

Department of Clinical Laboratory Sciences

Graduate Masters Theses

**Effect Of Anticonvulsants On MRP-2 Gene And Protein Expression.**

Lynnette Walters. MS, May 2002.

**ABSTRACT**

The canalicular multispecific organic anion transporter (MRP-2) reportedly is responsible for the biliary transport of the carboxylate forms of CPT-11 and SN-38, as well, the lactone and carboxylate forms of SN-38 glucuronide. MRP-2 (symbol: ABCC2; cMOAT in rats) is a member of the ATP-binding cassette transporters. MRP-2 is present in the apical membrane of the liver, kidney, intestine, blood-brain-barrier and expressed in the human lung, gastric and colorectal cancer cells. MRP-2 is a 1545 amino acid glycoprotein (190kDa) encoded by a distinct gene found on chromosome 10q24. MRP-2 has a sequence homology of 49% with the human multidrug resistance associated protein (MRP1). MRP-2 functions as a conjugate export pump mediation the unidirectional transport of bilirubin glucuronides, reduced folate and amphiphilic anions, particularly lipophilic substances conjugated with glutathione, glucuronide or sulfide.

Significant brain tumor concentrations of CPT-11 and SN-38 can be achieved in the SV-40 transformed (C57B1/6J- TgN (SV) 7 Bri) brain tumor mouse model. Preliminary data for clinical trials of patients with malignant brain tumor treated with CPT-11 indicated that enzyme inducing antiepileptic drugs (EIAEDs) such as phenytoin have profound effects on the pharmacokinetics (lower concentrations) and pharmacodynamics (decreased toxicity) of CPT-11. These effects cannot be totally explained by the influence of the EIAEDs on the induction of cytochrome P450-3A4, carboxylesterase or glucuronyltransferase. The effects of EIAEDs on MRP-2 have not been characterized. Reverse transcription polymerase chain reaction (RT-PCR) was utilized to determine the presence or absence of MRP-2 gene in HepG2 cells (reported to express MRP-2), in human peripheral mononuclear cells (PBMN), SV-40 murine transformed brain tumor cell line and in the U87 human derived brain tumor cell line. The effect of phenytoin (prototypic enzyme inducing anticonvulsant), valproic acid (non-EIAEDs), dexamethasone (steroid), and SN-38 (topo I inhibitor) on the expression of MRP-2 mRNA and protein content in HepG2 cells has been evaluated utilizing RT-PCR and Western blot analysis. RT-PCR for MRP-2 indicates the presence of mRNA in HepG2, PBMN, and the U87 cell lines. The murine SV-40 cells, all cells expressed glyceraldehydes -3-phosphate dehydrogenase (GADPH), a ubiquitous "house keeping" gene used for quantitative RT-PCR and to correct for variation in the PCR amplification. Western Blot analysis revealed that the MRP-2 protein was only expressed in the HepG2 cells. Although U87 cells carry the gene sequence for MRP-2, not protein expression was observed. Furthermore, incubation of the U87 cells with phenytoin for 24 hours did not induce expression of the MRP-2 protein. Confluent HepG2 cells were incubated with clinically achievable concentrations of phenytoin, valproic acid, dexamethasone and SN-38 for 0, 6, 12 and

24 hours. Messenger RNA was extracted and evaluated using RT-PCR. Neither increase, nor decrease in mRNA was observed. Respective protein samples were prepared at the same time as mRNA. Western Blot analysis did not indicate an increase or decrease of protein in the HepG2 cells. Under these experimental conditions, SN-38 phenytoin, valproic acid and dexamethasone at clinically achievable concentrations had no effect on MRP-2 gene and protein expression. Our in vitro results suggest that phenytoin, valproic acid and dexamethasone will not influence the hepatic elimination of SN-38 by the up-regulation of MRP-2.