Effect of Organic Halogen Compounds on the Structure and Reductase Activity of Rat Liver Microsomal Membranes (II)

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Recently, organochlorine compounds, which are produced in tap water during chlorination, have been suggested to be dangerous environmental pollutants, since they may show liver toxicity and carcinogenicity. These compounds seem to have a potent toxic effect on liver microsomes, but the interaction of these compounds with microsomal membranes is not clear. Previously, we observed the effect of CCl_4 on liver microsomal membranes using ^{31}P -NMR and spin-label techniques and found that orally administered CCl_4 fluidized the lipophilic region of the membranes. To compare the acute effects of trihalomethanes on the liver microsomes with that of CCl_4 , we investigated the effects of CHBr_2Cl and CHBr_3 on the structure and reductase activity of microsomal membranes by using the spin-label method.

CHBr_2Cl , CHBr_3 , and CCl_4 were administered orally at a dose of $1/3$ of LD_{50} to male wistar rats (5 weeks old). After 1, 3, or 6 h, the liver microsomes were prepared by the standard method, and labeled with following spin-labeled stearic acid derivatives; 5-, 12-, or 16- (*N*-oxyl-4', 4'-dimethyloxazolidine)- stearic acid and their methyl esters, (abbreviated as 5SLS, 12SLS, 16SLS, 5SLSM, 12SLSM, and 16SLSM, respectively). Electron spin resonance (ESR) spectra showed typical signals due to spin-probes in microsomal membranes. To estimate the membrane fluidity, we calculated the order parameter and the apparent rotational correlation time from the spectra of 5SLS and 12SLS, respectively.

No significant difference could be observed in the order

parameter among the CHBr_2Cl -, CHBr_3 - and CCl_4 -treated and control groups, suggesting that the dynamic properties in hydrophilic regions of the membranes may not be affected by these compounds. The correlation time decreased from 4 to 3.5 ns at 1 h after the administration of CHBr_2Cl or CHBr_3 and then approached to control level, while CCl_4 decreased the time to 2 ns between 1 and 24 h after administration. These findings indicate that CHBr_3 , CHBr_2Cl and/or their metabolites fluidize the lipophilic region of microsomal membranes, but the effects of CHBr_3 and CHBr_2Cl seem to be less than that of CCl_4 .

The ESR spectrum of 5SLSM in microsomal membranes was also changed by the oral administration of CHBr_3 or CHBr_2Cl . The spectrum consisted of two different signals, a rather isotropic signal and an anisotropic one. The ratio of the two signals indicates the population ratio of the spin-probe in non-bilayer regions and in lamellar regions. The transfer rate of 5SLSM from non-bilayer to lamellar regions increased at 6 h after administration of CHBr_3 but was hardly changed first 3 h. This effect was quite different from that of CCl_4 , since CCl_4 treatment decreased the rate during 24 h.

Nitroxide groups of spin-probes are known to be decreased as a result of reduction. The ESR signal of 5SLSM in membranes gradually declined in the presence of microsomes. The addition of NADPH accelerated the reduction but that of NADH did not, suggesting that nitroxide groups of this spin-probe are reduced by the NADPH-Cyt P-450 system. The administration of CCl_4 greatly decreased the reduction rate during 24 h after administration. CHBr_2Cl also decreased the rate at 6 h after administration, but the effect was much smaller than that of CCl_4 . On the other hand, CHBr_3 accelerated the reduction 6 h after administration, while the reduction was depressed in the first 3 h. The change of reduction rate by CHBr_3 seems to be related to the transfer rate of 5SLSM.