Endogenous Factors Involved in Enterohepatic Circulation of Methylmercury with Special Reference to Its Chemical Form

TSUTOMU URANO, AKIRA NAGANUMA and NOBUMASA IMURA

Department of Public Health, School of Pharmaceutical Sciences, Kitasato University, Minato-ku, Tokyo 108, Japan

Enterohepatic circulation of methylmercury (MM) has been recognized to play an important role in its retention, metabolism and toxicity in animals. The main form of MM in rat bile has been believed to be MM-glutathione (MM-GSH), but different results have also been reported, for example, MM-cysteine (Cys) or a low-molecular-weight substance other than MM-GSH and MM-Cys. However, most of the studies were not designed to eliminate the oxidation of non-protein sulfhydryls (NPSHs) which was reported to change the chemical form of the biliary mercury compounds during the analytical procedure. In the present study, we investigated the chemical form of MM in rat bile under conditions where the oxidation of NPSHs was strictly prevented. The intestinal absorption rate of MM compounds recognized in the bile was also examined, focusing on their roles in enterohepatic circulation of MM.

Male Wistar rats of various ages (4, 8, 12 and 22 weeks old) were anesthetized with sodium pentobarbital (50 mg/kg) and the bile ducts were cannulated with polyethylene tubing. Then, the rats were administered $5 \mu\text{mol/kg}$ of $^{203}\text{HgCl}$ i.v. and bile samples were collected in ice-chilled tubes containing EDTA solution from 2 to 3 h after the injection of MM. The chemical form of MM in the bile sample was analyzed using Sephadex G-15 and DEAE Sephadex A-25 columns. Concentrations of NPSHs such as GSH, Cys and cysteinylglycine (CysGly) were determined by HPLC.

Gel filtration and ion-exchange chromatography of the bile of rat exposed to methylmercuric chloride (MMC) revealed that MM-CysGly complex was the predominant chemical form of MM

at 4–22 weeks of age and the residual MM was found as MM-GSH. The ratio of MM-GSH to total MM in the bile tended to increase with age from 4 weeks until 12 weeks of age, reflecting the age-dependent increase in the ratio of GSH to total NPSHs in the bile. In the older rats, though the concentration of GSH in the bile was about three times higher than that of CysGly, most of the MM was bound to CysGly. This suggests that CysGly has a higher affinity to MM than GSH. In fact, when MM was mixed *in vitro* with GSH and CysGly in the same molar ratio as that in the bile, MM was found to be predominantly associated with CysGly. These results suggested that age-dependent changes in the chemical form of MM in rat bile reflected the age difference in the components of NPSHs in bile and also that no matter what chemical form of MM is excreted into the bile from the liver, it will be immediately re-equilibrated to NPSHs contained in the bile.

Then, we investigated the intestinal absorption rate of MM compounds recognized in the bile by means of direct injection of MM compounds into a ligated intestinal segment of 8-week-old rat. Intestinal absorption of MM was measured 30 min after the injection of MMC (400 nmol/rat) alone or premixed with Cys, CysGly or GSH (800 nmol/rat). MM complexed with NPSHs was absorbed more efficiently than MMC. The intestinal absorption rate of MM-CysGly was the same as that of MM-Cys and 1.5 times higher than that of MM-GSH. The results indicated that MM-CysGly, the major component of rat bile, may play an important role in the enterohepatic circulation of MM.

The combination of acivicin, a potent inhibitor of γ -glutamyltranspeptidase (GGT), and probenecid, a blocker of organic anion transport, showed a significantly stronger inhibitory effect than acivicin alone on the absorption rate of MM-GSH, which is another major MM compound in rat bile. These data raise the possibility that there are at least two systems for intestinal absorption of MM-GSH, *i.e.* GGT dependent and independent systems.