Effects of Anticholinergic Bronchodilators on Mucociliary Transport and Airway Secretion

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Abstract—The effects of atropine, ipratropium and oxitropium on the mucociliary clearance were studied in pigeons and rabbits. The normal mucociliary transport (MCT) in pigeons was inhibited after the treatment by any of the three drugs at concentrations of $10^{-4}$ to $10^{-3}$ g/ml. These three drugs markedly inhibited eserine-induced MCT acceleration at a lower concentration than ACh-induced acceleration. The normal airway secretion was inhibited only by atropine in rabbits. The results indicate that neither oxitropium nor ipratropium depress the normal mucociliary clearance, but atropine may depress it under some conditions. Additionally, we suggest that these anticholinergic drugs might selectively affect the mucociliary transport modulated by endogenous ACh.

In recent years, anticholinergic agents as antiasthmatic drugs have aroused considerable attention since the involvement of the vagal mechanism in bronchial constriction was emphasized. Mills and Widdicombe (1) and Dekock et al. (2) considered that histamine-induced airway constriction was mediated by the vagus. Yu et al. (3) and Gold et al (4) found that allergen-induced airway constriction was inhibited by atropine or vagotomy.

Oxitropium, a newly developed anticholinergic drug, was proved to inhibit bronchial constriction induced by several agents, similarly to ipratropium, and demonstrated to have a higher specificity towards bronchial smooth muscles (5, 6). These anticholinergic agents, however, have been little investigated for their effects on mucociliary clearance, which is an important pulmonary defense mechanism. Thus, we performed the present study to examine the effects of these drugs on the mucociliary transport (MCT) system in pigeons and airway secretion in rabbits.

Materials and Methods

1. Effects on mucociliary transport

1.1. Animals: Groups of 5 to 7 pigeons of either sex (body weight: 300–350 g) were used.

1.2. Apparatus and operation: Aside from the conventionally used wooden cubiform chamber (7), a newly designed chamber was used. It was a transparent acrylic cylindrical chamber (110 mm in diameter, 150 mm in height) that allowed us to easily observe the subjects. The plate of one end of the chamber had an opening adjusted in shape and size to the thoracic level transverse section of the pigeon. The opening was covered with a lid when the pigeon was not inserted. The other end of the chamber had two openings for spraying and the air supply, respectively, and they each had silicone plugs. The upper wall of the chamber was opened 5 mm wide and 25 mm long for direct observation of the trachea of the pigeon in the chamber.

Surgical operation and other procedures were performed as described previously (8). In brief, a pigeon was fixed in supine position. The trachea was exposed, incised, and opened 4 mm wide and 25 mm long to directly observe the tracheal mucosa. Within a minimal length of time after the surgical operation, the head to breast portion of the pigeon was
inserted into the observation chamber which was maintained at a temperature of ca. 37°C and humidity of ca. 100% with a humidifier connected to the chamber via the opening for the air supply. This cylindrical chamber provided a stable experimental condition, increasing the easiness and accuracy of the measurement of the MCT rate.

The drugs were dissolved in water and directly nebulized on the tracheal mucosa for 2 min via a pump-driven glass nebulizer. The pump was conditioned to displace 50 ml and to revolve at 120 rpm.

The site at which the MCT was most stable was observed to measure the time taken by an exact 10 mm transport of the fine particles of cork placed on the mucosa.

The effectiveness of the drugs was evaluated by expressing the means of the post-drug MCT rate measured for 60 min as a percentage of the means of the pre-drug MCT rate measured for 20 min.

2. Effects on airway secretion

2.1 Animals: Male New Zealand White rabbits weighing 1.9 to 2.6 kg were used in groups of 6 to 8 animals each. They were housed in stainless steel or aluminum cages and allowed free access to pellet diet and water.

2.2 Apparatus and operation: The method we had previously developed (9–11) was used. Animals were anesthetized with urethane (1.1 g/kg, i.p.). The trachea was exposed, and the ventral half of the trachea was transversely incised with minimized bleeding along the circular tracheal cartilage. One end of a specially designed r-shaped cannula for collecting the respiratory tract fluid (RTF) was inserted into the trachea, and the other end of the cannula was connected with a humidifier which supplied air (1 l/min/animal) maintained at 38°C and ca. 100% of humidity. The animal was allowed to breathe spontaneously. The third end of the cannula was inserted into a reservoir to collect RTF by the postural drainage.

Drugs were dissolved in physiological saline, and their aqueous solutions of various concentrations were prepared. The solution of a given concentration was placed in a 1-ml glass nebulizer connected to an appropriate glass adapter, and sprayed into the trachea via a cannula. The spraying conditions were: 50 ml of displacement, 60 rpm, and ca. 1 min of spraying.

The amount of RTF was measured for a total of 12 hr that consisted of a 3-hr pre-drug phase and a 9-hr post-drug phase. The results were compared with those obtained from the control animals treated with physiological saline.

3. Test compounds

Atropine sulfate was purchased from Wako Pure Chemicals.

Ipratropium bromide and oxitropium bromide were kindly supplied by Nippon Boehringer Ingerheim Co., Ltd. Their chemical structures are shown in Fig. 1.

4. Statistical analysis

The results of each experiment were evaluated by the unpaired Student's t-test. Significance was attributed to probability values of <0.05.

Results

1. Effects on mucociliary transport: As
shown in Fig. 2, atropine was inactive at $10^{-5}$ g/ml, but significantly decreased the normal MCT rate to $92.0 \pm 2.1$ and to $87.7 \pm 6.1\%$ of the control at concentrations of $10^{-4}$ and $10^{-3}$ g/ml, respectively. Ipratropium inhibited the MCT rate to $90.5 \pm 3.4$, $91.4 \pm 2.0$ and $79.5 \pm 8.5\%$ at the respective concentrations of $10^{-5}$, $10^{-4}$ and $10^{-3}$ g/ml. Oxitropium was inactive at $10^{-5}$ g/ml, but at $10^{-4}$ and $10^{-3}$ g/ml, it significantly inhibited the MCT rate to $87.6 \pm 2.8$ and to $82.6 \pm 2.9\%$, respectively.

These three drugs were evaluated for their effects on ACh- and eserine-induced MCT acceleration. Effects of ACh and eserine on MCT rate are shown in Fig. 3. Both agents concentration-dependently facilitated the MCT rate. Concentrations of ACh and eserine to cause a 50% increase in comparison with the distilled water-treated control group were $2.77 \times 10^{-2}$ and $1.36 \times 10^{-3}$ g/ml, respectively. ACh at $10^{-1}$ g/ml and eserine at $10^{-2}$ g/ml, which were used to examine the effects of anticholinergic drugs on their facilitated reactions, caused approximately a 70% increase in the MCT rate (Fig. 3), and the increase lasted for 50 min (data not shown). The following results were compared with those obtained from the distilled water-pretreated, stimulant-treated control groups. As shown in Fig. 4, MCT acceleration induced by ACh was concentration-dependently inhibited by the pretreatment of each anticholinergic drug at $10^{-4}$ and $10^{-3}$ g/ml. In the meanwhile, MCT acceleration induced by eserine was significantly inhibited by atropine, ipratropium and oxitropium at $10^{-5}$ g/ml to $61.8 \pm 8.3$, $82.5 \pm 10.7$ and $76.5 \pm 9.0\%$, respectively, and also inhibited by each drug even at $10^{-6}$ g/ml (Fig. 5).

2. Effects on airway secretion: Neither oxitropium nor ipratropium, both at $4 \times 10^{-9}$ to $4 \times 10^{-5}$ g/ml, inhibited airway secretion. Atropine, however, significantly inhibited
airway secretion at $4 \times 10^{-7}$ and $4 \times 10^{-5}$ g/ml (Fig. 6).

Discussion

In the present study, the effects on MCT rate made no significant difference among the three anticholinergic drug. This finding is supported by the report that oxitropium and ipratropium had no specific selectivity to muscarinic receptor subtypes ($M_1$, $M_2$), similarly to atropine (12). In high concentrations, these three drugs caused a similar and slight inhibition of the normal MCT. Iravani and Melville initially reported that ipratropium did not change the ciliary beat frequency, but later they changed their description to that it was "mildly ciliodepressive" (13). The present observation supports their later description. Muller et al. investigated the effects of ipratropium on MCT in $^{99m}$Tc-inhaling subjects by the scintigraphic technique and found that this drug had no effect (14). Although the difference of the present result from their finding is not clear, we suppose that it may be due to the doses of drugs and/or the species difference of the involvement of the vagus on MCT.

Pretreatments with atropine, ipratropium and oxitropium markedly inhibited ACh- and eserine-induced MCT acceleration. The inhibitory effects of the drugs on eserine-induced MCT acceleration were shown at lower concentrations than those required to inhibit ACh-induced acceleration. Eserine increases

![Fig. 5. Effects of atropine ($\triangle$), ipratropium ($\Box$) and oxitropium ($\bigcirc$) on eserine-induced MCT rate. Results are shown as a percentage of the MCT rate in the distilled water-treated group. The values show means with S.E. (bars) from 6 experiments. *P<0.05 vs. distilled water-treated group.](image)

![Fig. 6. Effects of atropine, ipratropium and oxitropium on the output volume of respiratory tract fluid (RTF) in rabbits. The values are shown as a percentage of the volume in the physiological saline-treated group. Open, hatched and dotted columns represent atropine-, ipratropium- and oxitropium-treated groups, respectively. The values show the means with S.E. (bars) from 6 experiments. *P<0.05 vs. distilled water-treated group.](image)
endogenous ACh which, in turn, promotes MCT. These nebulized anticholinergic drugs could inhibit the effect of endogenous ACh on the airway. This finding may explain the effectiveness of anticholinergic drugs on the bronchial constriction due to the involvement of vagal mechanisms (15).

MCT has been shown to fail in the absence of mucus, but can be restored by placing autologous or heterologous mucus on the epithelium (16). Thus, to examine the influence on the mucociliary clearance, the study of airway secretion also must be performed conventionally. In the present study, we showed the inhibitory effect of atropine alone on normal airway secretion in rabbits using the common method. This finding was opposed to the present results on the effects on MCT rate and the report that the anticholinergic activities of oxitropium and ipratropium were stronger than that of atropine when parenterally administered and that these drugs could not distinguish between muscarinic receptor subtypes (12). Although the exact explanation for these discrepancies is difficult, they may be due to a species difference and/or a yet undefined mechanism induced by atropine.

In conclusion, the present findings suggest that neither oxitropium nor ipratropium influenced the normal mucociliary clearance, but atropine might inhibit it under some conditions.

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