggests that probiotic strains might preserve gut integrity in several pathological conditions and that the mechanisms underlying this effect might be systemic, i.e. more than manipulation of intestinal microflora alone [17,20].

Inflammatory cellular infiltrate, present even in healthy-looking skin of patients with AD [2] is an inexhaustible source of reactive oxygen species (ROS). Inflammation is also responsible for the release of highly redox-active iron from ferritin and transferrin [15,16], which markedly enhance free radical related reactions, including peroxidation of lipids [16,21]. Previously, higher concentrations of iron in AD patients' dermis have been reported [22]. In patients supplemented with antioxidative *L.fermentum* ME-3, iron levels in the skin decreased significantly. Additional explanation for decreased skin iron content might be improved control of absorption of free iron from the gut due to improvement of intestinal mucosal barrier status. The suppression of oxidative reactions requiring the participation of free iron was confirmed by lesser lipid peroxidation as shown by the fall of DC amounts in the skin and oxLDL levels in the blood of patients.

The normal redox state of the cells largely depends on the capacity of antioxidant defenses, and is well characterized by the ratio of oxidized to reduced glutathione (GSSG/GSH ratio). Glutathione, a tripeptide consisting of glutamate, cysteine and glycine is primarily known as potent antioxidant and detoxifier [23]. After removal of peroxides, GSH oxidizes to oxidized glutathione (GSSG), and therefore, in case of OxS increases in GSSG/GSH ratio follows. GSSG is toxic and in normal circumstances GSSG/GSH ratio in the cells is kept very low, *i.e.* < 0.01 in erythrocytes [24], and close to 1:10 in the skin [25-27]. Increased glutathione redox ratio refers to insufficient redox-control over pro-oxidative mechanisms. In this study, GSH levels in the skin were practically stable, and the improvement of GSSG/GSH ratio resulted from the decrease of toxic GSSG amounts.

In contrast, the increase in GSH levels that occurred in patients' blood might be especially important in AD. Apart from being the principal cellular antioxidant, GSH has a number of other functions, including the balance of Th1 and Th2 cells, and the reduction of the production of IgE and IgG_4 by B cells [28].

Although subjects' numbers were limited in this study, our results demonstrate that adults suffering even from mild AD are under greater oxidative burden. Most of the atopic dermatitis patients regularly use emollients to improve barrier-disrupted skin. Regular use of probiotics might be a useful adjunct to the management of patients with atopic dermatitis.

Acknowledgements

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