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REVIEW

Thiazolidinediones – Tools for the Research of Metabolic Syndrome X

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Summary

The resistance to insulin (insulin resistance, IR) is a common feature and a possible link between such frequent disorders as non-insulin dependent diabetes mellitus (NIDDM), hypertension and obesity. Pharmacological amelioration of IR and understanding its pathophysiology are therefore essential for successful management of these disorders. In this review, we will discuss the mechanisms of action of thiazolidinediones (TDs), a new family of insulin-sensitizing agents. Experimental studies of various models of IR and an increasing number of clinical studies have shown that TDs normalize a wide range of metabolic abnormalities associated with IR. By improving insulin sensitivity in skeletal muscles, the adipose tissue and hepatocytes, TDs reduce fasting hyperglycaemia and insulinaemia. Furthermore, TDs markedly influence lipid metabolism – they decrease plasma triglyceride, free fatty acid and LDL-cholesterol levels, and increase plasma HDL-cholesterol concentrations. Although TDs do not stimulate insulin secretion, they improve the secretory response of beta cells to insulin secretagogues. TDs act at various levels of glucose and lipid metabolism – ameliorate some defects in the signalling cascade distal to the insulin receptor and improve glucose uptake in insulin-resistant tissues *via* increased expression of glucose transporters GLUT1 and GLUT4. TDs also activate glycolysis in hepatocytes, oppose intracellular actions of cyclic AMP, and increase intracellular magnesium levels. TDs bind to peroxisome proliferator activating receptors γ (PPARγ), members of the steroid/thyroid hormone nuclear receptor superfamily of transcription factors involved in adipocyte differentiation and glucose and lipid homeostasis. Activation of PPARγ results in the expression of adipocyte-specific genes and differentiation of various cell types in mature adipocytes capable of active glucose uptake and energy storage in the form of lipids. Furthermore, TDs inhibit the pathophysiological effects exerted by tumour-necrosis factor (TNFα), a cytokine involved in the pathogenesis of IR. These effects are most likely also mediated by stimulation of PPARγ. In mature adipocytes, PPARγ stimulation inhibits stearoyl-CoA desaturase 1 (SCD1) enzyme activity resulting in a change of cell membrane fatty acid composition. Apart from their metabolic actions, TDs modulate cardiovascular function and morphology independently of the insulin-sensitizing effects. TDs decrease blood pressure in various models of hypertension as well as in hypertensive insulin-resistant patients, and inhibit proliferation, hypertrophy and migration of vascular smooth muscle cells (VSMC) induced by growth factors. These processes are considered to be crucial in the development of vascular remodelling, atherosclerosis and diabetic organ complications. TDs induce vasodilation by blockade of Ca^{2+} mobilisation from intracellular stores and by inhibition of extracellular calcium uptake *via* L-channels. Furthermore, TDs interfere with pressor systems (catecholamines, renin-angiotensin system) and enhance endothelium-dependent vasodilation. A key role of TDs effects in vascular remodelling is played by inhibition of the mitogen-activated protein (MAP) kinase pathway. This signalling pathway is important for VSMC growth and migration in response to stimulation with

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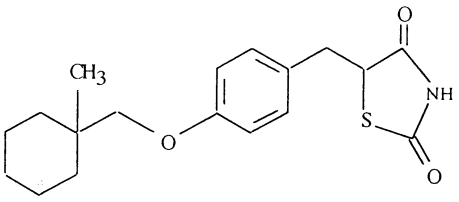
tyrosine-kinase dependent growth factors. In addition to the vasoprotective mechanisms mentioned above, troglitazone, the latest representative of this pharmacological group, possesses antioxidant actions comparable to vitamin E. In summary, TDs have the unique ability to attack mechanisms responsible for metabolic alterations as well as for vascular abnormalities characteristic for IR. Therefore, TDs represent a powerful research tool in attempts to find a common denominator underlying the pathophysiology of the metabolic syndrome X. A recently reported link between MAP kinase signalling pathway and PPARγ transcriptional activity suggests that this research direction is promising.

Key words
Non-insulin dependent diabetes mellitus – Insulin resistance – Hypertension – Thiazolidinediones – Troglitazone.

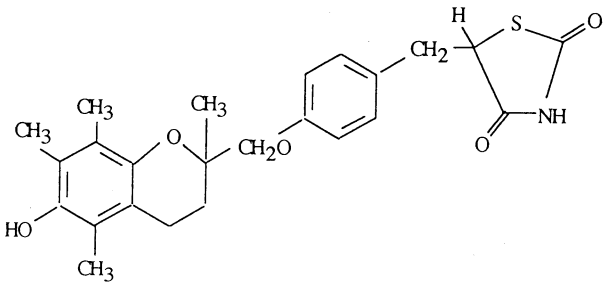
1. Introduction

Insulin resistance is defined as a state in which a given insulin concentration produces less than the expected bioeffect. Two metabolic defects usually cooperate in insulin-resistant states. Firstly, an insuficient response of hepatocytes to insulin actions leads to unopposed gluconeogenesis and increased production of lipoproteins by the liver. Second, the defect resides in peripheral tissues such as skeletal muscles or adipose tissue resulting in impaired insulin-stimulated glucose utilisation. Elevated plasma levels of free fatty acids and substrates for gluconeogenesis are also typical features of insulin resistance. Insulin resistance is a hallmark of non-insulin dependent diabetes mellitus (NIDDM) and often accompanies other common disorders such as hypertension, coronary heart disease or obesity. Association of these disorders with an atherogenic lipid profile, microalbuminuria, procoagulant state and other abnormalities is usually referred to as syndrome X or metabolic syndrome. This close association suggests common underlying mechanisms of these disorders. In this review, we will discuss the pharmacological effects and mechanisms of actions of thiazolidinediones (TDs), a novel group of compounds with a strong insulin-sensitizing effect. Considering the wide range of beneficial metabolic effects of these compounds, it is likely that detailed knowledge of the mechanisms of their pharmacological actions will contribute considerably to understanding the pathogenesis of insulin resistance. Furthermore, recent observations suggest that, in addition to their metabolic actions, TDs also influence vascular biology. Thus, independently of their metabolic actions, TDs may represent a new approach to organ and vascular protection in NIDDM and hypertension.

The first representative of this group, ciglitazone, discovered in 1982, was followed by the synthesis of other derivatives – englitazone, pioglitazone, and troglitazone. All share a common thiazolidine-2-4-dione structure (Fig. 1) which is responsible for the majority of pharmacological actions. Troglitazone was synthesized by alpha-tocopherol chain substitution in an attempt to produce a drug which would also have an antioxidant effect.



CIGLITAZONE



TROGLITAZONE

Fig 1. *Chemical structure of ciglitazone and troglitazone.*

2. Metabolic Actions of Thiazolidinediones

2.1. Effect of thiazolidinediones on glucose and lipid metabolism in experimental and clinical studies

Thiazolidinediones reduce hyperglycaemia and elevated plasma insulin levels in various experimental models of insulin resistance and NIDDM such as obese-diabetic yellow KK mice, Zucker fatty rats, old

Sprague-Dawley rats, *ob/ob* mice, spontaneously diabetic Chinese hamsters, fructose-fed rats, obese insulin-resistant Rhesus monkeys and Watanabe heritable hyperlipidaemic rabbits (Chang *et al.* 1983, Fujita *et al.* 1983, Fujiwara *et al.* 1988, Yoshioka *et al.* 1993, Kemnitz *et al.* 1994, Lee *et al.* 1994, Saku *et al.* 1997). Using euglycaemic hyperinsulinaemic clamp studies performed in insulin-resistant animals, it has been demonstrated that TDs restore the ability of insulin to suppress hepatic glucose production (Lee *et al.* 1994, O'Rourke *et al.* 1997) and enhance peripheral glucose uptake (Lee *et al.* 1994). *In vitro* studies with isolated adipose tissue and skeletal muscles confirmed that the *in vivo* effects mentioned above are associated with a marked increase in insulin sensitivity. TDs also improve the beta cell response to insulin secretagogues (Masuda *et al.* 1995). However, in contrast to sulphonylurea derivatives, TDs do not stimulate insulin secretion. Changes in the general characteristics of carbohydrate metabolism, such as plasma glucose, triglyceride and insulin levels in response to treatment with TDs, have been demonstrated only in insulin-resistant animals, whereas no effect was observed in normal or insulin-deficient animals (Fujita *et al.* 1983). These observations highlight one specific feature of these agents, namely a lack of hypoglycaemic activity in euglycaemic animals despite the potent sensitization of insulin action. Yet, using more sensitive methods, it is also possible to detect an improvement in peripheral and hepatic actions of insulin in normal animals (Lee and Olefsky 1995). The magnitude of the pharmacological effect of TDs on the carbohydrate metabolism is closely related to the plasma insulin levels, the most prominent effect being observed in models with the highest insulinaemia.

Table 1. Serum triglyceride concentrations in hereditary hypertriglyceridaemic (HTG) rats fed for 14 days a high-sucrose diet, without (HS diet) or with troglitazone (HS diet + TRO) in the satiated state and after overnight fasting (Vrána *et al.*, unpublished data).

	Fed (mmol/l)	Fasted (mmol/l)
n	13	14
HS diet	10.9±1.3	5.8±0.6
HS diet + TRO	5.2±0.5*	2.9±0.4*

*Data are given as means ± S.E.M.; *p<0.001 HS diet + TRO vs. HS diet*

Alterations in lipid metabolism, in particular elevated levels of triglycerides (TG) and non-esterified fatty acids (NEFA), and a decrease in HDL-cholesterol, often accompany NIDDM and other insulin-resistant states. In various insulin-resistant experimental models, including a model of severe hereditary hypertriglyceridaemia (Table 1), TDs markedly reduce plasma TG and NEFA levels (Fujita *et al.* 1983, Fujiwara *et al.* 1988, Kemnitz *et al.* 1994, Lee *et al.* 1994, Kaumi *et al.* 1996, Sreenan *et al.* 1996). This is achieved by inhibition of TG or VLDL synthesis in the liver (Fujiwara *et al.* 1988) as well as by increased clearance in the periphery (Kaumi *et al.* 1996). The hypolipidaemic effect of TDs is not, however, limited to insulin-resistant animals and has also been observed in streptozotocin diabetic rats (Shimabukuro *et al.* 1996). This shows that the effect of TDs on plasma lipids and lipoproteins may not be fully dependent on insulin-sensitization and suggests that these compounds exert their metabolic actions *via* multiple pharmacological mechanisms. Furthermore, Castle *et al.* (1992) showed that TDs also decrease plasma LDL-cholesterol levels and induce a rise in HDL-cholesterol in diabetic insulin-resistant KKA mice. Unlike TG or VLDL, these parameters are not so closely related to insulin metabolism. Putting this together, experimental studies have provided convincing evidence that TDs ameliorate all important metabolic derangements associated with insulin resistance.

The wide range of beneficial effects of TDs on glucose and lipid metabolism, observed in experimental animals, has been recently confirmed in a series of clinical double-blind, placebo-controlled studies in patients with NIDDM (Iwamoto *et al.* 1996a, 1996b, Kumar *et al.* 1996, Ghazzi *et al.* 1997), impaired glucose tolerance (Berkowitz *et al.* 1996, Antonucci *et al.* 1997) and obesity (Nolan *et al.* 1994). All these studies were performed with troglitazone (TRO), the only thiazolidinedione which has so far been approved for clinical trials. In addition to the studies in patients with frequent disorders mentioned above, TRO has also been proven to be effective in rare disorders associated with insulin resistance. In a polycystic ovary syndrome, which is characterized by defects in insulin action, insulin secretion, ovarian steroidogenesis and fibrinolysis, TRO improved insulin sensitivity and beta-cell response to a challenge with glucose. The normalization of plasma levels of steroid hormones resulted in improved reproductive functions in some patients. Moreover, treatment with TRO was associated with a marked reduction of the plasminogen activator inhibitor (PAI-1) which could be expected to improve the fibrinolytic response to thrombosis in these subjects (Dunaif *et al.* 1996, Ehrmann *et al.* 1997). In Werner's syndrom, a rare inherited form of insulin resistance due to defective signalling distal to the insulin receptor, TRO ameliorates glucose intolerance mediated by increased insulin sensitivity assessed by glucose tolerance tests or the euglycaemic clamp (Takino *et al.* 1994, Izumino *et al.* 1997).

2.2. Mechanisms of metabolic actions

An intensive search for molecular mechanisms responsible for pharmacological actions of TDs suggests that these agents are able to act at various levels of glucose and lipid metabolism. TDs may ameliorate some defects in the signalling cascade distal to the insulin receptor. *In vitro*, pioglitazone completely prevented glucose-induced impairment of the insulin receptor kinase activity suggesting that the drug might reverse the processes which are critical for the glucose-induced desensitization of insulin receptor (Maegawa *et al.* 1993). Administration of the same drug to obese Wistar rats resulted in a reversal of the decline in insulin receptor tyrosine kinase and insulin-stimulated phosphatidylinositol 3-kinase activities in skeletal muscles (Hayakawa *et al.* 1996). In insulin-resistant obese KKA mice, pioglitazone restored the expression of genes coding for proteins involved in the receptor signalling cascade (Bonini *et al.* 1995). Although the activation of insulin receptor signalling events is not considered to be one of the most important insulin-sensitizing mechanisms, it may play a major role in ameliorating receptor alterations due to hyperglycaemia, the so-called glucose toxicity (Saltiel and Olefsky 1996). An improvement of glucose uptake in insulin-resistant tissues such as skeletal muscle and fat, a distinguishing feature of TDs is associated with increased expression of glucose transporters GLUT1 and GLUT4 (Ciaraldi *et al.* 1990, Hofmann *et al.* 1991, Sandouk *et al.* 1993). These increases may not, however, be the result of a direct effect of TDs on glucose transporter gene promoters. Tafuri (1996) reported that increased expression of these transporters occurred secondarily to the acceleration of fat cell differentiation (see below). The activation of glucose transporters occurs not only at these typical sites of insulin resistance but also in the beta cells (Masuda *et al.* 1995). This phenomenon may explain an improvement in beta cell secretory response after the thiazolidine treatment (Cavaghan *et al.* 1997). In addition to their effects on glucose transporters, TDs may influence glucose metabolism by activation of glycolysis in hepatocytes (Murano *et al.* 1994), by interference with intracellular actions of cyclic AMP which usually opposes insulin actions (Sizer *et al.* 1994), and by increasing intracellular magnesium levels (Nadler and Scott 1994).

At least some important metabolic effects of TDs seem to be mediated by binding of these compounds to specific nuclear target structures known as peroxisome proliferator activating receptors (PPAR), members of the steroid/thyroid hormone receptor superfamily of transcription factors involved in adipocyte differentiation and glucose and lipid homeostasis (Schoonjans *et al.* 1996). Thus far, three major PPAR family member have been identified, α, γ and β. PPARα, known to be a receptor for widely used fibrate class of lipid-lowering drugs, mediates the regulation of lipoprotein gene expression. The current view is that TDs are high-affinity ligands for the PPARγ (Lehman *et al.* 1995). Activation of these receptors results in expression of adipocyte-specific genes and differentiation of various cell types in mature adipocytes capable of active glucose uptake and energy storage in the form of lipids (Sandouk *et al.* 1993, Teboul *et al.* 1995, Gimble *et al.* 1996). It has also been shown that TDs-induced stimulation of the PPARγ represses the *ob gene* (De Vos *et al.* 1996). This recently discovered gene (Zhang *et al.* 1994c) is exclusively expressed by adipocytes and plays an important role in energy metabolism (Frederich *et al.* 1995). This finding is rather surprising since the *ob gene* protein product, leptin, induces weight loss, reduces food intake, increases energy expenditure, and normalizes elevated insulin and glucose levels in ob/ob mice (Weigle *et al.* 1995). This factor also increases insulin sensitivity in normal rats (Sivitz *et al.* 1997).

In accordance with the observation showing the PPARγ-mediated repression of *ob gene*, Nolan *et al.* (1996) reported that troglitazone attenuated leptin production from human adipocytes *in vitro* and abolished the increment in leptin production induced by prolonged exposure to insulin *in vivo* without any change in plasma leptin levels. This is in contrast to the documented insulinomimetic properties of TDs and observations demonstrating an improvement in insulin sensitivity in normal rats. Most likely, there is no important physiological relationship, however, further studies are needed to explore this possibility.

It has been shown that tumour necrosis factor α (TNFα) is synthesized and secreted from adipocytes and that elevated tissue TNFα mRNA and protein expression is a common feature of genetically obese/insulin-resistant rodents (Hotamisligil *et al.* 1993) and obese humans (Kern *et al.* 1995). This factor causes insulin resistance *in vitro* (Hotamisligil *et al.* 1995) and *in vivo* both in humans and rodents (Van Der Poll *et al.* 1991, Miles *et al.* 1997). *In vitro*, TNFα interferes with insulin receptor signalling by decreasing insulin receptor tyrosine kinase activity (Hotamisligil *et al.* 1995), blocks adipocyte differentiation and expression of fatty acid binding protein and GLUT4 in these cells (Szalkowski *et al.* 1995). TDs inhibit the pathophysiological effects exerted by TNFα and thus improve insulin sensitivity *in vitro* and *in vivo* (Ohsumi *et al.* 1994, Szalkowski *et al.* 1995, Miles *et al.* 1997, Peraldi *et al.* 1997). These pharmacological effects are most likely mediated *via* stimulation of PPARγ (Ohsumi *et al.* 1994, Szalkowski *et al.* 1995, Peraldi *et al.* 1997). As has been postulated, the thiazolidinedione effects mediated *via* PPARγ deal with adipocyte cell precursors causing their differentiation and expression of specific genes. However, it is of interest whether TDs also possess some PPARγ-mediated actions in mature adipocytes. In a recent report (Kurebayashi *et al.* 1997), TRO as well as pioglitazone inhibited

stearoyl-CoA desaturase 1 (SCD1) enzyme activity by repressing the SCD1 gene expression in fully differentiated 3T3-L1 adipocytes. This enzyme catalyzes desaturation of palmitic and stearic acid leading to the formation of palmitoleic and oleic acid, important constituents of membrane phospholipids. Alterations in the ratio of stearic acid to oleic acid influences cell membrane fluidity, which has been implicated in insulin action (Spector and Yorek 1985), and the TDs-induced change in cell membrane fatty acid composition may directly improve the efficacy of the hormone.

The key role of PPARγ in mediating thiazolidinedione pharmacological actions has been recently challenged by the fact that its expression is highest in the adipose tissue and much lower levels have been detected in skeletal muscle (Braissnt *et al.* 1996) which are the major site of insulin resistance. Furthermore, TRO also exerts antidiabetic actions in aP2/DTA mice (Burant *et al.* 1997). In this particular model, the brown and white fat tissue was eliminated by genetic manipulation and the animals fed a normal diet had suppressed leptin levels, hyperlipidaemia, hyperglycaemia, and elevated insulin levels suggestive of insulin-resistant diabetes. Although TRO cannot act *via* its adipose tissue-specific mechanisms in this model, treatment with this drug normalized glucose tolerance and alleviated metabolic abnormalities. Interestingly, PPARγ levels in the liver of the aP2/DTA mice were not changed by the TRO treatment. It is therefore possible that, apart from the above discussed mechanisms, there are some other so far unidentified modes of thiazolidinedione action operating especially in skeletal muscles and possibly also in the liver.

3. Vascular Actions of Thiazolidinediones

The association of insulin resistance and hypertension has been well documented in numerous epidemiological studies. Interventions leading to improvement of insulin sensitivity, such as body weight loss, are associated with a decrease in blood pressure. Provided that the treatment with TDs ameliorates insulin resistance, it could also be expected to reduce elevated blood pressure. As will be shown in the following part of this review, this hypothesis has been tested in experimental as well as in clinical conditions. Furthermore, there is increasing evidence suggesting the ability of these agents to modulate the function and morphology of vascular smooth muscle cells (VSMC) independently of the insulin-sensitizing effects.

3.1. Effects of thiazolidinediones on blood pressure and cardiovascular morphology in experimental and clinical studies

TDs decrease blood pressure in various models of insulin resistance and hypertension. This effect was reported in Zucker obese rats (Pershadsingh *et al.* 1993, Yoshioka *et al.* 1993), fructose-fed rats (Buchanan *et al.* 1995, Chen *et al.* 1996), and obese monkeys (Kemnitz *et al.* 1994). In these models, the blood pressure-lowering effect was associated with an improvement of insulin sensitivity and was comparable to the effects of ACE inhibitors and AT^1 angiotensin receptor blockers in fructose-fed rats (Chen *et al.* 1996). In contrast to the above observations, TDs did not influence blood pressure in another insulin-resistant, severely hypertensive model – the spontaneously hypertensive rat (SHR) (Katayama *et al.* 1994). This suggested that the blood pressure-lowering effect of TDs may not always be linked to insulin-sensitization. Indeed, Zhang *et al.* (1994b) compared the effects of pioglitazone on the blood pressure in salt-sensitive Dahl rats, an insulin-resistant hypertensive model, with a hypertensive model with normal insulin sensitivity (Goldblatt hypertensive rat). Pioglitazone decreased blood pressure in both models, however, insulin sensitivity was improved only in the Dahl rat. Metformin, a traditional insulin sensitizer, did not influence blood pressure in any of these models.

Recent experiments have been focused on another very important area of thiazolidinedione vascular action. *In vitro* studies have shown that TDs inhibit the proliferation, hypertrophy and migration of VSMC induced by numerous growth factors including insulin (Dubey *et al.* 1993, Law *et al.* 1996). These processes are considered to be crucial in the development of vascular remodelling, atherosclerosis and diabetic organ complications. Relevance of these *in vitro* observations has been confirmed *in vivo* studies. Law *et al.* (1996) reported an inhibitory effect of TRO on neointima formation following balloon vascular injury. The vasoprotective effect of TDs and their role in the prevention of diabetic complications have also been suggested by Yoshimoto *et al.* (1997) in genetically obese diabetic rats. In addition to the decrease in blood pressure, treatment with pioglitazone prevented glomerular injury, renal arteriolosclerosis and aortic medial wall thickening in these rats. Similar to the blood pressure-lowering effects, the TDs-induced organ protection is not limited to insulin-resistant models and is not necessarily associated with improvement of insulin sensitivity. Shimabukuro *et al.* (1996) demonstrated beneficial cardioprotective effects of TRO on cardiomyopathy associated with streptozocin diabetes in the rat. This diabetic model is characterized by low or absent plasma insulin due to beta cell damage and normal insulin sensitivity. In addition to other organ complications, streptozotocin-diabetic rats demonstrate ultrastructural heart changes such as alterations in the myofibrils, mitochondria, nuclear membrane and cytoplasm associated with functional impairment. In this model, TRO partially normalized the increased basal heart rate, cardiac output, and postischaemic functional deficit. Beneficial

effects of TRO treatment were also evident after ultrastructural analysis of cardiomyocytes.

In contrast to its metabolic effects, the influence of TRO on blood pressure in insulin-resistant patients are less convincing. Although some authors have observed blood pressure decreases in obese (Nolan *et al.* 1994) and NIDDM patients (Ogihara *et al.* 1995, Ghazzi *et al.* 1997), other authors have reported negative results (Iwamoto *et al.* 1996a,b, Kumar *et al.* 1996, Antonucci *et al.* 1997). The absence of the TRO effect on blood pressure does not, however, exclude the possibility that this agent possesses antihypertensive actions. The majority of clinical studies has so far been designed to test the metabolic actions of TRO as primary endpoints. Further clinical studies, focusing primarily on the cardiovascular effects and on the possibility of preventing diabetic complications and atherosclerosis are needed to elucidate this issue.

3.2. Mechanisms of vascular actions

Besides their metabolic actions, TDs may influence vascular tone and morphology at various levels. Pershadsingh *et al.* (1993) demonstrated that ciglitazone prevented platelet-derived growth factor (PDGF)-induced mobilisation of intracellular calcium in the VSMC. Buchanan *et al.* (1995) described that pioglitazone treatment significantly reduced the contractile responses of aortic rings to norepinephrine, vasopressin and potassium chloride. The blunting was mediated by a blockade of extracellular calcium uptake by the VSMC. This suggested an ability of TDs to act as calcium channel blockers. Indeed, TDs have been shown to inhibit L-type Ca^{2+} channels (Zhang *et al.* 1994a, Song *et al.* 1997). It should be noted that the same type of channels is inhibited by a widely used dihydropyridine class of antihypertensive agents. There is evidence that extracellular glucose leads to increased intracellular calcium levels which may contribute to cardiovascular complications associated with hyperglycaemia (Barbagallo *et al.* 1995). Therefore, by ameliorating intracellular Ca^{2+} overload in the VSMC, TDs may cassualy influence one of the mechanisms implicated in the development of hypertension in NIDDM. Hyperglycaemia also induces changes in cardiomyocytes resulting in diabetic cardiomyopathy. This complication is characterized by prolonged relaxation due to defective clearance of intracellular Ca^{2+}. In addition to the effects on Ca^{2+} homeostasis in the VSMC, TRO also prevents the effects of high glucose on cardiomyocytes (Ren *et al.* 1996).

TDs also modulate vascular pressor responses *in vivo*. Pretreatment with pioglitazone inhibited pressor responses to norepinephrine and angiotensin II in salt-sensitive Dahl rats (insulin-resistant model), but not in normal Sprague-Dawley rats (Kotchen *et al.* 1996). *In vitro* data presented in the same paper suggested that this phenomenon could be at least partly explained by pioglitazone-induced enhancement of endothelium-dependent vasodilation. The antihypertensive effects of pioglitazone in fructose-fed SHR were associated with a decrease in urinary excretion of catecholamines and plasma renin activity suggesting that TDs may modulate activities of these pressor systems (Uchida *et al.* 1997).

The capability of TDs to modulate VSMC growth has been suggested by several studies mentioned in a previous section. A crucial mechanism likely to be responsible for these effects operates downstream to the growth factor receptor. TDs inhibit tyrosine-kinase dependent growth factor action on VSMC: insulin and epidermal growth factor (EGF) (Dubey *et al.* 1993), and basic fibroblast growth factor and platelet-derived growth factor (PDGF) (Law *et al.* 1996). One common signalling pathway that is activated, when mitogenic growth factors engage their receptors, is the MAP kinase pathway. Engagement of the growth factor receptor activates the intracellular tyrosine kinase domain which phosphorylates receptor tyrosine residues that serve as specific binding sites for the recruitment of other signalling proteins. This event triggers a cascade of multiple serine-threonine protein kinases leading to activation of MAP kinase. MAP kinase modifies nuclear transcription factors and regulates the expression of genes, such as c-fos involved in VSMC growth (Davis 1993, Dudley *et al.* 1995). Law *et al.* (1996) and Graf *et al.* (1997) have recently proved that TRO inhibits the growth factor and angiotensin II signalling by inhibiting MAP kinase pathway. This signalling pathway has also been implicated in growth factor-mediated VSMC migration, another process which plays a role in the pathogenesis of atherosclerosis. In accordance with that, inhibition of MAP kinase by TRO resulted in inhibition of VSMC migration (Law *et al.* 1996).

Table 2. Serum concentrations of conjugated dienes (CD) and thiobarbituric acid reacting substances (TBARS) in HTG rats fed a high sucrose diet for 4 weeks, without (HS diet) or with troglitazone (HS diet+TRO) (Vrána *et al.*, unpublished data).

Treatment	CD (abs. U)	TBARS (nmol/ml)
n	13	14
HS diet	1.02±0.13	3.66±0.18
HS diet + TRO	0.54±0.03*	2.95±0.15*

*Data are given as means ± S.E.M.; * $p < 0.01$: HS diet + TRO vs HS diet*

Pharmacological actions of TDs resulting in activation of PPARγ and inhibition of MAP kinase offer some space for speculation. As has been recently reported by Camp and Tafuri (1997) in *in vitro* and *in vivo* studies, PDGF and EGF decrease ligand-activated PPARγ transcriptional activity *via* MAP kinase dependent phosphorylation. This finding suggests a link between the pathways mentioned above which are possibly involved in metabolic and vascular alterations associated with insulin resistance. A better understanding of the events acting at this level may contribute considerably to the effort for disclosing a common factor underlying metabolic and vascular abnormalities in insulin-resistant states.

As has already been mentioned, the chemical structure of troglitazone has been enriched by an antioxidant alpha-tocopherol moiety (Saltiel and Olefsky 1996). With respect to the evidence demonstrating the role of the oxidative stress in the pathogenesis of diabetic angiopathy, this modification could enhance vasoprotective properties of the drug. TRO inhibits oxidative modification of human low density lipoproteins (LDL) (Nagasaka *et al.* 1995, Noguchi *et al.* 1996) and its effect is comparable to alpha-tocopherol (Nagasaka *et al.* 1995). In another *in vitro* study assessing TRO's antioxidant actions, Cominacini *et al.* (1997) demonstrated a dose-dependent decrease in LDL and HDL oxidation induced by copper ions. Furthermore, the drug has been shown to be an even more potent radical scavenger than vitamin E. Fulgencio *et al.* (1996) suggested the possibility that the antioxidant effects, in particular inhibition of long-chain fatty acid oxidation resulting in inhibition of gluconeogenesis, contribute to metabolic effects characteristic for TRO. In an animal model of insulin resistance combined with severe hypertriglyceridaemia troglitazon markedly reduced the plasma concentrations of conjugated dienes and malonyldialdehyde (Table 2). However, the significance of these findings for prevention of diabetic vascular complications has to be tested in long-term studies.

4. Conclusion

In contrast to traditional insulin-sensitizing agents, TDs possess a unique ability to attack mechanisms responsible for the metabolic alterations as well as for vascular abnormalities characteristic for insulin-resistant states. Apart from the immense therapeutic potential which must, however, be further elucidated by testing the side effects of these agents, TDs represent a powerful research tool in the attempt to find common denominators underlying the pathophysiology of the metabolic syndrome X.

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