A cell-based assay using glutathione-depleted HepaRG cells for predicting drug-induced liver injury

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[Purpose] Drug-induced liver injury (DILI) is a major cause for termination of drug development and withdrawal of approved drugs from the market. Although toxic potential of compounds is often evaluated in in vitro, current in vitro systems generally have a low concordance with human hepatotoxicity. In the past years, human hepatoma HepaRG line has been shown to be a valuable tool to study the mechanism of DILI. However, high level of glutathione in the cell is considered to lessen the cytotoxicity by drugs. In this study, we investigated utility of a glutathione-depleted HepaRG cells in detecting toxicity caused by reactive metabolites.

[Methods] HepaRG and HepG2 cells were pre-incubated with 400 μM L-buthionine-S,R-sulfoximine (BSO) for 3 h, and then the cells were treated with 30 test compounds classified as withdrawn, boxed-warning, warning and safe at 1.6-, 6.4-, 25-, and 100-fold of the therapeutic maximum plasma concentration for 24 h. Cytotoxicity was evaluated by LDH assay. Sensitivity and specificity were calculated (cut-off = 70%) to evaluate the predictability of the assay.

[Results and Discussion] Cytotoxicity was strongly enhanced in glutathione-depleted HepaRG cells but not obviously in glutathione-depleted HepG2 cells. For example, co-treatment of boxed-warning drug flutamide with BSO decreased HepaRG cell viability from 82 % to 54 % , but did not affect HepG2 cell viability. Among 30 test compounds, BSO-treated HepaRG cells exhibited the highest sensitivity of 43%, while non-treated HepaRG cells was only 21%, both of them exhibited 100% specificity. BSO-treated HepG2 cells exhibited the sensitivity of 36 % and 100 % specificity, while non-treated HepG2 cells was only 21% and 92% specificity. These results indicate that glutathione-depleted HepaRG cell model is useful in predicting potential DILI risks caused by reactive metabolites.