ABSTRACT
The thiazolidinediones (TZDs) are a class of oral drugs used for the management of type 2 diabetes mellitus and act as ligands for the transcription factor Peroxisome Proliferator-Activated Receptor gamma (PPARγ). Rosiglitazone, an example of TZD, is an anti-diabetic agent acting as a potent insulin sensitizer and is used clinically to enhance insulin-stimulated glucose uptake in tissues. However, in spite of the beneficial effects of TZDs in management of type 2 diabetes, the safety of rosiglitazone has recently been called into question. The debate about the risks associated with rosiglitazone therapy heightened in 2007, when it was reported that rosiglitazone was associated with an increase risk of myocardial infarction and death from cardiovascular causes. The cardiovascular risk associated with rosiglitazone use remains to be further elucidated, but there are several reasonable hypotheses.

Keywords: Thiazolidinediones; Rosiglitazone; Type 2 Diabetes; Cardiovascular risk

INTRODUCTION
Type 2 diabetes (T2D) is the most common metabolic disorder worldwide (Goldstein, 2003); its pathogenesis involves insufficient insulin secretion and/or resistance to the action of insulin. Insulin resistance results in decreased glucose uptake by the peripheral tissues while glucose production by the liver is increased. High levels of insulin production as a result of insulin resistance lead to high levels of insulin in the blood, a condition called hyperinsulinemia. When insulin production reaches a peak, such high levels of insulin are not sustainable and ultimately β-cell insulin secretion fails. This leads to loss of glycemic control, and hence full-blown T2D. There is an increasing evidence of T2D in almost every population worldwide and epidemiological studies suggests that without proper prevention and control programmes, prevalence of the disease will continue to increase globally (Alberti et al., 2007).

Thiazolidinediones (TZDs) are a class of oral drugs used for the treatment of T2D. TZDs were discovered as a result of screening of compounds with lipid-lowering capacity and were noted to reduce hyperglycemia and hyperinsulinemia in rodent models of insulin resistance (IR) (Lebovitz and Banerji, 2001). At around the same time, the mechanism of action of TZDs was found to involve interaction with the transcription factor PPARγ, binding of the resultant complex to the PPARγ response element within the promoter of PPARγ target genes, and thus regulation of the expression of genes that play a role in lipid and glucose metabolism (Olefsky, 2000). TZDs that have been extensively studied in animal and human models include Troglitazone, Rosiglitazone and Pioglitazone. Troglitazone was the first TZD to be introduced, however because of its association with liver damage it was removed from the market in March 2000 (Gale, 2001), while rosiglitazone and pioglitazone were approved for clinical use in 1999 (Lebovitz and Banerji, 2001).
The negative impact of the high prevalence rate of T2D is associated with morbidity and mortality, as well as considerable public cost (Davis et al., 2006; Yach et al., 2006).

**Thiazolidinediones for the Treatment of Type 2 Diabetes**

As mentioned earlier, TZDs are a class of oral drugs used for the treatment of T2D. TZDs have been shown to enhance the ability of insulin to promote glucose transport into skeletal muscles and therefore lower glucose levels in the blood (Olefsky and Saltiel, 2000), and decrease hepatic glucose production (Maggs et al., 1998) while prolonging pancreatic β-cell function by preventing β-cells apoptosis (Higa et al., 1999). With regard to the other component of the metabolic syndrome, TZDs play a role in blood pressure regulation, vascular tone and endothelial function, all of which might directly and/or indirectly influence cardiovascular disease (St John et al., 2002; Mudalier, 2007). Enhancement of fibrinolytic activity system as a result of TZD treatment has also been shown in patients with type 2 diabetes (Kruszynska et al., 2000). TZDs’ other vascular effects include their ability to reduce the expression of endothelial adhesion molecules thereby limiting smooth muscle cell activation (Marx et al., 2001), while other important cardiovascular effects of TZDs include their ability to reduce elevated plasma triglycerides, increase plasma HDL cholesterol and decrease LDL cholesterol/HDL cholesterol ratio (Lebovitz and Banerji, 2001). Other studies suggested that TZDs could stimulate adipocyte differentiation and generate small adipocytes that are more insulin-sensitive than large adipocytes (Kahn et al., 2000). Consequently, TZDs may also have the potential to reduce visceral obesity, low-grade inflammation and microalbuminuria in patients with metabolic syndrome (Viberti, 2005).

Despite the aforementioned beneficial effects of TZDs as anti-diabetic agents, several studies have shown that they have adverse side effects. Weight gain due to fluid retention or oedema is among the adverse side effects of TZDs treatment (Lebovitz and Banerji, 2001). Inhibition of vascular smooth muscles and endothelial cells growth and migration has also been attributed to TZDs treatment (Hsueh and Law, 2001). In addition, risk of congestive heart failure limit the use of TZDs as first line treatment in diabetics (Nathan et al., 2006). However, TZDs have also been shown to increase the risk of myocardial infarction and death from cardiovascular causes (Nissen and Wolski, 2007) although this has been contradicted by other study (Singh et al., 2007). The effect of TZDs on negative cardiovascular effects will be discussed in subsequent section.

**Peroxisome Proliferator-Activated Receptors (PPARs)***

Peroxisome Proliferator-Activated Receptors (PPARs) are ligand-activated transcription factors that belong to the nuclear receptor super family. They are activated by specific ligands that play an important role in cell signalling. For more than a decade, PPARs have been extensively studied and shown to participate in maintenance of glucose and lipid homeostasis, differentiation and cellular proliferation (Delerive et al., 2001). The PPARs consists of three isotypes: alpha (PPARα), gamma (PPARγ) and beta/delta (PPARδ) (Cabrero et al., 2002). These three isoforms of PPAR are encoded by separate genes that perform different functions and are expressed in different manner in different tissues. PPAR alpha is highly expressed in liver, muscle, kidney and heart, where it stimulates the beta-oxidative degradation of fatty acids (Chinetti et al., 2000). PPAR gamma is mainly expressed in monocytes/macrophages, endothelial cells, smooth muscle cells, intestine and adipose tissues where it triggers adipocyte differentiation and promote lipid storage (Chinetti et al., 2000). PPARbeta is ubiquitously expressed in vascular cells. Transcriptional activation of PPARs involves heterodimerization with retinoid-X-receptor (RXR). The dimer binds to related DNA elements called PPAR response elements (PPRE) in the 5’ flanking region of target genes (Evan and Bruce, 2001).

**PPARγ***

Due to its broad range of physiological roles, PPARγ is the most extensively studied isoform of PPARs. In humans, PPARγ gene is located on chromosome 3p25 having 9 exons and extends over more than 100kb of the genome. PPARγ has three different protein isoforms: PPARγ1, PPARγ2, and PPARγ3. The N terminus of PPARγ2 contains 30 additional amino acids compared to PPARγ1 (Tontonoz et al., 1994). Most tissues however, have relatively low levels of PPARγ2. Although PPARγ2 and PPARγ3 are expressed in adipose tissues, PPARγ1 is expressed at higher levels. Studies have shown that PPARγ plays a vital role in cellular differentiation, insulin sensitivity, atherosclerosis and cancer. Apart from its effects in glucose homeostasis, PPARγ has also been shown to have other diverse metabolic and cardiovascular effects (Hsueh and Brummer, 2004). However, because of its actions to improve insulin sensitivity, most researches focused on PPARγ.

Several synthetic and naturally occurring compounds have been shown to bind to and activate PPARs. These synthetic ligands were shown to have high affinity for PPARγ. TZDs are one such ligands. TZDs bind directly to the ligand-binding domain of PPARγ but not PPARα or PPARδ, and hence function as receptor agonists (Smith, 2003). Other synthetic PPAR ligands include triterpinoid [2-cyano-3,12-divoalaenea-1,9-diene-28-oic acid] (Suh et al., 1999) and aryly-tyroline derivatives (Evan and Bruce, 2001). On the other hand, natural compounds such as fatty acids, eicosanoids and oxidized fatty acids are also shown to activate PPARs (Kliwer et al., 1995; Nagy et al., 1998).

**Rosiglitazone for the Treatment of Type 2 Diabetes***

Marketed by GlaxoSmithKline, Rosiglitazone is one of the anti-diabetic agents of the TZD class of antihyperglycemic drugs.
Currently, the drug exists in three different forms: Avandia (monotherapy), Avandamet (combination product containing rosiglitazone and metformin) and Avandaryl (combination product containing rosiglitazone and glimepiride). Rosiglitazone is a potent insulin sensitizer clinically used to enhance insulin stimulated glucose uptake by tissues and has been shown to restore normal insulin secretion in individuals with impaired glucose tolerance (Juhi et al., 2003). Addition of rosiglitazone to insulin treatment significantly improves glycemic control and was generally tolerated by diabetic patients (Raskin et al., 2001). Other studies showed that rosiglitazone could have effect on calcium signalling by affecting monocytyic calcium signalling processes which may give a level of guard against diabetic microangiopathy (Atkin, 2008). Platelets of type 2 diabetics showed significant hyperaggregability and increased thrombogenic potential (Watala et al., 2005). However, treatment with rosiglitazone increased platelet SERCA2 expression and Ca\textsuperscript{2+}-ATPase activity, decreased SERCA2 tyrosine nitration and normalized platelet intracellular calcium concentration (Randriamboavonjy et al., 2008). Rosiglitazone has also been shown to reduce MMP-9 serum levels in type 2 diabetic patients with coronary artery disease (Marx et al., 2003). MMPs are shown to be involved in the development of unstable plaques of arteriosclerotic lesions in patients with type 2 diabetes mellitus (Marx et al., 2003). Overall, the importance of rosiglitazone in the management of type 2 diabetes is well established.

**Rosiglitazone and Negative Cardiac Outcome**

The ability of rosiglitazone to reduce blood glucose and glycated haemoglobin levels in patient with type 2 diabetes was the basis of its approval. However, there has been considerable increase in debate regarding the risks and benefits of its use to treat diabetes. Meta-analysis by Nissen and Wolski suggested that rosiglitazone treatment is associated with a significant increase in the risk of myocardial infarction and with a borderline significant increase in the risk of death from cardiovascular causes (Nissen and Wolski, 2007). Nissen and Wolski’s meta-analysis was primarily based on small-scale short-term trials that were not intended to ascertain cardiovascular outcomes (Nissen and Wolski, 2007), nevertheless the study highlighted the urgent need for clarification of the role of rosiglitazone in increasing cardiovascular risk. In a related development, the rosiglitazone evaluated for cardiovascular outcome and regulation of glycaemia in diabetes (RECORD) interim analysis (Home et al., 2007) showed no evidence of any increase in cardiovascular or all-cause mortality. However the study found an increased risk of heart failure associated with rosiglitazone treatment (Home et al., 2007). Another meta-analysis by Singh et al., (2007) showed that 12 months of rosiglitazone administration in patients with impaired glucose tolerance or T2D is associated with a significant increase in risk of myocardial infarction and heart failure without a significant increase in the risk of cardiovascular mortality. On the other hand, more recent studies have held up the view that rosiglitazone should be used only alongside stronger warnings together with the use of informed consent (Rosen, 2010). However, it is important to note that in none of these studies was the mechanism by which rosiglitazone may cause the risk of death from cardiovascular causes (Nissen and Wolski, 2007), myocardial infarction and heart failure (Home et al., 2007; Singh et al., 2007) was elucidated. Besides the negative cardiac outcomes associated with rosiglitazone therapy, other detrimental side effects of rosiglitazone therapy include weight gain, increase in plasma volume, edema and increased plasma LDL-cholesterol concentration appear to be the classical effects of TZDs (Lebovitz and Banerji, 2001). As reported by Soroccau et al, (2004) rosiglitazone impacts negatively on bone remodelling by promoting osteoblast and osteocyte apoptosis (Soroccau et al., 2004). Rosiglitazone is also associated with reductions in markers of bone formation and reductions in bone mineral density (Grey et al., 2007).

**Rosiglitazone and Calcium Homeostasis**

Due to the cardiac problems associated with rosiglitazone, and the links between hypertension, cardiac dysfunction, microvascular diabetic complications (Advani et al., 2004; Belke et al., 2004; Singh et al., 2005) and disruptions in Ca\textsuperscript{2+} homeostasis, the effects of rosiglitazone on Ca\textsuperscript{2+} homeostasis within monocytes and cardiomyocytes take on increased importance. A small number of studies have shown that rosiglitazone can cause disruption in Ca\textsuperscript{2+} homeostasis. For example, treatment of cardiomyocytes with 10µM rosiglitazone upregulates the calcium pump enzyme of cardiomyocytes, sarco-endoplasmic reticulum ATPase (SERCA2a) and bring about altered intracellular calcium handling (Shah et al., 2004). In a related development, Randriamboavonjy et al., (2008) also demonstrated that treatment of megakaryocytes and platelets with rosiglitazone upregulates SERCA2b.

**Molecular Mechanism of Rosiglitazone-Induced Cardiac Failure**

Study within our research group had shown that rosiglitazone could induce the unfolded protein response (UPRs) in vascular smooth muscle cells (VSMC) and monocytes, but that this did not lead to cytotoxic apoptosis effects in these cells (Caddy et al., 2010). We also showed that rosiglitazone-induced upregulation of UPR target genes in monocytes and VSMC was sufficient to restore normal cell physiology and prove not to be cytotoxic in those cells. However, as the safety concerns reported for rosiglitazone apply specifically to negative cardiac outcomes (Nissen and Wolski, 2007; Rosen 2010), we extended these observation to cardiomyocytes (Issa, 2011) by investigating the effects of rosiglitazone on Ca\textsuperscript{2+} homeostasis, endoplasmic reticulum (ER) stress and cell viability and apoptosis in *in vitro* model system for cardiomyocytes (HL-1 cells).
The ER performs several important functions including post-translational modification, folding and assembly of newly synthesized secretory proteins and calcium homeostasis. Disturbing any of the ER functions may lead to imbalance between protein-folding load and the capacity of the ER, causing unfolded or misfolded proteins to accumulate in the ER lumen, a condition referred to as ER stress (Araki et al., 2003; Zhang and Kaufman, 2008). Cells have evolved a protective response to deal with the deleterious effects of ER stress called UPR (Patil and Walter, 2001; Ron and Walter, 2007) that aims to restore normal ER function, or lead to apoptotic cell death in conditions of prolonged ER stress (Szegedi et al., 2006). \([\text{[Ca}^{2+}]/\text{cyt}\] is tightly regulated for normal cell physiology. Factors that can interfere with calcium signalling processes may induce cell death by apoptosis (Kass and Orrenius, 1999). In addition, ER stress-related genes have been previously shown to be induced in samples of human and mouse heart failure, suggesting that ER stress may be involved in the pathogenesis and the development of heart failure (Okada et al., 2004). Thus, we hypothesised that rosiglitazone by interfering with ER calcium homeostasis can induce ER stress and UPR in HL-1 cardiomyocytes.

To test our hypothesis, the consequent effects of rosiglitazone on apoptosis were examined. Furthermore, the induction of different transcription factors involved in ER stress and UPR following rosiglitazone treatment of HL-1 cells were also investigated. The rosiglitazone concentrations selected are within the range the drug has been shown to exert molecular effects in myocytes of neonatal rat ventricles (Shah et al., 2004) and in monocytes (Caddy et al., 2008) and comparable to those approximating its pharmacological levels (Niemi et al., 2003). Treatment of HL-1 cells with rosiglitazone was associated with a significant increase in apoptosis and with a significant decrease in cell viability (Isa, 2011). Given that rosiglitazone causes significant cardiomyocyte apoptotic cell death (figure 1), it is important to elucidate the mechanisms by which rosiglitazone treatment may result in cardiomyocyte apoptosis. Thus, we elucidated the mechanism as being induction of ER stress and UPR (Isa, 2011).

![Bar graph showing the effects of treatment with DMSO (0.1%) or rosiglitazone (0.5 to 10µM) on HL-1 cells apoptosis (P<0.05 in all cases) (Isa, 2011).](image)

Figure 1: The effects of treatment with dmsos (0.1%) or rosiglitazone (0.5 to 10µM) on HL-1 cells apoptosis (P<0.05 in all cases) (Isa, 2011).

To further investigate the fact that ER stress is involved in rosiglitazone-induced apoptosis in cardiomyocytes, C/EBP homologous protein (CHOP) was investigated. CHOP is an important UPR gene that is barely detected under physiological conditions in proliferating cells but is upregulated in response to growth arrest signals and ER stress (Hotamisligil, 2005). Importantly CHOP is found to be induced in failing heart and cardiomyocytes apoptosis during the progression from cardiac hypertrophy to heart failure (Okada et al., 2004). We showed that CHOP mRNA is upregulated following rosiglitazone treatment of HL-1 cells (Isa, 2011). Upregulation of CHOP mRNA (Figure 2) strongly suggests that rosiglitazone treatment is associated with ER stress.
**CONCLUSION**

As reviewed by Kang and Izumo (2000), the etiology of heart failure involves numerous conditions but progressive loss of cardiac myocytes is largely the most significant. Myocytes apoptosis occurs during heart failure and may play a significant role in the development of heart failure. Apoptosis may perhaps cause cardiac dysfunction by numerous mechanisms. For example, loss of cardiomyocytes by apoptosis leads to loss of cardiac mass and cardiac remodelling as a result of alteration of neighbouring cardiomyocytes. Thus, it may be envisaged that the loss of cardiac mass as a result of apoptosis may lead to diminished pump power and disturbance in electric conductance that may also lead to arrhythmias. In view of our understanding that rosiglitazone causes significant cardiomyocytes apoptosis (Isa, 2011), we suggest that our study might help to explain why the interim meta-analysis of Nissen and Wolski (2007), the RECORD study and Singh et al’s (2007) meta-analysis all reported negative cardiovascular events (particularly heart failure) associated with rosiglitazone therapy.

**REFERENCES**


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