·论著(译文)·

Effects of Intracerebroventricular Injection of Recombinant Leptin on Food Intake, Body Weight and Blood Lipids in Diet-induced Obese Rats

LIU Qian-Qi, CHEN Rong-Hua, GUO Xi-Rong, FEI Li, GONG Hai-Xia

Department of Pediatrics, Second Clinical College, Nanjing Medical University, Nanjing 210029, *China*

Abstract: Objective To investigate the effects of intracerebroventricular injection of recombinant murine leptin on the food intake, body weight and blood lipids in diet-induced obese rats and normal rats. **Methods** Male SD rats were put on a standard diet (n = 15) and high-nutrition diet (n = 20) for 7 weeks. After 7 weeks, the rats received cannulas implanted into the right lateral ventricles. Allowed 7 days to recover, the rats received an intracerebroventricular injection of recombinant murine leptin (5 μg/rat) for 5 days. On each test day, the body weight and food intake were measured before the injection. Blood lipids were measured after the experiment. **Results** ① The body weight of the rats on the high-nutrition diet was more significantly increased than that in the normal rats. At the end of the 7th week, the body weight of each group was (342.05 ±39.27) g and (302.87 ±31.93) g ($P < 0.01$), with an increase of (270.00 ±39.99) g and (226.13 ±30.04) g ($P < 0.01$), respectively. In the diet-induced obese rats, the inhibition of body weight and food intake was observed after the intracerebroventricular leptin injection on the 1st day, more significant on the 5th day [body weight from (336.3 ±52.1) g to (287.9 ±53.4) g ($P < 0.01$), and a decrease of (48.4 ±17.9) g; food intake from (35.6 ±13.7) g to (21.1 ±11.8) g ($P < 0.01$), a decrease of (14.6 ±4.8) g]. But in the normal group, the effects were observed on the 3rd day [body weight from (294.5 ±29.9) g to (269.5 ±30.9) g ($P < 0.05$), a decrease of (25.0 ±17.8) g; food intake from (31.0 ±3.5) g to (25.6 ±3.6) g ($P < 0.05$), a decrease of (5.3 ±3.3) g]. ② In the obesity group with leptin administration, the levels of TC, TG and LDL-C decreased more significantly than those in the normal control group [TC (1.51 ±0.27) mmol/L vs. (2.22 ±0.36) mmol/L, $P < 0.01$; TG (0.43 ±0.06) mmol/L vs. (0.76 ±0.17) mmol/L; LDL (0.47 ±0.12) mmol/L vs. (0.86 ±0.20) mmol/L, $P < 0.01$]. But there was no obvious change of HDL-C in any of the groups. **Conclusions** Recombinant leptin could reduce the body weight and blood lipids, and inhibit the food intake in diet-induced obese rats after intracerebroventricular injection, and also had a certain effect on normal rats.

Key words: Leptin; Intracerebroventricular injection; Obesity; Rat

[CLC number] R-332 [Document code] A [Article ID] 1008-8830(2001)04-0464-05

With the improvement of living standard, obesity in childhood, the prevalence of which has been progressively increasing worldwide, is closely associated with the increased morbidity and mortality caused by several of the most common diseases in adults, including diabetes, hypertension, cardiovascular diseases and cancer[1]. The pathogenesis of obesity remains largely unknown, but research on its pathophysiology has recently intensified, largely because the cloning of the obese gene and the identification of its gene product, leptin, in 1994, by Zhang et al., opening a floodgate of research into the biological meaning of this 167 amino acid peptide[2]. Leptin, the product of the obese gene, is an adipocyte-derived cytokine and functions as a peripheral signal to the brain to regulate food intake, body weight and energy metabolism. In ob/ob mice, which are markedly hyperphagic and obese, the obese gene is mutated and no leptin is produced; when given leptin, they stop eating and lose weight[3]. As is reported, serum leptin concentration and the levels of ob mRNA in adipose tissues in obese humans or diet-induced obese

[Received] January 8, 2001; [Revised] June 30, 2001
[Introduction to the First Author] LIU Qian Qi (1972-), Female, M.D., Attending Physician.

rats or mice are elevated, and there is a strong positive correlation between serum leptin concentrations and the percentage of body fat, the body-mass index, which suggests that obesity may be associated with leptin resistance. Meanwhile, human obesity does not generally appear to be associated with mutations in the genes encoding leptin[4]. Mice with a mutation obese gene can be returned to normal weight by the administration of recombinant leptin, but its effects in diet-induced obese rats and normal rats are still unclear. Therefore, the aims of the present study were to investigate the effects of intracerebroventricular injection of recombinant murine leptin on body weight, food intake and blood lipids in diet-induced obese rats and normal rats, to study the mechanism of obesity and the future clinical therapy of recombinant leptin, and develop novel therapeutic approaches for obesity.

1 Materials and Methods

1.1 Preparation of obese rat models

Weanling Sprague-Dawley male rats (obtained from Jiangsu Experimental Animal Center) at 4～5 weeks of age were randomly divided into two groups, the normal group (n = 15, 75.40 g ±11.38 g) and the obese group (n = 20, 72.05 g ±12.53 g). They were housed individually in metabolic cages with a constant temperature (18～20 ℃) and 12 h of light and 12 h of darkness per day. The 15 rats in the normal groups were given a standard diet and the 20 in the obese group were fed with a high-nutrition diet for 7 weeks. Each kilogram of high-nutrition diet contained 80 % standard chow, 2 eggs, 12 % milk powder, 3 % grease, and 1 milliliter of cod-liver oil. Clean water was available ad libitum as reported by Qian Bo-Chu and modified[5].

1.2 Right lateral ventricular annulization

After 7 weeks, the rats in the normal and obese group were randomly divided into 2 groups again (the control group and the group with leptin administration), respectively. All rats except those in the normal and obese control group were implanted with a stainless steel cannula (external diameter = 0.8 mm, internal diameter = 0.5 mm, obtained from Professor Hu Gang) into the right lateral ventricle under sodium pentobarbital anesthesia (5 mg/100 g of body weight, administered i. p) as described by Noble CP[6]. Briefly, the rats were positioned and the cranial bone was exposed. The cannula was positioned 1.5 mm behind the bregma and 1.5～2.0 mm on the right of the midline and was lowered 3.5～4.0 mm under the cranial bone.

1.3 Administration

The rats were allowed to recover for approximately one week. Then they received an intracerebroventricular injection of 5 μg leptin at 10:00 per day for 5 consecutive days. On each experiment day, the body weight and food intake were measured before the first injection[7].

1.4 Blood lipids (TC, TG, HDL-C, LDL-C) measurement

At the time of sacrifice, blood samples were directly collected by cardiopuncture and centrifuged at 2000 rpm for 20 minutes. Serum was stored at -20 ℃. TC, TG, HDL-C were detected by using the standard kits and LDL-C = TC - (TG/5 + HDL-C) as described previously.

1.5 Statistic analysis

All the data were shown as mean(SE) for the groups of rats. The effects of leptin on food intake, body weight and blood lipids between the control and leptin-treated groups or normal and obese groups were analyzed using Student's *t* test and paired Student's *t* test.

2 Results

2.1 Changes in body weight 7 weeks after the high-nutrition and normal diet

During the experimental process, the rats' weight was measured weekly. There was no difference between the two groups before the experiment. From the second week to the end, the body weight of the rats given the high-nutrition diet was more significantly increasing than that of the normal rats. At the end of the seventh week, the body weight of each group was (342.05 ±39.27) g and (302.87 ±31.93) g ($P < 0.01$), with an increase of (270.00 ±39.99) g and (226.13 ±30.04) g ($P < 0.01$), respectively (Figure 1).

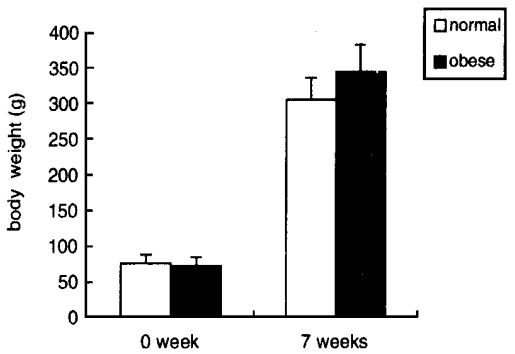

Figure 1 Changes in body weight 7 weeks after high-nutrition and normal diet ($\bar{x} \pm s$, g)

2.2 Changes in body weight and food intake after the leptin intracerebroventricular injection

In the diet-induced obese rats, the inhibition of body weight and food intake was observed on the first day after the intracerebroventricular leptin injection, and it was more significant on the fifth day [body weight from (336.3 ±52.1) g to (287.9 ±53.4) g ($P < 0.01$), with a decrease of (48.4 ±17.9) g; food intake from (35.6 ±13.7) g to (21.1 ±11.8) g ($P < 0.01$), with a decrease of (14.6 ±4.8) g]. But in the normal group, the effects were observed after 3 days [body weight from (294.5 ±29.9) g to (269.5 ±30.9) g ($P < 0.05$), with a decrease of (25.0 ±17.8) g; food intake from (31.0 ±3.5) g to (25.6 ±3.6) g ($P < 0.05$), with a decrease of (5.3 ±3.3) g](Table 1, 2).

Table 1 Changes in food intake after leptin intracerebroventricular injection ($\bar{x} \pm s$, g)

Group	n	Before Injection	After Injection					Decrease
			1 d	2 d	3 d	4 d	5 d	
Normal	6	31.0 ±3.5	27.3 ±5.6	28.5 ±3.5	25.2 ±4.3[a]	25.3 ±3.4[a]	25.6 ±3.6[a]	25 ±17.8
Obese	11	35.6 ±13.7	32.8 ±13.6[b]	30.9 ±13.5[b]	27.3 ±13.3[b]	23.8 ±12.3[b]	21.1 ±11.8[b]	48.4 ±17.9[c]

a vs Before Injection, $P < 0.05$; b vs Before Injection, $P < 0.01$; c vs Normal, $P < 0.01$

Table 2 Changes in body weight after leptin intracerebroventricular injection ($\bar{x} \pm s$, g)

Group	n	Before Injection	After Injection					Decrease
			1 d	2 d	3 d	4 d	5 d	
Normal	6	294.5 ±29.9	303.0 ±31.3	300.3 ±35.4	290.3 ±39.3	289.8 ±46.2	269.5 ±30.9[a]	25 ±17.8
Obese	11	336.3 ±52.1	329.9 ±59.8[a]	321.8 ±57.3[b]	309.5 ±55.2[b]	297.9 ±56.2[b]	287.9 ±53.4[b]	48.4 ±17.9[c]

a vs Before Injection, $P < 0.05$; b vs Before Injection, $P < 0.01$; c vs Normal, $P < 0.05$

2.3 Comparison of serum lipids in each group

In the obesity group with leptin administration, the levels of TC, TG, LDL-C decreased more significantly than those in the normal control group [TC (1.51 ±0.27) mmol/L vs. (2.22 ±0.36) mmol/L, $P < 0.01$; TG (0.43 ±0.06) mmol/L vs. (0.76 ±0.17) mmol/L; LDL (0.47 ±0.12) mmol/L vs. (0.86 ±0.20) mmol/L, $P < 0.01$]. But there was no obvious change in HDL-C in any of the groups (Table 3)

Table 3 Comparison of serum lipids in each group ($\bar{x} \pm s$, mmol/L)

Group		n	TC	TG	HDL-C	LDL-C
Normal	Control	5	1.54 ±0.19	0.37 ±0.09	0.97 ±0.19	0.49 ±0.21
	Leptin	6	1.57 ±0.42	0.31 ±0.06	0.93 ±0.30	0.58 ±0.21
Obese	Control	5	2.22 ±0.36	0.76 ±0.17	1.21 ±0.23	0.86 ±0.20
	Leptin	9	1.51 ±0.27[a]	0.21 ±0.03[a,b,c]	1.00 ±0.24	0.47 ±0.08[a]

a vc Obese Control, $P < 0.01$; b vs Normal Control, $P < 0.01$; c vs Normal Leptin, $P < 0.01$

3 Conclusions

As has been described previously, leptin plays an important role in various physiologic and pathophysiologic states, especially in the pathogenesis of obesity, and is thought to mediate the neuroendocrine response to food deprivation as a satiety factor. Leptin could reduce food intake, and increase energy expenditure to regulate body weight and body fat mass[2]. As reported by Van Heek[8], the high-fat diet-induced obese rats exhibited plasma levels of leptin and the expression of obese gene mRNA in adipose tissues raised, similar to the simple obese human[9]. So it suggested that high-level endogenous leptin failed to control body weight and food intake and leptin resistance can result in obesity. This study therefore examined whether high-nutrition diet-induced obese rats and normal rats could depress food intake and reduce body weight and blood lipid levels when injected into the lateral ventricle with recombinant leptin. This approach tests the anti-obesity effect of exogenous leptin at central sites, independent of any alternations in the transport of circulating leptin into the central nervous system. In this study, in the diet-induced obese rats with intracerebroventricular injection of recombinant leptin, the body weight and food intake were decreased on the first day, and more significant on the fifth day. Moreover, in the normal group, the inhibitive effects were observed 3 days after leptin administration.

Recent studies have suggested that leptin should act by a specific leptin receptor in the hypothalamus, then activate the JAK-STAT signaling pathway and alter the expression of many hypothalamic neuropeptides, such as neuropeptide Y (NPY) and Corticotropin releasing hormone (CRH)[10]. It is suggested that in this study, after an ICV bolus injection of leptin in obese and normal rats, leptin may permeate into the arcuate nucleus and paraventricular nucleus of the hypothalamus, then combine its receptor and regulate the expression of those target genes by the signaling pathway. Ob/ob mice are a genetic obese model of hyperphagia, hyperglycemia, obesity and high-level NPY in the arcuate nucleus. A bolus of leptin, given in an ICV or i.p. injection, can improve these symptoms and decrease the concentration of NPY[11]. This study demonstrates that exogenous recombinant leptin with central administration can reduce body weight and blood lipids and inhibit food intake to improve leptin resistance, and also have a certain effect on normal rats. It is possible that leptin resistance can be explained by the abnormal expression of leptin receptor isoforms, but enough leptin can regulate the expression and distribution of leptin receptor isoforms in the hypothalamus, resulting in a reduction in body weight in diet-induced obese rats. This study also demonstrates that the secretion of serum lipids (TC, TG, LDL-C) in the rats given a high-nutrition diet was more significantly increasing than that in the normal rats. The increase of blood lipids was partly attributable to the addition of grease in the diet and the increase of body weight, which could increase the intake of cholesterol and saturated fatty acid in the liver to raise the synthesis of cholesterol, triglyceride and VLDL, and inhibit the synthesis and activity of LDL receptor[12]. In addition, an injection of leptin by ICV decreased the secretion of serum lipids (TC, TG, LDL-C) in high-nutrition diet-induced obese rats, similar to the normal control and leptin-treated rats. It is possible that leptin could not only reduce the body weight, improve the metabolism of lipids, but also enhance the insulin sensitivity, eliminate the insulin resistance to inhibit the synthesis of VLDL and increase the clearance of VLDL and LDL[13]. However, in this study, no significance in HDL-C in the obese and normal rats was found. These data are not in line with other studies showing the changes of HDL-C. These apparent differences need further study in the future.

With further studies on leptin mechanism, exogenous leptin will open new possibilities for the treatment of obesity.

Acknowledgements

This study was supported by a grant (BK99136) from the Natural Scientific Fund of Jiangsu Scientific and Technological Committee.

References

[1] Li J Y. Pay attention to prevent adult cardiovascular disease during childhood [J]. Chin J Pediatr, 1994, 32(3): 137 - 138.

[2] Zhang YY, Proenca R, Maffei M, et al. Positional cloning of the mouse obese gene and itshuman homologue [J]. Nature, 1994, 372(4): 425 - 432.

[3] Halaas JL, Gajiwala KS, Maffei M, et al. Weight - reducing effects of the plasma protein encoded by the obese gene [J]. Science, 1995, 269(28): 543 - 546.

[4] Robert VC, Madhur KS, Mark LH. Serum immunoreactive - leptin concentration in normal - weight and obese humans [J]. N Engl J Med, 1996, 334(5): 292 - 295.

[5] Qian BC. A method for preparation of obese rat model. Chin Pharmalology Bulletin, 1993, 9(1): 75 - 76.

[6] Noble CP. A single and rapid method for injecting 3H - norepinephine into the lateral ventricle of the brain [J]. Life Sci, 1967, 6(3): 281 - 291.

[7] Cusin I, Rohner - Jeanrenand F, Stricker - Knongrod A, et al. The weight - reducing effect of an intracerebroventricular bolus injection of leptin in genetically obese fa/fa rats [J]. Diabetes, 1996, 45(10): 1446 - 1450.

[8] Van Heek M, Compton DS, France CF, et al. Diet - induced obese mice develop peripheral, but not central, resistance to leptin [J]. J Clin Invest, 1997, 99(1): 385 - 390.

[9] Masuzaki H, Ogawa Y, Isse N, et al. Human obese gene expression: Adipocyte - specific expression and regional differences in the adipose tissue [J]. Diabetes, 1995, 44(7): 855 - 858.

[10] White DW, Kuropatwinski KK, Devos R, et al. Leptin receptor (OB - R) signaling [J]. J Bio Chem, 1997, 272(7): 4065 - 4071.

[11] Stephens TW, Basinski M, Bristow PK, et al. The role of neuropeptide Y in the antiobesity action of the obese gene product [J]. Nature, 1995, 377(12): 530 - 532.

[12] Zhao SP. The works of clinial serum Lipids [M]. Changsha: HuNan Sience and Technology Publishing Company, 1997, 2 - 14.

[13] Masuzaki H, Ogawa Y, Hosoda K, et al. Augmented expression of the obese gene in adipose tissue from rats fed high - fat diet [J]. BBRC, 1995, 216(1): 355 - 358.

(本文编辑:孙晓玲)