Biochemical Pharmacology xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Involvement of pregnane X receptor in the impaired glucose utilization induced by atorvastatin in hepatocytes

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ARTICLE INFO

Article history:
Received 4 October 2015
Accepted 20 November 2015
Available online xxx

Keywords:
Diabetes
Atorvastatin
Pregnane X receptor
Glucose transporter 2
Glucokinase
Glucose tolerance

ABSTRACT

Accumulating evidences demonstrated that statins impaired glucose utilization. This study was aimed to investigate whether PXR was involved in the atorvastatin-impaired glucose utilization. Rifampicin/PCN served as PXR activator control. Glucose utilization, glucose uptake, protein levels of GLUT2, GCK, PDK2, PEPCK1 and G6Pase in HepG2 cells were measured. PXR inhibitors, PXR overexpression and PXR siRNA were applied to verify the role of PXR in atorvastatin-impaired glucose utilization in cells. Hypercholesterolemia rats induced by high fat diet feeding, orally received atorvastatin (5 and 10 mg/kg), pravastatin (10 mg/kg) for 14 days, or intraperitoneally received PCN (35 mg/kg) for 4 days. Results showed that glucose utilization was markedly inhibited by atorvastatin, simvastatin, pitavastatin, lovastatin and rifampicin. Neither rosuvastatin nor pravastatin showed the similar effect. Atorvastatin and pravastatin were selected for the following study. Atorvastatin and rifampicin significantly inhibited glucose uptake and down-regulated GLUT2 and GCK expressions. Similarly, overexpressed PXR significantly down-regulated GLUT2 and GCK expressions and impaired glucose utilization. Ketoconazole and resveratrol attenuated the impaired glucose utilization by atorvastatin and rifampicin in both parental and overexpressed PXR cells. PXR knockdown significantly up-regulated GLUT2 and GCK proteins and abolished the decreased glucose consumption and uptake by atorvastatin and rifampicin. Animal experiments showed that atorvastatin and PCN significantly elicited postprandial hyperglycemia, leading to increase in glucose AUC. Expressions of GLUT2 and GCK in rat livers were markedly down-regulated by atorvastatin and PCN. In conclusion, atorvastatin impaired glucose utilization in hepatocytes via repressing GLUT2 and GCK expressions, which may be partly due to PXR activation.

1. Introduction

Diabetes is often accompanied by hypercholesterolemia, which is thought to promote the development of atherosclerotic complications, thus to lower low density lipoprotein-cholesterol is of vital importance in reducing cardiovascular risk. Statins, 3-hydroxy-3-methylglutaryl enzyme A reductase inhibitors, are frequently administered to diabetic patients and have showed their efficacy in prevention of atherosclerotic cardiovascular disease events. However, most recently, the Food and Drug Administration in the United States has added an adverse event warning to statin labels, stating that statins have been associated with increased glycosylated hemoglobin and fasting blood glucose levels. Although multiple meta-analyses have been published to examining the topic [1–7], these investigations often yielded conflicting results. For example, West of Scotland Coronary Prevention study suggested a protective effect of pravastatin in preventing the development of diabetes [8], but this protective effect was not corroborated by clinical trials of simvastatin [9], pravastatin [10] and atorvastatin [11]. A review of 16 studies [12] from patients receiving pravastatin 10–40 mg/day (three trials,

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; PCN, pregnenolone-16α-carbonitrile; PXR, pregnane X receptor; GLUT2, glucose transporter 2; GCK, glucokinase; PDK2, pyruvate dehydrogenase kinase isoenzyme 2; PEPCK1, phosphoenolpyruvate carboxykinase 1; G6Pase, glucose 6-phosphatase; DMEM, Dulbecco's Modified Eagle's Medium; TC, total cholesterol; TG, triglyceride; LDL-C, low density lipoprotein-cholesterol; HDL-C, high density lipoprotein-cholesterol; FBG, fasting blood glucose; OGTT, oral glucose tolerance test; HFD, high-fat diet; AUC, area under the concentration–time curve.

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http://dx.doi.org/10.1016/j.bcp.2015.11.023
0006-2952/

$n = 164$), atorvastatin 10–40 mg/day (five trials, $n = 315$), rosuvastatin 10–40 mg/day (five trials, $n = 419$), and simvastatin 10–80 mg/day (five trials, $n = 369$) showed that when pooled as a class, statins had no significant impact on insulin sensitivity. However, when comparing the individual statins it was observed that pravastatin significantly improved insulin sensitivity while simvastatin worsened it. Yamakawa et al. reported that 3-month treatment with atorvastatin significantly increased glycaemic parameters in diabetic patients, but neither pravastatin nor pitavastatin had the adverse effect on glycaemic control [13]. All these results indicate that the risk of new-onset diabetes or worsened glycaemic control may be dependent on types of statins.

Several studies have demonstrated that statins may affect blood glucose balance via different pathways. Lovastatin [14] was considered to disrupt early events in insulin signaling and simvastatin [15] was reported to suppress glucose-induced insulin release from the rat islet β-cells. Impaired glucose metabolism by atorvastatin may be due to reduction in the insulin-induced tyrosine phosphorylation of IRS-1 and serine/threonine phosphorylation of Akt or decrease in the glucose uptake by adipocytes [16]. A recent study also revealed that simvastatin increased serum glucose levels via inducing G6Pase in gluconeogenesis [17]. However, these findings are not sufficient to account for the differential metabolic effects of distinct statins on glucose metabolism. Potential mechanisms that statins induce new-onset diabetes or worsen glycaemic control were not fully characterized.

Statins are known to activate the pregnane X receptor (PXR; NR1I2) [18–20]. Recent studies have demonstrated that PXR activation mediates drug-induced development of hyperglycemia [17,21]. Activation of human PXR was reported to increase levels of glucose 6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase 1 (PEPCK1) mRNA, which were evidenced by the findings that rifampicin treatment impaired oral glucose tolerance in both tuberculosis patients and healthy volunteers [21,22] and that 4-day treatment with rat PXR agonist pregnenolone 16α-carbonitrile (PCN) elicited postprandial hyperglycemiain rats [21]. Several studies have verified roles of PXR in glucose metabolism using $PXR^{-/-}$ allele mice [23,24]. Liver plays an important role in the homeostasis of glucose, involving in the transport, storage, production and metabolism of glucose. Glucose transporter 2 (GLUT2) is the main glucose transporter in mammalian liver [25]. A report showed that PCN treatment elicited postprandial hyperglycemia via repressing expression of GLUT2 mRNA in liver of rats [21]. Glucokinase (GCK) and pyruvate dehydrogenase kinase isoenzyme 2 (PDK2) are another two critical regulators of glucose metabolism in hepatocytes [26,27], mRNA of which were also reported to be decreased by PCN [21]. All these results indicate that these targeted proteins may be involved in the PXR-mediated homeostasis of glucose.

The aim of the study was, firstly to compare effects of six statins (atorvastatin, simvastatin, pitavastatin, lovastatin, rosuvastatin and pravastatin) on glucose utilization using HepG2 cells. Secondly, atorvastatin (positive) and pravastatin (negative) were selected to investigate whether statins impaired glucose utilization was involved in PXR activation in HepG2 cells. Overexpressed human PXR cells (HepG2-NR1I2), PXR inhibitors (ketoconazole and resveratrol) and siRNA-transfected PXR cells were applied to further verify role of PXR in atorvastatin-impaired glucose utilization. These findings were further verified using primary hepatocytes of hypercholesterolemia (HFD) rats induced by high-fat diet feeding. The impaired homeostasis of glucose by atorvastatin was also performed in HFD rats. Corresponding targeted proteins such as GLUT2, GCK, PDK2, PEPCK1, G6Pase and CYP3A, as well as nuclear protein PXR were also measured using Western blots.

2. Materials and methods

2.1. Chemicals

Atorvastatin calcium, simvastatin, pitavastatin calcium, lovastatin, rosuvastatin calcium, pravastatin sodium, rifampicin, dexamethasone and ketoconazole were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Atorvastatin calcium (used for animal experiment) and insulin were purchased from Meilun Biological Technology Co., Ltd. (Dalian, China). Dulbecco's Modified Eagle's Medium (DMEM, high glucose; DMEM, low glucose; DMEM, no glucose, no glutamine, no phenol red) and non-essential amino acids (MEM, 100X) were obtained from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS), trypsin (0.25%)-EDTA and insulin-transferrin-selenium (ITS) were purchased from GIBCO (Grand Island, NY, USA). Phloretin, sodium pyruvate and hexokinase colorimetric assay kit were bought from SigmaAldrich (MO, USA). Hepes, resveratrol and phenylmetthyl sulfonylfluoride (PMSF) were purchased from J&K Chemical (Shanghai, China). 2-[*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose (2-NBDG), Silencer® Pre-Designed siRNA (NR1I2), Silencer® Negative Control siRNA, Lipofectamine® RNAiMAX Transfection Reagent and Opti-MEM® I Reduced Serum Medium were purchased from Molecular Probes (Invitrogen, Burlington, Ontario, Canada). Pregnenolone-16α-carbonitrile (PCN) was obtained from Santa Cruz Technology (Santa Cruz, CA, USA). Kits for glucose, total cholesterol (TC), triglyceride (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) content detection were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Radioimmunoprecipitation (RIPA) lysis buffer and bicinchoninic acid (BCA) kit for protein content were purchased from Beyotime Institute of Biotechnology (Nanjing, China). Nuclear Extract Kit was purchased from KeyGen Biotech (Nanjing, China). Distilled water was prepared using a Milli-Q system (Millipore, Bedford, MA, USA). All the other chemicals used were commercially available and of analytical grade.

2.2. Animal

Male SprageDawley (SD) rats, weighing 100–110 g, from SinoBritish Sippr/BK Laboratory Animal Ltd. (Shanghai, China), were housed under controlled environmental conditions with a temperature of $23 \pm 1\,^{\circ}C$ and a relative humidity of $50 \pm 10\%$ with 12 h light/dark cycle. The rats were allowed free access to food and water. The experiments were carried out in accordance with guidelines on the Care and Use of Animals developed by the National Advisory Committee for Laboratory Animal Research. All animals received humane care and their use was approved by the Animal Ethics Committee of China Pharmaceutical University (No. CPU-PCPK-13211010324).

2.3. Cell culture

HepG2 cells obtained from Chinese Academy of Medical Sciences and HepG2-NR1I2 cells, a HepG2-derived stable cell line that expresses human PXR (Viewsolid Biotech Co., Ltd., Beijing, China), were cultured in high glucose Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% nonessential amino acids, antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin), 2 mM L-glutamine, and 3.7 g/L of $NaHCO_3$ in a humidified incubator of 5% CO_2 and 95% air atmosphere at $37\,^{\circ}C$.

Primary hepatocytes of normal rats and HFD rats were isolated with a twostep perfusion method described previously [28]. Cell

Please cite this article in press as: Z. Ling, et al., Involvement of pregnane X receptor in the impaired glucose utilization induced by atorvastatin in hepatocytes, Biochem Pharmacol (2015), http://dx.doi.org/10.1016/j.bcp.2015.11.023