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European Journal of Pharmacology 403 (2000) 157–161

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Short communication

Effect of dimethylthiourea, a hydroxyl radical scavenger, on cigarette smoke-induced bronchoconstriction in guinea pigs

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Received 14 July 2000; accepted 17 July 2000

Abstract

Cigarette smoke exposure causes bronchoconstriction in guinea pigs by stimulating cholinergic and excitatory nonadrenergic, noncholinergic (eNANC)-nerves in vagus system. The aim of this study is to elucidate the role of hydroxyl radical (OH^-), contained in cigarette smoke, in bronchoconstriction. Anaesthetized animals were exposed to 80 puffs of smoke for 4 min. Pretreatment with dimethylthiourea, a OH^- scavenger, significantly inhibited cigarette smoke-induced bronchoconstriction. To investigate its site of action, effects of dimethylthiourea were examined on vagally mediated bronchcoconstriction by electrical stimulation and on the bronchoconstriction by intravenous acetylcholine and neurokinin-A. Dimethylthiourea did not inhibit bronchoconstriction evoked by vagal stimulation, acetylcholine or neurokinin-A. These results suggest that dimethylthiourea inhibits cigarette smoke-induced bronchoconstriction by scavenging the smoke-derived OH^-, but not by inhibiting airway nerve function.

Keywords: Vagus nerve; C-fiber; Cholinergic nerve; Acetylcholine; Neurokinin-A

1. Introduction

Acute exposure to cigarette smoke provokes bronchoconstriction in guinea pigs. It has been suggested that multiple mediators, including tachykinins, acetylcholine, and thromboxane, are involved in this bronchoconstriction (Hong et al., 1995; Lee et al., 1995; Matsumoto et al., 1996a,b). The most important pathway is that cigarette smoke likely stimulates the capsaicin-sensitive C-fibers in the airway, which not only causes the cholinergic bronchoconstriction by vagal reflex, but also induces the tachykinin-mediated bronchoconstriction by axon reflex (Hong et al., 1995; Matsumoto et al., 1996a). The latter component is known as excitatory nonadrenergic, noncholinergic (eNANC)-bronchoconstriction.

Despite those progressive investigations, it remains uncertain which constituents of cigarette smoke are responsible for the bronchoconstriction. Previous studies reported that nicotine, in a particular phase of cigarette smoke, elicits bronchoconstriction by stimulating afferent nerves, the central nervous system, and efferent nerves (Hartiala et al., 1984; Lee et al., 1989; Hong et al., 1995). In addition, a nicotine-free vapor phase of cigarette smoke stimulates the C-fiber endings to release tachykinins (Lundberg et al., 1983; Lei et al., 1995). This vapor phase contains abundant reactive oxygen radicals, including superoxide anion, hydrogen peroxide, and hydroxyl radical (OH^-) (Church and Pryor, 1985). In particular, OH^- is an extremely reactive oxygen metabolite, and shown to activate airway C-fibers (Lai and Kou, 1998). Thus, OH^- is proposed as a potent initiator of cigarette smoke-induced bronchoconstriction.

To clarify the role of OH^-, we examined the effect of dimethylthiourea in guinea pigs acutely exposed to cigarette smoke. Dimethylthiourea is a non-enzymatic scavenger of OH^- and has very little effect on other reactive oxygen species (Fox et al., 1983; Wasil et al., 1987). Previous studies reported that dimethylthiourea markedly inhibited neurogenic plasma exudation induced by exposure to capsaicin or cigarette smoke in guinea pig airways (Lai, 1990; Lei et al., 1995). However, an extremely high dose of

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00511-2

dimethylthiourea (1.5 g/kg) needs to be administered intravenously for scavenging OH^- in vivo, which has left a possibility that such a high dose of dimethylthiourea might affect various sites of neuro-effector pathway for the bronchoconstriction, independent of its expected property as a OH^- scavenger. To address this issue, we investigated the effect of dimethylthiourea on the vagally mediated bronchoconstriction induced by electrical stimulation, and on the bronchoconstriction by intravenous acetylcholine and neurokinin-A.

2. Materials and methods

2.1. Study protocol

Hartley guinea pigs weighing 500–600 g (Kyudo, Kumamoto, Japan) were anaesthetized intraperitoneally with 50 mg/kg pentobarbital sodium. The animals were intubated via tracheostomy and mechanically ventilated with a respirator (Model 683, Harvard Apparatus, South Natick, MA, USA) at a constant tidal volume of 7 ml/kg and a rate of 60 breaths/min. Pulmonary insufflation pressure was measured by a differential pressure transducer (Model TP-603T, Nihon Kohden, Tokyo, Japan) connected to a side-arm in the expiratory limb of the ventilation tubing. A catheter was introduced into the jugular vein to administer drugs. Since our recent study has revealed that cigarette smoke-induced bronchoconstriction is markedly attenuated by the α_2 and β_2 adrenergic nervous system (Matsumoto et al., 1998), all the following experiments were performed in the presence of yohimbine (0.5 mg/kg) and propranolol (1 mg/kg). These drugs were administered intravenously 10 min before the application of bronchoconstrictive stimuli.

2.1.1. Protocol 1

Animals were randomly divided into two groups (five per group): vehicle control group (0.9% saline, 2 ml/kg) and dimethylthiourea-treated group (1.5 g/kg in 2 ml/kg of 0.9% saline). After the baseline pulmonary insufflation pressure was determined, vehicle or dimethylthiourea solution was intravenously infused over 10 min. Animals were exposed to cigarette smoke, 10 min after the administration, as described previously (Matsumoto et al., 1996a,b, 1998). Briefly, cigarette smoke was supplied by a smoke generator using a volume-controlled respirator (Model 681, Harvard Apparatus) set at a constant tidal volume of 3.5 ml and a rate of 60 breaths/min. Smoke from a cigarette directly connected to the ventilation circuit was delivered to each animal in 10 consecutive breaths, followed by 20 breaths of fresh air. This procedure was repeated for 4 min. Thus, the animals were intermittently exposed to a total of 80 puffs of cigarette smoke over 4 min. Cigarettes were purchased from Japan Tobacco (Tokyo, Japan). Each cigarette contained 2.7 mg of nicotine and 26 mg of tar.

2.1.2. Protocol 2

Animals were randomly divided into two groups (five per group) consisting of a control group (0.9% saline, 2 ml/kg) and a dimethylthiourea-treated group (1.5 g/kg in 2 ml/kg of 0.9% saline). After the baseline pulmonary insufflation pressure was determined, cervical vagus nerves were carefully exposed and kept covered with 0.9% saline-dampened gauze until the stimulation. The nerves were stimulated electrically at 5 Hz, 5 V, 5 ms for 150 s, 10 min after the vehicle or dimethylthiourea infusion. This stimulation setting was found to be optimal for inducing the degree of bronchoconstriction similar to that induced by cigarette smoke. The stimulation was first applied to one group, in the absence of atropine, to evaluate the whole bronchoconstrictive activity of vagus nerves. Then, to evaluate eNANC-bronchoconstriction, the stimulation was applied to the other group in the presence of atropine (1 mg/kg), which was administered intravenously 10 min before the stimulation.

2.1.3. Protocol 3

Animals were divided into two groups, the same as in protocol 2, 10 min after the vehicle or dimethylthiourea infusion. The animals were administered acetylcholine or neurokinin-A as follows: 1 ml/kg of 0.9% saline was given intravenously for 5 s, and the subsequent value of pulmonary insufflation pressure was used as the baseline. Acetylcholine or neurokinin-A solution (1 ml/kg) was administered intravenously for 5 s, separated by 5-min intervals. The concentration of agents was increased for each series of administration. Pulmonary insufflation pressure was monitored for 5 min after each administration, and the maximum value was plotted against the agent concentration.

2.2. Materials

The drugs used in this study were dimethylthiourea (Sigma, St. Louis, MO, USA) atropine sulfate (Tanabe Pharmaceutical, Osaka, Japan), propranolol hydrochloride (Zeneca Pharmaceutical, Osaka, Japan) and pentobarbital sodium (Abbott Laboratories, North Chicago, IL, USA). Aliquots of acetylcholine (Sigma) and neurokinin-A (Peptide Research, Osaka, Japan) were stored at -20°C and diluted in 0.9% saline on each day of experimentation. Yohimbine hydrochloride (Sigma) was dissolved in acid saline (0.9% NaCl with HCl added to a pH of 2.6).

2.3. Statistical analysis

Data are expressed as the means $\pm$ SEM. Values are expressed as the percentage of the baseline. Time-course curves for pulmonary insufflation pressure during the exposure to smoke and dose–response curves during acetylcholine or neurokinin-A challenge between groups were

assessed by two-way analysis of variance (ANOVA). The comparison of vagally mediated bronchoconstriction among groups was performed with a Kruskal–Wallis test. A level of $P < 0.05$ was accepted as statistically significant.

3. Results

Vehicle-treated, cigarette smoke-exposed animals exhibited bronchoconstriction, as evidenced by increased pulmonary insufflation pressure to $266 \pm 44\%$ of baseline (Fig. 1A). Dimethylthiourea pretreatment significantly inhibited the bronchoconstriction, as $135 \pm 13\%$ of baseline ($P < 0.05$).

Fig. 1B showed the effect of dimethylthiourea on the bronchoconstriction induced by electrical stimulation of vagus nerves. The vagal stimulation increased pulmonary insufflation pressure to $286 \pm 21\%$ of baseline in vehicle-treated animals in the absence of atropine. This bronchoconstriction was not affected by dimethylthiourea ($280 \pm 8\%$ of baseline pulmonary insufflation pressure; $P = 0.89$). To evaluate eNANC bronchoconstriction, the vagal stimulation was applied in the presence of atropine. Atropine significantly reduced the degree of bronchoconstric-

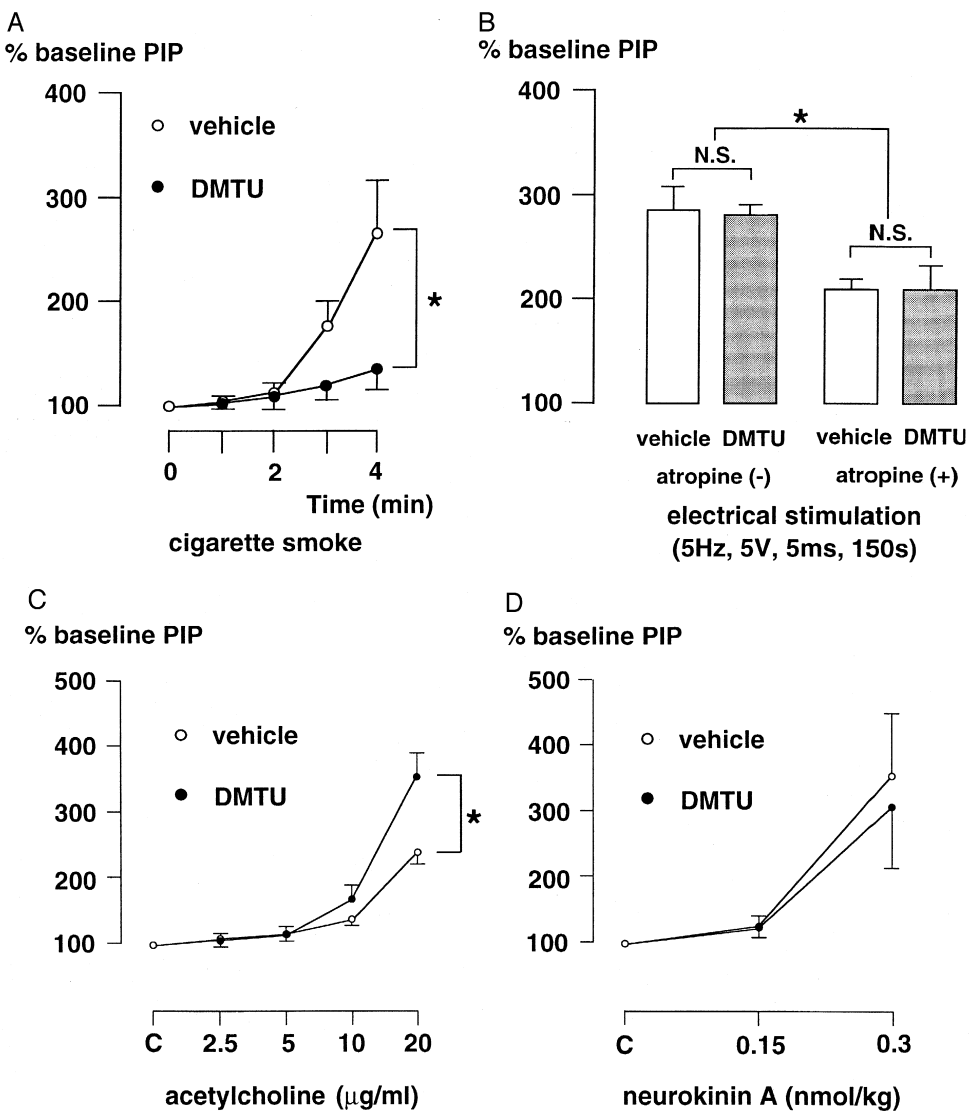

Fig. 1. A; Cigarette smoke exposure caused bronchoconstriction shown by the increase in pulmonary insufflation pressure (PIP). Dimethylthiourea (DMTU) pretreatment significantly inhibited this bronchoconstriction. B; The stimulation of vagus nerves at 5 Hz, 5 V, 5 ms for 150 s, increased pulmonary insufflation pressure in vehicle-treated animals, which was not affected by dimethylthiourea. Atropine pretreatment significantly reduced the degree of bronchoconstriction in vehicle-treated animals. The remaining increase in pulmonary insufflation pressure was regarded as excitatory nonadrenergic, noncholinergic bronchoconstriction, which was not affected by dimethylthiourea. C; The administration of acetylcholine increased pulmonary insufflation pressure, which was significantly enhanced by dimethylthiourea. D; The administration of neurokinin-A increased pulmonary insufflation pressure, which was not affected by dimethylthiourea. Values represent the mean ± SEM for five animals. $^{*}P < 0.05$, NS; not significant.

tion in vehicle-treated animals ($286 \pm 21\%$ of baseline without atropine vs. $208 \pm 8\%$ of baseline with atropine, $P < 0.05$). Thus, the remaining increase in pulmonary insufflation pressure above the baseline was regarded as eNANC bronchoconstriction. Again, the bronchoconstriction was not affected by dimethylthiourea ($208 \pm 21\%$ of baseline pulmonary insufflation pressure; $P = 0.90$).

Finally, the effect of dimethylthiourea on intravenous acetylcholine or neurokinin-A was examined (Fig. 1C,D). The administration of acetylcholine at 20 μg/ml increased pulmonary insufflation pressure to $240 \pm 9\%$ of baseline in vehicle-treated animals. This bronchoconstriction was significantly enhanced by dimethylthiourea, as $353 \pm 31\%$ of baseline ($P < 0.05$). While the administration of neurokinin-A at 0.3 nmol/kg increased pulmonary insufflation pressure to $357 \pm 88\%$ in vehicle-treated animals. This was not affected by dimethylthiourea, as $305 \pm 84\%$ of baseline ($P = 0.71$).

4. Discussion

In the present study, dimethylthiourea inhibited the bronchoconstriction induced by exposure to cigarette smoke in guinea pigs. Dimethylthiourea is known as a specific scavenger of OH^-. Previous investigations showed that dimethylthiourea inhibited neurogenic plasma exudation induced by exposure to capsaicin or cigarette smoke in guinea pig airways (Lai, 1990; Lei et al., 1995). In those studies, dimethylthiourea did not inhibit either plasma exudation by electrical stimulation of vagus nerves or by intravenous substance P. Those results suggested that dimethylthiourea may elicit its action by scavenging OH^- in cigarette smoke, but not by inhibiting the neural function of C-fibers. In the present study, dimethylthiourea also did not affect either, eNANC bronchoconstriction or intravenous neurokinin-A-induced bronchoconstriction. This result strongly supports the above possibility, although the activation of eNANC nerves and the following neurokinin-A release, compose an important pathway of cigarette smoke-induced bronchoconstriction (Lee et al., 1995; Matsumoto et al., 1996a,b).

Cholinergic pathway is another important component of cigarette smoke-induced bronchoconstriction (Hong et al., 1995; Matsumoto et al., 1996a,b, 1998), however, the effect of dimethylthiourea on that pathway is not yet known. Thus, we examined whether or not dimethylthiourea affects the bronchoconstriction induced by electrical stimulation of vagus nerves or by intravenous acetylcholine. On the electrical stimulation in the absence of atropine, pulmonary insufflation pressure increased to $286 \pm 21\%$ of baseline in control animals. In the presence of atropine, the increase in pulmonary insufflation pressure was significantly reduced to $208 \pm 8\%$ of baseline, thus, the subtracted 80% of change in pulmonary insufflation pressure may be regarded as the cholinergic component. This component did not seem to be affected by dimethylthiourea pretreatment. An unexpected result was that dimethylthiourea rather enhanced the bronchoconstriction induced by acetylcholine. This enhancement may be acetylcholine-specific, because it was not observed in neurokinin-A-induced bronchoconstriction. Taking this finding into account, the lack of inhibition by dimethylthiourea on vagally mediated bronchoconstriction in this study, may not preclude the possibility that dimethylthiourea might inhibit acetylcholine release from cholinergic nerves. This inhibitory effect might be masked by the observed postjunctional enhancement. However, dimethylthiourea had no net effect on the vagally mediated cholinergic component of cigarette smoke-induced bronchoconstriction, after all.

In summary, cigarette smoke-induced bronchoconstriction was markedly inhibited by dimethylthiourea, a OH^- scavenger. While dimethylthiourea did not affect vagally mediated bronchoconstriction, rather, it enhanced bronchoconstriction to acetylcholine but not to neurokinin-A. These results suggest that dimethylthiourea inhibits cigarette smoke-induced bronchoconstriction through the mechanism by scavenging OH^- derived from cigarette smoke.

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