INTRODUCTION

Drug-induced liver toxicity has been one of the most frequent reasons for restriction or withdrawal of an approved medicinal product from the market. Tolcapone, a nitrocatechol O-methyltransferase (COMT) inhibitor has been associated with increased risk of drug-induced liver injury, and it is, therefore, essential to predict the potential risk of other nitrocatechol drug candidates to induce hepatotoxicity. Entacapone has not been associated with liver toxicity. Opicapone, a third generation novel nitrocatechol COMT inhibitor, was specifically designed as a hydrophilic 1,2,4-oxadiazole analogue with a pyridine N-oxide residue at position 3 and to provide high inhibitory potency while avoiding cell toxicity. In the present study, the potential hepatotoxicity risk of opicapone, as compared to tolcapone and entacapone was evaluated.

AIM

This study evaluated the potential hepatotoxicity risk of opicapone, as compared to tolcapone and entacapone.

MATERIALS & METHODS

Cells: Fresh human hepatocytes (5 donors) were plated onto 24-well collagen plates with a matrigel layer (cat. 03757-PL/15). HepaRG cells were obtained from Lonza (Walkersville MD). HepaRG cells were maintained in liver medium (Lonza) containing 10% FCS, 1% penicillin/streptomycin (1000/1 mg/ml) and amphotericin (0.075 µg/ml). HepaRG cells were cultured in Williams’ E media supplemented with 5 µM FPP and 25 mM HEPES. HepaRG cells were subjected to the amplification and differentiation protocol as described by the manufacturer. HepaRG cells were kept in differentiation media (hatched bars) or tolcapone (horizontal dashed line) for 24 days and used for experiments within 7 days.

Calcium AM cell viability assay: Fresh hepatocyte cell viability was measured using calcein-AM, a nonfluorescent cell permeant dye that, in live cells, is converted by intracellular esterases to green fluorescent calcium, as described.

ATP depletion: The ATP content of HepaRG cells and human cryopreserved hepatocytes was determined using the ATPlite assay system (Perkin Elmer) according to manufacturer instructions.

Caspase 3/7 activation: Caspase 3 activity was measured using the Caspase 3 Assay Kit (Sigma) according to manufacturer instructions.

Mitochondrial membrane potential (MMP) evaluation: MMP was determined using the fluorescent dye JC-1. In brief, cryopreserved hepatocytes and HepaRG cells were washed with HBSS and stained with 100 µg JC-1/15 µl for 1 h at 37°C in the dark. Cells were washed with HBBS and incubated with test compounds (5, 10, 50, 100 µM) for 2 h. Negative controls (no compound) and positive controls (0.175, 0.5, 1.25, 5.0 µM (Sigma)) were performed in parallel. After incubation, cells were washed with HBBS and fluorescence was measured.

Glutamate dehydrogenase (GSH) assay: Liver tissue was homogenized (1:10 w:v) in ice-cold 0.1 M sodium phosphate buffer (pH 7.4). The homogenate was centrifuged at 5000 rpm for 10 min at 4°C. The supernatant was used for the determination of GSH. The tissue GSH level was determined by calorimetric assay (Sigma) according to manufacturer instructions.

RESULTS

Opicapone did not affect the viability of human primary hepatocytes (Fig. 1) whereas tolcapone reduced human hepatocyte viability and altered cellular morphology.

Opicapone was the least potent compound in decreasing both MMP and ATP content in human primary hepatocytes with IC50 values (Table 1) of, respectively, 74.8 µg/ml (181 µM) and 40.5 µg/ml (96 µM), whereas for tolcapone the values obtained were, respectively, 7.9 µg/ml (29 µM) and 6.8 µg/ml (25 µM). In HepaRG cells MMP IC50 values for opicapone and tolcapone were of 500 µM and 120 µM, respectively.

Neuroprotective property with no liver injury, the drug compounds were subjected to the neuronal induction test (Fig. 2), caspase activation (Fig. 3) or glutathione depletion (Fig. 4), whereas tolcapone (200 µM) significantly compromised cell viability and depleted glutathione.

No GSH-adducts of opicapone were detected in liver microsomes, whereas GSH-adducts from tolcapone and entacapone metabolites were found (Fig. 5).

CONCLUSION

Opicapone appears to be devoid of toxicity effects in human hepatocytes and in HepaRG cells under the conditions tested.

BIBLIOGRAPHY