Mild Exercise Suppresses Exacerbation of Dermatitis in NC/Nga Mice: Correlation with β-endorphin Levels

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Abstract
Atopic dermatitis (AD) is known to be affected by mild or strong stress. However, the mechanism underlying this phenomenon is unclear. This study analyzed the mechanism(s) responsible for the influence of different levels of stress on AD. Specific pathogen-free (SPF) and conventional NC/Nga mice were used for the studies. Conventional mice, but not SPF mice, spontaneously develop dermal symptoms similar to that of patients with AD. Two types of stress, mild (20 m/min for 60 min) or strong (25 m/min for 90 min) exercise were applied using a treadmill four times per day. The symptoms of the conventional group were strongly exacerbated by strong exercise but ameliorated by mild exercise. The plasma concentration of β-endorphin was increased by mild exercise. The transsepidermal water loss of strong exercise in the conventional mice was higher than that of the no-exercise conventional mouse group. The levels of collagen IV in conventional group were unchanged by mild exercise, but decreased by strong exercise. The level of matrix metalloproteinase-9 was suppressed by mild exercise in the conventional groups, and elevated further by strong exercise. In addition, the expression of the µ-opioid receptor was increased on the mast cell surface of the conventional mice that were subjected to mild exercise. These observations suggest that exercise-induced stress significantly affects the symptoms of AD concomitant with the levels of β-endorphin. This hormone might control the collagen IV degradation from mast cells, and thus affect the barrier function of the skin.

Keywords: Atopic dermatitis; β-endorphin; µ-opioid receptor; Collagen IV; Matrix metalloproteinase-9

Abbreviations: AD: Atopic Dermatitis; TEWL: Transepidermal Water Loss; MMP-9: Matrix Metalloproteinase-9; BM: Basement Membrane

Introduction
Atopic dermatitis (AD) is a chronic, recurrent, and pruritic skin disorder usually beginning in childhood, with the typical clinical feature brings skin dryness. Previous studies have revealed that the water content of the stratum corneum is decreased in patients with AD [1,2]. In addition, atopic skin shows a defective barrier function, as measured by the transepidermal water loss (TEWL), in both involved and uninvolved skin [2-4]. In normal healthy skin, the TEWL is increased when the epidermal water content is high. In contrast, in AD patients, the TEWL is increased despite normal or low epidermal water content [4]. Furthermore, in AD patients, although the skin appears to be clinically normal, the water content is lower than that of normal healthy skin [5-7]. In addition, it has been reported that in lesional atopic skin, the expressions levels of filaggrin and involucrin, which are associated with keratinocyte differentiation, are lower than those in normal healthy skin [8,9].

NC/Nga mice were established as an inbred strain from Japanese fancy mice in 1957, and have recently been shown to spontaneously develop AD-like dermatitis with immunoglobulin E hyperproduction in air-uncontrolled, conventional circumstances [10,11]. In our previous study using NC/Nga mice, we showed that the AD-like symptoms were exacerbated by strong stress and reduced by mild stress [12]. In addition, the plasma level of β-endorphin was increased in mice with a mild stress load [12]. This suggested the possibility that β-endorphin contributed to the reduction of the AD-like symptoms.

In this study, to examine the mechanism underlying the AD-like symptom improvement and the potential involvement of β-endorphin, we examined the water maintenance (an indication of the barrier function) of the skin and its relationship to β-endorphin.

Materials and Methods

Animals
Conventional and specific pathogen-free (SPF) NC/Nga male mice (7 weeks old) were purchased from SLC (Hamamatsu, Japan). They were housed in rooms with a 12-h light/12-h dark cycle, and all animals were allowed free access to laboratory chow (CE-2, Oriental Yeast Co., Tokyo, Japan) and water ad libitum during the experiments. These animals were subjected to experiments according to the animal care regulations of Osaka City University Medical School. Conventional, but not SPF mice, spontaneously started to exhibit symptoms characteristic of AD at the age of 7 weeks old. The mice were divided into six experimental groups (n = 10) and then were used at same time. Six groups set up the following groups, 1; no exercise by SPF mice, 2; mild exercise by SPF mice, 3; strong exercise by SPF mice, 4; no exercise by conventional mice, 5; mild exercise by conventional mice, 6; strong exercise by conventional mice, respectively. In addition, each experiment was repeated three times.

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Voluntary and forced exercise protocols

The details of the protocol were described in a previous article [12]. In brief, to condition animals to the device for exercise, they were placed on a resting treadmill for 10 min, followed by running at 5 m/min for 10 min and then at 10 m/min for 10 min. On day 2, animals were placed in an uphill inclined device (at 10°) at a speed of 20 m/min for 60 min or 25 m/min for 90 min to provide mild or strong stress, respectively. The latter conditions elicited strong fatigue (mice were unable to move due to exhaustion) while the former conditions provided only mild stress (no appreciable effect on their behavior) based on an analysis of their behavior for the 60 min after the exercise. These exercise programs were carried out every other day (for a total of 4 exercise sessions).

Evaluation of the inflammatory score

The symptoms of dermatitis in these animals were evaluated on day 9, at their rostral skin and the severity of edema, erythema and hemorrhage was scored (0, none; 1, slight; 2, moderate; 3, severe) as described previously [13].

Assessment of epidermal barrier function

The TEWL were measured using a Tewameter TM300 (Courge and Khazaka Electronic DmbH, Cologne, Germany) on day 10. The TEWL was recorded with the probe placed on the dorsal skin around the scapula. It was measured repeatedly for appropriate durations of time, and two values were adopted when the difference between them was less than 20%.

Analysis of peptide hormones in plasma

Under light isoflurane anesthesia, blood samples were obtained by cardiac puncture 24 h after the final exercise session. Plasma samples were obtained by centrifugation and analyzed for peptide hormone levels. The plasma levels of β-endorphin were determined by using ELISA kits according to manufacturer’s instructions. The ELISA kit for β-endorphin was obtained from Phoenix Pharmaceuticals (CA, USA).

Preparation and staining of skin samples

The skin specimens were fixed in phosphate-buffered paraformaldehyde (4%), embedded in frozen Tissue-Tek, OCT compound, and cut into 5 μm thick sections. The sections of the skin was washed in PBS and then were subsequently incubated overnight at 4°C with a rabbit anti-collagen type IV (1:100) polyclonal antibody (Abcam, Tokyo, Japan), rabbit anti matrix metalloproteinase (MMP)-9 (1:50) polyclonal antibody (Abnova., Taipei, Taiwan), goat anti-mast cell tryptase (1:50) polyclonal antibody (Santa Crus Biotechnology Inc., Santa Cruz, CA), or rabbit anti opioid receptor μ (1:100) polyclonal antibody (ENZO Biochem., New York, NY). The sections were then washed in PBS and incubated at room temperature for 2 hours with FITC-conjugated anti-rabbit immunoglobulin, TRITC-conjugated anti-rabbit immunoglobulin, or TRITC-conjugated anti-goat immunoglobulin (1:30; Dako Cytomation, Glostrup, Denmark). The expression levels of collagen IV, MMP-9, mast cell tryptase, and opioid receptor μ were evaluated immunohistochemically using a fluorescent microscope.

Statistical analysis

All data were presented as the means ± SD derived from 10 animals. The results obtained from two animal groups were analyzed by either Student’s t-test or an ANOVA using a computer software package. Differences were considered to be significant when p<0.05.

Results

Effect of exercise on dermatitis and the plasma levels of β-endorphin

Conventional mice exhibited symptoms characteristic of AD, including edema, erythema and haemorrhage of their rostral skin. The dermal symptoms were ameliorated in the animal group that was subjected to the mild exercise. In contrast, the symptoms deteriorated in the animal group that was subjected to the strong exercise. The symptoms of dermatitis were not apparent in the SPF group regardless of the exercise program. In addition, the plasma levels of β-endorphin increased markedly when the symptoms similar to AD became apparent in the conventional group. Although the plasma levels of β-endorphin were not affected by exercise in the SPF group, the levels further increased by the mild exercise, but were suppressed by strong exercise, in the conventional group (Figure 1).

![Figure 1: An AD-like skin lesion from a conventional NC/Nga mouse (a). Conventional mice were subjected to strong or mild exercise. In the mice subjected to the strong exercise load, the skin symptoms worsened compared to the conventional mice with no exercise burden, but the symptoms were reduced in the mice with a mild exercise load. The SPF NC/Nga mice did not develop any eczematous lesions. The dermatitis score (b) for the rostral back was assessed after the final episode of treadmill running (0, none; 1, slight; 2, moderate; 3, severe). This scoring was based on the severity of erythema/haemorrhage and pilosis. (c) Mild exercise in mice increased the plasma β-endorphin levels in comparison with the no exercise mice, and strong exercise decreased the levels in comparison with the no exercise mice. Values are the means ± SD derived from 10 animals. *P<0.05 compared with the no exercise SPF or conventional NC/Nga mice.](image-url)
Effect of exercise on the epidermal barrier function

The epidermal barrier function was determined by measurement of the TEWL (Figure 2-b) and the expression of collagen IV (Figure 2-a). In the mild exercise group of conventional mice, the TEWL was not significantly different from that of the SPF group, but it significantly increased in the strong exercise group of conventional mice. In addition, the TEWL of the strong exercise group was higher than the no-exercise conventional group. The expression of collagen IV was significantly lower in the conventional group than in the SPF group. In contrast, the decrease in the level of collagen IV was suppressed by mild exercise, but they decreased further by strong exercise.

Effect of exercise on the expression of MMP-9 in the dorsal skin

The expression of MMP-9 was higher in the conventional group than in the SPF group. The elevated level of MMP-9 was suppressed by mild exercise in the conventional groups, and it increased further by strong exercise (Figure 3).

Effect of exercise on the expression of mast cell tryptase and µ-opioid receptor

To understand the pathological significance of exercise stress, we analyzed the properties associated with decreased collagen IV (increased MMP-9) by examining the populations of various mast cells and examining the µ-opioid receptor expression level. An immunohistochemical analysis revealed that exercise stress load of conventional mice increased the level of mast cell tryptase in the dorsal skin. However, the expression of the µ-opioid receptor was higher in the conventional mice with the mild exercise stress load than in the mice subjected to strong exercise (Figure 4). In addition, in the mice subjected to mild exercise, the µ-opioid receptor expression was associated with the mast cells.

Discussion

This present study demonstrated that the AD-like symptoms of the conventional mice were exacerbated by strong exercise but ameliorated by mild exercise. In addition, the levels of β-endorphin in conventional mice were decreased in the strong stress group in comparison to the non-stress control group, and increased in the mild stress group. Furthermore, the expression of the µ-opioid receptor was increased in the mild stress group.

Plasma β-endorphin serves as an independent and important factor influencing pruritus in AD. Opioid peptides and their µ-specific receptors modulate the function of calcium channels specifically on unmyelinated c-fibers of the central nervous system, and thus, they are thought to be involved in the central itch-regulatory mechanism [14]. Some reports suggest that endogenous opioid peptides may be involved as central mediators of itch [15,16]. β-endorphin, belonging...
to the endogenous opiate family, is generated upon stimulation of the pituitary-adrenal axis after stress [17,18]. The present study also demonstrated that, although the plasma levels of β-endorphin and the expression of the µ-opioid receptor in the dorsal skin were significantly higher in the conventional group than in the SPF group, mild exercise increased the plasma level of β-endorphin and the expression of µ-opioid receptor in the skin, while strong exercise decreased the plasma level of β-endorphin, the expression of the µ-opioid receptor, and exacerbated the symptoms of AD. These results suggest that the different strengths of the exercise modulated the plasma levels of β-endorphin and the expression of the µ-opioid receptor to affect the symptoms of dermatitis.

Another possible mechanism was suggested by the fact that the number of mast cells is increased in AD. Mast cell granules contain several mediators (TNF-α, PGE2, and serine proteases) that could directly or indirectly modulate the degradation of the extracellular matrix or activate the proenzyme form of MMPs [19,20]. Previous studies have suggested that mast cell tryptase can activate MMP-1 through the activation of pro MMP-3 [21,22]. Another study indicated that tryptase could also activate pro MMP-9 [23]. In addition, tryptase has previously been reported to cleave fibronectine in the pericellular matrix of fibroblasts, resulting in separation of the dermal-epidermal junction [20,24]. Although the mast cells are not in direct contact with the basement membrane (BM), the tryptase released as a result of AD could reach this zone, resulting in direct cleavage of the BM, and the barrier function of the skin decreased. As a result, it was thought that an increase of the quantity of water transpiration and exacerbation of inflammation were induced in the conventional mice.

In this study, one of the most important characteristic features of the mast cells was the expression of the µ-opioid receptor. The expression of the µ-opioid receptor increased on the surface of the mast cells in the conventional NC/Nga mice which were burdened by mild stress, and the inflammation of their skin was reduced. Although we could not demonstrate a β-endorphin-induced secretion of inflammatory mediators in the skin, we provided direct evidence for a β-endorphin-mast cell interaction based on our observation of a significant decrease in the tryptase concentrations in the skin.

The current results showed that there was hormonal regulation, supporting the idea that mild exercise is effective for ameliorating the symptoms of AD. In the atopic group, we were able to demonstrate a β-endorphin-mast cell interaction, which reduced the allergic inflammatory response. Our present findings suggest the possibility of new targets that can be used to prevent and/or treat AD.

References


