EFFECT OF D-GALACTOSAMINE/LIPOPOLYSACCHARIDE TREATMENT ON MITOCHONDRIAL GLUTATHIONE S-TRANSFERASE OF RATS
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Simultaneous administration of D-galactosamine(D-GalN)/lipopolysaccharide(LPS) induces a useful model of fulminant hepatic failure and septic shock. Recent studies revealed that D-GalN/LPS induced liver injury was mainly caused by oxidative/nitrosative stress. Efficient protection systems involving specific enzymes have emerged throughout dynamic evolution but not known well. In this study, we examined the relative contribution of mitochondrial glutathione S-transferase (mtMGST1) on D-GalN/ LPS induced liver injury in rats, which catalyses the conjugation of glutathione (GSH) to toxic metabolites of lipid peroxidation. The results showed that reactive oxygen/nitrogen species in the liver were increased significantly by D-GalN(600mg/kg, s.c)/LPS(0.5 μg/kg, i.p) treatment after 24h. The mitochondrial oxidative/nitrosative stress was also confirmed by an increase of lipid peroxidation and of iNOS expression in mitochondria. D-GalN/LPS treatment increased significantly mtMGST1 activity as compared with saline treatment whereas the cytosolic GST activity was decreased. In addition, alkylation of the sole cysteine residue of mtMGST1 by N-ethylmaleimide increased the activity to 5.7-6.8 fold in saline treatment, but to 2.0-2.5 fold in D-GalN/LPS treatment. The activity increased by D-GalN/LPS treatment was reversed partially by dithiothreitol whereas mtMGST1 dimer was not observed. These results suggest that mtMGST1 is activated by D-GalN/LPS treatment through, at least, modification of the sulfhydryl group.

BUTYRYLCHOLINESTERASE AND ERYTHROCYTE ARYLESTERASE ARE RESPONSIBLE FOR GABEXATE HYDROLYSIS IN HUMAN BLOOD
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Gabexate (GB), which is widely used for the treatment of acute pancreatitis and disseminated intravascular coagulation, has been reported to be hydrolyzed by human serum albumin. However, other enzymes responsible for GB hydrolysis in human blood remain unclear. In this study, we examined in vitro metabolism of GB with human blood. Kinetic analysis indicated that $V_{max}$ and $K_m$ values were 1750 nmol/min/ml and 529 μM for plasma, and 10600 nmol/min/ml and 7360 μM for erythrocytes, respectively. The activity of human plasma and albumin was inhibited by 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) (40 and 84% inhibition at 5 mM, respectively), indicating the involvement of albumin in the plasma GB hydrolysis. Furthermore, the plasma activity was inhibited by phenylmethylsulfonyl fluoride (PMSF), diisopropyl fluorophosphate (DFP) and ethopropazine (15, 34 and 27% inhibition at 0.1 mM, respectively). The erythrocyte activity was decreased by DTNB (56% inhibition at 5 mM), while little or no inhibition of this activity was seen with PMSF, DFP and BW284C51. Butyrylcholinesterase (BChE) from human serum showed GB hydrolytic activity with $V_{max}$ of 363 nmol/min/mg protein and $K_m$ of 263 μM. These results suggest that, in addition to albumin, BChE and erythrocyte arylesterase are responsible for GB hydrolysis in human blood.