Benefit of Pitavastatin plus Sitagliptin Combination Treatment on Hyperinsulinemia and Fatty Liver Damage in Obese Mice

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Abstract

Obesity contributes to the development of insulin resistance and the subsequent incidence of Type 2 Diabetes Mellitus (T2DM). The effects of co-administration of sitagliptin (dipeptidylpeptidase-4 inhibitor) and pitavastatin (lipid-lowering drug) on hyperinsulinemia and fatty liver damage accompanied by obesity in the preclinical stages of T2DM have not yet been investigated. We aimed to evaluate the additive effects of co-administration of sitagliptin and pitavastatin on hyperinsulinemia and fatty liver damage in obese ob/ob mice. Normal male C57BL/6j mice and obese ob/ob mice were divided into four groups, each receiving different drugs: standard mice chow, n = 8 (Normal); ob/ob mice with vehicle, n = 8 (Control); sitagliptin, n = 8 (SG); pitavastatin + sitagliptin, n = 8 (SG + PS). At the end of the study period (8 weeks), blood samples were drawn from all mice to determine Glycated Hemoglobin (HbA1c), serum glucose, insulin, triglyceride, total cholesterol, High-Density Lipoprotein (HDL) cholesterol, Aspartate-Aminotransferase (AST), Alanine Aminotransferase (ALT) and leptin levels. The SG+PS group had significantly (P < 0.05) lower levels of HbA1c, serum insulin, total cholesterol, AST, and ALT, and liver weight ratio, than did the Control group. And also, the SG+PS group had significantly (P < 0.05) higher levels of leptin than did the SG group. Body weight and serum glucose levels in the SG+PS group did not significantly decrease compared to those in the Control group. Serum triglyceride and HDL-cholesterol levels were unchanged among the four groups. These results suggest that combination treatment with sitagliptin plus pitavastatin may be a promising therapeutic approach in the management of T2DM because it has a beneficial effect on the pathophysiological processes of obesity.

Keywords: Pitavastatin; Sitagliptin; Obesity; Mice

Introduction

Recent evidence indicates that obesity and its related metabolic abnormalities increase the risk of developing cardiovascular and hepatic diseases because these conditions are associated with increased levels of fasting plasma triglycerides, Low-Density Lipoprotein (LDL) cholesterol, blood glucose, and insulin, and blood pressure, and decreased levels of HDL cholesterol [1,2].

Statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors) are used for the treatment of hyperlipidemia accompanied by obesity, and have been shown to reduce the risk of major cardiovascular events in a prospective meta-analysis [3]. Pitavastatin is a statin that has been reported to ameliorate severe steatohepatitis by enhancing hepatic free acid β-oxidation activity in aromatase-deficient (Ar−/−) mice [4]. It has also been shown to inhibit hepatic fibrosis in a Non-Alcoholic Steatohepatitis (NASH) model in rats [5]. Therefore, statins such as pitavastatin effectively prevent the development of obesity-related hyperlipidemia. On the other hand, Dipeptidyl Peptidase-IV inhibitors (DPP-IV inhibitor), such as sitagliptin, have been used in recent years to improve postprandial glycemic control in T2DM [6]. Furthermore, sitagliptin was shown to improve steatohepatitis by increasing the insulin sensitivity and improving the lipid profiles of High Fat Diet (HFD) induced obese rats [7].

Although statins are generally safe and well tolerated, some evidence suggests that they are associated with an increased occurrence of new-onset Diabetes Mellitus (DM) [8]. However, the benefits of statins outweigh the increased risk of DM in people with Cardiovascular Disease (CVD) and those who are at a high risk of CVD [9]. On the other hand, the benefits of early statin therapy for obesity-related hyperinsulinemia and fatty liver damage during the preclinical stages of T2DM remain to be established. Therefore, further study of the effects of co-administration of Sitagliptin and pitavastatin on obesity-related hyperinsulinemia and fatty liver damage is required to reveal the clinical potential of this treatment regimen. We aimed to evaluate the combination effect of pitavastatin and Sitagliptin co-administration on the prevention of T2DM in obese mice.

Methods

Animals

In this study, male C57BL/6JHamSlc-+/+ mice and C57BL/6JHamSlc-ob/ob mice (Japan SLC Inc., Shizuoka, Japan) that weighed 18-22 g and 38-42 g (both 7 weeks of age) were used. We used young adult mice for long treatment periods. The mice were maintained on a standard powder diet (MF® diet;
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Oriental Yeast, Tokyo, Japan) for 1 week. They had free access to chow and water and were kept in a room maintained at 22°C ± 2°C with a 12 h light/dark cycle (diurnal time, 08:00–20:00 hours). All experimental procedures were conducted according to the Osaka Ohtani University Guidelines for the Care and Use of Laboratory Animals.

Drugs

Sitagliptin phosphate hydrate was purchased from MSD KK (Tokyo, Japan). Pitavastatin calcium was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Treatment of animals and sample preparation

The mice were randomly divided into 4 groups; mice in the normal group were maintained on standard chow. The composition of the standard chow (energy, 360 kcal/100 g) was 7.7% water, 23.6% protein, 5.3% fat, 2.9% fiber, and 54.4% nitrogen-free extracts. Obese mice were randomly divided into the following 3 subgroups: untreated (Control), treated with sitagliptin (SG group), or treated with pitavastatin and sitagliptin (SG+PS group). Sitagliptin in SG group mice was administered orally once a day (10 mg/kg/day) presuspended with 5% carboxymethyl cellulose solution. In the SG + PS group, pitavastatin and sitagliptin were administered orally once a day (3 mg/kg/day and 10 mg/kg/day, respectively) presuspended in 5% carboxymethyl cellulose solution. The mice had access to chow and tap water ad libitum. The body weight of the mice and food intake per cage were measured on a weekly basis. At the end of 8 weeks, the mice were anesthetized under diethyl ether and blood samples were collected from the inferior vena cava; serum was separated by centrifugation. After being sacrificed, the livers of each mouse were excised and rinsed with cold saline; the tissues were then weighed and stored at 80°C.

Analytical methods

Blood glycated hemoglobin (HbA1c) was measured using a commercial reagent (Medidasu HbA1c S, Sanwa Kagaku Kenkyusho Co., Ltd.). Serum AST, ALT and glucose levels were determined using the commercial reagents G0T+GPT CII-Test Wako, and Glucose CII-Test Wako, respectively (Wako Pure Chemical Industries Ltd., Osaka, Japan). Serum insulin and leptin levels were measured with ELISA kit (Morinaga, Yokohama and Yanaihara Institute Inc., Shizuoka, Japan, respectively). Serum triglycerides, HDL-cholesterol, and total cholesterol levels were determined using the commercial reagents Triglyceride E-Test Wako, HDL-Cholesterol E-Test Wako, and Cholesterol E-Test Wako, respectively, which were also purchased from Wako Pure Chemical Industries Ltd.

Statistical analysis

Experimental data are expressed as the mean ± standard deviation. Statistical analysis of the differences between the mean values was performed using Tukey’s multiple comparison test and an unpaired Student's t test with a significance level of P < 0.05.

Results

The mean body weight of obese mice was significantly higher than that of the Normal group (P < 0.05) during the experimental period. The body weights of the SG and SG + PS group showed a tendency to decrease compared with those in the Control group at the end of 8 weeks (Figure 1). The liver wet weight/body weight ratio in the Control group mice significantly increased compared with that in the Normal group mice (P < 0.01), while this ratio decreased significantly in the SG and SG+PS group mice compared with the Control group mice (P < 0.05; Figure 2).

The HbA1c levels of the SG+PS group mice were significantly decreased compared with those of the Control group mice (P < 0.05; Table 1). Serum glucose and insulin levels in the Control group mice were significantly increased compared with those of the Normal group mice. Serum insulin levels in the SG + PS group only mice were significantly decreased compared with those of the Control group mice (P < 0.05; Table 1). In this study, we did not perform Oral Glucose Tolerance Test (OGTT), because we assumed that the glucose disposal before and after an oral glucose load would be improved from these data on HbA1c and serum insulin levels. Serum total cholesterol levels were significantly increased in the Control group mice in comparison to the Normal group mice (P < 0.01), while serum total cholesterol decreased significantly in the SG and SG+PS group mice compared with the Control group mice (P < 0.05; Table 1). However, there were no significant differences in serum triglyceride and HDL-cholesterol levels between the 4 groups (Table 1). We did not measure the LDL-cholesterol in this study as HDL-cholesterol and triglyceride levels did not change in this study. So we presumed that LDL-C levels were proportional to total cholesterol levels based on the Friedewald equation.

LDL cholesterol = total cholesterol – HDL-cholesterol – (triglycerides/5)

Serum AST and ALT levels in Control group mice were significantly increased compared with those of the Normal group mice (P<0.01 for both), while these values decreased significantly in the SG+PS group mice in comparison to the Control group mice (P<0.05; Table 1) and ALT levels in SG+PS mice decreased significantly compared with SG mice (P<0.05; Table 1). And also, The SG and SG+PS group mice had significantly higher levels of leptin than did the Control group (P<0.05).

Discussion

Sitagliptin and pitavastatin monotherapy have each effect in animal model. Sitagliptin ameliorated lipid profile changes induced by atherogenic diet in rabbits that accompany the insulinotropic effect [10] and it also improve hepatic steatosis by increasing insulin sensitivity [7]. And also, sitagliptin inhibited gluconeogenesis and hepatic glucose production in high fat-induced obese rats due to decreased glycerol availability [11]. On the other hand, pitavastatin inhibited hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats [5]. However, the effects of co-administration of DPP-IV inhibitor and statin
in obese model animals have not been reported. One study in healthy subjects showed that the atorvastatin plus sitagliptin combination improved both blood glucose levels and cholesterol concentrations, without clinically relevant adverse events [12].

In this study, we investigated the combination effects of sitagliptin and pitavastatin on obesity related metabolic abnormalities in obese mice. The results of the present study indicate that co-administrated with sitagliptin and pitavastatin can ameliorate the development of hepatic impairment (fatty liver or chronic hepatitis stage) in obese mice by decreasing serum AST, ALT, and liver weight ratio along with decreasing serum total cholesterol levels. Furthermore, increased HbA1c and hyperinsulinemia were improved by the beneficial effects of sitagliptin and pitavastatin co-administration in obese mice. And
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Table 1: Biochemical analysis of blood and serum in the experiment mice.

<table>
<thead>
<tr>
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<th>Normal</th>
<th>Control</th>
<th>SG</th>
<th>SG+PS</th>
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<tr>
<td>HbA1c Serum (%)</td>
<td>&lt;4.20</td>
<td>6.24 ± 1.23</td>
<td>5.63 ± 0.85</td>
<td>5.18 ± 0.57*</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>176 ± 32</td>
<td>347 ± 145&lt;sup&gt;**&lt;/sup&gt;</td>
<td>335 ± 101&lt;sup&gt;**&lt;/sup&gt;</td>
<td>263 ± 64&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Insulin (µU/L)</td>
<td>1.70 ± 0.99</td>
<td>18.5 ± 1.27&lt;sup&gt;**&lt;/sup&gt;</td>
<td>16.3 ± 3.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>15.4 ± 3.05&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>49.0 ± 12.7</td>
<td>174.5 ± 23.7&lt;sup&gt;**&lt;/sup&gt;</td>
<td>114.8 ± 44.3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>118.3 ± 30.1&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>22.3 ± 11.4</td>
<td>17.0 ± 6.9</td>
<td>19.9 ± 8.0</td>
<td>14.0 ± 8.4</td>
</tr>
<tr>
<td>HDL-Cholesterol (mg/dL)</td>
<td>20.4 ± 9.9</td>
<td>15.9 ± 5.4</td>
<td>18.9 ± 7.7</td>
<td>15.4 ± 4.5</td>
</tr>
<tr>
<td>AST (mg/dL)</td>
<td>35.1 ± 19.6</td>
<td>205.4 ± 57.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>155.0 ± 50.8&lt;sup&gt;**&lt;/sup&gt;</td>
<td>114.4 ± 50.7&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>ALT (mg/dL)</td>
<td>17.8 ± 2.1</td>
<td>143.7 ± 43.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>156.0 ± 57.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>68.4 ± 32.6&lt;sup&gt;**&lt;/sup&gt;</td>
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Data are mean ± standard deviation. P < 0.05, *P < 0.01 compared normal group, †P < 0.05 compared with control group and ‡P < 0.05 compared with SG group. SG-Sitagliptin; PS-Pitavastatin; AST-Aspartate amino transferase; ALT-Alanine amino transferase.

It was also found that the combination benefit of pitavastatin plus sitagliptin treatment was larger on a hyperinsulinemia and fatty liver damage in mice compared with sitagliptin only treatment.

An increase of dietary intake amount in leptin-deficient mice directly causes an energy imbalance. Excess accumulation of liver lipids (enlarged liver) results in the generation of harmful substances such as reactive oxygen species and can lead to increased fatty liver damage [13]. Serum AST and ALT levels as liver damage marker were significantly reduced in mice treated with pitavastatin and sitagliptin (Table 1). In addition, pitavastatin and sitagliptin administration resulted in decreased liver weight in obese mice. The combination of pitavastatin and sitagliptin contributed to the improvement of hyperinsulinemia and liver damage, and hypocholesterolemic effects. Obesity model animals such as C57BL/6J-ob/ob mice revealed hyperglycemia, insulin resistance and hypercholesterolemia. NASH is considered to be the hepatic manifestation of metabolic syndrome. In our mice steatohepatitis model, serum cholesterol concentrations were correlated with serum AST and ALT levels. In addition, hypercholesterolemia is an independent predictor of human NASH severity [14].

It is unclear whether sitagliptin or pitavastatin predominantly improved hyperinsulinemia that is prevalent in obesity over a long period. Reduction of adiponectin in adipose tissue has been associated with insulin resistance, dyslipidemia, and atherosclerosis in humans [15]. Although we did not measure serum adiponectin levels, an increase in serum leptin levels in the SG+PS group mice could be positively correlated with inflammation in adipose tissues which induces hyperinsulinemia [5]. Leptin regulates food intake, the development of hyperinsulinemia (insulin resistance) and dyslipidemia by a paracrine and/or endocrine function. However, the direct effects of sitagliptin or pitavastatin on alterations of adiponectin and leptin require further study.

In this study, we showed the attenuating effects of sitagliptin and pitavastatin co-administration on fatty liver damage associated with hyperlipidemia in obese mice. These findings may be of clinical benefit with regard to the prevention of steatohepatitis, which is an independent predictor of the risk of cardiovascular events in obese individuals with hyperinsulinemia (insulin resistance). Further investigations are needed to verify the benefits of this combination and to determine the long-term treatment effects of these drugs in obese mice. In conclusion, co-administration of pitavastatin and sitagliptin compared with sitagliptin only may offset an increased risk of developing T2DM in obese individuals with metabolic syndrome.

References

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