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Effect of gender, dose and time on 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT)-induced hepatotoxicity in Fischer 344 rats

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Abstract

1. The thiazolidinedione (TZD) ring present in drugs available for type II diabetes may contribute to hepatic injury. Another TZD ring-containing compound, 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT), produces liver damage in rats. Accordingly, the effects of gender, dose and time on DCPT hepatotoxicity were therefore evaluated.
2. Male rats were more sensitive to DCPT (0.4–1.0 mmol/kg by i.p. administration) as shown by increased serum alanine aminotransferase (ALT) levels and altered hepatic morphology 24 h post-dosing. Effects in both genders were dose-dependent. In males, DCPT (0.6 mmol/kg) produced elevations in ALTs and changes in liver sections 3 h after dosing that progressively worsened up to 12 h. DCPT-induced renal effects were mild.
3. We conclude that male rats are more susceptible to DCPT hepatotoxicity and that damage occurs rapidly. DCPT primarily affects the liver and may be a useful compound to investigate the role of the TZD ring in hepatic injury. However, the gender dependency and rapid onset of DCPT hepatotoxicity require further investigation.

Keywords

hepatotoxicity; thiazolidinediones; 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione; rats

1. Introduction

Troglitazone (Fig. 1) was the first drug of its class to be approved in the USA for the treatment of type II diabetes and became available in 1997. Clinical studies showed that troglitazone effectively lowered hypertriglyceridemia, hyperglycemia and hyperinsulinemia (Schwartz et al., 1998). This agent exerts its antihyperglycemic effects by interacting with the gamma subtype of the peroxisome proliferator-activated receptor (PPAR- γ) (Hauner, 2002). PPAR- γ is a member of the nuclear receptor subfamily that controls transcription of a number of genes involved in glucose and lipid metabolism (Willson et al., 2000). As a result, troglitazone increases insulin sensitivity in skeletal muscle, liver and adipose tissues (Sahi et al., 2003).

Whereas troglitazone offered significant clinical benefits, it was withdrawn from the US market three years following introduction after it was associated with hepatotoxicity, including approximately 90 cases that required liver transplantation or resulted in death (Graham et al., 2003). Damage seen with troglitazone was mostly hepatocellular with occasional instances of mixed hepatocellular-cholestatic-type injury or a predominant cholestatic reaction. Liver biopsies from patients with troglitazone-induced hepatotoxicity typically showed zone 3 necrosis and hepatocyte dropout with rare foci of bridging necrosis to triads (Gitlin et al., 1998; Kohlroser et al., 2000). Infiltration of inflammatory cells around the necrotic lesions has also been reported in some cases (Gitlin et al., 1998; Kohlroser et al., 2000). Two similar PPAR- γ antagonists (rosiglitazone and pioglitazone) are still available for clinical use. A common structural feature in all three glitazones is the 2,4-thiazolidinedione (TZD) ring. Although limited in number and severity, there are reports of liver injury attributed with the use of rosiglitazone (Forman et al., 2000; Gouda et al., 2001; Bonkovsky et al., 2002) and pioglitazone (Maeda, 2001; May et al., 2002; Marcy et al., 2004). Periodic monitoring of hepatic enzymes, such as alanine aminotransferase (ALT), is recommended for patients taking these drugs and they are not indicated for use in patients with pre-existing liver disease (Scheen, 2001). The mechanism of hepatotoxicity of these drugs remains unknown. Several studies have shown that metabolic activation, involving an initial cytochrome P450-mediated sulfoxidation step, can occur in the TZD ring of the glitazones (Kassahun et al., 2001; Tetley et al., 2001; and He et al., 2004; Baughman et al., 2005; Álvarez-Sánchez et al., 2006), although whether this is a factor in hepatotoxicity is currently controversial (Masubuchi, 2006). Further investigations into glitazone-induced liver damage have been hampered by the lack of normal animal models for toxicity studies (Sharyo et al., 2001; Chojkier, 2005; Ong et al., 2007).

During a structure-activity relationship study into nephrotoxicity of the agricultural fungicide N-(3,5-dichlorophenyl)succinimide (NDPS, Fig. 1), we found that 3-(3,5-dichlorophenyl)-2,4-thiazolidinedione (DCPT, Fig. 1) was hepatotoxic in rats (Kennedy et al., 2003). Biotransformation of NDPS is an important factor in its toxicity (Rankin, 2004); whether this is true for DCPT is under investigation. NDPS and DCPT differ only in the nature of the cyclic imide group (succinimide ring vs. TZD ring, respectively) that is attached to the dichlorobenzene (DCB) ring (Fig. 1); however this change led to a switch in target organ from the kidney to the liver. Although DCBs themselves are potentially hepatotoxic in rats (Stine et al., 1991), we do not believe that this is a factor with DCPT, since NDPS and other DCB ring-containing compounds did not exert liver damage in our animal model (Kennedy et al., 2003). DCPT produced hepatotoxicity 48 h after administration to male rats and the damage was characterized by swelling of hepatocytes and centrilobular necrosis with infiltration of neutrophils. Similar to the glitazones, DCPT contains a TZD ring; however, DCPT produces liver damage in a common laboratory rodent species. In contrast, glitazone toxicity is not observed in wild-type animals (Sharyo et al., 2001; Chojkier, 2005; Ong et al., 2007). Although troglitazone is no longer available for the treatment of type II diabetes, rosiglitazone and pioglitazone remain in clinical use. Furthermore, TZD rings are also present in a series of aldose reductase inhibitors that may be beneficial in reducing diabetic complications (Bruno et al., 2002; Rakowitz et al., 2006). Therefore, the possibility of continued human exposure to drugs that contain a TZD ring remains. Since DCPT may be a useful model compound to evaluate TZD ring-induced liver injury, it is important to fully characterize the selectivity and nature of its toxicity.

In the present report we extended our previous work by conducting a DCPT dose response study using male and female rats. The males were more susceptible to DCPT-induced hepatotoxicity, which could be due to differences in biotransformation of the compound. In contrast to its effects on the liver, DCPT did not produce any marked effects on rat kidney. We are ultimately interested in determining the mechanism by which DCPT produces liver

injury and this should be done at time points before the damage is fully established. Therefore, a time course study for the onset and duration of DCPT-induced hepatotoxicity was also conducted in male rats. Evidence of mild liver damage was seen within 3 h of DCPT administration. This suggests that, if DCPT metabolism is important for the observed effects on the liver, biotransformation of this compound into a hepatotoxic species occurs rapidly.

2. Methods

2.1 Chemicals/Reagents

Ethyl 2-mercaptoacetate and 3,5-dichlorophenylisocyanate and were purchased from Alfa Aesar (Ward Hill, MA, USA). DCPT was synthesized and recrystallized using the method described by Kennedy et al. (2003); purity was established by sharp melting point (m.p. 169.5–170.5 °C; lit. mp. 164.5–165.5 °C, Fujinami et al., 1971). Bio-Rad protein assay kit was purchased from Bio-Rad Laboratories (Hercules, CA, USA) and was used to assess urine protein levels using bovine serum albumin (BSA) as standard. Sodium pyruvate, 2,4-dinitrophenylhydrazine, DL-alanine and α -keto-glutaric acid were all products from Sigma Chemical Company (St. Louis, MO, USA). Serum samples were originally analyzed using the alanine aminotransferase (ALT, No. 505-P) and blood urea nitrogen (BUN, No. 640-5) assay kits from Sigma Chemical Company. These kits were later discontinued by the manufacturer. In all subsequent experiments, the ALT assay was conducted using a procedure similar to that described in the Sigma kit. The BUN assay was performed using a kit (No. B7551-120) obtained from Pointe Scientific (Detroit, MI, USA). Validation experiments were conducted to ensure that the assay results from the different kits were comparable.

2.2 Animals

Age-matched female and male Fischer 344 rats (ca. 145–150 g and 200–210 g, respectively) were purchased from Charles River Laboratories (Wilmington, MA, USA). Rats were housed in standard stainless steel hanging cages under a 12 h light/dark cycle at ca. 22 °C and 45–50% relative humidity. The animals were given one week to acclimate prior to being used in any experiments. Food (laboratory rodent diet #5001, PMI Foods, Inc., St. Louis, MO, USA) and water were freely available unless otherwise noted. The Institutional Animal Care and Use Committee of the University of the Sciences in Philadelphia approved all experiments described in these studies.

2.3 In Vivo Toxicity

The experimental method is a modification of our previously published 48 hour procedure (Kennedy et al., 2003). Animals were randomly divided into groups (N = 4 rats per group). After the initial acclimation period, rats were transferred to individual stainless steel metabolism cages (Allentown Caging Equipment Co., Allentown, NJ, USA) and given an additional two days to acclimate before use. On the first day of the experiment (prior to administration of DCPT) urine was collected for 6 h to measure baseline protein content. During this time period, food and water were removed to avoid dilution and contamination of the urine samples. Food and water were returned at the end of the urine collection period. Animals were administered DCPT on the second day of the experiment as described below. The i.p. route of administration was chosen for comparison to our previous studies (Kennedy et al., 2003).

In the dose response study (24 h duration), male and female rats received DCPT (0.2, 0.4, 0.6 and 1.0 mmol/kg, i.p. in corn oil) or corn oil only (4 ml/kg, i.p.). Due to very poor solubility characteristics in corn oil, DCPT could not be administered at doses higher than

1.0 mmol/kg. Immediately after dosing, urine was collected for 6 h for assessment of protein content. As described above, food and water were removed during this period, but were returned to the rats immediately afterwards. Twenty four hours after administration of DCPT or corn oil, the animals were anesthetized with isoflurane and a blood sample was obtained by cardiac puncture for assessment of ALT and BUN levels. While still under isoflurane anesthesia, the rats were euthanized by cervical dislocation. The kidneys and livers were removed, weighed, and the right kidney and a section of liver were fixed in 10% formalin for later histological analysis. Tissue sections were prepared and stained with hematoxylin and eosin by American Histolabs, Inc. (Gaithersburg, MD, USA). Slides were coded and read by a person blinded to the code. Body weights, food/water intake and the total amount of urine excreted over the 24 h period following administration of DCPT or corn oil were also measured.

To evaluate the onset of liver damage, male rats were administered a single dose of DCPT (0.6 mmol/kg, i.p. in corn oil) or corn oil (4 ml/kg, i.p.). This dose reproducibly produces hepatotoxicity, but has minimal effects on the kidneys in rats. The experimental design was similar to above (collection of urine, blood, tissue samples, etc.) except that animals were euthanized 1, 3, 6, 12 and 24 h after DCPT administration.

2.4 Statistical Analyses

Statistical analyses were conducted using SigmaStat (version 3.1, Systat Software, Inc., San Jose, CA, USA). Results are expressed as means $\pm$ standard errors (N = 3–4). The data were analyzed by a one way ANOVA, Student's *t*-test or the corresponding non-parametric tests. When significance was detected in the ANOVA, Student-Newman-Keuls or Dunn's post hoc tests were used to isolate the groups. Differences in the means were considered significant when $p < 0.05$.

3. Results

3.1 Dose Response Study (Males/Females)

The lowest dose of DCPT (0.2 mmol/kg) did not produce a significant change in serum ALT levels in either gender when compared to the respective corn oil controls (Fig. 2). In contrast, ALT levels were elevated approximately 9-, 11- and 20-fold in males at the 0.4, 0.6 and 1.0 mmol/kg DCPT doses, respectively. Serum ALTs were also increased (4- to 12-fold) in females beginning at 0.4 mmol/kg DCPT (Fig. 2); however the values were significantly lower than those in the males that received the same doses. Compared to corn oil-treated animals (Table 1), DCPT treatment produced a statistically significant, but small, elevation in BUN concentration at the highest dosage (1.0 mmol/kg) in males. Slight increases in BUNs were observed in females at the two highest doses of DCPT. There were no gender-dependent differences in BUN levels except at the 0.6 mmol/kg dose.

No significant changes were observed in urine volume or urine protein content with any DCPT dose compared to the corn oil controls in male or female rats (Table 1). Urine protein content was uniformly lower in the females than in the males; however, this gender-based difference in rats has been previously reported (Hong et al. 1998; Hong et al., 2001) and was not unexpected. Body, liver and kidney weights were not significantly altered at any dose of DCPT in male rats (Table 2). At the lowest dose of DCPT female rat body weight was decreased; however, this was not significantly different from the pre-dosing value in these animals (data not shown). Kidney weights in the 0.2 mmol/kg female group were elevated compared to the corn oil controls and same dose males. DCPT resulted in decreased food consumption by the rats, whereas water consumption was not altered in any treatment group (data not shown).

Liver sections from corn oil-treated animals appeared normal (Figs. 3A and 3B). Hepatocytes were healthy in appearance, arranged in a cord-like fashion radiating out of the central veins. When compared to the controls, liver sections from male and female rats exhibited no or little damage at the two lowest doses of DCPT examined (0.2 mmol/kg, not shown; 0.4 mmol/kg, Figs. 3C and 3D). Necrosis, small areas of metabolic changes and the presence of inflammatory cells were noted in male rat livers at 0.6 mmol/kg DCPT (Fig. 3E). At the highest DCPT dose (1.0 mmol/kg) areas of necrosis, the start of an inflammatory response and the infiltration of neutrophils were observed in liver sections from male rats (Fig. 3G). Compared to the male rats, the female rat livers showed markedly less inflammation and necrosis at all doses of DCPT. Liver sections from female rats that received 0.6 mmol/kg DCPT (Fig. 3F) were comparable to the corresponding corn oil controls (Fig. 3B). Furthermore, female rats dosed with 1.0 mmol/kg DCPT showed only minor hepatocyte swelling (Fig. 3H).

Kidney morphology in male rats was not significantly different from corn oil controls (Fig. 4A) at all doses of DCPT (1.0 mmol/kg dose shown in Fig. 4C). However, in female rats, proteinaceous material was present in the collecting tubules in both corn oil and DCPT-treated animals (data not shown). Female rat kidney sections showed slight damage with lower doses of DCPT, whereas sporadic necrotic proximal tubular cells were evident at the highest dose (data not shown). Compared to corn oil controls (Fig. 4B), swelling of the proximal tubules, as well as the glomerular space, was also noted in females (1.0 mmol/kg dose shown in Fig. 4D).

3.2 Time Course Study in Male Rats

Compared to the corn oil controls, DCPT treatment did not produce any changes in serum ALT levels 1 h post-dosing in male rats (Fig. 5). The ALT levels were significantly elevated 3 h after administration of DCPT and continued to rise up to the 12 h time point (Fig. 5). As in the dose-response study, serum ALTs were still elevated 24 h post-dosing. Compared to the corn oil control animals, DCPT treatment produced diuresis 6, 12 and 24 h after administration (Table 3). Urine protein levels were significantly greater (1.5–3.5-fold) in DCPT-treated rats at the 6 and 12 h time points only (Table 3). An insufficient amount of urine was obtained 1 and 3 h after DCPT administration for analysis. In contrast, there were no significant differences noted in any of the other parameters measured (BUN levels, body weights or liver/kidney weights) between corn oil- and DCPT-treated male rats 1, 3, 6, 12 or 24 h post-dosing (Tables 3 and 4).

No morphological differences were observed in liver sections between the corn oil or DCPT treated male rats 1 h after administration (Figs. 6A and 6B). Compared to the corn oil treatment group (Fig. 6C), liver sections from the 3 h time point group treated with DCPT exhibited slight damage around the central vein (Fig. 6D). Following DCPT treatment, nuclei of hepatocytes were not as round as in the controls. The cytoplasm appeared granular and vacuoles begin to appear. Cells appeared to be in an early stage of injury. Liver sections taken from male rats 6 h and 12 h (Figs. 6E and 6G) after administration of corn oil appeared normal. However, the cytoplasm was lacy in appearance and some vacuolization was noted in hepatocytes 6 h after administration of DCPT (Fig. 6F). Hepatic damage was more evident in DCPT-treated animals 12 h post-dosing (Fig. 6H) than at the earlier time points. Cytoplasm in the cells around the central vein was condensed and irregularly stained, showed larger vacuoles and exhibited a very lacy appearance. One animal at the 12 h time point that was treated with DCPT exhibited moderate steatosis (data not shown). Nuclei were very condensed and the sinusoids were occluded. At the 24 h time point, DCPT-treated animals exhibited pockets of centrilobular necrosis with infiltration of neutrophils into the connective tissue (data not shown). Other areas of the liver showed minor cell death. In many instances, nuclear lysis and nuclear swelling had occurred.

Kidney sections from DCPT-treated male rats exhibited minimal damage 1, 3, 6, 12 and 24 h post-dosing (not shown). Proximal tubules showed minor swelling with proteinaceous deposits accumulating in the lumen. Scattered distal tubules and collecting tubules were slightly dilated, which could have contributed to the increased urinary flow seen in the rats that received DCPT.

4. Discussion

The mechanism of hepatotoxicity of the glitazones, and whether the TZD ring is involved, is unknown. However, two of these drugs remain in clinical use for the treatment of type II diabetes. TZD rings are also found in prototype aldose reductase inhibitors that may be useful in treating complications associated with diabetes (Bruno et al., 2002; Rakowitz et al., 2006). Thus, there is the potential for continued human exposure to TZD ring-containing compounds. Unfortunately, *in vivo* investigations into glitazone-induced hepatic injury have been hampered by the lack of normal, wild-type animal models for toxicity studies (Sharyo et al., 2001; Chojkier, 2005; Ong et al., 2007). DCPT also has a TZD ring as part of its structure, but unlike the glitazones, it produces liver damage in a common strain of rats (Kennedy et al., 2003). Thus, DCPT may be a useful compound for evaluating the potential role of the TZD ring in chemically-induced hepatotoxicity. Our previous structure activity relationship study was limited to a single time point (Kennedy et al., 2003). To further characterize DCPT-induced liver injury we have extended those studies to include a dose-response relationship in male and female rats, and a time course for the onset of hepatotoxicity in males.

The clinical and histological data suggest that liver damage occurs in male rats at doses of 0.4 mmol/kg DCPT and above (Figs. 2 and 3). In contrast, the lowest dose examined (0.2 mmol/kg) produced no apparent effects on rat liver. At 24 h post administration there was a correlation between increasing dose of DCPT, elevations in serum ALT levels (Fig. 2) and morphological changes (Fig. 3). Similar observations have been reported for other hepatotoxins, such as carbon tetrachloride (Janakat and Al-Merie, 2002) and 2,3,7,8-tetrabromodibenzo-p-dioxin (Ohbayashi et al., 2007) and could be due to greater hepatic exposure to the compounds with increasing dose. Liver weight was not a reliable predictor of toxicity from this compound (Table 2). DCPT-induced hepatic damage in male rats consisted primarily of centrilobular (zone 3) necrosis (Fig. 3), which is consistent with the type of liver injury seen in some diabetic patients that used troglitazone (Gitlin et al., 1998; Kohlroser et al., 2000). The zonal nature of troglitazone- and DCPT-induced hepatotoxicity could indicate a role for cytochrome P450 (CYP) enzymes in the generation of a toxic species, since these enzymes are predominantly centrilobular in location (Lindros, 1997). In fact, metabolic activation by CYPs is the most frequent mechanism of hepatocellular injury (Cullen, 2005). Furthermore, there is evidence that CYP-mediated biotransformation in the TZD ring of all three glitazones can lead to the formation of reactive intermediates (Kassahun et al., 2001; Tettey et al., 2001; and He et al., 2004; Baughman et al., 2005; Álvarez-Sánchez et al., 2006). *In vitro* glutathione (GSH) trapping experiments may help determine if DCPT can also undergo metabolic activation in its TZD ring.

Female rats were not as susceptible as males to the hepatotoxic effects of DCPT at comparable doses. Even at the highest DCPT dose examined (1.0 mmol/kg) the effects on serum ALT levels in the females were less pronounced than in the males (Fig. 2) and this was paralleled by the absence of severe hepatic damage on morphological examination (Fig. 3). The most common basis for these gender-dependent effects in rats involves differences in biotransformation (Czerniak, 2001). In fact, male rats generally metabolize drugs and chemicals at higher rates than female rats, although this can depend on the substrate and

enzyme(s) involved (Mulder, 1986; Mugford and Kedderis, 1998). For example, male Sprague-Dawley rats were more susceptible to liver damage from the commonly used drug acetaminophen than females and this was attributed to differences in biotransformation between the sexes (Raheja et al., 1983). Likewise, a gender-specific difference in hepatic CYP isozyme expression may be responsible for the increased metabolic activation of clivorine, a hepatotoxic pyrrolizidine alkaloid, in male versus female rat liver microsomes (Lin et al., 2003). Thus, it is possible that conversion of DCPT to a putative hepatotoxic metabolite may occur more extensively or selectively in male rat liver than in female rat liver, although this will require further investigation.

The onset of DCPT-induced (0.6 mmol/kg) hepatic effects in male rats was rapid. Liver sections exhibited slight damage as early as 3 h after dosing, which was more evident at the 6, 12 and 24 time points. Elevations in serum ALT levels were generally consistent with changes in hepatic morphology and increased over time up to 12 h (Figs. 5 and 6). ALT values at the 24 h time point were comparable to what we observed with 0.6 mmol/kg DCPT in the dose response study (Figs. 2 and 5). Although the mechanism of DCPT-induced hepatotoxicity is not known, the rapid onset of hepatic injury would not necessarily be inconsistent with the formation of a toxic metabolite. For example, acetaminophen (APAP) undergoes CYP-mediated oxidation to form the reactive intermediate N-acetyl-para-aminobenzoquinone imine, which is thought to mediate liver damage by covalent binding (Nelson, 1995). Another well known hepatotoxicant, carbon tetrachloride (CCl₄), is metabolized by CYPs to the trichloromethyl free radical that is responsible for cell membrane injury (Nelson, 1995). Although both of these latter chemicals require metabolic activation to produce hepatotoxic species, liver damage occurs rapidly after administration. For example, Limaye and co-workers (2003) reported that animals treated with a non-lethal dose of CCl₄ showed marked increases in plasma ALT levels within 3 h of administration of the compound. Furthermore, serum ALTs were elevated within 12 h of APAP dosing in rats (Raheja et al., 1983). Conceivably, rapid liver uptake and biotransformation of DCPT to a hepatotoxic species may occur with DCPT as well.

In spite of its close structural similarity to the known nephrotoxicant NDPS (Fig. 1; Rankin, 1982; Rankin et al., 1984; Kennedy et al., 2003), DCPT did not produce any marked effects on rat kidney morphology in males (Fig. 4). Modest changes in BUN levels (1.0 mmol/kg dose only, Table 1) and urine volume/protein levels (0.6 mmol/kg, Table 3) could indicate that DCPT produces mild kidney damage in male rats. Morphological disturbances in female kidneys appeared to be more marked than those in males (Fig. 4); however, whether or not this indicates a gender-based difference in nephrotoxicity will require further investigation. The proteinaceous deposits that we observed in some rat kidney sections were found in both control and DCPT-treated animals, and we do not believe that they are of toxicological significance. Since body weights were not altered by DCPT treatment (Tables 2 and 4) it seems that this compound is not systemically toxic to the animals. Thus, the results of our dose-response and time course studies have clearly shown that the liver is the primary target for DCPT-induced toxicity, especially in male rats. It is conceivable that the specific hepatotoxicity of DCPT reflects differences in hepatic versus renal biotransformation; however, this remains to be determined.

In conclusion, these studies suggest that DCPT has a rapid onset of hepatotoxicity in male Fischer 344 rats. DCPT produces predominantly centrilobular damage which is consistent with the hepatic injury reported for troglitazone. We observed a marked disparity in sensitivity between male and female rats, and one possible explanation for this finding is differential metabolism between the two genders. The fact that liver damage occurs within 3 h of DCPT administration in males suggests that, if biotransformation is required, a hepatotoxic metabolite must be generated rapidly from this compound. However, the

mechanism behind DCPT-induced hepatic injury and whether metabolism is involved requires further investigation. Since DCPT produces predominantly liver damage in laboratory rats it may serve as a useful model compound to study the potential role of the TZD ring in chemically-induced hepatotoxicity.

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NDPS

DCPT

Figure 1.
Structures of troglitazone, NDPS and DCPT.

Figure 2.

Effect of DCPT dose on ALT levels in male and female rats. Animals received either corn oil (4 ml/kg) or DCPT. ALT levels were assessed 24 hours after dosing. Values indicate means $\pm$ standard errors (N=4). An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group in the same gender. A dagger (†) indicates that the value in female rats is significantly different ($p < 0.05$) from the same dose in male rats.

Figure 3.

Effect of DCPT dose on liver histology in male and female rats. Animals received either corn oil (4 ml/kg) or DCPT. Liver sections were obtained 24 hours after dosing. Representative sections are shown for (A) male rats, corn oil; (B) female rats, corn oil; (C) male rats, 0.4 mmol/kg DCPT; (D) female rats, 0.4 mmol/kg DCPT; (E) male rats, 0.6 mmol/kg DCPT; (F) female rats, 0.6 mmol/kg DCPT; (G) male rats, 1.0 mmol/kg DCPT; and (H) female rats, 1.0 mmol/kg DCPT. Black arrow heads define an area of necrosis. White arrow heads define areas of inflammation and neutrophil infiltration. The white arrows indicate examples of swollen hepatocytes. Sections were stained with hematoxylin-eosin. Magnification is 400 $\times$.

Figure 4.

Effect of DCPT dose on kidney histology in male and female rats. Animals received either corn oil (4 ml/kg) or DCPT. Kidney sections were obtained 24 hours after dosing. Representative sections are shown for (A) male rats, corn oil; (B) female rats, corn oil; (C) male rats, 1.0 mmol/kg DCPT; and (D) female rats, 1.0 mmol/kg DCPT. Black arrow heads define a swollen glomerulus. White arrow heads point to swollen proximal tubules. Sections were stained with hematoxylin-eosin. Magnification is 400 $\times$.

Figure 5.

Effect of DCPT on serum ALT levels in male rats 1, 3, 6, 12 and 24 hours after dosing.

Animals received either corn oil (4 ml/kg) or DCPT (0.6 mmol/kg). Values indicate means $\pm$ standard errors (N=3-4). ALT data from one rat (corn oil, 12 h time point) was omitted due to hemolysis in the serum sample. An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group at the same time point.

Figure 6.

Effect of DCPT on liver histology in male rats 1, 3, 6 and 12 hours after dosing. The 24 h time point is not shown, but morphological changes were comparable to those seen in the dose response study. Animals received either corn oil (4 ml/kg) or DCPT (0.6 mmol/kg). Representative sections are shown for (A) 1 h, corn oil; (B) 1 h, DCPT; (C) 3 h, corn oil; (D) 3 h, DCPT; (E) 6 h, corn oil; (F) 6 h, DCPT; (G) 12 h, corn oil; and (H) 12 h, DCPT. Black arrow heads indicate examples of granular cytoplasm. White arrow heads indicate examples of vacuoles. The white arrow points to a hepatocyte with condensed cytoplasm. Sections were stained with hematoxylin-eosin. Magnification is 400x.

The effect of corn oil (4 ml/kg) or DCPT (0.2, 0.4, 0.6 or 1.0 mmol/kg) on rat blood urea nitrogen concentration, urine volume and urine protein content.

Table 1

DCPT Dose (mmol/kg)	Blood Urea Nitrogen (mg/dl)		24 hour Urine Volume (ml)		6 hour Urine Protein Content (mg)	
	Male	Female	Male	Female	Male	Female
corn oil	23.1 ± 1.8	34.6 ± 1.5 [†]	4.9 ± 0.8	9.8 ± 0.5	1.4 ± 0.2	0.12 ± 0.02
0.2	25.7 ± 3.3	30.4 ± 1.5 [*]	12.1 ± 2.1	8.0 ± 1.3	3.3 ± 1.1	0.47 ± 0.17
0.4	20.1 ± 1.1	29.6 ± 4.0 [*]	20.6 ± 5.5	16.6 ± 0.6	2.4 ± 0.5	0.13 ± 0.04
0.6	20.1 ± 2.9	40.4 ± 1.1 ^{*†}	21.2 ± 6.4	17.0 ± 2.7	2.7 ± 0.4	0.13 ± 0.01
1.0	43.9 ± 5.9 [*]	55.7 ± 10.4 [*]	17.4 ± 3.1	14.0 ± 2.1	3.2 ± 0.5	0.42 ± 0.13

Values indicate means ± standard errors (N=4).

An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group in the same gender.

A dagger (†) indicates that the value in female rats is significantly different ($p < 0.05$) from the same dose in male rats.

Table 2

The effect of corn oil (4 ml/kg) or DCPT (0.2, 0.4, 0.6 or 1.0 mmol/kg) on rat body, liver and kidney weights.

DCPT Dose (mmol/kg)	Body Weight (g)		Liver Weight (g/100 g body weight)		Kidney Weight (g/100 g body weight)	
	Male	Female	Male	Female	Male	Female
corn oil	218.0 ± 10.5	140.3 ± 2.7	3.55 ± 0.21	3.82 ± 0.08	0.40 ± 0.01	0.37 ± 0.02
0.2	210.0 ± 7.9	114.5 ± 2.8*	3.81 ± 0.18	4.22 ± 0.12	0.38 ± 0.01	0.45 ± 0.01*†
0.4	196.8 ± 12.2	144.3 ± 2.1	3.96 ± 0.18	4.07 ± 0.19	0.38 ± 0.01	0.38 ± 0.01
0.6	203.4 ± 3.3	136.5 ± 3.5	3.55 ± 0.08	3.66 ± 0.05	0.40 ± 0.01	0.39 ± 0.01
1.0	204.8 ± 2.3	132.0 ± 4.6	3.81 ± 0.24	3.71 ± 0.14	0.44 ± 0.00	0.41 ± 0.01

Values indicate means ± standard errors (N=4).

An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group in the same gender.

A dagger (†) indicates that the value in female rats is significantly different ($p < 0.05$) from the same dose in male rats.

Table 3

The effect of corn oil (4 ml/kg) or DCPT (0.6 mmol/kg) on rat blood urea nitrogen concentration, urine volume and urine protein content 1, 3, 6, 12 and 24 hours post-dosing.

Time Post-dosing (h)	Blood Urea Nitrogen (mg/dl)		Urine Volume (ml)		Urine Protein Content (mg)	
	Corn oil	DCPT	Corn oil	DCPT	Corn oil	DCPT
1	26.5 ± 2.0	22.5 ± 1.4	n.d. ^a	n.d. ^a	n.d. ^a	n.d. ^a
3	22.7 ± 1.6	21.8 ± 2.8	1.0, 1.0 ^b	1.5 ± 0.4	1.14, 1.94 ^b	2.6 ± 0.3
6	24.0 ± 0.6	17.7 ± 1.4	0.9 ± 0.2	2.9 ± 0.4 [*]	2.8 ± 0.1	4.2 ± 0.5 [*]
12	19.2 ± 2.3	20.3 ± 4.5	0.9 ± 0.1	3.6 ± 0.3 [*]	0.7 ± 0.2	2.6 ± 0.2 [*]
24	24.6 ± 2.6	28.2 ± 2.5	4.8 ± 0.6	14.0 ± 2.1 [*]	1.3 ± 0.4	2.5 ± 0.7

Values indicate means ± standard errors (N=3-4). BUN data from one rat (corn oil, 12 h time point) was omitted due to hemolysis in the serum sample.

An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group at the same time point.

^aNot detectable, no urine was obtained at the one hour time point in either treatment group

^bUrine was only obtained from two animals and the actual urine volumes are shown. The 3 h time point data was not included in the statistical analyses.

Table 4

The effect of corn oil (4 ml/kg) or DCPT (0.6 mmol/kg) on rat body, liver and kidney weights 1, 3, 6, 12 and 24 hours post-dosing.

Time Post-dosing (h)	Body Weight (g)		Liver Weight (g/100 g body weight)		Kidney Weight (g/100 g body weight)	
	Corn oil	DCPT	Corn oil	DCPT	Corn oil	DCPT
1	217.5 ± 5.4	204.5 ± 3.3	4.04 ± 0.11	3.63 ± 0.17	0.40 ± 0.01	0.416 ± 0.004
3	193.3 ± 5.9	208.5 ± 5.3	3.26 ± 0.26	3.34 ± 0.14	0.38 ± 0.01	0.41 ± 0.02
6	214.8 ± 3.3	203.8 ± 3.3	3.51 ± 0.09	3.59 ± 0.08	0.39 ± 0.01	0.39 ± 0.01
12	206.3 ± 3.1	198.3 ± 2.8	3.35 ± 0.10	4.99 ± 1.32	0.37 ± 0.01	0.40 ± 0.01
24	196.7 ± 4.1	181.2 ± 6.9	3.85 ± 0.24	3.83 ± 0.06	0.37 ± 0.01	0.40 ± 0.01

Values indicate means ± standard errors (N=4).

An asterisk (*) indicates that the value is significantly different ($p < 0.05$) from the corn oil control group at the same time point.