INTRODUCTION

Previously we reported that increased resazurin reduction to resorufin and/or decreased removal of resorufin in response to carboxylated drug candidates is consistent with reactive acyl glucuronides (RAGs) generated from parent drugs in HepG2 cells (Hari Singh et al., 2015). NOO1 appears to be the critical enzyme inhibited by RAGs in HepG2 cells, resulting in inhibition of resorufin fluorescence removal, with the increased fluorescence providing an easy, convenient screen for evaluating compounds for RAG activity (Hari Singh et al., 2015). However, HepG2 cells are metabolism deficient and although enough glucuronidation is present to detect RAGs, the sensitivity of the resazurin assay is low. In the present study, we have taken advantage of the much higher metabolic capacity (glucuronidation and Cyp activities) in freshly isolated primary rat hepatocyte cultures, to examine the sensitivities of three NSAIDs in the RAG resazurin assay. Diclofenac, difluorinated and flufenamic acid all produced RAGs at concentrations similar or far below their therapeutic concentrations. At low concentrations, diclofenac (as the other two NSAIDs examined) is well known to be metabolized by Cyps rather than glucuronidated. Cyp inhibitors reduce diclofenac cytotoxicity by diverting diclofenac away from its cytotoxic metabolites to the formation of RAGs (Kretz-Rommel and Boelsterli, 1993). We observed potentiation of the resorufin fluorescence intensity with Cyp inhibitor ketocanozole (15 μM) at two concentrations of diclofenac, which are close to its therapeutic concentration (consistent with Kretz-Rommel and Boelsterli, 1993). The sensitivity of the assay to difluorinated and flufenamic acid were not similarly increased by ketocanozole. We used SKF 525A (40 μM, pre-incubated for an hour) as a non-selective inhibitor of Cyp 450 enzymes and observed a huge potentiation of the resorufin fluorescence intensity at various concentrations of diclofenac and attributed it to better Cyp inhibition.

CONCLUSION

Potentiation of resorufin fluorescence intensity by diclofenac is observed by several Cyp inhibitors and this suggests that inhibition of the Cyp metabolism diverts more of the diclofenac away from its cytotoxic metabolites to more RAGs (complimentary to Kretz-Rommel and Boelsterli’s observation with diclofenac cytotoxicity). It has been pointed out that SKF 525A is metabolized to a potent diclofenac antagonist but more important from the perspective of the resazurin/resorufin assay to its ability to inhibit the mRNA expression for the SKF 525A. The SKF 525A thus turns RAGs which might interfere with the removal of resorufin, thereby allowing measurement that are commonly being observed.

Evaluating Reactive Acyl Glucuronides Formation from Diclofenac using a Resazurin/Resorufin Assay with Primary Rat Hepatocytes

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