

Introduction

A diversity of chemicals elicit marked hepatomegaly when administered to rodents. Many, if not all of these agents exert their liver growth effects *via* activation of specific transcription factors of the nuclear hormone receptor super family (e.g. PPAR, PXR, CAR). The hepatomegaly is characterised by both hypertrophy and hyperplasia; the latter being due to increased cell replication and decreased apoptosis.

The present studies were designed to investigate species differences in the “liver growth” effects of PPAR (Peroxisome Proliferator Activated Receptor) agonists. Wy14643 was used as a PPAR α “selective” agonist and rosiglitazone and troglitazone were selected as PPAR γ “selective” agents.

Methods

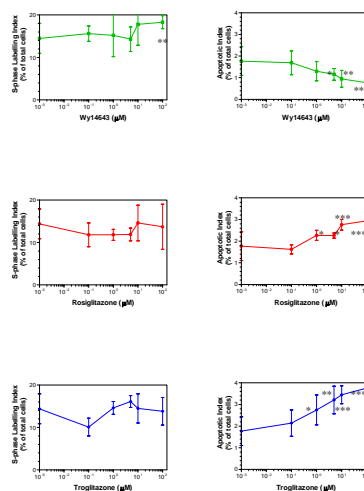
- Male Dunkin Hartley guinea pigs (~ 350 to 400g) and male Sprague-Dawley (CrI: CD BR) rats (~ 200-250g) were used.
- Rats and guinea pigs were terminally anaesthetised (pentobarbital) and hepatocytes were isolated by *in situ* perfusion.
- Viabilities (trypan blue exclusion) of the hepatocyte preparations were in excess of 90% for both species.
- Three independent human hepatocyte preparations (UK Human Tissue Bank) were used: Donor 1, 78 year old male, resection for tumour removal (89% viable); Donor 2, 59 year old female, resection for tumour removal (62% viable); Donor 3, 56 year old male, resection for tumour removal (81% viable).
- Hepatocytes were cultured in Leibowitz CL15 medium, with the addition of 5 mg/L vitamin C (for guinea pigs and humans only), for 4 hours, the media were changed and the cells then exposed to rosiglitazone, troglitazone and Wy14643. The media were replenished daily for a further 3 days.
- S-phase (replicative DNA synthesis) was determined immunocytochemically following the incorporation of BrdU into hepatocyte nuclei over the last 2 days of culture. Data were expressed as a labelling index (% of total cells that have incorporated BrdU). EGF (25ng/mL) was included as a positive control in some cultures.
- The apoptotic index of the cultures was determined using the dye Hoechst 33258 and fluorescent microscopy. Data were expressed as % apoptotic cells. Transforming growth factor β_1 (TGF β_1 , 5ng/mL) was included as a positive control in some cultures 24 hours before harvest.
- Student's t-Test (2-sided) was performed on the data; * = statistically different from control at p<0.05; ** = p<0.01; *** = p<0.001.

Results

- In rat hepatocytes Wy14643 stimulated S-phase at all concentrations above 0.1 μ M. Rosiglitazone and troglitazone increased the labelling index at the lower concentrations examined, but as the concentration increased the stimulation of S-phase was lost (Figure 1).
- Wy14643 inhibited apoptosis in a dose-dependent manner in rat hepatocytes. A dose-dependent stimulation of apoptosis was seen following exposure of rat hepatocytes to rosiglitazone and troglitazone (Figure 1).

- All three compounds decreased the labelling index in human hepatocytes, with rosiglitazone and troglitazone completely inhibiting S-phase (Figure 3). This was not accompanied by overt cytotoxicity (data not shown).
- In two of the donor human hepatocytes cultures (1 and 3), rosiglitazone and troglitazone stimulated apoptosis. Wy14643 had no effect.

Figure 1. S-phase and Apoptosis in Cultured Guinea Pig Hepatocytes



Labelling Index for EGF (25ng/ml) = 27 \pm 3.6 %
Apoptotic Index with TGF β (5ng/mL) = 3.2 \pm 0.49 %

Figure 1. S-phase and Apoptosis in Cultured Human Hepatocytes

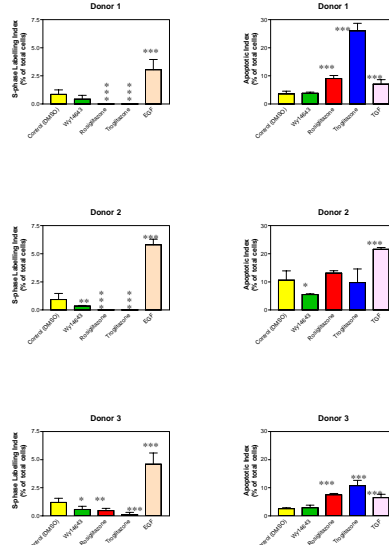
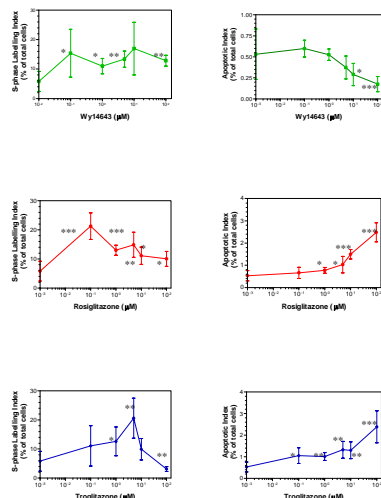


Figure 1. S-phase and Apoptosis in Cultured Rat Hepatocytes



Labelling Index with EGF (25ng/ml) = 20 \pm 5.4 %
Apoptotic Index with TGF β (5ng/mL) = 3.1 \pm 0.40 %

Summary

PPAR Agonist	Rat	Guinea Pig	Human
	Labelling Index (S-phase)		
Alpha	increased	no change	decreased
Gamma	biphasic effect	no change	decreased
Apoptotic Index			
Alpha	decreased	decreased	no change (2/3)
Gamma	increased	increased	increased (2/3)

- Guinea pig hepatocyte S-phase was unaffected by Wy14643, rosiglitazone and troglitazone (Figure 2). A slight inhibition of apoptosis was seen following exposure to Wy14643; however rosiglitazone and troglitazone caused marked increases in apoptotic index.