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The influence of culture medium composition on drug metabolising enzyme activities of the human liver derived Hep G2 cell line

H. Doostdar*°+, S.J. Duthie*, M.D. Burke+, W.T. Melvin° and M.H. Grant*

*Clinical Pharmacology Unit, Departments of Medicine & Therapeutics, °Biochemistry and + Pharmacology, University of Aberdeen, Polwarth Building, Foresterhill, Aberdeen AB9 2ZD, Scotland

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When grown in the standard Dulbecco's medium the human liver derived Hep G2 hepatoma cell line shows only 10–20% of the cytochrome P-450-dependent mixed function oxidase (MFO) activity of freshly isolated human adult hepatocytes. However, the MFO activities and, to a lesser extent, the activities of UDP-glucuronyltransferase and glutathione-*S*-transferase can be increased by altering the composition of the growth medium. Modified Earle's medium was more effective in this respect than Williams' E medium and increased the *O*-dealkylations of ethoxyresorufin, benzyloxyresorufin and pentoxyresorufin 50-, 30- and 10-fold, respectively.

Cytochrome P-450; UDP-glucuronyltransferase; Glutathione; Glutathione-*S*-transferase; (Human Hep G2 hepatoma cell, Cell culture)

1. INTRODUCTION

There have been a number of reports recently suggesting that human hepatoma cell lines which retain differentiated parenchymal functions may provide an in vitro system for studying drug metabolism and cytotoxicity directly in man. Certain cell lines, including Hep G2, Hep 3B and SK-Hep-1, can carry out cytochrome P-450-dependent mixed function oxidase (MFO) and conjugation reactions [1–4] and are capable of activating benzo(*a*)pyrene, aflatoxin B_1 and cyclophosphamide to cytotoxic and mutagenic metabolites [2,5–7].

The drug metabolising enzyme activities of the human liver derived hepatoma cell line, Hep G2, have recently been compared with those in freshly isolated human adult hepatocytes [8]. MFO activities towards three substrates were found to be 5–10-fold lower in the Hep G2 cells grown in the standard Dulbecco's medium than in the hepatocytes, whereas the activities of the conjugating enzymes, GT and GST were similar in the two cell types [9]. Hep G2 is a highly differentiated cell line which has retained many of the specialised functions normally lost by hepatocytes in culture [10]. The loss of MFO activities in Hep G2 cells may have occurred either as a consequence of the original transformation of the hepatocytes to tumour cells or, alternatively, as a result of being grown in inappropriate culture conditions.

MFO activities are known to be unstable during primary culture of hepatocytes from both animals and man [11–13], and are markedly influenced by the culture conditions, for example medium and substratum composition [12,14]. The addition of hormones, nicotinamide, metyrapone and the exclusion of L-cysteine and L-cystine from the culture medium have all been reported to improve the maintenance of MFO activities in cultured rat hepatocytes [11,15,16]. Albumin production in

Correspondence (present) address: M.H. Grant, Department of Physiology and Pharmacology, Strathclyde University, Royal College, 204 George Street, Glasgow G1 1XW, Scotland

Abbreviations: EROD, ethoxyresorufin *O*-dealkylation; MROD, methoxyresorufin *O*-dealkylation; PROD, pentoxyresorufin *O*-dealkylation; BROD, benzyloxyresorufin *O*-dealkylation; MFO, mixed function oxidase; GT, UDP-glucuronyltransferase; GST, glutathione-*S*-transferase; GSH, reduced glutathione; CDNB, 1-chloro-2,4-dinitrobenzene

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Huh 6 human hepatoma cells has been shown to respond to culture conditions and was increased when the cells were grown on fibronectin or laminin substrata [17]. The aim of this study was to determine whether drug metabolising enzyme activities, particularly those of MFO, in Hep G2 cells could be modified by altering the composition of the growth medium. The enzyme activities of cells grown in Dulbecco's medium were compared with those of cells grown in Williams' E or modified Earle's, the two media which were the most effective for maintaining the cytochrome P-450 content of cultured rat hepatocytes [12].

2. MATERIALS AND METHODS

2.1. *Materials*

Flow Laboratories supplied the Dulbecco's and Williams' E media and fetal calf serum was purchased from Gibco. 1-Naphthol, 1-naphthol glucuronide, bilirubin, UDP-glucuronic acid, NADPH and bromobenzene were obtained from Sigma. Resorufin, methoxyresorufin, ethoxyresorufin, pentoxyresorufin and benzyloxyresorufin were synthesized as described by Burke and Mayer [18]. The Hep G2 cell line was obtained from Professor C.N. Hales, Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge.

2.2. *Culture of Hep G2 cells*

Hep G2 cells were routinely grown in Dulbecco's modification of Eagle's medium containing 10% (v/v) fetal calf serum and split 1:3 every 7 days (seeding density 3.5×10^6 cells in a 75 cm^2 flask). When the growth medium was altered, the cells were allowed to grow for at least 7 days in the new medium before enzyme activities were measured. The medium was renewed routinely 3 and 6 days after passage and all enzyme activity measurements were carried out on confluent cells 7 days after subculture. The composition of modified Eagle's medium was as described previously [19] except that 0.25 mM L-cysteine was added.

2.3. *Incubation of Hep G2 cells for toxicity experiments*

Suspensions of Hep G2 cells were prepared as described

Table 1

Drug metabolising enzyme activities and glutathione content of Hep G2 cells cultured in different media

	Reaction rate		
	Dulbecco's medium	Williams' E medium	Earle's medium
MROD[a]	1.0 ± 0.1 (3) (100%)	2.7 ± 0.3* (5) (270%)	49.8 ± 5.9* (3) (498%)
EROD[a]	2.7 ± 0.2 (3) (100%)	6.1 ± 0.5* (5) (226%)	127.1 ± 12.4* (3) (4700%)
BROD[a]	0.7 ± 0.1 (3) (100%)	1.7 ± 0.4* (5) (243%)	20.7 ± 3.0* (3) (2957%)
PROD[a]	0.4 ± 0.05 (3) (100%)	0.8 ± 0.2 (5) (200%)	3.0 ± 0.1* (3) (750%)
1-Naphthol GT[b]	0.57 ± 0.05 (5) (100%)	0.68 ± 0.15 (3) (119%)	1.03 ± 0.05* (6) (181%)
Bilirubin GT[b]	1.10 ± 0.24 (6) (100%)	1.67 ± 0.15 (6) (152%)	4.51 ± 0.38* (3) (410%)
CDNB GST[b]	7.20 ± 0.20 (3) (100%)	6.72 ± 0.28 (3) (93%)	16.13 ± 1.11* (3) (224%)
GSH content[c]	18.02 ± 1.08 (3) (100%)	15.85 ± 0.39 (3) (88%)	29.70 ± 2.98* (3) (165%)

[a] Reaction rate results are expressed in pmol/min per mg protein
[b] Reaction rate results are expressed in nmol/min per mg protein
[c] Reaction rate results are expressed in nmol/mg protein

The values are means ± SE, with the numbers of experiments in parentheses. The values obtained in cells cultured in Williams' E and modified Earle's media were expressed as a percentage of those obtained in cells cultured in Dulbecco's medium and the percentages are shown in brackets. * $P < 0.05$, by one way analysis of variance followed by Dunnett's test. Significance values refer to differences in enzyme activities and GSH content between cells grown in Dulbecco's medium and those grown in the other two media

previously [9] and incubated in 50 ml round bottomed flasks in Krebs-Henseleit buffer, pH 7.4, containing 10 mM Hepes at 37°C under an atmosphere of 95% O_2:5% CO_2. The effect of 10 mM bromobenzene on cell viability was assessed using Trypan blue exclusion. In some experiments 0.5 mM metyrapone was included in the incubations as an inhibitor of the MFO system.

2.4. *Analytical methods*

Enzyme activities were measured on cell homogenates prepared in 0.1 M sodium phosphate buffer, pH 7.6, as described previously [9]. MFO activities were measured by the *O*-dealkylation of MROD, EROD, PROD and BROD, which are metabolised by different isoenzymes of cytochrome P-450 in the rat [20], as described previously [9]. GST activity was measured using 50 μM CDNB in the presence of excess reduced glutathione (1 mM) by direct spectrophotometry as described by Habig and Jakoby [21]. Intracellular GSH was determined fluorimetrically [22] after scraping the cultured cells into 6.5% (w/v) trichloroacetic acid. GT activity was measured either using 1-naphthol (50 μM) in the presence of 0.5 mM UDP-glucuronic acid and 5 mM $MgCl_2$ by continuous fluorescence detection of the glucuronide formed [23] or using 0.4 mM bilirubin in the presence of 4 mM UDP-glucuronic acid and 10 mM $MgCl_2$ by the diazotization procedure as described in [24].

3. RESULTS

The activities of MFO, GT and GST and the GSH content of Hep G2 cells grown in Dulbecco's, Williams' E and modified Earle's media are shown in table 1. The lowest MFO activities were in the cells grown in Dulbecco's medium, while the culture in Williams' E medium resulted in an approx. 2-fold increase in all the activities. Compared with Dulbecco's medium, cultures in the modified Earle's medium increased MROD activity 50-fold, EROD 47-fold, BROD 30-fold and PROD 10-fold. The activities of GT and GST were also increased by culture in the modified Earle's medium, although the effect of medium composition on the activities of these two enzymes was not as marked as the effect on MFO. GSH content was 2-fold higher in cells grown in modified Earle's medium.

Fig.1 shows that, compared with the response of cells grown in Dulbecco's medium, the toxicity of bromobenzene was markedly increased in cells grown in Williams' E medium and was greatest in those grown in modified Earle's medium. The toxicity of bromobenzene to Hep G2 cells grown in Williams' E medium was almost completely prevented by the presence of 0.5 mM metyrapone.

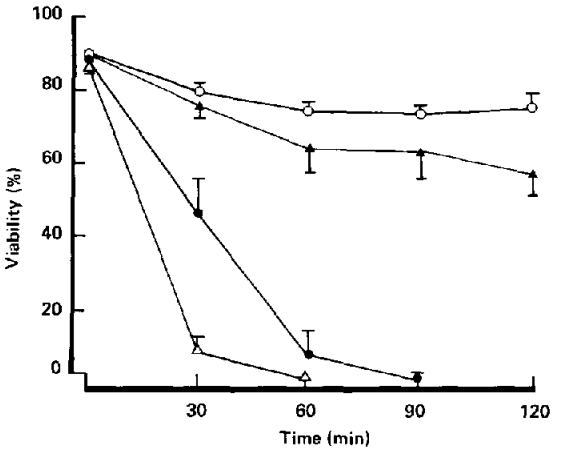

Fig.1. The effect of 10 mM bromobenzene on the viability of Hep G2 cells grown in Dulbecco's (○, $N = 2$), Williams' E (●, $N = 3$) and modified Earle's (△, $N = 3$) media. (▲) The effect of 0.5 mM metyrapone on the toxicity of bromobenzene in cells grown in Williams' E medium ($N = 3$). Error bars represent either the SE, where $N > 2$, or the range, where $N = 2$.

4. DISCUSSION

The results shown here suggest that the absence of MFO activities in the Hep G2 cell line is due to a large extent to the use of inappropriate culture conditions for growing the cells.

The modified Earle's medium increased the MFO activities expressed by Hep G2 cells in a substrate specific manner, exerting the greatest effect on EROD and MROD, which are probes for a 3-methylcholanthrene induced form of cytochrome P-450 in the rat [20]. There was least effect on PROD activity, which is a probe for a phenobarbitone induced cytochrome P-450 in rat liver. This medium is supplemented with hydrocortisone (10^{-4} M) and 5-aminolaevulinic acid (10^{-4} M), and these supplements were thought to be responsible for its efficiency in maintaining the level and function of cytochrome P-450 in cultured hepatocytes [12,13] and Hep G2 cells. However, the addition of hydrocortisone and 5-aminolaevulinic acid to either Dulbecco's or Williams' E medium did not significantly increase MFO activities (not shown).

The presence of L-cysteine or L-cystine in modified Earle's medium had previously been found to be detrimental to the maintenance of cytochrome P-450 content and MFO activities in

cultured rat hepatocytes [15], and these amino acids were replaced by L-methionine. However, Hep G2 cells cannot use L-methionine for GSH synthesis [9] and do not survive in modified Earle's medium without L-cysteine. We have found that, in contrast to hepatocytes, the presence of L-cysteine in modified Earle's medium improved the expression of MFO, GT and GST activities in the Hep G2 cells approx. 2-fold (not shown).

Bromobenzene is metabolised to a cytotoxic epoxide metabolite by the MFO system [25] and the increase in bromobenzene-induced toxicity to Hep G2 cells observed in Williams' E and Earle's medium reflects the increased levels of MFO activity in these media. The toxicity of bromobenzene to the Hep G2 cells was prevented by including 0.5 mM metyrapone, an inhibitor of the MFO system, in the incubations.

The effects of medium composition on the activities of GT and GST were less dramatic than on the MFO system. The conjugation enzyme activities were also more stable than the MFO activities in cultured rat and human hepatocytes and in these cultures also they are relatively unaffected by changes in medium composition [13,26]. The increase in GST activity observed in Hep G2 cells grown in modified Earle's medium was not simply due to the increase in the intracellular GSH, since the GST activities were measured in cell homogenates in the presence of added excess GSH.

Human hepatoma cell lines, such as Hep G2, have previously been thought to contain only low activities of the major drug metabolising enzymes. However, with the demonstration, in this paper, that the MFO activities, and to a lesser extent the GST and GT activities, of Hep G2 cells can be manipulated by medium composition, these cells become a powerful tool for investigating the potential hepatic metabolism and cytotoxicity of xenobiotics in man in vitro.

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