The Effect of Tulobuterol Tape on Histamine-Induced Bronchoconstriction in Conscious Guinea Pigs: Long Duration of Action

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ABSTRACT—The objective of the present study was to determine the action duration of tulobuterol tape within a 24-hr period in conscious guinea pigs. The bronchoconstriction induced by histamine-inhalation was significantly inhibited by tulobuterol tape in comparison with its placebo tape 8 and 12 hr after binding, and the inhibitory rate was $50 \pm 11\%$ and $35 \pm 13\%$, respectively. Twenty-four hours after binding, the inhibitory effect of tulobuterol tape gradually diminished, but the inhibitory rate was maintained at $30 \pm 14\%$. These results suggest that tulobuterol tape has a long lasting bronchodilatory action.

Keywords: Tulobuterol tape, β_2 stimulant, A patch formulation

Tulobuterol (α -[(*tert*-butylamino)methyl]-*o*-chlorobenzyl alcohol) hydrochloride is a β_2 -adrenoceptor stimulant that has played an important role in the treatment of bronchial asthma over the last 18 years. Recently, the patch formulation of tulobuterol using a technique of the transdermal delivery system has been developed. We previously studied the effects of tulobuterol tape on airway resistance, heart rate and blood pressure in anesthetized guinea pigs and dogs, and we reported that tulobuterol tape had a long lasting bronchodilatory action (up to 8 hr) and did not affect the cardiovascular system (1). In that report (1), we further revealed that the bronchodilatory action of tulobuterol tape lasted much longer than that of intraduodenally administered tulobuterol hydrochloride in anesthetized dogs. According to these previous data, we confirmed the usefulness of the patch formulation from the view points of efficacy and also adverse reactions. These confirmations including the action duration for up to 22 hr were also obtained by the precious investigations in humans (2, 3). However, we could not test the action duration of tulobuterol tape over 8 hr in anesthetized animals. Thus in the present study, we investigated the action duration of tulobuterol tape within a 24 hr period in conscious guinea pigs.

Male Hartley guinea pigs weighing 382–532 g obtained

from Charles River Japan, Inc. (Atsugi) were used. The animals were kept in an air-conditioned room and were given standard chow and water ad libitum. Tulobuterol tape, which included 2 mg of tulobuterol, and its placebo tape, 10 cm² in size, were obtained from Nitto Denko (Ibaraki), and histamine dihydrochloride was purchased from Nacalai Tesque (Kyoto).

One day prior to the experimental day, the backs of the animals were completely shaven using hair clippers and depilatories. Before the tape was placed on their backs on the experimental day, the basal resistance volume (RV) was measured by the method of Pennock et al. (4) using two-chambered, whole-body plethysmography (M.I.P.S., Osaka). Briefly, guinea pigs were placed in a perspex, whole-body plethysmography, with their necks extending through the partition between the two chambers and respiration-related pressure changes were measured in the "head-chamber" and "body-chamber" using pressure transducers. The phase shift between the thoracic (from "body-chamber") and mouth (from "head-chamber") volumes was measured by a respiratory analyzer. RV was measured with a data-logging analyzer with the formula below:

$$\tan \theta = W \times R \times C$$

where θ equals the phase difference between the thoracic and mouth flow signals; W equals $2\pi \times$ respiratory fre-

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quency; R equals pulmonary resistance; and C equals compressibility of lung volume:

$$C = V / \{(P_{\text{atm}} - 47) \times 1.36\}$$

where V equals thoracic gas volume, P_{atm} equals barometric pressure in millimeters of mercury, and 1.36 equals the factor to convert millimeters of mercury to centimeters of H_2O ; therefore:

$$RV = \{(P_{\text{atm}} - 47) \times 1.36 \times \tan \theta\} / \{2\pi \times \text{respiratory frequency}\}$$

The RV was averaged during the 10-breath-period. At 8, 12 or 24 hr after 2 pieces of tulobuterol tape or its placebo tape were placed on the backs of animals, an aerosol from a 0.03% solution of histamine was driven into the "head-chamber" for 30 sec using an ultrasonic nebulizer (Omron, Tokyo). The RV was measured for 2 min according to the method described above. The difference between the RV value (ΔRV) after histamine-inhalation and the basal value was calculated. Three experiments were independently performed and were designated as the 8, 12 and 24 hr-Exp. In each experiment, a placebo tape group was established. All experiments were performed in a blind manner.

All values were expressed as the mean and S.E.M. Statistical analysis was performed by a Student's *t*-test for unpaired observations between tulobuterol tape and the placebo tape groups. The data were expressed as the percentage values, where the ΔRV in each placebo tape group was 100.

The results are shown in Fig. 1. The bronchoconstriction induced by histamine-inhalation was significantly inhibited by tulobuterol tape (4 mg) in comparison with its placebo tape after binding for 8 or 12 hr, and the inhibitory rate was $50 \pm 11\%$ ($n=20$, $P=0.022$) and $35 \pm 13\%$ ($n=20$, $P=0.029$), respectively. Between the 12 and 24 hr-Exp., the inhibitory effect of tulobuterol tape gradually diminished, but the inhibitory rate was maintained at $30 \pm 14\%$ ($n=20$, $P=0.091$). The values of ΔRV in the placebo tape groups of the 8, 12 and 24 hr-Exp. were 25.8 ± 5.4 , 35.1 ± 4.5 and 33.5 ± 5.9 $\text{cmH}_2\text{O}/\text{sec}$ ($n=20$), respectively. No significant difference was observed among these values (one-way ANOVA).

In our previous study using anesthetized guinea pigs (1), we could not test the action duration of tulobuterol tape over 8 hr since the bronchoconstriction in anesthetized animals could not be reproducibly maintained.

Fig. 1. Effect of tulobuterol tape on histamine-induced bronchoconstriction in conscious guinea pigs (transdermal administration). Open column: placebo tape (20 cm^2), closed column: tulobuterol tape (20 cm^2) including 4 mg of tulobuterol. Data represent the mean and S.E.M. of 20 animals. The statistical probability (P value) is indicated on the top of each closed column (vs each open column).

No study had previously observed the effect of a β_2 -adrenoceptor stimulant for over 8 hr in anesthetized guinea pigs. In the present study, we investigated the action duration of tulobuterol tape for up to 24 hr in conscious guinea pigs by the method of Pennock et al. (4) using two-chambered, whole-body plethysmography. As a result, we confirmed that tulobuterol tape acts for up to 24 hr. These results and the previous data (1) suggest that tulobuterol tape exhibits long lasting bronchodilatory action without affecting the cardiovascular system.

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