

MODULATORY EFFECT OF PERIOSTRACUM CICADAE AND BETULAE CORTEX EXTRACTS ON THE ACTIVATION OF ATOPIC DERMATITIS-RELATED ION CHANNELS ORAI1 AND TRPV3

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**Abstract**

**Background:** The cast-off shells of *Cryptotympana pustulata* (Periostracum Cicadae, PC) and the bark of *Betula platyphylla* (Betulae Cortex, BC) are used as traditional medicines for the treatment of skin diseases. This study was conducted to investigate the regulatory effects of PC and BC extracts on the activation of the ion channels, calcium release-activated calcium channel protein 1 (ORAI1) and transient receptor potential cation channel subfamily V member 3 (TRPV3).

**Materials and Methods:** Human HEK293T cells, co-overexpressing ORAI1/stromal interaction molecule 1 (STIM1) or overexpressing TRPV3, were treated with PC or BC extracts at 0.1 mg/mL. The changes in ORAI1 and TRPV3 activities were measured using a conventional whole-cell patch-clamp technique.

**Results:** PC and BC extracts significantly decreased ORAI1 activation in ORAI1-STIM1 co-overexpressing HEK293T cells and significantly increased TRPV3 activation in TRPV3 overexpressing cells, compared to that of 2-aminoethoxydiphenyl borate (2-APB, 100  $\mu$ M), a known agonist of TRPV3.

**Conclusion:** Our results suggest that PC and BC extracts have therapeutic potential to improve skin barrier abnormalities in atopic dermatitis via modulation of ORAI1 and TRPV3 activation.

**Keywords:** *Cryptotympana pustulata*; *Betula platyphylla* var. japonica; calcium channels; modulatory effect

**Abbreviations:** AD, atopic dermatitis; BAPTA, 1,2-Bis(2-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid; BC, Betulae Cortex; LTC4, leukotriene C4; NFAT, nuclear factor of activated T cells; ORAI-1, calcium release-activated calcium channel protein 1; PC, Periostracum Cicadae; TG, transglutaminase; TRPV3, transient receptor potential cation channel subfamily V member 3; TSLP, thymic stromal lymphopoietin; 2-APB, 2-aminoethoxydiphenyl borate; STIM1, stromal interaction molecule 1; IP<sub>3</sub>, inositol 1,4,5-trisphosphate.

**Introduction**

Periostracum Cicadae (PC), the cast-off shell of *Cryptotympana pustulata*, is used as an antifebrile, antiphlogistic, and spasmolytic agent, and is mainly prescribed for the treatment of allergic rhinitis in traditional

medicine (Yen et al., 2015). In addition, PC has been reported to have anti-inflammatory effects in the treatment of contact dermatitis (Kim et al., 2014), allergic rhinitis (Yen et al., 2015), and nephropathy (Shen et al., 2016).

Betulae Cortex (BC), the bark of *Betula platyphylla* var. japonica, is traditionally used for the treatment of dermatitis and chronic bronchitis, and its anti-osteoarthritis, anti-allergy, and anti-inflammatory effects have been studied (Oh et al. 2012).

In the past, skin barrier abnormalities were considered epiphenomena caused by inflammation and scratching, but it is now believed that skin barrier defects are important contributors to atopic dermatitis (AD) progression (Boguniewicz and Leung, 2011). Therefore, the prevention and treatment of AD should focus on skin barrier recovery and hyper-immunity suppression.

To form the skin barrier and activate T cells, an increase in intracellular calcium ions ( $\text{Ca}^{2+}$ ), via routes such as transient receptor potential cation channel subfamily V member 3 (TRPV3) and calcium release-activated calcium channel protein 1 (ORAI1), is a key factor leading to the initiation of intracellular signaling. TRPV3 is highly expressed in keratinocytes, and TRPV3 activation can mediate their terminal differentiation to form the skin barrier. ORAI1 is a major mechanism for intracellular  $\text{Ca}^{2+}$  increase in non-excitabile cells, T lymphocytes, and mast cells, which initiate the hyperimmune response (Lacruz and Feske, 2015). Therefore, agents that regulate these ion channels have been considered recently as novel therapeutics for alleviating AD (Di Capite et al., 2011).

Therefore, we investigated the modulatory effects of PC and BC extracts on the activation of the ion channels, ORAI1 and TRPV3.

## Materials and Methods

### Preparation of PC and BC extracts

The voucher specimens of dried cast-off shells of PC and BC bark were deposited in the Herbarium of the College of Korean Medicine under the registration numbers, BAE2015-PC and BAE2015-BC. PC and BC (200 g each) were extracted with 2 L 70% methanol for 3 h, filtered through Whatman Grade 1 filter paper (Sigma-Aldrich, St. Louis, MO, USA), and then lyophilized (yield: 1.6% and 8.9%, respectively). For experimentation, each lyophilized extract was dissolved in distilled water.

### Cell Culture

HEK293T (ATCC, Manassas, VA, USA) cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin (Life Technologies, Carlsbad, CA, USA). Cells were maintained in a humidified incubator containing 10%  $\text{CO}_2$  and 20%  $\text{O}_2$  at 37 °C.

### Transient Transfection and Treatment

HEK293T cells were transfected with a mammalian expression vector (pcDNA3.1; Life Technologies) containing TRPV3 (pReceiver-M02; GeneCopoeia, Rockville, MD, USA), hORAI1 (Origene Technologies, Rockville, MD, USA), or hSTIM1 (human stromal interaction molecule 1, Origene Technologies) genes using Lipofectamine Plus reagent (Life Technologies), according to the manufacturer's instructions. In all transfection studies, we co-transfected the HEK293T cells with a pEGFP-N1 plasmid (pcDNA3.1 construct: pEGFP-N1, 9:1) to allow selection of positive transfectants by green fluorescent protein expression. The cells were treated with PC and BC 70% methanol extracts at 0.1 mg/mL.

### Electrophysiology

To determine the effect of BC and PC on ORAI1 and TRPV3 activity, whole-cell patch clamping was used (Nam et al., 2016, 2017). All experiments were performed at room temperature. Briefly, cationic currents were recorded using an Axopatch 200B amplifier, and the acquired data were digitized using a Digidata 1440A (Molecular Devices, Sunnyvale, CA, USA), with a sampling rate of 10 kHz. The recorded currents were digitally filtered through a low-pass filter at 1 kHz, using pCLAMP 10.4 software (Molecular Devices). To measure the hTRPV3 currents, voltage clamp protocols were applied every 20 s from  $-100$  mV to 100 mV over 100 ms. The holding potential was applied at 0 mV. For measuring hORAI1 current, ramp-like pulses from  $-130$  to 70 mV over 100 ms were applied every 30 s at a +10 mV holding potential. All voltage and current trace data were analyzed using Clampfit software 10.4.

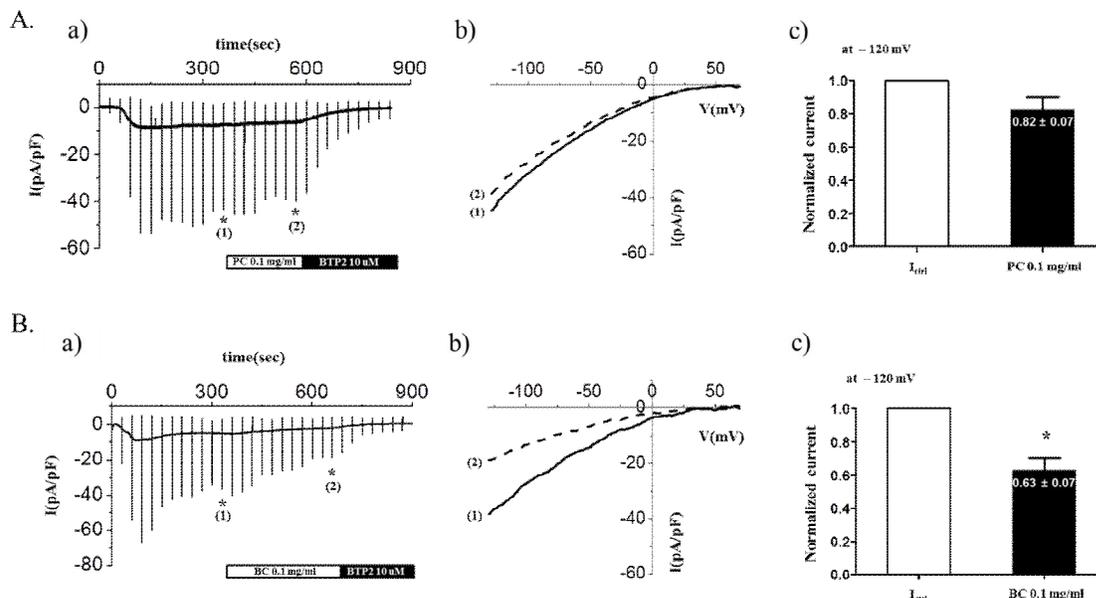
### Statistics

All data are expressed as mean  $\pm$  SEM. Data were analyzed by analysis of variance followed by paired Student's *t*-tests for comparisons between groups with Prism 6.0 (GraphPad, La Jolla, CA, USA) and Origin 8.0 (Microcal, Northampton, MA, USA). Probability values  $< 0.05$  were considered significant.

## Results and Discussion

### Effect of PC and BC Extracts on $I_{ORAI1}$ Activity

We investigated the effect of PC and BC extracts on the regulation of  $I_{ORAI1}$  activity in ORAI1/STIM1 co-overexpressing HEK293T cells using a whole-cell patch clamp technique.  $I_{ORAI1}$  was slowly activated in the presence of 20 mM 1,2-Bis(2-aminophenoxy)ethane- $N,N,N',N'$ -tetraacetic acid (BAPTA) and 20  $\mu$ M inositol 1,4,5-trisphosphate ( $IP_3$ ) in an internal solution with a whole-cell configuration [Fig. 1A (a)]. This weak, inwardly rectifying current, with reversal potential at approximately +50 mV is typical of  $I_{ORAI1}$  [Fig. 1A (b)]. To confirm the basal current, we applied the ORAI1 channel inhibitor, BTP2, at the end of the experiment. PC extract (0.1 mg/mL) in an extracellular solution slightly inhibited  $I_{ORAI1}$  by  $22 \pm 0.08\%$ ; however, the effect was not statistically significant ( $P < 0.12$ ) [Fig. 1A (c)]. We also performed the same experiment with BC extract. As shown in Fig1B, BC extract (0.1 mg/mL) significantly inhibited  $I_{ORAI1}$  by  $22 \pm 0.08\%$ . The aberrant skin barrier in AD is more susceptible to penetration by allergens and chemical irritants; this induces proliferation of keratinocytes and dendritic cells and production of proinflammatory cytokines, such as thymic stromal lymphopoietin (TSLP), which activates and recruits  $CD4^+$  T cells (Boguniewicz and Leung, 2011). In T cells, ORAI1 is activated via antigen receptors, increasing intracellular  $Ca^{2+}$  concentration, which subsequently activates nuclear factor of activated T cells (NFAT) to produce Th2 cytokines (IL-2, IL-4, and IL-13) (Srikanth and Gwack, 2013). In mast cells, the high-affinity IgE receptor activates ORAI1 channels, resulting in the release of proinflammatory mediators (histamine and leukotriene C4 ( $LTC_4$ )), which induces an allergic response (Di Capite et al., 2011). Therefore, agents that inhibit ORAI1 channel activation are potential therapeutics for alleviating AD.

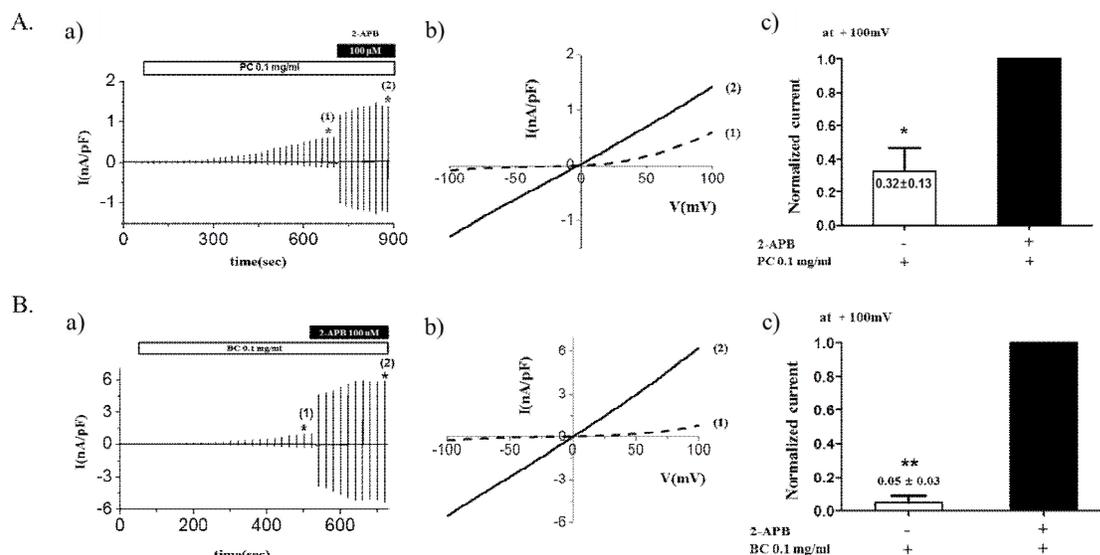


**Figure 1:** Inhibition of  $I_{ORAI1}$  by PC and BC extracts

(a) Typical chart trace recording of  $I_{ORAI1}$  activation and its inhibition by PC (A) or BC (B) extract. The numbers in parentheses indicate the steady state activation point of  $I_{ORAI1}$  (\*1) and the inhibition of  $I_{ORAI1}$  by PC or BP extracts (\*2). (b) Representative current-voltage ( $I/V$ ) curves corresponding to Fig. 1a (\*1) and (\*2). (c) Histogram of the normalized inward current at -120 mV. The current densities were normalized to peak  $I_{ORAI1}$ , which was set as one. P values  $< 0.05$  were considered significant (\* $p < 0.05$ ). Abbreviations: PC, Periostracum Cicadae; BC, Betulae Cortex; ORAI1, calcium release-activated calcium channel protein 1.

### Effect of PC and BC Extracts on $I_{TRPV3}$ Activity

TRPV3 is a vital player in the regulation of physiological skin homeostasis. The regulation of  $Ca^{2+}$  signaling by TRPV3 activation increases transglutaminase activity, which is important for maintenance of the skin barrier (Cheng et al., 2010). Therefore, we investigated the effects of PC and BC extracts on TRPV3 activation in hTRPV3-transfected HEK293T cells. PC extract significantly increased the activity of  $I_{TRPV3}$  (32%) compared to that of 2-APB, a potent agonist of TRPV3 (Fig. 2A). BC extract induced a slight but significant activation of  $I_{TRPV3}$  compared to that of 2-APB (5%) (Fig. 2B). TRPV3 mediates wound healing processes in epithelial cells, and TRPV3-knockout mice have been shown to have impaired skin barrier function with a loss of transglutaminase activity (Aijima et al., 2015).



**Figure 2:** Activation of  $I_{TRPV3}$  by PC and BC extracts

(a) Representative chart trace recording of  $I_{TRPV3}$  activation and its increase by PC (A) or BC (B) extract. After confirming the steady state  $I_{TRPV3}$  (\*1), 2-APB at 100  $\mu$ M was applied to determine the maximum current activation (\*2). (B) Current-voltage (I/V) curves corresponding to Fig. 2a (\*1), which represent  $I_{TRPV3}$  activation by the extracts and the maximal current of  $I_{TRPV3}$  in Fig. 2a (\*2). (c) Histogram of normalized outward current densities at +100 mV. 2-APB-induced  $I_{TRPV3}$  was set as one. P values < 0.05 were considered significant (\* $p$  < 0.05 and \*\* $p$  < 0.01). Abbreviations: PC, Periostracum Cicadae; BC, Betulae Cortex; TRPV3, transient receptor potential cation channel subfamily V member 3; 2-APB, 2-aminoethoxydiphenyl borate.

## Conclusion

Our results suggest that PC and BC extracts have therapeutic potential for the treatment of abnormal skin barrier pathologies with allergic immune reactions in AD, through modulation of ORAI1 and TRPV3 activity. To the best of our knowledge, this is the first study of the electrophysiological effects of natural medicines via the regulation of ion channel activity.

**Conflicts of interest:** The authors declare that there are no conflicts of interest.

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