INVESTIGATIONS INTO MECHANISMS OF PARACETAMOL-INDUCED TOXICITY USING IN VITRO SYSTEMS

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Abstract

The expression of toxicity in primary mouse hepatocyte monolayer culture to the model hepatotoxin, paracetamol (APAP), was found to be dependent on the composition of the extracellular medium. Control cultures, i.e. cultures not challenged with paracetamol, were observed to undergo a differential decline in normal cellular function. This decline was most apparent for the cells incubated in unmodified RPMI 1640 medium and was attenuated to differing degrees for cells incubated in the presence of more complex media, e.g. media incorporating added hormones and vitamins (HM-RPMI 1640) or the same media with an altered sulphhydril amino acid balance (CM-RPMI 1640). A paracetamol-induced cytotoxicity was more readily observed when hepatospecific function was maintained in vitro.

The glutathione depleting agents diethyl maleate (DEM) and bathionine sulphoximine (BSO) were used to investigate the role of glutathione (GSH) in paracetamol-induced cytotoxicity in primary mouse hepatocyte cultures. DEM did not augment a dose-related paracetamol-induced cytotoxicity. BSO pretreated cultures were observed to undergo a modest, non-additive, increase in paracetamol-mediated cellular toxicity.

The effect of polymorphonuclear myeloperoxidase (PMN-MPO) activity on paracetamol toxicity was investigated in the human hepatoma cell line, Huh7. The low basal PMN-MPO activity of Huh7 monolayer cultures was induced in vitro by treatment of cells with the cytochrome P-450 specific inducer, 3-methylcholangantran. The specific induction of cytochrome P-450 isozyme activity, observed as an increase in 7-ethoxyresoruflur demethylase (7-ECOD) activity in contrast to no induction of ethylmorphine N-demethylase activity, resulted in a dose-related cytotoxic response to paracetamol becoming evident. In contrast, no APAP-mediated cytotoxicity was observed in uninduced Huh7 cultures.

Investigations into the role of both extracellular and intracellular calcium in hepatotoxicity were undertaken in the primary mouse hepatocyte cell culture system. A dose-related biphasic increase in the free cytosolic calcium concentration occurred in cultures incubated in the presence of normal medium calcium. Initial rises in
free cytosolic calcium were observed prior to any measurable alteration in cellular function normally associated with cytotoxicity (increased LDH leakage and K⁺ efflux) and the extent of changes in the concentration of free cytosolic calcium positively correlated with the severity of the subsequent lesion. Incubations of primary mouse hepatocyte cultures in the presence of EGTA and promethazine did not result in any dose-related increases in free cytosolic calcium concentration and dose-related decreases in cellular viability to paracetalol were also abolished. These findings are discussed in relation to current speculations in the literature regarding the role of calcium in cellular toxicity.
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