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LONG TERM MAINTENANCE AND INDUCTION OF CYTOCHROME P-450 IN RAT LIVER CELL CULTURE

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A characteristic property of the hepatic, microsomal cytochrome P-450 linked drug metabolising system is its induction by many chemicals (ie its concentration is increased). Most inducing chemicals can be divided into two main classes which are typified by drugs such as phenobarbital and the polycyclic hydrocarbon benzanthracene (1). Although the induction of cytochrome P-450 mediated metabolism has been observed in rat liver cell culture (2-4) there is only one report of a concomitant increase in the concentration of cytochrome P-450 (5). An obstacle in demonstrating the induction of cytochrome P-450 has been the rapid loss of this cytochrome in rat liver cell culture (6,7). Thus when induction of cytochrome P-450 was demonstrated, its concentration was increased, by phenobarbital treatment, from 8% to 22% of the concentration found in the initial hepatocyte preparation (5).

Recently we have devised two culture systems that prevent the loss of cytochrome P-450 during the first 24 hours of culture. These are: (a) the addition of 0.5 mM metyrapone to the culture medium (8). (b) the use of a medium, "P-450 medium", without cystine and cysteine and supplemented with 100 μM 5-aminolevulinic acid (9).

The present work documents the effect of these culture systems on the concentration of cytochrome P-450 in hepatocytes cultured for up to 7 days and shows that treatment with phenobarbital or benzanthracene can increase the concentration of cytochrome P-450 to levels that are comparable to those found in the livers of rats treated with inducers.

MATERIALS AND METHODS

Isolation and culture of hepatocytes

Hepatocytes were isolated, as previously described (8), and 20 x 10^6 cells were cultured in 150 mm diameter petri dishes in 20 ml of either Williams medium E (Flow Labs, Irvine, Scotland) or "P-450 maintenance medium" (9). Both media contained 5% (v/v) foetal calf serum (Gibco Bicult Ltd., Paisley, Scotland), 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) per 100 ml medium. When the medium contained metyrapone (a gift from Ciba Laboratories, Horsham, Sussex, U.K.) it was directly dissolved in the medium. 4 hours after commencing the cultures the medium was changed, where indicated, to medium containing sodium phenobarbital (British Drug House, Poole, Dorset) or medium containing 1,2 benzanthracene (Sigma Chemical Co.). Sodium phenobarbital was directly dissolved in the culture medium. 1,2 Benzanthracene was added to the medium as a solution in dimethylformamide (British Drug Houses). The final concentration of dimethylformamide was 0.5% (v/v). The culture medium was then changed every 24 hours.

Cytochrome P-450 and protein was determined as previously described (10).

Treatment of rats with phenobarbital 180-200 gm male rats of the Porton derived Wistar strain were given drinking water containing 1 mg sodium phenobarbital/ml for 10 days as described by Marshall and McLean (11).

Table 1: Effect of hepatocyte culture conditions on the content of cytochrome P-450 and its induction by phenobarbital (PB).

Culture medium	Additions to culture medium		Cytochrome P-450 concn. (% initial) after culture for (hours)						
	0.5mM Metyrapone	2mM PB	24	48	72	96	120	144	168
	-	-	48 ± 8	32 ± 4	23 ± 8	22 ± 7	33 ± 8	28 ± 12	27 ± 6
Williams' medium E	-	+	49 ± 1	53 ± 10	71 ± 16	63 ± 11	52 ± 23	49	39
	+	-	95 ± 5	102 ± 7	101 ± 10	110 ± 10	124 ± 20	113 ± 16	96 ± 3
	+	+	94 ± 9	103 ± 16	166 ± 8	227 ± 16	184 ± 28	219 ± 4	204
"P-450 medium"	-	-	96 ± 10	68 ± 15	44 ± 11	33 ± 14	33 ± 8	28 ± 12	27 ± 6
	-	+	96 ± 11	77 ± 12	75 ± 7	74 ± 19	71 ± 16	63 ± 11	52 ± 20
	+	-	91 ± 15	103 ± 3	105 ± 8	93 ± 19	99	84	68
	+	+	101 ± 8	113 ± 14	157 ± 12	192 ± 2	188	201	184

The results are given as the average for cells prepared from two separate rat livers, except where given as mean ± S.D. (n = 3). The initial cytochrome P-450 concentration in isolated hepatocytes was 187 ± 25 pmoles/mg protein.

Table 2. Induction of cytochrome P-450 by culture medium containing 1,2 benzanthracene

Additions to Williams medium E		Cytochrome P-450 concn.(% initial) after culture for (hours)			
0.5 mM Metyrapone	0.05 mM 1,2 Benzanthracene	24	48	72	96
-	-	37	36	28	22
-	+	48	56	43	33
+	-	97	108	97	110
+	+	87	175	220	215

Hepatocytes were cultured in medium containing 1,2 Benzanthracene added in dimethylformamide or in medium containing dimethylformamide (0.5% v/v final). The results are given as the average for cells prepared from two separate rat livers. The initial cytochrome P-450 concn. in isolated hepatocytes was 168 pmoles/mg protein.

Table 3. Dose response relationship for the induction of cytochrome P-450 by benzanthracene or phenobarbital.

Concn. (mM) of inducer in Williams' medium E	Cytochrome P-450 concn.(% initial) after culture for 96 hours in medium containing	
	Benzanthracene	Phenobarbital
0.01	110	-
0.02	156	-
0.04	215	-
0.05	286	-
0.06	228	-
0.08	237	-
0.50	-	121
1.00	-	147
2.00	-	220
4.00	-	106 (cytotoxic)

Hepatocytes were cultured in Williams medium E containing 0.5 mM Metyrapone. Hepatocytes were treated with Phenobarbital or Benzanthracene after 4 hours of culture. The medium was then changed every 24 hours until the cultures were 96 hours old when their cytochrome P-450 content was determined. The initial cytochrome P-450 concn.in isolated hepatocytes was 191 pmoles mg protein.

RESULTS AND DISCUSSION

When hepatocytes are cultured in Williams' medium E (ie plus cystine and cysteine) they lose 60% of their cytochrome P-450 content during the first 24 hours (9) and during the next 144 hours there is a further loss of the cytochrome (Table 1). The culture of hepatocytes in "P-450 medium" prevents the loss of the cytochrome during the first 24 hours but between 48 and 168 hours there is a loss of cytochrome P-450 (Table 1). The addition of 2 mM phenobarbital, to either medium, retards the decline of the cytochrome such that after 72-96 h, hepatocytes cultured in the presence of phenobarbital contain two to three times the concentration of cytochrome P-450 found in the absence of phenobarbital. However the concentration of cytochrome P-450 in hepatocytes cultured in media containing phenobarbital does not increase above that found in the initial cell preparation, even at 96 hours (Table 1). In contrast the addition of 0.5 mM metyrapone to either Williams' medium E or "P-450 medium" prevents the loss of cytochrome P-450 over a 7 day (168 hour) culture period. Since metyrapone alone does not increase the concentration of cytochrome P-450 in cultured hepatocytes above that found in the initial cell preparation it does not appear to be an inducer. However, in the presence of metyrapone, phenobarbital is able to induce the cytochrome to levels that are higher than those found in the initial cell preparations (Table 1). The maximal concentration of cytochrome P-450 (458 ± 12 pmoles/mg protein) is found after 96 hours of culture in the presence of 2 mM phenobarbital (Table 1). This is comparable to that found in the livers of rats, treated chronically, with phenobarbital (see section on methods) where the concentration of cytochrome P-450 is 576 ± 53 (n=4) pmoles/mg protein.

Table 2 shows that benzanthracene, added to medium containing 0.5 mM metyrapone, is able, like phenobarbital, to induce cytochrome P-450 to concentrations above that found in the initial cell preparation. Maximal induction of cytochrome P-450, by benzanthracene, was observed after 72 hours of hepatocyte culture (Table 2) using 50 μM benzanthracene (Table 3). When cultured hepatocytes were treated with both benzanthracene and phenobarbital, at concentrations which separately produce maximal induction, cytotoxic effects were observed. Accordingly it was impossible to determine whether the two classes of inducers produced additive effects in liver cell culture. Nevertheless the present work shows that the culture of hepatocytes in media containing 0.5 mM metyrapone not only prevents the loss of cytochrome P-450 from hepatocytes for up to 7 days of culture but also permits classical inducers, such as phenobarbital and benzanthracene, to increase the concentration of the cytochrome above that found in the initial cell preparations. The culture of hepatocytes in medium containing metyrapone could therefore prove a useful model for determining the inducing effects of the many chemicals in current therapeutic use.

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