EVALUATION OF THE TOXICITY OF A SUBSTITUTED 2,4-THIAZOLIDINEDIONE MOIETY TO ISOLATED RAT HEPATOCYTES: RELEVANCE TO GLITAZONE TOXICITY

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Troglitazone (TGZ), a 2,4 thiazolidinedione (TZD) anti-diabetic agent, has been associated with hepatotoxicity in type II diabetic patients. The mechanism of toxicity has not yet been established. However, it has been reported (Kennedy *et al*., 2003) that the incorporation of a sulphur atom in the cyclic imide structure of N-(3,5-dichlorophenyl)succinimide (NDPS), analogous to the 2,4-TZD moiety in TGZ, resulted in hepatotoxicity. In this study we have examined the relative *in vitro* hepatotoxicity of 3-(3,5-dichlorophenyl)-2,4,thiazolidinedione (DCPT), which contains the 2,4-TZD moiety, and that of its structural analogue NDPS.

NDPS and DCPT were synthesised using a modification of the method of Fujinami et al (1971) and characterised by NMR and mass spectrometry. Hepatocytes were prepared from male Sprague-Dawley rats (180-220g), and cell viability was measured using Trypan Blue exclusion. Preparations with initial cell viability above 80% were used in all experiments. Cells were incubated for 3 hours with NDPS and DCPT at (0µM, 100µM, 500µM and 1mM in dimethylsulphoxide (0.1% (v/v)) at 37^{o}C in an atmosphere of 95%O_2/5%CO_2). Samples were taken at regular time intervals (0, 15, 30, 60 90, 120, 180 minutes) for the measurement of viability, reduced glutathione (GSH) content and lactate dehydrogenase (LDH) activity in the extracellular medium.

Table 1. Viability, GSH content and LDH activity after exposure of hepatocytes to DCPT and NDPS for 180 minutes

Treatment (mM)	Viability (%)		GSH Content (nmol/10^6 cells)		LDH activity (nmol/min/10^6 cells)	
	DCPT	NDPS	DCPT	NDPS	DCPT	NDPS
0	58.0±2.0	58.5±3.8	28.8±4.1	40.2±7.3	1.5±0.3	1.5±0.3
0.1	56.0±7.4	56.5±3.4	32.5±5.4	37.7±5.7	1.3±0.3	1.5±0.3
0.5	49.0±1.9	50.0±4.7	27.8±5.3	33.8±7.7	1.2±0.3	1.3±0.2
1.0	46.0±2.0	49.5±6.9	28.2±3.9	31.6±7.8	1.2±0.3	1.3±0.3

Data are Mean ± S.E.M., n=4 separate experiments.

Statistical analyses (ANOVA followed by Dunnett's test) of the data (Table 1) obtained for hepatocytes exposed to DCPT and NDPS did not reveal significant differences in GSH content, LDH activity or cell viability over a 3h incubation period. These data indicate that the incorporation of a sulphur atom in the succinamide ring of NDPS to produce the corresponding 2,4 TZD (DCPT) does not result in an increase in hepatotoxic effects *in vitro*. This finding, together with our previous report on the lack of toxicity of the 2,4-TZD containing, rosiglitazone (Ball et al 2004), would suggest that a chemical mechanism of toxicity of TGZ (if feasible) might be a function of the whole molecule rather than the TZD moiety alone.

Kennedy E.L. *et al*. (2003) *Toxicol*., **186**, 79-91.
Fujinami A et al (1971) *Agric. Biol. Chem*. **35**, 1707-1719.
Ball A.J. *et al* (2004) *Toxicol*., **194**, 250-251.