Anxiolytic and Antidepressant Characteristics of Impaza

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Antibodies to endothelial NO synthase in ultralow doses exhibited anxiolytic and antidepressant effects and their efficiency after single and course treatment is not inferior to that of amitriptyline and diazepam. The psychotropic activity of ultralow-dose antibodies to endothelial NO-synthase is presumably one of important mechanisms of their efficiency in the treatment of erectile dysfunction.

Key Words: *erectile dysfunction; ultralow-dose antibodies to endothelial NO-synthase; anxiolytic, antidepressant effects*

Erectile dysfunction (ED) is a prevalent chronic disease characterized by failure to attain and/or maintain erection sufficient for coitus [6,7,10]. The development of ED was attributed solely to psychological problems, but now it is assumed that 80% ED cases had organic nature and develop as a complication of somatic diseases [3]. Psychological factors (depression, anxiety, and social phobia [9]) occupy a special place in the development of ED [6,8]. They can be the main causes of erectile disorders and be combined with organic causes of these disorders, which significantly impedes treatment of this disease [1]. Treatment of anxiety and depression in the presence of ED is difficult because traditional drugs can augment the risk of ED development [3]. Hence, the search for a drug stimulating erection and reducing depression and anxiety remains a pressing problem.

Preclinical and clinical trials of Impaza (Materia Medica Holding) containing ultralow-dose antibodies to endothelial NO-synthase (mixture of homeopathic dilutions C12+C30+C200) demonstrated its efficiency in the treatment of ED [3]. Here we studied the anxiolytic and antidepressant effects of Impaza on the models of depression and anxiety in rats.

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MATERIALS AND METHODS

The study was carried out on 2-3-month-old (n=240) outbred male rats (230-250 g).

Anxiolytic effect was studied using the elevated plus-maze (EPM) test (n=60, 5 min) [4] and Vogel conflict test (n=60, 10 min) [4,13]. The EPM consisted of four perpendicular arms 0.5 m long and 10 cm wide. Two contralateral arms had 40 cm high walls over their entire length, two other arms are open. The EPM is illuminated by two 20-W lamps at a height of 60 cm above the maze. The anxiolytic effect of the drugs was evaluated by the number and duration of excursions to the open arms. Vogel conflict test explores a conflict between drinking motivation and painful electric stimulation. The drinking fountain was placed in the common cage and not in the dark section, because the animals getting into a new setting instinctively hide in a dark arm and can find the fountain by chance, instead of purposeful search for satisfying the motivation. The anxiolytic effect was evaluated by the number of punished water licks (current

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TABLE 1. Anxiolytic effect of Impaza in Rats (*M*±*m*)

Test; parameter	Control		Diazepam		Impaza	
	single dose	course	single dose	course	single dose	course
EPM						
number of excursions to open arms	0.00±0.00	0.00±0.00	1.00±0.94*	0.90±0.57*	0.80±0.63*	1.50±0.71*
duration of stay in open arms, sec	0.00±0.00	0.00±0.00	26.46±12.83*	29.19±17.05*	24.88±12.24*	34.40±8.50*
Conflict situation after Vogel						
number of punished water gulps	306.00±115.29	247.2±30.3	473.60±141.24*	405.90±70.52*	460.10±92.97*	384.20±79.71*
number of crossed squares	17.30±7.26	19.00±2.91	10.50±3.34*	11.00±2.26*	20.70±2.98	19.70±2.45

Note. Here and in Table 2: *$p<0.05$ compared to control.

strength 0.25 mA) over 10 min and by the total motor activity (number of crossed 10×10 cm squares). The sedative effect of the drug was evaluated by reduction of motor activity.

Antidepressant effect of the drugs in rats was studied in the forced swimming test with free rotating wheels after Nomura (n=60) [11] and behavioral despair test after Porsolt (n=60) [12]. The severity of depression was evaluated by the number of wheel rotations and duration of immobilization. The observations were carried out over 10 min.

Forty minutes before testing, rats of 6 groups (10 per group) received a single dose or a course of Impaza in a dose of 4 ml/kg (5 days, twice daily) intragastrically, distilled water in the same volume (control), and the reference drugs: diazepam (seduxen; Gedeon Richter-Rus; 2 mg/kg, 4 ml/kg) in the studies of the anxiolytic effect or amitriptyline (Dalkhimfarm; 10 mg/kg, 4 ml/kg) in the studies of antidepressant activity.

RESULTS

A single dose and a course of Impaza and diazepam exhibited a clear-cut anxiolytic effect in EPM test and in conflict Vogel test; in the conflict test it manifested in a significant increase in the number of punished water licks in comparison with the control, while in EPM test by entries into open arms, while control rats did not venture into open arms. The number of excursions into open arms was 40% higher after a course of Impaza than after a course of diazepam (p=0.05; Table 1).

Single doses of Impaza and diazepam increased the number of punished water licks by 50.3 and 54.8%, respectively, courses of these drugs by 55.4 and 64.2%, respectively ($p<0.05$). In contrast to the Impaza group, in animals treated with diazepam motor activity decreased by 39.3% after a single dose and by 42.1% after a course of treatment ($p<0.05$ compared to the control), which indicated sedative activity of the drug (Table 2).

Forced swimming test with free rotating wheels after Nomura and behavioral despair test after Porsolt demonstrated pronounced antidepressant effects of amitriptyline and Impaza.

Single doses of these drugs reduced manifestations of depression in comparison with the control (the number of wheel rotations increased by 55.5 and 63.6%, respectively ($p<0.05$), while courses of treatment with these drugs reduced depression by 64.7 and 84.4%, respectively ($p<0.05$; Table 2).

The immobilization stage reflecting depression-like state in Porsolt's test appeared rapidly and lasted for some time.

Single doses of Impaza and amitriptyline decreased the duration of immobilization by 1.3 and 1.5 times, respectively ($p<0.05$). Courses of these drugs increased of immobilization episodes by 2 and 2.3 times, respectively, the duration of immobilization decreased by 1.5 and 1.7 times ($p<0.05$; Table 3).

Hence, single and course treatment with Impaza produced anxiolytic and antidepressant effects.

TABLE 2. Antidepressant Effect of Impaza: Time Course of Rat Behavior in Forced Swimming Test after Nomura (*M*±*m*)

Drug	Number of wheel rotations	
	single dose	course
Control	60.5±22.2	65.20±26.14
Amitriptyline	99.00±16.36*	120.40±17.32*
Impaza	94.10±19.53*	107.40±10.21*

TABLE 3. Antidepressant Effect of Impaza: Time Course of Rat Behavior in the Forced Swimming Test after Porsolt (*M*±*m*)

Drug	Single dose		Course	
	number of immobilization episodes	duration of immobilization, sec	number of immobilization episodes	duration of immobilization, sec
Control	2.80±1.14	407.35±93.50	1.80±0.79	437.39±55.29
Amitriptyline	5.10±1.66	273.51±89.35	4.20±0.92	260.06±54.04
Impaza	8.40±3.47	302.79±68.46	3.80±0.82	281.01±48.44

By anxiolytic activity Impaza was not inferior to diazepam (2 mg/kg) and, in contrast to this drug, had no sedative effect. Antidepressant activity of Impaza was somewhat lower than that of amitriptyline in a dose of 10 mg/kg. These effects of Impaza prompt its further studies in the treatment of patients suffering from ED of psychogenic or mixed origin.

REFERENCES

1. L. M. Gorilovskii and D. A. Lakhno, *Rus. Med. Zh.*, **13**, No. 10, 653-657 (2004).
2. I. I. Gorpinchenko and Ya. O. Miroshnikov, *Zdorovye Muzhchiny*, No. 2, 8-9 (2004).
3. D. Yu. Pushkar', *Spravochnik Poliklinich. Vracha*, No. 2, 55-58 (2004).
4. *Manual for Experimental (Preclinical) Studies of New Drugs*, Ed. R. U. Khabriev [in Russian], Moscow (2005).
5. S. A. Tarasov, Yu. L. Dugina, O. I. Epstein, and S. A. Sergeeva, *Signatura*, No. 2, 72-80 (2007).
6. A. A. Araujo, C. B. Johannes, H. A. Feldman, *et al.*, *AJE*, **152**, No. 6, 533-541 (2000).
7. T. J. Bivalacqua, M. F. Usta, H. C. Champion, *et al.*, *J. Androl.*, **6**, 17-37 (2003).
8. G. Corona, E. Mannucci, L. Petrone, *et al.*, *J. Androl.*, **27**, 86-93 (2006).
9. N. Hunt and S. McHale, *Endocrinol. Metab. Clin. North Am.*, **36**, 521-531 (2007).
10. T. F. Lue, *NEJM*, **342**, 1802-1813 (2000).
11. S. Nomura, J. Shimizu, M. Kinjo, *et al.*, *Eur. J. Pharmacol.*, **83**, Nos. 3-4, 171-175 (1982).
12. R. D. Porsolt, G. Anton, N. Blavet, and M. Jalfre, *Eur. J. Pharmacol.*, **47**, 379-391 (1978).
13. J. Vogel, B. Beer, and T. Clody, *Psychopharmacology*, **21**, 1-7 (1971).