Anti-inflammatory Effect of Theophylline in Rats and Its Involvement of the Glucocorticoid–Glucocorticoid Receptor System

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Abstract. Although theophylline has been suggested to have an anti-inflammatory effect, there have been few reports to show the in vivo effect and the mechanism of anti-inflammatory activity of theophylline experimentally. To reveal the anti-inflammatory activity of theophylline, we studied the effect of theophylline and its metabolites on carrageenan-induced edema in rat foot pad. Subcutaneous injection of theophylline (5–100 mg/kg) inhibited carrageenan-induced edema dose-dependently. Theophylline metabolites, that is, 1-methylxanthine, 3-methylxanthine, 1-methyluric acid, and 1,3-dimethyluric acid (equimolar dose to 50 mg/kg of theophylline), did not inhibit the edema significantly. The inhibitory effect of theophylline on carrageenan-induced edema disappeared by pretreatment with aminoglutethimide, an inhibitor of glucocorticoid synthesis and with mefepristone, an antagonist of the glucocorticoid receptor. These results suggest that theophylline itself has anti-inflammatory activity and the glucocorticoid–glucocorticoid receptor system is involved in the anti-inflammatory activity of theophylline.

Keywords: theophylline, anti-inflammation, carrageenan, glucocorticoid

Introduction

Theophylline is commonly used for the treatment of bronchial asthma because of its bronchodilator activity. Recent studies suggest that theophylline has not only bronchodilator activity but also anti-inflammatory activities. It has been reported that theophylline reduced the interleukin (IL)-5-prolonged survival of eosinophils (1) and inhibited the release of IL-4 in peripheral blood mononuclear cells collected from patients with bronchial asthma (2). In a clinical study on the steroid-dependent chronic asthma, it has been demonstrated that frequency of asthma symptoms and the doses of beta 2-adrenoceptor stimulants/steroids is less in the theophylline group than in placebo group (3). Another study showed that theophylline inhibited a delayed allergen challenge–related response in patients with asthma (4). In a placebo-controlled double-blind study on atopic bronchial asthma, theophylline inhibited both immediate and delayed responses of the bronchial mucosa to inhalation of allergen (5). Furthermore, theophylline may inhibit the production of various inflammatory mediators, and it is considered that the therapeutic effect of theophylline is based on its anti-inflammatory effect, as well as its bronchodilator activity (6).

So far, the anti-inflammatory activity of theophylline has been mainly studied on the cellular or clinical level, and there have been few reports to explain the mechanism of the in vivo anti-inflammatory activity of theophylline experimentally. In this study, we evaluated the in vivo anti-inflammatory effects of theophylline and the metabolites on the carrageenan-induced edema rate in rat foot pad. We also studied the mechanism of the
effect in relation to the glucocorticoid (GC)–GC receptor system.

Materials and Methods

Agents
Theophylline, 1-methylxanthine (1-MX), 3-methylxanthine (3-MX), 1-methyluric acid (1-MU), 1,3-dimethyluric acid (1,3-DMU), and pentoxifylline were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, USA). \(\lambda\)-Carrageenan was purchased from Wako Pure Chemical Co., Ltd. (Osaka). Other reagents used in this study were of analytical grade.

Experimental animals
Male Wistar rats (3-week-old) were supplied from Sankyo Labo Service Co. (Tokyo), and kept under a 12 h / 12 h light and dark condition for a week with free access to food and water and used in this study when they were 4-week-old (90 – 100 g). The ethical guidelines as described in the National Institutes of Health Guide for Care and Use of Laboratory Animals and Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society were followed throughout the experiments described. In addition, we carried out this study in accordance with “the Guide for the Care and Use of Laboratory Animals in Kyoritsu University of Pharmacy”.

Effects of theophylline on carrageenan-induced edema in the rat hind paw

All the experiments in this study were conducted using the blind method so that persons who determined the foot volume did not know the kind of treatment.

Theophylline was dissolved in physiological saline and administered subcutaneously in the neck of the rats. The foot volume was measured immediately before the administration of 1.0% \(\lambda\)-carrageenan (0 h). One h after theophylline administration, 0.1 ml of 1.0% \(\lambda\)-carrageenan in saline was subcutaneously administered in the foot pad of the rat left hind paw. The foot volume was measured 3 h (unless otherwise mentioned) after carrageenan administration with a plethysmometer (TK-101; Unicorn Co., Ltd., Chiba).

Pentoxifylline was also dissolved in 0.05 M NaOH /saline and administered subcutaneously in rats 1 h before the subcutaneous injection of carrageenan, and the foot volume of rat hind paw was measured as described above.

Effects of theophylline metabolites on carrageenan-induced edema
Theophylline and its metabolites (1-MX, 3-MX, 1-MU, and 1,3-DMU) in 0.05 M NaOH/saline were administered subcutaneously in rats, and the effect of these agents on carrageenan-induced edema rate was examined as described mentioned.

Effects of aminoglutethimide and mifepristone on anti-inflammatory effect of theophylline
To reveal the involvement of the GC–GC receptor system, the effect of aminoglutethimide, an inhibitor of GC production (7), and mifepristone, a reported GC-receptor antagonist (8), on the anti-inflammatory effect of theophylline was studied. Aminoglutethimide (25 mg /kg) and mifepristone (4 mg/kg) in 0.1 M NaCl saline were intraperitoneally administered 1 h before theophylline treatment. Then, theophylline (50 mg/kg) in 0.05 M NaOH saline was subcutaneously administered, and carrageenan was administered in the foot pad, and the foot volume was measured 3 h after the carrageenan injection.

Edema rate
We estimated carrageenan-induced edema and drug efficacy using the edema rate as an index:
Edema rate (%) = (foot volume at measurement time – foot volume at 0 h) / foot volume at 0 h × 100.

Statistical analyses
Data were subjected to analysis of variance using SPSS version 11.5J for Windows (SPSS Japan, Tokyo). Data were analyzed by one-way ANOVA followed by Dunnett’s multiple comparison tests. A probability \((P) \) of 0.05 or less was considered significant. Data values are each expressed as the mean \(\pm\) S.D.

Results
Carrageenan-induced edema in the rat foot pad and the effect of theophylline
Subcutaneous administration of 1.0% \(\lambda\)-carrageenan induced edema in the foot pad of rat hind paw. The maximum volume of the carrageenan-injected foot pad was obtained 3 h after the administration. The edema rate at 3 h after carrageenan administration was 70.0 \(\pm\) 5.7%. Pretreatment with theophylline (50 mg/kg) significantly suppressed the edema rate (Fig. 1), and the edema rate at 3 h after carrageenan-injection was 53.1 \(\pm\) 2.3% in theophylline-treated rats. As maximum increase of foot volume induced by carrageenan was obtained 3 h after the injection and the effect of theophylline was clearly demonstrated, we measured foot volume 3 h after carrageenan-injection in the following experiments.

Theophylline (5 – 100 mg/kg) was subcutaneously
administered one h before the carrageenan injection. Carrageenan was administered in the foot pad of the rat hind paw, and the food volume was measured 3 h after the carrageenan-injection. Pretreatment with theophylline decreased the carrageenan-induced edema rate in a dose-dependent manner (Table 1). Pentoxifylline (77.2 mg/kg, equimolar dose to 50 mg/kg theophylline) did not decrease the edema rate (51.8 ± 5.25%).

### Effects of theophylline and its metabolites on carrageenan-induced edema rate

Effects of theophylline and its metabolites on carrageenan-induced edema rate were studied. Theophylline (50 mg/kg) and theophylline metabolites (equimolar dose to 50 mg/kg theophylline) were pre-administered, and the foot volume was determined 3 h after carrageenan-injection. Theophylline significantly decreased the carrageenan-induced edema rate (42.4 ± 3.8%, Fig. 2), which was significantly lower than that value in control group (58.4 ± 5.0%, Fig. 2). However, pre-treatment with 1-MX, 3-MX, or 1-MU did not affect the edema rate (Fig. 2); and pre-treatment with 1,3-DMU slightly inhibited the edema rate (49.6%, not significant) (Fig. 2).

### Effects of aminoglutethimide and mifepristone on anti-inflammatory effect of theophylline

To reveal whether the mechanism of anti-inflammatory activity of theophylline involved the GC–GC receptor system, we examined the effect of aminoglutethimide, an inhibitor of GC production, and mifepristone, a reported antagonist of the GC receptor, on anti-inflammatory activity of theophylline.

Theophylline (50 mg/kg) significantly inhibited the carrageenan-induced edema rate (46.9 ± 6.4%). Pretreatment with aminoglutethimide (25 mg/kg) or mifepristone (4 mg/kg) completely abolished the anti-inflammatory activity of theophylline (Fig. 3).

### Discussion

Theophylline is still widely used for the treatment of bronchial asthma. Recent studies have suggested that theophylline exhibits both bronchodilator and anti-inflammatory activities (5, 6). However, there have been no reports to show the anti-inflammatory effect of theophylline clearly, especially in experimental animals. In the present study, we examined the anti-inflammatory actions of theophylline and its mechanism in vivo using carrageenan-induced edema rate in rat foot pad. The edema rate, which was calculated from carrageenan-induced increase of foot volume, was inhibited by pretreatment with theophylline in a dose-dependent manner (Fig. 1 and Table 1). This result suggests that theophylline has in vivo anti-inflammatory activity in experimental animals. Abdel-Salam et al. reported that pentoxifylline, not theophylline, inhibited carrageenan-induced edema rate in rats (9). Our present results showed that theophylline had anti-inflammatory activity and pentoxifylline had no effect on the edema. So far, we do not know the exact reason for this discrepancy between our present results and those reported by Abdel-Salam et al. (9). They administered theophylline 30 min before the carrageenan injection, whereas we administered the agent 1 h before the carrageenan injection. We previously studied the effect...
of theophylline and methylxanthines on endogenous GC production in mice (10). Intraperitoneal administration of theophylline (100 mg/kg) increased the serum GC levels and the peak level occurred at 1 h after theophylline administration. According to the results shown by Sato et al. (10), we administered theophylline 1 h before carrageenan injection. Furthermore, pentoxifylline did not increase the serum GC levels (10). These might be reasons for the discrepancy.

To reveal whether theophylline itself or its metabolites have anti-inflammatory activity, we examined the effects of theophylline metabolites (1-MX, 3-MX, 1-MU, and 1,3-DMU) as well as theophylline itself on carrageenan-induced edema rate. As shown in Fig. 3, 1-MX, 3-MX, or 1-MU did not affect the edema rate, whereas theophylline itself inhibited the edema rate. 3-MX, which is an active metabolite of theophylline (11), did not affect the edema rate, which suggests that the anti-inflammatory activity of theophylline is independent of the inhibitory activity of phosphodiesterase. 1,3-DMU slightly inhibited the edema rate (not significant). These results suggest that theophylline itself has anti-inflammatory activity through a mechanism other than the inhibition of phosphodiesterase activity.

GC is well known to be an endogenous product with strong anti-inflammatory activity, and the activity of GC is considered to be exerted through the binding to its receptor sites. Sato et al. reported that theophylline
(10 mg/kg) induced GC production in mice, and the theophylline-induced GC production was suppressed by pretreatment with dexamethasone. They suggested that this increase in the glucocorticoid levels is a mechanism of the anti-inflammatory activity of theophylline (10). It is important to reveal whether the anti-inflammatory activity of theophylline involved the GC–GC receptor system. Thus we studied the effect of aminogluthethimide, an inhibitor of GC production (7), and mifepristone, an antagonist of GC receptors (8), on the anti-inflammatory activity of theophylline. Pre-treatment with aminogluthethimide or mifepristone diminished the anti-inflammatory activity of theophylline (Fig. 3), which suggests the involvement of GC and the GC-receptor system in the anti-inflammatory activity of theophylline.

From our present results, we suggest that theophylline has anti-inflammatory activity in vivo that involves the GC–GC receptor system. We also suggest that theophylline is an effective drug for the treatment of bronchial asthma through not only bronchodilator activity but also anti-inflammatory activity.

References


