PHARMACOLOGY

Tissue Availability of Afobazole and Its Major Metabolites in Rats

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Intraperitoneally injected afobazole and its major metabolites are intensively distributed in highly vascularized tissues of rats, including the liver, spleen, and kidneys; the content of these compounds in moderately and poorly vascularized tissues (muscles and mesentery) is much lower. Afobazole and its metabolites possess intermediate ability to penetrate into the brain.

Key Words: *pharmacokinetics; afobazole; metabolites; tissue availability*

Selective anxiolytic afobazole was developed at the V. V. Zakusov Institute of Pharmacology and introduced into clinical practice [2,4,5].

Here we measured the contents of afobazole and its biological derivatives in organs and tissues of rats.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 200±20 g and obtained from the Stolbovaya nursery (Russian Academy of Medical Sciences). The animals were maintained in a vivarium under standard conditions and 12:12-h light/dark regimen. Aqueous solution of afobazole in a single dose of 25 mg/kg was injected intraperitoneally. The blood and organs were sampled 1, 2.5, 5, 15, 30, and 45 min and 1, 1.5, 2, and 3 h after afobazole injection (8 animals per point).

Afobazole and its metabolites were extracted from blood plasma and tissues with diethyl ester. Mass spectra of metabolites were recorded using an Agilent Technologies liquid chromatograph (model 1100) equipped with mass spectrometric and diode matrix detectors (G1322A degasser, G1311A pump, flow rate 0.7 ml/min). Afobazole-containing samples of rat plasma were analyzed by high performance liquid chromatography on a Perkin Elmer chromatograph, which consisted of a PE-290 isocratic pump, a PE-230 UV detector, and a computer with software for chromatogram analysis.

Chromatography was conducted using a Luna C-18 (2) analytic column (5 μ, 250×4.6 mm, Phenomenex). Detection was performed at 300 nm. Glycine buffer (pH 3.4) and acetonitrile (100:25 ratio) served as a mobile phase; flow rate was 1.5 ml/min.

Quantitative measurements were performed by the method of external standards. The linearity of this method is confirmed by average correlation coefficient 0.9994. The sensitivity limit was 100 ng/ml.

The main pharmacokinetic parameters were calculated by the model-independent method (M-IND software) [1]: $AUC_{0-\infty}$ (μg/ml/h or μg/g/h) is the area under the pharmacokinetic curve (area under the curve in a plot of concentration of drug or metabolite against time) after intraperitoneal treatment of rats. It is calculated from zero to infinity.

Tissue availability (f_T) was calculated as follows:

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TABLE 1. Tissue Availability (f_T) of Afobazole and Its Metabolites after Intraperitoneal Injection in a Single Dose of 25 mg/kg to Rats

Organ	Degree of tissue vascularization	Tissue availability			
		afobazole	M-6	M-7	M-11
Brain	Target organ	0.6	0.2	0.5	0.8
Liver	High	2.5	0.5	2.0	5.8
Spleen		1.5	0.4	1.4	3.3
Kidneys		1.5	0.5	3.0	3.4
Muscles	Intermediate	0.7	0.3	0.7	1.3
Mesentery	Low	0.2	0.2	0.4	0.6

$$f_T = AUC_{T0-\infty}/AUC_{P0-\infty},$$

where $AUC_{T0-\infty}$ is AUC in tissue; and $AUC_{P0-\infty}$ is AUC in blood plasma [3].

RESULTS

Afobazole was intensively metabolized in the organism of rats. Mass spectrometry of blood plasma revealed unchanged compound and 17 products of its biotransformation. The test compounds were numbered according to an increase in the retention time on a chromatographic column. Study of mass spectral characteristics and counter chemical synthesis allowed us to identify the major metabolite of afobazole (M-11) and metabolites M-6 and M7.

The distribution of afobazole and its metabolites was evaluated in organs and tissues responsible for their elimination and differing in blood supply, as well as in the brain (potential target for the drug). Afobazole and its metabolites were detected in all examined organs and tissues. The distribution of afobazole and its metabolites was characterized by significant heterogeneity (Table 1).

The measurement of absolute values of f_T for afobazole and its metabolites showed that the test compounds were intensively distributed in highly vascularized tissues of the liver, spleen, and kidneys. However, the content of these compounds in moderately and poorly vascularized tissues (muscles and mesentery) was much lower.

Metabolite M-6 penetrated into tissues less significantly than afobazole, M-7, and M-11. Tissue availability of afobazole and M-7 was evaluated in the liver-blood plasma (2.5 and 2.0, respectively), spleen-blood plasma (1.5 and 1.4, respectively), and kidneys-blood plasma systems (1.5 and 3.0, respectively). Tissue availability of M-6 in highly vascularized tissues did not exceed 0.5. Moderate penetration ability of M6 in tissues is related to its lower lipophilicity compared to more lipophilic afobazole, M-7, and M-11.

The difference between f_T for afobazole and metabolites M-7 and M-6 in moderately and poorly vascularized tissues was much lower than in highly vascularized tissues. Lipophilicity of the test compounds was probably insufficient for their penetration into these tissues.

Tissue availability of afobazole and M-11 was evaluated in the liver-blood plasma (2.5 and 5.8, respectively), spleen-blood plasma (1.5 and 3.3, respectively), kidneys-blood plasma (1.5 and 3.4, respectively), muscles-blood plasma (0.7 and 1.3, respectively), and mesentery-blood plasma systems (0.2 and 0.6, respectively). Tissue availability of M-11 in the brain (target organ) was 0.8. However, f_T for afobazole in the brain was 0.6. Hence, M-11 penetrated into the brain to a greater degree compared to afobazole. These data indicate that if M-11 has pharmacological activity, this compound plays a role in the pharmacological effect of afobazole.

The main substances with high value of f_T are characterized by relatively low content in blood plasma and rapid accumulation in tissues. Hence, f_T for these substances significantly exceeds 1. Values of f_T for substances with intermediate and high f_T correspond to 0.1-1.0 and <0.1, respectively.

We conclude that afobazole and its metabolites are characterized by intermediate ability to enter the brain.

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