DEPLETION OF THE GLUTATHIONE CONTENT OF ISOLATED LIVER CELLS BY HEPATOTOXIC DRUGS

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Isolated liver cells (hepatocytes) are very useful for biochemical studies of drug metabolism and drug toxicity (Moldeus et al 1978). As hepatic glutathione (GSH) plays a protective role in drug-induced liver injury (Gillette et al 1974) we have examined the effects of 4 hepatotoxic drugs on the GSH content of isolated rat hepatocytes.

Unstarved male Sprague-Dawley rats (ca. 200 g) were pretreated with phenobarbitone (80 mg/kg i.p.), cobaltous chloride ($CoCl_2$) (40 mg/kg s.c.) or saline (as control) daily for 4 days. Hepatocytes from these animals were prepared (Berry and Friend 1969) by perfusing the liver in situ with a recirculating oxygenated buffer containing 0.5% collagenase. The buffer (Krebs-Henseleit bicarbonate) also contained methionine (2.0 mM), serine (2.0 mM) and EGTA (10 mM) to maintain physiological levels of GSH. Hepatocyte suspensions (ca. 70 mg wet wt. of cells) in oxygenated buffer (now further supplemented with 10% calf serum and 10 units/ml heparin) (5 ml) were incubated at 37° with hepatotoxic drugs at varying concentrations (0-10 mM) and for various times (0-120 min). Reactions were stopped by adding trichloracetic acid (30%, 0.5 ml) and cooling (5°C). GSH in the supernatent of this cell lysate was measured using Ellman's reagent (Sedlak and Lindsay 1968).

Table 1 Concentrations of Drug Causing 50% Depletion ED_{50} of the GSH Content of Isolated Rat Hepatocytes in 1 hour

Drug	ED_{50} (mM)
Paracetamol	9.0
Cocaine	0.25
Dextropropoxyphene	0.27
Hycanthone	0.17

Physiological levels of GSH were found in hepatocytes from control and phenobarbitone treated rats (5.4 ± 0.5 and 4.8 ± 0.4 nmoles GSH/mg wet wt. cells respectively). Pretreatment with $CoCl_2$ caused a significant increase in GSH content (7.5 ± 0.5 nmole GSH/mg wet wt.). These levels could be maintained on incubation. However addition of a hepatotoxic drug caused a rapid fall in GSH content, generally complete within 30-60 min. This GSH depletion was dose- and time-dependent for all the 4 drugs listed in Table 1. The effects shown by paracetamol and by cocaine were enhanced by phenobarbitone and inhibited by $CoCl_2$ pretreatment. The depletions caused by dextropropoxyphene and by hycanthone could not be influenced significantly by pretreatment.

Our results indicate that the reactive toxic metabolites formed from paracetamol and from cocaine are products of the cytochrome P-450 system. In contrast those from dextropropoxyphene and from hycanthone must be produced by other, yet unestablished, metabolic pathways leading to drug toxicity (see Miller and Hulbert 1976). Furthermore this work shows that GSH depletion of hepatocytes is a good in vitro system for predicting the hepatotoxic potential of drugs and their analogues.

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