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Metabolism of troglitazone in hepatocytes isolated from experimentally induced diabetic rats

A. J. Meechan, C. Henderson, C. D. Bates, M. H. Grant and J. N. A. Tettey

Abstract

Troglitazone (TGZ), the prototype 2,4-thiazolidinedione antidiabetic agent, is associated with hepatotoxicity in patients with Type 2 diabetes. Although the mechanism of toxicity has not been established, alterations in the clearance of TGZ from in-vitro hepatocyte cultures through metabolic conjugation reactions are believed to modulate the toxicity of the compound. In this study, the metabolism of TGZ in freshly isolated hepatocytes from the fat-fed streptozotocin-treated rat model of Type 2 diabetes is described. Biochemical parameters such as cellular reduced glutathione content, content of cytochromes P450 and b_5, and the expression of glutathione-*S*-transferase α (subunits Y_a and Y_{c2}) were not affected by the induced diabetes. TGZ was metabolized primarily to a sulfonate, a quinone and a glucuronide in both control and experimentally diabetic animals. However, metabolism after induction of diabetes was characterized by a moderate increase in sulfation, a decrease in the elimination half-life of TGZ and the absence of the minor metabolites of TGZ, notably the glutathione adduct of the putative reactive intermediate ($m/z = 747$ $(M+H)^+$; $m/z = 745$ $(M-H)^-$).

Introduction

First generation 2,4-thiazolidinedione (TZD) antidiabetic agents have been associated with a number of hepatic injuries in patients treated for non-insulin dependent diabetes mellitus (Type 2 diabetes) (Scheen 2001; Gale 2001). While over 90 cases of severe hepatotoxicity, including 63 fatalities, resulted in the complete withdrawal of the prototype TZD troglitazone (TGZ) in 1999, rosiglitazone and pioglitazone have only been associated with few instances of liver toxicity (Al-Saman et al 2000; Forman et al 2000; Maeda 2001; May et al 2002).

TGZ is more toxic to rat and human hepatocytes than either rosiglitazone or pioglitazone (Elcock 1999; Baines et al 2001; Haskins et al 2001; Lloyd et al 2002). Chemical mechanisms have been proposed for this toxicity based on a CYP3A-dependent activation to electrophilic reactive metabolites (Kassahun et al 2001; Tettey et al 2001; Prabhu et al 2002; He et al 2004) and oxidation of a toxic quinone epoxide metabolite (Yamamoto et al 2002). However, there are doubts about the role of metabolic activation in the genesis of acute TGZ toxicity because the TZD moieties in the relatively non-toxic rosiglitazone and pioglitazone are also activated by a similar α-keto isocyanate reactive intermediate pathway described for TGZ (Kassahun et al 2001; Baughman et al 2005).

Cryopreserved human hepatocytes show a large variation in susceptibility to the toxicity of TGZ. Increased toxicity in these cells has been correlated to decreased glucuronosylation and reduced glutathione (GSH) conjugation (Kostrubsky et al 2000; Prabhu et al 2002). This has led to speculation that pre-existing diabetic pathologies may contribute to the toxicity of TGZ (Bova et al 2005). Some of the complications of diabetes are associated with an increase in production of reactive oxygen species and oxidative cellular damage. Whether a TGZ-induced weakening of the hepatocelluar defence to oxidative and carbonyl stress in diabetes, either alone or in combination with the presumed toxic effects of reactive metabolites, accounts for hepatotoxicity is unclear.

In this study, the metabolism of TGZ by hepatocytes from the fat-fed streptozotocin (STZ)-treated rat model of Type 2 diabetes (Reed et al 2000) was assessed. The levels of biochemical indicators, such as GSH and cytochrome P450, which are involved in the metabolism of TGZ (Tettey et al 2001), were measured, and the metabolite profile and elimination rate of TGZ in hepatocytes isolated from diabetic and non-diabetic rats livers were

Department of Pharmaceutical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, Scotland, UK

A. J. Meechan, C. D. Bates, J. N. A. Tettey

Bioengineering Unit, University of Strathclyde, 106 Rottenrow, Glasgow G4 0NR, Scotland, UK

C. Henderson, M. H. Grant

Correspondence: J. N. A. Tettey, Department of Pharmaceutical Sciences, University of Strathclyde, 27 Taylor Street, Glasgow G4 0NR, Scotland, UK. E-mail:justice.tettey@strath.ac.uk

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compared to improve our understanding of the possible alteration of TGZ metabolism in Type 2 diabetes.

Materials and Methods

Chemicals

TGZ (5-[4-(6-hydroxy-2,5,7,8-tetramethyl-chroma-2-ylmethoxy)-benzyl]-thiazolinedione-2,4-dione) was a gift from the Drug Safety Research group, University of Liverpool, UK. The Western blotting assay kit with purified goat anti-rabbit IgG alkaline phosphatase conjugate was obtained from Bio-Rad Laboratories Inc., (Hercules, CA, USA). Rabbit anti-rat glutathione-*S*-transferase (GST) α (subunits Y_a and Y_{c2}) and π (subunit Yf) antibodies were obtained from Professor John Hayes (Ninewells Biomedical Centre, Dundee University, UK). Unless otherwise stated, all chemicals and reagents were of analytical reagent grade or better and were obtained from Sigma-Aldrich (Poole, Dorset, UK). Solvents were obtained from VWR International Ltd, Lutterworth, UK and were of chromatographic or cell culture grade.

Isolation of rat hepatocytes

All experiments involving animals were conducted under Home Office project licence PPL 60/255. Studies were performed using suspensions of hepatocytes that had been freshly isolated from whole livers of male Sprague Dawley rats (180–200 g) by a two-stage collagenase perfusion (Moldeus et al 1978) following anaesthesia with 60 mg kg^{-1} intraperitoneal pentobarbitone sodium (Sagatal; Aventis, Dagenham, UK). The viability of hepatocytes was 80–85% by Trypan blue exclusion.

Induction of Type 2 diabetes

The fat-fed STZ-treated non-obese rat model of Type 2 diabetes was established as previously described (Reed et al 2000; Sawant et al 2004). Male Sprague Dawley rats (180–200 g starting weight) were fed a high-fat diet consisting of 40% fat, 41% carbohydrate and 18% protein. After 2 weeks, the animals (designated as FF/STZ) were treated with STZ (50 mg kg^{-1}) via the tail vein and used in experiments after 6–7 days. The controls were fat-fed animals without STZ treatment (FF) and normal chow-fed animals with and without STZ treatment (CF/STZ and CF, respectively). All animals had free access to both water and food during the study. Glucose levels were measured using an Accu-Chek Active blood glucose system (Roche Diagnostics, Indianapolis, USA) on blood obtained via cardiac puncture prior to hepatocyte isolation.

Effect of experimental diabetes on drug metabolizing enzymes

Cytochromes P450 and b_5

Measurements of cytochromes P450 and b_5 content were carried out as previously described (Watts et al 1995). The absorbance of CO-treated cell homogenates was compared with that of CO-treated and sodium dithionite reduced homogenates between wavelengths of 400 and 500 nm. The differences in absorbance between 450 and 469 nm were used with the extinction coefficient value of cytochrome P450 (E=91 mM^{-1} cm^{-1}) to calculate the concentration. Also, the differences in absorbance between 424 and 469 nm were used with the extinction coefficient value of cytochrome b_5 (100 mM^{-1} cm^{-1}) to calculate its concentration.

Glutathione-S-transferase α and π

The GST isoenzymes α and π were visualized by Western immunoblotting. Hepatocytes from livers of the variously pretreated rats were homogenized in 0.1 M sodium phosphate buffer pH 7.6 and the protein content determined by the method of Lowry et al (1951). Total protein (10 μg) was resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrophoretically transferred to a nitrocellulose membrane. Membranes were probed with rabbit anti-rat GST α (subunits Y_a and Y_{c2}) and π (subunit Y_f) antibodies and detected with purified goat anti-rabbit IgG alkaline phosphatase conjugate according to the manufacturer's instructions. The intensities of the bands (optical density mm^{-2}) were quantified using Scion Image software (Scion Corporation, MD, USA).

Metabolism studies

Hepatocytes were incubated in suspensions (2×10^6 viable cells mL^{-1}) with TGZ in Krebs-Henseleit buffer (pH 7.6) containing 12.5 mM HEPES (*N*-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonoic acid]) in 50-mL rotating round-bottomed flasks at 37°C and an atmosphere of 95%O_2/5%CO_2. The viability of the cells and their GSH content were measured in samples of cells incubated with 50 and 100 μM TGZ, whereas the metabolic profile was determined using only 100 μM TGZ. For the metabolic studies, incubations were terminated by flash-freezing in liquid nitrogen followed by freeze-drying (–60°C, 10^{-1} mbar). The freeze-dried material was extracted with acetonitrile (2×1 mL) and the resulting extract reduced to dryness under a stream of nitrogen at ambient temperature. The residue was reconstituted in 100 μL of acetonitrile/ammonium acetate buffer (40:60 v/v; 25 mM, pH 4.0) and a 40-μL aliquot was analysed by high performance liquid chromatography (HPLC). The recovery efficiency of TGZ (100 μM) from dead, non-metabolically active hepatocytes was 77.3±4.4% (mean±s.e.m., n=5).

Determination of the extent of TGZ metabolism

TGZ and its metabolites were separated on a Phenomenex C8 column (150×4.6 mm i.d., 5 μm particle size) fitted with a 4×3 mm i.d. guard column on an Agilent 1090 liquid chromatograph (Agilent Technologies, Waldbroon, Germany) at ambient temperature. The mobile phase, consisting of acetonitrile (solvent A) and 25 mM ammonium acetate pH 4 (solvent B) was delivered at a flow rate of 1 mL min^{-1} using a stepwise linear gradient of 20% A for 20 min followed by 20–65% A over 4 min. Eluates were monitored at 278 nm. The amount of TGZ remaining was determined over a time course of 0–3 h and the half-life (t) of elimination was computed for the different treatments using GraphPad Prism statistical software (GraphPad Software, San Diego, CA, USA). In the absence of an authentic standard of the principal metabolite TGZ sulfonate (TGZS), its extent of formation was determined based on the UV detector response of its corresponding chromatographic peak expressed as a percentage of that due to the starting concentration of TGZ (100 μM).

Liquid chromatography/mass spectrometry (LC/MS) analyses

Full-scanning data (m/z = 100–1000 Da) was obtained in positive and negative ion modes using an Agilent MSD single quadrupole mass spectrometer coupled to a Hewlett Packard 1050 liquid chromatograph (Agilent Technologies). Analytes were separated on a Thermo BDS Hypersil C18 column (100×2.1 mm i.d., 3 μm particle size) with a 4×3 mm i.d. C18 guard column. The mobile phase, consisting of acetonitrile (solvent A) and 25 mM ammonium acetate pH 4 (solvent B) was delivered at 0.3 mL min^{-1} using a stepwise linear gradient of 20% A for 20 min followed by 20–65% A over 4 min for determinations in positive ion mode. Determinations in negative ion mode were made using formic acid (0.1% v/v) instead of ammonium acetate buffer in the mobile phase. The conditions for acquisition of mass spectral data were as follows: drying gas (nitrogen) temperature 300°C, capillary voltage ± 3kV, fragmentor voltage 70V. Data analysis was carried out using Chemstation software (version 10.1; Agilent Technologies).

Determination of GSH

The GSH concentrations were measured fluorimetrically by the method of Hissin & Hilf (1976). Samples (0.5 mL) of the cell incubate were removed, centrifuged (50 *g*, 4 min) and resuspended in 0.5 mL 10% (w/v) trichloroacetic acid to extract the GSH. The suspension was then centrifuged to pellet the cellular debris, and 25 μL of the supernatant was used to measure GSH. The remaining cell pellet was suspended in 0.5 M sodium hydroxide and the protein content was measured using the method of Lowry et al (1951).

Statistical analysis

Results are expressed as mean ± s.e.m. One way analysis of variance, followed by the Dunnett's test was performed for multiple comparisons at a 5% significance level.

Results

Animals in the CF/STZ and FF/STZ treatment groups were characterized by extensive polyuria and polydipsia and had significantly ($P < 0.05$) higher blood glucose levels (20.83 ± 3.3 and 23.83 ± 5.6 mmol L^{-1}, respectively) than the CF and FF control groups (7.33 ± 0.5 and 8.9 ± 0.4 mmol L^{-1}, respectively).

The levels of hepatocyte cytochromes P450 and b_5, and the GST isoforms α and π were determined in the four groups. Hepatocyte cytochromes P450 and b_5 content and GST α protein expression were unaltered by the induction of experimental diabetes (Table 1). In addition, GST (subunit Y_f), a marker of hepatic injury, was not detected in hepatocytes from any of the four groups of animals.

The content of GSH in hepatocytes was measured before, and 3 h after, exposure to 50 μM and 100 μM TGZ. The induction of experimental diabetes (CF/STZ or FF/STZ) did not affect the content of GSH in cells as measured at the start of the experiments (Table 1). In addition, exposure of the cells to TGZ did not result in significant changes in GSH levels relative to control (data not shown). After exposure of the hepatocytes from the various treatment groups to TGZ (0, 50 and 100 μM) for 3 h, there were no significant differences in the viability of any of the groups (Figure 1).

The extent and nature of metabolism of the higher concentration of TGZ (100 μM) was assessed by HPLC and LC/MS, respectively. TGZ (retention time (t_R) = 19 min) and its metabolites, TGZ quinone (TGZQ; t_R = 16 min) and TGZS (t_R = 14 min) were detected by HPLC with UV detection. The metabolism of TGZ was characterized by an initial increase in the formation of TGZQ (0–15 min) followed by its disappearance over the 3-h incubation period. TGZS was the major metabolite after 3 h (based on UV response at λ = 278 nm). The amount of TGZ remaining in hepatocyte incubations was determined at specific intervals (0, 15, 30, 60, 90, 120 and 180 min) over the 3-h incubation period and the t½ computed for the different treatments (Figure 2). A significant decrease ($P < 0.05$) in t½ was observed with the CF/STZ controls and a slight decrease in t½ was observed for the FF and FF/STZ groups relative to the CF animals. The t½ data, obtained with a concentration of TGZ (100 μM) consistent with reported values in the liver in-vivo (Yamamoto et al 2001), would require further substantiation of the linearity of elimination kinetics in-vivo. The chromatographic peak for the major metabolite, TGZS, accounted for 15.1 ± 3.8, 26.3 ± 15.0, 20.0 ± 1.6 and 25.8 ± 14.0% (mean ± s.e.m., n = 3; Figure 3) of the detector response due to 100 μM TGZ for the CF, FF, CF/STZ and FF/STZ groups, respectively, after 3 h. There was a moderate increase in the formation of TGZS in hepatocytes from the

Table 1 Effect of animal pre-treatment on the content of cytochromes P450 and b_5, and the expression of glutathione-*S*-transferase (GST) α subunits Y_a and Y_{c2} as determined by Western immunoblotting, and the reduced glutathione (GSH) content

Treatment	Cytochrome P450 nmol (mg protein)$^{-1}$	Cytochrome b_5 nmol (mg protein)$^{-1}$	GST α subunit Y_a optical density mm^{-2}	GST α subunit Y_{c2} optical density mm^{-2}	GSH nmol (mg protein)$^{-1}$
CF	0.21 ± 0.01	0.16 ± 0.02	6.6 ± 1.6	6.1 ± 0.6	7.8 ± 1.2
FF	0.18 ± 0.01	0.16 ± 0.01	6.6 ± 0.3	6.9 ± 0.3	11.0 ± 3.6
CF/STZ	0.28 ± 0.09	0.18 ± 0.01	6.0 ± 1.1	6.3 ± 0.6	15.4 ± 3.4
FF/STZ	0.15 ± 0.03	0.19 ± 0.02	5.6 ± 0.7	6.1 ± 0.3	12.6 ± 1.4

CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment. Data are mean ± s.e.m., n = 3.

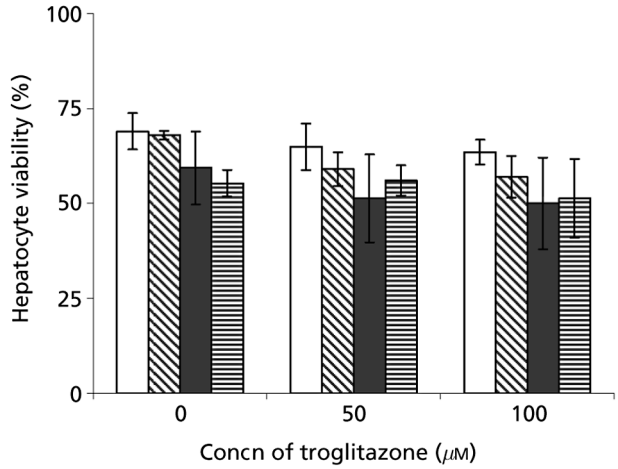

Figure 1 Effect of troglitazone (50 and 100 μM) on the viability of hepatocytes from CF (open column), FF (hatched column), CF/STZ (closed column) and FF/STZ (striped column) rats. Cell viability after incubation for 3 h was determined by the Trypan blue exclusion test. Data are mean ± s.e.m., n = 3. CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment.

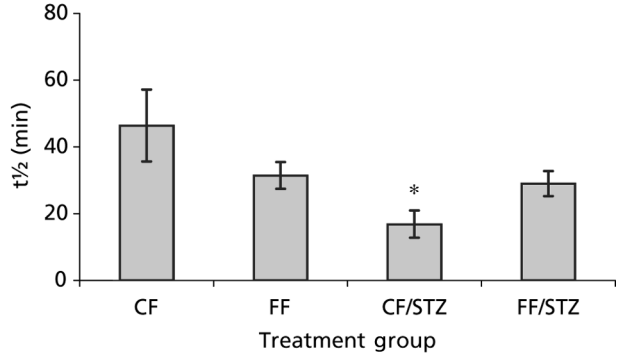

Figure 2 Half-life (t½) of the disappearance of troglitazone from hepatocyte incubations for control hepatocytes (CF) and hepatocytes from FF, CF/STZ and FF/STZ rats. Results are means ± s.e.m., n = 3. *$P < 0.05$, significantly different compared with CF control rats (analysis of variance followed by the Dunnett's multiple comparison test). CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment.

CF/STZ, FF/STZ and FF groups after 3 h compared with the CF control group (Figure 3).

An LC-MS extracted-ion chromatogram from a 3-h incubation of TGZ with hepatocytes from CF-treated rats (Figure 4A) showed the presence of unmetabolized TGZ ($t_R = 20.2$ min; m/z 442 for $[M+H]^+$), TGZQ ($t_R = 17.4$ min; m/z 475, $[M+NH_4]^+$), TGZS ($t_R = 13.1$ min; m/z 539, $[M+NH_4]^+$), TGZ glucuronide (TGZG; $t_R = 11.6$ min; m/z 635, $[M+NH_4]^+$) and TGZ glutathione adduct (TGZ-SG; $t_R = 11.2$ min; m/z 747, $[M+H]^+$) in hepatocyte incubations

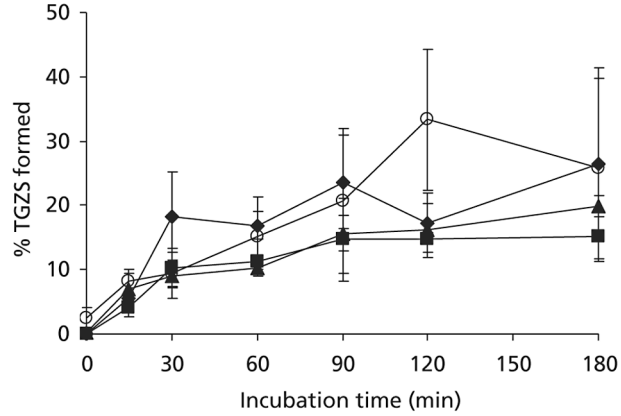

Figure 3 Comparison between the time courses of the formation of the major metabolite troglitazone sulfonate (TGZS) in control hepatocytes (CF; ■) and cells from FF (◆), CF/STZ (▲) and FF/STZ (○) rats over a 3-h incubation period with troglitazone (100 μM). Results are mean ± s.e.m., n = 3. CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment.

from FF and CF control animals. The extracted-ion chromatogram (Figure 4B) representative of incubations with hepatocytes from CF/STZ and FF/STZ-treated rats did not show the TGZ-SG adduct. The presence/absence of the TGZ-SG adduct was also confirmed in negative-ion mode ($t_R = 11.8$ min; m/z 745, $[M - H]^-$) as reported previously (Tettey at al 2001). In addition, ion current peaks corresponding to previously reported minor metabolic routes for TGZ, such as the hydroxyl-TGZS/benzoquinol sulfonate ($t_R = 22.5$ min, m/z 555, $[M+NH_4]^+$) and TGZ benzoquinol sulfonate ($t_R = 17.9$ min, m/z 557, $[M+NH_4]^+$) (Tettey et al 2001), were detected in incubations of TGZ with FF and CF controls but not in the FF/STZ and CF/STZ models.

Discussion

The FF/STZ rat model of Type 2 diabetes is characterized by the development of hyperglycaemia without significant changes in insulin levels compared with control animals (Reed et al 2000; Sawant et al 2004), and simulates the history and metabolic characteristics of patients with Type 2 diabetes (Reaven et al 1993). The degree of hyperglycaemia observed with the FF/STZ rats in this study was consistent with that described by Reed et al (2000). In this study, biochemical parameters involved in the metabolism of TGZ such as content of cytochromes P450 and b_5 and the GST isoenzymes α (subunits Y_a and Y_{c2}) and π (subunit Y_f) were measured to assess possible detrimental effects of the STZ treatment. GST π (subunit Y_f) serves as a useful marker of hepatocellular toxicity (Aliya et al 2003). The apparent non-perturbation of biochemical parameters after induction of experimental diabetes is consistent with the model described by Sawant et al (2004) and supports the use of the FF/STZ model in the present study.

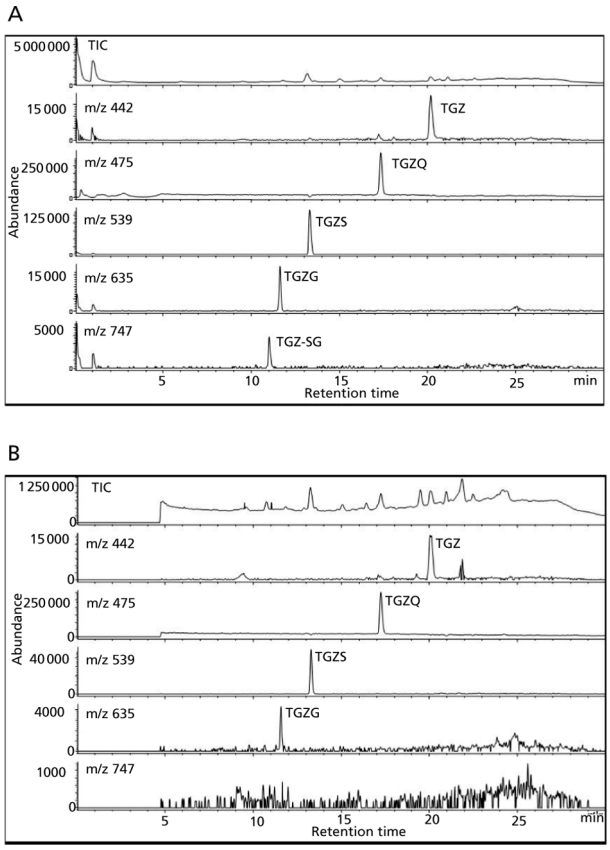

Figure 4 Representative liquid chromatography/mass spectrometry extracted-ion chromatograms of analyses of incubations of troglitazone (TGZ) with freshly isolated hepatocytes from control CF and FF (A) and diabetic CF/STZ and FF/STZ (B) rats showing TGZ (m/z 442 $[M+H]^+$) and its metabolites TGZ quinone (TGZQ; m/z 475 $[M+NH_4]^+$), TGZ sulfonate (TGZS; m/z 539 $[M+NH_4]^+$), TGZ glucuronide (TGZG; m/z 635 $[M+NH_4]^+$) and TGZ glutathione adduct (TGZ-SG; m/z 475 $[M+NH_4]^+$). TGZ-SG was not present in incubations with cells from CF/STZ and FF/STZ rats. Data are representative of three separate experiments per animal treatment. CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment.

TGZ shows dose-dependent toxicity in cultured rat hepatocytes and human hepatoma HepG2 cells, with IC50 values of 35 μM and 20 μM, respectively (Tettey et al 2001). However, freshly isolated hepatocytes from the variously treated rats in this study were not susceptible to the toxicity of the compound. No significant differences were observed between the viabilities of cells from animals with experimentally induced diabetes and those from control animals after exposure to TGZ. The activity of the microsomal cytochrome P450, responsible for the detoxification of many chemicals including TGZ, decreases significantly within the first 24–48 h of culture of hepatocytes (Grant et al 1985) to about 50–80% of the content in the intact liver (Paine 1990). The low levels of cytochrome P450 in the cultured hepatocytes and in the human hepatoma HepG2 cells might explain their susceptibility to TGZ toxicity. Suspensions of freshly isolated hepatocytes, though limited in terms of the duration of experiments (up to 4 h), are more similar in function to liver cells in-vivo.

The metabolism of TGZ in rats, both in-vivo and in-vitro, has been described using non-diabetic animals (Kassahun et al 2001; Tettey et al 2001; He et al 2004). The major pathways involve the cytochrome P450 mediated oxidation to TGZQ, sulfonation (TGZS) and glucuronosylation (TGZG) (Figure 5). Also, TGZ and TGZQ are metabolized to GSH conjugates in the rat in-vivo (Kassahun et al 2001; Tettey et al 2001) and by in-vitro reactive metabolite trapping experiments using liver microsomes (He et al 2004). In the present study, the conversion of TGZ to TGZQ, TGZS and TGZG by hepatocytes from all the treated animals was observed. However, previously reported minor metabolites, such as the hydroxyl-TGZS/benzoquinol sulfonate and TGZ benzoquinol sulfonate (Tettey el al 2001), and the putative GSH adduct of a reactive TGZ metabolite (m/z 747 $[M+H]^+$), were generated by hepatocytes from control animals (FF and CF) but not by those from FF/STZ and CF/STZ animals.

The presence of glutathione adducts of reactive metabolites is usually an indication of the ability of the metabolism system to detoxify potentially toxic electrophilic reactive intermediates. In the present experiments, there was no alteration in either GSH or GST content in the hepatocytes from STZ-treated rats. This suggests that the capacity to detoxify electrophilic metabolites by GSH was retained in cells from these animals, but that perhaps the lack of formation of the intermediate reactive metabolites themselves was the primary defect.

The t½ for the metabolism of TGZ showed a significant decrease with hepatocytes from CF/STZ compared with those from CF rats ($P < 0.05$). However, no significant differences were observed for t½ with hepatocytes obtained from the FF/STZ rats compared with those from either FF or CF rats. A moderate increase in sulfation of TGZ was observed with the CF/STZ, FF/STZ and FF groups, and the subsequent alteration in the balance between the pathways of metabolism might account for the absence of the minor metabolites in some (CF/STZ and FF/STZ) of these incubations. An increase in the activity of phenol sulfotransferase has previously been reported in hepatocytes from diabetic rats (Grant & Duthie 1987). The changes in the nature of metabolism and the small decrease in t½ for the elimination of TGZ in this rat model of Type 2 diabetes compared with the CF control is of toxicological significance in view of the observations of Kostrubsky et al (2000) and Prabhu et al (2002), who correlated the susceptibility of cryopreserved human hepatocytes to TGZ toxicity to a decrease in the formation of GSH adducts and glucuronides. In a previous study, we reported an increase in the toxicity of TGZ to human hepatocarcinoma HepG2 cells relative to cultured rat hepatocytes (Tettey el al 2001). It would seem that the lower metabolizing capacity of the former resulted in a prolonged exposure to unmetabolized TGZ with subsequent lethality.

Conclusions

The toxicity of TGZ to human cryopreserved hepatocytes has previously been correlated to decreased glucuronosylation and GSH conjugation (Kostrubsky et al 2000; Prabhu et al

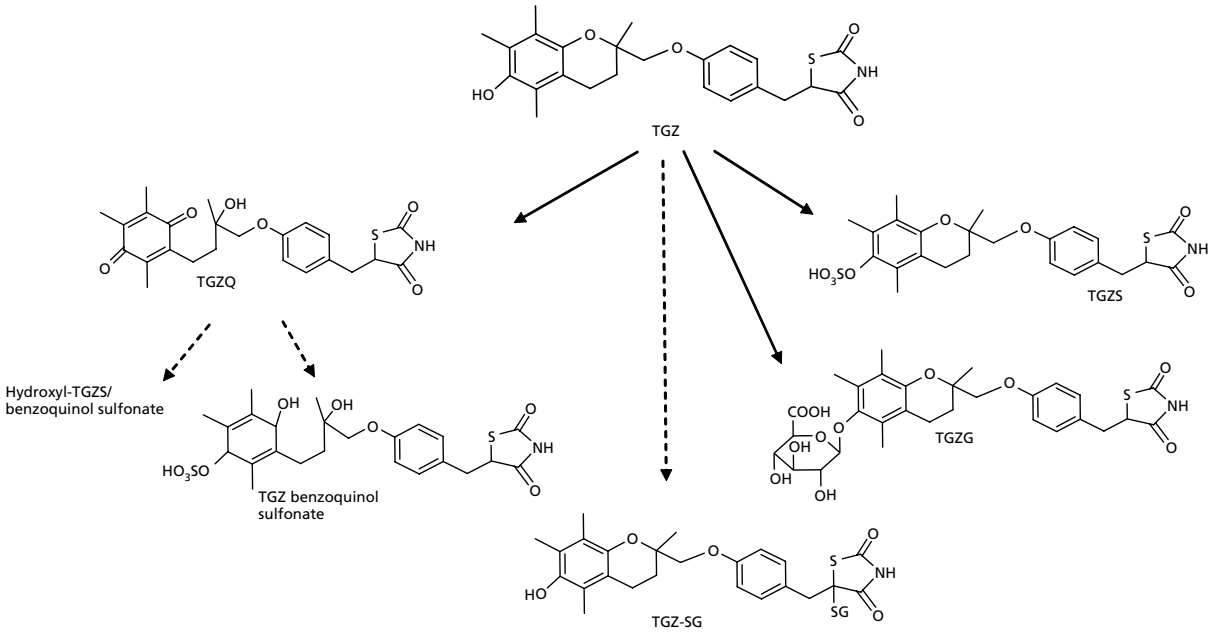

Figure 5 Metabolic pathway of troglitazone (TGZ; 100 μM) in freshly isolated rat hepatocytes after 3 h incubation. Metabolic pathways with dashed arrows were present with hepatocytes from CF and FF rats but not those from CF/STZ or FF/STZ rats. CF, chow-fed without streptozotocin (STZ) treatment; FF, fat-fed without STZ treatment; CF/STZ, chow-fed with STZ treatment; FF/STZ, fat-fed with STZ treatment.

2002). The present study has demonstrated that the induction of diabetes in rats results in perturbations in the nature and extent of metabolism of TGZ by isolated hepatocytes. This is shown by a moderate increase in sulfation and the absence of the TGZ-SG adduct without an alteration in either hepatocyte GSH or GST content. The moderate changes in the nature and extent of TGZ metabolism did not translate into acute toxic events, probably due to the metabolic competence of the freshly isolated hepatocyte system and the short (3 h) duration of exposure. However, in view of the association of TGZ toxicity with chronic administration of the compound, it might be prudent to extend these investigations to long-term in-vivo studies with the experimentally induced fat-fed diabetic rat model.

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