External Application of Horse Oil Alleviates Atopic Dermatitis Symptoms in Mice

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(Received December 3, 2017; December 10, 2017; Accepted December 17, 2017)

Abstract

Atopic dermatitis is characterized by skin barrier dysfunction, edema, and infiltration with various inflammatory cells. Horse oil, the purified form from horse fat, has been used for a long time by folk remedies on burn, wound, and various skin diseases. In this study, we investigated the improving effects of horse oil on atopic dermatitis induced by 0.3% of DNCB in mice. Horse oil B (oil phase, 100 mg/kg) significantly decreased serum IgE levels in experimental atopic dermatitis mice induced by DNCB. Also, horse oil A (cream phase, 100 mg/kg) and B alleviates atopic dermatitis symptoms (ear edema, inflammatory cell infiltration, and lymph node size). Overall, the effect of horse oil B was slightly better. These results suggest that horse oil can be a useful remedy to improve atopic dermatitis. (J Med Life Sci 2017;12(2):62–66)

Key Words: Horse oil, Atopic dermatitis, 2, 4-Dinitrochlorobenzene, Immunoglobulin E

INTRODUCTION

Atopic dermatitis (AD) is a chronic relapsing skin disease associated with intense pruritus and skin hyper-reactivity, which affects approximately 10–20% of children and 1–3% of adults worldwide. The skin lesions in AD patients are generally characterized by thickening of the papillary dermis, skin barrier dysfunction, epidermal hyperplasia, severe skin dehydration, parakeratosis, and various inflammatory cells hyperproliferation, which consist mainly monocytes, mast cells, basophils, and T cells.

Mast cells play an important role in allergic, innate immunity and anaphylactic reactions. Activated mast cells release a variety of inflammatory mediators such as cytokines, serotonin and histamine following cross-linking of their high affinity surface receptor (FcεRI) for immunoglobulin E (IgE) .

Horse oil, the purified form from horse fat, has been used for a long time by folk remedies on burn, wound, and various skin diseases. In recent years, numerous horse oil products are being sold in a variety of ways of on line, duty free shop, and local souvenir shop. Despite of so many products, the results of scientific research on horse oil are insufficient. In 1950 and 1952, Brooker et al., presented study results on the composition of horse oil . Anti-bacterial and anti-inflammatory effects of horse oil in HaCaT keratinocyte was reported .

Therefore, we aimed to investigate the effects of horse oil on the various symptoms in mouse atopic dermatitis.

MATERIAL AND METHODS

Reagents

Two types of horse oil (cream and oil) were obtained from Internet market, and hydrocort cream was obtained from Green Cross, Korea. Dinitrochlorobenzene (DNCB) was purchased from Tokyo Kasei Kogyo (Tokyo, Japan), and mouse IgE enzyme–linked immunosorbent assay (ELISA) kits was purchased from Biolegend (San Diego, CA). All other chemicals and reagents were of reagent grade.

Experimental animals

BALB/c mice (female, 7–weeks–old) were purchased from Orient Bio (Orient Bio Inc., Seongnam, Korea) and maintained under pathogen–free conditions in the animal facility of Jeju National University. All animal experiments were approved by the Jeju National University Animal Care
DNCB application to induce AD

Mice were divided into four groups (n=5 per group): saline (normal), AD (induction-only), AD + hydrocort cream, and AD + horse oil A and B. Mice were sensitized by applying 1% DNBC or vehicle on their abdomen as the first sensitization (day-7). Hydrocort cream was used as a positive control. On Day 0, mice were challenged again by applying 0.3% DNBC to the ears on every other day for up to 30 days. Starting on Day 12, the mice were treated with hydrocort cream (Green Cross, Korea) containing 2 mg/g hydrocortisone valerate and Horse oil (100 mg/kg) on their ears every other day. Ear thickness was measured on days 0, 12, 16, 20, 24, and 29. The mice were sacrificed on day 31.

Enzyme-linked immunosorbent assay (ELISA)

The levels of IgE in mouse serum were measured using ELISA kits (Biolegend, San Diego, CA) according to the manufacturer’s instructions.

Ear edema and histology

In the experimental AD mouse model, DNBC stimulation elicited ear edema, and ear thickness was measured using a Digital Thickness Gauge (Mitutoyo, Kawasaki, Japan). Ear tissues were fixed in 10% formalin, and then embedded in paraffin. Paraffin sections (3 µm each) were stained with hematoxylin and eosin (H&E).

Statistical analysis

Quantity One version 4.2.1 and Image-Pro plus version 4.5 software were used to transform images into numerical values. Student’s t-test and two-way analysis of variance were used to determine the statistical significance of differences between experimental and induction groups. Data are shown as mean ± standard deviation. P-values less than 0.05 were considered statistically significant.

RESULTS

Horse oil suppresses the expression of serum IgE

To induce experimental AD, mice were stimulated an initial sensitization with 1% DNBC on the abdomen. They were then re-sensitized by applying 0.3% DNBC to the ears on every other day for up to 30 days. Starting on day 12, the mice were received with hydrocort cream and horse oil (100 mg/kg) on their ears every other day. On day 31, all mice were sacrificed (Fig. 1). IgE is a crucial immunoglobulin for AD, as it is the major activator of mast cells, which release histamine, tryptase and cytokines. Therefore, we measured the levels of serum IgE in mice with experimental dermatitis by ELISA. The horse oil B-treated group showed significantly decreased levels of IgE compared with the induction group (mice exposed to DNBC but not treated sample) (Fig. 2).

Figure 1. DNBC-induced atopic dermatitis in mice.
Mice were sensitized by applying 1% DNBC or vehicle on their abdomen as the first sensitization (day-7). On day 0, mice were challenged again by applying 0.3% DNBC to the ears every other day for up to 30 days. Starting on day 12, the mice were treated with hydrocort cream and horse oil (100 mg/kg) on their ears every other day. The mice were sacrificed on day 31.

Figure 2. Horse oil suppresses the expression of serum IgE
After sacrifice, the IgE in mouse serum was measured by ELISA. Data are representative of 5 mice per group. (n = 5 mice per group). Values represent the mean ± SD. *** P < 0.001 compared to mice stimulated with DNBC alone (induction group).
Horse oil suppresses the development of experimental AD. We tested skin swelling as a measure of AD progression. We found that cutaneous edema in horse treated mice was reduced on day 31 compared with that observed in the induction-only mice (Fig. 3A and B). The skin lesions associated with AD are characterized by infiltration of various inflammatory cells. Therefore, we examined the effect of horse oil on the infiltration of inflammatory cells by H&E staining of ear tissue sections. Epidermal thickness and the degree of inflammatory cell infiltration were significantly lower in the horse oil-treated group than in the induction group (Fig. 3C). LNs have a crucial role in cell-mediated immunity by regulating the activity of T and B cells. Therefore, we examined morphologic changes in the LNs of AD mice. The LNs in mice in the induction-only group were quite swollen, whereas those in horse oil-treated mice were smaller (Fig. 3D).
Figure 3C

Figure 3D

Figure 3. Horse oil suppresses the development of experimental AD.
(A) Photos of the ears on day 31. (B) Ear thickness was measured on days 0, 12, 16, 20, 24, and 29. (C) Paraffin-embedded sections of ear tissue stained with hematoxylin and eosin. (D) The lymph nodes (LN) were photographed to record morphologic changes. (n = 5 mice per group). Scale bar = 0.1mm. Values represent the mean ± SD. **P < 0.001 compared to mice stimulated with DNBC alone (induction group).

Discussion

In this study, we aimed to elucidate the improving effects of horse oil on atopic dermatitis in mice. We utilized DNBC-stimulated AD mouse model to investigate the effects of horse oil. IgE is an important therapeutic target for allergy, and signaling through FceRI can release histamine, tryptase and cytokines from mast cells, which leads to potent induction of edema or itching. Therefore, we tested whether horse oil can decrease serum IgE hyper-production and cutaneous edema. The horse oil treatment reduced the levels of IgE and edema compared with the induction-only group. H&E staining of the ear tissue showed that horse oil treatment alleviated the infiltration of inflammatory cells compared with the induction-only group. The LNs play an important role in regulating the immune responses and contains a variety of immune cells. Also, enlarged LN means a lymph node-enlargement by abnormality of immune system. We investigated the morphologic features of the LNs in experimental AD model. The induction-only group had markedly enlarged LNs: the LNs from horse oil-treated mice were smaller compared with those from mice in the induction-only group. In summary, horse oil had significant inhibitory effects on various AD symptoms. So we are currently trying to identify the inter-relationships between horse oil and immune-modulatory cells. Our results suggest that suggest that horse oil can be a useful remedy to improve atopic dermatitis.
CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES