Toxic Effect of Single Treatment with Bromantane on Neurological Status of Experimental Animals

I. N. Iezhitsa, A. A. Spasov, L. I. Bugaeva, and I. S. Morozov

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Neurotoxicological profile of actoprotector bromantane was studied on rats using S. Irwin's protocol of multi-test observation. The drug in doses of 30-300 mg/kg stimulated and in doses of 600-9600 mg/kg suppressed behavioral activity. Spontaneous motor activity increased after single treatment with bromantane in doses of 30-300 mg/kg, did not change after treatment in doses of 600 mg/kg, and was inhibited after treatment in doses above 600 mg/kg. In doses of 300-600 mg/kg the drug reduced pain sensitivity threshold and in doses above 600 mg/kg elevated the pain threshold and tactile sensitivity and reaction to knock. Bromantane induced mydriasis in all studied doses; in doses above 10 g/kg the preparation induced blepharoptosis. In doses above 5 g/kg bromantane slightly increased respiration rate and depth (Kussmaul-like respiration). In some animals bromantane in high doses induced regurgitation, diarrhea, and polyuria. Rectal temperature decreased by 0.5-1°C after virtually all doses. Behavioral effects of bromantane in doses of 30 and 600 mg/kg were associated with stimulation of the central dopamine and suppression of muscarinic and nicotinic cholinergic structures, n-cholinolytic effects of bromantane was more pronounced at a dose of 30 mg/kg than at a dose of 600 mg/kg.

Key Words: *bromantane; actoprotectors; neurotoxicological profile; Irwin test; open field; actometer; pharmacological analysis; rats*

Toxicological studies are an important aspect in evaluation of the safety of new drugs. These multilevel studies are carried out on animals of different species. An important stage in these studies is evaluation of the neurological status of animals after drug administration. We evaluated the neurotoxicological profiles of actoprotector bromantane using Irwin's multiple test protocol [7].

MATERIALS AND METHODS

Experiments were carried out on 200 random-bred adult albino rats of both sexes (150-200 g). Neurotoxicological profile of bromantane was evaluated after a single dose by Irwin's method [6,7]. The drug was given orally in increasing doses: 30, 60, 150, 300, 600, 1200, 2400, 4800, and 9600 mg/kg.

After bromantane administration the rats were put into isolated boxes (15×25×15 cm). Each dose was studied on 5 males and 5 females.

The total status, state of the fur and mucosae were evaluated. Neuromuscular excitability was evaluated by the presence of convulsions, pareses, tremor, twitching, reflectory reactions to external stimuli: acoustic (knocking), tactile (touching), painful (irritation of the skin with sharp stillette) and presence of Schtraube syndrome. Tremor, convulsions, and posture reflexes were scored visually using a common scale [6,7]. Changes in the posture (prostration, rigidity) were evaluated in rats in common posture and uncommon (given) postures. The position of the limbs (flexion-extension) was compared to that of untreated controls; general muscle tone was evaluated by jerking back the fore

Department of Pharmacology, Volgograd Medical Academy. ***Address for correspondence:*** Farm@interdacom.ru. Iezhitsa I. N.

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paw after it was captured and by the abdominal muscle tone.

The intensity of head jerking during irritation of the cornea (corneal reflex) and acoustic meatus were recorded. Autonomic nervous system function was evaluated by the pupil size, upper eyelid ptosis, urination, defecation, salivation, piloerection, respiration type and rate, and skin color. Drug effects on metabolic processes and thermoregulation were studied by thermometry with TPEM-I electrothermometer. Rectal temperature in all animals was measured on day 1 of the experiment 0.5 h before and every 30 min during 12 h after the drug was given, after which thermometry was carried out twice a day (in the morning and evening).

Emotional (grooming, anxiety, fear, aggression, vocalization, alertness) and motor (locomotion, stereotypy, reactivity, passiveness) activities were studied. Excitability was evaluated by general motor activity and aggressiveness. The reactivity of animals was evaluated by their reaction to changes in the environment (placing onto an open table, *etc.*). Alertness was evaluated by the orientation reflexes (hand clapping served as the stimulus). Aggressiveness and fear were evaluated using careful standard manipulations with rats, for example touching with forceps.

After the animals received bromantane they were constantly observed for 12 h and then regularly in the morning and evening. The duration of the experiment was 2 weeks. The beginning, peak, course, type, and intensity of the drug action were determined for each dose. The spectrum of external changes caused by bromantane was evaluating at the peak of the effect using an 8-point score [7]. The increase of the score from 4 to 8 indicated intensification of the effect and the decrease from 4 to 0 meant suppression of the effect (4 points – normal score); normally absent or negligible signs were evaluated using an 8-point score (0 corresponded to the absence of intoxication signs).

In addition, 1 h after drug administration in doses of 30, 150, and 600 mg/kg, motor (vertical and horizontal), exploratory (peeping into holes), and emotional (number of defecations) activities were evaluated in the open field test [6]. Spontaneous motor activity was evaluated on a single-channel actometer (Ugo Basile, Italy) for 5 min. The effect of the test drug on mnestic functions was studied in the conditioned passive avoidance test [5].

The number of dead and survived animals was recorded every day for 2 weeks. LD_{50} was assessed by Litchfield—Wilcoxon method on a PC using LD_{50} software (version 1.03), allowing estimation of this parameter from the results of pharmacological trials by tests with alternative reaction.

RESULTS

The resultant LD_{50} values and classification of substance toxicity [4] allow us to refer bromantane to low toxic agents (LD_{50}>10,000 mg/kg).

A parabolic form of the dose-effect relationship was derived from the behavioral and neurological parameters of animals treated with bromantane (Table 1); only parameters deviating from the normal are shown.

During the first hour of observation motor activity of rats receiving the drug in doses of 30-300 mg/kg increased by 60-100%; no changes were observed in rats receiving 600 mg/kg bromantane. In higher doses bromantane suppressed motor activity. One hour after treatment with bromantane in doses of 30-300 mg/kg spontaneous motor activity still increased, while treatment with bromantane in doses of 600 mg/kg and higher suppressed it in a dose-dependent mode. Pain sensitivity threshold decreased in rats receiving bromantane in doses of 300-600 mg/kg and increased (together with tactile sensitivity threshold and reaction to knocking) in those receiving the drug in doses of 600 mg/kg and higher. Bromantane induced mydriasis in all studied doses; in doses of 10 g/kg and higher it induced blepharoptosis.

Low motor activity and reduced pain and tactile sensitivity thresholds were observed in rats receiving >1 g/kg bromantane and a slight increase of respiration rate and Kussmaul type changes were observed 1.5-3 h after drug administration. Some animals receiving bromantane in doses above 5 mg/kg developed regurgitation, diarrhea, and polyuria. Rectal temperature decreased by 0.5-1°C in all rats irrespective of the dose (Table 1).

Analysis of the psycho- and neurotoxic profiles of bromantane suggested that catecholamine- and cholinergic structures could be involved in the pathogenetic mechanism of acute poisoning. Atropine-like effects were observed after a single dose of bromantane: decreased salivary secretion and pupil dilatation. In low doses bromantane stimulated CNS functions (increased respiration rate and motor activity) and in high doses suppressed these functions (decreased respiration rate, rectal temperature, and motor activity). This can be explained by its effects on the central adrenergic, cholineric, and dopaminergic systems of the brain.

Bromantane stimulates dopamine release from presynaptic terminals [1], modulates (depending on the dose and duration of treatment) concentrations of serotonin and 3,4-dihidroxylphenylacetic acid in some brain structures of rats [2]. The central noradrenergic effect of bromantane is less pronounced than the above-mentioned effects and manifests at higher concentrations (500 μM) by blocking norepinephrine reuptake by isolated cerebral synaptosomes in rats [3].

Cholinergic components of the neurotropic effect of bromantane are poorly expressed and manifest at high doses [3].

In order to evaluate the involvement of central structures in the mechanism of neurotoxic effect of bromantane, we carried out a series of experiments in order to study its influence on the effects of phenamine, arecoline, and nicotine.

Phenamine effects were potentiated in rats receiving bromantane in doses of 30 and 600 mg/kg. The duration of "phenamine stereotypy" in rats after bromantane dose of 30 mg/kg increased by 40% and after 600 mg/kg by 60% ($p<0.05$); the duration of the motor excitation was shortened by 30 and 50% ($p<0.05$), respectively, in comparison with the control. Bromantane in a dose of 30 mg/kg shortened the latentcy by 42% and the duration of arecoline hyperkinesia by 33% in comparison with the control. The dose of 600 mg/kg had a more pronounced m-cholinergic effect and shortened the duration of arecoline tremor by 50%. On a model of nicotine tremor, bromantane in doses of 30 and 600 mg/kg showed antagonism towards nicotine effects, shortening the duration of nicotine tremor by 60-70% ($p<0.05$) and prolonging the latentcy by 30-80%. The duration of convulsive state differed negligibly from the control, while the latentcy of convulsions was prolonged significantly only after bromantane dose of 30 mg/kg.

A single dose of bromantane caused motor hyperactivity in both male and female rats. In the open field test motor activity of females increased and that of males decreased. Vertical motor activity of both females and males increased by 88% and the number of grooming acts increased by 100-120% after bromantane in a dose of 30 mg/kg ($p<0.05$). Other parameters of behavioral activity in the open field virtually did not differ from the control. Females developed a trend to a dose-dependent increase in all motor activity parameters, which was paralleled by reduction of emotional activity after bromantane in doses of 150 and 600 mg/kg. Males showed a slight reduction of horizontal and vertical motor activity 1 h after bromantane dose of 150 mg/kg and increase of these parameters after a dose of 600 mg/kg. Bromantane doses above 600 mg/kg gradually inhibited all types of activities and increased emotional activity both in females and males. It is noteworthy that during testing of females receiving bromantane in doses of 30, 150, and 600 mg/kg we distinguished a subgroup characterized by a high level of defecations and suppression of

TABLE 1. Effect of Single Oral Dose of Bromantane on the Psycho- and Neurotoxicological Profile of Experimental Animals

Parameter	Normal	Bromantane dose, mg/kg								
		30	60	150	300	600	1200	2400	4800	9600
Unconscious reactions										
alertness	4	4	4	4	4	4	3	2	0	0
passiveness	0	0	0	0	0	2	4	4	4	4
Emotional state										
grooming	4	5	5	6	6	4	3	2	1	0
Motor activity										
spontaneous	4	5	5	6	6	8	4	2	2	2
reaction to pain	4	4	4	4	6	6	4	4	4	4
reaction to knock	4	4	4	4	4	4	3	3	2	2
Autonomic nervous system status										
pupil size	4	4	4	4	6*	6*	6*	6*	6*	6*
upper eyelid ptosis	4	4	4	4	4	4	6	6	8	8
urination	0	0	0	0	0	1^{+}	1^{+}	2^{+}	2^{+}	2^{+}
defecation	0	0	0	0	0	2^{o}	4^{o}	4^{o}	4^{o}	4^{o}
temperature	4	4	4	3	3	3	3	3	3	3
skin color	4	4	4	4	3	3	3	3	3	3
respiration rate	4	4	4	4	2^{x}	3^{x}	6^{x}	6^{x}	5^{x}	5^{x}
salivation	4	4	4	4	4	4	4	4	6	6

Note. *Myosis, $^{+}$polyuria, ^{o}diarrhea, ^{x}Kussmaul type changes in respiration.

motor activity (both vertical and horizontal). Animals of this subgroup were more passive by grooming parameters and showed a more pronounced autonomic reaction.

These results suggest cholinolytic and dopamine-positive mechanism of changes in the motor behavior of rats under the effect of bromantane. It was shown previously that the specific pharmacological effect of bromantane, distinguishing it from classical psychostimulating agents, can be explained by its multicomponent effect on dopamine release in the striatum [1,2]. The cerebral serotoninergic system can also play a role in the formation of psychotropic effects of bromantane [1].

Bromantane promoted the formation of short-term memory traces in rats after a single dose of 30 mg/kg and virtually did not influence this process in doses of 150 and 600 mg/kg. The drug notably accelerated training and consolidation of the habit in animals and therefore stimulated better formation of the long-term memory trace.

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