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MECHANISMS OF AMELIORATION FOR CISPLATIN-INDUCED NEPHROTOXICITY BY DIURETIC DRUGS AND METHIMAZOLE IN RATS: EFFECTS ON PHARMACOKINETICS AND RENAL EXPRESSION OF PROTEINS AND mRNA
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[Purpose] Cis-Diamminedichloro-platinum (II) (cisplatin) is one of the most effective anticancer agents available, but adverse reactions such as nephrotoxicity and nausea frequently restrict the continuation of treatment. We have been studying the determining factors affected on the nephrotoxicity based on the pharmacokinetics of unchanged cisplatin. The objective in this study was to elucidate the amelioration mechanisms by methimazole and diuretics based on the pharmacokinetics and intracellular reactions forward to the elevation of blood urea nitrogen. [Methods] Cisplatin was intravenously bolus administered (5 mg/kg) via jugular vein to Wistar male rats pretreated with saline, methimazole, furosemide or mannitol. After 0.25, 0.5, 1, 2, 3, 4 or 5 days, the rats were sacrificed and ice-cold 0.9% NaCl was injected into the heart. The kidneys were excised quickly, weighed and homogenized with an 8-fold volume of lysis buffer. Unchanged cisplatin was determined by HPLC. The expression of cleaved caspase-3 and other proteins were determined by western blotting. The expression of mRNA level was determined by DNA microarray (CodeLink™ Rat Whole Genome Bioarray, Filgen, Inc.). [Results and Discussion] All drugs studied significantly reduced the cisplatin-induced nephrotoxicity. However, pharmacokinetics, such as clearance, renal distribution and binding to intrarenal organelle, were not significantly different by these drugs, indicating that these drugs may affect on the toxicodynamics. The cleaved caspase-3 level at 3 days after administration of cisplatin was significantly increased but the level was significantly reduced by these drugs. These results suggested that these drugs may affect the upstream of apoptosis induced by cisplatin. We also tried to analyze the effects of these drugs on the expression of mRNAs.

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SUPPLEMENTARY REACTION TO DRUG METABOLISM IN THE MULTIPLE HISTAMINE RECEPTOR GENE KNOCKOUT MICE
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[Purpose] Gene knockout mice were used in order to clarify the physiological roles. However, when the effect of drug was observed using gene knockout mice, the data on disposition of drug are often overlooked. We studied whether or not the changes in drug-metabolizing enzyme activities occur in the multiple histamine receptor gene knockout mice. [Methods] Female H1 receptor gene deficient mouse (H1-KO) was mated with H2 receptor-deficient mouse (H2-KO). The male H1/H2 double receptor knockout (H1/H2 DKO) mice were then mated with H3 receptor-deficient mouse (H3-KO; a gift from Dr. Lovenberg to generate the H1/H2/H3 triple knockout mice (H1/H2/H3 TKO). The metabolism of imipramine (N-demethylation, 2-hydroxylation and N-oxidation) was investigated in the liver microsomes of these knockout mice. In order to determine the concentrations of imipramine and its metabolites (desipramine, 2-hydroxydesipramine, 2-hydroxyimipramine and N-oxide), an analytical method using HPLC-PDAD was developed. [Results and Discussion] We were able to detect, simultaneously, six PCR products of each histamine receptor gene for KO and wild type H1, H2 and H3 receptor, respectively using QIAGEN Multiplex PCR kits. The formations to 2-hydroxyimipramine and desipramine of imipramine catalyzed by CYPs significantly increased in the liver microsomes of H1-KO, H2-KO and H1/H2/H3 TKO mice. Although, there was no significant difference in N-oxidation activity between wild type and these KO mice, the formation of N-oxide in H1-KO mice was analysed to be biphasic, indicating gene expression of different type of flavin-containing monoxygenase by knockout of H1 receptor gene [Conclusion] These results suggested that the gene knockout may cause supplementary reaction to drug metabolism.