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Original Article / 원저

The effect of *Periostracum Cicadae* on capsaicin-induced model of atopic dermatitis in rats

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Capsaicin으로 유도된 아토피 피부염 rat model에서 선태의 효과

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Abstract

Objectives : 선태는 아토피 피부염에서 소양증 완화를 위해 사용되고 있다. 본 연구에서는 면역계 및 신경계 손상을 일으킨 rat model에서 선태 추출물이 소양증 완화에 효과가 있는지 알아보고자 한다.

Methods : 출생 48시간 이내의 rat을 대상으로, capsaicin(50 mg/kg)을 피하 투여하였다. 임의로 선정된 12마리의 실험군에 3주 동안 선태 추출물(0.5g/kg)을 매일 경구 투여하였다. 이후 scratching behavior 와 dermatitis score를 측정하였다.

Results : 선태 투여군과 대조군에서 scratching number 와 dermatitis score의 차이가 없었다.

Conclusions : 위의 결과로부터 capsaicin으로 유발한 아토피 피부염 rat model에서 선태의 소양증 완화 효과가 없다는 것을 알 수 있었다. 아토피 피부염의 효과적인 치료를 위해 면역계 뿐만 아니라 신경계 손상 회복시키는 약물을 찾기 위한 더 많은 연구가 필요할 것으로 생각된다.

Key words : Pruritus; Atopic dermatitis; Rat model; Capsaicin; *Periostracum Cicadae*

Ⅰ. Introduction

Pruritus is the unpleasant sensation that strongly provokes the desire to scratch[1]. Although pruritus is an essential characteristic of atopic dermatitis and greatly impacts the quality of the patient's life, its pathophysiology is not completely understood. The interactions between the central and peripheral nervous system are related with pruritus in atopic dermatitis. Triggering factors induce the release of mediators (histamine, neuropeptides, tryptase and so on) from directly sensory nerve or indirectly mast cell and keratinocytes. The activation of itching related receptors by the mediators stimulates peripheral C fiber nerve endings of primary afferent neurons in the epidermis or dermis. And then the activated C fiber nerve endings are responsible for the transmission of the pruritic information to the central nervous system via dorsal root ganglia and spinal cord[2-4].

Likewise, because the pathophysiology of pruritus in atopic dermatitis is complex, it is difficult to set up an animal model similar to human atopic dermatitis. Generally, herb medicines have therapeutic effects by affecting various pharmacological actions. Therefore, many researchers who study the effects of herbs on atopic dermatitis mostly use skin sensitized model among several animal models because the models is induced by more complicated causes than targeted transgenic or knockout mouse model. In this study, we use an atopic dermatitis like phenotype in rats by capsaicin injection, one of the skin sensitized models. This model is distinctive because in this model, pruritus is caused by the manipulation of neuronal pathway which is neuronal damage, C-fiber degeneration, along with immunological abnormality[5].

Periostracum Cicadae is the cast-off shell of insects *Cryptotympana atrata* and *Cryptotympana dubia* in the family of *Cicadae*. It has been widely used for the treatment of pruritus, eczema, and atopic dermatitis in traditional korean medicine. Pharmacological studies on *Periostracum Cicadae* demonstrated biological properties such as antioxidant and anti-inflammatory activities[6-8]. Although there have been studies which prove the anti-inflammatory effect of *Periostracum Cicadae*, it is unknown whether *Periostracum Cicadae* has an effect on pruritus of atopic dermatitis like lesions in rats with both immunologic and neuronal damages. Therefore, in this study, we attempt to investigate a therapeutic effect of *Periostracum Cicadae* on pruritus in an atopic dermatitis-like phenotype in rats treated neonatally with capsaicin.

Ⅱ. Materials and Method

A. Materials

1. Preparation of Periostracum Cicadae extract

Periostracum Cicadae(china) was purchased from an oriental drug store (hepy, Jecheon-si,

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Chungbuk, Korea). The 2.4kg of dried *Periostracum Cicadae* was soaked in 24L of water for 24hr and then boiled for 2 hours. After filtering, it was concentrated and dried freeze for 5 days (6.25% yield, 150g). This lyophilized *Periostracum Cicadae* extract powder was then dissolved in distilled water.

2. Animals

One week prior to parturition, pregnant Sprauge-Dawley rats were gotten from Sam Tako (Osan-si, Gyeonggi-do) and delivered to laboratory and only male pups were used in this study. In order to raise all animals, a room was maintained on a 12 h light/dark cycle (light on at 08:00 h) and at 22-25 ℃, with free access to food and water. All experiments were approved by the Korea University College of Medicine Animal Research Policies Committee (KUIACUC-2009-134).

B. Methods

1. Classification of experimental groups

New born male rats were randomly divided into two groups : capsaicin (Cap)-injected, vehicle (Veh)-injected. Then capsaicin-injected rats were randomly assigned to the *Periostracum Cicadae* administration group (Drug group, n=12) and the control group (Drug-CON group, n=7).

2. Induction of Atopic dermatitis-like lesion

Atopic dermatitis-like lesion was induced by subcutaneous injection of 10 μl Capsaicin (0.5g/kg, 10% ethanol, 10% tween-80, 80% saline) in the dorsal part of rat pups within 48hours after birth[5]. To confirm whether the capsaicin injection worked properly, two nociceptive tests were carried out as previously reported[9]. We examined the expression of transient receptor potential vanilloid 1 (TRPV1) in S1 dorsal root ganglia (DRG) by immunologic staining at the point of three weeks after birth. Also, the tail-withdrawal latency time in response to noxious infrared radiant heat (IR90, IITC) stimulus was measured.

3. Oral administration of *Periostracum Cicadae*

Freeze dried *Periostracum Cicadae* extract powder was diluted with saline. From the first day of experiment, the solution was orally given daily for three weeks (0.5g/kg).

4. Scratching behavior

Once a week, the scratching behavior was observed in separate acryl chambers (36×22×15 ㎝) equipped with a mirror placed behind the chamber after 30 min for adjustment. Without presence of humans, scratching behavior of rats were recorded by a digital camcorder (Sony, Tokyo, Japan) for an hour and the video file was transferred to a computer in the lab and analyzed to quantify the rats' scratching behavior. A bout of recurring scratching strokes by the hind paw was regarded as one scratch.

5. Scoring of cutaneous lesion

The dermatitis score was measured once a week by the criteria (hair loss/ flare, abrasion/ bleeding, scarring) as previously reported[9](Table 1). For each different body part, a dermatitis rating scale was devised as follows: for head and

face 0 points (normal), 1 point (wispy fur), 2 points (alopecia and flare), and 3 points (bleeding or ulcerative lesion) / for ear 0 points (normal), 1 point (flare), 2 points (bleeding), and 3 points (loss of part of the ear due to scratching behavior). Skin lesion extent was roughly calculated with a standard extent being 25mm^2, because the average skin lesion was within 25(5㎜ ×5㎜)㎟, and multiplied by the severity of dermatitis lesion. The summed score was adopted as the dermatitis score for each rat.

6. Body weight

Body weight was measured weekly.

7. Statistical analysis

All data are presented as mean ± SEM. Statistical significance was analyzed using Student's t-test. A difference of $P < 0.05$ was considered to be statistically significant. All statistical analyses were performed using Sigma Stat (ver. 3.5, Systat Software Inc., IL, USA).

Ⅲ. Result

A. TRPV1 expression and tail-withdrawal latency

To confirm whether the capsaicin injection worked effectively, we examined transient receptor potential vanilloid 1 (TRPV1) expression level and tail-withdrawal latency time in response to noxious infrared heat. As shown in Figure 1A, in Cap-injected rats, TRPV1 expression in DRG neurons was decreased. In addition, in the tail-flick test, we observed that latency time in

Table 1. Evaluation of Cutaneous Lesion

Score	Skin condition	
	Face	Ears
0	Normal	Normal
1	Wispy fur	Flare
2	Alopecia and flare	Bleeding
3	Bleeding or ulcerative lesion	Loss of part of the ear

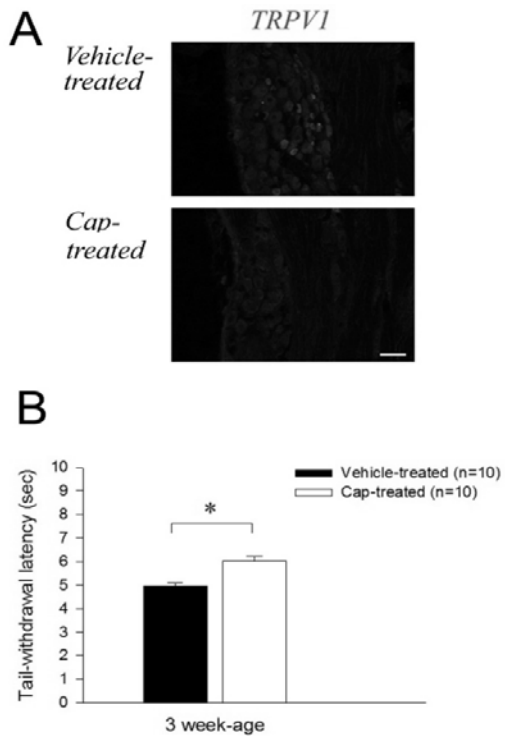

Fig. 1. The decrease of TRPV1-expressing DRG neurons and damaged nociception after capsaicin injection in neonatal rats. A. Few TRPV1-expressing neurons in the S1 DRG were shown in the capsaicin (Cap)-injected animals, compared to vehicle treated rats. B. In terms of the tail-withdrawn latency time, responding to a noxious infrared radiant heat(IR90, IITC) stimulus, the latency time was noticeably longer in the Cap-injected rats than the vehicle treated rats. *P<0.01 (t-test). Scalebar=100㎛.

Cap-injected rats (6.04±0.19 sec) is longer than that in Veh-injected rats (4.98±0.10 sec) (Fig. 1B).

B. Scratching behavior

After 3 weeks of *Periostracum Cicadae* administration, there was no significant difference in the number of scratches between Drug group and Drug-CON group (Table 2, Fig. 2A).

C. Dermatitis score

The skin lesion of Cap-injected rats are similar to those of atopic dermatitis. Cutaneous lesions were featured by prototypical dermatitis, such as alopecia, dryness, superficial erosion, deep excoriation, bleeding, and scarring. In three weeks after capsaicin injection, mild dermatitis appeared and between the 3-week and 6-week, symptoms of dermatitis got worse over time in all groups. There was a tendency to have lower dermatitis score in Drug group compared to Drug-CON group. However, there was no statistically significant difference ($P < 0.05$) (Table 3, Fig. 2B).

Table 2. Mean Values of Scratching Numbers After Drug (*Periostracum Cicadae*) Treated

	Drug group*	Drug-CON group†
Scratching numbers		
3 weeks	56.08±9.01	47.29±8.58
4 weeks	80.58±10.37	87.29±19.49
5 weeks	108.25±16.51	71±15.26
6 weeks	120.92±21.46	112.71±16.77

* Drug group: capsacin injection + administraion of *Periostracum Cicadae* extract
†Drug-CON group: capsacin injection + no treatment

D. Body weight

During the experiment, all rats (n=19) survived and their body weight increased consistent with their age. There was no notable difference between the groups and the Drug and Drug-CON rats looked clinically similar (Table 4, Fig. 2C).

Ⅳ. Discussion & Conclusion

Atopic dermatitis is one of the most common skin diseases and is mainly featured by severe pruritus, xerosis and visible eczematous skin lesions. Since the disease progresses chronically

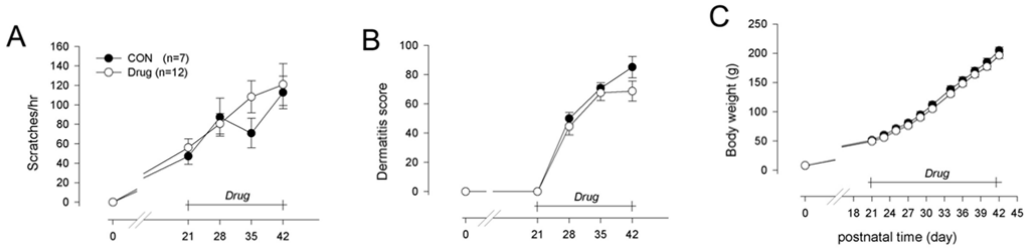

Fig. 2. The effect of *Periostracum Cicadae extract* (drug) on both the scratching behavior and atopic dermatitis-like skin lesion induced by neonatal capsaicin treatment.

A. The effect of drug on the scratching behavior. B. The effect of drug on the skin lesion. C. The effect of drug on weight gaining.

Table 3. Mean Values of Dermatitis Score After Drug (*Periostracum Cicadae*) Treated

	Drug group	Drug-CON group
Dermatitis score		
3 weeks	0±0	0±0
4 weeks	44.5±5.73	50±4.11
5 weeks	67.58±5.28	70.71±3.71
6 weeks	68.75±6.92	85.14±7.31

Table 4. Mean Values of Body Weight After Drug (*Periostracum Cicadae*) Treated

	Drug group	Drug-CON group
Body Weight		
0 weeks	8.00±0.25	7.86±0.34
3 weeks	49.42±2.59	51.43±3.72
4 weeks	90.42±4.14	94.71±5.48
5 weeks	147.75±4.16	153.57±5.95
6 weeks	196.50±5.30	204.86±5.83

and exerts a profound impact on the quality of life of patients and their families, many researchers have tried to develop therapeutic agents[10]. Recently, the use of natural products in the treatment of atopic dermatitis and the relief of symptoms, the pruritus particularly, has aroused great interest.

Periostracum Cicadae has been commonly used for treating dermatologic inflammation accompanying pruritus in traditional korean medicine. Its taste is sweet and character is cold. It is regarded as relating to lung, liver and eliminating wind and heat and so treats skin eruption, eye disease, allergic disease, convulsions and cancer[11,12]. Chemical studies have shown that it contains considerable amount of chitin, N, protein, organic acid, phenols, flavonoids, sterols, saccharide, lipid, essential oils, amino acids, ethanolamine and 24 different trace elements such as Al, Fe, Ca, Mg, P, Zn. Chitin is the precursor of chitosan and known to have anti-inflammatory effect. N-acetyldopamine dimmers extracted from *Periostracum Cicadae* were also proved to have antioxidant and anti-inflammatory effects[8,13]. In addition, although clinical trials have not yet been performed, animal models with atopic dermatitis proved the therapeutic effect of *Periostracum Cicadae*[14,15].

There are three types of atopic dermatitis mouse models : skin sensitized models, transgenic and knockout mouse models, and Spontaneous mouse models (NC/Nga mice). Since genes between rats and humans are not perfectly matched despite of considerable similarities, it is difficult to establish exactly the same models with human atopic dermatitis[16,17]. Researchers who study medicinal herbs mostly choose skin sensitized models. Since medicinal herbs express the therapeutic effect through various pharmacological actions, it is more suitable to use a animal model induced by complicated causes than specifically targeted transgenic and knockout mouse models. To make skin sensitized models, chemicals such as DNCB (dinitrochloro benzene), DNFB (dinitro fluorobenzene), PiCl (picryl chloride), and antigens such as house dusts, mite extract, OVA(ovalburnin) are used.

In this study, we set up skin sensitized-atopic

dermatitis rat model induced by subcutaneously injected capsaicin in neonatal rats. Since this model induces C-fiber degeneration, it is possible to verify whether the therapeutic effect of *Periostracum Cicadae* on atopic dermatitis is due to the regeneration of injured nerves apart from the immunologic mechanism. Capsaicin injection to newly born animals can make neurodegenerative change along the sensory pathway. The neurodegenerative change has been reported to possibly reach dorsal horn of spinal cord and spinothalamic tract, nucleus gracilis, and even sensory area in cerebral cortex. Once the neurodegenerative changes happen, an enormous amount of neuropeptides like Substance P is discharged, resulting in depletion of the neuropeptides in nerve endings which consequently brings in a long-term neurotransmission injury[18]. According to previous studies[5], we decided proper capsaicin dose, capsaicin administration method, experiment period and the number of rats. In addition, this model has the merit of no need for repetitive sensitization and excellent reproducibility even though pruritus usually goes through self-remission. Hence, atopic dermatitis similar to that of human can be obtained during the course of dermatitis[5,19,20].

It is well known that the senses of pain and itch share a common nervous transmission pathway and are evoked by hypersensitive peripheral or central nervous system. The ascending tract for nociception crosses over the center of the spinal cord and projected to sensory area of cerebral cortex and thalamus. Itching sensation pass a similar tract and the antagonism between pain and itch is known. Because of their antagonistic relationship of pain and itch, repeated noxious heat and scratching inhibit itch sensation[21,22]. Capsaicin injected rats feel less pain because of C fiber damage and feel itchy as an antagonistic action.

Although there have been studies to prove the therapeutic effect of *Periostracum Cicadae* on DNCB or DNFB induced dermatitis mice[14,15], there is no report of using capsaicin induced atopic dermatitis model in rats. Therefore, in this study, we set up an atopic dermatitis-like phenotype in rats by capsaicin injection and evaluated the therapeutic effect of *Periostracum Cicadae* on inhibiting pruritus by examining the changes in the scratching numbers and severity in dermatitis before and after the treatment. To clarify whether capsaicin injection was effective, we performed TRPV1 expression level tests and tail-withdrawal latency test in response to noxious infrared radiant heat. TRPV1 is largely in peripheral sensory neurons that are important for the detection of painful stimuli and activated by painful stimuli such as protons, ethanol and noxious heat (〉 42℃)[23,24]. As shown in our results, TRPV1 expression level was decreased and tail-withdrawal latency time was longer in capsaicin injected rats. This result indicates that capsaicin injected rats feel less pain and by antagonistic relationship between pain and itch, pruritus is induced. To investigate the anti-pruritic effect of *Periostracum Cicadae*, we examined the change of scratching behavior and dermatitis score after feeding *Periostracum Cicadae*. However, there was no statistically significant changes between *Periostracum Cicadae*

administered rats and control group. Although several studies proved that *Periostracum Cicadae* has anti-inflammatory effect, we did not obtain meaningful results in this study. This is because that the animal model in this study was induced by not only immunologic abnormality but also neurologic damage. It seems that the anti-inflammatory effect of *Periostracum Cicadae* might have been insufficient to suppress the itch sensation caused by neurologic damage. On the other hand, in our previous study, we proved that electoacupuncture alleviates pruritus in capsaicin-induced rat model of atopic dermatitis via the release of dynorphin[9]. Further studies need to find medicinal herbs having both anti-inflammatory and analgesic drug effect as the effect of electroacupuncture. Among medicinal herbs, *Coptis japonica*, *Scutellaria baicalensis*, *Cicadae periostacum*, *Sophorae radix*, *Rehmanniae radix*, *Lithospermi radix*, *Lonicera japonica*, *Kochiae fructus,* Xiao-Feng-San are known to inhibit peripheral itch related to histamine release and so they may be less effective in this model[25,26]. Whereas C*haenomeles sinensis, Asiasarum sieboldi, Brassica juncea, Evodia officinalis, Aremarrhenae asphodeloides, Bufo bufo gargarizans, Gardemia jasminoides, Piperlongum, Carthamus tinctorius, Piprus nigrum, Siegesbeckia glabrescens, Magnolia officinalis* are studied to have both analgesic and anti-inflammatory activities[27] and so they may be effective in this animal model. During the experiment, no statistically remarkable difference in the body weight measured in each groups. This implicates that capsaicin injection, or administration of *Periostracum Cicadae* extract didn't have a harmful effect on rat's general medical condition.

In summary, we report the first animal study undertaken to explore the anti-pruritus efficacy of *Periostracum Cicadae* on atopic dermatitis-like phenotype in rats induced by capsaicin injection. Although our results indicate that *Periostracum Cicadae* have no efficacy on atopic dermatitis induced by neurologic damage, further researches need to be performed to find medicinal herbs recovering nerve damage as well as alleviating symptoms of atopic dermatitis. So far, the researches about itching and pain have been conducted separately. However, considering common nervous transmission pathway between pain and itching, it would be more progressive if the two different sensations are studied integrally. Also, studies about atopic dermatitis need to take into account skin, immune system, nervous system, and nerve endings synthetically.

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