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PRELIMINARY COMMUNICATION

THE CONTROL OF GLUTATHIONE AND CYTOCHROME P-450 CONCENTRATIONS OF PRIMARY CULTURES OF RAT HEPATOCYTES

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The culture of rat hepatocytes is a popular model for the study of drug metabolism and toxicity (1,2). An obstacle to the use of liver cell culture for these studies has been the knowledge that the concentration of cytochrome P-450, a major system involved in activating drugs to reactive intermediates, falls by 60-70% during the first 24 hours of culture (3). Recently we reported that this loss could be prevented by culturing hepatocytes in a medium that does not contain cystine (4). However sulphur containing amino acids are precursors of glutathione which plays an important role in the detoxification of reactive metabolites (5,6). We have therefore examined the glutathione content of hepatocytes cultured with and without cystine.

MATERIALS AND METHODS

Isolation and culture of hepatocytes

Hepatocytes were isolated and 20 x 10^6 cells were cultured in 150 mm diameter petri dishes in 20 ml of the respective medium as previously described (7). The medium used was based on Earle's balanced salt solution (Gibco Biocult Ltd., Paisley, Scotland) containing 1 ml "BME vitamin solution x 100 strength" (Gibco Biocult Ltd) per 100 ml medium and amino acids (BDH, Poole, Dorset, U.K.) at the same concentration as found in RPMI 1640 (8). All media contained 5% foetal calf serum (Gibco Biocult), 10^{-6} M insulin, 10^{-4} M hydrocortisone-21-sodium succinate (both from Sigma Chemical Co., Poole, Dorset, U.K.) and 5 mg gentamicin (Flow Labs.Irvine, Scotland) per 100 ml medium.

"P-450 Maintenance Medium" constitutes the basic medium without cystine and supplemented with 100 μM 5-amino laevulinic acid (Sigma Chemical Co.) (4).

Cytochrome P-450 and protein were determined as previously described (9).

Glutathione (GSH) was determined as described by Hissin & Hilf (10). For this determination a 5% (w/v) homogenate of liver and isolated hepatocytes was made in 0.1 M sodium phosphate buffer containing 5 mM EDTA pH 8 by using an Ultra-Turrax TP18/11 blender for 10 secs. GSH was determined in cultured hepatocytes by scraping hepatocytes from

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2 x 150 mm diameter petri dishes into 5 ml 0.1 M sodium phosphate buffer containing 5 mM EDTA pH 8 and homogenising for 10 secs with an Ultra-Turrax blender. The addition of 50 µg GSH/ml buffer prior to homogenisation demonstrated that the homogenisation procedure caused no loss of GSH. GSSG, determined as in ref.11, could not be detected in homogenates of intact liver, isolated or cultured hepatocytes.

RESULTS AND DISCUSSION

Table 1 shows that the procedure used to isolate hepatocytes results in a 30-50% loss of glutathione ($p < 0.05$). The culture of these cells for 24 hours in a medium containing 0.2 mM cystine restores their glutathione content to the same concentration as found in intact liver (Table 1). However culture medium containing 0.2 mM cystine causes a 60% loss of cytochrome P-450 (Table 1). This loss, of cytochrome P-450, can be prevented by removing cystine from the culture medium (4) but hepatocytes cultured for 24 hours in medium without cystine have a glutathione concentration which is 30% of that found in intact liver (Table 1).

Table 1 Effect of hepatocyte isolation and culture conditions on their glutathione and cytochrome P-450 concentrations.

Treatment and sample origin	Glutathione concn. (µg/mg protein)	Cytochrome P-450 concn. (% intact liver)
Intact liver	9.5 ± 0.2	100
Isolated hepatocytes	6.6 ± 0.5*	100
Hepatocytes cultured for 24 hr in:		
medium + 0.2 mM cystine	8.3 ± 0.8	48 ± 4*
medium - cystine	3.7 ± 0.5*	90 ± 6

*denotes significantly different ($p < 0.05$) by Student's t-test, from intact liver. The results are means ± S.D. for individual values found in preparations derived from six separate rat livers (i.e. n = 6). The cytochrome P-450 concentration of intact liver was 155 ± 20 pmoles/mg protein.

These observations raise two interesting questions:-

Firstly, how can the potential of cultured hepatocytes, as a model system, to activate and detoxify chemicals be maintained at the same level as found in the intact liver? Reed & Beatty (12) have shown that methionine is a more efficient precursor of glutathione, in hepatocyte suspensions, than is cystine. Accordingly we examined the effect of increasing the methionine concentration of the "P-450 maintenance medium" (i.e. medium without cystine) (4) on the glutathione concentration of cultured hepatocytes. Table 2 shows that increasing the methionine

concentration from 0.1 mM to 0.5 mM maintains the glutathione concentration of hepatocytes cultured for 24 hours at the same level as found in intact liver. Thus the effect of removing cystine from the culture medium on the glutathione concentration of cultured hepatocytes can be overcome by increasing the methionine concentration of the medium.

The second question raised by the results shown in Table 1 is: is there a causal relationship between the concentration of glutathione and the maintenance of cytochrome P-450? Such a relationship is suggested because medium that maintains cytochrome P-450 results in a loss of glutathione and medium that maintains glutathione results in a loss of cytochrome P-450. However the results in Table 2 show that this is not the case since it is possible to maintain both cytochrome P-450 and glutathione.

These observations may be useful since they provide the opportunity to dissociate the involvement of glutathione and cytochrome P-450 in drug metabolism and toxicity.

Table 2 Effect of methionine on the concentration of glutathione and cytochrome P-450 in hepatocytes cultured for 24 hours in "P-450 maintenance medium".

Methionine concn.(mM)	Glutathione concn. (μg/mg protein)	Cytochrome P-450 concn. (% isolated cells)
0.1	4.0 ± 0.6*	85 ± 14
0.25	6.0 ± 0.2*	98 ± 6
0.5	11.0 ± 1.2	94 ± 7
1.0	12.0 ± 0.8	101 ± 12
2.0	11.2 ± 0.9	87 ± 10

*denotes significantly different ($p < 0.05$) by Student's t-test, from intact liver. The results are means ± S.D. for individual values found in preparations derived from three separate rat livers (i.e. n = 3). The cytochrome P-450 concentration of isolated cells was 166 ± 29 pmoles/mg protein.

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