GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Effects of glucose and disorders in lipid metabolism on cytokine levels and cognitive impairment in the olanzapine-induced obesity rat model

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Genet. Mol. Res. 14 (3): 11396-11402 (2015)
Received February 1, 2015
Accepted May 19, 2015
Published September 25, 2015
DOI http://dx.doi.org/10.4238/2015.September.25.7

ABSTRACT. The aim of the study was to explore the effects of increased levels of blood sugar and cytokines on impaired cognitive function in the olanzapine-induced obesity rat model. A total of 40 rats were randomly divided into 2 groups; the control and olanzapine groups (N = 20 per group). The control rats were fed regular food, while the olanzapine rats received olanzapine-enriched (1.2 mg/kg) food by gavage for 4 weeks to establish the olanzapine-induced obese rat model. Enzyme-linked immunosorbent assays were used to measure the serum levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP). Serum glucose content was measured by biochemical colorimetry. Learning and memory capacity was measured using a Y-maze, and the time before escape from a Morris water maze was recorded. Body weight and levels of blood glucose, lipids, TNF-α, IL-6, and CRP increased in the olanzapine group. In addition, the number of shocks received before reaching the learning

and memory standard and the time before escape from the Morris water maze were higher in the olanzapine group than in the control group. Olanzapine causes disorders in glucose and lipid metabolism. Increase in blood glucose promotes the toxicity of cytokines and leads to cognitive dysfunction in rats.

Key words: Olanzapine; Obese rat; Adipose-derived cytokine; Cognitive impairment

INTRODUCTION

Studies have reported that antipsychotic drugs cause abnormal glucose metabolism in patients with schizophrenia, including the induction and worsening of diabetes and diabetic ketoacidosis (Xu and Yu, 2010; Liu et al., 2011; Yi et al., 2013). The plasma levels of cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and C-reactive protein (CRP) are high in psychiatric patients, which may be one of the important causes of drug-induced obesity (An et al., 2006). In addition to their relationship with obesity, typical antipsychotic drugs block the dopamine D2 receptor, which can cause or worsen cognitive impairment. Atypical antipsychotics likely damage working memory via blockade of the dopamine D1 receptor. In the present study, we determined the interaction between adipose-derived cytokines and glucose metabolism, using an olanzapine-induced obesity rat model. Furthermore, we explored the role of elevated levels of blood sugar and cytokines in deteriorating cognitive function.

MATERIAL AND METHODS

Experimental animals

A total of 40 Sprague-Dawley (SD) male rats with body weight between 270-300 g were purchased from the Experimental Animal Center of Xinjiang Medical University [certificate of conformity for experimental animals: SYXK (new) 2011-0001]. Rats were housed separately under normal lighting with 50 to 70% relative humidity at 22°C and had free access to water and food. The SD rats were randomly divided into two groups, namely the control and olanzapine groups, with 20 rats per group. The design and execution of the experiments were reviewed and approved by the Animal Ethics Committee of The Four Hospital of Xinjiang.

Drugs and reagents

Olanzapine tablets were purchased from Eli Lilly Pharmaceutical Co., Ltd. The serum glucose, IL-6 assay, and TNF-α ELISA kits were purchased from Xian Baoxin Biotechnology. The serum CRP assay kit was purchased from Beckman (USA).

Olanzapine-induced obesity rat model

One week after the rats adapted to their feeding regimen, the olanzapine group was administered the drug (1.2 mg/kg) in their food. While the olanzapine-induced obesity model group was being established, rats in both the control and olanzapine groups had free access to

food and water. Rats were weighed every 2 days, and continuously fed for 4 weeks. When the average weight of the rats in the olanzapine group was 20% higher than the control group, we considered the model to be successfully established (Chandler et al., 2005).

Measurement of plasma lipids

Rats were fasted for 12 h and then anesthetized by an intraperitoneal injection of 3% sodium pentobarbital. Blood was collected in EDTA anticoagulant tubes for serum separation. The Beckman CX5 automatic biochemical analyzer was used for the determination of the levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C).

Measurement of fasting blood sugar (FBS)

Rats were fasted for 12 h and then anesthetized by an intraperitoneal injection of 3% sodium pentobarbital. Tail vein blood was collected for measurements of FBS. The measurements were performed in strict accordance with the kit instructions.

Y-maze test

Learning and memory were tested using the Y-maze (Shanghai Information Technology Co.), after gavage. The rats were placed in the maze and habituated to their new environment through free exploratory access to all the three arms, for 5 min. All tests were conducted randomly and initiated by placing the rats in the starting arm of the maze. The voltage was set at 75 V, with a delay of 5 s. After the electric shock, rats that immediately escaped to a safe area were considered to have chosen the correct response. Each measurement was followed by a 30 s break, and the rats were allowed to rest for 5 min after the tenth test session. Choosing the correct response nine times out of ten consecutive tests, was considered the basic standard for learning. We then recorded the number of shocks required for the animals to attain the learning standard. This experiment was repeated 24 h later, to test memory in the rats. Tests were carried out under quiet and dark conditions.

Morris water maze test

The Morris water maze video analysis system (Shanghai ZH-SBS Doctor Rat video software) was used for this test. Navigation test sets were performed as follows: training was conducted four times a day for 7 days, followed by a test on day 8. The time required for rats to find the hidden platform was recorded.

Statistical analysis

All data were analyzed by the Student *t*-test using the statistical package for the social sciences (SPSS) 13.0 software and are reported as means ± SD for N = 20 animals per group. Pearson correlation analysis was performed for the FBS, TNF-α, IL-6, and CRP, with $P < 0.05$ considered as statistically significant.

RESULTS

Effect of olanzapine on rat body weight

The weights of the rats in the olanzapine group increased significantly 2 weeks after gavage compared to the control group (>20% of the control group, $P < 0.01$). This result indicates the olanzapine-induced obese rat model was successfully established (Table 1).

Table 1. Comparison of body weights between olanzapine and control groups.

Group	N	1 week	2 weeks	3 weeks	4 weeks
Control	20	294.22 ± 26.64	310.22 ± 24.99	318.00 ± 24.37	330.78 ± 25.14
Olanzapine	20	322.9 ± 25.73	361.33 ± 24.19*	374.22 ± 24.07*	400.44 ± 23.57*

*P < 0.05 compared to control, N = 20, means ± SD (mg).

Effects of olanzapine on serum lipid and glucose

The olanzapine group showed significantly higher serum TG, TC, and LDL-C than the control group after 4 weeks of gavage ($P < 0.01$). HDL-C levels were lower than the normal control group ($P < 0.01$). FBS was higher than in the normal control group ($P < 0.01$, Table 2).

Table 2. Comparison of blood lipid and glucose in rats.

Group	N	FBS	TC	TG	HDL-C	LDL-C
Control	20	4.90 ± 0.08	1.75 ± 0.04	0.71 ± 0.03	2.57 ± 0.11	0.68 ± 0.04
Olanzapine	20	6.06 ± 0.13*	2.98 ± 0.09*	2.61 ± 0.09*	1.68 ± 0.04*	0.96 ± 0.06*

*P < 0.05 compared to control, N = 20, means ± SD (mM).

Olanzapine-induced changes in serum TNF-α, IL-6, and CRP

The olanzapine group showed significantly higher TNF-α, IL-6, and CRP levels after 4 weeks of gavage ($P < 0.01$, Table 3).

Table 3. Comparison of serum TNF-α, IL-6, and CRP levels.

Group	Cases	TNF-α (ng/mL)		IL-6 (pg/mL)		CRP (mg/mL)	
		1 week	4 weeks	1 week	4 weeks	1 week	4 weeks
Control	20	0.49 ± 0.03	0.59 ± 0.03	88.63 ± 12.35	96.58 ± 8.77	1.75 ± 0.06	1.86 ± 0.04
Olanzapine	20	0.58 ± 0.04	1.57 ± 0.04*	85.24 ± 7.54	127.47 ± 11.38*	1.84 ± 0.02	2.68 ± 0.06*

*P < 0.05 compared to control, N = 20, means ± SD.

Correlation analysis of serum TNF-α, IL-6, and CRP

Correlation analysis showed that both TNF-α and IL-6, are positively correlated with CRP ($P < 0.05$, Table 4).

Table 4. Correlation analysis of FBS and cytokines (hs-CRP, IL-6 and TNF-α).

Group	Cases	Correlation coefficient (r)	P
FBS/TNF-α	20	0.385	0.013
FBS/IL-6	20	0.260	0.005
FBS/CRP	20	1.280	0.045

Comparison of learning and memory

The behavioral tests showed that compared with the control group, the rats from the olanzapine group had significantly impeded learning and memory. In addition, the number of electric shocks delivered before the rats learned their escape route in the Y-maze increased. The time required for the olanzapine-treated rats to escape the Morris water test was also prolonged ($P < 0.01$, Table 5 and Figure 1).

Table 5. Cognitive ability of olanzapine-treated and control groups.

Groups	N	Y-maze test		Morris water maze test
		Learning ability (s)	Memory (times)	Escape time (s)
Control	20	50.25 ± 2.02	50.25 ± 2.05	23.54 ± 2.12
Olanzapine	20	82.65 ± 2.12*	82.65 ± 2.10*	62.20 ± 2.10*

*P < 0.05 compared to control.

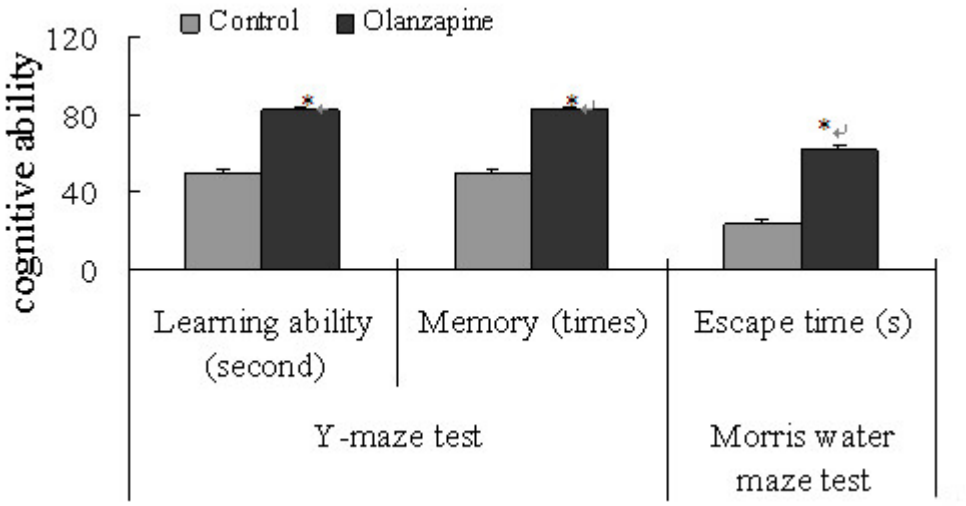

Figure 1. Cognitive ability of olanzapine-treated and control group. The number of shocks received before reaching the learning and memory standard and the time before escape from the Morris water maze were higher ($P < 0.05$) in the rats in the olanzapine group than those in the control group. *P < 0.05 compared to control group.

DISCUSSION

Previous studies have found that adipocytes secrete cytokines and many cytokine-like molecules, including leptin, adiponectin, and anti-hormones. Many other cytokines not ex-

pressed by fat tissues can also be secreted by adipocytes, including fat-derived cytokines such as TNF-α and IL-6. Studies have shown that adipocytes in obese individuals produce large amounts of biologically active TNF-α, however, the serum concentration of TNF-α decreases after weight loss (Nishimura et al., 2003). TNF-α is involved in the regulation of fat accumulation mainly by causing insulin resistance. It has been previously shown that TNF-α is an important mediator for pathogenic islet cells. Inflammatory cytokines such as IL-6, TNF-α, IL-1, and CRP have effects on islet β-cells, resulting in cytotoxicity (Wang et al., 2002). It was further confirmed that both healthy and type 2 diabetes patients show IL-6 gene expression in islet β-cells (Wang et al., 2009). In addition, studies have found that IL-6 is regulated by glucose, while high blood sugar can promote islet β-cells to secrete IL- 6 (Esposito et al., 2002). Heilbronn et al. (2001) showed that serum CRP is higher in obese patients than in patients with healthy body weight. In obese patients, insulin sensitivity is reduced, and its physiological role is inhibited, which increases the synthesis of CRP. Considerable recent evidence suggest that serum CRP is closely related to the development and prognosis of obesity, insulin resistance, and diabetes.

In this study, we found that obese rats persistently treated with the antipsychotic olanzapine continued to increase in weight. The weight gain resulted in disorders in blood glucose and lipid metabolism as well as increased serum TNF-α, IL- 6, and CRP. Meanwhile, the FBS in obese rats was positively correlated with the levels of TNF-α, IL-6, and CRP. With increased blood sugar, the levels of adipocyte cytokines were also increased. Blood glucose can regulate the levels of inflammatory cytokines in obese rats, thereby promoting cytotoxicity and causing chronic pathologies.

In this study, behavioral tests showed that learning and memory capacity are impaired in rats treated with olanzapine. The cognitive decline combined with increase in adipocyte-secreted inflammatory cytokines is complicated by the increase of blood sugar. These pathologies are presumably due to the direct neuronal damage caused by high blood sugar. However, there may also be the involvement of the indirect effects of inflammatory cytokines on the expression of certain neurotransmitters. This interference with neurotransmission causes structural damage to the brain, and changes in brain function, ultimately leading to mild cognitive impairment in the rats. Weight gain and metabolic disorders are common adverse drug reactions in patients treated with antipsychotics (Allison and Casey, 2001). Many studies have shown that antipsychotics such as olanzapine lead to obesity, hyperglycemia, and dyslipidemia (Henderson et al., 2000). High blood glucose can induce the proliferation of brain capillaries, causing increased permeability of the blood-brain barrier. The dysfunction in the blood-brain barrier increases the permeation of inflammatory cytokines and antibodies, which invade the neurons leading to decreased cognitive function (Purdon et al., 2001). Mogi et al. (2004) reported that cognitive test performance, positively correlates with glycated hemoglobin. Studies also confirmed that the use of antidiabetic drugs to control metabolic disorders in type 2 diabetes improves the working memory and cognitive function of patients (Ryan et al., 2006). This result indirectly reflects the effect of hyperglycemia on cognitive function (Ryan et al., 2006; Ergul et al., 2007). High blood glucose can damage blood vessels, induce metabolic abnormalities, impede neuronal calcium homeostasis, and cause changes in neurotransmitters. Furthermore, it can result in damage to cerebral blood vessels, caused by inflammatory responses. Collectively, these abnormalities lead to decreased understanding, processing, and information integration processes, which eventually cause reduction in the cognitive and processing capacity of patients.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by a grant (#G111310003) from the Project Science and Technology Development, Urumqi Municipal Science and Technology Bureau.

REFERENCES

Allison DB and Casey DE (2001). Antipsychotic-induced weight gain: a review of the literature. *J. Clin. Psychiatr.* 62: 22-31.

An B, Liu X, Dong X, and Qi SG (2006). Effect and clinical significance of clozapine on serum IL-2, IL-6 of schizophrenia. *Neurosci. Mental Health* 6: 14-17.

Chandler PC, Viana JB, Oswald KD, Wauford PK, et al. (2005). Feeding response to melanocortin agonist predicts preference for and obesity from a high-fat diet. *Physiol. Behav.* 85: 221-230.

Ergul A, Elgebaly MM, Middlemore ML, Li W et al. (2007). Increased hemorrhagic transformation and altered infarct size and localization after experimental stroke in a rat model type 2 diabetes. *BMC Neurol.* 7: 33.

Esposito K, Nappo F, Marfella R, Giugliano G, et al. (2002) Inflammation cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. *Circulation* 106: 2067-2072.

Heilbronn LK, Noakes M and Clifton PM (2001). Energy restriction and weight loss on very-low fat diets reduce C-reactive protein concentrations in obese, healthy women. *Arteriosceler. Thromb. Vasc. Biol.* 21: 968-970.

Henderson DC, Cagliero E, Cray C, Nasrallah RA, et al. (2000). Clozapine diabetes mellitus, weight gain, and lipid abnormalities: a five-year naturalistic study. *Am. J. Psychiatr.* 157: 975-978.

Liu W, Cai S and Hong XH (2011). The research progress of schizophrenia and diabetes comorbidity. *Chin. J. Behav. Med. Brain Sci.* 20: 958-960.

Mogi N, Umegaki H, Hattori A, Maeda N, et al. (2004). Cognitive function in Japanese elderly with type 2 diabetes mellitus. *J. Diabetes Complications* 18: 42-46.

Nishimura F, Iwamoto Y, Mineshiba J, Shimizu A, et al. (2003). Periodontal disease and diabetes mellitus: the role of tumor necrosis factor-alpha in a 2-way relationship. *J. Periodontol.* 74: 97-102.

Purdon SE, Labelle A and Boulay L (2001). Neuropsychological changes in schizophrenia after 6 weeks of clozapine. *Schizophr. Res.* 48: 57-67.

Ryan CM, Freed MI, Rood JA, Cobitz AR, et al. (2006). Improving metabolic control leads to better working memory in adults with type 2 diabetes. *Diabetes Care* 29: 345-351.

Wang G, Huang X and Wu G (2009). Relations of macrovascular lesions of type 2 diabetes to levels of serum CRP, IL-6, and TNF-a. *J. Hainan Med. Univ.* 15: 1012-1013.

Wang JL, Qian X, Chinookoswong NS, Lu J, et al. (2002). Polyethylene glycolated recombinant TNF receptor I improves insulitis and reduces incidence of spontaneous and cyclophosphamide-accelerated diabetes in nonobese diabetic mice. *Endocrinology* 143: 3490-3497.

Xu Y and Yu J (2010). Antipsychotic drugs and schizophrenia patients with abnormal glucose metabolism. *Med. J. Qilu.* 25: 469-470.

Yi F, Mei J, Su X, Mao JY, et al. (2013). Metformin for prevention of weight gain in patients with schizophrenia treated with second-generation antipsychotics: a meta-analysis. *Chin. J. Behav. Med. Brain Sci.* 22: 221-224.