PHARMACOKINETICS OF PLASMA AND URINE CLENBUTEROL IN MAN, RAT, AND RABBIT

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Therapeutic dose (20, 40 and 80 μg/man) of clenbuterol hydrochloride, a β2-adrenergic stimulant, was orally administered to healthy volunteers, and the unmetabolized drug in plasma and urine was determined by enzyme immunoassay. The plasma levels of clenbuterol reached the maximum value of 0.1, 0.2 and 0.35 ng/ml, respectively, in a dose-dependent manner within 2.5 h, which lasted for over 6 h after the administration. The half-life of clenbuterol in plasma was estimated to be about 35 h. When the drug was orally administered repeatedly to men twice a day, the plasma level reached the plateau within 4 d after the initial administration. At that time, the plasma levels of the unchanged form were 0.2 to 0.5 ng/ml and 0.5 to 0.6 ng/ml at doses of 20 and 40 μg/man, respectively. The bound ratio of the drug to plasma protein was estimated to be 89–98% at a single administration of 80 μg of the drug. The cumulative urinary excretion of unchanged compound corresponded to about 20% of the administered dose as measured at 72 h following a single oral administration. When clenbuterol hydrochloride was orally administered to rats at a dose of 2 μg/kg, the plasma level reached the maximum at about 1 h after the administration. In rabbits, the plasma concentrations reached the maximum value of about 0.2 and 0.8 ng/ml within 2 h following administration of clenbuterol hydrochloride at doses of 0.5 and 2 μg/kg, respectively. The half-life of clenbuterol in plasma was about 30 h in rats and about 9 h in rabbits.

Keywords—pharmacokinetics; clenbuterol; enzyme immunoassay (EIA); plasma; urine; human

INTRODUCTION

Clenbuterol hydrochloride, 4-amino-α-[(tert-butylamino)]methyl-3,5-dichlorobenzyl alcohol hydrochloride, a selective β2-adrenoreceptor stimulant, has been introduced by Keck et al.9 in 1972 as a potent bronchodilator for patients with bronchial asthma, chronic bronchitis and pulmonary emphysema.2–6 Its therapeutic dose is very small quantity, approximately 40 μg/man by a single dose.

The pharmacokinetic behavior and metabolic pathway of clenbuterol have been studied in men, dogs and rats with relatively high dose of the 14C-labelled compound.7–9 However, if one wishes to measure precisely the plasma level of clenbuterol following a clinical dose of this agent by oral administration (around 40 μg/man), a highly sensitive and specific analytical method is required. Although the development of radioimmunoassay (RIA) for this compound has been tried, a successful result is not obtained in terms of sensitivity capable of measuring plasma clenbuterol.9

In previous report,10 we have developed an enzyme immunoassay (EIA) method for this compound, which allows to detect as low as 1–500 pg/ml.

We here report results on the measurement of plasma and urinary concentration of the unchanged compound after single or repeated oral administrations of therapeutic doses of clenbuterol hydrochloride to healthy volunteers, rats and rabbits.
MATERIALS AND METHODS

1. Materials — Clenbuterol hydrochloride (NAB 365) and NA 1141 were supplied by Teijin Co. Ltd. (Tokyo) (Fig. 1). β-D-Galactosidase (β-Gal., from Escherichia coli, Grade VIII, 674 units/mg protein), human serum albumin (HSA, crystallized and lyophilized, essentially globulin free), bovine serum albumin (BSA, crystallized and lyophilized, essentially globulin free), 4-methylumbelliferone (4-MU) and 4-methylumbelliferyl-β-D-galactoside (4-MUG) were obtained from Sigma Co. Ltd. (St. Louis, Mo), Freund’s complete and incomplete adjuvants, from Difco Lab. (Detroit, Mich); polystyrene balls (diameter, 1/4 inch), from Ichico Co. Ltd. (Nagoya). ACS II scintillation cocktail, from RCC Amersham (London). All other chemicals from commercial sources were of analytical grade quality. Goat anti-rabbit immunoglobulin G (IgG) antibody was prepared in our laboratory by immunizing goats with 2 mg of purified rabbit IgG and complete Freund’s adjuvant.

2. Sample Preparation — 1) Human Plasma Sample: Twelve healthy male volunteers were subjected to this study. Medication and sampling of blood and urine from healthy volunteers were carried out in the Pharmacological laboratory in Hamamatsu University between February and April, 1980. Tablets each containing 10 µg of clenbuterol hydrochloride (offered from Teijin Co. Ltd.) were used. For a single administration, three different doses (20, 40 and 80 µg) were medicated and the compound was administered at 9:00 a.m. under hungry condition. For repeated administrations, two different doses (20 and 40 µg) were medicated, and the tablets were orally given twice a day at 9:00 a.m. under hungry condition and after dinner (9:00 p.m.), for 5 d. Twelve volunteers were separated into groups of 3 persons each according to the doses used. The mean age was 35 years old and the mean body weight was 63.9 kg. There were no considerable intergroup differences in terms of mean age and average body weight. Blood samples were taken just before and 1, 2, 3, 4, 6, 12, 24, 48 and 72 h after the administration in the clinical study of single administration.

During the treatment period, blood was sampled at 9:00 a.m. (just before administration) and 4 h after the administration (1:00 p.m.) every day, and at 9:00 a.m. on the 6th day. The samples (2.5 ml each) were immediately centrifuged to separate the plasma, which was frozen and stored until use for EIA.

To determine the percent binding of clenbuterol to plasma protein, 1 ml of plasma sample obtained from the subjects treated with 80 µg clenbuterol was centrifuged with a membrane filter (Amicon Centriflo Type CF25, U.S.A.) at 1500 rpm for 5 min and the filtrate was determined as free form in plasma.

2) Human Urine Sample: For the measurement of unchanged clenbuterol excreted in urine, following single administration of 20, 40 or 80 µg clenbuterol hydrochloride, urine samples were collected for 3 d.

3) Rat Plasma Sample: Sprague–Dawley male and female rats (9 weeks old) were used in the experiment. Groups of three animals of either sex were orally treated with clenbuterol hydrochloride dissolved in saline at a dose of 2 µg/kg. Those animals were fasted for 12 h before experiments. Two-hundred µl of blood samples were taken from cardiac puncture under anesthesia with ether at the indicated time. The plasma samples were used for EIA.

![Chemical Structures of Cleanbuterol and NA 1141](image-url)
4) Rabbit Plasma Sample: Male albino rabbits (3 months old) were separated into two groups of 3 animals each, and treated with clenbuterol hydrochloride at a dose of 0.5 or 2 µg/kg by a single oral administration. Blood samples were taken from the ear vein by 500 µl each at the indicated time following administration. The plasma samples were used for EIA.

3. Enzyme Immunoassay Procedure — One hundred µl of plasma was diluted with 4 volumes of 0.1 M phosphate buffered solution (pH 6.6) containing 0.1 M NaCl and 1 mM MgCl₂, then heated at 100 °C for 1 min, and centrifuged at 15000 rpm for 20 min. The obtained supernatant fluid was used as sample for EIA of clenbuterol. One hundred µl of the urine specimen was five-fold diluted with the same buffer and heated for 1 min. Aliquots of the sample were used as a sample for EIA of clenbuterol.

Clenbuterol in blood and urine was assayed by EIA based on the method of Yamamoto and Iwata, whose principle is schematically shown in Fig. 2. Specific antibody to clenbuterol and NA 1141-β-Gal were prepared by the method previously described.

One hundred µl of standard solution of clenbuterol hydrochloride in phosphate buffer (pH 6.6) containing 0.1 M NaCl and 1 mM MgCl₂ or the heat-treated sample solution was mixed with 50 µl of NA 1141-β-Gal solution (diluted 1:2000 with 0.1 M phosphate buffer (pH 6.6) containing 5% BSA, 0.1 M NaCl and 1 mM MgCl₂) and 50 µl of anti-clenbuterol serum (diluted 1:3200 with 0.1 M phosphate buffer (pH 6.6) containing 5% of BSA, 0.1 M NaCl and 1 mM MgCl₂) and incubated in a tube at 4 °C for 18 h. Then, 200 µl of 0.1 M phosphate buffer (pH 6.6) containing 0.1 M NaCl, 1 mM MgCl₂ and 2.5% BSA, and a

FIG. 2. Principles of EIA for Clenbuterol

-> clenbuterol specific antibody, ○-○ NA 1141 -β-Gal, ○ clenbuterol (sample or standard), >++ 2nd Ab: IgG Fr. of anti-rabbit IgG (goat).
second antibody immobilized polystyrene ball were added to each assay solution. After rocking the ball in the mixture for 5 h at room temperature, the ball was washed with 0.01 M phosphate buffer (pH 6.6) containing 0.1 M NaCl and 1 mM MgCl₂ and transferred to a tube holding 200 μl of 0.3 mM 4-MUG and 200 μl of the same buffer for measuring the activity of β-Gal bound to the polystyrene ball. After incubation for 1 h at 37 °C, 2.5 ml of 0.1 M glycine–NaOH buffer was added. The amount of the 4-MU liberated was determined by fluorescence spectrophotometry with an excitation wave-length at 360 nm and emission wave length at 450 nm. Each assay was performed by duplicate run.

RESULTS

1. Plasma Concentration of Clenbuterol in Man

1) Single Administration — Fig. 3 shows the time course of the mean plasma concentrations of clenbuterol after a single oral administration of clenbuterol HCl at a dose of 20, 40 or 80 μg to healthy volunteers. The plasma concentration reached the peak level within 2—3 h following administration. The values at the peak were 0.1, 0.2 and 0.35 ng/ml of plasma after the drug administration at doses of 20, 40 and 80 μg/man, respectively. The values at the peak increased in a dose-dependent manner. The half-life of clenbuterol in plasma was estimated to be about 35 h from the observed declining-curves.

2) Multiple Administrations — Fig. 4 shows the pharmacokinetics of clenbuterol in plasma after the repeated oral treatments of three healthy volunteers with clenbuterol hydrochloride at a dose of 20 or 40 μg. The drug was administered at 9:00 a.m. and 9:00 p.m. every day, and blood sample was taken twice: just before and 4 h after the administration through 5 d.

It was found that a steady state of the plasma concentration corresponding to 0.2—0.3 ng/ml, was reached in 4 d after the initiation of the treatment with 20 μg of clenbuterol hydrochloride. In 40 μg-treated group (two persons), the steady-state concentration was also achieved corresponding to 0.5—0.6 ng/ml, after 4 d. As a somewhat high concentration was observed in one of the three volunteers on day 3 after the administration the drug medication was ceased. Therefore,

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**FIG. 3. Plasma Levels of Clenbuterol after a Single Oral Administration to Human Volunteers**

Clenbuterol hydrochloride was orally administered to 9 volunteers divided into 3 groups each. Each point represents the mean ± S.E. of 3 men.

- △ — 80 μg/body, — O — 40 μg/body, — X — 20 μg/body.

**FIG. 4. Plasma Levels of Clenbuterol after Repeated Oral Administrations to Human Volunteers**

Clenbuterol hydrochloride was orally administered at a dose of 20 μg or 40 μg/body at 12 h intervals. Each point represents the mean of 2 to 3 volunteers.

- O — 20 μg, — X — 40 μg.
calculation was performed except the value from that person.

2. Plasma Protein Binding

Using plasma samples from healthy volunteers treated with a single administration of 80 µg of clenbuterol hydrochloride, free clenbuterol was estimated in order to know the plasma binding of the drug. The result was shown in Table I. It was found that approximately 96–98% clenbuterol was bound to plasma protein at the period of 1 to 24 h after administration.

3. Urinary Excretion in Healthy Volunteers

Table II shows the total amount of the unchanged compound eliminated by the renal route over a period of 72 h after a single administration of clenbuterol hydrochloride.

The percent excretion of three groups (20, 40 and 80 µg/man) was 21.6%, 17.7% and 20.3%, respectively, indicating no significant differences between three different groups.

**TABLE I. Plasma Protein Binding of Clenbuterol**

<table>
<thead>
<tr>
<th>Time after administration (h)</th>
<th>Clenbuterol in plasma (ng/ml)</th>
<th>Free clenbuterol in plasma (ng/ml)</th>
<th>Bound ratio to plasma protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.261±0.016</td>
<td>0.007±0.002</td>
<td>97</td>
</tr>
<tr>
<td>2</td>
<td>0.313±0.011</td>
<td>0.010±0.004</td>
<td>97</td>
</tr>
<tr>
<td>3</td>
<td>0.339±0.029</td>
<td>0.007±0.002</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>0.310±0.037</td>
<td>0.010±0.003</td>
<td>97</td>
</tr>
<tr>
<td>6</td>
<td>0.315±0.021</td>
<td>0.010±0.003</td>
<td>97</td>
</tr>
<tr>
<td>12</td>
<td>0.223±0.009</td>
<td>0.007±0.001</td>
<td>97</td>
</tr>
<tr>
<td>24</td>
<td>0.156±0.008</td>
<td>0.007±0.002</td>
<td>96</td>
</tr>
<tr>
<td>48</td>
<td>0.092±0.006</td>
<td>0.007±0.001</td>
<td>92</td>
</tr>
<tr>
<td>72</td>
<td>0.062±0.005</td>
<td>0.007±0.002</td>
<td>89</td>
</tr>
</tbody>
</table>

*The value represents the mean ± S.E.*

**TABLE II. Cumulative Urinary Excretion of Clenbuterol**

<table>
<thead>
<tr>
<th>Single dose</th>
<th>Volunteer</th>
<th>0—24 h (µg)</th>
<th>0—48 h (µg)</th>
<th>0—72 h (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 µg</td>
<td>M.N.</td>
<td>2.04 (10.2%)</td>
<td>2.76 (13.8%)</td>
<td>3.26 (16.3%)</td>
</tr>
<tr>
<td></td>
<td>K.M.</td>
<td>3.92 (19.6%)</td>
<td>4.75 (23.8%)</td>
<td>5.10 (25.5%)</td>
</tr>
<tr>
<td></td>
<td>H.A.</td>
<td>3.10 (15.5%)</td>
<td>3.95 (19.8%)</td>
<td>4.60 (23.0%)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.02 (15.1%)</td>
<td>3.82 (19.1%)</td>
<td>4.32 (21.6%)</td>
</tr>
<tr>
<td>40 µg</td>
<td>Y.M.</td>
<td>4.63 (11.5%)</td>
<td>5.93 (14.8%)</td>
<td>7.03 (17.6%)</td>
</tr>
<tr>
<td></td>
<td>A.S.</td>
<td>5.20 (13.0%)</td>
<td>6.66 (16.7%)</td>
<td>7.76 (19.4%)</td>
</tr>
<tr>
<td></td>
<td>A.M.</td>
<td>3.26 (8.2%)</td>
<td>5.56 (13.9%)</td>
<td>6.46 (16.2%)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>4.36 (10.9%)</td>
<td>6.05 (15.1%)</td>
<td>7.08 (17.7%)</td>
</tr>
<tr>
<td>80 µg</td>
<td>S.S.</td>
<td>10.80 (13.5%)</td>
<td>15.15 (18.9%)</td>
<td>19.20 (24.0%)</td>
</tr>
<tr>
<td></td>
<td>K.T.</td>
<td>8.16 (10.2%)</td>
<td>10.96 (13.7%)</td>
<td>13.75 (17.2%)</td>
</tr>
<tr>
<td></td>
<td>T.I.</td>
<td>14.90 (18.5%)</td>
<td>17.68 (22.1%)</td>
<td>18.63 (23.3%)</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>11.25 (14.1%)</td>
<td>14.60 (18.3%)</td>
<td>17.19 (21.5%)</td>
</tr>
</tbody>
</table>

*Total mean (13.4%) (17.5%) (20.3%)*
4. Plasma Concentration of Unchanged Compound in Rats

Fig. 5 shows the time course of the plasma concentration after a single oral administration of clenbuterol hydrochloride to male and female rats at a dose of 2 µg/kg. The maximum of plasma level was achieved 1 h after the drug administration in both sexes. The values were 0.08 ng/ml in males, and 0.11 ng/ml in females, but there was no significant sex difference in time profile of the whole plasma concentration. The half-life of the drug in plasma for both sexes was also estimated as approximately 30 h from the declining curves.

5. Plasma Concentration of Clenbuterol in Rabbits

Fig. 6 shows the time course of the plasma concentration of clenbuterol after a single oral administration of clenbuterol hydrochloride at a dose of 2 or 0.5 µg/kg to male rabbits. At these two dose levels, the plasma concentration reached the maximum at 2 h following the administration, whose level was 0.78 ng/ml at 2 µg/kg and 0.15 ng/ml at 0.5 µg/kg, respectively. The half-life of plasma concentration was about 10 and 8 h in the group treated with clenbuterol hydrochloride at doses of 2 and 0.5 µg/kg, respectively.

**DISCUSSION**

Clenbuterol was developed as a bronchodilator which acts selectively on β_{2}-receptor, and its pharmacodynamic behavior has been studied by means of radioimmunoassay to measure the plasma or urine concentration of clenbuterol after massive administration to animals,\(^6,7\) or by using \(^{14}\)C-labelled clenbuterol to humans.\(^9\)

In the present study, we examined the pharmacokinetics of plasma level of clenbuterol analyzed by EIA which established by Yamamoto and Iwata,\(^10\) following the therapeutic dose (20–80 µg/man) of clenbuterol HCl. It was observed that the plasma concentration of clenbuterol was dose-dependent and the half-life of the drug in plasma was about 35 h. These findings are well consistent with the result obtained in the experiments using \(^{14}\)C-labelled clenbuterol which demonstrated that 75% of the compound
detected in the blood at the maximal level are unchanged clenbuterol. Therefore, it was considered that most of clenbuterol in human blood exists as an unchanged form. When the drug was repeatedly administered, the plasma level of clenbuterol reached the plateau at day 4 following the initiation of administration. The steady-state level of 0.2—0.3 ng/ml was achieved after twice daily administration of 20 μg, while the level of 0.5—0.6 ng/ml was obtained after twice daily administration of 40 μg was repeatedly treated.

After the oral administration of clenbuterol hydrochloride it was excreted rapidly in urine within 24 h, and in 72 h about 20% of the administered dose was excreted in urine as an unchanged form. The study using 14C-clenbuterol shows that 43% of the total radio-activity administered was excreted as unchanged in 48 h-urine. These findings suggested that clenbuterol is relatively unsusceptible to metabolism and that it is eliminated via the renal route as unchanged form or conjugated forms such as glucuronide and sulfate.

When the drug was orally administered at a dose of 2 μg/kg to male or female rats, the plasma concentration of clenbuterol reached the maximum value of about 0.1 ng/ml at 1 h following the administration. The time (h) reaching to maximum concentration of clenbuterol in plasma coincides with that of the experiment with high dose (5 mg/kg) of 14C-labeled compound in rats. The plasma level of clenbuterol in dogs began to increase within 15 min after intraduodenal administration, indicating the rapid absorption of the drug from the intestine, and reached its maximum level within 60 to 90 min in a dose-dependent manner. The half-life of clenbuterol in plasma was about 30 h in rats as estimated from time course of the plasma concentration until 72 h following administration, while it was estimated to be about 8—10 h in rabbits. This apparently suggests that there is a species difference in pharmacodynamic behavior of clenbuterol. Therefore we believe that study of the metabolic fate of this drug in plasma is very meaningful to know its pharmacological action on humans.

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