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ENHANCEMENT OF BETA-NAPHTHOFLAVONE-INDUCED CYP1A1 mRNA EXPRESSION BY ANDROGRAPHOLIDE IS MODULATED BY ALTERED INTRACELLULAR GSH CONTENT.
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【Purpose】Andrographolide (Andro) is the major diterpenoid constituent of the plant Andrographis paniculata, which has been long used as a folk medicine for alleviating inflammatory disorders, hepatic protection and antiproliferative purpose of cancer. One of the mechanisms was proposed that Andro induced cell cycle arrest and apoptosis via the alteration of cellular redox status. Previously we reported that Andro enhanced the expression of CYP1A1 mRNA induced by β-naphthoflavone (βNF), a typical CYP1A1-inducer, in mouse hepatocytes in primary culture. Since intracellular redox status might alter the expression of mRNA, we investigated the influence of intracellular glutathione (GSH) content on the enhancing effect of Andro. 【Methods】 ddY mouse hepatocytes in monolayer culture were treated with Andro (25 µM), βNF (10 µM), or Andro + βNF, 24 h after seeding in the presence of either of GSH-content modulators, GSH, N-acetylcysteine (NAC), or buthionine sulfoximine (BSO). Total RNA was prepared 24 h later for determination of CYP1A1 mRNA expression by quantitative RT-PCR. 【Results and discussion】Elevation of GSH content by addition of GSH (5-20 mM) or NAC (0.3-3 mM) attenuated the enhancing effect of Andro on βNF-induced CYP1A1 mRNA expression; especially NAC at 3 mM completely reversed it. On the contrary, reduction of GSH by BSO (25-100 µM) augmented the enhancing effect of Andro. Present observations suggest that GSH might decrease reactive oxygen species (ROS) generated after βNF treatment, resulting in overwhelming suppressive effect of ROS on mRNA expression. The other possibility is that GSH might directly scavenge Andro by reacting with it.

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APPLICATION OF THE MULTIPLE REGRESSION APPROACH TO PREDICT THE CONTRIBUTION OF CYTOCHROME P450 (CYP) ISOZYMES IN THE DISCOVERY STAGE
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【Purpose】Identification of major elimination pathways in humans is important in promoting efficient drug discovery research. Given that a polymorphic drug-metabolizing enzyme is predominantly involved in drug elimination, significant interindividual differences may be observed between poor and extensive metabolizers1. Therefore, an approach to estimating the contribution of this polymorphic enzyme may lead to improvements in the clinical success rate of new chemical entities. Recent in vitro metabolism studies with human liver microsomes (HLMs) have enabled us to obtain metabolically stable compounds. However, these compounds hamper determination of the contribution of CYP isozymes using the substrate depletion method. 【Methods】Metabolically stable CYP2D6 substrates (amitriptyline (AT), imipramine (IP), desipramine (DP), and venlafaxine (VF)) were selected as model compounds. To screen the isozymes responsible for their metabolism, a substrate depletion assay using recombinant human CYP isozymes was conducted. Following this, multiple regression analysis was conducted between the isozyme specific activities and metabolites formation rates of these drugs in a panel of 21 HLMs. 【Results and Discussion】The fraction metabolized by CYP2D6 in HLMs was 40% and 70% for AT and IP, respectively, while that metabolized by CYP2D6 exceeded 90% for both DP and VF. Our findings were comparable to those of other studies2. Further, dramatic interindividual differences were observed with DP and VF in comparison with AT and IM, which showed only modest differences. 【Conclusion】The approach used in the present study may be useful in the drug discovery stage in cases where substrate depletion is insufficient to estimate metabolic clearance rate. 1) John P. Gibbs et al., Drug Metab Dispos 34:1516-1522.