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Short Comnunication

Inhibitory Effect of Theophylline and Methylxanthines on Carrageenan-induced Edema in Rats and their Structure-activity Relationship

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ABSTRACT

We studied the effect of theophylline and other methylxanthines (MXs) on carrageenan-induced edema in rat foot pad to reveal the structure-activity relationship (SAR) in the inhibitory activity on the edema. 1,3-Dimethylxanthine (theophylline) and 1,3,7-trimethylxanthine (caffeine) inhibited the edema, whereas mono-methylated xanthines, other di-methylated xanthines did not affect the edema. These results suggest that methyl groups at position 1 and 3 in MXs are essential for the inhibitory activity of MXs in carrageenan-induced edema. (Jikeikai Med J 2008 ; 55 : 15-8)

Key words : methylxanthine theophylline, carrageenan, rat, edema

INTRODUCTION

Theophylline (1,3-dimethylxanthine, 1,3-DMX), which is widely used for the treatment of bronchial asthma, has been reported to have anti-inflammatory activities[1-7] as well as bronchodilator activity. It is important to reveal whether theophylline and other methylxanthines have acute anti-inflammatory activity *in vivo*. To know the acute anti-inflammatory activity of theophylline and structure-activity relationship in acute anti-inflammatory activity of methylxanthines (MXs), we studied the effect of mono-, di- and tri-methylated xanthines on carrageenan-induced edema in rats.

MATERIALS AND METHODS

1. *Chemicals*

1-Methylxanthine (1-MX), 3-methylxanthine (3-MX), 7-methylxanthine (7-MX), 1,3-DMX, 1,7-dimethylxanthine (1,7-DMX), 1,9-dimethylxanthine (1.9-DMX), 3,7-dimethylxanthine (3,7-DMX, theobromine) and 1,3,7-trimethylxanthine (1,3,7-TMX, caffeine) were purchased from Sigma Chemical Co., Ltd. (MO, U.S.A.). λ-Carrageenan was purchased from Wako Pure Chemical Co. Ltd. (Osaka, Japan). Other reagents used in this study were of analytical grade.

2. *Animals*

Male Wistar rats (3 weeks old) were supplied from Sankyo Labo Service Co. (Tokyo, Japan), and kept under a 12 h/12 h light and dark condition for a

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week with free access to food and water. And rats (4 weeks old, 90–100 g) were used in this study. We carried out this study in accordance with "National Institutes of Health Guide for Care and Use of Laboratory Animals", "Use of Laboratory Animals and Guiding Principles for the Care and Use of Laboratory Animals approved by the Japanese Pharmacological Society" and "Guide for the Care and Use of Laboratory Animals in Kyoritsu University of Pharmacy".

3. Measurement of Edema rate

MXs were dissolved in 0.05M NaOH/saline, and subcutaneously administered in rats (injection volume was 0.1 mL/kg). One hour after the administration of MXs, 0.1 mL of 1%-λ-carrageenan in saline was injected subcutaneously in foot pat of rat hind paw. The foot volume was measured 3 hours after carrageenan injection with a plethysmometer (TK-101, Unicorn Co. Ltd., Chiba Japan). And edema rate was calculated according to the equation shown bellow: Edema rate=(foot volume at 3 hours after carrageenan injection—foot volume before carrageenan injection)/foot volume before carrageenan injection $\times 100$

4. Statistical Analysis

Data are expressed as means±standard deviation. 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.

Results and Discussion

Our preliminaly experiment showed subcutaneous administration of 1%-λ-carrageenan induced edema in foot pad of rat hind paw with a peak of edema at 3 hours after the administration (data not shown). Then, we measured foot volume 3 hours after carrageenan injection and calculated edema rate, to study the effect of MXs as well as theophylline on carrageenan-induced edema in rats. The edema rates in rats pretreated with 10, 25 and 50 mg/kg of theophylline were 57.7±13.3%, 53.9±7.9% and 52.1±4.6% respectively, whereas the rate in control rats was 65.9±8.1% ($n=7$). From these our preliminary results, we decided to use the 50 mg/kg of theophylline and equimolar doses of MXs to evaluate the inhibitory effect of MXs on carrageenan-induced edema.

In control rats (vehicle-injected rats), the edema rate 3 hours after carrageenan injection was 59.9±5.9%. Pretreatment with 1-MX, 3-MX and 7-MX (46.1 mg/kg, an equimolar dose of 50 mg/kg theophylline) did not affect the edema rate. Among di-methylated xanthines, pretreatment of 1,3-DMX (50 mg/

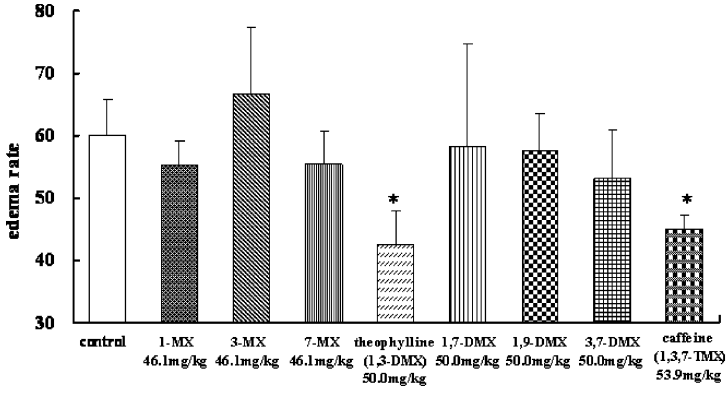
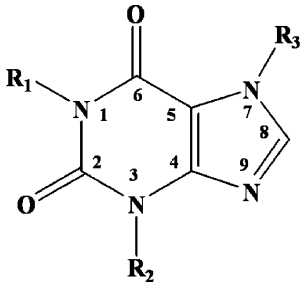
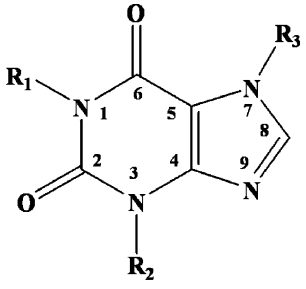
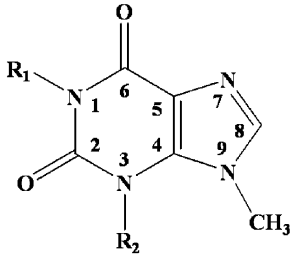
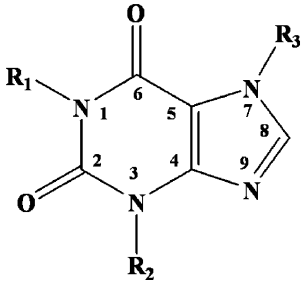


Fig. 1. Effect of theophylline and other methylxanthines on carrageenan-induced edema rate in rat foot pad. 1,3-DMX (theophylline, 50 mg/kg) and other methylxanthines (equimolar doses of 50 mg/kg theophylline) was subcutaneously administered in Wistar rats (male, 4 weeks old). One hour after the administration, carrageenan was injected subcutaneously in foot pad. Three hours after the injection of carrageenan, the foot volume was determined and edema rate was calculated. 1,3-DMX (theophylline) and 1,3,7-TMX (caffeine) significantly decreased edema rate. $n=5$–19. Mean±standard deviation. $^*p<0.01$ compared with control (Dunnett's test).

Table 1. Chemical structures of methylxanthines

			R1	R2	R3
Monomethylxanthines		1-methylxanthine	CH_3	H	H
		3-methylxanthine	H	CH_3	H
		7-methylxanthine	H	H	CH_3
Dimethylxanthines		1,3-dimethylxanthine (Theophylline)	CH_3	CH_3	H
		1,7-dimethylxanthine	CH_3	H	CH_3
		3,7-dimethylxanthine (Theobromine)	H	CH_3	CH_3
		1,9-dimethylxanthine	CH_3	H	–
Trimethylxanthine		1,3,7-trimethylxanthine (Caffeine)	CH_3	CH_3	CH_3

kg) significantly inhibited the rate (42.4±5.4%, $p<0.01$). On the other hand, 1,7-DMX, 1,9-DMX and 3,7-DMX, did not affect the rate. Pretreatment with 1,3,7-TMX (53.9 mg/kg, an equimolar dose of 50 mg/kg theophylline) significantly decreased the edema rate (45.0±2.3%, $p<0.01$) (Fig. 1, Table 1).

In this study, we demonstrated the SAR in the inhibitory activity of MXs on carrageenan-induced edema in rat foot pad. 1,3-DMX, and 1,3,7-TMX had inhibitory activity on the edema.

Recently, theophylline (1,3-DMX) have been reported to have anti-inflammatory activity in clinical studies[1–4] and *in vitro* studies[5–7]. To reveal the anti-inflammatory activity of theophylline and SAR of MXs, we studied the effect of theiphylline and various MXs on carrageenan-induced edema in rat foot pad.

As shown in Fig. 1 and Table 1, mono-methylated xanthines (an equimolar dose of 50 mg/kg thophylline) did not affect the carrageenan-induced edema. Among di-methylated xanthines, only 1,3-DMX reduced the edema. And 1,3,7-TMX (an equimolar dose of 50 mg/kg thophylline) had an inhibitory activity on the edema. Sato et al. reported that MXs, which have methyl groups at N-1 and N-3 nitrogens of xanthine, remarkably increased endogenous glucocorticoid levels in mice through the activation of hypothalamo-pituitary-adrenocortical axis[8]. The enhancement of endogenous glucocorticoid levels might be a mechanism of inhibitory activity of 1,3-DMX and 1,3,7-TMX on carrageenan-induced edema. These results suggest that methyl groups at position both1 and 3 in MXs are essential for the inhibition of carrageenan-induced edema.

In conclusion, we suggest that theophylline and caffeine have anti-inflammatory activity and methyl group at position 1 and 3 in xanthine is essential for their anti-inflammatory activity.

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