J. Tokyo Med. Univ., **65**(4) : 393-400, 2007

The anti-pruritic effects of oyster extract

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Abstract

Intractable pruritus unresponsive to anti-histamine (anti-HS), common anti-pruritic drugs that are commercially available, is typically observed in patients with uremic pruritus who are undergoing maintenance hemodialysis. Many of these patients have decreased zinc concentrations in the blood and epidermis. This finding suggests that zinc replacement therapy may be effective for uremic pruritus. In the present study, the anti-pruritic effects on atopic dermatitis (AD) of oyster extract (OE), which is abundant in zinc, were examined. To investigate the action of OE on skin inflammation and pruritus in patients with AD associated with intractable pruritus, the anti-pruritic effects were first examined by a topical application test using a pruritus-induced guinea pig model, which showed that the quantitative measurable pruritic behavior related to both general pruritus, which is responsive to anti-HS, and intractable pruritus (Experiment 1). Then, inflammation and pruritus were examined by an internal use test using the ICR mouse model (IgE-mIP-ICR mouse) of pruritus associated with IgE-mediated auricular skin inflammatory reactions (Experiments 2-4). In Experiment 1, immediately after intracutaneous administration of the pruritus-inducing substance, histamine (HS) or kallikrein (KK), 100 μl of normal saline containing 1% (w/v) OE or normal saline alone (control) was applied to the injection site. In Experiments 2-4, repeated doses of 200 mg/kg of OE in 0.3% CMC suspension or suspension alone (control) were given orally for 1 week before the start of the experiment. In Experiment 2, the cumulative duration of pruritus behavior within 2 hours was measured in the IgE-mIP-ICR mouse model, and the anti-pruritic effects of the test substance were examined. Inflammation and pruritus were assessed at 1, 4, 24, and 48 hours after elicitation (Experiments 3-4). Experiment 1 showed that OE significantly inhibited pruritus induced by both Hs and KK. Accordingly, the effects of the major components of OE, taurine (Tau), glycogen (Gly), and organic zinc (OZ) in normal saline containing 1% (w/v), on KK-induced intractable pruritus were examined to identify the major active ingredient in OE. As a result, only OZ showed a significant inhibitory action. Experiment 2 showed that both OE and OZ exhibited anti-pruritic effects and significantly reduced the cumulative duration of pruritic behaviors within 2 hours of DNFB application. Experiment 3 showed that OZ significantly inhibited inflammatory reactions in the IgE-mIP-ICR mice, which showed biphasic skin inflammation at 1 and 24 hours after elicitation. Experiment 4 showed that OZ significantly inhibited the cumulative duration of pruritic behaviors within 1 hour in IgE-mIP-ICR mice showing biphasic pruritus at 1 and 24 hours after elicitation.

Since OE and its active ingredient, OZ, significantly inhibited skin inflammation and pruritus in the AD animal model, they may be used as health food materials for treating inflammation and pruritus in AD patients as well as an effective medication for uremic pruritus in patients with chronic renal failure undergoing hemodialysis.

Received May 25, 2007, Accepted June 28, 2007
Keywords : Oyster extract, Zinc, Uremic pruritus, AD animal, Pruritus
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Introduction

In addition to pruritus in atopic dermatitis (AD), uremic pruritus in patients undergoing chronic hemodialysis due to chronic renal failure is well known as an intractable pruritus condition, which is unresponsive to commercially available anti-pruritic anti-histamine (anti-HS) drugs. Many uremic pruritus patients have decreased blood zinc concentrations[1] and the amount and activity of Cu, Zn-superoxide dismutase (SOD), which is distributed in the basal cell layer of the epidermis, are significantly decreased compared with cells positive for this enzyme in healthy subjects[2]. Similarly zinc concentrations in the blood and epidermis are also decreased in people with total parenteral nutrition[3]. Izumi et al. confirmed that zinc-deficient rats have low zinc concentrations in the blood and epidermis, as observed in uremic pruritus patients, as well as a reduced threshold intensity of itch receptors located on free nerve endings of C-fibers at the dermal-epidermal junction, which provokes the itch sensation[4]. These findings indicate that, when zinc becomes deficient in uremic pruritus patients and other organisms that suffer oxidative stress, in which decreased blood zinc concentrations decrease the zinc level in the basal cell layer of the epidermis, the number of Cu, Zn-SOD-positive cells present in the same cell layer further reduces. This appears to stimulate itch receptors located on free nerve endings of C-fibers at the junction between the adjacent epidermis and dermis, provoking itch sensation. Accordingly, we considered that pruritus in uremic pruritus patients could be suppressed by zinc supplementation[5]. In the present study, we investigated ingestion of oyster extract (OE), a food abundant in zinc, and its major components, taurine (Tau), glycogen (Gly), and organic zinc (OZ), on AD associated with intractable pruritus. To establish an AD animal model that is highly reproducible, the anti-pruritic effects were examined using the pruritus-induced guinea pig model[6], which can quantitatively determine general pruritus, which responds to anti-HS, and intractable pruritus, which does not respond to anti-HS (Experiment 1). Based on the results of a clinical study using this animal model, which showed that apple polyphenol improved skin symptoms, inhibited pruritus in AD patients[7][8] and improved pruritus in uremic pruritus patients undergoing maintenance hemodialysis[9], we consider that this pruritus-induced guinea pig model (Experiment 1) is a convenient and useful system that can quantitatively determine pruritus behavior and predict clinical effects. Further, since intractable AD and blood IgE levels correlate well[10] and AD is predisposed to produce IgE antibody by definition[11], actions on skin inflammation and pruritus in AD were investigated using the IgE-mediated inflammation ICR (IgE-mIP-ICR) mouse model[15] as an animal model that is highly reproducible because conditions are similar (Experiments 2 to 4). This model was prepared by replacing the BALB/c mouse in the model of biphasic inflammation associated with the IgE-mediated late phase reaction of Katayama et al.[12][13] with the ICR mouse[14], which is optimal to observe pruritus behaviors, and incorporating pruritus into dermatitis.

Materials and methods

Pruritus-inducing substances and other materials

In Experiment 1, HS hydrochloride produced by Wako Pure Chemical Industries, Ltd., (Osaka, Japan) and swine pancreas tissue KK (low molecular weight) produced by Merck Ltd., (Tokyo, Japan) were used to induce pruritus in guinea pigs. In Experiment 2, mouse monoclonal IgE (anti-DNP) from Seikagaku Corporation (Tokyo, Japan) was used to induce IgE-mediated auricular skin inflammatory reaction. Dinitrofluorobenzene (DNFB), acetone, and olive oil from Nakarai Chemicals Ltd., (Kyoto, Japan) were used. To measure auricular thickening, the Dial Thickness Gauge system from Mitsutoyo Corporation (Kanagawa, Japan) was used.

Experimental agents

OE and its major components, Tau, Gly, and OZ were provided by Miyashita Bio-Health Research Institute Co., Ltd. (Tokyo, Japan). The zinc content in OZ prepared from OE was 810 μg/g (810 ppm).

Laboratory animals

In Experiment 1, wild-type 8-week-old male Hartley guinea pigs (Hoshino Laboratory Animals, Saitama, Japan) were used. In Experiments 2-4, wild-type 4-week-old female ICR mice (Clea Japan, Inc., Tokyo, Japan) were used to examine the inflammatory reactions and pruritic behavior in the IgE-mediated auricular skin inflammation model. After 1 week of preliminary rearing at room temperature (23±2°C) and at a humidity of 55±10%, the animals were divided into specific groups. Guinea pigs and mice were fed with RC4 (Oriental Yeast Co., Ltd., Tokyo, Japan) and CE-2 (Clea Japan, Inc., Tokyo, Japan), respectively, and were provided with tap water ad libidum.

Experimental methods

Each experimental group consisted of 12 animals

a) HS or KK induced pruritus behavior in guinea pigs (Experiment 1)[6]

Guinea pigs were intracutaneously given 50 μl of a pruritus-inducing substance, HS hydrochloride (0.3 mg/

ml) or swine pancreas KK (25 U/animal), in the midventral region. Animal behavior was recorded using an overhead video camera for 2 hours immediately after the injection. From the video recording, the cumulative duration of pruritus behaviors, including turning to the injection site to scratch it with their teeth and scratching it with a hind leg, were measured (measurements were recorded in seconds within the 2-hour period). After intracutaneous administration of the pruritus-inducing substance, animals were assigned to receive 100 μl of normal saline containing 1% (w/v) OE, Tau, Gly or OZ, or saline alone (control) at the injection site.

b) Cumulative duration of pruritus behaviors in IgE-mIP-ICR mice within 2 hours (Experiment 2)[15]

ICR mice were passively sensitized with 0.5 ml anti-DNP IgE Ab (×7,680 as PCA titer), and 100 μl 0.15% DNFB in acetone/olive oil (4 : 1) was applied to the auricle of the animals to elicit biphasic pruritus 1 hour after sensitization. After elicitation, pruritic behaviors against the DNFB application site were video-recorded within 2 hours as in Experiment 1. The cumulative duration of pruritic behaviors was measured. Animals received repeated oral doses of 200 mg/kg OZ in 0.3% CMC suspension or 0.3% CMC suspension alone (control) for 1 week before the start of the experiment.

c) Changes in auricular skin inflammatory reactions in IgE-mIP-ICR mice over 48 hours (Experiment 3)[15]

DNFB solution was applied to the auricle of ICR mice sensitized with anti-DNP IgE Ab, as in Experiment 2, to elicit biphasic skin inflammation. At 1, 4, 24, and 48 hours after elicitation, auricular thickening (thickening based on auricular thickness before elicitation) at the DNFB application site was measured using a Dial Thickness Gauge. Animals received repeated oral doses of 200 mg/kg OE or OZ in 0.3% CMC suspension or 0.3% CMC suspension alone (control), as in Experiment 2.

d) Auricular pruritus behaviors in IgE-mIP-ICR mice over 48 hours (Experiment 4)[15]

DNFB solution was applied to the auricle of ICR mice sensitized with anti-DNP IgE Ab, as in Experiment 2, to elicit biphasic skin pruritus. Pruritic behaviors at the DNFB application site of the auricle were video-recorded for 1 hour at 1, 4, 24, and 48 hours after elicitation, as in Experiment 1. Animals received repeated oral doses of 200 mg/kg OZ in 0.3% CMC suspension or 0.3% CMC suspension alone (control), as in Experiment 2.

Statistical analysis

Results are reported as means±standard deviations. Analysis of variance was performed for group comparisons, and Student's t-test was performed for pairwise comparisons with the control group. A p-value less than 0.05 was considered to indicate a statistically significant difference.

Results

1. Pruritus behaviors in guinea pigs with pruritus induced by HS or KK (Experiment 1)

Figure 1 shows the total duration of pruritus behaviors induced by HS or KK in the OE group. Figure 2 shows the total duration of pruritic behaviors induced by KK in animals receiving Tau, Gly or OZ, the major components of OE. The total duration of pruritic behaviors induced by HS and KK was significantly lower in the OE group. The pruritic behavior lasted 11.4±5.4 s/2 h ($p<0.05$) with HS-induced pruritus

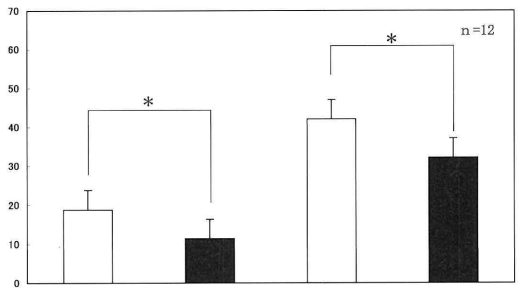

Fig. 1 Effects of topical oyster extract (OE) on total pruritic behavior induced by histamine (HS) or kallikrein (KK) in guinea pigs. Each column represents mean with standard deviation. Each group consisted of 12 guinea pigs. *$p<0.05$ (Student's t-test)
y-axis : Accumulated time of total pruritic behavior (s/2 h)
x-axis : left HS, right KK
open column : control group
solid column : OE group

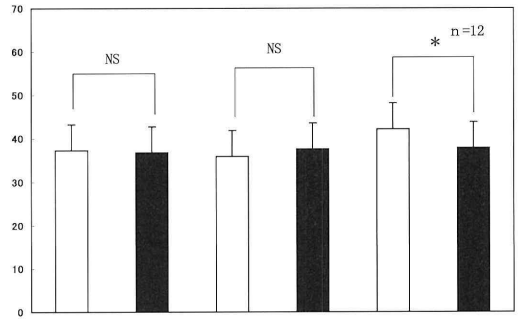

Fig. 2 Effects of topical taurine (Tau), glycogen (Gly) and organic zinc (OZ), major components containing into OE on total pruritic behavior induced by KK in guinea pigs. Each column represents mean with standard deviation. Each group consisted of 12 guinea pigs. *$p<0.05$ (Student's t-test)
y-axis : Accumulated time of total pruritic behavior (s/2 h)
x-axis : left Tau, middle Gly, right OZ
open column : control group
solid column : Tau group (left)
Gly group (middle)
OZ group (right)

compared with 18.8±7.2 s/2 h in the control group, and 32.1±4.1 s/2 h ($p<0.05$) in KK-induced pruritus compared with 42.0±6.0 s/2 h in the control group. The total duration of pruritic behaviors in animals receiving Tau, Gly, and OZ was 36.8±5.7 (ns), 37.6±6.0 (ns), and 37.8±4.2 s/2 h ($p<0.05$), respectively, compared with 37.3±7.4, 35.9±7.4, and 42.2±4.5 s/2 h in the respective control groups.

2. Cumulative duration of pruritic behaviors in IgE-mIP-ICR mice within 2 hours (Experiment 2)

Figure 3 shows the cumulative duration of pruritic behaviors within 2 hours in IgE-mIP-ICR mice, which received either OE or OZ. The duration of pruritic behavior was significantly lower in the OE and OZ groups (286.0±35.3 s/2 h [$p<0.01$] and 290.5±33.0 s/2 h [$p<0.01$], respectively), compared with 383.0±20.2 s/2 h, 396.2±35.3 s/2 h in the respective control groups.

3. Changes in auricular skin inflammatory reactions in IgE-mIP-ICR mice over 48 hours (Experiment 3)

Figure 4 and 5 show the changes in auricular skin inflammatory reactions over 48 hours in IgE-mIP-ICR mice prepared in Experiment 2, which received OE or OZ. In the control group, a clear biphasic pattern of inflammation was observed, with auricular thickening of 61.8±8.4, 24.6±3.4, 41.6±5.1, and 24.8±4.4 μm at 1, 4, 24, and 48 hours after DNFB elicitation. On the other hand, inflammation was significantly suppressed in the OE group at 1, 24, and 48 hours after DNFB elicitation, with auricular thickening of 41.1±4.7 ($p<0.01$), 21.2±3.3, 32.2±4.0 ($p<0.01$), and 20.1±2.8 ($p<0.05$) μm at 1, 4, 24, and 48 hours (Fig. 4). In the OZ group,

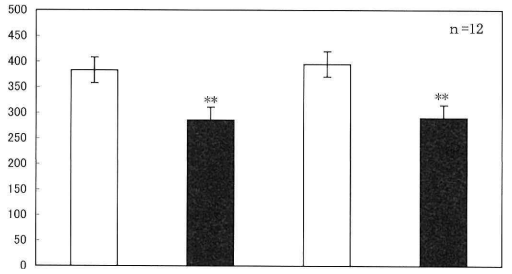

Fig. 3 Effect of oral OE or organic zinc (OZ), one of the major components of OE, pretreatment on cumulative duration time of pruritic behaviors versus control within 2 h after induction with DNFB in anti-DNP IgE Ab sensitized (PCA titer ×7,680) IgE mIP-ICR mice. Each column represents mean with standard deviation. Each group consisted of 12 mice. *$p<0.05$ (Student's t-test)
y-axis: Accumulated scratching time (s/2 h)
x-axis: left OE, right OZ
left open column: control group
left solid column: OE group
right open column: control group
right solid column: OZ group

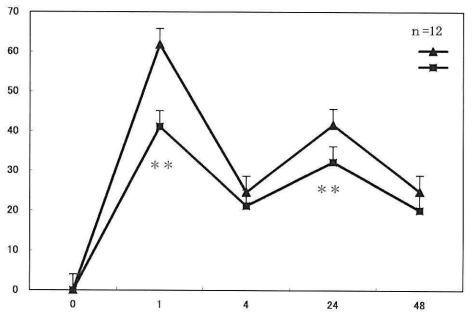

Fig. 4 Effect of oral OE pretreatment on increase of auricular thickness (μm) over 48 h after induction with DNFB after anti-DNP IgE Ab sensitization in mIP-ICR mice. Each value represents the mean with standard deviation. Each group consisted of 12 mice. **$p<0.01$ (Student's t-test)
y-axis: Increase of auricular thickness (μm)
x-axis: Time after induction (h)
triangles: control group
squares: OE group

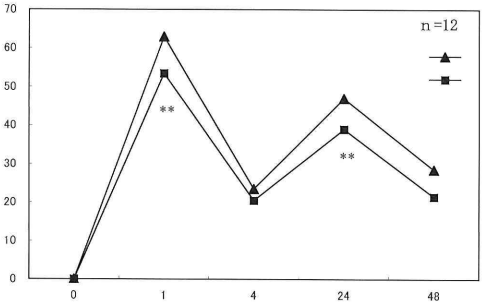

Fig. 5 Effect of oral OZ pretreatment on increase of auricular thickness (μm) over 48 h after induction with DNFB after anti-DNP IgE Ab sensitization in mIP-ICR mice. Each value represents the mean with standard deviation. Each group consisted of 12 mice. *$p<0.05$, **$p<0.01$ (Student's t-test)
y-axis: Increase of auricular thickness (μm)
x-axis: Time after induction (h)
triangles: control group
squares: OZ group

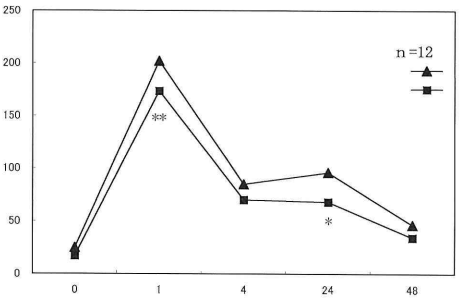

Fig. 6 Effect of oral OZ pretreatment on cumulative duration time (s/1 h) of pruritic behaviors over 48 h after induction with DNFB after anti-DNP IgE Ab sensitization in mIP-ICR mice. Each value represents the mean with standard deviation. Each group consisted of 12 mice. *$p<0.05$, **$p<0.01$ (Student's t-test)
y-axis: Accumulated scratching time (s/1 h)
x-axis: Time after induction (h)
triangles: control group
squares: OZ group

inflammation was significantly suppressed at 1 and 24 hours after DNFB elicitation, with auricular thickening of 53.1±5.2 ($p<0.01$), 20.6±2.5 ($p<0.05$), 38.6±4.1 ($p<0.01$), and 21.9±2.7 ($p<0.01$) μm at 1, 4, 24, and 48 hours compared with 62.5±3.6, 23.2±2.2, 45.9±4.6, and 28.3±3.1 μm in the control group (Fig. 5).

4. Cumulative duration of pruritic behaviors in IgE-mIP-ICR mice over 48 hours (Experiment 4)

The cumulative duration of pruritic behaviors over 48 hours in the IgE-mIP-ICR mice were compared between the OZ group and the control group, and the pruritic behaviors are shown in Fig. 6. In the control group, a clear biphasic pruritus behavior pattern was observed, with duration of behavior of 204.7±20.6, 85.2±19.5, 94.3±30.7, and 46.5±19.1 s/1 h at 1, 4, 24, and 48 hours after DNFB elicitation. On the other hand, pruritus behaviors were significantly lower at 1 and 24 hours in the OZ group, with duration of behavior of 169.3±26.3 ($p<0.01$) and 69.6±25.6 ($p<0.05$) s/h, respectively.

Discussion

AD is an allergic disease that is not completely cured in adulthood, and the incidence of adult AD is increasing every year. In clinical practice, steroid and topical immunosuppressants, such as FK506, are used as standard therapy, while the use of anti-histamine with an anti-allergic action is recommended as combination therapy for pruritus[11]. However, the flare of dermatitis and the associated recurrence of pruritus often fall into a vicious cycle, and dermatologists claim that, if pruritus can be suppressed, the disease will not worsen. To overcome this problem, it is critical to identify materials that can prevent intractable pruritus, which cannot be suppressed by anti-histamine. Meanwhile, uremic pruritus is well known as an intractable pruritus condition in patients undergoing hemodialysis as a result of chronic renal failure other than pruritus in AD. Uremic pruritus patients have decreased blood zinc concentrations[1], and the amount and activity of Cu, Zn-SOD distributed in the basal cell layer of the epidermis are significantly decreased compared with Cu, Zn-SOD-positive cells in healthy subjects[2]. Izumi et al. showed that zinc-deficient rats, which were prepared by feeding a zinc-deficient diet, have decreased zinc concentrations in the blood and epidermis. As the result, a reducd threshold of the itch receptors located on free nerve endings of C-fibers at the dermal-epidermal junction, provoking the itch sensation[4] Therefore, it was considered that in patients undergoing hemodialysis as a result of uremic pruritus could be suppressed by zinc supplementation[5].

Accordingly, in the present study we investigated the effect of OE, a food abundant in zinc, and its major components, Tau, Gly, and OZ, on AD associated with intractable pruritus.

First, we confirmed the anti-pruritic effect using the pruritus-induced guinea pig model[6] as an animal model that is highly reproducible because the conditions are similar (Experiment 1). This animal model can be used to quantitatively determine general pruritus, which responds to anti-HS, and intractable pruritus, which does not respond to it. So far, in this series of studies, we have explored health food materials, apple polyphenol and grape seed polyphenol,which also suppress intractable pruritus. Of these, clinical results with apple polyphenol showed improvements in skin symptoms and suppression of pruritus in patients with AD, representative intractable pruritus, and improvement of pruritus in hemodialysis patients[7-9]. Therefore, we consider that this pruritus-induced guinea pig model[6] used in Experiment 1 is a convenient and useful system, which can be used to quantitatively determine pruritus behavior and to predict clinical effects. Furthermore, since severe AD and blood IgE levels correlate well[10], and AD is predisposed to produce IgE antibody[11], the action on skin inflammation and pruritus in AD was investigated using the IgE-mediated inflammation ICR (IgE-mIP-ICR) mouse model[15] which was used in Experiments 2-4. This model was prepared by replacing the BALB/c mouse in the model of biphasic inflammation associated with IgE-mediated late phase reaction of Katayama et al.[12] and Otoyama[13] with the ICR mouse[14], which is optimal to observe pruritus behavior, and incorporating pruritus into dermatitis to obtain an animal model that is highly reproducible because conditions are similar.

The anti-pruritic effects of OE and its major components, Tau, Gly and OZ were first examined using the pruritus-induced guinea pig model[6] (Experiment 1). As a result, OE significantly suppressed pruritus induced by both HS with behaviors lasting 11.4±5.4 s/2 h ($p<0.05$) compared with 18.8±7.2 s/2 h in the control group and with KK-induced pruritus with behaviors lasting 32.1±4.1 s/2 h ($p<0.05$) compared with 42.0±6.0 s/2 h in the control group (Fig. 1). We then examined the effect of Tau, Gly, and OZ on KK-induced pruritus. Only OZ significantly suppressed KK-induced pruritus, with behaviors lasting 37.8±4.2 ($p<0.05$) s/2 h compared with 42.2±4.5 s/2 h in the control group (Fig. 2). We examined the anti-pruritic effects using the IgE-mIP-ICR mouse model[15] (Experiment 2). The pruritic duration of behavior was significantly lower in both OE and OZ groups with values of 286.0±35.3 s/2 h ($p<0.01$), and 290.5±33.0 s/2 h ($p<0.01$). On the other hand, clear pruritus behavior was observed, with values of 383.0±20.2 s/2 h, 396.2±35.3 s/2 h in the respective control groups (Fig. 3).

Finally, the effect on dermatitis (Figs. 4, 5) in both the OE and OZ groups and cumulative pruritic behavior (Fig. 6) in the OZ group over 48 hours were examined using the IgE-mIP-ICR mouse model described above. The control group showed a clear biphasic inflammation pattern in skin inflammation, with auricular thickening of 61.8±8.4, 24.6±3.4, 41.6±5.1 and 24.8±4.4 μm at 1, 4, 24 and 48 hours after DNFB elicitation, respectively, whereas inflammation was significantly suppressed in the OE group at 1, 24, and 48 hours after DNFB elicitation, with auricular thickening of 41.1±4.7 ($P<0.01$), 21.2±3.3, 32.2±4.0 ($P<0.01$) and 20.1±2.8 ($P<0.05$) μm at 1, 4, 24, and 48 hours, respectively (Fig. 4). Inflammation was significantly suppressed at 1 and 24 hours after DNFB elicitation in the OZ group, with auricular thickening of 53.1±5.2 ($P<0.01$), 20.6±2.2 ($P<0.05$), 38.6±4.1 ($P<0.01$), and 21.9±2.7 ($P<0.05$) μm at 1, 4, 24 and 48 hours compared with 62.5±3.6, 23.2±2.2, 45.9±4.6 and 28.3±3.1 μm in the respective control groups. Both immediate pruritus (first peak) and late phase reaction (second peak) were suppressed in both the OE (Fig. 4) and the OZ groups (Fig. 5). In addition, cumulative pruritic behavior over 48 hours was examined in the OZ group, using the IgE-mIP-ICR mouse model[15]. Here, the control group showed a clear biphasic pattern in pruritus, with auricular thickening of 204.7±20.6, 85.2±19.5, 94.3±30.7 and 46.5±19.1 μm at 1, 4, 24 and 48 hours after DNFB elicitation, respectively, whereas cumulative pruritic behavior was significantly suppressed at 1, 24, and 48 hours after DNFB elicitation, with auricular thickening of 169.3±26.3 ($P<0.01$), 71.3±15.3, 69.6±25.6 ($P<0.05$) and 34.8±15.3 μm at 1, 4, 24, and 48 hours, respectively (Fig. 6). These results were consistent with the results in the pruritus-induced guinea pigs.

We previously reported successive condensation products, proanthocyanidine, apple polyphenol and grape seed polyphenol, which are health foods[6][15], as materials that suppress intractable pruritus in the pruritus-induced guinea pig model[6] as well as late phase reaction and intractable pruritus in evaluation of effectiveness on immediate inflammation, which is represented by the first peak and general pruritus as well as the second peak, which is represented by late phase reaction and intractable pruritus in the IgE-mIP-ICR mouse model[15]. Since uremic pruritus, an intractable pruritus condition, is improved by zinc supplementation[5], OE, which is abundant in zinc, and its major components, Tau, Gly and OZ, were investigated in the pruritus-induced guinea pig model[6] and the IgE-mIP-ICR mouse model[15], which was prepared by incorporating pruritus in dermatitis in the present study as in previous reports. Both general pruritus and intractable pruritus were suppressed as with apple polyphenol and grape seed polyphenol, which are proanthocyanidine. Therefore, it is considered that, in chronic renal failure, the oxidative stress, which results in a decrease in blood zinc concentrations[1] decreases zinc localized in the basal cell layer of the epidermis[3], resulting in a lower level and activity of Cu, Zn-SOD distributed in this cell layer[2]. This process lowers the itch threshold of itch receptors present on free nerve endings of C-fibers at the junction between the adjacent epidermis and dermis, thus provoking an itch sensation[4]. Epidermis in the skin is designed to protect the body from oxidation stress such as UV radiation. Vitamins with antioxidation effects and radical scavengers such as Cu, Zn-SOD play a role in this defensive system[16]. Threrefore, the proanthocyanidine apple polyphenol, is considered to suppress the peripheral itch receptors through potential antioxidation stress effect[17], also appears to suppress peripheral pruritus by increasing of Cu, Zn-SOD activity in epidermis[18].

References

1) Chvapil M : Effect of zinc on cells and biomembranes. Med Clin Nor Am **60** : 799-812, 1976
2) Toyama K : The relationship between skin superoxide dismutase and pruritus in chronic renal failure. (In Japanese) The St. Marianna med J **21** : 550-560, 1993
3) Kitazume H, Takagi Y, Okada A, Kawashima Y : Tissure distribution of zinc during total parenteral nutrition (TPN) and zinc-free diet fed rats—(II)—. (In Japanese) Trace Metal Metabolism **11** : 49-56, 1983
4) Izumi H, Mori H, Uchiyama T, Kuwazuru S, Ozima Y, Nakamura I, Taguchi S : Sensitization of nociceptive C-fibers in zinc-deficient rats. Am J Physiol **268** (*Regulatory Integrative Comp Physiol* **37**) R1423-R1428, 1995
5) Sanada S, Kuze M, Yoshida O : Beneficial effect of zinc supplementation on pruritus in hemodialysis patients with special reference to change in serum histamine levels. (In Japanese) Acta urol Jpn **33** (12) : 1955-1959, 1987
6) Taguchi S, Ishihama S, Kano T, Yamanaka T, Matsumiya T : Evaluation of antipruritic effect of apple polyphenols using a new model of pruritus. J Tokyo Med Univ **60** : 123-129, 2002
7) Sasai M, Yamamoto A, Kanda T, Kojima T, Taniuchi S, Hattori K, Yoshijima S, Kino M, Ono A, Aoki T, Kobayashi Y : a double blind study to evaluate the effectiveness of polyphenols ointment from unripe apples in treating atopic dermatitis. (Abstract in Japanese) Jpn J Pediatr Alle Clin Immunol **10** : 373, 1996
8) Kojima T, Akiyama H, Sasai M, Taniuchi S, Goda Y, Toyoda M, Kobayashi Y : Anti-allergic effect of

apple polyphenol on patients with atopic dermatitis : a pilot study. Allergol Intl **49** : 69-73, 2000

9) Sato S, Watanabe C, Murakami K, Mimuro T, Tanaka Y : Effectiveness of apple polyphenols cream in treating pruritus in hemodialysis patients. (Abstract in Japanese) J Jpn Soc Dial Ther **32** : 886, 1999
10) Jones HE, Inouye JC, McGerity L, Lewis CW : Atopic disease and serum immunoglobulin-E. Brit J Dermatol **92** : 17-25, 1975
11) Furue M, Furukawa F, Hide M, Takehara K : Guidelines for therapy for atopic dermatitis 2004. (In Japanese with English abstract) Jpn J Dermatol **114** : 135-142, 2004
12) Katayama I, Tanei R, Yokozeki H, Nishioka K, Dohi Y : Induction of eczematous skin reaction in experimentally induced hyperplastic skin of Balb/C mide by monoclonal anti-DNP IgE antibody : possible implications for skin formation in atopic dermatitis. Int Arch Allergy Appl Immunol **93** : 148-154, 1990
13) Otoyama K : Evaluation of immuno-modulators on anti-DNP IgE antibody mediated biphasic cutaneous reaction in mice. (In Japanese with English abstract) Kitasato Med **24** : 24-30, 1995
14) Inagaki N, Nagao M, Igeta K, Kawasaki H, Kim JF, Nagai H : Scratching behavior in various strains of mice. Skin Pharmacol Appl Skin Physiol **14** : 87-96, 2001
15) Kano T, Taguchi S, Ishihama S, Yamanaka T, Terada T, Matsumiya T : Effects of grape seed extract on skin inflammation and pruritus in an animal model sensitized by monoclonal anti-DNP IgE antibody in ICR mice. J Tokyo Med Univ **65**(2) : 120-127, 2007
16) Miyachi Y : Photooxidative damade and superoxide dismutase activity in skin. Cytoprotection Cytobiol **5** : 226-229, 1988
17) The Nikka Distilling Co. Ltd., Japan : unpublished data, 1994
18) Takeuchi M, Fukaya Y, Abe M, Masutani M, Ueda H : Immunohistochemical localization of superoxide dismutase in normal human skin, psoriasis vulgaris and atopic dermatitis. Skin Res **34** : 7-14, 1992

牡蠣肉エキスの鎮痒作用

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【要旨】 代表的な市販鎮痒薬の抗ヒスタミン剤が奏功しない難治性掻痒の疾患のひとつに、慢性腎不全による血液透析患者の皮膚掻痒症 (uremic pruritus) があり、本症の多くは血中および表皮中の亜鉛濃度が低下している。このことから、血液透析患者の皮膚掻痒症には亜鉛補充療法が有効と考えられている。本研究では、亜鉛を多量に含む牡蠣肉エキス (OE : oyster extract) のアトピー性皮膚炎 (AD) に対する鎮痒作用について検討した。まず、OE の発痒モルモットモデルでの外用塗布試験による鎮痒作用 (実験 1)、次に、IgE 介在性の耳介皮膚炎症反応を伴う ICR 掻痒 (IgE-mIP-ICR) マウスモデルでの炎症と掻痒を内服試験で検討した (実験 2-4)。実験 1 の発痒モルモットモデル試験ではヒスタミン (HS) またはカリクレイン (KK) で発痒したモルモットで鎮痒作用を検討した。次に、実験 2-4 では、OE は 200 mg/kg (0.3% CMC 懸濁) を惹起物質 Dinitrofluorobenzene (DNFB) 塗布前に 1 週間連続経口投与し、対照には同懸濁液 OE 同様に DNFB 塗布前に 1 週間連続経口投与した。また、実験 2 では、IgE-mIP-ICR マウスモデルで 2 時間累積掻痒行動を確認すると共に被験物質の鎮痒作用を検討した。実験 3-4 では、同モデルでの炎症及び掻痒に対する 48 時間経時的変化および被験物質の作用を検討した。実験 1 では、OE は 1% (w/v) OE の生理食塩液、対照には溶剤の生理食塩液を発痒物質の皮内投与部位に投与直後塗布 (100 μl) した。実験 2-4 では、OE は 200 mg/kg (0.3% CMC 懸濁) を DNFB 塗布前に 1 週間連続経口投与し、対照には同懸濁液 OE 同様に DNFB 塗布前に 1 週間連続経口投与した。その結果、実験 1 では、OE は HS と KK の両発痒に対して有意に抑制した。そこで、OE 中の有効成分を探索する目的から OE の主成分であるタウリン (Tau : taurine)、グリコーゲン (Gly : glucogen) および有機亜鉛 (OZ : organic zinc) の各 1% (w/v) 含有の生理食塩液について、難治性掻痒の KK 発痒に対して検討した結果、OZ のみが有意な抑制を示した。実験 2 では、OE および OZ は DNFB 塗布後の 2 時間累積掻痒行動を共に有意に抑制し、鎮痒効果を示した。実験 3 では、OE および OZ について検討し、惹起 1, 24 時間後に二峰性の皮膚炎症を示す IgE-mIP-ICR マウスの炎症反応を有意に抑制した。実験 4 では、OZ が惹起 1, 24 時間後に二峰性の皮膚炎症を示す IgE-mIP-ICR マウスの 1 時間累積掻痒行動を有意に抑制した。

以上の結果より、OE およびその有効成分の OZ が AD 動物モデルの皮膚の炎症と掻痒のいずれも優位に抑制したことから、AD 患者の炎症と掻痒に有効な健康食品素材であると同時に、慢性腎不全の血液透析患者の皮膚掻痒症 (uremic pruritus) に有効である可能性を示した。

〈キーワード〉 牡蠣肉エキス、亜鉛、皮膚掻痒症、AD 動物、掻痒