

## Original article

# Inhibitory effect of Taemyeongcheong on allergic reactions

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## ABSTRACT

Taemyeongcheong (TMC) is Korean traditional extracted drink with various health ingredients. TMC has been used to treat hepatic damage, obesity, gastritis, and colitis. However, the role of TMC on allergic reaction has not been studied yet. In this study, we investigated the anti-allergic effects of TMC against a compound 48/80-induced systemic anaphylactic reaction and IgE-mediated passive cutaneous anaphylaxis (PCA). TMC significantly inhibited the compound 48/80-induced systemic anaphylactic reaction and IgE-mediated PCA reaction. Furthermore, TMC reduced the levels of interleukin (IL)-1 $\beta$ , IL-4, IL-13, and vascular endothelial growth factor in the serum of mice under PCA reaction. Taken together, these results suggest that TMC can play a useful role as an anti-allergic agent.

**Keywords** Taemyeongcheong, systemic anaphylactic reaction, passive cutaneous anaphylaxis, cytokines

## INTRODUCTION

Allergy affects a significant health problem in modern life and has been developed through complex interactions between the environment and genes. Unfavorable eating habits could affect allergens sensitization or raise the risk of developing allergic symptoms (Heffler et al., 2016).

Mast cell plays leading parts in the pathogenesis of allergic inflammatory reaction in all mammalian tissues (Heger et al., 2014). Mast cells are strongly associated with early stage of allergic reactions and immediate hypersensitivity (El-Agamy, 2012; Galli and Tsai, 2012). Mast cell activated by interaction of immunoglobulin E (IgE) and its receptor, Fc $\epsilon$ RI, released inflammatory cytokines and chemical mediators (Han et al., 2012; Tkaczyk et al., 2004). Jeong et al. (2001) reported that as a typical model for the mast cell-dependent immediate allergic reactions, the anti-IgE antibody induces passive cutaneous anaphylaxis (PCA). Mast cell was also activated by compound 48/80. Compound 48/80 is not only known to be a latent inducer of mast cell degranulation but also responsible for anaphylactic reactions in release of histamine and other chemical mediators (Bronner et al., 1987). Activated mast cells can release several Th2 cytokines, such as interleukin (IL)-1 $\beta$ , IL-4, IL-13, and vascular endothelial growth factor (VEGF) (Kawakami et al., 2009). Cytokines produced from mast cells increase the pro-inflammatory cytokine production by other immune cells (Galli and Tsai, 2012). These reactions contribute to the aggravation and expansion of the inflammatory reactions (Galli and Tsai, 2012). Therefore, the modulation of cytokine production can distribute a useful remedial strategy for allergic inflammatory diseases (Choi et al., 2007).

Taemyeongcheong (TMC) is a Korean folk functional drink

based on herbs, including *Saututus chinesis* (Lour.) baill, *viscum album* L., *Houttuynia cordat*, etc. (Yi et al., 2015). In ancient Korean medical books, such as Sangayorok and Donguibogam, these herbs were used as medicinal properties since Joseon Dynasty (Kim, 2012). These are continuously used as functional foods due to their anti-inflammatory, antioxidant, and hepatic-protective activities (Yin et al., 2011). Recent studies have reported the preventive effects of TMC on gastritis, colitis, hepatic damage, and obesity Kim, 2012; Yi et al., 2015). However, the role of TMC in allergic reactions has not been reported. In this study, we investigated the regulatory effects of TMC on *in vivo* allergic models.

## MATERIALS AND METHODS

### Materials

Compound 48/80, anti-dinitrophenyl (DNP) IgE, Evans blue, DNP-human serum albumin (HAS), dexamethasone (Dex), and ketotifen were purchased from Sigma Chemical Co. (St. Louis, MO, USA); Anti-mouse IL-4 antibody from BD Pharmingen (Torreyana Road, San Diego, CA, USA); Anti-mouse IL-1 $\beta$ , IL-13, and VEGF antibodies from R&D Systems (Minneapolis, MN, USA); TMC atopy medicine (ato-cream, ato-beverage, ato-mist) from Gahwa Well Food Co (Yongin, Gyeonggi, Republic of Korea).

### Animals

Male ICR mice (4 weeks old) were purchased from the Dae-Han Experimental Animal Center (Eumsung, Chungbuk, Republic of Korea). The animals were maintained under conventional conditions and experiments were performed under approval from the animal care committee of Kyung Hee University (KHUASP(SE)-15-118).

### Preparation of TMC-atopy medicines

Each ingredient in TMC was processed by Korean traditional method, beopje. Dried herbs were controlled in order of wash, steam, dehydrated, parch, and then dehydrate. Composition of

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Received May 13, 2016; Accepted May 21, 2016; Published May 31, 2016

doi: <http://dx.doi.org/10.5667/tang.2016.0015>

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**Table 1.** Composition of TMC-atopy medicine

TMC-atopy medicine type	Composition
Tc	Viscum album, Cirsium, Glycyrrhiza glabra L., Zingiber officinale, Berchemia berchemiifolia, Fraxinus rhynchophylla Hance, Salicornia herbacea L., Momordica charantia L., Saururus chinensis (Lour.) Baill., Houttuynia cordat, TMC extract
Tb	Bambuseae leaf, Camellia sinensis O.Kuntze, Atractylodes ovata (Thunb.) DC, Saururus chinensis (Lour.) Baill, Viscum album, Houttuynia cordat, Cornus officinalis, Capsella bursa-pastoris, Rumex crispus L, Cordyceps sinensis, TMC extract
Tm	Viscum album, cirsium, Glycyrrhiza glabra L., Zingiber officinale, Berchemia berchemiifolia, Foeniculum vulgare, Bambuseae leaf, Camellia sinensis O.Kuntze, Ilex paraguayensis, Mentha piperascens (Malinv.) Holmes, Momordica charantia L., Saururus chinensis (Lour.) Baill., Houttuynia cordat, TMC extract

TMC, Taemyeongcheong; Tc, TMC ato-cream; Tb, TMC ato-beverage; Tm, TMC ato-mist; TMC, Taemyeongcheong.

TMC-atopy medicines, such as ato-cream (Tc), ato-beverage (Tb), and ato-mist (Tm) is shown in Table 1. The voucher specimens (ato-cream, voucher No. 20150520; ato-beverage, voucher No. 20150521; ato-mist, voucher No. 20150522) were deposited in the herbarium in the Gahwa Well Food Co.

#### Compound 48/80-induced systemic anaphylactic reaction

Mice were given an intraperitoneal injection of the mast cell degranulator compound 48/80 (8 mg/kg). Tb was orally administered 1 h before the injection of compound 48/80. The mortality was measured on the basis of control mice that died about 16 min after the compound 48/80-injection.

#### Passive cutaneous anaphylaxis reaction

PCA reaction was performed according to a previous report (Kim et al., 2013).

#### Enzyme-linked immunosorbent assay (ELISA)

IL-1 $\beta$ , IL-4, IL-13, and VEGF levels from serum of PCA-induced mice were measured by ELISA method according to the manufacturer's specifications (R & D system and BD Pharmingen).

#### Statistical analysis

Data are expressed as the mean  $\pm$  Standard Error of Mean (SEM, n=5). Statistical evaluation of the results was performed by an independent *t*-test and a one-way ANOVA with a least significant difference post hoc test. All statistical analyses were performed using SPSS v12.00 statistical analysis software (SPSS Inc.). The results were considered significant at a value of  $p < 0.05$ .

**Table 2.** Effect of TMC on compound 48/80-induced systemic anaphylactic reaction

Treatment	Dose (mg/kg)	Compound 48/80	Mortality (%)
None (DW)	-	+	100
Tb	10	+	0
Ketotifen	10	+	20

The groups of mice were orally administered with DW or TMC ato-beverage 1 h before compound 48/80 injection (n =5, the total number of mouse/group). The compound 48/80 solution was intraperitoneally given to the groups of mice. Mortality (%) is presented as the 'number of dead mice / total number of experimental mice x 100'. DW, distilled water; Tb, TMC ato-beverage; TMC, Taemyeongcheong.

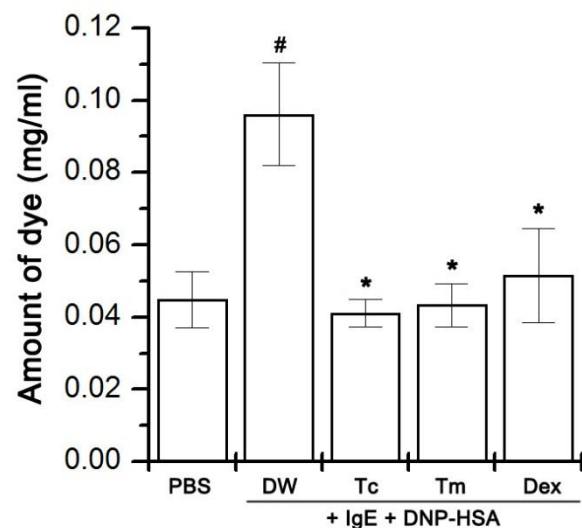
## RESULTS

#### Effect of TMC on compound 48/80-induced systemic anaphylactic reaction

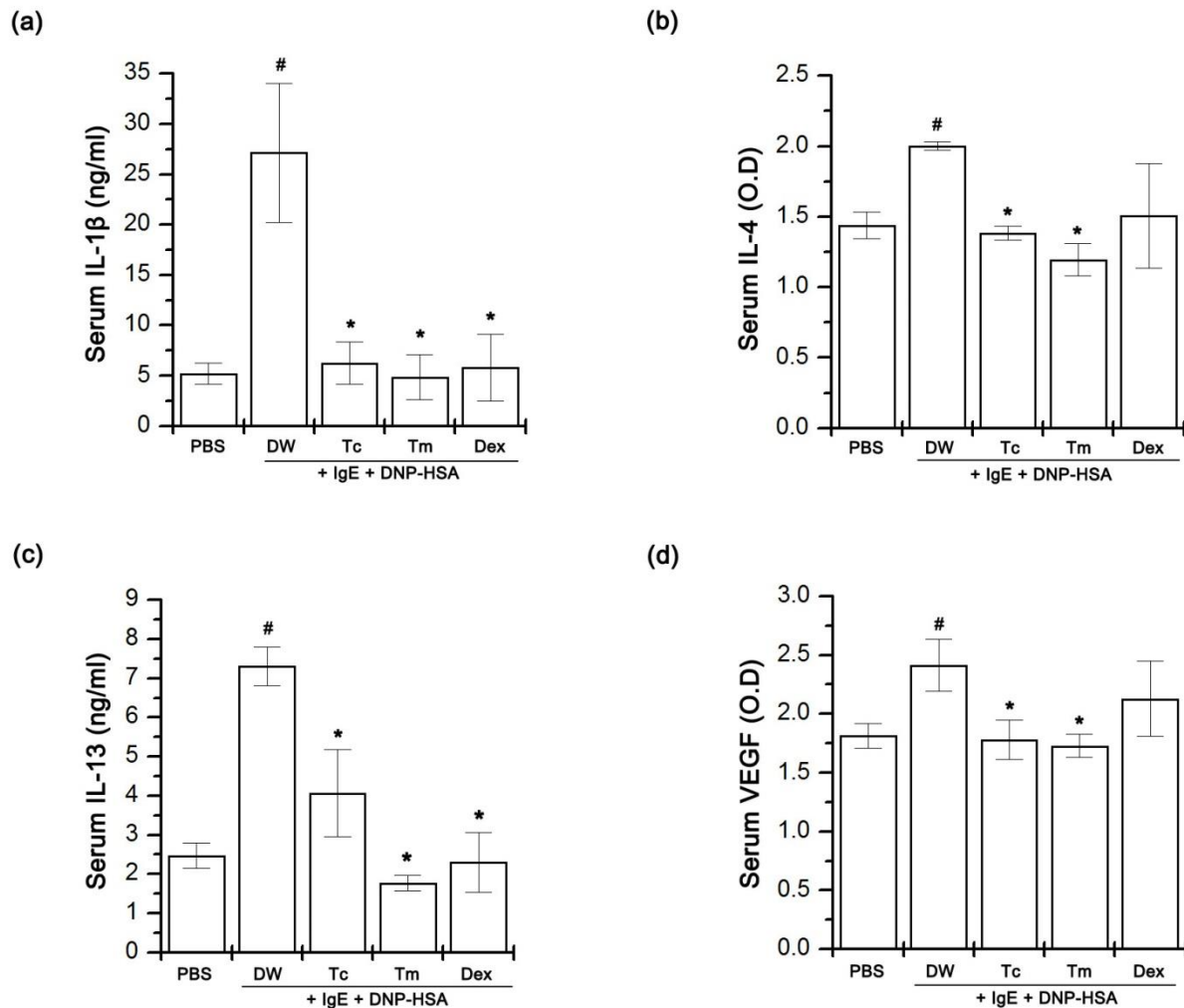
To investigate anti-allergic effect of TMC in anaphylactic reaction, we first used the murine model for systemic anaphylactic reaction. Tb was orally administered 1 h before the compound 48/80 injection. As shown in Table 2, Tb completely suppressed the compound 48/80-induced mortality. Ketotifen, known as an anti-histamine drug, was used as a reference agent (Kim et al., 2011). Ketotifen also inhibited the compound 48/80-induced mortality (Table 2).

#### Effect of TMC on PCA reaction

One of the most important murine models of anaphylaxis in allergic reactions is PCA (Wershil et al., 1987). When Tc and Tm were intradermally administered on the dorsal skin of mice, the IgE-induced PCA reaction was significantly inhibited ( $p < 0.05$ ; Fig. 1). In addition, Dex, known as an anti-inflammatory



**Fig. 1.** Effect of TMC on PCA reaction. Tc (20 mg/site) and Tm (110  $\mu$ l/site) were administered on dorsal skin of mice 1 h before the challenge with DNP-HSA. Each amount of dye was measured at 620 nm in ELISA reader and expressed as the mean  $\pm$  SEM. # $p < 0.05$ ; significantly different from the PBS-administrated mice. \* $p < 0.05$ ; significantly different from the DW-administrated mice. DNP-HSA, dinitrophenyl-human serum albumin; DW, distilled water; Tc, TMC ato-cream; Tm, TMC ato-mist; Dex, Dexamethasone; TMC, Taemyeongcheong.



**Fig. 2.** Effect of TMC on levels of Th2 cytokines under PCA reaction. Tc (20 mg/site) and Tm (110  $\mu$ l/site) were administered on dorsal skin of mice 1 h before the challenge with DNP-HSA. The levels of cytokines in the serum under the PCA reaction were measured by ELISA method. Data was expressed as the mean  $\pm$  SEM. # $p$  < 0.05; significantly different from the PBS-administrated mice. \* $p$  < 0.05; significantly different from the DW-administrated mice. DNP-HSA, dinitrophenyl-human serum albumin; DW, distilled water; Tc, TMC ato-cream; Tm, TMC ato-mist; Dex, Dexamethasone; TMC, Taemyeongcheong.

drug, was used as a reference agent (Jeong et al., 2003) and it is also significantly inhibited IgE-induced PCA reaction ( $p$  < 0.05; Fig. 1).

#### Effect of TMC on Th2 inflammatory cytokines under PCA reaction

Th2 inflammatory cytokines contribute to the aggravation and expansion of the allergic inflammatory reactions (Galli and Tsai, 2012). Therefore, the modulation of cytokines can distribute a useful remedial strategy for allergic inflammatory diseases (Choi et al., 2007). Blood from mice under PCA reaction was taken to clarify the effect of TMC on IgE-induced inflammatory cytokine production. The levels of IL-1 $\beta$ , IL-4, IL-13, and VEGF in the serum were measured using ELISA method. Tc and Tm significantly reduced the levels of IL-1 $\beta$ , IL-4, IL-13, and VEGF (Fig. 2a-d;  $p$  < 0.05). Dex significantly reduced the IL-1 $\beta$  and IL-13 levels (Fig. 2a and c;  $p$  < 0.05), whereas did not reduced the IL-4 and VEGF in the serum under PCA (Fig. 2b and d;  $p$  < 0.05).

#### DISCUSSION

Mast cells are immune cells in tissues of all mammals. They release a various chemical mediators that contribute to inflammatory symptoms of allergic disorders. Compound 48/80, the condensed product of N-methoxyphenylamine with formaldehyde, is well known as one of the most potent mast cell degranulator (Theoharides et al., 1985). Compound 48/80 releases the histamine, inflammatory cytokines, and other chemical mediators that cause anaphylactic symptoms from mast cells (Bronner et al., 1987; Jiang et al., 2007). Ketotifen is a second generation histamine H2 blocker that has been used to manage allergic disorders. In addition to histamine receptor antagonism, ketotifen has inhibitory effect on release of allergic mediators from mast cell (Hei et al., 2008). In this research, we observed that TMC inhibited the compound 48/80-induced systemic anaphylactic reaction as much as ketotifen did. Therefore, these results indicate that TMC exerts anti-allergic effects. And we can assume that TMC may exhibit anti-allergic effect through regulating mechanism of histamine actions and mast cell-mediated allergic reactions.

Upon degranulation, mast cell releases mediators including histamine and some cytokines (Wang et al., 2009). Aggregations of cell surface-specific receptors for IgE by

specific antigen caused secretory response of mast cells (Alber et al., 1991; Metzger et al., 1986). Cross-linking with antigen-specific IgE and FcεRI stimulate mast cells and promotes the immediate release of preformed mediators as well as the synthesis of delayed phase cytokines (Matcalfe et al., 2009). Thus, PCA has widely been used to investigate sensitivity to allergens and agent efficacy in inhibiting allergic reaction (Lin et al., 2001). Dex is one of steroidal anti-inflammatory drugs and it is an effective treatment for inflammatory diseases and PCA by decreasing levels of inflammatory cytokines (Kim et al., 2013). In this study, IgE-mediated PCA reaction and cytokines levels were inhibited by administration of TMC as much as dex did.

TMC contains many traditional medicines and others. TMC mainly consists of three different herbs. Each of them or their components has already been reported to have an effect on the symptoms of allergy. *Saututus chinensis* (Lour.) baill (SC), *viscum album* L. (VA), and *houltuynia cordat* are main active compounds in TMC. SC is the herb that used for treating various skin diseases including atopic dermatitis (Choi et al., 2008) and it has been reported that SC could possess a number of biological effects, such as anti-allergic and anti-inflammatory (Scalbert et al., 2000; Parr et al., 2000). VA has been reputed against hypertension, arthritis, and immune system stimulation (Orhan et al., 2006). *Houttuynia cordat* has anti-allergic effect and suggested to be therapeutic applications of in inflammatory allergic diseases through inhibition of cytokines (Han et al., 2009). These three main active compounds in TMC have anti-allergic effects. Therefore, as an extracts them, we could assume that TMC might inhibits allergic reaction. Taken together, it is conceivable that TMC decrease the immediate type allergic reactions of initial phase by regulating degranulation of mast cells. Therefore, we can deduce that TMC significantly inhibits allergic reactions.

In conclusion, the present study shows regulatory effects of TMC on allergic in vivo models, compound 48/80-induced systemic anaphylaxis and anti-DNP IgE-induced PCA reaction. The levels of Th2 cytokines, such as IL-1β, IL-4, IL-13, and VEGF were significantly reduced by TMC in the serum of mice under the PCA reaction. Therefore, these results suggest that TMC might be useful for the treatment of allergic reactions. However, further study is needed to clarify the detailed mechanism by TMC on allergic reactions.

## ACKNOWLEDGEMENTS

None.

## CONFLICT OF INTEREST

The authors have no conflicting financial interests.

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