# Excretion of Himantane and Its Metabolites with the Urine and Feces in Rats

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The levels of himantane and its metabolites in daily urine and feces of rats were measured after intraperitoneal and oral dose of 25 mg/kg. The injected dose of the initial substance and 1.3% its metabolites were eliminated with excrements within 24 h after administration via both routes 0.23%.

**Key Words:** *experimental pharmacokinetics; himantane; excretion*

A potential antiparkinsonian drug himantane, N-(2-adamantyl)-hexamethylene iminohydrochloride, has been created at V. V. Zakusov Institute of Pharmacology [1].

We measured himantane and its metabolites in the daily urine and feces of rats by mass spectrometry and calculated the percentage of injected dose of the initial compound and products of its biotransformation in excrements.

## MATERIALS AND METHODS

The study was carried out on outbred albino male rats (200±30 g) from Stolbovaya Breeding Center of the Russian Academy of Medical Sciences. The animals were kept under standard vivarium conditions at 12:12 h light:dark regimen. The animals were fasting for 12 h before experiment. The drug dissolved in water was injected intraperitoneally and administered orally in a single dose of 25 mg/kg to 8 rats. The animals were kept in individual cages for collection of the urine and feces. Daily urine and feces were collected.

Potassium hydroxide (0.2 ml 2 M solution) and 20-fold volume of diethyl ether were added to experimental urinary samples (1.0 ml). The mixture was shaken on a mechanical horizontal shaker for 15 min. Extraction was carried out twice. The ether extract was collected and evaporated to dry residue in nitrogen flow. Glucurone-conjugated fractions of himantane metabolites were measured in the urine after its pre-incubation at 37°C with 3000 U/ml β-glucuronidase (from *E. coli* K12; Roche) for 1.5 h. KOH (0.2 ml 2 M solution) was added to the resultant mixture and extraction was carried out twice in 20-fold volume of diethyl ether.

The feces was dried in dry hot cabinet at 50°C. A specimen of 0.5 g was fragmented, homogenized in 2 ml water, and then processed similarly as the urine specimens. Dry residue was dissolved in 1 ml acetonitrile and 15 μl aliquots were put in the chromatographer injectors.

Himantane and its metabolites were measured in animal excretions by HPLC with a mass spectrometric detector on an Agilent Technologies chromatographer (series 1200).

The measurement conditions were as follows. Analytical column C18 – 150×2.1 mm (5-μ particles). Mobile phase: solution A and solution B in 1:1 proportion. Solution A: 25 ml 0.1 M ammonium acetate (Merk) and 2.5 ml concentrated formic acid (Merk) were put into a 500-ml flask and deionized water (Merk) was added to bring the volume to 500 ml. Solution B: 25 ml 0.1 M ammonium acetate (Merk) and 2.5 ml concentrated formic acid (Merk) were put into a 500-ml flask and extrapure acetonitrile (Merk) was added to bring the volume to 500 ml. The mobile phase was delivered at 0.4 ml/min, nitrogen at 12 liter/min, evaporator temperature 350°C. Detection was carried out in the positive ionization mode by complete ionic current, chromatographic analysis at ambient temperature (22-24°C) [2].

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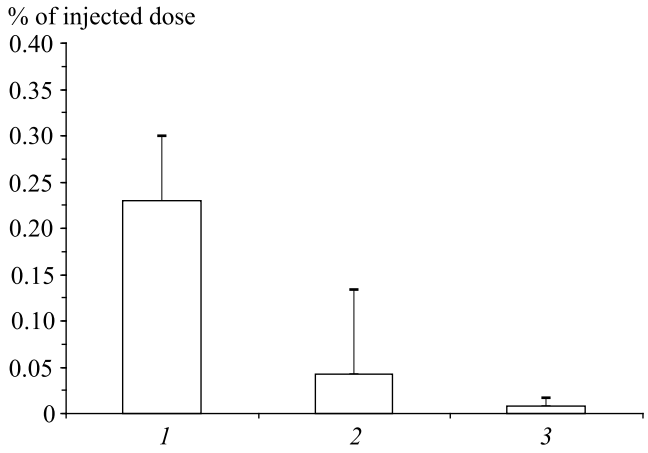


**Fig. 1.** Levels of himantane in daily urine (*1*) and feces (*2*: intraperitoneal injection; *3*: oral intake) after its administration in a dose of 25 mg/kg via different routes ($\bar{x}$±SD).

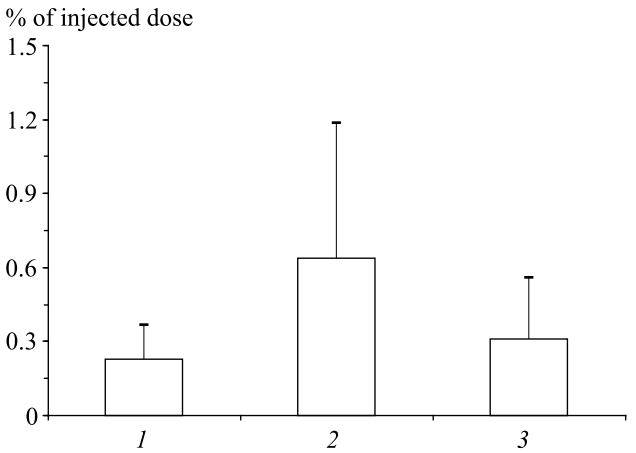


**Fig. 2.** Content of himantane and its metabolites in urine after intraperitoneal injection in a dose of 25 mg/kg ($\bar{x}$±SD). *1*) unchanged substance; *2*) metabolite 250 m/z; *3*) metabolite 266 m/z.

## RESULTS

Himantane was detected in the rat urine and feces in extremely low amounts after intraperitoneal and oral administration. The substance content was 0.23% of injected dose in daily urine and 0.27% dose in daily feces after intraperitoneal injection, while after oral intake 0.022% of the dose was detected (Fig. 1).

Since himantane level in feces was by 5.2 higher after intraperitoneal injection than after oral intake, we conclude that the drug was completely absorbed from the gastrointestinal tract into systemic circulation.

In addition to unchanged himantane (m/z 234), the urine and feces contained its biotransformation products. The studied compounds were designated by increase of the molecular ion weight to charge proportion: metabolite 250 m/z and metabolite 266 m/z. The increase of metabolite weights by 16 and 32, respectively, suggested the formation of hydroxy derivatives [3]. A counter chemical synthesis should be carried out in order to detect their precise structure.

Compound 250 m/z could be regarded as the main metabolite in the urine and feces, as its concentrations were high in all specimens of excrements (Figs. 2, 3). The level of metabolite 250 m/z after intraperitoneal injection was 1.9 times lower than after oral dose (Fig. 3). A similar trend was noted for metabolite 266 m/z (3.9 times lower). These results indicated a pronounced "first passage effect" of himantane passing through the liver after oral administration.

The levels of metabolites 250 m/z and 266 m/z virtually did not increase ($p \leq 0.95$) after addition of β-glucuronidase to urinary samples and hydrolysis. It seemed that himantane metabolites did not form glucurone conjugates.

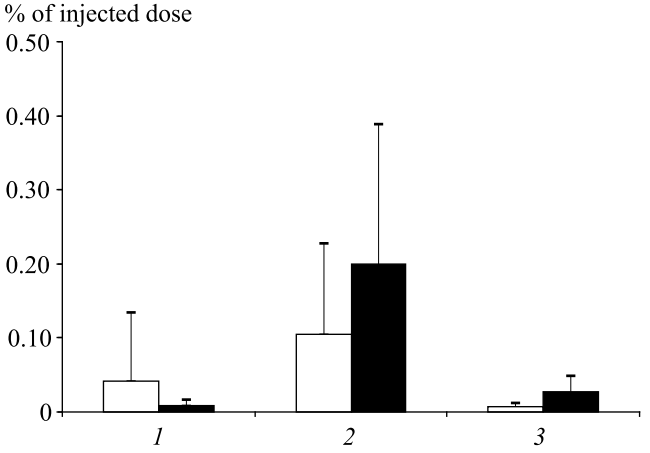


**Fig. 3.** Content of himantane and its metabolites in feces after intraperitoneal (light bars) and oral (dark bars) dose of 25 mg/kg ($\bar{x}$±SD). *1*) unchanged substance; *2*) metabolite 250 m/z; *3*) metabolite 266 m/z.

The results confirmed the data indicating that adamantane derivatives were eliminated by forming hydroxylated metabolites [3].

Analysis of excretion of himantane and its metabolites with the urine and feces in rats demonstrated that the drug was almost completely eliminated by biotransformation in the liver: the summary excretion of metabolites with the urine over 24 h was 10-fold higher than excretion with feces; the main direction of metabolism was presumably hydroxylation.

## REFERENCES

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