Cytotoxicity of the thiazolidinedione compounds troglitazone and pioglitazone in human isolated hepatocytes

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Introduction

The thiazolidinedione compounds troglitazone and pioglitazone represent a new class of drugs used in the treatment of type II (non-insulin dependent) diabetes mellitus (Subramaniam, 1999). Recently, there have been several reports of hepatic injury associated with troglitazone, leading to its withdrawal from world markets (Kohlroser *et al.*, 2000; Warner-Lambert Press Release, 2000). In the present study, we evaluated the effect of troglitazone and pioglitazone upon release of LDH, ALT and AST, as markers of acute cytotoxicity in human freshly isolated hepatocytes. Acetylsalicylic acid was also studied, as it has been previously shown to cause cytotoxicity in rat hepatocytes (Shrivastava *et al.*, 1992).

Methods

Hepatocytes were isolated from liver wedges by two-step collagenase digestion (Strom *et al.*, 1982), re-suspended in William's E medium and cultured on collagen-coated 96-well plates or 8-well slide chambers for 10-12 h. The donor details of the liver samples are shown in Table 1. Hepatocytes were treated with troglitazone, pioglitazone and acetylsalicylic acid in serum-free, basic Williams E medium for 24 h. Control and solvent (DMSO) treated cultures were also prepared. Cell supernatants were removed and assayed for LDH, ALT and AST release using standard kits. Hepatocytes cultured in 8-well slide chambers were fixed in 30% methanol, and the H&E method of cell staining was applied. Statistical analysis of the data was performed using a one-way ANOVA and Dunnett's post-test.

Donor	Age	Sex	Surgical Procedure
1	53	Female	Excision of small end of liver
2	65	Female	Right hepatectomy
3	75	Male	Right hepatectomy

Table 1. Donor details of human liver samples

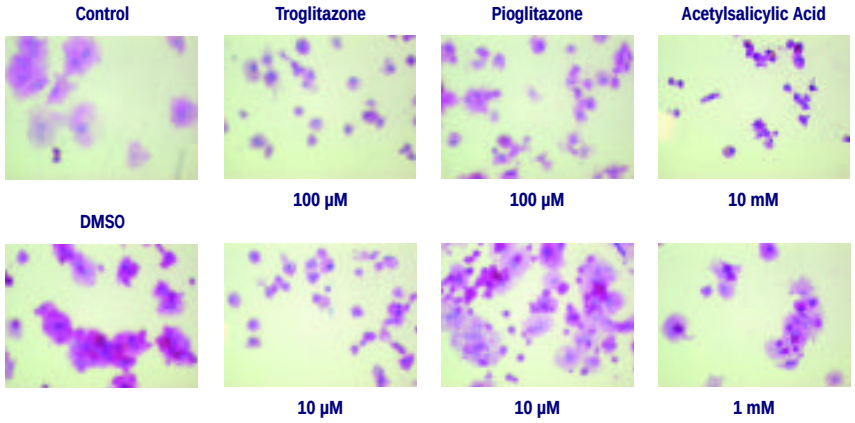

Figure 2. H&E staining of hepatocytes (x400 magnification) from donor 1 treated with troglitazone, pioglitazone, acetylsalicylic acid and DMSO (1%).

References

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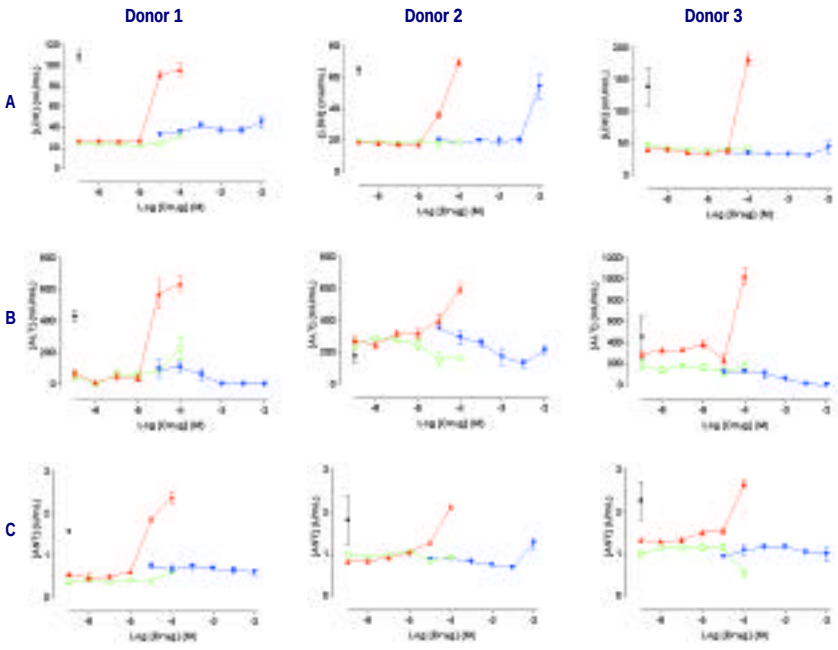

Figure 1. Effect of troglitazone (▲), pioglitazone (□), acetylsalicylic acid (▼) and 1% Triton X-100 (◆) upon A) LDH; B) ALT and C) AST release. Data are the mean ± SEM from three donors.

Results

Troglitazone caused significant release of LDH, ALT and AST at 100 µM in cells from all donors when compared to control values (Figure 1, $P < 0.01$). In the case of LDH, release by troglitazone was similar to that observed with Triton X-100, consistent with cell lysis. However, the effects of Triton X-100 on ALT and AST release were less marked or consistent. Pioglitazone caused release in only one donor at 100 µM and acetylsalicylic acid produced significant release of LDH and AST, but not ALT in one donor at 10 mM ($P < 0.01$). The rank order of potency for enzyme release was troglitazone > pioglitazone >> acetylsalicylic acid. H&E staining identified morphological characteristics indicating cell lysis with 100 µM and 10 µM troglitazone, 100 µM pioglitazone, and 10 mM acetylsalicylic acid (Figure 2).

Conclusions

These data demonstrate for the first time that the glitazone compounds caused differential effects on markers of cell integrity consistent with acute cytotoxicity in human isolated hepatocytes. Further studies will be required to characterise the observed phenomena.

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