OSMOTIN: A NEW ADIPONECTIN AGONIST, IN TYPE-II DIABETES AND OBESITY

Vandit R. Trivedi 1, Mehul R. Chorawala 1, Gaurang B. Shah 1

1K. B. Institute of Pharmaceutical Education & Research, Kadi Sarva Vishwavidyalaya, Sector-23, Gandhinagar, India.

*Corresponding author’s E-mail: vandit2781@yahoo.com

Accepted on: 29-06-2012; Finalized on: 31-08-2012.

ABSTRACT

Osmotin, a member of PR-5 (pathogenesis related-5) plant protein, has three dimensional structure similar to mammalian adiponectin and overlaps each other well. It is also reported that it activates AMPK in mammalian C2C12 myocytes via adiponectin receptor. Thus osmotin can act as adiponectin receptor agonist and thus its efficacy in type II diabetes was evaluated. Osmotin was isolated from salt-adapted tobacco leaves cell suspensions. Two weeks of high fat diet and STZ (35 mg/kg; i.p) produces obesity and insulin resistance syndrome similar to human Type-II diabetes in SD rats. From day 15 to day 28, treatment with osmotin-I (5mg/kg; i.v), osmotin-II (5mg/kg; i.v) and pioglitazone (10mg/kg; p.o) were given. A range of parameters, including body weight, serum glucose, serum insulin, serum triglyceride and total serum cholesterol, were measured to evaluate its antidiabetic activity. Both osmotin-I and osmotin-II possess antidiabetic activity as shown by decreased body weight, serum triglyceride and total serum cholesterol. Also serum glucose and serum insulin were low after oral glucose load. These effects were comparable to pioglitazone. Both osmotin I and II shows beneficial effects in hyperinsulinaemia and insulin resistance in HFD and STZ induced diabetic rats, probably by acting as adiponectin agonist causing increased fatty acid transport and increased expression of PPARa.

Keywords: Osmotin, adiponectin, type II diabetes, high fat diet.

INTRODUCTION

Obesity is commonly associated with type 2 diabetes, coronary artery disease, and hypertension, and the coexistence of these diseases has been termed the metabolic syndrome 24. Insulin resistance is a key feature of these diseases and is defined as a state that requires more insulin to obtain the biological effects achieved by a lower amount of insulin in the normal state. 4. Insulin resistance and obesity are associated with cardiovascular disorders. 5. However molecular basis of this association is still unclear.

Adipose tissue, a storage site for excess energy, is regarded as highly versatile endocrine gland, secreting large number of bioactive molecules collectively called adipokines or adipocytokines. These adipokines include free fatty acid, adiponin, leptin, plasminogen activator inhibitor-1, resistin, TNF-α, and adiponectin. These adipose-tissue-derived factors act either in a paracrine manner to enable local inflammation in adipose tissue or in an endocrine manner to induce insulin resistance and vascular dysfunction. Abnormal production of adipokines has recently been proposed to be a key mechanism that links obesity to type II diabetes and various vascular complications.

Although most adipokines impair insulin sensitivity and promote vascular diseases, adiponectin appears to possess antidiabetic, anti-atherogenic and anti-inflammatory activities. Adiponectin is a protein produced primarily by adipocytes, but its production is decreased in obese or overweight subjects. Adiponectin is positively correlated with protective factors such as HDL Cholesterol, and inversely related to many of the negative risk factors related to cardiovascular diseases and Type 2 Diabetes such as increased adiposity, triglycerides, high blood pressure and hyperinsulinemia in adults and youth. Lindsay et al., (2002) reported that a low concentration of plasma adiponectin is strongly correlated with reduced insulin sensitivity, and individuals with high concentrations of adiponectin were less likely to develop Type II diabetes than those with low concentrations. The adiponectin receptors Adipor1 and Adipor2 have been cloned. These receptors contain seven transmembrane domains, but are structurally and functionally distinct from other known GPCRs (G-protein-coupled receptors). Adiponectin upon interaction with adiponectin receptors, AdipoR1 and AdipoR2, conditions sensing of energy status, fatty acid oxidation and glucose transport. According to this adiponectin hypothesis, a therapeutic strategy for type 2 diabetes, metabolic syndrome, and cardiovascular diseases may include the up-regulation of plasma adiponectin, up-regulation of adiponectin receptors, or the development of Adipor agonists. There are no currently available therapeutic strategies that have been shown to mimic the actions of adiponectin in activating its receptors. A recent in vitro study has identified osmotin, a member of the PR-5 (pathogenesis related-5) family of plant defence proteins, as a potential adiponectin receptor agonist.

Osmotin is a member of the PR-5 family that was originally identified as the predominant protein that accumulated in tobacco cells as a function of osmotic regulation of plasma adiponectin, up-regulation of adiponectin receptors, or the development of Adipor agonists. In Saccharomyces cerevisiae by signaling suppression of cellular stress responses via RAS2/cAMP. The gene, ORE20/PHO36 in yeast, encodes a seven transmembrane domain receptor-like protein, homologous to mammalian adiponectin receptors, and is involved in yeast lipid and...
phosphate metabolism\textsuperscript{31}. X-ray crystallographic studies revealed that both globular adiponectin and osmomin consist of antiparallel $\beta$-strands arranged in the shape of a $\beta$-barrel. The domain I (lectin-like domain) of osmomin can be overlapped with adiponectin, suggesting that the two proteins share the lectin-like domain\textsuperscript{18}. Osmomin can induce AMP kinase phosphorylation in mammalian C2C12 myocytes via adiponectin receptors\textsuperscript{20}. This strongly suggests that further research examining similarities between adiponectin and osmomin function may facilitate efficient screening of potential adiponectin receptor agonists.

Thus, enhancing or mimicking adiponectin action through modulation of expression and/or function of the adiponectin receptors may be a novel and promising therapeutic strategy for insulin resistance, type 2 diabetes, obesity and the metabolic syndrome. The present study was undertaken to examine the above hypothesis that osmomin may be effective as adiponectin type II diabetes produced using high fat diet and low dose streptozotocin.

**MATERIALS AND METHODS**

**Isolation and purification of osmomin**

Osmomin was isolated from salt-adapted tobacco cell suspensions (Nicotiana tabacum L) as described by Singh et al.\textsuperscript{22}. Briefly, the two isoforms of osmomin (osmomin-I and osmomin-II) were isolated after homogenization of salt adapted tobacco cells in phosphate buffer followed by ammonium sulfate fractionation, CM Sephadex cation-exchange chromatography and cationic preparative HPLC separation. Purified fractions of both osmomin-I and osmomin-II were labeled and homogeneity of osmomin preparations was confirmed using SDS-PAGE (12%). For pharmacological studies, both fractions of osmomin were dissolved in normal saline. Optimum dose of osmomin was determined to be 5 mg/kg from the pilot studies carried out in our laboratory.

**Chemicals**

Streptozotocin (STZ) was purchased from Sigma, USA. The feed ingredients such as casein, dl-Methionine, yeast extract, cholesterol, lard and vitamin and mineral were procured from commercial sources.

**Animals**

Sprague Dawley (SD) rats (150–170 g) were procured from the central animal facility of the Institute. The animals were housed in standard polypropylene cages and maintained under controlled room temperature (22±2 °C) and humidity (55±5%) with 12:12 h light and dark cycle. All the rats were provided with commercially available rat normal pellet diet (NPD) (Pranav Agro, Baroda) and water ad libitum, prior to the dietary manipulation. The study was approved by the institutional animal ethics committee and procedures followed according to the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India.

**Study protocol and Treatments**

The rats were allocated into two dietary regimens consisting of 6 and 54 rats by feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) ad libitum, respectively, for the initial period of 2 weeks\textsuperscript{28}. The composition and preparation of HFD were as described by Srinivasan et al., 2004\textsuperscript{28}. After the 2 weeks of dietary manipulation, a subset of HFD fed rats were divided into five groups of 6 animals each. Group II received only HFD till the completion of the study. On day 15, groups III, IV, V and VI animals were injected intraperitoneally (i.p.) with single dose of STZ (35 mg kg\textsuperscript{-1}) dissolved in 0.05M citrate buffer (pH-4.5) while group IV, V and VI received osmomin-I (5 mgkg\textsuperscript{-1}, i.v.), osmomin-II (5 mgkg\textsuperscript{-1}, i.v.) and pioglitazone (10 mgkg\textsuperscript{-1}, p.o.) respectively from day 15 to day 28. NPD fed rats were given only citrate buffer as vehicle. The body weight and biochemical estimations (total serum cholesterol, serum triglyceride and oral glucose tolerance test\textsuperscript{28} (serum glucose and serum insulin) were carried out just before and at day 14 (before animal allotment). The feed and water intake of the animals were also measured. The rats were allowed to continue to feed on their respective diets until the end of the study.

**Collection of blood and analytical methods**

Blood was collected from retro-orbital plexus of the rats under light ether. The serum was separated and was analyzed for glucose (GOD-POD), triglycerides (GPO-POD) and total cholesterol (CHOD-POD) levels using commercially available colorimetric diagnostic kits (Span Diagnostics, Surat, India).

**Statistical Analysis**

The results are expressed as mean±S.E.M. The unpaired Student’s t-test was used for analyzing the data between two groups where as one-way ANOVA followed by multiple comparison tests (Tukey’s test) was employed if there were more than two groups. A value of p<0.05 was considered statistically significant.

**RESULTS**

**Features of HFD-fed insulin-resistant rats**

Table 1 illustrates that the feeding of HFD for 2 weeks resulted in significant increase (p<0.05) in body weight as well as triglycerides and total cholesterol levels in rats as compared to NPD-fed rats.

**Effect of STZ and test drugs on HFD-fed insulin-resistant rats**

STZ administration results into significant changes in various parameters (Table 2) as measured on day 28. The body weight was significantly reduced when STZ was administered to HFD fed rats, which was still considerably higher than NPD fed rats. Treatment with osmomin-I,
osmotin-II and pioglitazone also showed similar decrease in body weight. In addition, there was a significant (p<0.05) increase in basal STG and STC in HFD+STZ rats when compared to HFD fed and NPD fed rats alone.

**Serum glucose and insulin responses to oral glucose loading**

Feeding of high fat diet for 2 weeks produces a condition of insulin resistance similar to human. Injection of STZ (35 mg kg\(^{-1}\), i.p.) after 2 weeks of dietary manipulation significantly (p<0.05) increased serum glucose in HFD rats, thus producing the frank hyperglycemia. Oral glucose tolerance test was carried out on day 0, day 14 and day 28 to determine the effect of STZ and various treatments on HFD fed rats. After 14 days, there was significantly higher serum glucose (fig. 1A) and serum insulin (fig. 1B) in HFD group as compared to NPD fed rats (p<0.05).

Treatment with osmotin-I, osmotin-II and pioglitazone was started from day 15 and continued till day 28. There was significant (p<0.05) decrease in serum glucose (fig. 2A) and serum insulin (fig. 2B) in both osmotin treatment groups as compared to HFD-STZ rats, which was still higher than pioglitazone treated rats. It was observed that both osmotin-I and osmotin-II showed similar effects on all biochemical parameters as well as body weight.

### Table 1: Effect of HFD on body weight and biochemical parameters in rats after two weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPD fed</th>
<th>HFD Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>175 ± 1.77</td>
<td>214.1 ± 2.17*</td>
</tr>
<tr>
<td>Serum triglyceride (STG) (mg dl(^{-1}))</td>
<td>97.98 ± 2.92</td>
<td>211.95 ± 2.93*</td>
</tr>
<tr>
<td>Serum total cholesterol (STC) (mg dl(^{-1}))</td>
<td>99.62 ± 6.33</td>
<td>190.93 ± 2.71*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05 vs. NPD group (n=6)

### Table 2: Effect of STZ and Osmotin on body weight and biochemical parameters in rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NPD fed</th>
<th>HFD Fed</th>
<th>HFD + STZ</th>
<th>Osmotin-I</th>
<th>Osmotin-II</th>
<th>Pioglitazone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>169.17 ± 2.94</td>
<td>293.67 ± 4.37*</td>
<td>207.00 ± 3.46(^d)</td>
<td>194.17 ± 3.16</td>
<td>202.83 ± 2.98</td>
<td>191.17 ± 3.40</td>
</tr>
<tr>
<td>STG (mg dl(^{-1}))</td>
<td>116.49 ± 4.01</td>
<td>270.94 ± 5.30*</td>
<td>309.97 ± 6.14(^d)</td>
<td>252.62 ± 5.20(^^)</td>
<td>260.46 ± 6.19(^^)</td>
<td>213.64 ± 4.98(^^)</td>
</tr>
<tr>
<td>STC (mg dl(^{-1}))</td>
<td>97.66 ± 3.47</td>
<td>222.93 ± 3.45*</td>
<td>240.69 ± 6.52(^d)</td>
<td>174.56 ± 4.54(^^)</td>
<td>183.61 ± 4.99(^^)</td>
<td>154.30 ± 3.66(^^)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. * * * p<0.05 vs. NPD group (n=6); ^p<0.05 vs. HFD+STZ group (n=6)

**Figure 1:** Effect of HFD on serum glucose and serum insulin after oral glucose load. Values are mean ± SEM.

**Figure 2:** Effect of test drugs on serum glucose and serum insulin after oral glucose load. Values are mean ± SEM.
Obese subjects had significantly lower plasma adiponectin concentrations than non-obese subjects, although adiponectin derives exclusively from adipose tissue. Adiponectin increases insulin sensitivity in the liver and skeletal muscle and reduces atherosclerosis. In addition to these effects, adiponectin also seems to have pleiotropic effects, particularly in relation to metabolic syndrome. High fat diet induced insulin resistance associated with obesity is a major risk factor for diabetes and cardiovascular diseases, the prevalence of which is increasing sharply. There are no currently available therapeutic strategies that have been shown to mimic the actions of adiponectin in activating its receptors. Narasimhan et al., (2005) has identified a novel protein, Osmotin, a member of the PR-5 (pathogenesis related-5) family of plant defence proteins, as a potential adiponectin receptor agonist.

Osmotin is a member of a large PR-5 protein family, which is both ubiquitous (fruits and vegetables, etc.) and diverse. PR-5 proteins are also extremely stable and may remain active even when in contact with the human digestive or respiratory systems. It is reported that osmotin activates AMPK via adiponectin receptor in mammalian C2C12 myocytes and as well as three dimensional structure of osmotin and adiponectin are similar and overlap each other. Thus it can be suggested that similarities between osmotin and adiponectin may facilitate development of potential adiponectin receptor agonists and may be used as novel therapeutic strategy for insulin resistance and Type II diabetes. In the present study, we have isolated osmotin from salt adapted cultured tobacco cell and have made an effort to evaluate its efficacy in Type II diabetes and insulin resistance induced by HFD and low dose streptozotocin.

The HFD was prepared (58% calories as fat), such that it causes insulin resistance in rats over a short period of time. Thus, the feeding of HFD for a period of 2 weeks produced rats with insulin resistance syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia as shown in table 1 and fig. 1. It was observed that administration of STZ in HFD fed rats has increased the basal levels of serum glucose as consequence of insulin resistance. HFD has been shown to increase level of triglyceride and total cholesterol level. Also it was observed that due to decreased TG content, there was decrease in serum insulin levels which in turn caused decrease in serum glucose level as shown in fig.2A and 2B. Thus our results are consistent with above mentioned hypothesis. However, it was also observed that both osmotin-I and osmotin-II shows similar efficacy.

Adiponectin increases the expression level of PPARα in vivo. Thus osmotin, like adiponectin, presumably via PPARα activation at least in part, leads to decreased TG content in the liver and skeletal muscle and increases insulin sensitivity. Other studies have demonstrated that adiponectin modulates insulin sensitivity by stimulating glucose utilization and fatty acid oxidation via phosphorylation and activation of AMPK in muscle and liver. Osmotin is also reported to activate AMPK in mammalian C2C12 myocytes. It can be thus said that osmotin may stimulate β-oxidation and glucose uptake via AMPK and increase fatty acid combustion and insulin sensitivity.