Topical royal jelly alleviates symptoms of pruritus in a murine model of allergic contact dermatitis

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ABSTRACT

Background: Royal jelly is widely used as a health tonic, especially in Asia. Royal jelly is commonly used in cosmetics as well as in dietary supplements and beverages. Little is known, however, about the pharmacologic efficacy of topical royal jelly. Therefore, we investigated the antipruritic activity of topical royal jelly on chronic pruritus in experimental allergic contact dermatitis in mice. **Materials and Methods:** Hairless mice (HOS: HR-1), with chronic allergic contact dermatitis induced by 5 weeks of repeated application of 2,4,6-trinitro-1-chlorobenzene (TNCB) to the entire back skin were treated topically with royal jelly (0.01% or 1%) for 5 weeks after sensitization with TNCB. The effects of royal jelly on pruritus and inflammation were evaluated by measurement of scratching behavior and skin inflammation score, respectively. **Results:** Repeated application of TNCB to the back skin of mice elicited frequent scratching behavior immediately and 24h after challenge. Topical royal jelly (0.01% or 1%) and betamethasone (0.01%) significantly ameliorated this chronic pruritus throughout the experimental period. The level of nerve growth factor mRNA in back skin was increased in the mice with dermatitis and reduced by betamethasone, but not by royal jelly. **Conclusion:** The inhibitory effect of royal jelly on chronic pruritus may occur through different mechanisms from those of betamethasone. Topical application of royal jelly, as used in cosmetics, might be beneficial for the alleviation of chronic pruritus.

Keywords: Allergic contact dermatitis, chronic pruritus, hairless mice, royal jelly, topical application

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INTRODUCTION

Royal jelly is a secretion of the hypopharyngeal and mandibular glands of young worker honey bees (*Apismellifera*). It is a creamy, yellow-white, acidic material, slightly pungent in odor and taste. About half of its dry weight consists of protein; the other components are free amino acids, fatty acids, sugars, vitamins, and minerals. Recently, a protein with a molecular mass of 57,000 in royal jelly, called royalactin, was shown to induce the differentiation of honeybee larvae into queens. Royalactin increased body size and ovary development and shortened developmental time in honeybees.[1] Royal jelly is widely used as a health tonic, and it has a much larger market in Asia than in the USA or Europe. In Asia it is commonly found in cosmetics, dietary supplements, and beverages.

Many pharmacologic studies have confirmed that royal jelly has antitumor,[2] antifatigue,[3] and antihypertensive effects.[4] In contrast to human data, it was reported that oral royal jelly inhibits skin lesions in NC/Nga mice,[5] and suppresses allergic reactions by improving the Th1-Th2 balance in 2,4-dinitrophenylated keyhole limpet protein (DNP-KLH) immunized mice.[6] These studies were performed using oral, but not topical application, of royal jelly. However, oral royal jelly consumption has been linked to acute asthma, anaphylaxis, and death, especially in patients with asthma and atopic dermatitis(AD).[7-15]

The market for topical royal jelly, which is used in cosmetics and moisturizing agents, is very large, especially in Asia. Topical application of royal jelly causes fewer side-effects than oral intake. As royal jelly contains many kinds of nutrients such as amino acids, fatty acids, vitamins, and minerals, vital nutrients can be provided for maintaining and improving skin conditions by topical application. However, there is little information about the efficacy of topical royal jelly based on scientific evidence.

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We have all experienced the uncomfortable sensation of pruritus that causes us to scratch for relief. Often, scratching can intensify itching and cause further damage to the skin, a phenomenon termed the "itch–scratch cycle".[16,17] Chronic pruritus is associated with many diseases, the most common being chronic renal insufficiency, cholestatic liver diseases, and AD.[18,19] There are also many patients with pruritic diseases who experience pruritus without any skin lesions. Irrespectively of the cause of itching, skin dryness worsens each type of pruritus. Thus, all patients should regularly moisturize their skin.[20] As mentioned earlier, royal jelly is widely used in cosmetics and moisturizing agents in Asia. Therefore, we focused on the antipruritic effect of topical royal jelly.

In the present study, we investigated the antipruritic activity of topical royal jelly using a mouse model with moderate dermatitis and chronic pruritus.

MATERIALS AND METHODS

Animals

All experiments and procedures were approved by the Chiba University Institutional Animal Care and Use Committee.

Female hairless mice (HOS: HR-1, 6 weeks of age) were obtained from Japan SLC Inc. (Hamamatsu, Japan) and housed under controlled lighting (0700–1900h) and temperature (24°C) conditions with food and water available *ad libitum*. All animals were maintained under these conditions for 1 week before the start of experiments.

Reagents and drugs

Protease-treated royal jelly (royal jelly) was supplied as a dried powder by Yamada Bee Farm Corporation (Okayama, Japan). Betamethasone valerate (betamethasone) were obtained from Sigma Chemical Co. (St. Louis, MO). Hydrophilic ointment was obtained from Maruishi Pharmaceutical Co. (Osaka, Japan). 2,4,6-trinitrochlorobenzene (TNCB) was obtained from Tokyo Chemical Co. (Tokyo, Japan) and used for both sensitization and elicitation of response.

Sensitization and challenge procedure

The experimental protocols employed are illustrated in Figure 1.

TNCB was dissolved in acetone/olive oil (3:1). On day -7, hairless mice were sensitized by application of 100μl 1.2% TNCB solution to the entire back skin, and on day 0 animals were challenged with 50μl/10g body weight 1.5% TNCB solution. TNCB was then applied repeatedly to the same site every second day until day 32.

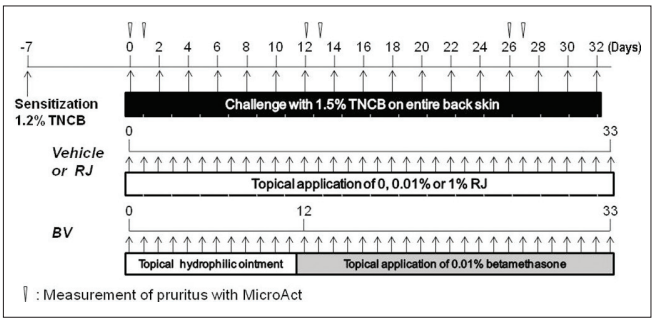

Figure 1: Schedule for the elicitation of chronic allergic contact dermatitis and application of reagents to test the effect of topical application of royal jelly RJ: royal jelly; BV: betamethasone valerate

Acetone/olive oil (3:1) was repeatedly applied as a negative control.

Drug treatment

Royal jelly (0.01 and 1mg/site/day) and betamethasone (0.01mg/site/day) were mixed in hydrophilic ointment at concentrations of 0.01, 1, and 0.01wt%, respectively. Vehicle (hydrophilic ointment) and royal jelly were applied daily at 100μl to the entire back skin from day 0 to 33, whereas betamethasone was applied daily from day 12 to 33. On the day of TNCB challenge, each drug was administered 4h prior to the TNCB challenge.

Evaluation of skin inflammation

To test the effectof topical royal jelly, the severity of dermatitis was evaluated on days -7, 1, 5, 13, 19, 27, and 33 in accordance with the scoring criteria described below. The inflammation score (minimum 0, maximum 12) was defined as the sum of the individual scores, graded as: 0, no symptoms; 1, mild; 2, moderate; 3, severe, for each of the four symptoms of edema, erythema/hemorrhage, excoriation/erosion, and scaling/dryness.

Evaluation of pruritus

Pruritus was evaluated by automatic counting of the bouts of scratching behavior using MicroAct (Neuroscience Inc., Tokyo, Japan) as reported previously.[21]

The scratching bouts were counted for 2h immediately after and 24h after TNCB challenge.

Histopathological analysis

After evaluation of pruritus on day 33, skin specimens were fixed in 10% buffered formalin and immersed in a series of 10%, 20%, and 30% sucrose, followed by embedding in Optimal Cutting Temperature (OCT) compound (Sakura Fine Technical Co., Ltd., Tokyo, Japan) for histological examination. Frozen sections 8 μm thick were then cut, thoroughly rinsed, and stained with hematoxylin–eosin for standard histopathological observation.

Expression of nerve growth factor mRNA in skin

To evaluate the effect of topical royal jelly, the back skin of hairless mice was sampled after evaluation of pruritus on day 33. Each specimen was homogenized, and the total ribonucleic acid (RNA) was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany). Complementary deoxyribonucleic acid (cDNA) was prepared from RNA by reverse transcription using PrimeScript RT reagent Kit (Takara Bio Inc., Shiga, Japan). Real-time quantitative polymerase chain reaction (PCR) was performed using a Step One TM Real Time PCR system (Applied Biosystems Inc., Carlsbad, CA) using SYBR Premix Ex Taq for mouse glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) (forward 5'-AACGACCCCTTCATTGAC-3' and reverse 5'-TCCACGACATACTCAGCAC-3') and mouse nerve growth factor (NGF) (forward 5'-TGCCAAGGACGCAGCTTTC-3' and reverse 5'-TGAAGTTTAGTCCAGTGGGCTTCAG-3') in accordance with the manufacturer's instructions (Takara Bio Inc.). Results are expressed as the relative mRNA level corrected with reference to GAPDH mRNA as an internal control.

Statistical analysis

All data are presented as mean±SEM. Statistical significance was analyzed using Dunnett's method for multiple comparisons or the Student's *t*-test. Differences at $P<0.05$ were considered statistically significant. All statistical analysis was conducted using StatLight software (Yukms Co. Ltd., Tokyo, Japan).

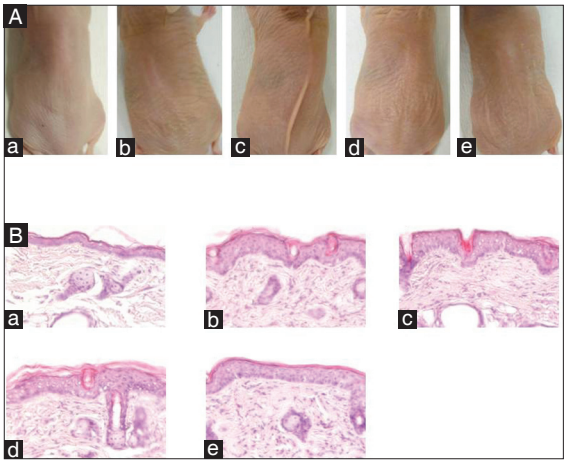

Figure 2: Surface features. (A) and a section of back skin stained with hematoxylin-eosin. (B) Hairless mice were sensitized with 100µl of 1.2% TNCB over the entire back skin, and then 50µl/10g body weight of 1.5% TNCB solution or acetone/olive oil (3:1) (as a negative control) was repeatedly applied to the same site every second day, from day 0 to day 32. Vehicle (hydrophilic ointment) and royal jelly were applied daily from day 0 to day 33, whereas betamethasone was applied daily from day 12 to day 33. This photograph of the mice was taken on day 33. (a): acetone/olive oil (Nil); (b): TNCB-treated (Veh); (c): royal jelly (0.01%); (d): royal jelly (1%); (e): betamethasone valerate (0.01%)

RESULTS

Effects of topical royal jelly on inflammation score

Repeated application of TNCB induced mild skin lesions on the back skin of the treated mice, and the inflammation score of TNCB-treated vehicle control animals remained less than 4 on day 33 [Figures 2 and 3]. Topical application of royal jelly and betamethasone did not significantly affect the inflammation score throughout the experimental period [Figure 3].

Effects of topical royal jelly on number of scratching bouts

Repeated application of TNCB evoked a significant increase in the number of scratching bouts in the vehicle group, compared with acetone/olive oil-treated mice (Nil), and the scratching count reached 330 immediately after the challenge on day 26. Repeated topical application of royal jelly suppressed this increase, and significant suppression was observed immediately after the challenge on days 12 and 26, and also 24h after the challenge on day 12 [Figure 4]. Topical application of betamethasone also significantly suppressed the number of scratching bouts immediately after the challenge on day 12, and 24h after the challenge on day 12.

Effects of topical royal jelly on ngf mrna level

To investigate the mechanism of the antipruritic effect of royal jelly, we determined the levels of NGF mRNA in lesional back skin of hairless mice. The level of NGF was increased about 2.5-fold relative to the vehicle-treated control group. Topical royal jelly did not affect this increase [Figure 5]. In contrast, topical betamethasone decreased the level of NGF mRNA.

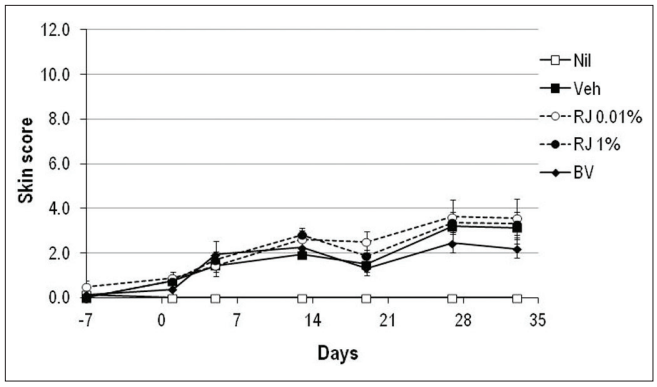

Figure 3: Effect of royal jelly on clinical score of skin symptoms induced by repeated application of TNCB. Vehicle (hydrophilic ointment) and royal jelly were applied daily from day 0 to day 33, whereas betamethasone was applied daily from day 12 to day 33. Skin score was determined at 24h after application of TNCB. Nil: acetone/olive oil treatment; Veh: TNCB treatment; RJ: royal jelly (0.01% and 1%); BV: betamethasone valerate (0.01%). Values represent mean±SEM (n=7-8)

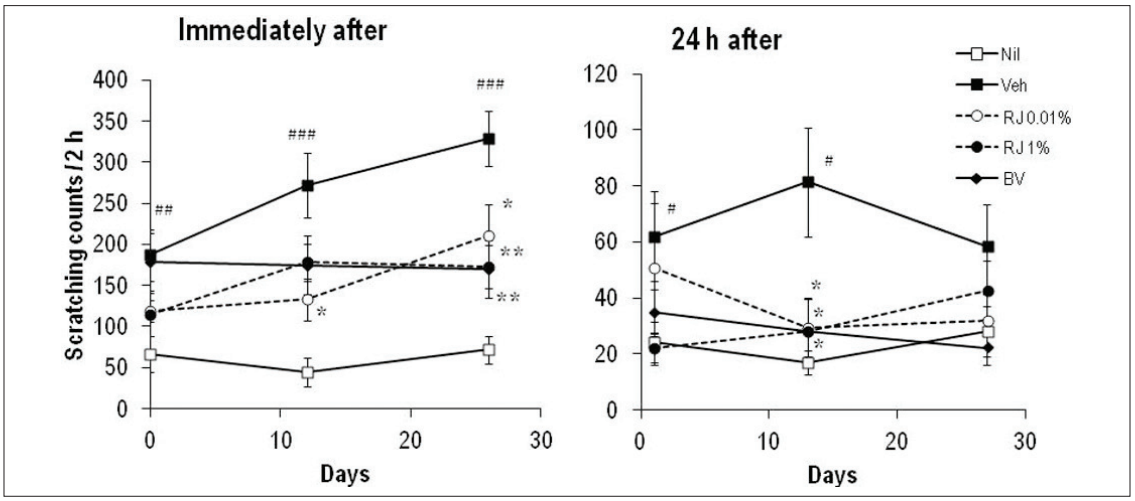

Figure 4: Effect of royal jelly on scratching counts induced by repeated application of TNCB. Vehicle (hydrophilic ointment) and royal jelly were applied daily from day 0 to day 33, whereas betamethasone was applied daily from day 12 to day 33. The number of bouts of scratching behavior was counted for 2h immediately after and 24h after TNCB challenge. Nil: acetone/olive oil treatment; Veh: TNCB treatment; RJ: royal jelly (0.01% and 1%); BV: betamethasone valerate (0.01%). Values represent mean±SEM (n = 7-8). #P <0.05, ##P <0.01, ###P <0.001 compared with Nil (Student's t-test), *P <0.05 compared with vehicle (Dunnett's test)

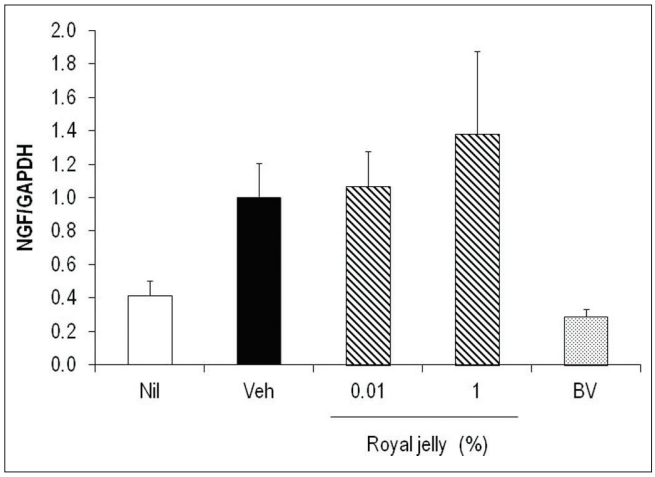

Figure 5: Effect of royal jelly on NGF mRNA levels in mouse skin. Expression of NGF mRNA levels were determined by real-time quantitative PCR, and normalized to the corresponding GAPDH mRNA levels. Nil: acetone/olive oil treatment; Veh: TNCB treatment; BV: betamethasone valerate (0.01%). Values represent mean±SEM (n = 6-8)

DISCUSSION

There are many kinds of cosmetics that contain royal jelly. For example, they are foundations, toners, moisturizing lotions, creams, emulsions, beauty essences, face wash, etc. Because these products are not used for medication, but are used for skincare, we investigated the effect of topical application of royal jelly using a mouse model with moderate dermatitis and chronic pruritus rather than severe dermatitis. We created the pruritus model, which shows continuous scratching behavior at 24h after TNCB challenge with moderate dermatitis, by adjusting the type of ointment used and the timing between the application of ointment and TNCB. Topical royal jelly had a significant antipruritic effect in this model both at low (0.01%) and high (1%) concentrations; this effect was equivalent to that of betamethasone. The concentration of royal jelly used in this study was almost equivalent to the concentration in cosmetic products on the market if the concentration of extract was converted to the weight of dry powder. These findings show that topical application of royal jelly in cosmetics may have an inhibitory effect on chronic pruritus.

Pruritus is defined as an sensation that, if sufficiently strong, will provoke scratching or the desire to scratch.[22] It is the most common symptom in dermatology, one that can occur with or without concomitant visible skin changes. A new, two-step classification of pruritus has recently been proposed by the International Forum for the Study of Itch (IFSI).[23] The IFSI classification distinguishes three groups of pruritic individuals. The second group is termed "pruritus on primary nondiseased, noninflamed skin". This term has multiple interpretation, such as pruritus without underlying origin, pruritus without any skin changes, pruritus in systemic diseases without any initially visible skin changes, pruritus characterized by the "absence of specific cutaneous lesions of an itching dermatosis" or even pruritus in the elderly. For the treatment of pruritus on noninflamed skin of this group, corticosteroids such as betamethasone are not suitable. Irrespectively of the cause of itching, these patients should regularly moisturize their skin. Therefore, the cosmetics and moisturizing agents, which contain royal jelly, may be helpful for the treatment of pruritus classified in this group.

The ends of primary afferent C fibers are mainly distributed

in the basal laminae of normal skin. However, these nerve fibers are observed at higher densities in the epidermis of skin of patients with AD. It was reported that NGF, which is released from keratinocytes in the skin and plays an important role in the extension of nerve fibers, is significantly increased in AD patients.[24-26] The sensory nerve fiber sprouting into the epidermis induced by NGF decreases pruritus threshold in AD skin.[27,28] Moreover, a similar pathologic condition was also reported in the lesional skin of NC/Nga mice.[28-30] Therefore, we focused on the production of NGF to investigate the mechanism of the antipruritic effect of topical application of royal jelly. After repeated application of TNCB, skin NGF mRNA was significantly increased on day 33. Betamethasone reduced this increase in NGF mRNA expression; this represents one of the mechanisms of the antipruritic effect of betamethasone. In contrast, topical royal jelly did not have any clear effect on the expression of NGF mRNA. Therefore, we assumed that the antipruritic effect of topical royal jelly is due to a different mechanism from that of betamethasone. Although further investigation is needed to clarify the mechanism of the antipruritic effect of topical royal jelly, it seems that topical application of royal jelly, as used in cosmetics, might be beneficial for the alleviation of chronic pruritus.

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