Excretion of Afobazole and Its Metabolites with Urine and Feces in Rats

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The amount of afobazole and identified metabolites was measured in the urine and feces of rats after intraperitoneal and peroral administration of the drug in a dose of 25 mg/kg. Over 1 day after intraperitoneal or peroral treatment with afobazole, urine and feces contained 0.1% initial compound (from administered dose) and 42.1% metabolites.

Key Words: *excretion; afobazole; metabolites*

Selective anxiolytic afobazole (2-(2-morpholinoethylthio)-5-ethoxybenzimidazole dihydrochloride) was synthesized at the V. V. Zakusov Institute of Pharmacology [2,3,8].

This work was designed to identify afobazole metabolites in the urine and feces of rats using synthetic standards. Moreover, the ratio of afobazole and its biological derivatives was measured in waste products (from administered dose of initial compound).

MATERIALS AND METHODS

Experiments were performed on male outbred albino rats weighing 200±20 g from Stolbovaya nursery (Russian Academy of Medical Sciences). The animals were maintained in a vivarium (V. V. Zakusov Institute of Pharmacology) under standard conditions and 12:12-h light/dark cycle. Afobazole in a single dose of 25 mg/kg (aqueous solution) was injected intraperitoneally or given perorally.

Afobazole and its metabolites were extracted from blood plasma and tissues with diethyl ester. A 20-fold volume of diethyl ether was added to 1 ml urine sample. The mixture was shaken for 15 min. Glucuronoconjugated fractions of afobazole metabolites in the urine were assayed after preincubation with β-glucuronidase (3000 U/ml) at 23°C for 24 h. A 20-fold volume of diethyl ether was added. The mixture was shaken for 15 min.

Fecal samples were dried in a hot-air sterilizer at 50°C. A weighted sample of feces was ground and homogenized in 1 ml pure water. A 20-fold volume of diethyl ether was added to the homogenate. The mixture was shaken for 15 min. Extraction was performed 2 times.

Mass spectra of metabolites were recorded on an Agilent Technologies liquid chromatograph (model 1100). Afobazole-containing samples of rat urine and feces were studied by high performance liquid chromatography on a Perkin Elmer chromatograph, which consisted of a PE-290 isocratic pump, PE-230 ultraviolet detector, and computer (software for chromatogram analysis). Chromatography was performed as described previously [1].

RESULTS

Daily urine samples contained ≥0.07% afobazole (from administered dose). Daily fecal samples contained 0.05 and 0.01% of the administered intraperitoneal and peroral dose, respectively (Fig. 1).

The amount of afobazole in rat feces was 5-fold higher after intraperitoneal injection (as compared to peroral administration). Hence, this compound is completely absorbed from the gastrointestinal tract into the systemic circulation.

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Products of afobazole biotransformation were also identified in rat urine and feces. Synthetic standards were used to measure the concentration of 5 metabolites, including M-3 (hydroxylated by the benzimidazole ring), M-6 (oxidized by the sulfur heteroatom), M-7 (hydroxylated by the aliphatic radical), M-11 (oxidized by the morpholine fragment), and afobazole sulfone (Figs. 2 and 3).

Chromatograms of the urine from treated rats had numerous unidentified metabolite peaks, which were absent in control samples.

M-3 is the major metabolite of afobazole in rat urine and feces. M-3 concentration in the urine 17-foldsurpassed that in fecal samples (Figs. 2 and 3). M-3 concentration in urine samples increased by 2.4 times after addition of β-glucuronidase and hydrolysis. The concentration of nonconjugated and conjugated M-3 in 24-h urine samples was 16.7 and 23.2% of administered afobazole dose, respectively. The urine included considerable amounts of M-7 and afobazole sulfone. However, the amount of other metabolites in 24-h urine (M-6 and M-11) and fecal samples (M-6, M-7, M-11, and afobazole sulfone) was low (Figs. 2 and 3).

Our results confirm previous data that benzimidazole derivatives are excreted in the form of benzimidazole ring-hydroxylated metabolites (conjugated and nonconjugated compounds) [5,6].

M-7 includes hydroxyl in the aliphatic radical of the benzimidazole ring. This metabolite probably does not form the glucuronoconjugate, since the concentration of M-7 in urine samples remains unchanged after hydrolysis with β-glucuronidase. Over 1 day after treatment with afobazole, the amount of identified compounds in urine and fecal samples was 40 and 1.3% of the administered dose, respectively. Our results are consistent with published data on the excretion of benzimidazole derivatives. For example, 80% of omeprazole dose are excreted in the urine (metabolites). Only a small part of afobazole is excreted with feces [7]. Similar results were obtained for lansoprazole and pantoprazole [6,9].

Analysis of the excretion of afobazole and its metabolites shows that elimination of afobazole in rats is related to biotransformation in the liver. Total excretion of afobazole metabolites with urine is 30-fold higher than with feces. Biotransformation of this compound mainly includes the formation of metabolites that are hydroxylated by the aromatic ring of the benzimidazole cycle.

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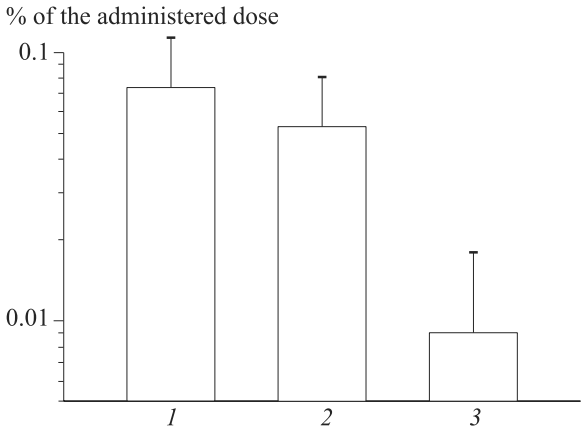

Fig. 1. Afobazole concentration in 24-h urine samples after intraperitoneal and peroral administration of this compound. Urine after intraperitoneal injection of afobazole (*1*); feces after intraperitoneal injection of afobazole (*2*); and feces after peroral administration of afobazole (*3*).

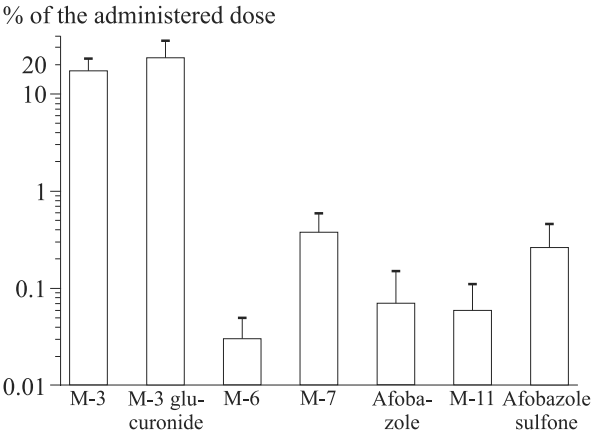

Fig. 2. Concentration of afobazole and its metabolites in 24-h urine samples after intraperitoneal injection of this compound.

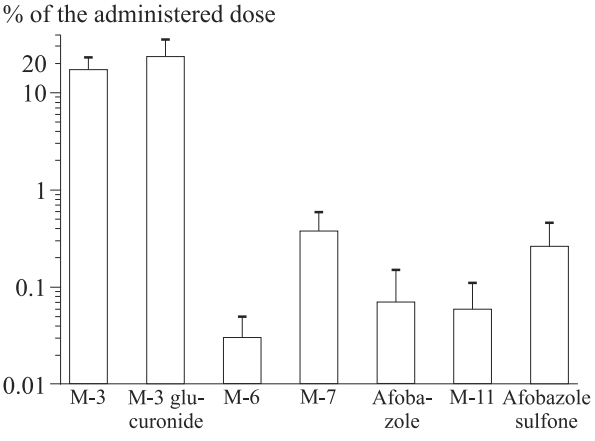

Fig. 3. Concentration of afobazole and its metabolites in 24-h fecal samples after intraperitoneal injection (light bars) and peroral administration of this compound (dark bars).

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