Angiotensin type-1 receptor blockade with losartan increases insulin sensitivity and improves glucose homeostasis in subjects with type 2 diabetes and nephropathy

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Abstract

Background. A growing body of evidence supports the concept that treatment with the newer angiotensin type-1 receptor blockers (ARBs) improves glucose homeostasis under conditions wherein it is impaired. Controversy exists, however, regarding the ability of losartan, an older ARB, to exert comparable improvement. The present study was undertaken to evaluate the effects of losartan on glucose homeostasis in subjects with type 2 diabetes and nephropathy.

Methods. Twenty-seven subjects with type 2 diabetic nephropathy were enrolled in this prospective, randomized, controlled study. Losartan (100 mg daily) or the calcium channel blocker amlodipine (10 mg daily) was administered for a period of 3 months. Fasting blood glucose, serum insulin and C-peptide concentrations were measured at baseline and at the end of the study. Oral glucose tolerance tests were performed to evaluate insulin sensitivity and \( \beta \)-cell responsiveness. Insulin resistance was measured using the homeostasis model assessment of insulin resistance (HOMA-IR).

Results. Fasting blood glucose, HbA1c, AUC glucose, and urinary protein values were significantly decreased in the losartan group as compared with the amlodipine group (\( P < 0.05 \)). Furthermore, C-peptide concentrations, the insulin sensitivity index, and the insulin-to-glucose ratio were significantly increased after 3 months of therapy with losartan as compared to amlopidine (\( P < 0.05 \)). Reductions of fasting insulin concentrations and HOMA-IR were also observed for the losartan group; however, reductions were not significant when compared with the amlopidine group.

Conclusion. In addition to reducing urinary protein excretion, losartan at 100 mg daily increases insulin sensitivity and improves glucose homeostasis in subjects with type 2 diabetic nephropathy.

Keywords: angiotensin type-1 receptor blocker (ARB); glucose homeostasis; insulin; losartan; nephropathy; type 2 diabetes

Introduction

Angiotensin II (Ang II), the main effector peptide of the renin–angiotensin system (RAS), is implicated in the development of vascular, cardiac, and renal pathologies. Several lines of evidence suggest that Ang II impairs insulin sensitivity \[1,2\]. Furthermore, angiotensin type-1 receptor (AT1R) blockers (ARBs) have recently been demonstrated to exert beneficial effects on glucose and lipid metabolism in adipocytes and adipose tissue \[3\]. Findings from recent clinical trials support the hypothesis that suppression of the RAS, either by inhibition of angiotensin-converting enzyme (ACE) \[4\] or blockade of the AT1R \[5\], substantially lowers the risk for type 2 diabetes. In the Heart Outcomes Prevention Evaluation (HOPE) trial, a 34% reduction in relative risk for the development of type 2 diabetes was observed after treatment with agents that suppress the RAS \[6\]. Similarly, in the Losartan Intervention For Endpoint Reduction in Hypertension (LIFE) study, the incidence of type 2 diabetes was found to be reduced by 25% in patients treated with losartan as compared with other antihypertensive agents \[5\]. Additionally, blockade of the AT1R has been shown to improve insulin sensitivity in animal models of insulin resistance \[7\]. However, the mechanisms underlying the insulin-sensitizing and antidiabetic effects of the ARBs have not been defined.

The nuclear hormone receptor peroxisome proliferated-activated receptor-\( \gamma \) (PPAR-\( \gamma \)) is known to serve an important function in the preservation of insulin sensitivity \[8\]. Certain findings implicate this receptor in the actions of the ARBs. For example, telmisartan and irbesartan have been observed to increase PPAR-\( \gamma \) activity at low doses and to promote PPAR-\( \gamma \)-dependent differentiation in adipocytes \[3,9\].
However, not all ARBs appear to possess PPAR-γ-activating properties. Based on findings of in vitro studies, it was proposed that the observed differences among PPAR-γ-activating ARBs are attributable to their physicochemical properties and that high lipophilicity is required to obtain sufficiently high penetration rates for effective binding to intracellular PPAR-γ [10]. At high concentrations, losartan was found to activate PPAR-γ in a manner similar to that of partial PPAR-γ agonists [9]. Nonetheless, it is unclear whether losartan improves insulin sensitivity in hypertensive patients [11–13]. Furthermore, several recent studies have yielded conflicting results regarding the ability of the ARBs to lower HbA1c in type 2 diabetics with nephropathy [14–19]. The present investigation was undertaken to ascertain whether treatment with losartan increases insulin sensitivity, improves fasting and postprandial glucose homeostasis, and lowers HbA1c in patients with type 2 diabetes and nephropathy.

**Subjects and methods**

**Subjects**

Eligibility criteria included age ≥20 years, a fasting plasma glucose concentration of 3.3–9.0 mmol/l, a 2 h plasma glucose concentration of 7.5–13 mmol/l in response to an oral glucose tolerance test (OGTT), and a body mass index (BMI) ≥17 kg/m². Nephropathy was defined clinically by the presence on two occasions of a ratio of urinary albumin to urinary creatinine from a first morning specimen of at least 300 mg/24 h, or by a 24 h urinary protein concentration ≥500 mg. Patients were excluded if they had received a diagnosis of type 1 diabetes or non-diabetic renal disease. Twenty-seven subjects with type 2 diabetes and nephropathy were randomly divided into two groups: losartan (n = 14) and amlodipine (n = 13). Throughout the study, patients received their conventional anti-hypertensive medications (β-blocking agents, diuretics, α-blocking agents) but did not receive angiotensin-1-converting enzyme (ACE) inhibitors or ARBs other than losartan. Individuals in both groups received injections of 70/30 mixed human insulin twice daily, before breakfast and supper. The 70/30 insulin injection was initiated at 0.2 units/kg and adjusted to achieve a fasting blood glucose concentration ≤9.0 mmol/l without frequent occurrence of hypoglycemia. These subjects were maintained at this insulin dosage for 3 months unless hypoglycemia occurred frequently. Losartan was administered at 100 mg daily, with this dose fixed for the entire study period. The study was approved by the Ethical Committee of the No.3 People’s Hospital of Shanghai Jiao Tong University, and all patients gave their informed consent.

**Fasting glucose, insulin and C-peptide concentrations**

Fasting blood glucose, fasting serum insulin, and C-peptide concentrations were measured at baseline and at the end of the study. Blood glucose concentrations were determined using an automated glucose analyser, and serum insulin was measured using a competitive chemiluminescent immunoassay. C-peptide was measured using a double-antibody C-peptide kit (reference range of 0.33–3.76 ng/ml).

**Insulin sensitivity, β-cell responsiveness and insulin resistance**

Prior to initiation of the study (baseline) and after 3 months of therapy, an OGTT was performed to assess insulin sensitivity and β-cell responsiveness. The OGTT was initiated with 75 g glucose, and blood samples were withdrawn at 30 min intervals (0, 30, 60, 90 and 120 min) for the determination of glucose and C-peptide concentrations and for measurement of the insulin response.

Insulin sensitivity and β-cell responsiveness to glucose administration were estimated from the OGTT [20,21]. Insulin sensitivity was estimated as 1/(fasting insulin, or by the insulin sensitivity index (ISI), which was calculated as 1/(fasting insulin × fasting glucose). ISI and 1/fasting insulin were highly correlated at baseline (Spearman’s r = 0.98).

Insulin secretion was estimated by two methods: (i) the corrected insulin response (CIR) = 30 min insulin/[30 min glucose–3.89 mmol/l], and (ii) the insulin-to-glucose ratio (IGR) = (30 min insulin–fasting insulin)/(30 min glucose–fasting glucose). CIR and IGR were highly correlated at baseline (Spearman’s r = 0.96). Additionally, area under the curve (AUC) analyses for glucose and C-peptide concentrations were employed for further evaluation of the status of insulin secretion, and AUC glucose and C-peptide concentrations were calculated using trapezoidal integration. Insulin resistance was determined using the homeostasis model assessment (HOMA-IR) [22] according to the following formula:

\[
\text{HOMA-IR} = \frac{\text{fasting serum glucose (mmol/l)}}{\text{fasting insulin (µIU/ml)}} \times 22.5.
\]

**Statistical methods**

Data are expressed as means ± SD, with the exception of skewed data, which are expressed as median (range). After testing for data normality (Kolmogorov–Smirnov statistics), baseline comparisons and differences in changes from baseline to the end of treatment between the losartan and control groups were evaluated using the unpaired Student’s t-test for Gaussian data and using the Wilcoxon rank sum test for non-Gaussian data. The chi-square test was also performed for categorical data. P-values <0.05 were considered statistically significant. The SAS 8.0 statistical package was utilized for all analyses.

**Results**

**Participants and adherence**

The losartan treatment group comprised 14 participants, and the amlodipine control group comprised 13 participants. The groups were well matched for sex, age, BMI, duration of diabetes and 24 h urinary protein concentrations (Table 1). All participants...
completed the 3-month treatment. Fasting values for blood glucose, HbA1c, insulin, and C-peptide did not differ between the two groups at baseline (Tables 2 and 3).

**Responses to treatment**

Changes from baseline to the end of treatment in fasting blood glucose and HbA1c values were significantly different between the losartan and control groups \((P = 0.01)\) for fasting blood glucose and \(P = 0.02\) for HbA1c. Subjects receiving losartan exhibited a 0.83 mmol/l decrease in fasting blood glucose and a 0.55% decrease in HbA1c (Table 2). The effects of the treatments on insulin sensitivity and \(\beta\)-cell responsiveness were determined from the OGTT and from insulin assays conducted at baseline and after 3 months. Glucose concentrations, measured at 30 min intervals throughout the OGTT, were significantly lower at all measurement times for subjects treated with losartan for 3 months as compared with these subjects at baseline \((P \leq 0.01);\ Figure 1\). In contrast, the glucose tolerance curves for the control group at baseline and after 3 months of treatment with amlodipine were comparable (Figure 1). In comparison with the amlodipine control group, patients in the losartan treatment group displayed significant decreases in AUC glucose values and urinary protein concentrations. AUC glucose values declined from 1306 mmol/l/120 min at baseline to 1007 mmol/l/120 min at the end of the treatment period, and urinary protein concentrations decreased from 755 mg/24 h at baseline to 655 mg/24 h at the end of the treatment period \((P < 0.01)\) (Table 3). Decreases in fasting insulin concentrations and in insulin resistance as measured by HOMA-IR were also observed for subjects treated with losartan; however, these decreases were not statistically significant. An improvement in AUC C-peptide values was also observed for the losartan treatment group: these values increased from 186.4 ng/ml 120 min at baseline to 235.9 ng/ml 120 min after 3 months of therapy \((P < 0.01)\). Furthermore, significant increases in the ISI and IGR were observed in the losartan group as compared with the control group, for which increases were not observed \((P = 0.02 \text{ for the ISI and } P < 0.01 \text{ for the IGR})\). After 3 months of therapy, the ISI increased from 0.77% at baseline to 1.44%, and the IGR increased from 5.81 at baseline to 12.34 in the losartan group.

**Discussion**

It has been recognized for many years that Ang II, a potent vasoconstrictor, possesses the capacity to impair insulin action and provoke glucose
intolerance [1,2]. Additionally, inhibition of Ang II action is currently believed to result in improved insulin sensitivity and β-cell responsiveness to glucose under various conditions. Findings from in vitro and in vivo studies have revealed that two newer ARBs, telmisartan and irbesartan, have the potential to improve insulin sensitivity and β-cell responsiveness [9,23]. However, controversy has arisen regarding the ability of losartan to produce comparable effects. For example, in normotensive offspring of hypertensive parents, losartan at a dose of 50 mg/day did not increase insulin sensitivity [11]. Furthermore, in mildly hypertensive patients, losartan administered at 50–100 mg/day for 4 weeks failed to alter insulin sensitivity or glucose metabolism [12]. In contrast, investigation of the effects of losartan on insulin-mediated glucose uptake and substrate oxidation in insulin-resistant patients with hypertension

**Table 3.** Metabolic parameters at baseline and at the end of treatment for the two groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Losartan (n = 14)</th>
<th>Control (amlodipine) (n = 13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC glucosea (mmol/l 120min)</td>
<td>1305.96 ± 181.94</td>
<td>1277.88 ± 105.99</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>1007.14 ± 95.01</td>
<td>1293.35 ± 101.88</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>−298.82 ± 152.01</td>
<td>15.46 ± 20.39</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>Fasting insulina (µIU/ml)</td>
<td>24 (10, 55)</td>
<td>24 (13, 61)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>14.5 (6, 37)</td>
<td>22 (14, 36)</td>
<td>0.03**</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>−14 (−47, 20)</td>
<td>0 (−25, 23)</td>
<td>0.14</td>
</tr>
<tr>
<td>30 min insulinb (µIU/ml)</td>
<td>42.5 (28, 89)</td>
<td>45 (23, 95)</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>44.5 (23, 106)</td>
<td>36 (24, 89)</td>
<td>0.48</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>−6 (−34, 61)</td>
<td>−4 (−12, 29)</td>
<td>0.81</td>
</tr>
<tr>
<td>AUC C-peptidea (ng/ml 120min)</td>
<td>186.40 ± 60.18</td>
<td>188.91 ± 64.74</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>235.96 ± 61.49</td>
<td>190.66 ± 60.35</td>
<td>0.07</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>49.56 ± 34.79</td>
<td>1.75 ± 6.24</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>HOMA-IRb</td>
<td>8.83 (1.47, 21.76)</td>
<td>7.76 (3.73, 22.23)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>4.34 (1.23, 10.52)</td>
<td>7.26 (4.54, 13.12)</td>
<td>0.04**</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>−3.86 (−19.3, 4.33)</td>
<td>0 (−9.1, 4.79)</td>
<td>0.10</td>
</tr>
<tr>
<td>Urinary protein concentrationb (mg/24 h)</td>
<td>755 (680, 980)</td>
<td>770 (680, 1020)</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>655 (380, 760)</td>
<td>790 (380, 920)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>−160 (−370, −30)</td>
<td>−40 (−190, 200)</td>
<td>&lt;0.01**</td>
</tr>
<tr>
<td>Insulin sensitivity indexa (%)</td>
<td>0.77 ± 0.72</td>
<td>0.63 ± 0.25</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>1.44 ± 1.02</td>
<td>0.63 ± 0.20</td>
<td>0.01*</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>0.68 ± 0.93</td>
<td>−0.01 ± 0.28</td>
<td>0.02*</td>
</tr>
<tr>
<td>Insulin-to-glucose ratioa</td>
<td>5.81 ± 6.05</td>
<td>6.7 ± 4.98</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>12.34 ± 7.03</td>
<td>6.26 ± 4.86</td>
<td>0.03*</td>
</tr>
<tr>
<td>Change from baseline to end of treatment</td>
<td>6.54 ± 7.31</td>
<td>−0.44 ± 5.09</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

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*Values are presented as means ± SD.

*Values are presented as medians (range).

*Significant difference between the two groups; two-sample t-test.

**Significant difference between the two groups; Wilcoxon rank sum test.

Fig. 1. Blood glucose concentrations following oral administration of 75 g glucose (OGTT). Measurements were performed for the losartan and amlodipine control groups at baseline and after 3 months of therapy. Samples were withdrawn for blood glucose measurements before glucose administration and every 30 min thereafter through 2 h. Findings are presented as means ± SD. Blood glucose concentrations were significantly lower at every time post-administration of glucose for the losartan group after 3 months of treatment as compared with baseline (*P ≤ 0.01 for all time points; two-sample t-test). However, glucose tolerance curves for the control group at baseline, for the control group after 3 months of treatment with amlodipine, and for the losartan group at baseline were comparable.
disclosed that losartan enhanced the rates of glucose clearance and disposal of whole body glucose in these patients [13]. Losartan was found to improve both insulin action and non-oxidative glucose metabolism. In addition, treatment of hypertensive patients with losartan was found to be associated with less peripheral vascular hypertrophy and higher insulin sensitivity [24].

In normotensive, normoalbuminuric type 1 diabetic patients, losartan (50 mg/day) was found to reduce glomerular hyperfiltration and to improve basal and insulin-stimulated glucose oxidation [25]. Additionally, administration of losartan orally to diabetic rats was observed to improve insulin sensitivity, to promote a significant decrease in insulin concentrations, and to reduce elevations in fasting and fed glucose concentrations [26]. In a more recent study [27], losartan was found to improve glucose-induced insulin release and proinsulin biosynthesis selectively in db/db mice. Although the insulin sensitivity of their peripheral tissues was not affected, oral losartan treatment delayed the onset of diabetes and reduced hyperglycemia and glucose intolerance in these mice. In contrast to these findings, fasting plasma glucose, free plasma insulin, and insulin resistance were reported to be significantly reduced in hypertensive patients with metabolic syndrome after 3 months of therapy with telmisartan (80 mg/day), but not with losartan (50 mg/day) [28]. The discrepancies in these findings may be the consequence of different dosages of losartan. It has been observed that administration of losartan at 100 mg daily is significantly more effective than at 50 mg daily with respect to reduction of albuminuria and lowering of systolic, diastolic, and mean arterial blood pressure in type 1 diabetic patients with nephropathy [29].

To our knowledge, investigations of the effects of losartan on insulin sensitivity and β-cell responsiveness in subjects with type 2 diabetic nephropathy have not been previously conducted. Findings of the present report reveal that losartan reverses abnormalities in glucose metabolism comparable with telmisartan and irbesartan when administered to patients with type 2 diabetic nephropathy. Improvement was evidenced by a variety of parameters. In particular, treatment with losartan was found to lower fasting glucose concentrations, postprandial glucose concentrations and HbA1c values, and to increase C-peptide concentrations, insulin sensitivity, and insulin/glucose ratios in these subjects.

Currently, ARBs represent the only evidence-based treatment strategy for patients with type 2 diabetes and proteinuria [30,31]. Three major studies, the findings of which were published in 2001, provided most of this evidence: the Irbesartan Diabetic Nephropathy Trial (IDNT) [15], the IRbesartan in patients with type 2 diabetes and Microalbuminuria Study (IRMA2) [16] and the Reduction in End points in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) [32]. Similarly to ACE inhibitors in type 1 diabetes, the ARBs were found to significantly reduce microalbuminuria in microalbuminuric hypertensive type 2 diabetic subjects. The ARBs also reduced the numbers of type 2 diabetic patients with nephropathy who reached a combined outcome of doubling of baseline serum creatinine, end-stage renal failure or all-cause mortality. Approximately 2–4 combined outcome events were prevented per 100 years of therapy. Since comparable benefits were not observed with conventional antihypertensive agents, these benefits were concluded to result from blockade of the RAS.

Concerns regarding the findings of the RENAAL study were subsequently raised [33]. It was noted that the placebo group included more patients with angina, myocardial infarction and lipid disorders than did the losartan group. Furthermore, information on glucose homeostasis was disregarded, and data regarding hypoglycemic therapy were not included in a multivariate analysis. Further analysis of metabolic parameters as predictors of risk in the RENAAL study was therefore performed [17]. Although losartan did not adversely affect glycemic control, no improvement in HbA1c values was observed. Additionally, in the IDNT and IRMA2 trials HbA1c values were not found to be lowered in response to irbesartan. It is unclear why HbA1c values were reduced in the present study but not in these larger, older studies. It is conceivable that the patient populations of the RENAAL, IDNT, and IRMA2 trials differed somewhat from the population of the present study with respect to the extent of their illness as manifested by their baseline HbA1c values. In the RENAAL and IRMA2 studies in particular, baseline HbA1c values ranged from 8.1–8.5 in contrast to baseline values of 6.5–6.7 in the present study. In the presence of more advanced diabetes, patients may be less responsive to the glucose-lowering effects of the ARBs. It should also be noted that, in contrast to the present study, which utilized six different methods to evaluate glucose homeostasis, HbA1c was the only parameter of glucose homeostasis investigated in these older studies.

In agreement with the findings of the present study, other investigators have observed that ARBs possess the capacity to lower HbA1c values in type 2 diabetic patients with nephropathy and/or hypertension. For example, treatment of 422 hypertensive microalbuminuric type 2 diabetic subjects for 6 months with losartan was found to lower both fasting glucose and HbA1c values [14]. Baseline HbA1c values for subjects of this study were lower than for those of the RENAAL and IRMA2 studies. In more recent studies, administration of irbesartan to a large number of hypertensive type 2 diabetic patients for more than 3 months [18] and of valsartan to a smaller number of similar subjects for 12 weeks was found to lower HbA1c values significantly. It may be relevant that the RENAAL, IDNT and IRMA2 trials were conducted for longer periods (2–3 years). Whether the beneficial
effects of ARBs on HbA1c are time-dependent in type 2 diabetic patients with nephropathy remains to be determined.

In the present study, a prominent reduction in fasting glucose concentrations was observed after 3 months of therapy with losartan, but not with amlodipine. Furthermore, following oral administration of glucose to the losartan-treated subjects, blood glucose values were substantially lower at 90 and 120 min as compared with values observed at baseline. The improvement in postprandial hyperglycemia after losartan treatment could have resulted from improved β-cell performance and/or enhanced tissue sensitivity to insulin. Consistent with the observed reductions in blood glucose concentrations, AUC glucose values decreased significantly and simultaneously with significant increases in AUC C-peptide values. These findings support an improved insulin secretory response in losartan-treated subjects with type 2 diabetic nephropathy.

The mechanisms responsible for the improvements of glucose homeostasis by losartan as described in the present study remain to be determined. Whether the observed improvements in insulin release and β-cell function by losartan are related to the observed reduction of hyperglycemia by this agent is also unclear. Based on the observations of other investigators, it is conceivable that inhibition of the RAS of pancreatic islets [34] or adipose tissue [35], activation of PPAR-γ-dependent gene expression in adipose tissue [8], and/or increased expression of adiponectin [36] mediate(s) the beneficial effects of losartan on glucose homeostasis in type 2 diabetes with nephropathy. Further investigations are required to ascertain whether any or all of such mechanisms are involved.

In summary, findings of the present study reveal that treatment with losartan at 100 mg daily increases insulin sensitivity, enhances β-cell responsiveness to glucose, and improves glucose homeostasis in subjects with type 2 diabetes and nephropathy. The mechanisms whereby losartan exerts its beneficial effects in these subjects remain to be determined.

Conflict of interest statement. None declared.

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