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Original Paper

Atypical Antipsychotic Drug Olanzapine Deregulates Hepatic Lipid Metabolism and Aortic Inflammation and Aggravates Atherosclerosis

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Key Words
Olanzapine • Hepatic lipid metabolism • Hyperlipidemia • Inflammation • Atherosclerosis

Abstract
Background/Aims: Olanzapine, an atypical antipsychotic drug, has therapeutic effects for schizophrenia. However, clinical reports indicate that patients taking atypical antipsychotic drugs are at high risk of metabolic syndrome with unclear mechanisms. We investigated the effect of olanzapine on atherosclerosis and the mechanisms in apolipoprotein E-null (apoE$^{-/-}$) mice. ***Methods:*** ApoE$^{-/-}$ mice were used as *in vivo* models. Western blot analysis was used to evaluate protein expression. Conventional assay kits were applied to assess the levels of cholesterol, triglycerides, free cholesterol, cholesteryl ester, fatty acids, glycerol, and cytokines. ***Results:*** Daily treatment with olanzapine (3 mg/kg body weight) for four weeks increased mean arterial blood pressure and the whitening of brown adipose tissue in mice. In addition, olanzapine impaired aortic cholesterol homeostasis and exacerbated hyperlipidemia and aortic inflammation, which accelerated atherosclerosis in mice. Moreover, lipid accumulation in liver, particularly total cholesterol, free cholesterol, fatty acids, and glycerol, was increased with olanzapine treatment in apoE$^{-/-}$ mice by upregulating the expression of de novo lipid synthesis-related proteins and downregulating that of cholesterol clearance- or very low-density lipoprotein secretion-related proteins. ***Conclusion:*** Olanzapine may exacerbate atherosclerosis by deregulating hepatic lipid metabolism and worsening hyperlipidemia and aortic inflammation.

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Introduction

Schizophrenia is a persistent and disabling psychotic disorder that may be attributed to multiple factors including genetic and environmental aspects and social processes [1, 2]. Schizophrenic patients may display abnormal social behavior and be out of touch with reality [1, 2]. The most common symptoms of schizophrenia include delusions, hallucinations and disorganized thoughts, which are divided into three categories: positive, negative, and cognitive [3].

The dopamine hypothesis of schizophrenia is the most widely discussed theory in psychiatry and suggests that a disturbed and hyperactive transduction of the dopaminergic signal contributes to schizophrenia symptoms [4, 5]. Targeting this pathway are typical antipsychotic drugs, also called first-generation antipsychotic drugs, which are reported to effectively attenuate positive symptoms of schizophrenia by blocking dopamine D_2 receptors on postsynaptic neurons [6]. However, because of their high affinity for dopamine D_2 receptors, typical antipsychotic drugs are associated with a high risk of extrapyramidal motor adverse effects [7].

Recently, atypical antipsychotic agents (second-generation antipsychotic drugs) prescribed for schizophrenia, such as olanzapine and clozapine, were found to incur less risk for extrapyramidal motor side effects because they only partially act as agonists at dopamine D_2 and 5-HT_{1A} receptors [8, 9]. However, evidence has increasingly revealed an association of atypical antipsychotic agent use and the risk of metabolic syndromes including obesity, diabetes, and hyperlipidemia but the mechanism remains unclear [10–12].

The liver is the major organ for both cholesterol production and cholesterol excretion from the human body [13, 14]. The balance between the delivery of apoB-containing lipoproteins (chylomicron remnants, very low-density lipoprotein [VLDL], intermediate-density lipoprotein and LDL) from the liver to peripheral tissues and high-density lipoprotein (HDL)-mediated reverse-cholesterol transport from tissues back to the liver is a crucial mechanism for maintaining the appropriate levels of circulating cholesterol [15]. Increased circulating levels of LDL or decreased HDL-mediated cholesterol transport may result in hyperlipidemia, the greatest risk factor for the initiation and progression of atherosclerosis [16, 17].

Atherosclerosis is the leading cause of death in developed countries and is characterized by excessive cholesterol deposition and persistent inflammation within the artery wall resulting from the interaction of oxidized LDL, immune cells, and vascular cells [18, 19]. Evidence accumulating during the past decades suggests that a deregulated inflammatory response and impaired cholesterol metabolism as a result of genetic factors, clinical drug treatment, and environmental toxins accelerate the progression of atherosclerosis [20–22]. However, less is known about the interlocking biology of olanzapine prescribed for schizophrenia and hepatic lipid metabolism or the development of atherosclerosis. Further investigation delineating the effect and molecular mechanisms of olanzapine in the metabolism of cholesterol by the liver and in atherosclerosis is warranted.

Given the impact of atypical antipsychotic agents on the development of metabolic diseases, we aimed to characterize the effect and molecular mechanisms of the use of the atypical antipsychotic drug olanzapine for atherosclerosis. We investigated first the effect of olanzapine on hyperlipidemia, aortic inflammation and the development of atherosclerosis in apolipoprotein E-deficient ($apoE^{-/-}$) mice and secondly whether olanzapine affected hepatic lipid metabolism and the possible molecular mechanisms. Olanzapine may deregulate hepatic lipid metabolism, increase hyperlipidemia, and worsen aortic inflammation, ultimately accelerating the development of atherosclerosis.

Materials and Methods

Reagents

Olanzapine was from Cayman Chemical (Ann Arbor, MI, USA). Rabbit antibodies for inducible nitric oxide (iNOS), CD36, scavenger receptor (SR)-BI, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), liver X receptor β (LXRβ), lysosomal acid lipase (LAL), cholesterol 7-alpha-hydroxylase (CYP7A1), ATP-binding cassette sub-family G member 5 (ABCG5), ABCG8, fatty acid synthase (FAS), acetyl-coenzyme A (CoA) carboxylase (ACC), liver-type fatty acid binding protein (L-FABP), diacylglycerol O-acyltransferase 1 (DGAT1), and long-chain-fatty-acid-CoA ligase 1 (ACSL1), goat antibodies for SR-A, sterol regulatory element-binding protein 2 (SREBP-2), low-density lipoprotein receptor (LDLR), acyl-CoA:cholesterol acyltransferase 2 (ACAT2), DGAT2, apolipoprotein B (apoB), and microsomal triglyceride transfer protein (MTP), and mouse antibody for SREBP-1 were from Santa Cruz Biotechology (Santa Cruz, CA, USA). Rabbit antibodies for intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and ABCG1, mouse antibodies for ABCA1, LXRα, and carnitine palmitoyltransferase Iα(CPT1α, and rat antibody for F4/80 were from Abcam (Cambridge, MA, USA). Mouse antibodies for tubulin and apoAI were from Sigma-Aldrich (St. Louis, MO, USA). Rabbit antibody for LDL receptor-related protein 1 (LRP1) was from Novus (Littleton, CO, USA). ELISA kits for cytokines were from R&D systems (Minneapolis, MN, USA). Cholesterol and triglyceride assay kits were from Randox (Crumlin, Co. Antrim, UK). Total cholesterol, free cholesterol, cholesteryl ester, triglyceride fatty acid, and glycerol fluorometric assay kits were from BioVision (Milpitas, CA, USA).

Mice

The investigation conformed to the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, eighth edition, 2011), and all animal experiments were approved by the Animal Care and Utilization Committee of National Yang-Ming University. ApoE$^{-/-}$ mice were purchased from Jackson laboratory (Bar Harbor, ME, USA). Mice were housed in barrier facilities, and maintained on a 12-h light/12-h dark cycle. Temperature (22 °C) and humidity (40–60%) of the vivarium were tightly controlled. Four to five mice were group-housed per cage and fed a regular chow diet containing 4.5% fat by weight (0.02% cholesterol) (Newco Distributors, Redwood, CA, USA). Four-month-old male apoE$^{-/-}$ mice received daily treatment with olanzapine (3 mg/kg body weight) or vehicle (oil) by gastric gavage for four weeks following previous reports [23, 24]. At the end of the experiment, the body weight and mean arterial pressure (MAP) of mice were measured and then mice were euthanized with CO_2. White (WAT) and brown adipose tissue (BAT) were isolated from the inguinal and gonadal white fat depots and interscapular brown fat depots of mice, respectively. WAT and BAT were weighted and then subjected to histological analysis or stored at -80 °C. Isolated aortas and livers were homogenized and lysates were subjected to western blot analysis.

Histological examination

Heart, liver, WAT, and BAT blocks were cut into 8-μm sections and subjected to histological examination. Deparaffinized sections were stained with hematoxylin and eosin (H&E) and viewed under a Motic TYPE 102M microscope (Motic Images, Xiamen, China). Quantification of the atherosclerotic lesions and content of cellular or non-cellular composition were analyzed by using Motic Images Plus 2.0.

Western blot analysis

Aortas and livers were lysed in immunoprecipitation (50 mmol/L Tris, pH 7.5, 5 mmol/L EDTA, 300 mmol/L NaCl, 1% Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, 10 μg/mL leupeptin, and 10 μg/mL aprotinin). Aliquots of lysates were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and transblotted onto Immobilon-P membrane, blocked with 5% skim milk for 1 h at room temperature, then incubated with primary antibodies overnight followed by the corresponding secondary antibody for 1 h. The protein bands were detected by using an enhanced chemiluminescence kit (PerkinElmer, Boston, MA, USA) and quantified using ImageQuant 5.2 (Healthcare Bio-Sciences, PA, USA).

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Measurement of inflammatory cytokines

The levels of pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-6, monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 2 (MIP-2) in aortas were measured by using ELISA kits.

Serum lipid profile analysis

Blood was collected by cardiac puncture. After clotting and centrifugation, serum was isolated and levels of cholesterol, HDL-cholesterol (HDL-c) and triglycerides in serum were measured by using Spotchem EZ SP 4430 (ARKRAY, Inc., Kyoto, Japan).

Determination of hepatic lipids

The levels of total cholesterol, free cholesterol, cholesteryl ester, triglycerides, fatty acids, and glycerol were measured using fluorescence assay kits (BioVision, Milpitas, CA, USA).

Statistical analysis

Data are presented as mean ± SEM. Data from mice were evaluated by parametric tests. The unpaired *t*-test was used to compare two independent groups. SPSS v20.0 (SPSS Inc., Chicago, IL, USA) was used for the analyses. Differences were considered statistically significant at $P < 0.05$.

Results

Effect of olanzapine on body weight, blood pressure and fat tissues in apoE$^{-/-}$ mice

Previous studies have reported the increased incidence of obesity, hypertension and type-2 diabetes; these are all important risk factors for atherosclerosis in schizophrenia patients who take olanzapine [10]. To elucidate the possible effect of olanzapine on atherosclerosis, we used hyperlipidemia- and atherosclerosis-prone apoE$^{-/-}$ mice as our *in vivo* model. We first investigated the effect of olanzapine on body weight, blood pressure and adiposity in these mice. Daily treatment with olanzapine for four weeks did not alter body weight but increased MAP as compared with vehicle-treated apoE$^{-/-}$ mice (Fig. 1A, B). Additionally, olanzapine decreased the weight of BAT without affecting that of WAT in apoE$^{-/-}$ mice (Fig. 1C, D). The number of adipocytes in WAT was increased but their size was decreased in

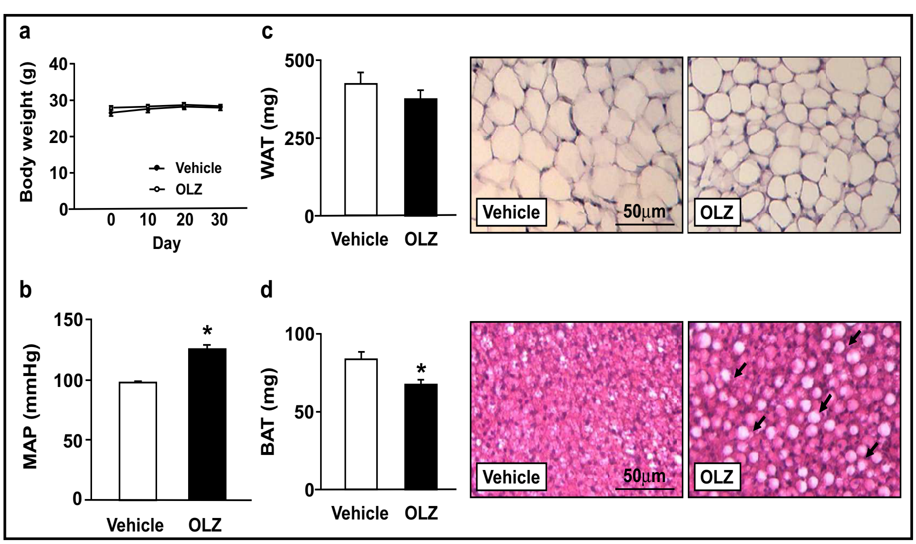

Fig. 1. Effect of olanzapine on body weight, blood pressure, and fat tissue of apoE$^{-/-}$ mice. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A, B) Body weight and mean arterial pressure (MAP). (C, D) Weight and histology of white adipose tissue (WAT) and brown adipose tissue (BAT). Scale bar = 50 μm. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle. Arrows indicate the size of lipid droplets in BAT of OLZ-treated mice.

olanzapine-treated versus vehicle-treated mice (Fig. 1D). Compared with vehicle treatment, olanzapine significantly increased the size of lipid droplets in BAT (whiting) in apoE$^{-/-}$ mice (Fig. 1D). These findings suggest that olanzapine may have a detrimental effect on blood pressure and the fat content of adipose tissues.

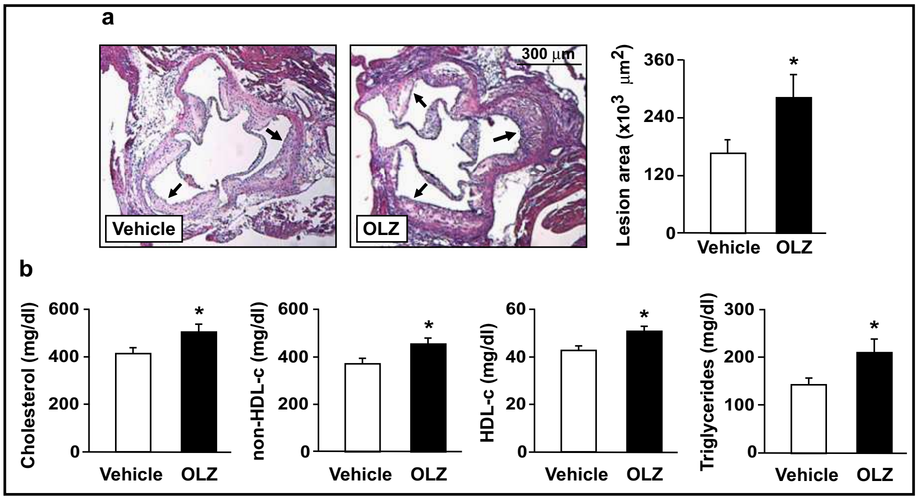

Fig. 2. Olanzapine exacerbates atherosclerosis lesion size in apoE$^{-/-}$ mice. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A) Atherosclerotic lesions at aortic roots were stained with H&E. (B) Serum levels of cholesterol, non-high-density lipoprotein cholesterol (non-HDL-c), HDL cholesterol (HDL-c) and triglycerides. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle. Scale bar = 300 μm

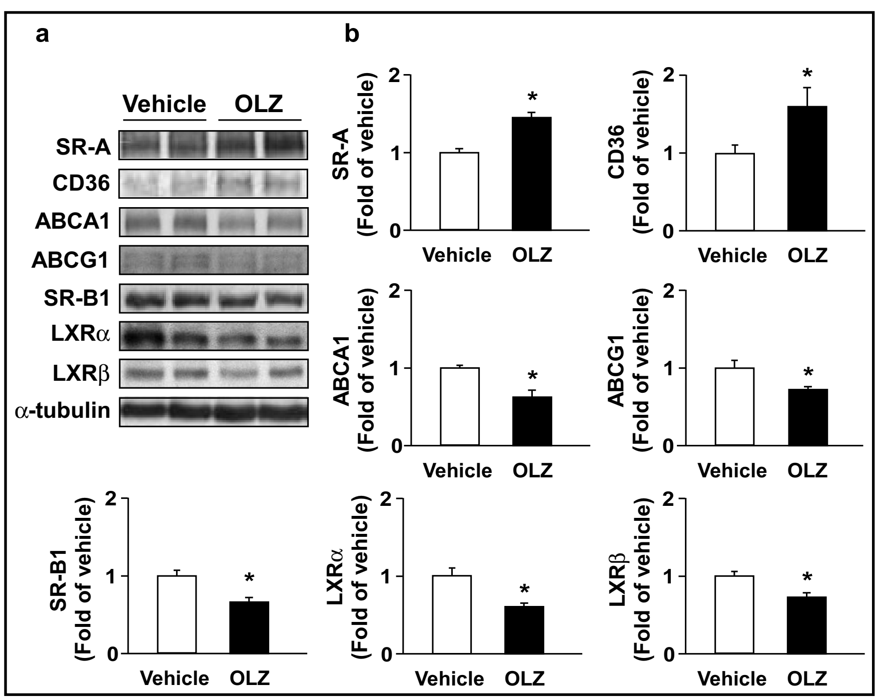

Fig. 3. Olanzapine upregulates scavenger receptors but downregulates reverse cholesterol transporters in apoE$^{-/-}$ aortas. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A, B) Western blot analysis of protein levels of SR-A, CD36, ABCA1, ABCG1, SR-BI, LXRα, LXRβ and α-tubulin in aortas of apoE$^{-/-}$ mice. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle.

Olanzapine exacerbates atherosclerosis and hyperlipidemia in apoE$^{-/-}$ mice

We next examined the effect of olanzapine on lipoid profile and the progression of atherosclerosis in apoE$^{-/-}$ mice. Compared with vehicle treatment, daily olanzapine treatment greatly increased the size of atherosclerotic lesions at the aortic sinus in apoE$^{-/-}$ mice (Fig. 2A). Furthermore, serum levels of total cholesterol, non-HDL-c, HDL-c and triglycerides were significantly increased in olanzapine-treated apoE$^{-/-}$ mice (Fig. 2B), which suggests that olanzapine worsens hyperlipidemia and enhances the development of atherosclerosis.

Olanzapine deregulates cholesterol flux and aggravates inflammation in apoE$^{-/-}$ mouse aorta

An imbalance in vascular cholesterol flux is crucial for the initiation and progression of atherosclerosis [25]. We therefore examined the effect of olanzapine on the protein expression of scavenger receptors and cholesterol transporters in atherosclerotic aortas of apoE$^{-/-}$ mice. Treatment with olanzapine significantly upregulated SR-A and CD36 but downregulated ABCA1, ABCG1 and SR-BI as well as LXRα and LXRβ (Fig. 3A, B), the two key transcript factors for gene regulation of lipid metabolism [26, 27]. Hence, olanzapine may increase cholesterol accumulation in aortas by increasing cholesterol uptake but decreasing cholesterol efflux in atherosclerotic aortas. However, inflammation within atherosclerotic lesions plays a central role in the progression of atherosclerosis [28, 27]. Therefore, we investigated whether olanzapine promotes the inflammatory response during atherogenesis. Compared with vehicle treatment, olanzapine increased levels of F4/80 (macrophage cell marker), iNOS, VCAM-1, TNF-α, IL-6 and MIP-2 without changing those of ICAM-1, IL-1β and MCP-1 in apoE$^{-/-}$ aortas (Fig. 4A, B). All these are important pro-atherogenic molecules in the development of atherosclerosis [28, 29]. Collectively, olanzapine may aggravate the progression of atherosclerosis by deregulating cholesterol homeostasis and the inflammatory response.

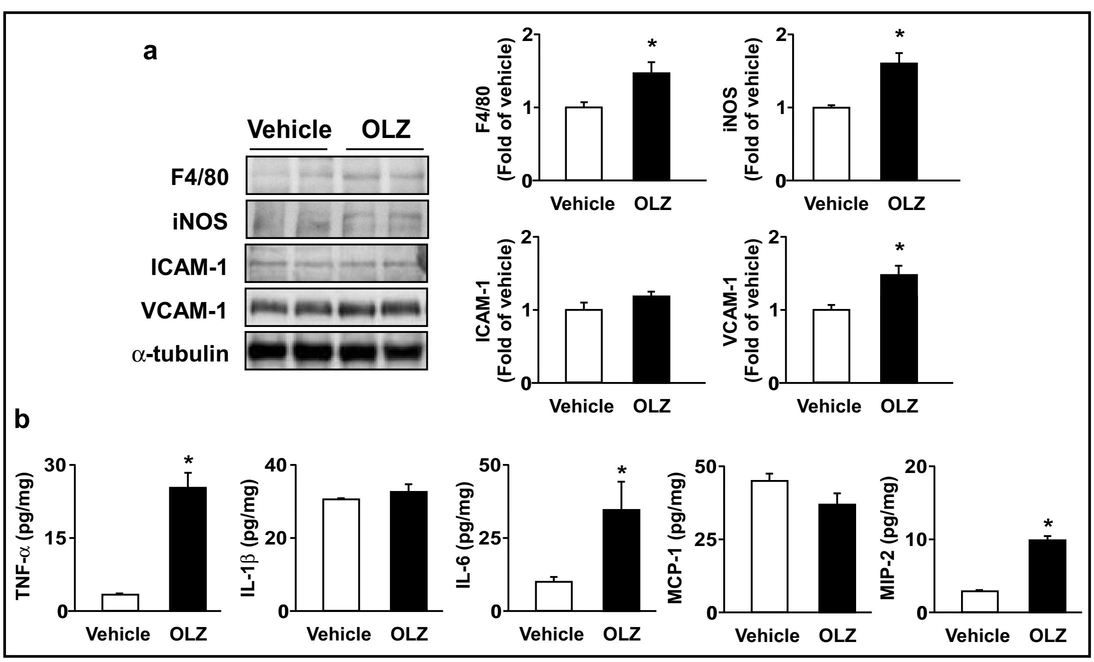

Fig. 4. Olanzapine aggravates inflammation in apoE$^{-/-}$ aortas. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A) Western blot analysis of protein levels of F4/80, iNOS, ICAM-1, VCAM-1 and α-tubulin in aortas of apoE$^{-/-}$ mice. (B) ELISA of aortic levels of TNF-α, IL-1β, IL-6, MCP-1 and MIP-2. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle.

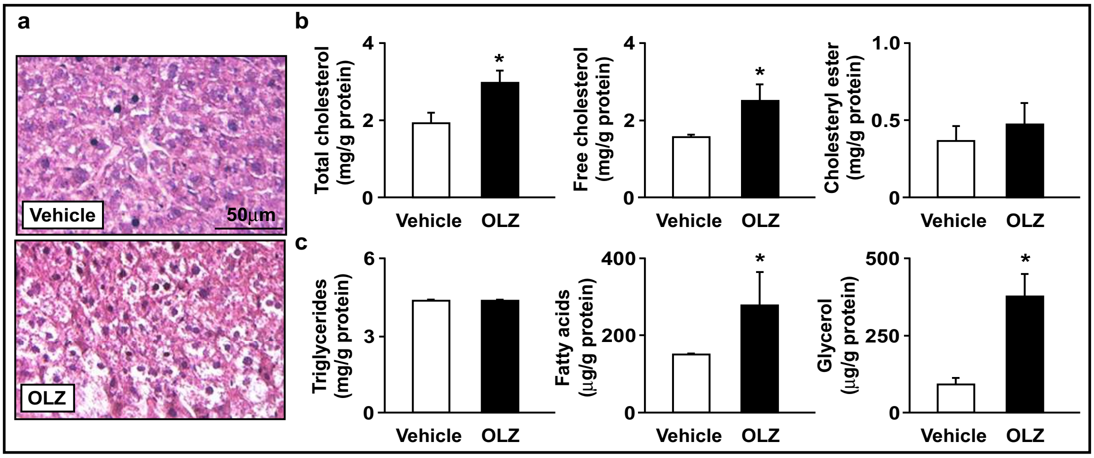

Fig. 5. Olanzapine increases lipid accumulation in apoE$^{-/-}$ liver. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A) Representative H&E staining of liver tissue. Scale bar = 50 μm (B) Hepatic levels of total cholesterol, free cholesterol and cholesteryl ester. (C) Hepatic levels of triglycerides, fatty acids and glycerol in apoE$^{-/-}$ mice assessed by fluorometric assay kits. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle.

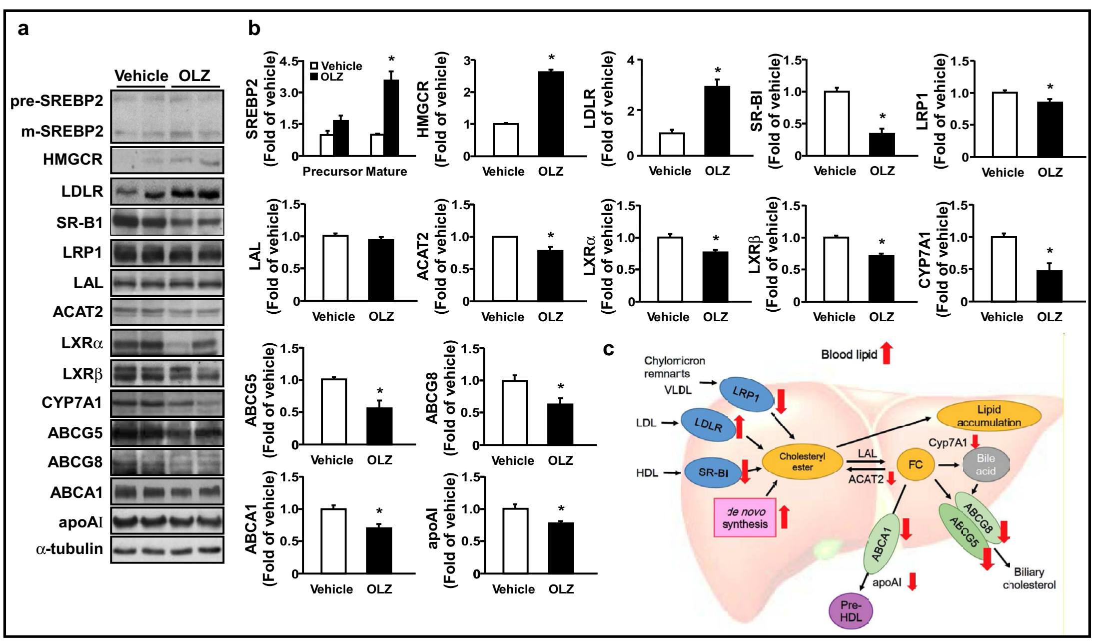

Fig. 6. Effect of olanzapine on de novo cholesterol synthesis-, lipoprotein metabolism-, bile acid metabolism-, and cholesterol efflux-related protein expression in apoE$^{-/-}$ liver. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A, B) Western blot analysis of protein levels of SREBP2, HMGCR, LDLR, SR-BI, LDLR, LRP1, LAL, ACAT2, LXR LXRβ, CYP7A1, ABCG5, ABCG8, ABCA1, apoAI and α-tubulin in the liver of apoE$^{-/-}$ mice. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle.

Olanzapine increases lipid accumulation in apoE$^{-/-}$ mouse liver

The liver is the major organ for lipid metabolism in the human body [30]. Disruption of hepatic lipid homeostasis leads to lipid accumulation in the liver or hyperlipidemia [31]. We examined the effect of olanzapine on hepatic lipid metabolism and the potential mechanism(s). Daily treatment with olanzapine for four weeks increased the hepatic levels of total cholesterol, free cholesterol, fatty acids and glycerol but not of cholesteryl ester and triglycerides in apoE$^{-/-}$ mouse liver (Fig. 5A–C).

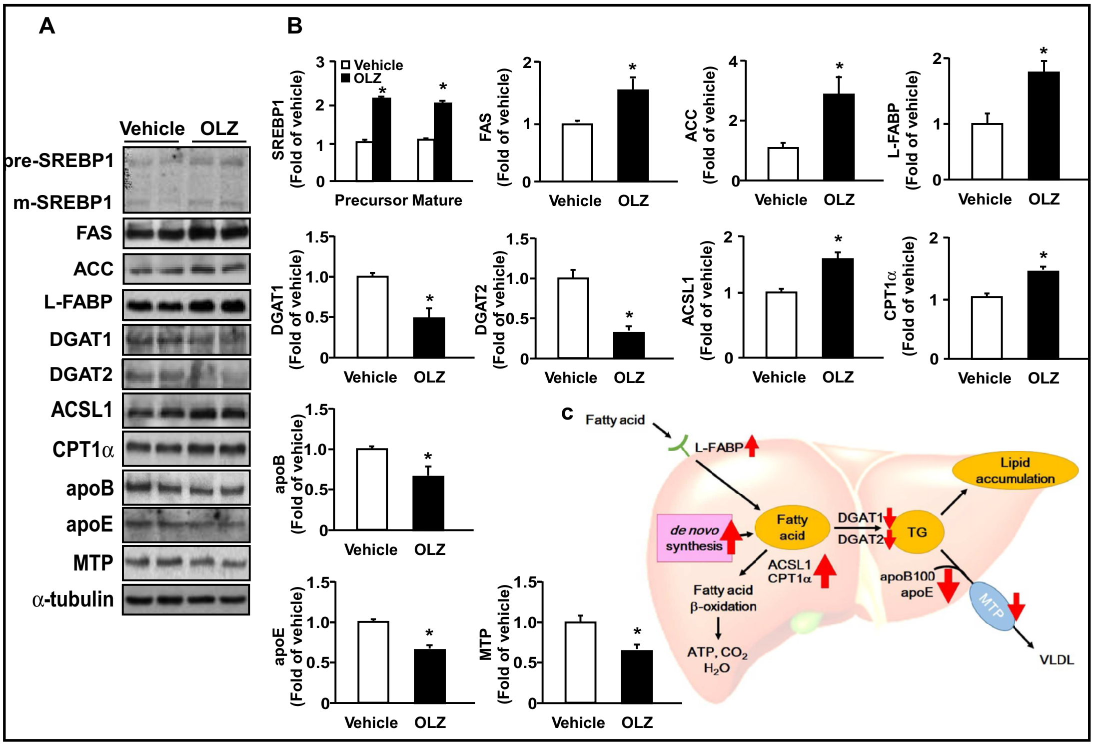

Fig. 7. Effect of olanzapine on de novo fatty acid synthesis-, triglyceride synthesis-, β-oxidation of fatty acids- and VLDL secretion-related protein expression in apoE$^{-/-}$ liver. Four-months-old male apoE$^{-/-}$ mice were orally administered olanzapine (OLZ, 3 mg/kg/day) or vehicle (oil) for four weeks. (A, B) Western blot analysis of protein levels of SREBP1, FAS, ACC, L-FABP, DGAT1, DGAT2, ACSL1, CPT1α, apoB, apoE, MTP and α-tubulin in the liver of apoE$^{-/-}$ mice. Data are mean ± SEM from nine mice. * P<0.05 vs. vehicle.

Olanzapine deregulates hepatic cholesterol and triglyceride metabolism in apoE$^{-/-}$ mice

Hepatic cholesterol and triglyceride metabolism are crucial factors for maintaining the homeostasis of the lipid pool [13, 14]. Thus, the effects of olanzapine on hepatic de novo lipogenesis, lipoprotein metabolism, triglyceride synthesis, and secretion, as well as fatty acid oxidation were investigated. We demonstrated that olanzapine increased the expression of the de novo cholesterol synthesis-related proteins SREBP2 and HMGCR, and of LDLR, the receptor for LDL internalization, but decreased that of SR-BI and LRP-1, the receptors for HDL or chylomicron remnants and VLDL uptake, respectively [32]. Moreover, the expression of cholesterol esterification- and clearance-related proteins including ACAT2, LXRα, LXRβ, CYP7A1, ABCG5, ABCG8, ABCA1, and apoAI was decreased with olanzapine treatment (Fig. 6A, B). These results suggest that olanzapine upregulates de novo synthase-related proteins and downregulates esterification- and clearance-related proteins, thereby leading to the accumulation of cholesterol in the liver (Fig. 6C).

We further examined whether triglyceride metabolism was affected by olanzapine. Olanzapine upregulated de novo fatty acid synthesis-related proteins SREBP1, ACC and FAS but downregulated the triglyceride synthesis-related proteins DGAT1 and DGAT2 (Fig. 7A, B). Furthermore, VLDL secretion-related proteins, including apoB100, apoE and MTP were downregulated with olanzapine treatment. The expression of proteins associated with β-oxidation, such as ACSL1 and CPT1α was increased with olanzapine treatment (Fig. 7A, B). As a whole, the data suggest that olanzapine increases de novo synthesis of fatty acids but decreases the conversion of fatty acid to triglycerides, leading to the elevation of fatty acid in the liver, ultimately increasing β-oxidation and decreasing VLDL secretion (Fig. 7C).

Collectively, daily olanzapine treatment exacerbates aortic inflammation, aggravated hyperlipidemia, deregulated hepatic lipid metabolism and, ultimately, worsens atherosclerosis in apoE$^{-/-}$ mice (Fig. 8).

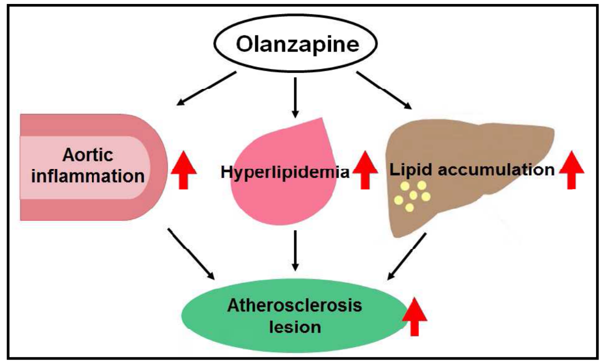

Fig. 8. Proposed model of mechanisms by which olanzapine accelerates the progression to atherosclerosis. Olanzapine exacerbates aortic inflammation, aggravates hyperlipidemia and deregulates hepatic lipid metabolism of apoE$^{-/-}$ mice, thereby accelerating atherosclerosis.

Discussion

Patients with schizophrenia who are taking atypical antipsychotic agents are known to be at high risk for metabolic syndrome, a well-known risk factor for atherosclerosis and related cardiovascular diseases [10–12]. However, the effect of atypical antipsychotic agents on atherogenesis remains elusive. In this study, we provide new insights into the effect of an atypical antipsychotic agent, olanzapine, on the pathogenesis of atherosclerosis and the underlying molecular mechanism. Chronic treatment with olanzapine for four weeks deregulated hepatic lipid metabolism and exacerbated hyperlipidemia and aortic inflammation of apoE$^{-/-}$ mice, ultimately aggravating atherosclerosis. The use of atypical antipsychotic agents in schizophrenia may have detrimental effects on the development of atherosclerosis.

However, our knowledge about the molecular mechanism behind the pro-atherogenic effect of olanzapine is less detailed. We used a hyperlipidemia mouse model to investigate the mechanism. We found that olanzapine could deregulate the adiposity of BAT as evidenced by the increased whiting of BAT, which agreed with previous findings that olanzapine upregulated aP2 expression, the marker of WAT adipocytes, but downregulated UCP1 expression, the marker of BAT adipocytes, in adipose tissues of rats [33, 34]. To date, the detailed mechanism underlying the whiting of BAT is not fully understood. Recently, Shimizu et al. reported that the phenotype of BAT whiting in mice is characterized by mitochondrial dysfunction, lipid accumulation, and downregulation of vascular endothelial growth factor, and might be attributed to obesity-induced capillary rarefaction and hypoxia in BAT [35]. However, how olanzapine induces the whiting of BAT is still elusive. Further investigation delineating the effect and molecular mechanisms of olanzapine in the BAT is warranted.

Intriguingly, treatment with olanzapine did not affect the body weight of apoE$^{-/-}$ mice within our four-week experimental period, which, however, was inconsistent with the previous findings reporting that treatment with olanzapine increased the body weight of human patients and experimental rats over longer experimental periods [34, 36]. Our data agree with Albaugh et al. and Shertzer et al., who found that treatment with olanzapine did not alter body weight in the mouse model [37, 38]. Although the exact mechanism is unclear, the reason for the discrepancies between our study and previous studies may be the difference in species or the genetic background of experimental animals or the length of the experimental period.

Besides the pro-obesity effect of olanzapine, we also found that it increased the MAP of apoE$^{-/-}$ mice, which agrees with clinical observations that patients under olanzapine or clozapine treatment run an increased risk of hypertension [12]. Although the exact mechanism is not known, hypertension is considered one of the major risk factors for the development of atherosclerosis in humans [39, 40]. Angiotensin II, the major player in hypertension, increases oxidative stress, induces endothelial dysfunction and promotes the proliferation and migration of smooth muscle cells, thereby leading to the progression of atherosclerotic lesions in experimental animal studies [41, 42]. However, whether the increased blood pressure induced by olanzapine contributes to the exacerbation of atherosclerosis and its underlying molecular mechanism(s) remains unclear. Further studies investigating the molecular mechanism underlying the hypertensive effect of olanzapine are warranted.

With olanzapine treatment, the hyperlipidemia of apoE$^{-/-}$ mice was exacerbated as evidenced by the increases in serum levels of total cholesterol, non-HDL-c, HDL-c and triglycerides. It is well established that dietary fats and those from de novo lipogenesis in the liver are the two main sources for circulating total cholesterol and triglycerides, which are carried in circulation as VLDL. Elevated circulating total cholesterol and triglycerides may be attributed to increased lipid synthesis, or decreased lipid clearance, or both in the liver [13–17]. In this study, no difference was found in food intake between vehicle-treated mice and olanzapine-treated mice (data not shown). Our findings demonstrated that olanzapine significantly decreased the protein expression of LRP1, the receptor for VLDL and chylomicron remnants in the liver, suggesting that impaired LRP1-mediated lipid clearance may be one of main causes for higher plasma total cholesterol and triglycerides in olanzapine-treated mice.

Deregulation of the macrophage-mediated cholesterol metabolism and inflammation in the artery wall is crucial in the initiation and progression of atherosclerosis [25]. Mainly, excess cholesterol accumulated in macrophages is primarily due to uncontrolled uptake of oxidized LDL or impaired cholesterol efflux [25]. We found that olanzapine upregulated the expression of SR-A and CD36 but downregulated that of ABCA1, ABCG1 and SR-BI. In addition to the critical effect of hyperlipidemia in the initiation of atherosclerosis, the progression of atherosclerotic lesions is determined by the inflammatory response within the artery wall [28, 29]. Interestingly, our results showed that olanzapine downregulated the aortic expression of ABCA1/ABCG1, 2 important regulators for cholesterol efflux from peripheral tissues to HDL or apo A-I [25–27]. Theoretically, the plasma level of HDL-c should be lower in olanzapine-treated mice than in vehicle-treated mice. However, our results also showed that olanzapine markedly decreased the hepatic level of SR-BI protein, the HDL receptor for the selective uptake of cholesterol ester from circulating HDL, and thus promoted the excretion of cholesterol into bile, leading to the elimination of excess cholesterol from the human body [26, 27]. We thus thought that the increase in plasma HDL-c driven by olanzapine might be attributed to the impairment of the SR-BI-mediated uptake of cholesterol ester from HDL.

Notably, we demonstrated that olanzapine promoted aortic inflammation, as evidenced by increased levels of F4/80 (a cell marker of macrophage), iNOS, VCAM-1, TNF-α, IL-6 and MIP-2 in aortic lysates, albeit inconsistent with the findings by Sugino et al., who reported that atypical antipsychotics including olanzapine suppressed lipopolysaccharide-induced acute systemic inflammation in mice [43]. Our results suggest that the local inflammatory response within atherosclerotic lesions rather than systemic inflammation plays an important role in the olanzapine-induced progression of atherosclerosis in apoE$^{-/-}$ mice. One possible explanation for the discrepancy between our study and previous research may be the difference in murine disease models. In view of the function of these proteins, olanzapine may disturb cholesterol homeostasis and regulation of the inflammatory response, thereby accelerating atherosclerosis in apoE$^{-/-}$ mice. Despite our unique findings, the detailed mechanisms by which olanzapine modulates lipid metabolism and the inflammatory response within atherosclerotic lesions merit further study.

Liver-mediated lipid metabolism plays a central role in controlling whole-body lipid homeostasis, including cholesterol and triglycerides with fluxes in dietary, circulating and peripheral local lipid pools [32, 44]. Under physiological conditions, the lipid content of liver is tightly controlled by the integration of several aspects of metabolic pathways, including de novo lipogenesis, β-oxidation of fatty acids, VLDL synthesis, lipoprotein internalization from circulation and bile acid synthesis [45–48]. Our findings that treatment with olanzapine significantly increased the lipid content of the liver, particularly total cholesterol, free cholesterol, fatty acids, and glycerol, suggested the unfavorable effect of olanzapine on hepatic lipid metabolism in hyperlipidemic apoE$^{-/-}$ mice. This observation agrees with previous reports of atypical antipsychotic agents disturbing hepatic lipid metabolism [48]. Nevertheless, the potential mechanism underlying the deregulation of lipid homeostasis by atypical antipsychotic agents remains poorly understood.

We subsequently asked how olanzapine affected hepatic lipid metabolism. Olanzapine activated SREBP-1 and -2 and the expression of FAS, ACC, HMGCR and LDLR, all crucial regulators in the de novo synthesis of hepatic triglycerides and cholesterol [26, 32]. Moreover, olanzapine decreased the uptake of lipoproteins from circulation, as evidenced by the downregulation of SR-BI, or LRP1, which are HDL or chylomicron remnants and VLDL receptors, respectively [32]. This deregulation of the lipoprotein metabolism may contribute to the elevated levels of circulating cholesterol and triglycerides. More importantly, olanzapine decreased the protein expression of LXRα, apoAI, ABCA1, CYP7A1, ABCG5 and ABCG8, all implicated in hepatic cholesterol efflux and bile acid metabolism [32, 49]. In terms of function, the downregulation of these proteins by olanzapine is likely one of the mechanisms for the olanzapine-increased accumulation of lipids in the liver. Notably, the protein expression of ACSL1 and CPT-1α, two key regulators in the β-oxidation of fatty acids [50, 51], was increased. Although the detailed mechanism was unclear, this response may be a compensatory mechanism for the olanzapine-increased fatty acid accumulation in the liver. Moreover, we found that the elevated free cholesterol could be toxic to cells in the liver as shown by increased numbers of apoptotic cells in the livers of olanzapine-treated mice (data not shown). Overall, congruous data are observed in the alteration of key molecules regulating lipid metabolism, which demonstrates again the detrimental effects of olanzapine on hepatic lipid metabolism. The disturbed hepatic lipid metabolism appeared to be the key event for the olanzapine-induced exacerbation of hyperlipidemia and subsequent progression of atherosclerosis in apoE$^{-/-}$ mice.

Nevertheless, our study contains several limitations because we did not use a schizophrenia animal model for experimental analysis. A specific schizophrenia mouse model with hyperlipidemia is not available, so we could not clarify the effects of atypical antipsychotic agents on lipid homeostasis under these pathological conditions. In addition, the cardiovascular significance of atypical antipsychotic agents in translational or clinical medicine is not fully clarified. We do not have clinical data to support our observations from animal studies. To this end, further investigations describing the implications of atypical antipsychotic agents for hepatic lipid metabolism and cardiovascular diseases are warranted.

Atherosclerosis is a complex, progressive disease process with multiple etiologies [18–22]. It is well established that hypertension, hyperlipidemia, and inflammation are crucial risk factors for the development of atherosclerosis [25, 28, 29, 40]. In this study, we found that olanzapine increases mean arterial pressure, exacerbates hyperlipidemia, and deregulates aortic inflammation and cholesterol metabolism; all of these events induced by olanzapine could accelerate the progression of atherosclerosis.

Conclusion

In summary, our study provides advanced evidence that olanzapine has multifaceted effects on hepatic lipid metabolism by increasing de novo lipogenesis, by decreasing lipoprotein internalization, cholesterol clearance, and efflux, and by reducing VLDL secretion, which exacerbate lipid accumulation in the liver and hyperlipidemia as well as the progression of atherosclerosis. Also, we discovered a link between atypical antipsychotic agents, hepatic lipid metabolism and cardiovascular biology, which broadens the clinical implications of atypical antipsychotic agents for the development of cardiovascular diseases.

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Disclosure Statement

The authors have no conflicts of interest to declare.

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