Experimental Study of the Effects of Dietressa, a New Weight-Reducing Drug

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The capacity of a new drug containing ultra-low doses of antibodies to cannabinoid receptor type 1 (Dietressa) to reduce body weight gain in mice on a high-calorie diet was evaluated, possible mechanisms of drug action were analyzed, and its safety (abuse potential in the reaction of self-stimulation) was evaluated. Dietressa was not inferior to sibutramine in reducing body weight gain in mice and exhibited no abuse potential.

Key Words: *Dietressa; cannabinoid receptors, obesity; sibutramine; self-stimulation*

Overweight and obesity are among the most serious public health problems in developed countries [4,5,9]. They are the major risk factors for cardiovascular disease, dyslipidemia, type 2 diabetes mellitus, *etc*. [4,6]. Treatment of obesity, as well as other chronic diseases, can not be confined to only short-term medication, but should last for a long time, enough to reach and maintain healthy weight, and should include complex changes in life style, diet, and pharmacotherapy.

Weight-reducing drugs can be divided into several groups: drugs regulating appetite (phentermine, diethylpropion, fluoxetine, sibutramine); drugs preventing absorption of fat (orlistat), and those enhancing energy expenditure and thermogenesis (ephedrine, caffeine). Cannabinoid receptor type 1 is one of the most promising targets in developing new drugs to treat the obesity [8], as the endocannabinoid system plays a significant role in the pathogenesis of obesity, regulation of feeding behavior, and fat accumulation [2,3,7].

The purpose of the work was to evaluate the ability of Dietressa, a new drug containing ultra-low doses of antibodies to cannabinoid receptor type 1, to reduce body weight (BW) gain in mice on a high-calorie diet. Possible mechanisms of drug action such as reduction in food intake and increased locomotor activity (LA) were analyzed. Sibutramine (Meridia) was used as the reference drug.

Complete toxicological study of Dietressa showed its safety, but taking into account the fact that the drug affects the endocannabinoid system, we also evaluated its abuse potential.

MATERIALS AND METHODS

Impact of Dietressa on the dynamics of BW gain and feeding behavior was assessed in 40 male C57Bl/6 mice (age 2 months, weight 14.3-15.6 g). Thirty mice received standard pelleted feed (GOST R 50258-92) in combination with high-energy additives (cream cheese, sour cream, cottage cheese, lard, *etc*.). Total caloric value of food consumed by mice was at least 4400 kcal/kg. The test preparations were administered intraperitoneally over 20 weeks of high-calorie diet: distilled water (control, 0.4 ml/mouse/day; n=10), sibutramine (10 mg/kg/day; n=10), and Dietressa (0.4 ml/mouse/day; n=10). Every 4 weeks, BW was determined and daily food intake and LA in the open field test were evaluated [1]. The results were compared with a group of intact mice (n=10) receiving only standard food (GOST R 50258-92) with calorie content of 3611 kcal/kg.

Abuse potential of Dietressa was assessed in 20 white outbred male rats (age 6-8 months, weight 230-

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TABLE 1. Time Course of BW (g) in Male Mice Treated with Dietressa or Sibutramine Intraperitoneally for 20 Weeks (*M*±*m*)

Term of study, weeks	Standard diet	High-calorie diet		
		control	sibutramine	dietressa
At baseline	15.10±0.31	14.30±0.37	15.40±0.43	15.60±0.34
4	18.98±0.27 (25.7)	19.63±0.30 (37.3)	19.26±0.36 (25.1)	19.44±0.32 (24.6)
8	21.41±0.28 (41.8)	21.63±0.23 (51.3)	21.44±0.49 (39.2)	21.08±0.33 (35.1)
12	22.60±0.27 (49.7)	22.97±0.26 (60.6)	22.64±0.38 (47.0)	21.82±0.32* (39.9*)
16	23.59±0.31 (56.2)	23.91±0.31 (67.2)	22.91±0.33 (48.8)	22.41±0.34** (43.7**)
20	24.90±0.39 (64.9)	25.15±0.43 (75.9)	23.34±0.38** (51.6**)	23.59±0.49* (51.2**)

Note. Values in brackets indicate the percent change from baseline. *$p<0.05$, **$p<0.01$ compared with controls.

250 g) receiving injections of distilled water (control; n=10) or Dietressa (n=10) intraperitoneally in a dose of 2.5 ml/kg for 5 days. Abuse potential was estimated by the reaction of self-stimulation (RSS) of the lateral hypothalamus. Before drug administration, nichrome electrodes were stereotaxically implanted in the ventrolateral hypothalamus under ketamine anesthesia by the following coordinates: AP=3.7 mm from the intersection point of bregma and sagittal suture; L=0.7 mm from the sagittal suture; H=8.8-8.9 mm. RSS was recorded in the Skinner box with mounted lever connected to an automatic counter. Rectangular alternating current pulses (40-100 μA) were delivered through the electrodes into rat brain upon lever pressing; pulse duration 0.1-0.5 sec was adjusted individually for each rat. RSS rate was registered within 5 days before, during, and after drug administration (number of lever pressings per 60 minutes was registered), with two-day pauses between each of these intervals.

RESULTS

On all time points, BW gain in mice receiving high-calorie food was higher than in mice receiving standard chow (Table 1). Dietressa and sibutramine reduced BW gain in mice during the entire observation period. Four weeks after the start of treatment, BW gain in sibutramine and Dietressa groups was by 12.2 and 12.7% below the control, respectively; after 8 weeks by 12.1 and 16.2%, after 12 weeks by 13.6 and 20.7% ($p<0.05$), after 16 weeks by 18.4 and 23.5% ($p<0.05$), and after 20 weeks by 24.3 and 24.7% ($p<0.01$), relatively (Table 1). The decrease in BW gain was more pronounced in Dietressa group and reached a significant difference from the control after 12 weeks vs.

TABLE 2. Time Course of Average Daily Food Consumption (g/10 g BW) by Mice Treated with Dietressa or Sibutramine Intraperitoneally for 20 Weeks

Group		4 weeks	8 weeks	12 weeks	16 weeks	20 weeks	Mean for the entire study period (*M*±*m*)
Standard diet		1.55	1.36	1.45	1.48	1.47	1.46±0.03
High-calorie diet	control	1.52	1.62	1.56	1.62	1.63	1.59±0.02+
	sibutramine	1.46	1.15	1.00	1.62	1.39	1.32±0.11*
	dietressa	1.46	1.31	1.34	1.81	1.73	1.53±0.10

Note. *$p<0.01$ compared with the control; +$p<0.05$ compared with mice on standard diet.

TABLE 3. Impact of Dietressa or Sibutramine on the Horizontal Movement in Open Field Test (*M*±*m*)

Term of study, weeks	Standard diet	High-calorie diet		
		control	sibutramine	dietressa
At baseline	80.57±2.58	80.57±2.58	80.57±2.58	80.57±2.58
4	66.00±3.62	96.60±7.01^{+}	92.80±5.22^{++}	98.10±3.73^{++}
8	70.10±5.89	103.30±4.79^{++}	99.10±7.48^{+}	72.10±7.77**
12	55.10±5.44	104.10±7.52^{++}	138.00±12.65*$^{+++}$	80.20±4.66*$^{++}$
16	54.10±4.69	84.10±6.04^{+}	137.80±13.28**$^{++}$	89.50±5.22^{++}
20	61.40±5.53	101.00±8.77^{+}	96.90±7.63^{+}	76.50±7.28*

Note. *p<0.05, **p<0.01 compared with the control; ^{+}p<0.01, ^{++}p<0.001 compared with mice on standard diet.

20 weeks in sibutramine group). The effects of drugs became similar by experimental week 20.

Mice on high-calorie diet consumed more food than those on standard diet (p<0.05; Table 2). Sibutramine decreased food consumption at all terms, while Dietressa just before the 12th experimental week.

Initially, LA in the open field was tested in all mice before they were divided into groups. In the control group, LA in mice on high calorie diet was higher than in mice on standard diet during the entire period of observation (p<0.01; Table 3). In sibutramine group, LA after 4, 8 and 20 weeks did not differ from the control, while after 12 and 16 weeks it was significantly higher (by 32.6 and 63.9%; p<0.05). In contrast, LA in Dietressa group did not differ from the control after 8, 12, 16 and 20 weeks and was below the control by 23% after 12 weeks (p<0.05; Table 3).

Investigation of possible abuse potential showed that RSS did not differ from the control and initial values (Table 4). It should be noted that we observed no withdrawal effect (manifesting in increased frequency of RSS); on the contrary, RSS frequency decreased by 15.0-35.2% compared to baseline values (p<0.05) and by 32.3-44.3% compared to the control (p<0.05).

TABLE 4. Impact of Dietressa on the Time Course of RSS Rate in Rats (*M*±*m*)

Days		Control	Dietressa
Before drug administration	1	242.80±15.84	188.30±27.20
	2	219.00±34.16	219.30±25.60
	3	206.80±33.77	199.40±20.40
	4	211.60±31.58	206.20±23.20
	5	235.30±28.85	202.30±27.30
During drug administration	8	208.20±26.85	185.40±26.40
	9	248.20±22.62	187.50±26.30
	10	259.60±33.41	187.80±24.90
	11	306.50±29.35	150.70±25.30^{+++}
	12	239.90±28.09	142.00±28.90^{+}
After drug administration	15	256.50±22.90	126.70±27.70**$^{++}$
	16	231.20±26.48	132.10±30.50**$^{+}$
	17	229.60±30.20	126.90±24.70**$^{+}$
	18	214.70±25.63	133.10±25.70**$^{+}$
	19	230.90±26.34	129.70±22.80**$^{++}$

Note. **p<0.01 compared with experimental day 1; ^{+}p<0.05, ^{++}p<0.01; ^{+++}p<0.001 compared with controls.

This attests to reduced self-stimulation (activation of positive evaluation) in rats and indicates that the drug can reduce the formation of dependence.

Thus, the capacity of Dietressa to reduced BW gain in rats on high-calorie diet is not inferior to the comparator drug sibutramine. At the same time, BW reduction caused by sibutramine is due to lower feeding activity and increased LA, while the effect of Dietressa is apparently linked with the metabolic processes (prevention of fat absorption, increased fat utilization, *etc.*) and requires further study. Dietressa also demonstrated the absence of narcotic potential in RSS. Given the absence of the damaging effect of the drug revealed in toxicological studies, it is possible to confirm its safety.

Thus, experimental studies show that Dietressa is an effective and safe drug for the treatment of overweight and obesity.

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