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PRELIMINARY COMMUNICATIONS

THE CONCENTRATION OF CYTOCHROME P-450 IN HUMAN HEPATOCYTE CULTURE

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INTRODUCTION

A problem with the study of drug metabolism and toxicity in experimental animals is extrapolating the relevance of the results to man. It is unethical to determine the toxic properties of chemicals by dosing these to humans but cell cultures prepared from human tissues could circumvent this problem. Liver cell culture is a popular model system to study mechanisms of hepatotoxicity in experimental animals (for reviews see 1 and 2). However, a limitation of this model is the rapid loss of cytochrome P-450 during the first 24 hours of hepatocyte culture (3-5). This is an obstacle since cytochrome P-450 mediated metabolism frequently determines the toxicity and carcinogenicity of many natural as well as synthetic chemicals (6).

In rat hepatocyte culture the loss of cytochrome P-450 can be prevented by including metyrapone or 1,2-cyanophenylimidazole (7) in the culture medium. Little is known about the effect of the culture conditions on the concentration of cytochrome P-450 in human hepatocytes and where results have been reported these are only on a few donors (8-10). The present study reports our experience with hepatocyte cultures prepared from seven human individuals and therefore, to the best of our knowledge, represents the largest study to be reported. The results show that, in contrast to hepatocyte cultures prepared from experimental animals (3,4) the majority (i.e. five) of the cultures prepared from human liver do not lose cytochrome P-450 during the first 24 hours of culture. Furthermore, where the loss of cytochrome P-450 did occur in culture the addition of metyrapone or 1,2-cyanophenylimidazole to the culture medium, which prevents the loss of the cytochrome in rat hepatocyte culture (7), also prevents the loss of the cytochrome in cultured human hepatocytes.

MATERIALS AND METHODS

Isolation of hepatocytes. Liver samples were obtained from renal transplant donors who had met traumatic death and were maintained on life support systems until the kidneys were removed. Donors were not receiving any chronic drug treatment but were given such drugs as necessary while on life support systems. After removal of the kidneys a section of liver

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(approximately 100 g) was removed from the organ such that the section had only one cut face. In order to remove as much of the entrapped blood as possible, the liver section was perfused with Hank's balanced salt solution (HBSS from Gibco Europe) via the larger vessels on the cut surface, using a 20 ml syringe and a flow rate of ca 20 ml/min. The procedure to isolate hepatocytes commenced within 30 minutes of removal of the liver section from the donor. A smaller section (approx 30 g) was cut, ensuring that the section had only one cut face and this piece was used for the isolation of hepatocytes. A vessel was selected, so that a cannula with a male luer end-piece fitted snugly into it. Too large a vessel resulted in leakage of perfusate, too small a vessel resulted in the vessel being damaged and both resulted in poor perfusion. The liver section was perfused with Ca- and Mg-free HBSS at a temperature of $37^{o}C$ and a flow rate of 50 ml/min for 15 minutes. The perfusate was run to waste. The liver was perfused at 50 ml/min with HBSS containing 2.5 mM $CaCl_2$ and 0.05% (w/v) collagenase (Boehringer) for 20-30 minutes. The liver was then transferred to a sterile crystallization dish containing 50 ml of HBSS with 2.5% (w/v) bovine serum albumin (fraction V, Sigma Chemical Co) and the capsule stripped off with forceps. The piece of liver was manually shaken with the forceps for 2-3 minutes to liberate the hepatocytes from the tissue. The undigested pieces of liver were removed and the cell suspension was gently shaken in a $37^{o}C$ water bath for 10 minutes. The cell suspension was then filtered through a 125 µm nylon mesh and the filtrate centrifuged at 80g for 3 minutes. The cell pellet was washed twice with 40 ml Waymouth MB 752/1 medium containing 10% (v/v) foetal calf serum (Gibco Europe) and 10^{-6} M insulin plus 10^{-4} M hydrocortisone-21-sodium succinate (both from Sigma Chemical Co). The cell yield varied between 5 and $20x10^{6}$ cells/g liver with a viability of 70-90% as assessed by trypan blue exclusion which is in agreement with other workers (9,10). The hepatocytes were cultured in 100 mm diameter plastic Petri dishes at a density of $7.5x10^{6}$ cells/10 ml Waymouth MB 752/1 medium containing the additions described above and referred to in Table 1.

Biochemical determinations: 25% (w/v) liver homogenates were used for the preparation of the microsomal fraction and determination of cytochrome P-450 was as described by McLean and Day (14). Protein was determined as previously described (12). Homogenates of freshly isolated and cultured hepatocytes were prepared in "Emulgen buffer" as described before (5).

RESULTS AND DISCUSSION

Table 1 shows the age, sex and cause of death of the human donors studied as well as their individual concentration of hepatic cytochrome P-450. The first, obvious, conclusion from the data presented in Table 1 is that there is a large variation in hepatic cytochrome P-450 content between human individuals (e.g. the range is from 32-166 pmoles/mg homogenate protein). We believe the values of cytochrome P-450 determined in whole homogenates of human liver to be reliable since there is a good correlation (correlation coefficient r=0.90) between the concentration of cytochrome P-450 found in homogenates and the microsomal fraction. Furthermore, perfusion of the liver and its dissociation into a single cell suspension which removed all of the haemoglobin entrapped within the liver, which can interfere with the P-450 determination also results in a good correlation (r=0.85) between values determined in liver homogenates and isolated hepatocytes. Finally, comparison of the results obtained with microsomal fractions prepared from human liver are also in good agreement with the values found in isolated cells (r=0.94). Thus, it may be concluded that the concentration of cytochrome P-450 found in the seven liver homogenates, (mean $\pm$ S.D.= 94 $\pm$ 42 pmoles/mg protein) is lower than found in liver homogenates prepared from Porton

Table 1. CYTOCHROME P450 CONTENT OF THE LIVERS OF KIDNEY TRANSPLANT DONORS AND IN HEPATOCYTE CULTURES PREPARED FROM THE DONORS

Our donor no.	Age	Sex	Cause of death	Cytochrome P-450 content (pmoles/mg protein) in:			Cytochrome P-450 (% of value in isolated hepatocytes) content after 24 hr culture with:		
				liver homogenate	microsomal fraction	isolated hepatocytes	no treatment	0.5 mM metyrapone	0.25 mM CPI
8	14	M	traffic accident	65	219	52	104	94	117
2	16	F	traffic accident	92	290	94	60	n.d.	n.d.
3	17	M	traffic accident	109	254	66	94	121	n.d.
5	21	M	traffic accident	84	308	112	85	84	96
6	27	F	brain tumour	166	404	163	44	91	76
7	30	F	brain haemorrhage	112	259	67	99	n.d.	n.d.
9	40	M	traffic accident	32	122	43	96	104	71

The results above are the average of duplicate determinations of cytochrome P-450. Liver homogenates and their microsomal fraction were prepared as described in the methods section. Hepatocytes were isolated and cultured for 24 hours in Waymouth MB 752/1 medium containing 10% foetal calf serum, 10^{-6} M insulin, 10^{-4} M hydrocortisone-21-sodium succinate plus 50 µg gentamicin/ml and either 0.5 mM metyrapone or 0.25 mM 1,2-cyanophenylimidazole (CPI). M= male; F= female; n.d.= not determined.

derived Wistar rats (12) (mean ± S.D.= 173 ± 15 pmoles/mg protein). The data in Table 1 is ranked by the age of the donor, not by the date of donation, and shows no obvious relationship between the donors age and their hepatic cytochrome P-450 content. The mean value for females (123 ± 38 pmoles/mg protein, n=3) was higher than found in the livers of male donors (73 ± 32, n=4). Cytochrome content was also determined in liver homogenates prepared from two additional donors (50 year old male and 16 year old female) which were not used for hepatocyte preparation and were found to be 54 and 129 pmoles/mg homogenate protein respectively. Inclusion of these results produces a mean of 69 ± 29 for males (n=5) and 125 ± 31 for females (n=4). However, nothing is known of the eating, drinking, smoking and drug taking habits of the donors.

The results in Table 1 show that in accord with studies in rat hepatocyte culture the procedure used to isolate human hepatocytes did not result in the loss of cytochrome P-450. In contrast to our experience with rat hepatocyte cultures (5) the results show that hepatocyte cultures prepared from five out of the seven human donors studied dit not lose cytochrome P-450 when cultured for 24 hours. Hepatocytes prepared from donor number 6 lost 60% of their cytochrome P-450 content during the first 24 hours of culture. However, the concentration of hepatic cytochrome P-450 in this donor was much higher than that found in the other donors. Guillouzo (personal communication) has found that hepatocytes isolated from a donor who died of barbiturate intoxication contained similar high concentrations of cytochrome P-450 and that hepatocyte cultures prepared from this individual lost cytochrome P-450. Perhaps cytochrome P-450 in our donor no. 6 was induced. Nevertheless, the results in Table 1 show that the addition of 0.5 mM metyrapone to the culture medium, which is known to prevent the loss of cytochrome P-450 in rat liver cell culture, can also prevent the loss of the cytochrome when it occurs in human hepatocyte culture. In conclusion, the present work shows that human hepatocytes in culture do not, generally, lose cytochrome P-450 and therefore should be useful for the identification of pathways of drug metabolism in man (e.g. see 13) as well as for the study of mechanisms of hepatotoxicity and possible chemotherapy (e.g. see 14).

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