MOLECULAR & CELLULAR TOXICOLOGY, Vol. 3, No. 2, 127-131, June 2007

Prevention of Olanzapine-induced Toxicities of Weight Gain and Inflammatory Reactions by Coadministration with Green Tea or its Major Component Phenolic Epigallocatechin 3-Gallate in Mouse

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Accepted 15 May 2007

Abstract

Chronic treatment with olanzapine (OLZ), an atypical antipsychotic drug, is associated with the adverse effects of weight gain, hyperglycemia and/or hypertriglyceridemia. Green tea or epigallocatechin gallate (EGCG), one of the most abundant green tea polyphenols, significantly reduces or prevents an increase in glucose levels, lipid markers and/or body weight. We hypothesized that combined treatment with OLZ and green tea extract (GTE) or EGCG may prevent body weight gain and increase of the lipid markers. ICR male mice weighing an average of 30.51 g (n=32) at the beginning of the experiment were used. OLZ, OLZ+GTE and OLZ+EGCG were administered for 27 d in the drinking water, and then the levels of fasting glucose, nitric oxide (NO), and a typical lipid marker triglyceride (TG) were determined in plasma. The body weight and food intake were also compared. The chronic treatment of OLZ increased the average body weight compared with that of controls. In the presence of GTE or EGCG, the OLZ-induced increase in body weight was significantly prevented. Furthermore, in the OLZ group, the plasma levels of glucose, NO and TG were significantly increased, whereas GTE or EGCG prevented these increases. These results implicate that OLZ may induce systematic inflammatory reaction, and suggest that GTE or EGCG can protect against OLZ-induced weight gain, hyperglycemia and hypertriglyceridemia.

Keywords: Schizophrenia, Olanzapine, Green tea, Weight gain, Triglyceride, Nitric oxide

Obesity is common in schizophrenia patients taking antipsychotic drugs such as olanzapine (OLZ) and clozapine, and people with schizophrenia appear to be at increased risk for certain obesity-related conditions, including type 2 diabetes and cardiovascular disease[11].

Antipsychotic drug-induced weight gain has a number of meaningful clinical implications, especially in the long-term management of schizophrenia. In addition to its effect on general health risks, weight gain has a major impact on the subjective acceptance of these drugs and thus on compliance[8,21]. Second generation antipsychotic drugs (atypical antipsychotic agents) are gradually replacing the older generation of antipsychotic drugs because they produce fewer extrapyramidal effects and may be effective in alleviating the negative symptoms of schizophrenia[22]. However, second generation antipsychotic agents can cause large increases in body weight in both adolescent and adult patients, with the putative mechanisms focusing on histamine and 5-hydroxytryptamine (serotonin; 5-HT) antagonism and being associated with increases in leptin levels; clozapine and OLZ appear to cause the most weight gain[16,25]. In addition to weight gain, concerns about hypertriglyceridemia with currently available novel antipsychotic drugs were raised in 1995 with the identification of four cases among patients treated with clozapine[10]. Recent reports have also documented a mean increase in fasting serum triglycerides associated with both long- and short-term treatments with OLZ[17,20]. Recently, Choi *et al.*[4] reported that the increased serum nitric oxide (NO) concentration correlates significantly with a high concentration of body fat in obese humans, although the obesity was not induced by the drug therapy. This interesting result might be explained by the inhibitory effect of overproduced NO on lipoprotein lipase activity in lipolysis as suggested by Gaudiot *et al.*[9] and Uchida *et al.*[23].

Green tea may enhance health because it reduces the incidence of cancer in various experimental models, is a potent antioxidant, and modulates serum cholesterol concentrations[18]. Green tea also has effects on body weight[1,14], and recent reports suggest that epigallocatechin gallate (EGCG), one of the most

abundant green tea polyphenols, significantly reduces or prevents an increase in body weight in lean and obese male and female Zucker rats[14,15].

Therefore, it was postulated that combined OLZ treatment with GTE or EGCG would differ from OLZ monotherapy with respect to the effects on body weight and serum levels of lipid markers, nitric oxide and glucose. To test this hypothesis, we performed a longitudinal study of body weight and serum lipid markers in four groups of male rats.

Effects of GTE or EGCG on Weight Gain and Food Intake Changed by OLZ Treatment

Alterations in body weight, food and water intake, plasma lipid marker (TG), and inflammatory mediator (NO) were examined in mice treated with an atypical antipsychotic agent (OLZ), OLZ plus green tea extract (GTE), and OLZ plus EGCG, and in a control group treated with water (CON).

The average weight of animals was 30.51 g (n=32) before the drug administration. As shown in Figure 1, the body weight changes according to the time variables among the four groups were significantly different (P=0.022). Although the body weight at the later stages had increased in the OLZ group, the OLZ-induced increase of body weight over the whole period was not significantly different (P=0.819). At the late time points, the average body weight in the OLZ group at 18, 21 and 27 d had increased at significantly higher (P=0.041, 0.002 and 0.029, respectively) than that of the CON group at the same stages. In the presence of GTE and EGCG, the OLZ-induced increase in body weight was significantly less than the increase in the OLZ group (P=0.038 and P=0.032, respectively). In particular, the effect of GTE was statistically significant at early and late stages compared (3, 6 and 27 d, $P<0.05$ or $P<0.01$), whereas EGCG had significant effect at 27 d ($P<0.05$) (Figure 1).

Mean food intake values among the three groups included OLZ+GTE or OLZ+EGCG group were not statistically different (P=0.554 or P=0.952, respectively). Furthermore, a little higher (99.91±25.60 g, median value: 99.20) without statistical significance

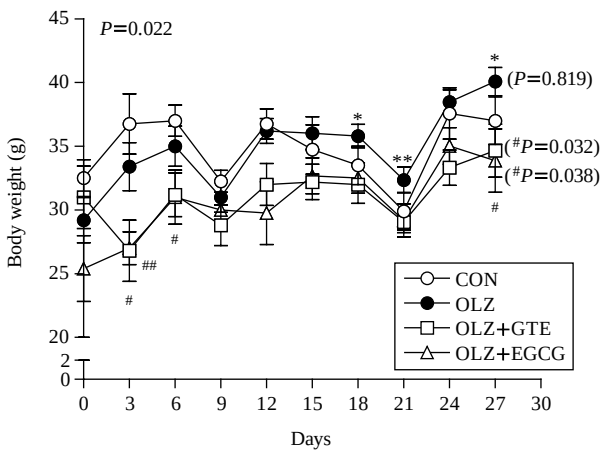

Figure 1. Effects of olanzapine (OLZ) treatment and cotreatment with OLZ and green tea extract (GTE) or EGCG on the changes of body weight. Values are shown as means± SD (n=5 per group). *$P<0.05$, **$P<0.01$ *vs* CON group, #$P<0.05$, ##$P<0.01$ *vs* OLZ group. The differences in body weight changes among the total four groups (P=0.022) and the increases in body weight according to the time variables ($P<0.0001$) were also statistically significant.

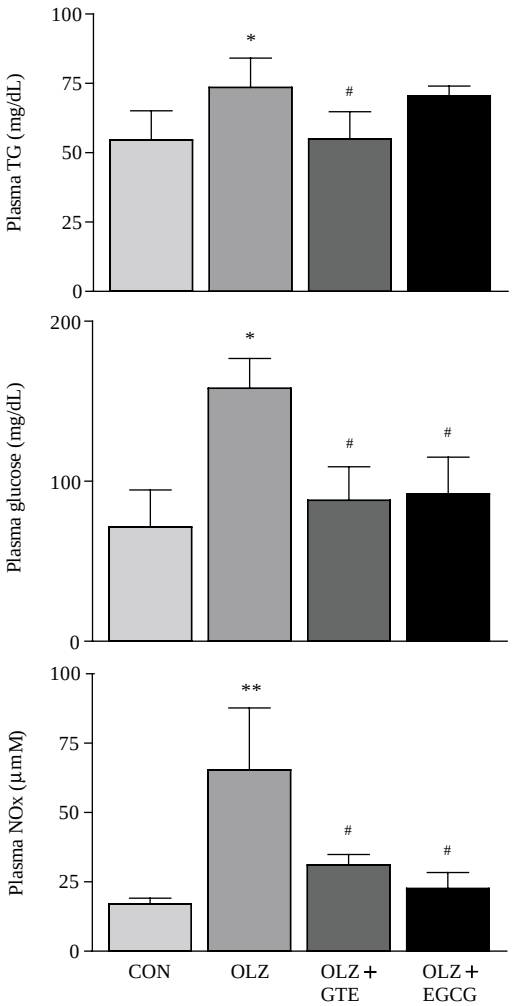

Figure 2. Effects of olanzapine (OLZ) treatment and cotreatment with OLZ and green tea extract (GTE) or EGCG on the changes in plasma levels of (A) triglyceride (TG), (B) glucose and (C) nitric oxide metabolites (NOx). Values are shown as means±SD (n=5 per group). *$P<0.05$, **$P<0.01$ vs CON group, #$P<0.05$ vs OLZ group.

in the group treated with OLZ only, whereas it was similar to the control group's intake in the other groups. Drink water intake was similar in all groups without significant differences (range of 24.6-30.4 mL per cage).

Effects of GTE or EGCG on the Plasma Levels of TG, Glucose and NOx Increased by OLZ Treatment

As shown in Figure 2, the plasma levels of TG, glucose and NOx were significantly increased in the OLZ-treated group ($P < 0.05$). In the OLZ-GTE and OLZ-EGCG groups, the increases in plasma levels of TG, glucose and NOx were significantly less than in the OLZ group ($P < 0.05$) (Figure 2). These results suggest that GTE or EGCG may protect from OLZ-induced weight gain, hyperglycemia and hypertriglyceridemia.

Discussion

Our study is, to the best of our knowledge, the first to investigate the inhibitory effects of green tea extract (GTE) and EGCG and to evaluate the biochemical underpinnings in the context of an atypical antipsychotic drug, olanzapine (OLZ)-induced weight gain and increase of lipid marker, glucose and nitric oxide (NO) levels in ICR male mice.

The weight gain associated with several antipsychotic drugs, most notably the newer atypical compounds OLZ and clozapine (CLZ), introduces problems with compliance and morbidity in the treatment of schizophrenia. Although the mechanisms underlying this process are not fully understood, some researchers have suggested that the weight gain may be attributable to the drug's high 5-HT2c and H1 antagonistic effects[13,22]. CLZ and OLZ have also been shown to increase serum levels of leptin, which acts as a feedback signal from the adipose tissue and may play a role in the pathophysiology of obesity. Leptin levels increase exponentially with body mass index or percentage of body fat[2]. This finding was confirmed by a recent clinical study[8].

The extent of weight gain reported in this study is indeed worrisome. During the 27 d experiment, body weight increased by an average of 32.0% in mice treated with OLZ monotherapy. Compared with the mice given OLZ only, however, the rate of weight gain was significantly lower in mice treated with OLZ-GTE. Interestingly, the feeding behavior was a little higher in the OLZ-treated group, but not different in other groups.

Recent reports have documented that green tea is a potent antioxidant[6], and has an effect on body weight[3,5] and energy expenditure[7]. EGCG, a major component of GTE, also significantly reduces or prevents the weight increase in Zucker rats[14,15]. In addition, our study suggests that GTE and EGCG have inhibitory effects on OLZ-induced weight gain. Our findings have great clinical implications, as no previous studies have been published on the inhibitory effect of GTE or EGCG in drug-induced obesity. If our findings can be replicated and applied to clinical situations, it would be helpful to management of schizophrenia with OLZ. Furthermore, we reported that the effects of green tea consumption on the pharmacokinetics of the antipsychotics that cause weight gain like clozapine[26].

In this experiment, we also observed an interesting behavior, in which the mice of all groups progressively removed the foil covering placed around the drinking bottles to protect the OLZ from light, except for two groups. This behavior was not observed from the beginning in OLZ with GTE and after two weeks in groups treated with OLZ-EGCG. Although we cannot explain this observation, possible effects may be predicted from the characteristics of EGCG, which penetrates easily through the blood-brain barrier.

Our results, increased triglyceride and glucose levels in animals treated with OLZ, are in accordance with previous reports showing that OLZ can cause hyperglycemia and hypertriglyceridemia in patients with schizophrenia[17,19]. Interestingly, GTE or EGCG prevented the increase of OLZ-induced plasma levels of TG and glucose; in contrast with the previous reports, we could not find changes in other lipid markers (e.g., total cholesterol, low density lipoprotein, high density lipoprotein, data not shown). Furthermore, OLZ treatment induced an increase in the serum concentration of NO metabolites, as reported in obese people taking no medication[4]. This increase in NO was completely prevented by cotreatment with GTE or EGCG, as shown in Figure 2. Thus, we suggest that OLZ-induced weight gain may also correlate with an increase in *in vivo* NO, because the inhibition of NO production by GTE or EGCG can promote lipolysis, as suggested by Gaudiot *et al.*[9]. Recently, we have examined the microarray technology which is widely applied to elucidate the broad mechanism of drug-induced toxicology, by profiling gene expression in transcriptional and translational scales[27,28].

In conclusion, OLZ can induce weight gain and increase serum levels of TG, glucose and NO in animals. GTE or EGCG can prevent these adverse effects. To verify our findings, well-designed further investigations in clinical situations are warranted.

Methods

Animals

ICR male young mice (SPF laboratory. Inha University, Korea) weighing 30.51±1.69 g (range, 28.5-32.2 g, n=32) at the beginning of the experiment were used. Animals were maintained in a temperature (22±2.0°C)-controlled room, air humidity 65±2.5% with a 12-h light-dark cycles, and housed eight per large cage with free access to food and water. This drinking water treated for experimental groups was included with OLZ, OLZ+GTE, or OLZ+EGCG. All experiments were approved by the Ethical Committee on Animal Experiments.

Experimental Procedures

Mice were randomly allocated to the four groups described below. In general, water feeding volume of one mouse was estimated as average 6.1 mL/d (4.9-7.3 mL/d). For drug treatment, thus, OLZ at concentration of 1.0 g/mL in water (200 mL), corresponding to about 1.6 mg/kg per day (ZYPREXA®, Eli-Lily Co. Ltd., Basingstroke, England) per cage (n=8: about 200 g/kg/d per mouse) was administered to the mice every day via drinking water for 27 d. In human, daily dosage of this drug is variable from 5 mg to 20 mg (about 80-320 g/kg/d). Green tea (SullocCha®, Pacific Co. Ltd., Jincheon, Korea) with a constant and rich concentration of EGCG (EGCG: 57.1±11.4 mg/g; caffeine: 18.6±3.9 mg/g; n=30 packs) was used to produce GTE by extracting 1 g of dry green tea with 120 mL of water at 100°C for 5 min. EGCG purchased from Sigma Chemical Co. (St. Louis, MO, USA) was added with concentration of 80 g/mL to the OLZ. Therefore, the first group was used as the control (CON), and the second group (OLZ) served to assess the effects of OLZ on *in vivo* related values with weight gain. The third (OLZ-GTE) and fourth (OLZ-EGCG) groups were used to assess the respective effect of GTE and EGCG on OLZ-induced alterations of the related values. We determined the related values with weight gain like body weight, food intake, feeding efficiency, plasma levels of a typical lipid marker [triglyceride (TG)] and inflammatory mediator [nitric oxide (NO)], and glucose levels.

Individual weight was estimated after fasting for 4 hr every 3 d, and food and water intake per cage was recorded from the remaining volumes every 3 d, and animals of all groups were killed 8 hr after the administration on the last day. Plasma levels of the typical related marker TG (milligrams per deciliter) and glucose (milligrams per deciliter) were determined with automatic clinical analyzer model 747 (Hitachi High-Technologies Corporation, Kobe, Japan). Plasma concentration (micromolar) of systemic nitric oxide metabolites (NOx) indicating the *in vivo* inflammatory NO production, which correlates significantly with body mass index (BMI) in humans[4], was measured by a NADPH-dependent nitrate reductase assay and Griess method[12,24].

Statistical Analysis

All statistical procedures were performed using SAS (Ver. 6.12) or PRISM (Ver. 3.0) softwares. General linear modeling procedures were used for the comparison among the groups, and repeated measure of ANOVA was used for the analysis of body weight increment. Bonferroni's multiple comparison test was performed as post-tests. In addition, the significance of the difference for food intake and feeding efficiency was assessed by one-way ANOVA test. The significance between groups in plasma levels for lipid markers, inflammatory mediators and glucose were compared by unpaired Student-*t* test. Statistical significance was confirmed at a P value < 0.05 or < 0.01.

Acknowledgements

This work was supported by an intramural grant from the Inha University (INHA 22053-01).

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