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Original Article

Tannic acid, a dopamine receptor agonist, ameliorates periodontitis, atopic dermatitis and psoriasis in animal models

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ABSTRACT

Signaling *via* dopamine receptors affect IL-17 secretion from Th17 cells, as shown in our previous studies. Tannic acid (TA) is a herbal polyphenol containing a galloyl group with anti-inflammatory and anti-viral properties. In our recent previous study, we found that i) TA is an agonist of the dopamine D2L receptor (D2LR); ii) TA enhances IL-10 secretion from lipopolysaccharide (LPS)-stimulated mouse splenocytes; iii) TA also enhances IL-10 but reduces IL-17 secretion from anti-CD3/CD28 antibody-stimulated splenocytes. In addition, iv) administration of TA to mice with experimentally induced neutrophilic colitis strikingly suppresses weight loss, colon shrinkage, and IL-17 secretion from mesenteric lymph node lymphocytes. Because IL-17 is one of the essential cytokines to induce neutrophilic inflammation, we in this study tested the effect of TA on other neutrophilic inflammation models in rodents, such as carrageenan-induced periodontitis, 2,4,6-trinitrochlorobenzene-induced atopic dermatitis, and imiquimod-induced psoriasis. We successfully showed that i) TA is effective in reducing the resorption of the alveolar bone in periodontitis model and in atopic dermatitis model; ii) TA and gallic acid are effective in easing psoriasis model as well as neutrophilic inflammation; and iii) not only TA but also related compounds such as gallic acid, epicatechin gallate, and epigallocatechin gallate are D2LR agonists. Therefore, TA and related compounds are effective in *in vivo* animal models for neutrophilic inflammation.

KEYWORDS: tannic acid, dopamine receptors, neutrophilic inflammation, periodontitis, atopic dermatitis, psoriasis, IL-17.

INTRODUCTION

Tannins are categorized into four major groups [1]: gallotannins, which are also known as tannic acid (TA); ellagitannins; complexes of TA and ellagitannins; and condensed tannins. TA is hydrolyzable and is the most abundant tannin. TA consists of five polygalloyl esters binding radially to one glucose molecule.

TA has been prescribed to treat digestive diseases, such as gastroenteritis [2, 3] and acute diarrhea [4]. TA can also react with protein in the mucosa, to form a thin layer that protects from inflammatory damage [5]. In addition, TA reportedly suppresses inflammation [6, 7], cancer cell proliferation [8], and viral proliferation in host cells [9].

It has been suggested that the anti-inflammatory effects of TA are induced through the scavenging of radicals (antioxidant effect) [10] and inhibition of the expression of inflammatory mediators such as cytokines [6], inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) [11].

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It has been shown that TA inhibits protein phosphatase 1 (PP1) and 2A (PP2A) in epithelial cells [11]. However, the molecular mechanisms through which TA inhibits cell signaling are largely unclear.

In our recent previous study, we showed that TA is a dopamine (DA) D2L receptor (D2LR) agonist. The DA receptor (DAR) family consists of five cognate receptors, D1R, D2R, D3R, D4R, and D5R. D2R has two isoforms, D2SR (short isoform) and D2LR (long isoform). The five DARs belong to a G-protein-coupled receptor family of class seven-transmembrane domain receptors, and are sub-categorized as D1-like receptors (D1-like-Rs) (D1R and D5R) and D2-like receptors (D2-like-Rs) (D2R, D3R, and D4R), carrying opposite effects on cytosolic cAMP level.

In neuronal cells, stimulation of D1-like-Rs triggers cAMP synthesis, which in turn activates protein kinase A. By contrast, stimulation of D2-like-Rs triggers cAMP degradation. DARs are expressed on various immune cells as well, and DA itself is also produced by these cells [12, 13]. It has been suggested that DA modulates the cytokine profile during inflammatory immune responses [13] and facilitates T-helper (Th) 2 and Th17 differentiation [12, 14]. DAR antagonists have anti-inflammatory effects [15], and D1R antagonists in particular have eased various inflammatory autoimmune diseases [14, 16-19], including dextran sodium salt (DSS)-induced mouse model of colitis. D2R knockout mice showed inflammatory responses in the central nervous system [20]. These observations collectively indicate that TA, acting *via* DARs, would also be expected to modulate the cytokine profile during inflammatory immune responses.

In this study, we tested the effect of TA on other neutrophilic inflammation models in rodents, such as atopic dermatitis, imiquimod-induced psoriasis and carrageenan-induced periodontitis. We show that TA and gallic acid are effective in easing psoriasis as well as neutrophilic inflammation. TA was also effective in atopic dermatitis model and in reducing bone adsorption in periodontitis model. Therefore, TA seems to be effective in *in vivo* animal models for neutrophilic inflammation.

METHODS

Reagents

TA, epicatechin gallate, epigallocatechin gallate, and gallic acid were purchased from Wako (Osaka, Japan). To make a stock solution for cell culture, TA and gallic acid were dissolved in RPMI medium containing 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 50 μM 2-mercaptoethanol (R10 medium). λ−carrageenan and pramipexole were purchased from Wako and SIGMA, respectively.

Carrageenan-induced rat periodontitis model

Animals were housed in appropriate animal care facilities at Saitama Medical University, and handled according to international guidelines for experiments with animals. All experiments in this study were approved by the Animal Research Committee of Saitama Medical University. Five-week-old male Wistar rats were obtained from Japan SLC (Shizuoka, Japan). While the rats were under anesthesia, buccal and lingual gingiva were exfoliated using an explorer. A silk ligature wire was cut to a length of the mesiodistal distance of the mandibular second molar and immersed in 1% carrageenan (CA group) [21]. The CA/TA group was treated with 1% carrageenan and 1% TA (n = 4). PBS was used as a mock-treatment. Then, they were inserted into the periodontal pocket once a week for 3 consecutive weeks. Four weeks after the first operation, the heads of rats were scanned by micro-CT 35 (SCANCO Medical, Switzerland). After making 3D images, the length of the perpendicular line from the connective line between the buccal and lingual cemento-enamel junction to the alveolar margin was measured.

2,4,6-trinitrochlorobenzene (TNCB)-induced mouse atopic dermatitis model

NC/Nga mice were obtained from Japan SLC (Shizuoka, Japan) and housed in a specific pathogen-free environment. TNCB solution (2%) was applied to the skin of the back (150 μl) on days 0, 3, & 7 to induce atopic dermatitis. On the same day, the TA group (n = 3) was treated with 1% TA-containing ointment. The control group was mock-treated. On day 10, clinical score was evaluated using the method described elsewhere [22].

Imiquimod (IMQ)-induced mouse psoriasis model

Mice were depilated on the back skin 2 days before the treatment and then treated daily with either IMQ cream containing 5% IMQ (Mochida Pharmaceutical, Japan) or sham cream on the ears for 5 or 6 consecutive days. On day 8, ear thickness (μm) was measured. In treatment groups, 4% pramipexole, 4% TA- or 4% gallic acid-containing cream was used. Paraffin-embedded tissue sections of the ears were stained with hematoxylin and eosin to assess neutrophilic inflammation and hyperkeratosis (New histo-blood science laboratory, Japan).

Biochemical analysis

cAMP modulation was determined using the HitHunter cAMP XS+ assay (Discoverx, Fremont, CA). The cAMP Hunter CHO-K1 DRD2L (Long Isoform) Gi Cell Line (Discoverx) was seeded in a total volume of 20 μl in white-walled, 384-well microplates and incubated at 37 °C for the appropriate times before testing. For agonist determination, the cells were incubated with the sample in the presence of forskolin at EC_{80} to induce a response. After overnight incubation, the medium was aspirated from the cells and replaced with 10-μl HBSS and 10 mM HEPES. Stock solutions of the test compounds or DA were diluted to 4× in an assay buffer (Discoverx) immediately before use, and 5 μl of the 4× dilution was added to the cells, followed by the addition of 5 μl of 4× forskolin. The cells were then incubated at 37 °C for 30 min. Following incubation, an assay signal was generated by incubation with 5 μl of cAMP XS+ Ab reagent (Discoverx) and 20 μl of cAMP XS+ ED/CL lysis cocktail (Discoverx). After incubation for 1 h at room temperature, 20 μl of cAMP XS + EA reagent (Discoverx) was added, and the cells were incubated for 2 h at room temperature. Microplates were read on a PerkinElmer Envision following chemiluminescent signal generation. The compound activity was analyzed using the CBIS data analysis suite (ChemInnovation). The percent agonist activity relative to the maximum effect of DA was calculated as follows: (mean RLU of control buffer - mean RLU of test sample)/(mean RLU of control buffer - mean RLU of the maximum response by DA) × 100, where the control buffer was Opti-MEM (Thermo Fisher Scientific, Waltham, MA) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mM L-glutamine, and 1% BSA.

Statistical analysis

The values are expressed as the mean ± SD. The data were analyzed using Student's two-tailed *t*-test. P values of <0.05 were considered to indicate statistical significance.

RESULTS AND DISCUSSION

Carrageenan-induced rat periodontitis model

A silk ligature wire was immersed in 1% carrageenan (CA group) or with 1% carrageenan and 1% TA (CA/TA group). Then, they were inserted into the periodontal pocket. Four weeks after the first insertion, the heads of rats were scanned by micro-CT and the length of the perpendicular line from the connective line between the buccal and lingual cemento-enamel junction to the alveolar margin was measured. As shown in Figure 1, resorption of the alveolar bone induced by carrageenan was effectively suppressed by TA. Carrageenan is a high-molecular-weight sulfated polygalactan used to improve the texture of commercial food products. Its use increased markedly during the last half century, although carrageenan induces inflammation in rheumatological models and in intestinal models of colitis [23]. It induces nuclear translocation of NFκB followed by chemo-attraction of neutrophils [24]. Regarding the resorption of the alveolar bone in periodontitis, RANKL produced by Th17 cells and the direct effect of neutrophils have been reported by others [25-27].

TNCB-induced mouse atopic dermatitis model

TNCB solution was applied to the skin of the back of NC/Nga mice to induce atopic dermatitis. TA group was treated with 1% TA-containing ointment. Ten days after the first treatment, clinical score was evaluated using the method described elsewhere [22]. As shown in Figure 2, TA efficiently suppressed the clinical score of TNCB-induced skin lesions. This mouse model for atopic dermatitis well represents human skin lesions; i.e., eosinophilic inflammation accompanied

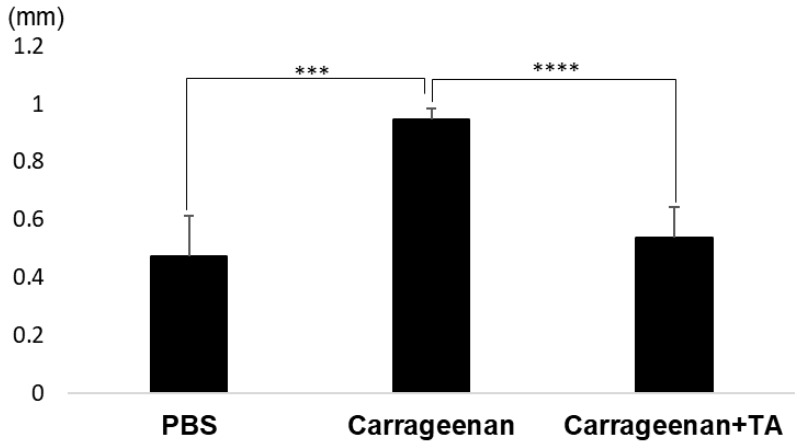

Figure 1. Carrageenan-induced rat periodontitis model.
A silk ligature wire was immersed in 1% carrageenan (CA group) or with 1% carrageenan and 1% TA (CA/TA group). Then, they were inserted into the periodontal pocket, followed by the estimation of the length of the perpendicular line from the connective line between the buccal and lingual cemento-enamel junction to the alveolar margin. ***: $P < 0.005$; ****: $P < 0.001$.

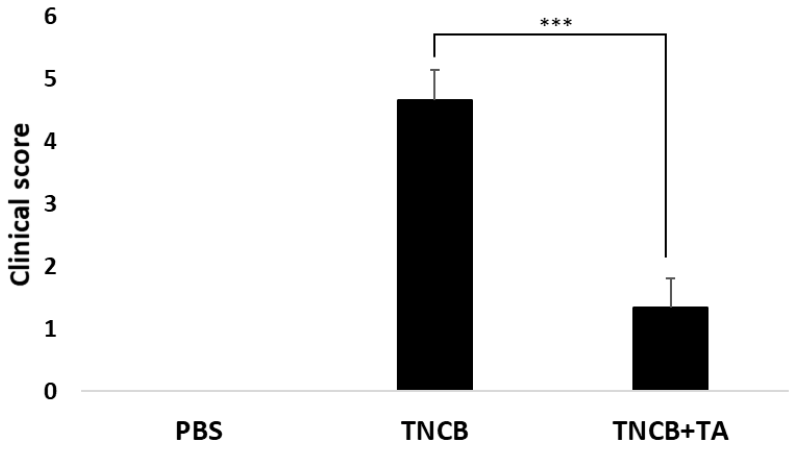

Figure 2. 2,4,6-trinitrochlorobenzene (TNCB)-induced mouse atopic dermatitis model.
TNCB solution was applied to the skin of the back of NC/Nga mice. TA group was treated with 1% TA-containing ointment. Clinical score was evaluated as described in 'METHODS'.
***: $P < 0.005$.

by infiltration of neutrophils. Furthermore, IL-17A reportedly induces Th2 responses in this model [28, 29].

IMQ-induced mouse psoriasis model

Mice were treated with either IMQ cream containing 5% IMQ or sham cream and ear thickness (μm) was measured. In treatment groups, 4% pramipexole, 4% TA- or 4% gallic acid-containing cream was used. As shown in Figure 3A, TA successfully suppressed the effect of IMQ in a dose-dependent manner. Figure 3B shows that not only TA but also gallic acid were effective in easing IMQ-induced psoriasis. As shown in Figure 3C, neutrophil infiltration and hyperkeratosis of the skin was induced by IMQ, which was efficiently suppressed by TA. The pathology of psoriasis is strongly dependent on IL-17A. Keratinocytes can be activated on exposure to IL-17A and other inflammatory cytokines and secrete chemokines

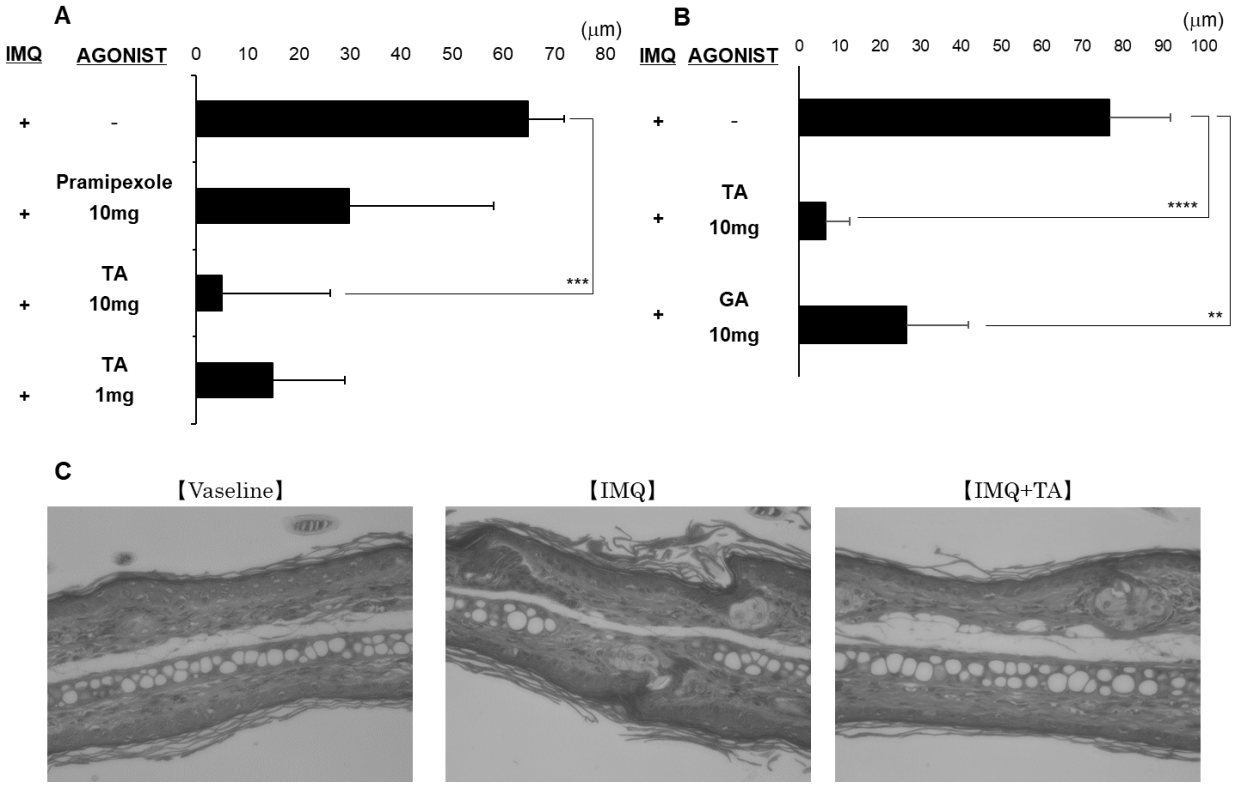

Figure 3. Imiquimod-induced mouse psoriasis model.
Mice were treated with either IMQ cream containing 5% IMQ or sham cream and ear thickness (μm) was measured. In treatment groups, 4% pramipexole, 4% TA- or 4% gallic acid-containing cream was used (A and B). Paraffin-embedded tissue sections of the ears were stained with hematoxylin and eosin (C).
**: P < 0.01; ***: P < 0.005; ****: P < 0.001.

such as CXCL-1 in inflamed skin. In psoriasis and its IMQ-induced mouse model, a strong skin infiltration of neutrophils and monocytes was observed. In this relation, Moos *et al*. [30] found that deletion of IL-17 receptor in keratinocytes reflected the full-body deletion of the receptor, resulting in reduced dermatitis development. Since activated Th17 cells are found in the IMQ model [31], it is conceivable that IL-17A produced by such Th17 cells acts on IL-17 receptor-positive keratinocytes to produce neutrophil-attracting chemokines such as CXCL-1, which functionally corresponds to IL-8 in humans.

Not only TA but also gallic acid, epicatechin gallate, and epigallocatechin gallate are D2LR agonists

In our previous studies, TA was shown to be a D2LR agonist by using a cell line that exogenously overexpressed D2L and led to an increase in intracellular cAMP level in response to agonism of the receptor. The cAMP level was determined using a competitive immunoassay. Because gallic acid was also effective in psoriasis model, we tested the biochemical features of gallic acid and other related compounds. As shown in Table 1, at a concentration of 100 μM, all four compounds showed clear agonism, although the degrees may correlate with their molecular mass. It is thereby possible to conclude that not only TA but also gallic acid, epicatechin gallate, and epigallocatechin gallate are D2LR agonists, both from biological and biochemical viewpoints.

CONCLUSION

Tannic acid and related compounds are effective in *in vivo* animal models for neutrophilic

Table 1. Activity of compounds harboring galloyl groups on the D2L receptor.

Compound name	Concentration (μM)	% Activity
Tannic acid	10	97.2
	100	111.8
Gallic acid	10	-3.1
	100	26.6
Epicatechin gallate	10	24.7
	100	91.5
Epigallocatechin gallate	10	37.4
	100	100

Percentage agonist activity relative to maximum response by dopamine. All analyses were performed in duplicate. The percent activity was calculated from the mean values.

inflammation, such as periodontitis, atopic dermatitis and psoriasis in animal models.

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AUTHORS' CONTRIBUTIONS

R.T. performed the experiments. M.K., T.S., and S.M. conceived and designed the experiments. R.T. and S.M. wrote the manuscript. All authors discussed the results and commented on the manuscript.

CONFLICT OF INTEREST STATEMENT

S.M. is an employee of iMmno, Inc. The other authors have no conflicts of interest.

ABBREVIATIONS

LPS, lipopolysaccharides; DA, dopamine; TA, tannic acid; D2LR, dopamine D2L receptor; TNCB, 2,4,6-trinitrochlorobenzene; IMQ, imiquimod.

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