Influence of Corticosterone on Tracheal Mucociliary Transport in Pigeons

Hirofumi KAI, Satoko YAMAMOTO, Kazuo TAKAHAMA and Takeshi MIYATA
Department of Pharmacological Sciences, Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan
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Abstract—The effects of corticosteroids on tracheal mucociliary transport (MCT) were examined in pigeons. Intramuscular administration of corticosterone had no effect at 1.0 mg/kg, while at a larger dose of 5.0 mg/kg, it slightly, but significantly increased the MCT rate. Metyrapone significantly decreased the MCT rate, and the inhibitory action was blocked by 1.0 mg/kg corticosterone. The present study suggested that corticosteroids modulated the mucociliary clearance, especially under some diseases associated with a decreased level of endogenous corticosteroids.

Airway mucociliary clearance is an important defense mechanism that serves to remove viscous sputum and inhaled substances from the lung (1, 2).

A stimulating effect on mucociliary transport has been attributed to a number of different pharmacologic agents: adrenergic agents, cholinergic agents, biologically active amines and methylxanthines (1). Many of these drugs may be of therapeutic value in disease states associated with an impairment of mucociliary transport.

Systemic corticosteroids ameliorate bronchial obstruction and facilitate expectoration in patients with asthma and chronic bronchitis, although they do not alter sputum viscosity (1). Direct exposure of the bronchial mucosa to prednisolone results in mild cilioexcitation (3), whereas the topical corticosteroid beclomethasone dipropionate had no effect on tracheal mucosa velocity in conscious sheep (1). Thus, the contribution of corticosteroids to mucociliary transport remains unclear.

In the present study, the effects of exogenous and endogenous corticosteroids and metyrapone, an adrenal 11-β steroid hydroxylase inhibitor, on tracheal mucociliary transport in pigeons were investigated.

Urethanized pigeons of either sex weighing 250–350 g were used.

The mucociliary transport rate was measured according to the method of Miyata et al. (4–6). The pigeon was fixed on its back, and its trachea was exposed according to the usual method. Blood vessels and connective tissue over the trachea were separated carefully over 2 cm from approximately 1 cm below the larynx. Two threads were tightly laid under the exposed trachea, with a distance of 2 cm between the threads, so that the part of the trachea used for the test was kept level. An incision of approximately 2 cm was made, and traumatic margins were clipped with celifers and fixed with the traumatic opening slightly opened. As soon as the operation was over, the pigeon was inserted through the opening into an observation box. The observation box kept the pigeon tracheal mucosa under the same temperature (38°C) and humidity (approximately 100%) as in vivo by the use of a humidifier. Several particles of cork, of 80–120 μm in diameter, previously floated on physiological saline, were placed on the caudal side of the tracheal mucosa to select the part which carried the particles the fastest. Measurement was performed at the same site throughout the experiment and started when the cork transport velocity to move 10 mm became constant.
Corticosterone (Sigma) dissolved in 30% polyethylene glycol solution and diluted with saline was administered intramuscularly, and metyrapone (Sigma) dissolved in saline was administered intraperitoneally. The control group was given 1.0 ml/kg of vehicle.

In a separate experiment, the arterial blood at 30 min after administration was obtained from the pigeons, and plasma corticosterone levels were measured using high performance liquid chromatography (HPLC). The extraction of corticosterone in the plasma was performed according to the method of Tamura et al. (7). Plasma (0.5 ml) added with 100 ng methylprednisolone (Sigma) as an internal standard was extracted with a solution containing 1 ml distilled water, 0.1 ml isooamyl alcohol, 0.5 ml 0.1 N NaOH and 10 ml methylene chloride. After the upper phase was removed, the solution was washed twice with 1 ml 0.1 N NaOH and with 1 ml distilled water, and then evaporated to dryness by an evaporator under reduced pressure at 40°C. The residue was dissolved in 0.5 ml 10% methanol and washed with 2 ml n-hexane. After evaporation to dryness under the same conditions as described above, the residue was dissolved in 20 μl methanol and subjected to HPLC.

HPLC was performed with a μBondapack C18 reverse-phase column (4 mm x 30 cm, Waters Assoc.), mobile phase solvent: 0.5% acetate solution/acetonitrile (70:30), flow rate: 1 ml/min, UV detection: at 243 nm. The average recovery of corticosterone was 40±5%. The lowest detectable amount was 5 ng/ml.

Student’s t-test for unpaired observations was used for statistical analysis. A P value <0.05 was considered significant.

First the effects of corticosterone on normal MCT rate were examined. Corticosterone had no effect at 1.0 mg/kg, while at the larger dose of 5.0 mg/kg, it slightly, but significantly increased the MCT rate at 10-30 min (Fig. 1). Prednisolone also had no effect at 1 mg/kg (data not shown). The influence of metyrapone treatment and the effect of simultaneous administration of 1 mg/kg of corticosterone on the MCT rate are shown in Fig. 1. Metyrapone at a dose of 150 mg/kg, which remarkably reduced endogenous corticosterone levels, significantly decreased the MCT rate over 20 min to 60 min after administration. The effect reached its maximum about 40 min later and then gradually recovered. Simultaneous administration of 1.0 mg/kg corticosterone with metyrapone blocked the metyrapone inhibition on MCT rate.

Table 1 shows corticosterone levels in the plasma of pigeons at 30 min after administra-

![Fig. 1. Effects of corticosterone and metyrapone on MCT rate in pigeons.](image)
Corticosterone is a main corticosteroid in the plasma of pigeons. Metyrapone prominently decreased the level of corticosterone in the plasma, and the decreased levels of corticosterone were recovered by the treatment with 1.0 mg/kg corticosterone.

The present study shows the involvement of corticosteroids in the mucociliary clearance system.

The mucociliary clearance is modulated by mucus components as well as the ciliary motion. Corticosteroids increased, although to a small degree, the rate of ciliary beat (3) and reduced the mucus secretion from human airway in vitro (8). These findings using a high dose of drug may support the present results, especially the increase of the MCT rate at the large dose of 5.0 mg/kg of corticosterone, while some problems such as differences in species and/or in the method for application of drugs (e.g., in vitro, in vivo) remain to be clarified.

Many biological materials, especially pulmonary surfactants, also influence the mucociliary function (1). We previously reported that surface active phospholipid, a main component of the surfactant, was a protective factor of the mucociliary clearance from the acid-induced inhibition (9). Also, Schlimmer et al. (10) proposed the hypothesis that phospholipids play an important role in MCT by preventing the agglomeration and adhesion of mucus particles. Pulmonary surfactant production is accelerated by the exposure to exogenous corticosteroids in the lung of fetal animals. This action is mostly due to the production of a fibroblast-pneumocyte factor, which subsequently acts on type 2 pneumocytes and stimulates surfactant synthesis (for review see Ref. 11). Sosenko et al. (12) have indicated that adrenal blockade with metyrapone delayed the maturation of the surfactant in fetal rats. In addition, we found that the secretion of surfactant depended on the level of endogenous corticosterone even in adult mice (13). Although the production and levels of pulmonary surfactant were not determined in the present study, it is reasonable to speculate that the endogenous corticosterone may regulate the mucociliary function through surfactant secretion.

Metyrapone is a very effective inhibitor of cytochrome P-450 (14). Cytochrome P-450 is related to metabolism as well as synthesis of corticosterone (15). Thus, the higher level of corticosterone in plasma after administration of corticosterone in the presence of metyrapone compared to the level of corticosterone in the absence of metyrapone may be due to the inhibition of metabolism of corticosterone by metyrapone.

Our preliminary study showed that the plasma corticosterone level was low in the chronic bronchitic rats in which MCT function was depressed.

Consequently, corticosteroids may act on the pulmonary defense system involving mucociliary clearance, particularly under some diseases associated with a decreased level of endogenous corticosteroids. However, further studies are undoubtedly necessary to elucidate the mechanism of action and the pathophysiological significance of corticosteroids in the mucociliary clearance.

References

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