Troglitazone, an Insulin Sensitizer, Increases Forearm Blood Flow in Humans
Shinichiro Fujishima, Yusuke Ohya, Yoshito Nakamura, Uran Onaka, Isao Abe, and Masatoshi Fujishima

To test whether troglitazone, a thiazolidinedione insulin sensitizer, increases the peripheral blood flow, the changes in forearm blood flow (FBF) were evaluated by venous occlusion plethysmography in 11 lean healthy male volunteers (age range, 24 to 39 years) after a single oral dose of 200 mg of troglitazone. Forearm vascular resistance (FVR) was calculated from FBF and blood pressure. Two hours after the dose, FBF increased from 3.66 ± 0.31 to 4.81 ± 0.57 mL/100 mL/min (P < .01), and FVR decreased from 24.7 ± 2.2 to 20.2 ± 2.2 units (P < .01), whereas both these values did not change during the control recordings obtained without troglitazone. Blood pressure, blood glucose levels, and serum immunoreactive insulin levels did not change significantly during the observation period. Serum concentrations of nitrate ions decreased from 27.0 ± 3.5 mmol/L to 23.1 ± 2.7 mmol/L (P < .01) after the administration. These results suggest that troglitazone increases muscular blood flow through vasodilation induced by a mechanism other than the correction of hyperinsulinemia or the increase in nitric oxide. The present study provides the first evidence that troglitazone dilates the vasculature in humans. Am J Hypertens 1998;11:1134–1137 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Thiazolidinedione, antidiabetic agent, peripheral blood flow.

Thiazolidinedione derivatives, such as troglitazone and pioglitazone, which increase insulin sensitivity in target tissues and correct hyperinsulinemia,1,2 have recently been reported to lower blood pressure (BP),1,3 although conflicting results exist.2 Because hyperinsulinemia could contribute to the pathogenesis of hypertension under some circumstances,4 the decrease in plasma insulin levels by thiazolidinediones would lower BP. On the other hand, thiazolidinediones have been shown in vitro to attenuate vasoconstriction5 as well as inhibit L-type Ca2+ currents in vascular smooth muscle cells.6–8 These direct vascular effects may also contribute to the depressor effects of thiazolidinediones. However, no studies have examined whether thiazolidinediones directly dilate the vasculature in humans. Because troglitazone is not dissolved in saline or any other detergents that can be safely injected to human, intravenous or intraarterial injection of this drug is not possible. Therefore, we measured forearm blood flow (FBF) and forearm vascular resistance (FVR) in healthy volunteers before and after administration of a single oral dose of troglitazone. We also measured the concentrations of blood glucose, serum insulin, and serum nitrate ions (NO3−) to assess the underlying mechanisms.

METHODS

Subjects Eleven Japanese male volunteers (age range, 24 to 39 years) were studied. They were healthy, normotensive, and not obese (body mass in-
TABLE 1. CHANGES IN VARIOUS PARAMETERS AFTER ADMINISTRATION OF TROGLITAZONE AND IN THE CONTROL TEST

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
</tr>
</thead>
<tbody>
<tr>
<td>Troglitazone</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>FBF (mL/100 mL/min)</td>
<td>3.66 ± 0.31</td>
<td>4.13 ± 0.28</td>
<td>4.25 ± 0.45</td>
<td>4.57 ± 0.52†</td>
<td>4.81 ± 0.57†</td>
</tr>
<tr>
<td>FVR (U)</td>
<td>24.7 ± 2.2</td>
<td>21.5 ± 1.8*</td>
<td>22.1 ± 2.4</td>
<td>21.0 ± 2.2*</td>
<td>20.2 ± 2.2*</td>
</tr>
<tr>
<td>SBP/DBP (mm Hg)</td>
<td>115 ± 4/66 ± 4</td>
<td>115 ± 5/67 ± 4</td>
<td>117 ± 6/67 ± 5</td>
<td>118 ± 4/68 ± 4</td>
<td>120 ± 6/68 ± 5</td>
</tr>
<tr>
<td>PR (beats/min)</td>
<td>59 ± 2</td>
<td>61 ± 2</td>
<td>59 ± 2</td>
<td>61 ± 2</td>
<td>61 ± 2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FBF (mL/100 mL/min)</td>
<td>4.29 ± 0.23</td>
<td>—</td>
<td>3.98 ± 0.21</td>
<td>—</td>
<td>3.82 ± 0.24</td>
</tr>
<tr>
<td>FVR (U)</td>
<td>19.8 ± 1.1</td>
<td>—</td>
<td>21.4 ± 1.4</td>
<td>—</td>
<td>22.2 ± 1.4</td>
</tr>
<tr>
<td>SBP/DBP (mm Hg)</td>
<td>113 ± 3/66 ± 3</td>
<td>—</td>
<td>113 ± 2/66 ± 3</td>
<td>—</td>
<td>112 ± 2/66 ± 3</td>
</tr>
<tr>
<td>PR (beats/min)</td>
<td>59 ± 2</td>
<td>—</td>
<td>58 ± 1</td>
<td>—</td>
<td>59 ± 2</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < .01 vs before the administration. FBF, forearm blood flow; FVR, forearm vascular resistance; SBP, systolic blood pressure; DBP, diastolic blood pressure; PR, pulse rate.

dex, 19.5 to 26.7 kg/m²). All subjects were nonsmokers, and were not taking medications. Their fasting blood glucose level (3.36 to 5.06 mmol/L) and hemoglobin A1c level (4.3% to 5.1%) were normal. Informed consent was obtained from all subjects before the study. The study protocol was approved by the ethics committee of our department.

Study Protocol The study was performed with subjects in the supine position after an overnight fast. Room temperature was maintained at approximately 25°C. Subjects took a 200-mg troglitazone tablet with 100 mL of water at 10:00 am after 30 min of bedrest. FBF, BP, and pulse rate (PR) were measured every 30 min from 10:00 am to noon. Blood samples were taken at 60-min intervals during the same period from a superficial vein of the left forearm to determine concentrations of blood glucose, serum immunoreactive insulin, and serum NO³⁻. Control data for FBF, BP, and PR for each subject were obtained every 60 min after taking 100 mL of water in the same condition on a separate day.

Measurement The FBF of the right arm was measured with mercury-in-Silastic strain-gauge plethysmography by a venous occlusion technique, as we have previously reported.9 FBF (measured as milliliters per 100 milliliters of forearm tissue per minute) was calculated from the rate of increase in forearm volume while venous return was being prevented by a cuff on the upper arm inflated with 40 mm Hg pressure. Circulation to the hand was arrested throughout the measurement by inflating a cuff around the wrist with 200 mm Hg pressure. FBF was measured eight times on each occasion and averaged. FVR was calculated by dividing the mean BP by the FBF. These values are expressed as arbitrary units throughout this report. Relative values of FBF and FVR, with the values at 10:00 am considered as 100%, were also calculated.

BP and PR in the left arm were measured by an automatic sphygmomanometer (BPM-300, Nippon Colin Co. Ltd., Komaki, Japan) using an oscillometric method. Measurements were obtained twice on each occasion and averaged.

Immunoreactive insulin levels were measured by radioimmunoassay. Glucose levels in the whole blood were measured by glucose oxidase method. Serum concentrations of NO³⁻ were measured by high-performance liquid chromatography, as described previously.10

Statistical Analysis Values are expressed as means ± SEM. The time-dependent changes in various parameters in response to troglitazone and in the control series were examined by analysis of variance (ANOVA) with repeated measures, followed by Duncan’s multiple comparison test. Two-factor repeated-measures ANOVA was used to evaluate statistical significance between the troglitazone response and the control values. Single comparisons between the values after administration of troglitazone and the corresponding control were made by the paired t test. A value of P < .05 was considered as statistical significance.

RESULTS

The FBF increased and FVR decreased after the administration of troglitazone. Neither FBF nor FVR changed significantly during the control recordings (Table 1). Relative changes in the FBF and FVR are shown in Figure 1. The increase in the relative FBF and the decrease in the relative FVR after taking troglitazone were significant compared with the control values (P < .01). There were no correlations between the
increase in relative FBF at 120 min and the baseline values of serum insulin, blood glucose, BP, or FBF (data not shown).

The BP and PR did not change significantly both after the administration of troglitazone and in the control recording. There were no significant differences in BP and PR between the day when troglitazone was taken and the day of the control tests (Table 1).

After the administration of troglitazone, there were no significant changes in blood glucose (before, 4.52 ± 0.16 mmol/L; 60 min after, 4.50 ± 0.13 mmol/L; 120 min after, 4.45 ± 0.13 mmol/L) and serum immuno-reactive insulin levels (before, 34.2 ± 3.7 pmol/L; 60 min after, 26.9 ± 3.1 pmol/L; 120 min after, 30.9 ± 4.0 pmol/L). Serum concentrations of NO$^{3-}$ significantly decreased after the administration of troglitazone (before, 27.0 ± 3.5 mmol/L; 60 min after, 24.8 ± 3.1 mmol/L; 120 min after, 23.1 ± 2.7 mmol/L; P < .01).

**DISCUSSION**

The present results show that the oral administration of troglitazone increases FBF in healthy subjects. Thiazolidinediones are reported to have direct vascular effects in vitro. For example, the micromole concentrations of the agent inhibit the contraction of isolated arteries, and also inhibit the Ca$^{2+}$ current in single vascular smooth muscle cells.\(^5\) Because pharmacokinetic studies after a single oral administration of troglitazone showed that plasma concentration ranged from 0.6 to 2.7 mmol/L,\(^1\) the agent could exert the direct vascular actions after an oral administration. The time course of FBF observed in the present study is consistent with that of the plasma concentration of troglitazone in a pharmacokinetic study;\(^1\) the plasma concentration reached a maximum level 60 min after a single oral dose and the half-life was about 9 h in healthy subjects.

Thiazolidinediones appear to act mainly on vascular smooth muscle cells to dilate the vasculature rather than the vascular endothelium, because the inhibitory action of pioglitazone persisted after removal of the endothelium\(^5\) and application of the nitric oxide (NO) inhibitor l-nitroarginine methyl ester.\(^7\) On the other hand, it was reported that incubation with insulin plus pioglitazone augments the vasodilation induced by acetylcholine,\(^12\) suggesting that pioglitazone augments the endothelium-dependent vasodilation mediated by insulin. In the present study, serum concentrations of NO$^{3-}$ decreased after the administration of troglitazone. Therefore, it is unlikely that the vasodilation induced by troglitazone is explained solely by the increase in production of NO. The decrease in the serum NO$^{3-}$ levels might be attributable to the changes in intracellular Ca$^{2+}$ concentrations or activities of nitric oxide synthase in vascular endothelial cells.\(^13\) In addition, FBF increased and FVR decreased without changes in the serum concentrations of insulin or glucose. Thus, improvement of hyperinsulinemia or glucose metabolism may not be associated with the increased FBF when troglitazone is acutely administered.

The blood pressure did not change after a single oral dose of troglitazone in the present study, although the FBF increased and FVR decreased. These blood pressure findings are comparable with those in a previous report in healthy volunteers.\(^1\) Compensatory mechanisms against a reduction of peripheral vascular resistance may maintain the blood pressure; for example, the pulse rate slightly increased in the present study, although it was not statistically significant. In hypertensive subjects with insulin resistance, Nolan et al\(^1\) and Ogihara et al\(^3\) reported that 8 to 12 weeks of chronic administration of troglitazone decreased the blood pressure. Because the subjects in the present study were not hypertensive and did not seem to have insulin resistance, a study in subjects with such abnormalities is required to determine whether direct vascular actions of troglitazone decrease the blood pressure.

Insulin-mediated glucose uptake occurs principally in skeletal muscle. Stimulation of skeletal muscle blood flow by insulin is impaired in insulin-resistant individuals, and this impairment could be associated with insulin-induced glucose uptake in insulin-sensitive tissues.\(^4\) Indeed, some vasodilating antihypertensive agents have been shown to improve insulin sensitivity.\(^14,15\) The increase in FBF by troglitazone, which reflects an increase in muscular blood flow, may improve insulin sensitivity.

In conclusion, the present study showed that troglitazone increases the peripheral blood flow and decreases the peripheral vascular resistance in healthy humans. We may hypothesize that these effects could
contribute not only to a lowering of blood pressure but also an improvement of insulin sensitivity. Such possibilities should be tested in further studies.

REFERENCES


