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Original Article

Atorvastatin delays the glucose clearance rate in hypercholesterolemic rabbits

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ABSTRACT

The administration of statin might increase the risk of new-onset diabetes in hypercholesterolemic patients based on the recent clinical evidence. However, the causal relationship must be clarified and confirmed in animal experiments. Therefore, we mimicked hypercholesterolemia by feeding rabbits a high-cholesterol diet (HCD) and performed 16 weeks of atorvastatin administration to investigate the effect of statin on glucose metabolism. The intravenous glucose tolerance test showed that plasma glucose levels in the statin-treated rabbits were consistently higher and that there was a slower rate of glucose clearance from the blood than in HCD rabbits. The incremental area under the curve for glucose in the statin-treated rabbits was also significantly larger than in the HCD rabbits. However, there was no significant difference between the two groups in the intravenous insulin tolerance test. The glucose-lowering ability of exogenous insulin was not impaired by statin treatment in hypercholesterolemic rabbits. The administration of a single dose of statin did not affect glucose metabolism in normal rabbits. The statin also significantly increased the levels of high-density lipoprotein cholesterol, alanine aminotransferase and aspartate transaminase and decreased plasma levels of total cholesterol, triglycerides and low-density lipoprotein cholesterol in the hypercholesterolemic rabbits, whereas it did not affect plasma levels of glucose and insulin. The current results showed that atorvastatin treatment resulted in a significant delay of glucose clearance in hypercholesterolemic rabbits, and this rabbit model could be suitable for studying the effects of statin on glucose metabolism.

1. Introduction

Statins, which are 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase inhibitors, are the first-line drugs for treating hyperlipidemic patients because statins are to not only able reduce plasma low-density lipoproteins cholesterol (LDL-C) levels through inhibition of cholesterol synthesis but also show anti-inflammatory effects, representing so-called pleiotropic functions. However, accumulated evidence from clinical trials suggests that statins might increase the risk of new-onset diabetes. The West of Scotland Coronary Prevention Study (WOSCOPS) showed that patients who used pravastatin at 40 mg daily exhibited a 30% lower risk of developing diabetes than those in the placebo control group [1,2]. Subsequently, the Prospective Study of Pravastatin in the Elderly at Risk (PROSPER) demonstrated the use of pravastatin at 40 mg daily in an elderly population (average age 75 years) resulted in a 32% increase in the incidence of diabetes, particularly when the characteristics of insulin-resistance syndrome were present [3]. Additionally, a 26% increase in new-onset diabetes was observed in the two-year Justification for the Use of Statins in Primary Prevention: Intervention Trial Evaluating Rosuvastatin (JUPITER) [4]. In the hypertensive arm of the Anglo-Scandanavian

Abbreviations: HCD, high cholesterol diet; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; LDL-C, low-density lipoproteins cholesterol; NOD, non-obese diabetic; IVGTT, intravenous glucose tolerance test; IVITT, intravenous insulin tolerance test; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; ALT, alanine aminotransferase; AST, aspartate transaminase; AUC, area under the curve; ApoE, apolipoprotein E; CETP, cholesteryl ester transfer protein; HE, hematoxylin–eosin staining; SD, standard deviation.

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Cardiac Outcome Trial – Lipid Lowering Arm (ASCOT-LLA) trial, a 10 mg dose atorvastatin was not found to increase the incidence of new-onset diabetes [5]. However, atorvastatin increased hemoglobin A1c versus placebo in the Collaborative Atorvastatin Diabetes Study (CARDS) and caused a significant 34% increase in new-onset type 2 diabetes the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial [6,7].

Combined with the clinical evidence, a meta-analysis also led to the conclusion that statins may increase the risk of new-onset diabetes [8,9]. However, the causal relationship between administration of statin and new-onset diabetes must be clarified and confirmed through in vivo experiments. Atorvastatin does not decrease or delay diabetes onset in mouse models with streptozotocin-induced diabetes and spontaneous non-obese diabetic (NOD) [10]. Consistent with the findings in these mice, statins, and especially atorvastatin, may influence glucose tolerance in mildly streptozotocin-induced diabetic rats, without alterations of insulin secretion [11]. Nevertheless, atorvastatin + pioglitazone showed a promising therapeutic effect in the management of high-fat diet-induced type 2 diabetes mellitus in rats [12]. Although rodent models have provided insights into the effects of statins on new-onset diabetes, rabbits may be more suitable for the study of statins because their lipoprotein metabolism is similar to that of humans but different from that of the most widely used mice [13,14].

In this study, we mimicked the status of hypercholesterolemic patients by feeding rabbits with a high-cholesterol diet and attempted to investigate the hypothesis of whether the administration of atorvastatin could affect glucose metabolism. The current study showed that atorvastatin treatment resulted in a significant delay of glucose clearance in hypercholesterolemic rabbits.

2. Materials and methods

2.1. Animals, diets and experimental procedures

All rabbits were provided by the Laboratory Animal Center of Xi'an Jiaotong University. Twenty male rabbits (16 weeks old) were randomly divided into two groups, which were fed a diet containing either 0.3% cholesterol, as a high-cholesterol diet (HCD) (control group, $n = 10$), or 0.3% cholesterol plus 0.05% atorvastatin (atorvastatin-treated group, $n = 10$) for 16 weeks. All rabbits were fed under a restricted diet intake (100 g/rabbit per day) and given free access to water. Atorvastatin calcium was purchased from Sequoia Research Products Ltd. (Pangbourne, UK). To test whether atorvastatin administration could affect glucose metabolism immediately, in ten normal diet-fed rabbits, the intravenous glucose tolerance test (IVGTT) and intravenous insulin tolerance test (IVITT) were performed before and after the administration of a single dose of atorvastatin (4.1 mg/kg, this dose≈80 mg/day in human). The experimental protocols were approved by the Laboratory Animal Administration Committee of Xi'an Jiaotong University and were performed according to the Guidelines for Animal Experimentation of Xi'an Jiaotong University and the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 2011).

2.2. Measurement of plasma parameters

After overnight fasting, blood samples were collected from the rabbits via the auricular. The blood samples were stored on ice and centrifuged (3000 rpm, 15 min, 4 °C) to obtain plasma. Plasma glucose, triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), alanine aminotransferase (ALT) and aspartate transaminase (AST) levels were measured using commercial assay kits (Biosino Bio-technology and Science Inc., Beijing, China). The plasma insulin assay was conducted using enzyme-linked immunoassay insulin kits (Yanaihara Institute Inc, Shizuoka, Japan).

2.3. Glucose and insulin tolerance tests

For the evaluation of glucose metabolism, rabbits were fasted overnight, and the IVGTT was performed as previously described [15]. A bolus of glucose (0.6 g/kg body weight) was injected through the ear vein, and a blood sample was collected through the ear artery at 5, 10, 15, 20, 30, 45, 60, 75 and 120 min. Blood glucose was immediately measured using a blood glucose meter (One-touch Ultra, Johnson & Johnson, USA). The IVITT was performed by injecting rabbits that had been fasted with a bolus of insulin (1.0 U/kg body weight, Eli Lilly) through an ear vein; blood glucose was analyzed as above. The incremental area under the curve (AUC) was calculated according to the trapezium rule [16].

2.4. Adipose tissue contents in rabbits

After 16 weeks of feeding HFSD, the rabbits were sacrificed, and adipose tissues from the whole body were carefully removed, weighed, and the data were expressed as total body fat weight (g) or a percentage of body weight. We divided adipose tissues into (i) subcutaneous adipose tissue including fat from the inguinal, axilla, and scapular regions, and (ii) visceral adipose from the abdominal cavity, mesenterium, and retroperitoneal fat [17].

2.5. Histological examinations and immunohistochemistry analysis

After sacrificing the rabbits, the adipose tissue, liver, kidney, spleen, heart, lung, pancreas and bone marrow were removed, weighed and fixed in a 10% neutral buffed formalin solution for subsequent morphological analysis. These tissues were embedded in paraffin, cut into 4-μm sections and stained with hematoxylin–eosin (HE) staining for light microscopy examination.

2.6. Statistical analysis

The results are expressed as the means ± SD. Statistical analysis was performed using either Student's t test for data with an equal F value or Welch's t test when the F value was not equal. Fisher's Exact Probability Test was performed for a table of frequency data. A P value of less than 0.05 was considered statistically significant.

3. Results

3.1. Plasma lipids

As shown in Fig. 1, the plasma levels of TC, TG, LDL-C and HDL-C were measured at the beginning, middle and the end of a 16-week period. Statin treatment decreased the plasma levels of TC, TG and LDL-C compared with the HCD group (Fig. 1a–c). The statin also significantly increased HDL-C levels, and the lipid-lowering effects of statin were basically attributed to the reduction of non-HDLs (Fig. 1d). During the experiment, there was no difference in food consumption and body weight between the two groups (data not shown).

3.2. Plasma glucose, insulin, ALT, AST and organ weight

Plasma glucose was significantly lower in statin-treated rabbits compared with control rabbits after feeding HCD for 16 weeks (Fig. 2a). However, insulin levels were not significantly different between the two groups (Fig. 2b). The 0.3% cholesterol diet did not