Disposition of $^3$H–KAA–276 after Intratracheal Administration to Male Rats

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Summary: The absorption, distribution, and excretion of KAA–276 were studied in male rats after intratracheal administration of $^3$H–KAA–276.

1. The radioactivity level in plasma reached a maximum at 5 min after intratracheal administration of $^3$H–KAA–276 to male rats at a dose of 30 μg/kg. The AUC at this dose was 17.8 ng eq. of KAA–276•hr/ml, which accounted for about 100% of the AUC observed after intravenous administration at the same dose. The $C_{\text{max}}$ and the AUC were increased in a dose-dependent manner at the dose range of 10 to 100 μg/kg.

2. The radioactivity levels in the lungs, trachea, liver, pituitary, kidneys, urinary bladder, and mandibular gland were high after administration of $^3$H–KAA–276 to the rats. Low levels were detected in the central nervous system, eyeballs, and testes.

3. The excretion of radioactivity in the urine and feces was 17.8% and 82.1% of the dose, respectively, by 168 hr after administration. The excretion of radioactivity in the bile was 75.3% of the dose within 48 hr. Moreover, almost 40% of the biliary excreta was re-absorbed and excreted in the bile and urine.

Key words: KAA–276, Antihistamine, Trachea, Absorption, Distribution, Excretion, Rat

Introduction

KAA–276, 1-[1-(4-fluorophenylmethyl)-1H-benzimidazole–2-yl]-5-[2-[4-(2-carboxyethyl)phenyl]ethyl]-1,5-diazacyclooctane sulfate (Fig. 1), a novel, non-sedative histamine H₁-receptor antagonist, is currently under development for the treatment of bronchial asthma by inhalation.

Recently, a new generation of orally administered histamine H₁-receptor antagonists, such as terfenadine¹–³, astemizole⁴–⁶, ebastine⁷, and loratadine⁸, has been developed and classified as non-sedative antihistamines. These compounds are different from classical antihistamines that cause side effects on the central nervous system and have atropine-like effects⁹.

Although KAA–276 is similar to astemizole in chemical structure, the administration route distinguishes KAA–276, given by inhalation, from astemizole and the other antihistamines, which are given by oral administration.

The compound selectively inhibits $^3$H–mepyramine binding to guinea pig cerebellum preparations and has a washout resistant property in guinea pig ileum in vitro. In vivo, this compound inhibits histamine- or antigen-induced bronchoconstriction in rats and guinea pigs when inhaled in a small amount, in which case there is no inhibition of histamine-induced cutaneous reaction or other systemic pharmacological actions. This in vivo effect is produced promptly following inhalation and continues at least for several hours. No noteworthy toxic signs were observed in 2-week oral and inhalation toxicity tests in rats and beagles.

In the present study, the absorption, distribution, and excretion of KAA–276 were investigated in male rats after single intratracheal administration of the $^3$H-labeled compound to obtain data on its metabolic fate.

Materials and Methods

1. Labeled Compound

The free form of $^3$H–KAA–276 (Fig. 1, Lot No. TP–366) was synthesized at Daiichi Pure Chemicals Co., Ltd. The specific activity of $^3$H–KAA–276 was 4.72 GBq/mg; and the radiochemical purity, determined by high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC), was more than 97%.

2. Animals

Male Wistar rats (SPF, Charles River Japan), 7–8 weeks old and weighing 204–271 g, were used. The rats, housed at a temperature of 23±2°C and a humidity of
55±15%, were given a standard diet (MF; Oriental Yeast Co. Ltd.) and water. The animals were fasted overnight before and for 4 hr after dosing, but were given water ad libitum.

3. Drug Administration

³H-KAA-276 prepared by the addition of H₂SO₄ to the free form was diluted with non-labeled KAA-276, and was administered to rats as an isotonic saline solution. The pH of the drug solution was about 6.

For the intratracheal study, the rats were anesthetized lightly with sodium pentobarbital, and a hole was made with a needle in a site just superior to the bifurcation of the trachea. A polyethylene cannula (SP-10; Natsume Seisakusho Co., Ltd.) was then introduced through this hole, and the drug was injected into the trachea via the cannula. The doses of administered drug were 10, 30, and 100 µg/kg; and the volume was 0.4 ml/kg.

In oral and intravenous studies, the dose of administration was 30 µg/kg; and the volume was 2 ml/kg.

4. Measurement of Radioactivity

Radioactivity in each sample was counted with a liquid scintillation counter (LSC-903; Aloka). The counting efficiency was corrected by the channel ratio method using an external standard source.

5. Plasma Concentration

After intratracheal, oral, or intravenous administration of ³H-KAA-276, blood samples were collected at various intervals from a tail vein into heparinized tubes. The plasma (50 µl) obtained by centrifugation was dried at 40°C for more than 24 hr (the dry method), after which it was combusted in a sample oxidizer (Tri-Carb 306; Packard). The radioactivity in each sample was counted after addition of 14 ml of Monophase-S (Packard).

6. Distribution Experiment

After intratracheal administration of ³H-KAA-276 (30 µg/kg), each rat was anesthetized with ether; and a blood sample from the abdominal aorta was taken into a heparinized tube. After the animal had been killed by exsanguination, the tissues or organs were isolated immediately. The samples were thenweighed, and the radioactivity was determined by the same procedure employed for the plasma samples (the dry method).

7. Excretion in Urine and Feces

After intratracheal administration of ³H-KAA-276 (30 µg/kg), each rat was kept separately in a glass metabolism cage. Urine and feces were collected separately at various intervals for up to 168 hr. All air exiting from the chambers was passed through a trap filled with distilled water. The rats were killed by ether treatment at 168 hr after dosing. The urine sample was diluted with water to 100 ml, and the fecal sample was homogenized in 300 ml of water. The carcass was dissolved by heating under reflux for 96 hr in 500 ml of 0.5 M sodium hydroxide and 20 ml of toluene. The carcass solution was diluted with water to 1000 ml. Radioactivity in an aliquot of each sample solution was counted directly after addition of 10 ml of Atomlight (Du Pont NEN Research Products) for evaluation of total radioactivity excreted (the wet method). Radioactivity in an aliquot of the trapped water from expired air was counted directly after addition of 10 ml of Hionic-fluor (Packard). In addition, the radioactivity in an aliquot of urine and feces samples was determined by the same procedure as used for plasma and tissues samples (the dry method).

8. Excretion in Bile

The bile duct and trachea were cannulated with polyethylene cannula under ether and sodium pentobarbital anesthesia, and ³H-KAA-276 (30 µg/kg) was then injected into the trachea. Thereafter the animals were placed in Bollman cages. Bile, urine, and feces were collected at various intervals up to 48 hr after administration. The biliary sample was diluted with water to 50 ml. Radioactivity in an aliquot of solution was counted after addition of 10 ml of Hionic-fluor (Packard). Radioactivity in urine and feces was determined as described above (the wet method). The rats were killed by ether treatment at 168 hr after administration, and the gastrointestinal contents were removed. The radioactivity of the gastrointestinal contents was counted by the wet method. The radioactivity of the carcass was determined as described above. In addition, the radioactivity in an aliquot of bile sample was determined similarly as for the plasma and tissues samples (the dry method).

9. Entero-hepatic Circulation

³H-KAA-276 (30 µg/kg) was injected into the trachea of bile duct-cannulated rats, and the bile was collected into an ice-cold container up to 6 hr. The bile (0.821 MBq/8 ml/kg) was then injected into the duodenum of other bile duct-cannulated rats. The rats were placed in Bollman cages; and the bile, urine, and feces were collected at various intervals up to 48 hr after administration. The rats were killed by ether treatment at 48 hr, and the gastrointestinal contents were removed. The radioactivity in the urine, feces, gastrointestinal contents, and carcass was counted as described above (the wet method). In addition, the radioactivity in an aliquot of bile samples was determined by the dry method.

**Results**

1. Comparison of the Drug Absorption by Different Dosage Routes

Fig. 2 and Table I show the radioactivity concentrations (as equivalents of free form KAA-276, the same below) in plasma and pharmacokinetic parameters after
intratracheal, intravenous, and oral administration of \( ^3\text{H}-\text{KAA-276} \) (30 µg/kg) to male rats. The radioactivity concentration in the plasma after intratracheal administration of \( ^3\text{H}-\text{KAA-276} \) reached a maximum concentration \( C_{\text{max}} \) of 11.61 ng eq. of KAA-276/ml at 5 min after dosing. Then it declined with a half-life of 1.64 hr (2-8 hr).

After intravenous administration, the maximum radioactivity concentration was observed to be 16.13 ng eq. of KAA-276/ml at 2 min and declined with a half-life of 2.13 hr. The \( \text{AUC}_{(0-24\ hr)} \) after intratracheal or intravenous administration was 17.8 or 18.0 ng eq. of KAA-276 hr/ml, respectively, i.e., nearly the same. On the other hand, by oral administration the concentration of \( ^3\text{H}-\text{KAA-276} \) reached \( C_m \) (2.75 ng eq. of KAA-276/ml) at 30 min after dosing and declined with a half-life of 4.10 hr. The \( \text{AUC}_{0-8 hr} \) was 4.6 ng eq. of KAA-276 hr/ml, equivalent to about 30% of the \( \text{AUC}_{0-8 hr} \) after intravenous administration.

### Table I Pharmacokinetic parameters of radioactivity in plasma from male rats after administration of \(^3\text{H}-\text{KAA-276}\)

<table>
<thead>
<tr>
<th>Route</th>
<th>Dose (µg/kg)</th>
<th>( C_{\text{max}} ) (ng eq. of KAA-276/ml)</th>
<th>( T_{\max} ) (hr)</th>
<th>( \text{AUC}_{(0-24 hr)} ) (ng eq. of KAA-276 hr/ml)</th>
<th>( \text{AUC}_{(0-24 hr)} ) (ng eq. of KAA-276 hr/ml)</th>
<th>( t_{1/2(0-24 hr)} ) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intratracheal</td>
<td>10</td>
<td>4.47±0.63</td>
<td>0.03±0.00</td>
<td>4.6±0.5</td>
<td>5.1±0.3</td>
<td>1.57±0.30</td>
</tr>
<tr>
<td></td>
<td>Intratracheal</td>
<td>30</td>
<td>11.61±1.39</td>
<td>0.08±0.00</td>
<td>15.7±2.3</td>
<td>17.8±3.2</td>
</tr>
<tr>
<td></td>
<td>Intratracheal</td>
<td>100</td>
<td>51.62±11.30</td>
<td>0.03±0.00</td>
<td>52.0±5.6</td>
<td>56.9±6.9</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>30</td>
<td>16.13±1.53</td>
<td>(0.03)</td>
<td>15.6±1.3</td>
<td>18.0±1.4</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>30</td>
<td>2.75±0.34</td>
<td>0.50±0.00</td>
<td>4.6±0.2</td>
<td></td>
</tr>
</tbody>
</table>

Each value represents the mean±S.D. of three animals.

Data were obtained by the dry method.

### 2. Relationship Between Dose and Absorption

Fig. 3 and Table I show the radioactivity concentrations in the plasma and pharmacokinetic parameters after a single intratracheal administration at doses of 10, 30, and 100 µg/kg. \( \text{AUC}_{0-24 hr} \) and \( C_{\text{max}} \) were increased dose dependently. The \( \text{AUC}_{(0-24 hr)} \) after dosing with 10 µg/kg was 29% of that after dosing with 30 µg/kg; and the \( \text{AUC}_{(0-24 hr)} \) after the administration of 100 µg/kg was 3.2 times larger than that after administration of 30 µg/kg. The relationship between dose and \( \text{AUC}_{(0-24 hr)} \) was linear in a range of 10 to 100 µg/kg.

### 3. Tissue Distribution of \(^3\text{H}-\text{KAA-276}\)

Table II shows the radioactivity concentration in tissues after intratracheal administration of \( ^3\text{H}-\text{KAA-276} \) (30 µg/kg) to male rats. Concentrations of radioactivity peaked at 15 min in all tissues except for testes, intestinal tract, and urinary bladder. At 15 min after dosing, the radioactivity concentrations in the lungs, trachea,
and liver were highest and 15 to 37 times higher than their plasma concentration (10.087 ng eq. of KAA-276/ml). The radioactivity concentrations in the thyroid, pituitary, kidneys, esophagus, stomach, and pancreas were 4.6–8.5 times higher than the plasma concentration. Radioactivity equal to the plasma level was detected in the urinary bladder, skeletal muscle, thymus, large intestine, caecum, skin, and blood. In the epididymis, fat, eyeball, testes, cerebellum, spinal cord, and cerebrum, the radioactivity concentration was lower than the plasma one (4–44%).

At 1 hr after administration, the radioactivity concentra-
trations in the lungs, ileum, jejunum, liver, mandibular gland, urinary bladder and trachea were more than 10 times higher than the plasma concentration, and those in the hardarian gland, kidneys, esophagus, caecum, and sublingual gland were 5 times higher. Then the radioactivity gradually decreased in most of the tissues.

At 24 hr after administration, the radioactivity in the mandibular gland was 10% of the maximum level. The concentration in other tissues, except the intestinal tissues, was less than 5% of the maximum or below the detection limit.

At 168 hr after administration, the radioactivity con-
centrations in trachea, liver, and kidneys were less than 1% of their maximum. In the other tissues, the radioactivity concentrations were below the detection limit.

4. Excretion in Urine and Feces

Table III shows the cumulative excretion of radioactivity in the urine, feces, and expired air after intratracheal administration of $^3$H-KAA-276 (30 µg/kg) to male rats. The excretion of radioactivity in the urine was 16.9% of the dose within 24 hr, 17.4% within 48 hr, and 17.8% within 168 hr. The excretion of radioactivity in the feces was 51.6% within 24 hr, 80.0% within 48 hr, and 82.1% within 168 hr. The cumulative radioactivity from expired air trapped in water was 0.2% of the dose within 168 hr. The residual radioactivity in the carcass at 168 hr after dosing was 0.4% of the dose.

The percentage of tritiated water was 5.1% of the radioactivity in urine within 168 hr of dosing; however, no tritiated water was detected in feces within this time period.

5. Excretion in Bile

Table IV Cumulative excretion of radioactivity in bile, urine, and feces after intratracheal administration of $^3$H-KAA-276 to bile duct-cannulated male rats (dose: 30 µg/kg)

6. Entero-hepatic Circulation

Bile was collected 0-6 hr after intratracheal administration of $^3$H-KAA-276 to bile duct-cannulated male rats at a dose of 30 µg/kg. The bile was then injected into duodenum of other bile duct-cannulated rats.

Radioactivity was excreted in the bile in an amount equal to 14.4% of the dose within 4 hr, 32.5% within 24 hr, and 33.4% within 48 hr. The excretion of radioactivity in urine and feces, determined simultaneously, was 18.1% and 3.8% of the dose within 48 hr after dosing, respectively. The residual radioactivity in the gastrointestinal contents and the carcass at 48 hr after dosing was 3.2% and 1.1% of the dose, respectively.

The percentage of tritiated water was 5.8% of the radioactivity in the bile within 48 hr of dosing.
Table V Cumulative excretion of radioactivity in bile, urine, and feces from bile duct-cannulated male rats intraduodenal injection of bile sample (0-6 hr) obtained from bile duct-cannulated male rats after intratracheal administration of H-KAA-276 (dose: 30 µg/kg)

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Excretion of radioactivity (% of dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bile</td>
</tr>
<tr>
<td>0–1 hr</td>
<td>4.5±1.9</td>
</tr>
<tr>
<td>0–2 hr</td>
<td>8.9±2.2</td>
</tr>
<tr>
<td>0–4 hr</td>
<td>14.4±2.5</td>
</tr>
<tr>
<td>0–6 hr</td>
<td>19.6±2.6</td>
</tr>
<tr>
<td>0–8 hr</td>
<td>23.0±1.5</td>
</tr>
<tr>
<td>0–24 hr</td>
<td>32.5±2.9</td>
</tr>
<tr>
<td>0–48 hr</td>
<td>33.4±2.9</td>
</tr>
</tbody>
</table>

(0–48 hr) (32.6±3.4)
Gastrointestinal contents (at 48 hr) 4.1±0.8
Carcass (at 48 hr) 1.0±0.4

Each value represents the mean±S.D. of three animals.
The values in parentheses were obtained by the dry method.
N.S.: No sample

Discussion

The radioactivity concentration in the plasma reached its maximum within a few minutes after the intratracheal administration of H-KAA-276 to male rats. The AUC after intratracheal administration was the same as that after intravenous administration (Table I). Moreover, the absorption rate from the trachea was extremely fast, because almost the entire amount of radioactivity was excreted into the bile within 4 hr after dosing (Table IV). To investigate the dose dependency in the drug absorption, we used the dose range of 10 to 100 µg/kg, which is the pharmacologically effective dose range. The Cmax and the AUC increased dose-dependently, and a good correlation (r²=0.9994) was observed between the dose and the AUC.

In the distribution study, the highest concentration was observed in the administration sites, the trachea and the lungs, at the first sampling point after the administration (15 min, Table II). Although most of tissues showed many times higher concentration than the plasma level, the radioactivity was distributed poorly in the brain, eye balls, and testes. The data suggested that KAA-276 penetrated poorly through the blood-brain barrier. Although the radioactivity was observed in the caecum and large intestine, the concentration was almost equal to the plasma level, and also the muscle level. This may be due only to the systemic circulation. The elimination in most tissues was rapid, whereas higher concentrations remained in the target tissues, the trachea and lung tissue, which included the bronchi, within 24 hr after the administration.

Some radioactivity was found in the esophagus and the stomach. As an explanation for this, a small amount of the drug injected into the trachea might have been transported towards the pharynx by the ciliary action in the respiratory tract, and then passed into the gastrointestinal tract. The radioactivity level of the esophagus and the stomach, however, represented less than 5% of the total radioactivity. These results show that most of the drug administered via the trachea passed into the bronchi and possibly into the alveolar sacs, where it was absorbed well and rapidly.

The excretion of radioactivity in urine and feces was almost completed within 48 hr after intratracheal administration, and the ratio of radioactivity in the feces at that time was more than 80% of the dose (Table III). In bile duct-cannulated rats at 48 hr after intratracheal administration, the radioactivity was recovered mainly in the bile (75.3%, Table IV). In investigating the enterohepatic circulation, we found that at least 36% of the biliary excreta was re-absorbed, of which about 90% was re-excreted in the bile (Table V). The ratio of radioactivity in the enterohepatic circulation was estimated to be about 30% of the dose. These results show that KAA-276 was excreted mainly in bile, passed through the enterohepatic circulation, and consequently was excreted in the feces.

A large portion of the drug in the systemic circulation was extensively converted to metabolites oxidized on the carboxyethyl moiety and to conjugates. These relatively inactive metabolites were identified in rat bile (data not shown). When the drug was administered orally, the bioavailability would be low due to the first-pass metabolism by the liver. For this reason, we assume that the unchanged KAA-276 (active form) concentration in the target tissues, the trachea and the bronchus, would not reach its effective level after oral administration without side effects. Therefore, local administration, such as intratracheal and nasal administration, will be advantageous over oral administration.

This study shows that intratracheal administration is able to deliver KAA-276 efficiently to the target tissues, the trachea and the bronchus. Thus, intratracheal administration, including inhalation, is a particularly useful administration route for KAA-276 in the treatment of bronchial asthma.

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References
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