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MOLECULAR-BIOLOGICAL PROBLEMS OF DRUG DESIGN AND MECHANISM OF DRUG ACTION

PHARMACOKINETICS OF INTRAVENOUSLY AND INTRAPERITONEALLY ADMINISTERED MAFEDINE SODIUM IN MICE

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Peculiarities of the pharmacokinetics of mafedine sodium salt upon intraperitoneal (i.p.) and intravenous (i.v.) administration to laboratory mice were studied. A high-performance liquid chromatography (HPLC) method for its quantitative determination was proposed. Mafedine sodium salt was found to have a short biological elimination half-life after both i.p. and i.v. administration (15.73 and 6.98 min, respectively) and high i.p. bioavailability (~94%). Features of the alpha-2-adrenomimetic activity of mafedine sodium salt could be elucidated by further searches for and investigations of its metabolites and their pharmacokinetics.

Keywords: mafedine sodium, pharmacokinetics, HPLC.

Mafedine [6-oxo-1-phenyl-2-(phenylamino)-1,6-dihydropyrimidin-4-ol] is a pyrimidine derivative with hypotensive activity due to activation of central alpha-2-adrenergic receptors [1].

Mafedine (as the base) in series of experimental investigations in mice, rats, and rabbits showed slower, more uniform, and longer hypotensive activity than clonidine for various administration modes [1]. Alpha-2-adrenergic receptor agonists are currently of only passing interesting as agents for correcting cardiovascular disorders, despite their high hypotensive efficacy. However, experimental and clinical trials using preparations of this drug class are ongoing because of their possible use in neurological practice. For example, clonidine and lofexidine were shown to be effective for treating heroin and methadone dependence [2]. Guanfacine could be an effective replacement for selective serotonin reuptake inhibitors used to treat agitation and hyperarousal in PTSD patients [3]. Dexamedetomidine is considered a promising analgesic adjuvant [4]. The neuroprotective properties of central alpha-2-adrenomimetics are highly interesting to researchers and should be mentioned separately [5-7]. Results

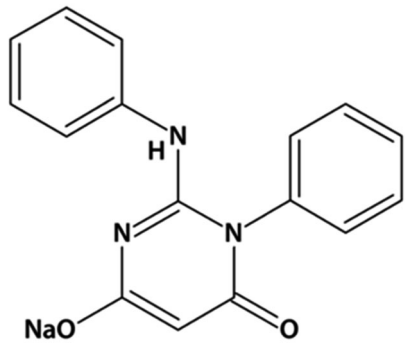

Fig. 1. Structural formula of mafedine sodium salt.

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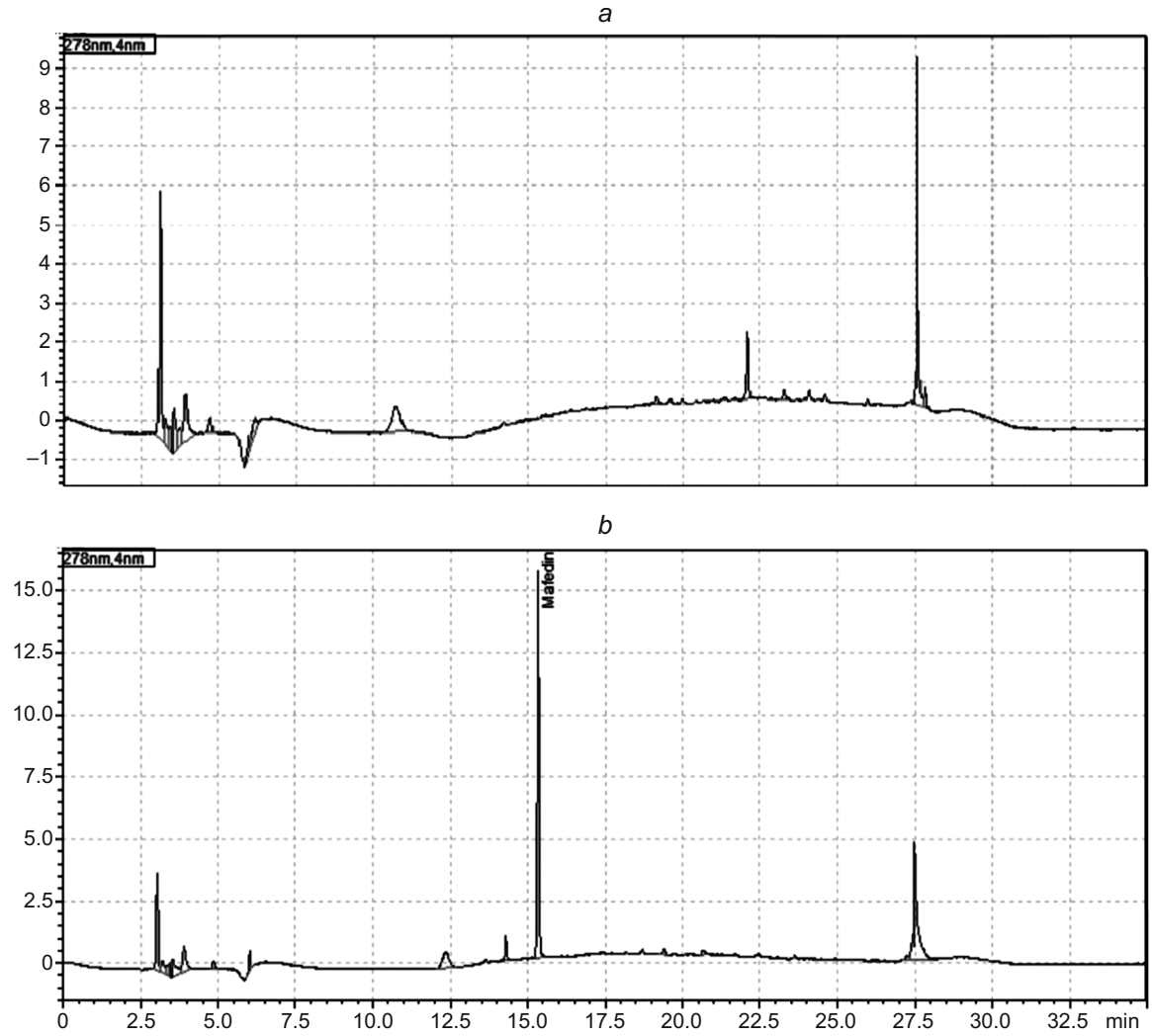

Fig. 2. Chromatograms of intact blood plasma (*a*) and blood plasma with mafedine at a concentration of 0.12 mg/mL (*b*).

from a meta-analysis of the use of dexmedetomidine that included nine randomized placebo-control studies involving 879 ischemic brain-injury patients provided the most convincing argument in favor of studying this group as agents for neuroprotection [8]. The drug showed the ability to suppress release of proinflammatory mediators and neuroendocrine hormones, to maintain intracranial homeostasis, and to reduce the amount of brain damage. The neuroprotectant activity of mafedine sodium salt was demonstrated by modelling traumatic brain injury in rats [9]. Regular administration of mafedine decreased the size of the damaged brain area and the intensity of inflammation processes in the damaged area with an overall reduction of the neurological deficit in injured animals.

TABLE 1. Time-programmed Gradient for Chromatographic Analysis

Time, min	Eluent in mobile phase, %
0	10
5	10
18	100
21	100

TABLE 2. Main Pharmacokinetic Parameters of Mafedine Sodium After Intraperitoneal and Intravenous Administration

Parameter	i.p.	i.v.
$T_{1/2}$, min	15.73	6.98
T_{max}, min	5.00	1.00
C_{max}, mg/mL	0.05	0.15
$AUC_{0\rightarrow\infty}$, mg/mL · min	1.59	1.69
Cl_{tot}, mL/min	6.29	7.04

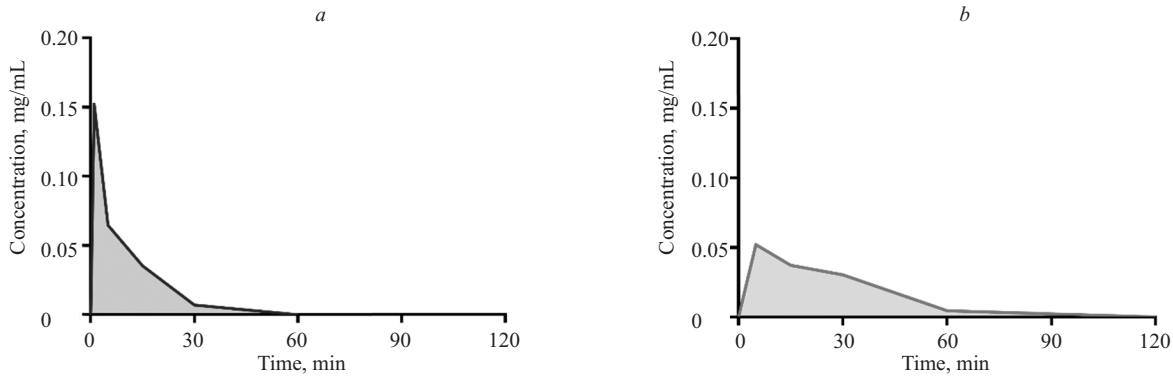

Fig. 3. Change of mafedine sodium concentration in mouse blood plasma after intravenous (*a*) and intraperitoneal administration (10 mg/kg) (*b*).

The pharmacokinetic properties of mafedine base and its sodium salt have not been evaluated despite its long history of use. The peculiarities of its pharmacokinetics must be understood for further studies of mafedine sodium salt as a neuroprotective agent so that the most effective administration regime can be chosen. HPLC with UV detection is a universal method available to most laboratories for quantitative analysis as compared to, for example, mass spectrometry. Also, chromatographic methods are widely used to analyze pyrimidine derivatives in pharmacokinetic studies and for therapeutic drug monitoring [10]. Thus, the goal of the present research was to study the peculiarities of the pharmacokinetics of mafedine sodium salt after intraperitoneal (i.p.) and intravenous (i.v.) administration to mice using HPLC with UV detection.

EXPERIMENTAL PART

Mafedine sodium salt (Fig. 1) is more soluble than the base and was synthesized at the Department of Organic Chemistry, St. Petersburg State Chemical and Pharmaceutical University (SPSCPU).

The standard was mafedine sodium substance obtained via chemical synthesis at the Laboratory of Organic Synthesis, SPSCPU.

Mafedine sodium was quantitatively determined in blood plasma using a Prominence LC-20 high-performance liquid chromatograph (Shimadzu, Japan) with an SPD-M20A diode-array detector at 278 nm and an SIL-20A autosampler. Analyses were made over a SUPELCOSIL LC-18 reversed-phase column (250 × 4.6, 5 μm) at 40°C. Samples (2 μL) were injected by the autosampler.

The studies were performed in gradient mode (Table 1). Mobile phase "A" consisted of deionized H_2O with trifluoroacetic acid (TFA, 0.1% v/v); mobile phase "B", MeCN with TFA (0.1% v/v). The flow rate was 1 mL/min. The retention time under these conditions was 15.5 ± 0.38 min (Fig. 2*b*). Mafedine sodium was quantitatively analyzed in plasma by absolute calibration of peak areas.

The experiments used white inbred female mice (18 – 20 g) obtained from Rappolovo Laboratory Animal Nursery (Leningrad Oblast). Animals were fed a complete feed (Laboratorkorm, RF) and water according to requirements of GOST 2874–82 "Drinking water" with access to feed and water *ad libitum*.

Mafedine sodium salt was injected i.p. and i.v. at a dose of 10 mg/kg. Animal blood was collected from the retro-orbital sinus at 5, 15, and 30 min and 1, 2, 4, 8, 12, and 24 h after i.p. administration and at 1, 5, 15, and 30 min and 1, 2, 4, 8, 12, and 24 h after i.v. administration. Four animals were used at each point. Withdrawn blood was centrifuged at 4,000 rpm for 10 min in a Sigma 2-16 PK centrifuge (Sartorius AG, Germany). The obtained plasma was transferred into sterile Eppendorf microtubes and stored at –40°C for subsequent analysis.

Proteins were precipitated and bound mafedine sodium was released from the blood plasma using MeCN (J. T. Baker, Poland); TFA (chemically pure, Chemical Line, Russia); and deionized H_2O obtained using an Arium mini purification system (Sartorius, Germany) as mobile-phase components.

A calibration curve was constructed by preparing six standard solutions of mafedine sodium of concentrations 5.64, 28.19, 56.37, 84.55, and 225.48 μg/mL that were analyzed to create a calibration curve for the dependence of compound concentration of its chromatographic peak area. The resulting regression equation was:

$$y = 511.13x,\ R^2 = 0.9992.$$

Pharmacokinetic parameters were calculated using the MS Excel PK Solver 2.0 add-in and the time dependence of the mean mafedine sodium concentration after a single i.p. or i.v. administration. The maximum concentration in blood plasma (C_{max}), time to reach the maximum concentration after administration (T_{max}), elimination half-life ($T_{1/2}$), and total clearance (Cl_{tot}) were determined from individual plots of the time dependence of the mafedine-sodium blood-plasma concentration. The area under the pharmacokinetic concen-

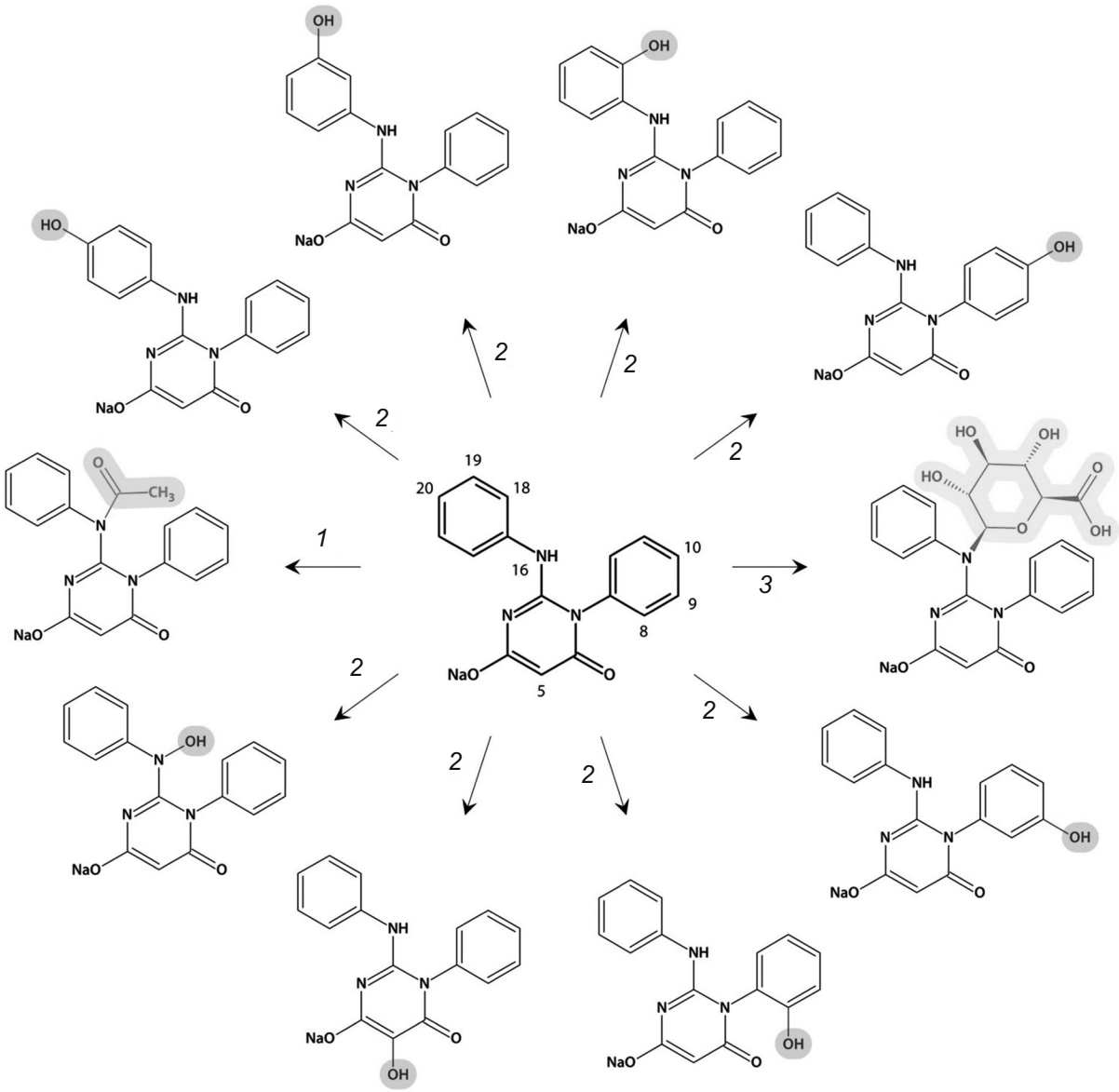

Fig. 4. Probable metabolic pathways of mafedine sodium: acetylation (*1*), hydroxylation (*2*), and glucuronidation (*3*).

tration–time curve ($AUC_{0(?)}$) was calculated by a trapezoidal method. The bioavailability of mafedine sodium after i.p. administration was defined as $AUC_{\text{i.p.}}/AUC_{\text{i.v.}}\cdot 100\%$.

RESULTS AND DISCUSSION

It was found that potentially interfering substances had no effect on the determination of mafedine sodium. Figure 2 shows typical chromatograms of intact mouse blood plasma (a) and an extract of blood plasma containing mafedine sodium (0.12 mg/mL) (b).

An analysis of the pharmacokinetic parameters showed that the peak plasma concentration of mafedine sodium in mice after i.v. injection was reached after 1 min (i.e., practically momentarily); after i.p. injection, after 5 min. The elimination half-life for these administration modes were 6.98 and 15.73 min, respectively. The absolute bioavailability of mafedine sodium after i.p. injection was calculated as ~94% based on the *AUC* values. Table 2 lists the main pharmacokinetic parameters after i.p. and i.v. injection.

The time dependences of the mafedine-sodium concentration in blood plasma were plotted using the pharmacokinetic results (pharmacokinetic concentration–time curves) for i.v. and i.p. injection (Fig. 3).

It is noteworthy that the obtained short elimination half-lives of mafedine sodium after i.p. and i.v. administration (15.73 and 6.98 min, respectively) contradict somewhat results of previously conducted pharmacological studies. For

example, the duration of the hypotensive effect of mafedine base after i.v. injection to rats at a dose of 1 mg/kg was shown to be up to 7 h and greater [1]. Later, screening tests at an i.p. dose of 2.5 mg/kg produced analogous results (unpublished data). One possible explanation of this phenomenon could be the pharmacological action of not the starting molecule but its possible metabolites. It could be argued in addition to this hypothesis that the two most characteristic alpha-2-adrenergic receptor agonists, i.e., clonidine and dexmedetomidine, exhibit hypotensive activity at doses (μg/kg) several orders of magnitude less than that (mg/kg) of mafedine. According to a computer prediction of possible metabolites using the Way2Drug online resource (www.way2drug.com), mafedine sodium could be hydroxylated at the 5-, 8-, 9-, 10-, 16-, 18-, 19-, or 20-position during the first biotransformation phase. Conjugation with acetic or glucuronic acid groups at the 16-N atom is possible in the second phase (Fig. 4).

Thus, a method for quantitative determination of mafedine sodium in mouse blood plasma using HPLC with UV detection was proposed for the first time in the present work. The obtained pharmacokinetic curves showed that the compound has a short elimination half-life upon i.p. and i.v. administration, which contradicts its lengthy hypotensive activity. Therefore, future research should address the search for and study of metabolites of mafedine sodium with alpha-2-adrenergic receptor activity.

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