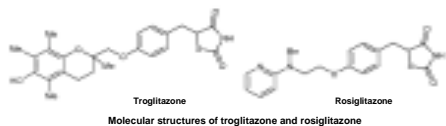


A Comparison of Cytotoxicity in Troglitazone vs Rosiglitazone in Cryopreserved Human Hepatocytes

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Troglitazone and Rosiglitazone Structures



Introduction

- Troglitazone and rosiglitazone are thiazolidinediones indicated for the treatment of type 2 (noninsulin dependent) diabetes mellitus. Their cellular action is believed to be mediated by the peroxisome proliferator-activated receptor γ (PPAR γ).
- Troglitazone was introduced in the market in January, 1997, and was withdrawn from the market in March, 2000, due to reports of its association with fulminant hepatic failure.
- Rosiglitazone was the second thiazolidinedione marketed in the US and was approved by the FDA in May, 1999. Rosiglitazone continues to be used and is considered safe. As of this writing, there are two reported cases associating rosiglitazone with severe hepatocellular injury.
- This study represents part of our comprehensive program to develop screening assays for human hepatotoxicity using isolated human hepatocytes as an experimental system.

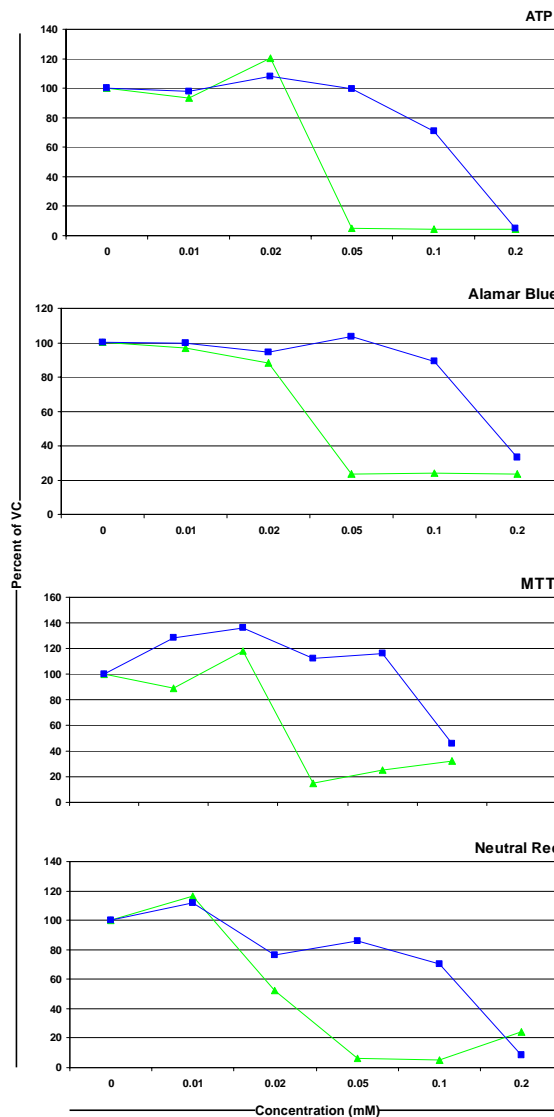
Materials and Methods

- Cryopreserved human hepatocytes** from 36 separate donors in our human hepatocyte bank were used for toxicity screening. The cells were isolated, cryopreserved and thawed using previously published procedures (Loretz *et al.*, 1989; Li *et al.*, 1992; 1999; Lu C and Li AP, 2001).
- Troglitazone and rosiglitazone** were obtained from Parke-Davis (Ann Arbor, MI). The final troglitazone concentrations used in the incubations were 0 (vehicle control; VC), 10, 20, 50, 100 and 200 μ M. DMSO was used as solvent. The final DMSO concentration was 0.1% v/v. Incubations of hepatocytes with drug and control solutions were carried out at 37°C, 5% CO₂ in saturating humidity for 2 hours.
- Cell viability** of the hepatocytes from all incubations was measured as a function of **adenosine triphosphate (ATP) production** using the ATP Lite-M kit from Packard Marketing (Groningen, The Netherlands). **MTT metabolism** was measured using MTT reagent purchased from Sigma (St. Louis, MO). **Alamar Blue** was purchased from Bio Source International (Camarillo, CA). **Neutral Red** uptake was measured using the Neutral Red Assay Kit (Sigma, St. Louis, MO).

Conclusions

- Both troglitazone and rosiglitazone cause dose-dependent cytotoxicity in human hepatocytes.
- Results with all donors show that rosiglitazone is consistently less cytotoxic than troglitazone. The results are consistent with clinical observations regarding the human hepatotoxic potential of these two drugs.
- Cryopreserved human hepatocytes represent a useful system for testing human cytotoxicity. The use of multiple donors allows a better definition of hepatotoxic potential, as well as providing initial information on individual differences in toxicity.

Results



EC50 Values (mM)					
Donor	Age	Sex	Race	Troglitazone	Rosiglitazone
105	59	Male	Caucasian	67.1	87.4
108	59	Male	Asian	105.0	106.3
109	69	Male	Caucasian	128.6	128.6
111	59	Female	Black	55.7	88.8
113	61	Female	Caucasian	91.4	132.2
114	47	Female	Caucasian	82.7	88.1
118	48	Female	Caucasian	142.8	233.8
119	48	Female	Caucasian	90.7	191.4
122	42	Male	Hispanic	71.9	124.4
129	N/A	N/A	N/A	92.2	196.4
130	2	Female	Caucasian	77.8	111.7
133	59	Female	Hispanic	81.5	125.3
59	33	Male	Caucasian	92.6	101.7
61	38	Male	Caucasian	63.7	62.6
62	46	Male	Caucasian	63.6	73.1
66	48	Male	Caucasian	95.1	146.2
70	57	Male	Caucasian	52.0	95.3
71	23	Male	Hispanic	83.4	103.2
75	15 mo.	Male	Caucasian	92.2	196.4
86	73	Female	Caucasian	76.3	135.9
88	84	Female	Caucasian	95.6	142.7
89	56	Male	Caucasian	99.6	57.0
90	51	Female	Caucasian	78.0	162.2
91	72	Female	Caucasian	139.4	139.4
95	43	Male	Caucasian	64.9	138.8
CNV	38	Male	Caucasian	93.2	133.8
DRL	44	Male	Caucasian	78.1	103.8
EFA	16	Male	Caucasian	63.2	78.9
ETR	51	Female	Caucasian	85.6	117.9
EVY	63	Male	Caucasian	69.3	105.8
GNG	45	Female	Caucasian	85.3	105.4
HRK	66	Female	Caucasian	115.2	124.5
KMI	45	Male	Caucasian	101.3	103.7
MQF	49	Female	Caucasian	57.8	123.3
MYO	59	Female	Caucasian	91.7	91.7
TVC	58	Female	Caucasian	91.7	74.4

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