

# Differential Vasoactive Effects of the Insulin Sensitizers Rosiglitazone (BRL 49653) and Troglitazone on Human Small Arteries In Vitro

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BRL 49653 (rosiglitazone) and troglitazone are thiazolidinedione insulin-sensitizing agents, which are undergoing clinical evaluation as treatments for NIDDM. Potential side effects of thiazolidinediones include edema and hemodilution. Although the underlying mechanisms are presently unclear, animal and human studies have demonstrated a vasodilator action of troglitazone, which could in theory cause fluid retention. This *in vitro* study compared the direct vasodilator effects of troglitazone and BRL 49653 in small arteries ( $n = 44$ ) from human subcutaneous fat. In arterial rings with a functioning endothelium and precontracted with norepinephrine (NE;  $6 \mu\text{mol/l}$ ), troglitazone ( $n = 22$  vessels), but not BRL 49653 ( $1\text{--}100 \mu\text{mol/l}$ ), caused a concentration-related relaxation ( $69.4 \pm 5.2\%$  at  $100 \mu\text{mol/l}$ ;  $P < 0.01$ ). In the presence of indomethacin (IM;  $10 \mu\text{mol/l}$ ;  $n = 12$ ), this vasorelaxant effect of troglitazone was abolished ( $P < 0.01$  vs. troglitazone alone) and replaced by enhanced vasoconstriction ( $58.5 \pm 39.5\%$  over the NE baseline) similar in magnitude to that produced by troglitazone vehicle (ethanol) alone ( $n = 16$ ; NS vs. ethanol vehicle). By contrast, BRL 49653 ( $100 \mu\text{mol/l}$ ;  $n = 22$ ) and an equivalent volume of ethanol alone ( $n = 12$ ) caused similar degrees of vasoconstriction ( $18.7 \pm 14.6$  and  $22.5 \pm 8.0\%$ , respectively; NS). In the presence of IM ( $10 \mu\text{mol/l}$ ;  $n = 10$ ), the vasoconstrictor effect of BRL 49653 was enhanced ( $41.5 \pm 14.4\%$ ), although not significantly (NS vs. BRL 49653 alone or ethanol alone). Additional studies in Wistar rat arteries showed a similar vasodilator effect of troglitazone that was not inhibited by L-NAME ( $100 \mu\text{mol/l}$ ). The  $\alpha$ -tocopherol moiety alone had no vasorelaxant effect at concentrations up to  $300 \mu\text{mol/l}$ . Thus, in human arterial resistance vessels *in vitro*, BRL 49653 does not possess the direct, IM-sensitive vasorelaxant action of troglitazone. This vasodilation could, in theory, permit transmission of systemic pressure to the capillary bed. *Diabetes* 47:810–814, 1998

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Received for publication 16 June 1997 and accepted in revised form 26 January 1998.

ACh, acetylcholine; IM, indomethacin; NE, norepinephrine; PG, prostaglandin; PPAR, peroxisome proliferator-activated receptor; PSS, physiological salt solution.

There is growing interest in the potential use of the thiazolidinedione insulin-sensitizing agents, e.g., troglitazone, BRL 49653, and pioglitazone, as therapy for patients with NIDDM (1,2). In addition, the potential of troglitazone to prevent progression from impaired glucose tolerance to NIDDM is being studied in the Non-Insulin-Dependent Diabetes Primary Prevention Trial (3).

Thiazolidinediones enhance insulin sensitivity in animal models of insulin resistance (3–6), and reduction in the compensatory hyperinsulinemia is thought to be a consequence of this. More recently, troglitazone has been shown to have similar insulin-sensitizing effects in human NIDDM (7) and in obese, insulin-resistant, but nondiabetic subjects (2).

Troglitazone and pioglitazone have been shown to lower elevated arterial pressure in rat models of hypertension (8–10), and a blood pressure-lowering effect has been observed with troglitazone in humans (2).

Pioglitazone and troglitazone have been reported to cause dilation of peripheral blood vessels in animal studies (4,10). Skin blood flow was increased by subcutaneous injection of troglitazone in dexamethasone-induced diabetic obese Zucker rats and control Wistar rats; however, no such effect was seen with pioglitazone or BRL 49653, and the troglitazone-induced increase in skin blood flow was inhibited by indomethacin (IM) (4). Troglitazone increased prostaglandin (PG) $I_2$  production from isolated aortic rings and increased PGI $_2$  and PGE $_2$  production by 3T6 fibroblasts (4), further suggesting that the increased skin blood flow was mediated by locally generated vasodilator PGs. Pioglitazone has been shown to lower blood pressure when given to Sprague-Dawley rats, and it has a direct effect to blunt the contractile response of aortic rings to norepinephrine (NE), arginine vasopressin, and KCl *in vitro*; this effect of pioglitazone was absent when Ca $^{2+}$  was removed from the medium (10). Further work by Zhang et al. (11) suggested that the vascular effects of pioglitazone are due to inhibition of the depolarization-induced inward flux of Ca $^{2+}$  into vascular smooth muscle cells. These data suggest that both troglitazone and pioglitazone possess properties other than binding to and activating the peroxisome proliferator-activated receptor (PPAR)- $\gamma$ , the molecular target now widely believed to be central to the insulin-sensitizing action of the thiazolidinediones (12,13).

The possibility that side effects of thiazolidinediones, unrelated to their primary action as insulin-sensitizing agents, will also be present in humans is a subject of considerable scientific and therapeutic interest. In the case of troglitazone, the

only thiazolidinedione for which substantial clinical data are published, there are suggestions that mild fluid retention, manifested as edema, is found in a variable proportion of patients (14–18). It is possible that these effects have a hemodynamic origin, as some patients exhibit a fall in mean arterial pressure and calculated peripheral resistance, as well as an increase (presumably baroreceptor-reflex mediated) in cardiac index and stroke volume index (19).

In the present study, we have compared troglitazone and BRL 49653 for their ability to relax small arteries from human subcutaneous adipose tissue, as such an action may expose the capillary bed to the systemic blood pressure.

## RESEARCH DESIGN AND METHODS

Small resistance arteries ( $n = 44$ ; diameter  $\sim 250 \mu\text{m}$ ) were dissected from subcutaneous fat removed from patients ( $n = 7$ ) undergoing laparotomy for cholecystectomy under general anesthetic. All patients were otherwise healthy and receiving no medication preoperatively, received the same anesthetic agents (thiopentone sodium induction and isoflurane inhalation maintenance), and gave written informed consent. The study had approval by the Royal Liverpool University Hospital Trust Ethics Committee.

Acetylcholine (ACh), NE, and IM (Sigma, Poole, U.K.) were made up as fresh base solutions in physiological salt solution (PSS; composition [in mmol/l]: NaCl 119, KCl 4.7,  $\text{CaCl}_2 \cdot 2.5$ ,  $\text{MgSO}_4 \cdot 1.17$ ,  $\text{NaHCO}_3$  25,  $\text{KH}_2\text{PO}_4$  1.18, EDTA 0.026, glucose 5.5) for each experiment. Troglitazone and BRL 49653 (SmithKline Beecham) were dissolved in 96% ethanol.

The subcutaneous fat was immediately placed in cold PSS ( $4^\circ\text{C}$ ) that had been gassed with 5%  $\text{CO}_2$  in  $\text{O}_2$ . Arteries were dissected free of fat and connective tissue and mounted on two 40- $\mu\text{m}$  diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, U.K.), based on the principle of the Mulvany myograph (20), and gassed continuously with 5%  $\text{CO}_2$  in  $\text{O}_2$ .

After 1-h equilibration, the length-tension characteristics for each vessel were determined using the law of Laplace ( $P = T/r$ ; where  $P$  is the transmural pressure,  $T$  is the tension, and  $r$  is the vessel radius). Each vessel was then adjusted to the normalized diameter, i.e., that which it would achieve at rest in vivo under a transmural pressure of 100 mmHg. This has previously been shown to be the diameter at which the greatest force is generated (21). The myograph computer calculated the target tension each vessel should develop in response to a maximal stimulus.

Each vessel was constricted twice using KPSS (in which the NaCl of PSS was replaced by 125 mmol/l KCl) for 2 min, followed by a 10-min rest period. Any vessel failing to reach its predetermined target tension was discarded.

Endothelial viability was assessed by precontracting vessels with 6  $\mu\text{mol/l}$  NE; when a plateau tension was achieved ( $\sim 2$  min), the vessels were relaxed by exposure to a stepped ACh concentration in the range of 10 nmol/l to 10  $\mu\text{mol/l}$ . Vessels

failing to achieve at least 65% relaxation with ACh were assumed to be damaged and were discarded.

After a 30-min rest period, the vessels were again precontracted with 6  $\mu\text{mol/l}$  NE and then exposed either to troglitazone ( $n = 22$ ) or to BRL 49653 ( $n = 22$ ; 1–100  $\mu\text{mol/l}$ ), added to the bath in half-log-molar increments. Subsequently, to further study the possible mechanism of troglitazone-induced vasorelaxation, on completion of the relaxation-response curve to these agents, a number of the vessels were washed three times and incubated for 15 min in drug-free PSS. IM (10  $\mu\text{mol/l}$ ) was subsequently added; the vessels were incubated for a further 15 min; and then the cycle of precontraction with NE, stepped addition of thiazolidinedione and washing to remove the drugs, was repeated. Finally, after 60 min of incubation in drug-free PSS, the vessels were precontracted with NE and exposed to ethanol vehicle alone, added in volumes equivalent to those used during earlier stepped addition of thiazolidinedione, to determine the vasoactive effects attributable to ethanol vehicle alone.

**Wistar rat arteries.** In addition, we studied the effects of BRL 49653 and troglitazone in mesenteric arteries from 10-week-old, out-bred male Wistar rats ( $n = 6$ ) in the same system. After being primed twice with KPSS, vessels were contracted first with KPSS and then exposed to BRL 49653 or troglitazone (1–100  $\mu\text{mol/l}$ ). After washing out the bath, vessels were then contracted with 6  $\mu\text{mol/l}$  NE and again exposed to BRL 49653 or troglitazone first in the absence and then in the presence of  $\text{N}^G$ -nitro-L-arginine methyl ester (L-NAME) (100  $\mu\text{mol/l}$ ). In calcium-free PSS (i.e., PSS without the addition of  $\text{CaCl}_2$ ), the arteries were contracted with 6  $\mu\text{mol/l}$  NE and then exposed to BRL 49653, troglitazone, or ethanol vehicle. After contraction with 6  $\mu\text{mol/l}$  NE, arteries were exposed to  $\alpha$ -tocopherol (Sigma), freshly dissolved in distilled water, 100–300  $\mu\text{mol/l}$ .

**Statistical analysis.** Results were analyzed by Scheffé's multiple analysis, two-way analysis of variance using Arcus Pro-II computer software (Medical Computing, Aughton, U.K.). Differences were considered statistically significant when  $P < 0.05$ . Data are presented throughout as means  $\pm$  SE.

## RESULTS

**Human arteries.** There was no significant difference in diameters between vessels assigned to exposure to troglitazone ( $278.5 \pm 14.6 \mu\text{m}$ ) or to BRL 49653 ( $263.6 \pm 18.7 \mu\text{m}$ ;  $P = 0.4$ ). Moreover, vessels in the two groups displayed comparable endothelium-dependent relaxation when exposed to ascending concentrations of ACh. The maximum relaxation response was  $77.7 \pm 7.6\%$  for the vessels assigned to BRL 49653 and  $78.1 \pm 5.2\%$  for those subsequently exposed to troglitazone (NS between groups; Fig. 1).

Addition of troglitazone (22 vessels) induced a rapid-onset (20–30 s latency), concentration-related relaxation response over the range 10–100  $\mu\text{mol/l}$ . For each addition of troglitazone to the bath, responses plateaued after approximately 2 min. At the highest concentration, the relaxation was  $69.4 \pm 5.2\%$  (Fig. 2). In the presence of IM ( $n = 12$ ), the vasorelaxant action of troglitazone was abolished, and instead there was further constriction of the arteries ( $58.5 \pm 39.5\%$  at 100  $\mu\text{mol/l}$ ;  $P < 0.01$  vs. troglitazone alone). This increment in vascular tone was not significantly different from that seen with ethanol vehicle alone ( $n = 16$ ; Fig. 2).

In contrast to troglitazone, increasing concentrations of BRL 49653 did not cause vasorelaxation ( $n = 22$ ; Fig. 3), only further vasoconstriction ( $18.7 \pm 14.6\%$  above baseline) similar to that caused by ethanol alone ( $n = 12$ ;  $22.5 \pm 8.0\%$ ; NS). In the presence of IM, the vasoconstrictor response to BRL 49653 was slightly but not significantly augmented ( $n = 10$ ;  $41.5 \pm 14.4\%$  above the NE baseline; NS vs. BRL 49653 alone or ethanol alone).

**Wistar rat arteries.** BRL 49653, as in the human vessels, caused no relaxation of either KPSS or NE-precontracted arteries in either PSS or calcium-free PSS (data not shown).

Troglitazone caused relaxation of both KPSS and NE-precontracted arteries between 1 and 100  $\mu\text{mol/l}$ , with a maximum of  $96.3 \pm 1.0\%$  relaxation (Fig. 4). The presence of L-NAME (100  $\mu\text{mol/l}$ ) had no effect on the relaxation, with a

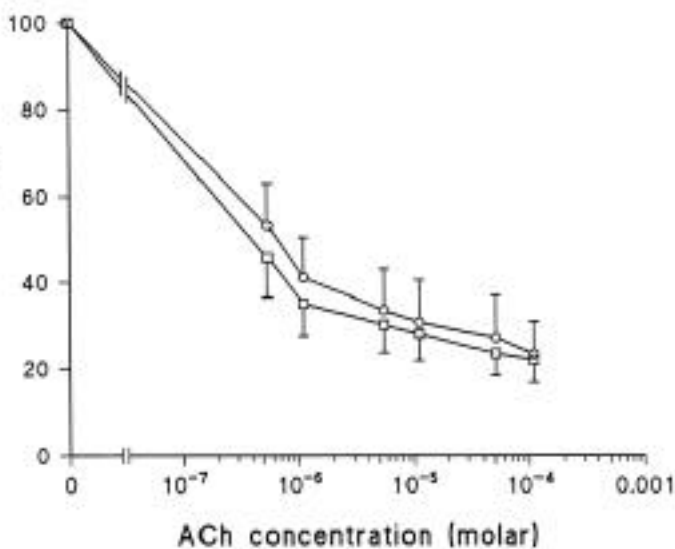


FIG. 1. ACh dose-relaxation curves in NE-precontracted arteries before exposure to BRL 49653 or troglitazone. ○, BRL 49653 ( $n = 22$ ); □, troglitazone ( $n = 22$ ). NS between curves.

maximum  $94.5 \pm 0.9\%$  relaxation with  $100 \mu\text{mol/l}$  troglitazone (Fig. 4). In calcium-free PSS, troglitazone caused a similar degree of relaxation: maximal  $94.8 \pm 1.5\%$  with  $100 \mu\text{mol/l}$  troglitazone (Fig. 4).

There was no relaxation observed with  $\alpha$ -tocopherol at concentrations up to  $300 \mu\text{mol/l}$ ; higher concentrations resulted in the compound coming out of solution in the organ bath.

## DISCUSSION

In the present study, troglitazone caused concentration-related relaxation of NE-precontracted human subcutaneous resistance arteries in vitro, and the response was inhibited by the cyclooxygenase inhibitor IM. These data suggest that in human small arteries, troglitazone-induced vasodilation is PG mediated. The observations in this study are consistent with the earlier report that troglitazone increases skin blood flow in both normal rats and dexamethasone-induced diabetic obese Zucker rats and that this effect is also prevented by IM (4). In rat aortic rings, troglitazone has been shown to augment production of  $\text{PGI}_2$  (4), although no attempt was made in the present study to identify the vasodilator prostanoid. Song et al. (22) have recently demonstrated that in the tail artery from Wistar rats, troglitazone decreased NE-induced contractile responses by a mechanism not dependent on nitric oxide production but associated with inhibition of  $\text{Ca}^{2+}$  currents in cultured vascular smooth muscle cells. These results do suggest some similarity between the effects of troglitazone on blood vessels from the rodent and humans in vitro. We also showed no effect of L-NAME on troglitazone-induced relaxation in rat vessels; however, our vessels also relaxed after NE-precontraction in calcium-free PSS, suggesting that a mechanism other than that causing closure of smooth muscle calcium channels is in operation. It may be that more than one mechanism explains the vasodilation seen with troglitazone and thus the apparent difference between our observation and those of Song et al. (22); however, like them we propose that the mechanism does not involve nitric oxide release. There is no evidence from our study to suggest that the  $\alpha$ -tocopherol moiety of troglitazone is vasoactive, even at concentrations threefold higher than the maximum concentration of troglitazone studied.

Interestingly, there is also evidence of a vasorelaxant action of troglitazone in humans (19). In that study, designed to detect cardiac hypertrophy by echocardiography in NIDDM patients during prolonged treatment with troglitazone (800 mg per day), there was evidence of a fall in calculated peripheral resistance at 12, 24, 36, and 48 weeks. Our results demonstrating a vasodilator effect of troglitazone on human resistance arteries in vitro provide a possible explanation for the results observed in vivo during long-term therapy. It is unknown at present whether the vasodilator action of troglitazone in humans can be antagonized by cyclooxygenase inhibition in vivo.

Blood flow in the microvascular circulation is normally controlled by a variety of neural, humoral, and paracrine mechanisms and has been extensively studied in IDDM. Hemodynamic abnormalities observed in the microcirculation of patients with IDDM include an increase in blood flow in the retina, kidney, and skin; this increase may predate the development of established microvascular complications.

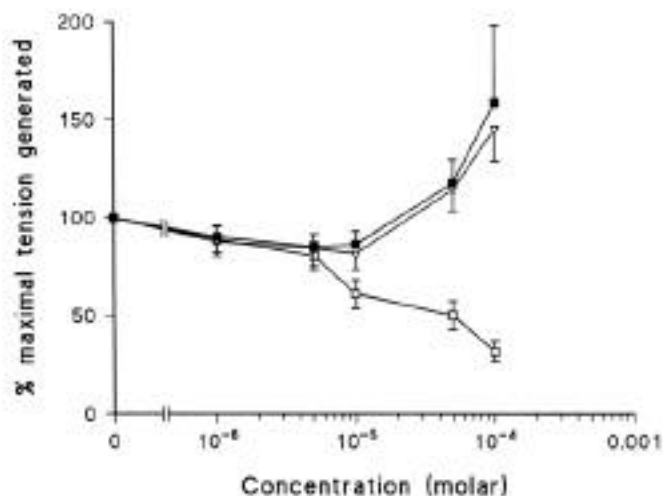


FIG. 2. Troglitazone dose-response curve, with and without IM ( $10^{-5}$  mol/l).  $\square$ , troglitazone;  $\blacksquare$ , troglitazone and IM ( $P < 0.01$  vs. troglitazone alone);  $\nabla$ , ethanol vehicle (NS vs. troglitazone and IM).

Microcirculatory function is less easily studied early in NIDDM, as there is often a long preclinical phase before the diagnosis of diabetes is made. Basal capillary pressure is not raised, and it has been proposed that the microvasculature is relatively protected from the effects of transmitted systemic blood pressure by changes in the precapillary resistance arteries, possibly due to the insulin-resistant state that precedes overt NIDDM (23–25). Although a vasodilator and thus blood pressure-lowering effect of the thiazolidinediones may be beneficial, it could theoretically be disadvantageous to dilate the precapillary resistance arteries and allow transmission of arterial blood pressure to the capillary circulation, with possible acceleration of microangiopathy.

Insulin has been shown to act as a vasodilator in a number of vascular beds (26,27), and this appears to be due to an effect of insulin to stimulate nitric oxide release (28–30).

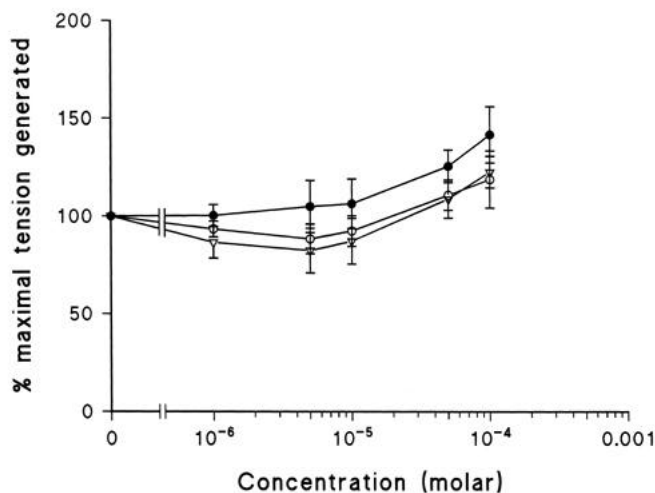


FIG. 3. BRL 49653 dose-response curves, with and without IM, compared with vehicle alone.  $\circ$ , BRL 49653;  $\bullet$ , BRL 49653 and IM ( $10^{-5}$  mol/l);  $\nabla$ , ethanol vehicle alone.

Insulin-induced vasodilation is impaired in subjects with insulin resistance due to obesity (31), NIDDM (32), and essential hypertension (33). It is possible that thiazolidinediones may lower blood pressure by enhancing any tonic vasodilator response to insulin, thereby reducing peripheral arterial resistance independent of, and possibly in addition to, any direct vasodilator action. In addition, by reducing plasma insulin levels, these drugs may reduce the potential blood pressure-raising actions of insulin, such as renal sodium reabsorption (8) and increased sympathetic activity (34,35).

It has been suggested that peroxisome proliferators could bind to fatty acid binding protein and displace fatty acids, which are then able to activate PPAR (36). If this were the case, then troglitazone may particularly stimulate arachidonic acid release, which would act as a substrate for cyclooxygenase, with enhanced PG production and the vasodilation observed in this study. However, it is not clear why this is not also seen with BRL 49653.

From this study, BRL 49653 does not exhibit vasorelaxant properties in human resistance arteries in vitro. Because no hemodynamic data have yet been published on BRL 49653, it is not possible to say whether the results observed in vitro translate to an absence of vasodilation in vivo. For the present, our findings constitute an interesting distinction between the drugs; however, it is premature to say whether these differences will also form a basis for distinguishing between the agents in terms of adverse events or theoretical benefits or disadvantages in the treatment of patients with NIDDM.

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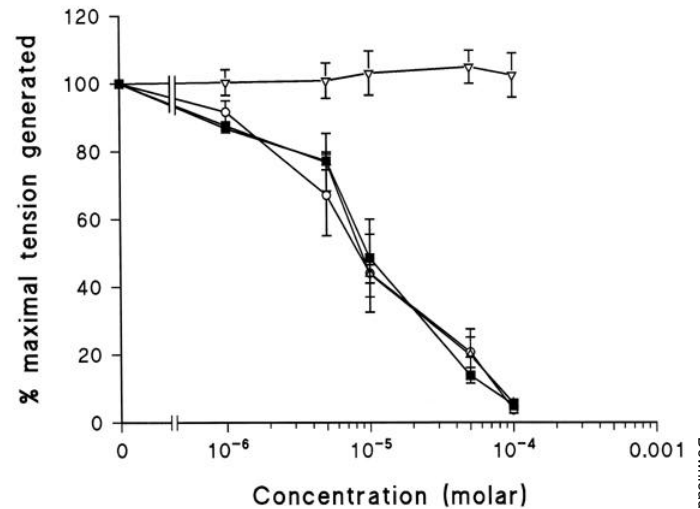


FIG. 4. Wistar rat mesenteric arteries. ■, troglitazone dose-response curve in PSS; △, troglitazone dose-response curve in calcium-free PSS; ○, troglitazone dose-response curve with 100  $\mu$ mol/l L-NAME in PSS; ▽, ethanol vehicle in calcium-free PSS.

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