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Metformin improves lipid metabolism disorders through reducing the expression of microsomal triglyceride transfer protein in OLETF rats

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ABSTRACT

Objective: This study aimed to investigate the role of MTP on lipid metabolism disorders in insulin-resistant rats and the potential mechanism through which metformin can improve lipid metabolism disorders.

Methods: 30 OLETF rats served as research subjects and 18 LETO rats of the same strain served as the control group (LETO group). After the first oral glucose tolerance test (at 8-week-old), 6 rats were randomly killed from each group. The remaining 24 OLETF rats were randomly divided into untreated group (OLETF group) and treated group (OLETF/M group, cured with metformin). By the end of the 10th and 20th week of treatment, MTP in the liver was measured for all rats in the study.

Results: All OLETF rats exhibited diabetic phenotypes at 18-week-old, with their triglyceride level higher than in LETO rats at the same age. In OLETF rats, MTP level in the liver was higher than in LETO rats at 18-week-old, and the difference was significant at 28-week-old [(13.79 ± 1.47) vs. (8.20 ± 1.14), $p < 0.05$]. Treatment with metformin for 20 weeks decreased triglyceride [(1.06 ± 0.23) vs. (2.20 ± 0.62) mmol/L, $p < 0.05$] and total cholesterol [(1.90 ± 0.19) vs. (2.36 ± 0.14) mmol/L, $p < 0.05$] in OLETF rats. Metformin also decreased MTP level in the liver [(7.65 ± 1.31) vs. (13.79 ± 1.47), $p < 0.01$].

Conclusions: MTP may be associated with the lipid metabolism disorder in OLETF rats and metformin could improve lipid metabolism through reducing the expression of MTP.

1. Introduction

Atherosclerosis-induced ischemic stroke, coronary heart disease, and lower limb ischemic necrosis are common and serious complications of diabetes [1,2]. Lipid metabolism disorders associated with diabetes elevate blood lipids and their deposition in the arterial intima, which initiate pathological changes of atherosclerosis [3–5].

The liver plays an important role in lipid metabolism, and intervention of the lipid metabolism process in the liver is an effective way to mitigate lipid metabolism disorders and prevent atherosclerosis [6].

Besides apolipoprotein B (apoB), another important lipid transfer protein, microsomal triglyceride transfer protein (MTP), participates in the transfer of triglycerides and the assembly of very low density lipoproteins (VLDLs) [7–9]. The

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major functions of MTP are to accelerate transport of triglycerides, cholesterol and phospholipid, to facilitate construction of cellular and subcellular membranes, and to limit the assembly and secretion of ApoB-containing lipoproteins. During the assembly of lipoproteins, MTP is involved in lipid transport. Triglycerides are transported into the ER lumen by MTP and bind to newly-synthesized ApoB, which are then transported extracellularly [10]. In addition, MTP reduces the affinity of apoB for ions [11]. Disorders that interrupt the level and activity of MTP can directly affect the metabolism of lipids and lipoproteins, which can lead to lipid metabolism disorders and cardiovascular disease. Gene mutations in MTP have shown to be linked to genetic diseases, such as β-hyperlipoproteinemia.

Therefore, the study about MTP has important clinical significance, although MTP is rarely studied. In addition, we have observed, metformin, as the first-line therapy [12,13] had improved the lipid metabolism disorders in patients with type 2 diabetes [14,15], Whether the effect of metformin on the lipid metabolism disorders is related to MTP remains to be seen.

Otsuka Long Evans Tokushima Fatty (OLETF) rats, established by Kawano and his co-worker, is a type 2 diabetes animal model with greedy, less-moving, obesity, spontaneous long-term hyperglycemia, hyperinsulinemia, insulin resistance, hyperlipidemia, diabetic nephropathy, and so on. These characteristics and pathogenesis are very similar to human type 2 diabetes mellitus [16]. Now, OLETF rats were widely used in animal studies of type 2 diabetes [17,18]. Therefore, in this paper, OLETF rats were used as the research object, Long Evans Tokushima Otsuka (LETO) rats for the same strain were used as control, the expression levels of MTP in liver tissue of OLETF rats and LETO rats were observed.

This experiment was approved by the Animal Care and Use Committee of Peking University First Hospital.

2. Materials and methods

2.1. Animals

4-week-old male OLETF rat and LETO rat were purchased from the Research Institute of Japan Otsuka pharmaceutical company. Animal were maintained in the SPF condition with a room temperature (23 ± 2 °C) and moisture (55 ± 10%) controlled facility, 12 light/dark cycle, and were given free access to food and water. 3–4 rats lived in one cage. From 8-week-old, one rat lived one cage. The body weight of rat was measured once a week. Beginning at 8 weeks of age, rats were fed normal chow diet; meanwhile, the water was changed into 100 g/sucrose (China National Medicines Corporation Ltd.) Group (Fig. 1): 30 OLETF rats served as our research subjects (OLETF group) and 18 LETO rats of the same strain served as our control group (LETO group). After the first oral glucose tolerance test (at 8-week-od), 6 rats were randomly selected and killed from each group. The remaining 24 OLETF rats were randomly divided into untreated group (OLETF group) and treated group (OLETF/M group). The rats in OLETF/M group were gavaged with 300 mg/(kg·d) metformin hydrochloride (The raw material powder was a gift from Beijing Double-Crane Pharmaceutical). The other rats were gavaged with 6 mL/(kg·d) pure water.

2.2. Oral glucose tolerance test (OGTT)

OGTT was performed at 8, 18, 28-week-old respectively, the body weight, lipid and insulin level in serum were detected at the same time.

From 4 PM of the day before the experiment, all rats stopped eating food, started drinking pure water freely. At 8 AM of the day of the experiment, we weighted the rats and obtain about 1 ml blood from the orbital angular vein of rats, Then all rats were fed 50% glucose solution (4 ml/kg) by Gavage and recorded time. Respectively after the recorded time 1 h, 2 h, we obtained about 1 ml blood again from the orbital angular vein of rats.

2.3. Determination of biochemical parameters

Blood glucose (BG) was measured by glucose oxidase method, using the German Roche BIOSEN C-LINE semi-automatic biochemical analyzer; Serum insulin was measured by radioimmuno-assay. Blood samples at 4 °C after 3000 r/min centrifugation, measured serum insulin. Insulin was measured by Beijing boreal Institute of Technology insulin radioimmuno-assay kit; plasma total cholesterol (Total Cholesterol, TCHO) and triglycerides (Triglyceride, TG) were detected by the U.S. BecKmanCX-5 automatic biochemical analyzer, the batch CV < 4%, inter-assay CV < 8%.

2.4. Liver total protein extracts

After OGTT, six rats at 8, 18 and 28-week-old were randomly selected from each group, respectively. These selected rats were killed by intraperitoneal injection of 2% pentobarbital, 40 mg/kg. Immediately, take the liver tissue into liquid nitrogen and subsequently stored at −80 °C refrigerator. Add pre-chilled Radio Immuno Precipitation Assay (RIPA) lysis buffer, 1 ml per 100 mg tissue [Biyuntian Biological Co. Ltd. P0013B, 50 mM Tris (pH 7.4), 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% Sodium dodecyl sulfate (SDS), and sodium orthovanadate, sodium fluoride, ethylene diamine tetraacetic acid (EDTA), leupeptin, and other inhibitors used within a few minutes before added phenylmethyl sulfonylfluoride (PMSF), PMSF to a final concentration of 1 mM]. After electric homogenizer, 4 °C, 14000 rpm centrifuged for 5 min, take the supernatant and measured protein concentration using the bicinchoninic acid (BCA) method. Then the samples were stored in buffer with 6 × SDS-polyacrylamide gel electrophoresis (PAGE) (Biyuntian Biological Co., Ltd. P0015F), 50 ug per aliquot. Mix and 95 °C water bathe for 5 min and stored at −80 °C.

2.5. Western blot hybridization

(1) SDS-PAGE vertical electrophoresis: 50 ug of total protein in the sample mixed with 6 × SDS-PAGE sample buffer, the protein preparation completed after mixing again and 95 °C water bath for 5 min. The sample was cen-