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Effect of fudosteine, a new cysteine derivative, on mucociliary transport

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Abstract

We examined the effect of fudosteine ((−)-(*R*)-2-amino-3-(3-hydroxypropylthio)propionic acid) on the mucociliary transport (MCT) rate in quails. The MCT rate was estimated by ash transport velocity on the tracheal mucosa of quails. Fudosteine (500 mg kg^{-1}, p.o.) did not affect the normal MCT rate. However, topical application of fudosteine to the tracheal mucosa dose-dependently protected the impairment of the MCT rate caused by exposure to cigarette smoke. The results suggest that fudosteine may participate in the defence mechanism in the respiratory tract against irritant gases.

Introduction

We found that fudosteine ((−)-(*R*)-2-amino-3-(3-hydroxypropylthio)propionic acid) (Figure 1) may have beneficial activity against airway clearance. For example, fudosteine inhibited the number of goblet cells increased by lipopolysaccharide or β-stimulant in airway epithelium (Takahashi et al 1998a, b), and increased serous secretion involving chloride ion in broncho-alveolar lavage of rats (unpublished data).

Mucociliary transport (MCT) is an airway defence mechanism that serves to remove inhaled substances from the respiratory tract (Braga 1989). Tobacco smoke, particularly cigarette smoke, is one of the most common air pollutants, recognized as being the major cause of various bronchopulmonary changes, such as chronic bronchitis and emphysema (Lundgren & Shelhamer 1990; King 1998). Experimental evidence of the ability of cigarette smoke to affect MCT has been obtained in various animal models (Wanner 1988). For example, Durak et al (1996) reported that cigarette smoke affects lung clearance of aerosolized ^{99m}Tc in rabbits. We also reported that cigarette smoke decreased the MCT velocity of ash on the trachea of pigeons (Miyata et al 1988). So far, little is known about the effect of fudosteine on MCT. This study was performed to investigate the effect of fudosteine in normal conditions on cigarette-smoke-induced impairment in MCT.

Materials and Methods

Animals

Quails of either sex, weighing 60–120 g, were purchased from Kyudo Co. Ltd (Fukuoka, Japan) and allowed free access to food and water.

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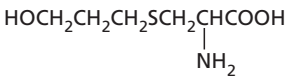

Figure 1 Chemical structure of fudosteine.

Drugs

Fudosteine was synthesized in our laboratories. Commercial sources of materials and reagents were as follows: sodium carboxymethylcellulose (CMC-Na) from Nakarai Tesque (Nagoya, Japan), urethane from Sigma Chemical Co. (St Louis, MO). Fudosteine was suspended in 0.5% CMC-Na and orally administered to quails at a volume of 1 mL kg^{-1}.

Measurement of MCT

The MCT rate was measured according to the method described previously (Miyata et al 1988; Tai et al 1997). This is a useful method to evaluate the effect of mucoactive drugs on MCT (Tai et al 1999a, b). Briefly, the quails were anaesthetized with urethane (1.6 g kg^{-1}, i.p.). The trachea was exposed according to the usual method. Blood vessels and connective tissues were separated carefully over the anterior trachea over 2 cm from approximately 1 cm below the pharynx. As soon as the operation was over, the head and neck of the quail was inserted through the opening into an observation box. The observation box (capacity, 1000 mL) kept the quail tracheal mucosa under the same temperature (38 ± 1°C) and humidity (approximately 100%) as in-vivo, by using a humidifier. After the quail was habituated to the condition of the observation box (30 min), several particles of ash were placed on the caudal side of the tracheal mucosa to select the part that carried the particles fastest. The measurement was started when the ash transport velocity became constant and the time taken for the ash particles to move 2 mm on the tracheal mucosa was measured. After the stability of the MCT rate was confirmed, each drug suspended in 0.5% CMC-Na was orally administered via a catheter, and the MCT measured continuously. In some experiments, the trachea of the quails was exposed to the diluted smoke of an unfiltered cigarette (High Lite, Japan Tobacco Inc., Japan) using an artificial ventilator (1 mL of smoke diluted with 50 mL of air, 15 rev min^{-1}, for 1 min). The topical application of drug to tracheal mucosa was performed by nebulizing the dissolved drug solution (1 mL) in distilled water with a nebulizer made of glass, using an artificial ventilator (50 mL × 120 rev min^{-1}, for 2 min). The drug inhalation was completed 1 min before the exposure to the cigarette smoke. The effectiveness of each drug was evaluated by expressing the time-course of percentage changes in the MCT rate after drug administration or exposure, which was obtained from comparison with the mean value of the MCT rate during 20 min before the treatment.

Statistical analyses

Data are expressed as mean ± s.e.m. The statistical significance of differences between control and test groups was determined by the unpaired Student's *t*-test or the Aspin-Welch test after the F-test. In the experiment using cigarette smoke, statistical evaluations were performed by a one-way analysis of variance or the Kruskal-Wallis test after Bartlett's test, followed by Dunnett's test for multiple comparisons. Differences were considered significant when $P < 0.05$.

Results

The normal MCT rate varied among quails and ranged between 17.3 and 24.6 mm min^{-1}, although the MCT rate was fairly stable and reproducible in the same quail. The MCT rate changed slightly for 60 min after application of 0.5% CMC-Na. Similarly, the oral administration (500 mg kg^{-1}) of fudosteine did not affect the normal MCT until 60 min after drug administration (Figure 2). Cigarette-smoke exposure produced secre-

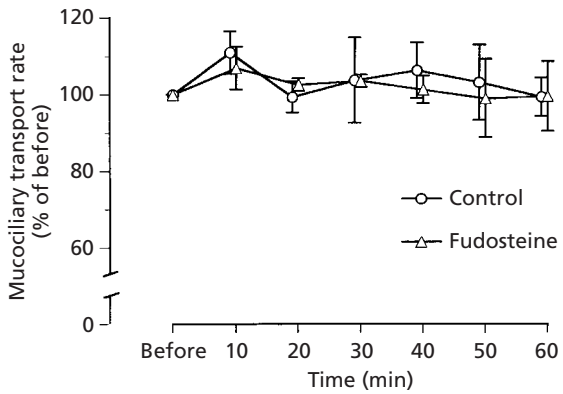

Figure 2 Effects of fudosteine on normal mucociliary transport rate in quails. Each drug (500 mg kg^{-1}) was orally administered after stable condition was established. The time-course of % changes in the mucociliary transport rate after drug administration, which was obtained from comparison with the mean value of the mucociliary transport rate during 20 min before the treatment, is shown. The results represent the mean ± s.e.m., n = 3.

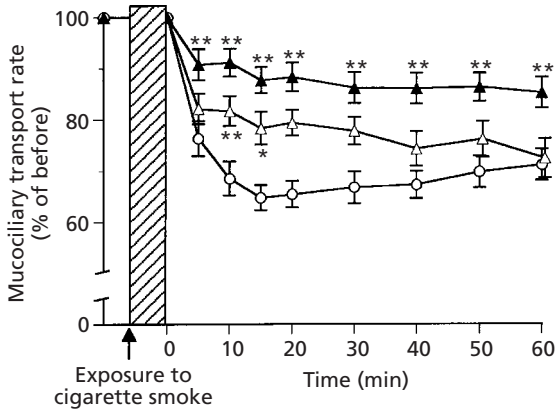

Figure 3 Effect of fudosteine on mucociliary transport impaired by cigarette-smoke exposure in quails. The drug inhalation was completed 1 min before the exposure to the cigarette smoke. The time course of % changes in the mucociliary transport rate after cigarette smoke exposure, which was obtained from comparison with the mean value of the mucociliary transport rate during 20 min before the treatment, is shown. ○, Control; △, fudosteine 1 mg mL^{-1}; ▲, fudosteine 10 mg mL^{-1}. The results represent the mean ± s.e.m., n = 6–8. $*P < 0.05$, $**P < 0.01$ vs control (Dunnett's test).

tion of thick mucus and prominently reduced the MCT rate in all quails (Figure 3). The maximum reduction in the MCT rate was 35.2% in the 15 min after cigarette smoke exposure. Pre-treatment with fudosteine dose dependently protected against the impairment of MCT rate and secretion of thick mucus by cigarette-smoke exposure (Figure 3).

Discussion

In this study, fudosteine inhibited the impairment of MCT induced by cigarette-smoke exposure, although fudosteine did not affect normal MCT. While further studies will be needed to evaluate the influence of fudosteine on normal MCT rate when it is topically applied and against the impairment of MCT rate when it is orally administered, we propose the following two speculations for the mechanism of action.

We have already reported that fudosteine increased the free *N*-acetylneuraminic acid (NANA) in the broncho-alveolar lavage fluid (BALF) of SO_2-induced bronchitic rats (Takahashi et al 1995). NANA exists in respiratory-tract fluid and sputum, and possesses anti-inflammatory properties, as was found in carrageenan-induced rat paw oedema and pleurisy tests (Görög & Kovács 1978). Moreover, it is known that NANA inhalation inhibits cigarette-smoke-induced MCT impairment (Miyata et al 1988). Thus, our data suggest that the inhibition by fudosteine against the impairment of the MCT induced by cigarette smoke may be at least in part, a reaction via endogenous NANA.

Another speculation is based on the finding that ciliary movement is dependent, at least in part, on ion (especially chloride ion) transport through the airway epithelium (Tamaoki et al 1991). It is known that cigarette smoke inhibits active ion transport in the canine tracheal epithelium (Welsh 1983). We found that fudosteine increased chloride secretion in the BALF of rats (unpublished data). Thus, the mechanism of protection by fudosteine against the impairment of MCT following cigarette-smoke exposure, may involve influence on the ion transport in the airway epithelium.

Impairment of mucociliary function may be caused by structural abnormalities of the respiratory cilia, by alterations in the properties of the mucus or by impairment of ciliary beat frequency (CBF). Ciliary dysfunction and impairment of MCT by short-term exposure to cigarette smoke have been demonstrated in rats, rabbits, cats and dogs (Wanner 1977). We are planning to investigate the effect of fudosteine against CBF.

In conclusion, fudosteine may possess beneficial activity against the impairment of MCT by irritant gases. In addition, Hernandez et al (1994) reported that mucoactive drugs, including cysteine derivatives, prevent airway hyperreactivity and inflammation induced by cigarette-smoke exposure. Consequently, our results suggest that fudosteine may be characterized as a mucoactive drug.

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