

TOXICOLOGICAL EVALUATIONS FOR ANTIMALARIAL LEAD OPTIMIZATION USING PRIMARY CULTURES AND A HUMAN HEPATOCYTE CELL LINE

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Lead compounds from different families are being evaluated by the U.S. Army for development as candidate antimalarial drugs. The objectives of this study were to compare toxicological evaluations of antimalarial leads in primary cultures and the HC-04 human hepatocyte cell line. HC-04 cells have proliferated freely in serum and hormone-supplemented medium after 4 years in continuous culture and are free of non-hepatocellular cells. HC-04 cells support complete development of *Plasmodium falciparum* exoerythrocytic stage and have been established as an *In vitro* model to study antimalarial drug and vaccine development. Commercially available human hepatocytes and HC-04 cells were subcultured in 96 well plates at 40,000 cells/cm². 72-hour toxicity tests were started by adding culture medium containing drugs with medium replacement every 24 h. Drugs stock solutions were prepared in organic solvents at 100X the higher concentration tested. Control wells received culturing medium containing 1% organic solvents. Cellular toxic responses were evaluated using two different cell viability assays: 1. Propidium iodide that binds double strand DNA of affected cells with permeable membranes; and 2. CCK-8 which is a water soluble tetrazolium salt that produces a water soluble formazan dye upon reduction by cellular dehydrogenases. Both methods were simple and easy to conduct with similar sensitivity; however, the CCK-8 method was a 2 step-method with no washes incorporated. Work in progress includes the evaluation of known *in vivo* hepatotoxins such as acetaminophen, Cu, dimethylformamide and microcystin. Lead compounds from the same family of drugs will be ranked according to the LC50 for both primary cultures and HC-04 human hepatocyte cell line. This study provides insight for lead optimization of antimalarial drugs by assisting further design and chemical synthesis of new analogs with desired attributes, and by building databases for modeling and structure activity relationships.

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