Troglitazone Improves Endothelial Dysfunction in Patients with Insulin Resistance

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Insulin resistance is a possible major metabolic cause of atherosclerosis. Endothelial dysfunction is commonly found in patients with insulin resistance, and primary treatment of insulin resistance with troglitazone should improve such endothelial dysfunction. Thus, the effects of troglitazone on endothelial function were investigated. Thirteen non-diabetic male subjects with hyperinsulinemic response to oral glucose load (n=7) and normal (n=6) subjects were investigated. Flow-mediated dilatation (FMD) of the brachial artery was examined by high resolution ultrasonography before and after the administration of troglitazone of 400 mg for 4 weeks. In insulin resistant subjects, fasting glucose (4.9±0.3 to 4.7±0.3 mmol/L, p<0.05), insulin (45±30 to 25±15 pmol/L, p<0.05) and response to oral glucose load (AUC glucose : 15.0±3.5 to 13.0±2.2 mmol · h/L, p<0.05 ; AUC insulin : 965±560 to 475±275 pmol · h/L, p<0.05) were significantly reduced. FMD was significantly improved in insulin resistant subjects. A significant negative correlation was observed between FMD and AUC insulin (r = -0.64, p< 0.05). The present study demonstrates that FMD is impaired in insulin resistant subjects, and troglitazone improves the blunted vascular response and impaired insulin response. This finding suggests that primary treatment of insulin resistance could prevent the development of atherosclerosis by improving endothelial dysfunction. J Atheroscler Thromb, 2000 ; 7 : 159-163.

Key words : Insulin resistance, Flow mediated dilatation, Endothelial function, Troglitazone
Methods

Subjects
This study included 13 non-diabetic male subjects, consisting of 7 patients with hyperinsulinemic response (insulin resistant) to oral glucose load and 6 patients with normal response (normal subjects). The hyperinsulinemic response was defined by the area under the curve (AUC) of insulin levels greater than 540 pmol \cdot h/L in a standard 75 g oral glucose tolerance test (greater than the mean \pm 1SD in subjects with normal glucose tolerance in our database, n =271). Each subject received troglitazone 400 mg twice daily for 4 weeks. They were asked to maintain constant diet and physical activity levels throughout the study. Other drugs, including aspirin, \( \beta \)-blockers, ACE inhibitors, calcium antagonists and statins, were kept at a constant dose. None of the patients were taking nitrates, probucol, fibric acid derivatives or other antidiabetic agents. The standard 75 g oral glucose tolerance test was performed at baseline and 4 weeks. Blood samples for glucose and insulin were drawn before glucose load and at 30, 60 and 120 minutes afterwards. Subjects received full verbal and written explanations of the nature and purpose of the study, and gave informed consent. This study was approved by the local ethical committee.

Protocol
FMD and nitroglycerin (NTG) induced dilatation of the brachial artery were performed at baseline and 4 weeks after the start of troglitazone treatment. Measurements were performed according to Celermajer et al. (15) with some modifications. Examinations were made in the morning (8:00AM to 9:00AM) after at least 12 hours fasting and in a quiet temperature-controlled (22°C to 24°C) room. Subjects were kept in bed for at least 15 minutes to stabilize their condition before the examination. The diameter of the artery was measured by high-resolution, two dimensional ultrasonography (Sonos 2500; Hewlett Packard) with a 7.5-MHz linear array transducer.

The left brachial artery was scanned over a longitudinal section 3 to 5 cm above the elbow, and the arm was kept in the same position throughout the study. A pneumatic tourniquet was placed around the forearm distal to the target artery and inflated to 250 mmHg for 5 minutes, then deflated suddenly. Reactive hyperemia was observed after the sudden deflation. After 15 minutes, a sublingual NTG spray (300 mg; Myocol Spray, Toa Eiyo Co.) was administered for measurement of the NTG induced dilatation. Scanning was done 5 times (before inflation, during inflation, 5 minutes after deflation, just before the NTG administration and 5 minutes after the NTG administration). The ultrasound images were recorded on S VHS videotape. The diameter of the brachial artery was measured from the anterior to the posterior interface and synchronized with R-wave peaks on the ECG. The diameter changes caused by FMD and NTG were expressed as the percentage change relative to the diameter on the initial resting image.

To minimize the operator variability this study was done with one operator who skilled at this procedure.

Laboratory tests
Fasting blood samples were taken at baseline and after 4 weeks. Serum insulin level was measured by the double antibody technique (Dainabot, Tokyo, Japan). Plasma glucose concentrations were determined by the glucose oxidase method (Kainos, Tokyo, Japan). Glycosylated hemoglobin (HbA1c) was measured by high performance liquid chromatography with a normal range of 4.3-5.8% (TOHSOH, Tokyo, Japan). Serum total cholesterol, triglycerides and HDL cholesterol levels were measured using standard enzymatic methods (Kainos, Tokyo, Japan) and LDL cholesterol values were calculated using Friedewald’s formula.

Peak particle diameter (PPD) of LDL was determined by gradient-gel electrophoresis according to Krauss and Burk with some modifications. In brief, electrophoresis was performed on 2.5% to 16% gradient polyacrylamide gel (Biocraft, Tokyo, Japan). The gels were prerun in 90 mM Tris, 80 mM boric acid, and 2.5 mM EDTA, pH 8.3 at 150 V for 20 minutes. The samples (20 \( \mu L \)) were applied (40% sucrose, 1% bromophenol blue) to each lane on the gel. Electrophoresis was performed at 150 V for 18 hours at 4°C. The gels were stained with 0.04% Oil Red O in 60% ethanol for 24 hours, and fixed with 50% methanol plus 7% acetic acid containing Coomassie Brilliant Blue. After destaining with 25% ethanol plus 9% acetic acid, the gels were scanned using a densitometric image analyzer. The calibration curve was determined from high molecular-weight standards (thryoglobulin (17.0 nm) and apoferritin (12.2 nm)) and known diameter sample (25.7 nm). The estimated diameter for the major peak in each scan was termed the PPD.

The lipid peroxide levels in plasma were determined by measuring thiobarbituric acid reactive substances (TBARS) using a lipid-peroxide test kit (Wako Junyaku Co., Osaka Japan). This result was expressed as the malondialdehyde equivalent (nmol/ml).

Statistical analysis
Values are given as mean \( \pm SD \). The area under the curve (AUC) for insulin and glucose was calculated using the trapezoidal rule. Single variable comparisons for baseline and troglitazone treatment results used the paired t test. Comparison of groups used the unpaired t test. Linear regression analysis was used to compare two continuous variables. All probability values for the statistical significance are two-tailed.
Results

All the subjects completed this study. Troglitazone was well-tolerated by all subjects, without serious side-effects including elevation in serum transaminases (data not shown).

Table 1 shows baseline characteristics and changes in metabolic variables. The insulin resistant subjects were significantly older than normal subjects, and the estimated body mass index (BMI) was slightly larger but no subjects were obese. Weight and blood pressures did not change during the study. Fasting glucose and insulin levels and responses to oral glucose load did not change in normal subjects. In contrast both fasting glucose and insulin levels, and the response to oral glucose load were significantly reduced (p<0.05) after treatment with troglitazone for 4 weeks in the insulin resistant subjects. No significant changes occurred in total cholesterol, triglyceride or LDL cholesterol levels in control and insulin resistant subjects. However, HDL-cholesterol level was significantly increased after treatment with troglitazone in insulin resistant subjects.

Table 2 shows the baseline and changes of the PPD and plasma TBARS levels caused by treatment with troglitazone for 4 weeks. No significant changes were observed in normal subjects. However, PPD was significantly increased (p<0.01) and plasma TBARS was significantly reduced (p<0.05) in the insulin resistant subjects.

FMD of the brachial artery after reactive hyperemia was significantly impaired in the insulin resistant subjects compared to normal subjects at the baseline (Fig. 1). A significant negative correlation was observed between the FMD and AUC insulin of response to glucose load at the baseline (r = -0.64, p<0.05) (Fig. 2). No significant changes in NTG induced dilatation occurred in either group of subjects (Fig. 3), but FMD was significantly increased by troglitazone for 4 weeks in the insulin resistant subjects (Fig. 3).

Discussion

The present study demonstrated that the FMD of the brachial artery was impaired in insulin resistant subjects, and that troglitazone improved both the blunted vascular response and the insulin response.

The blunted response to reactive hyperemia in subjects
with the hyperinsulinemic response supports an association between insulin resistance and endothelial dysfunction. Troglitazone treatment resulted in a significant reduction in the insulin response to an oral glucose load only in the subjects with the hyperinsulinemic response, suggesting an improvement in insulin sensitivity. Assuming a link between insulin resistance and endothelial dysfunction, troglitazone could ameliorate this early process in atherosclerosis, primarily by improving insulin sensitivity.

Hypercholesterolemia and atherogenic lipid profiles are considered to be two causes of endothelial dysfunction. Troglitazone treatment also improved the atherogenic lipid profiles. This study found significant increases in HDL cholesterol level and PPD of LDL. These favorable metabolic changes may also contribute to the significant increase in FMD.

We cannot exclude the possibility that troglitazone directly influences the impaired endothelial function. Interestingly, troglitazone contains an α-tocopherol structure that has antioxidant properties. The antioxidant probucol improves endothelial dependent vascular relaxation via reducing vascular oxidant in cholesterol-fed rabbits (17). Antioxidative effects of troglitazone may be one of the mechanisms for improving FMD. Plasma TBARS levels were reduced in both insulin resistant and control subjects, but was statistically significant only in the insulin resistant subjects. Therefore, the antioxidative effects may be based on both the direct effects of troglitazone and the improvement of insulin sensitivity, including the beneficial lipid profile.

We cannot specify the main mechanism of troglitazone for improving the endothelial dysfunction. The above possibilities may all contribute to the improvement of endothelial dysfunction by troglitazone treatment in patients with insulin resistance. We need further studies to clarify these findings.

The present study appears to contrast to a recently published study by Tack et al. (18) that failed to demonstrate any beneficial effects of troglitazone on endothelial function in the obese subjects. They found that troglitazone treatment improved neither vascular response to
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Troglitazone, an endothelial dependent vasodilator, nor insulin induced vasodilatation, and concluded that troglitazone had no effects on endothelial function. However, the obese subjects included in their study had normal responses to acetylcholine even at baseline. Therefore, their findings agree with ours in that troglitazone does not affect the vascular response in healthy subjects with normal endothelial function.

In conclusion, insulin resistant subjects show blunted FMD of the brachial artery, and troglitazone improves this impaired vascular response. This finding suggests that primary treatment of insulin resistance could prevent the development of atherosclerosis by improving the endothelial function. Further prospective studies are urgently needed to determine whether troglitazone therapy results in a better clinical outcome in such patients.

References