A novel method for evaluating the potential of reactive intermediates formation by using $^{35}$S-cysteine and $^{14}$C-cyanide as nucleophilic trapping reagents

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Metabolic activation of drugs to form reactive intermediates has been known to be responsible for various drug-induced adverse reactions. A conventional assay to investigate the potential reactivity of metabolites to microsomal proteins using radiolabeled drug materials has been established (Evans et al., 2004, Chem. Res. Toxicol.). We present a novel semi-quantitative in vitro procedure to evaluate the bioactivation potential of drug candidates without chemical synthesis of radiolabeled compounds. In this method, $^{35}$S-cysteine and $^{14}$C-sodium cyanide were used as nucleophilic trapping reagents to capture reactive metabolites and the radiolabeled adducts were quantified as radiochromatographic areas following the injection onto RI-HPLC. The reliability of this method was assessed using structurally diverse investigational compounds developed in Banyu by comparing RI area from this surrogate assay with the results from conventional assay for covalent binding to proteins. The results of $^{35}$S-cysteine trapping suggested that the radiochromatographic area of $^{35}$S-cysteine adducts with the reactive metabolites showed moderate correlations with the results of conventional assay, while those of $^{14}$C-cyanide trapping showed no correlations. The combination assay using both $^{35}$S-cysteine and $^{14}$C-cyanide improved the correlations between surrogate and conventional assays. These data indicated that the method provided semi-quantitative information on the potential for bioactivation of drug candidates leading to covalent binding to protein, and the method would be a feasible tool for the preliminary prioritization of drug candidates at an early stage of drug discovery.

DETERMINATION OF REACTIVE METABOLITES IN VITRO USING GLUTATHIONE TRAP METHOD

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[Introduction] Drug metabolism is basically a detoxication process. However, reactive metabolites formed by phase I drug metabolism sometimes cause idiosyncratic adverse effects by binding to biologically important macromolecules. Glutathione (GSH) plays an important role for the elimination of such reactive metabolites in vivo. Qualitative analyses to detect reactive metabolites have been reported by employing the binding of GSH to the reactive species. In the present study, we examined this GSH trap method, aiming at quantitative analysis of reactive metabolites formed in an in vitro drug metabolizing system. [Method] Test compounds were incubated with human liver microsomes, NADPH regenerating system, glutathione-S-transferase and tritium labeled GSH. The metabolic reaction was terminated by adding organic solvent for the extraction. The supernatant solution obtained by the extraction was then dried and resuspended in the mobile phase for HPLC. For the quantitation of reactive metabolites formed, the eluent was split into two lines, leading to a radioactivity detector (RAD) and a tandem mass spectrometer (MS/MS), respectively. [Results and Conclusion] Preliminary qualitative analyses showed acetaminophen, verapamil, quinidine and flutamide were positive compounds giving reactive metabolites. For the negative control, ibuprofen was used. In the quantitative analysis using RAD and MS/MS, tritiated GSH conjugates were found in all positive compounds (acetaminophen > flutamide > quinidine > verapamil). Species difference of GSH adduct formation was also examined by using flutamide. The present GSH trap method is promising for evaluating reactive metabolite formation of test compounds.