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HEPG2 CELLS: AN *IN VITRO* MODEL FOR P450-DEPENDENT METABOLISM OF ACETAMINOPHEN

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SUMMARY The human hepatoma cell line, HepG2, retains many cellular functions often lost by cells in culture. This research examined the constitutive bioactivation of acetaminophen and P450-dependent activity in microsomes from HepG2 cells and the effect of 0.1% acetone pretreatment on these activities. Low levels of acetaminophen bioactivation, P450 IIE1 activity, and P450 IA1-IA2 activity were demonstrated in non-induced HepG2 microsomes. Acetone increased acetaminophen bioactivation and IIE1-dependent metabolism but not P450 IA1-IA2-dependent activity. Thus, HepG2 cells may provide an *in vitro* model for assessing human xenobiotic metabolism of acetaminophen and other drugs.

HepG2, an immortalized human hepatoma cell line, retains many cellular functions often lost by primary hepatocytes such as expression of hepatocyte-specific cell surface receptors and synthesis and secretion of plasma proteins (1-3). These cells also retain cytochrome P450-dependent mixed function oxidases and glucuronic acid and sulfate conjugation activities involved in Phase I and Phase II metabolism of xenobiotics (1).

Acetaminophen was used to study reactive metabolite-dependent toxicity because of its widespread usage, potential for toxic overdose in man, and because it is a prototype hepatotoxin (4). Though the mode of toxic action of acetaminophen has yet to be determined, it is widely accepted to be dependent on the bioactivation of acetaminophen to a reactive metabolite, N-acetyl-p-benzoquinone imine, a metabolism primarily carried out by cytochrome P450 IIE1 and IA2 (5,6). The binding of N-acetyl-p-benzoquinone imine to

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cellular proteins is a good marker of acetaminophen toxicity; previously this laboratory has prepared specific antisera capable of demonstrating the 3-(cystein-S-yl)-acetaminophen adduct (3-Cys-acetaminophen) in various immunoassays (7).

The present research examined the constitutive acetaminophen bioactivation and P450 activity of HepG2 cells using the isoform specific P450-dependent activities, 7-ethoxyresorufin O-deethylase (8), 4-nitrophenol hydroxylase (9) and dimethylnitrosamine N-demethylase (10), and the effect of acetone, a specific inducer of IIE1 that increases acetaminophen toxicity (6), on these activities.

MATERIALS AND METHODS

Chemicals

All reagents and biochemicals, unless otherwise noted, were obtained from Sigma Chemical Co., St. Louis, MO. Cell culture materials were obtained from GIBCO (Grand Island, NY)

Cell Cultures

HepG2 cells obtained from Dr. Barbara B. Knowles (Wistar Institute of Anatomy and Biology, Philadelphia, PA) were maintained at 37°C with a 5% CO_2 atmosphere in Eagle's Minimum Essential Medium supplemented with 10% calf serum, L-glutamine, penicillin (1000 IU/ml), and streptomycin (100 μg/ml). Cells were grown to confluency in 150 x 25 mm petri plates, and grown for 10 additional days with or without 0.1% HPLC-grade acetone (J.T. Baker, Phillipsburg, NJ) in complete medium. Medium was replenished or suplemented approximately every 3 days.

Microsome preparation

Cells were harvested by scraping and washed (1X Hanks' Balanced Salt Solution), suspensions were collected in 50 ml tubes and centrifuged at 1000 x g for 10 min. The supernates were discarded and cell pellets were washed with a microsomal preparation buffer consisting of 50.0 mM Tris (pH 7.4) + 15.0 mM KCl and homogenized in a 10 ml Kjeldahl tube using a Teflon-coated tissue grinder. Microsomes were prepared from cell homogenates as described previously (11); pellets and the resulting supernates were separated and assayed immediately. Total protein was determined according to Lowry *et al.* (12) with bovine serum albumin as a standard. Total cytochrome P450 in microsomes was determined using the differential spectrophotometric method of Omura and Sato (13), as modified by Ghersi-Egea *et al.* (14).

Acetaminophen-Bioactivation by HepG2 microsomes

HepG2 microsomes (200-300 μg protein) were incubated with 1.0 mM acetaminophen and an NADPH regenerating system (11). After a 30 min incubation at 37°C, the reactions were halted by the addition of 200 μl of 20% SDS. Samples were dialyzed against PBS, concentrated under vacuum, and adjusted to 150 μg protein/ml with PBS. 3-Cys-acetaminophen protein adducts were detected using a competitive particle concentration fluorescence immunoassay as previously described (15).

P450 activity in HepG2 microsomes

P450 IA-family activity (7-ethoxyresorufin O-deethylase) in HepG2 microsomes was estimated by the method of Burke *et al.* (8) using a substrate concentration of 4.0 μM. P450 IIE1 activity was assayed in HepG2 microsomes (approximately 300 μg protein) by assessing 4-nitrophenol hydroxylase and dimethylnitrosamine N-demethylase activities using substrate concentrations of 100.0 μM and 400.0 μM, respectively (9, 16).

Statistics

Means of measured activities were compared by analysis of variance using the SAS statistical package for PC (SAS Institute, Cary, NC).

RESULTS AND DISCUSSION

Table 1 summarizes the metabolic activities of microsomes from both untreated and acetone-treated HepG2 cells. A typical microsomal preparation was conducted with 40 plates of confluent cells and yielded from 1.3 - 1.5 mg microsomal protein. Microsomes from either treatment contained approximately equal amounts of total P450.

HepG2 microsomes were capable of bioactivating acetaminophen which was trapped as 3-cys-acetaminophen protein adduct. 7-ethoxyresorufin O-deethylase activity was identical in microsomes from acetone-treated or untreated cells. Low K_m 4-nitrophenol hydroxylase and dimethylnitrosamine N-demethylase activity was measured in microsomes from control HepG2 cells. Microsomes from acetone-treated cells bioactivated significantly greater amounts of acetaminophen than controls ($p \leq 0.05$, $n=4$); incubations from acetone-treated microsomes contained 7 times as much 3-Cys-acetaminophen. Acetaminophen is metabolized into its reactive metabolite, N-acetyl-p-benzoquinone imine, by both P450 IIE1 and IA2. While the acetaminophen bioactivation in control microsomes is attributable to both isoforms, the increased activation in acetone-treated cells is most likely the result of increased P450 IIE1.

Previous studies have demonstrated HepG2 cells have constitutive P450 activity of the P450 IA family (1,17,18). The constitutive P450 IIE1 activity in HepG2 cells, as measured by dimethylnitrosamine N-demethylase, is approximately 20% that reported in human liver microsomes (19). The generally low metabolic activity in HepG2 cells has been attributed to dedifferentiation of the cells to a fetal phenotype (1,20,21). This is consistent with the report that P450 IIE1 and its mRNA are not apparent in the fetus, occur at low levels in neonates, but increase post-partum (22).

Table 1. Effect of acetone treatment (0.1% for 10 days) on microsomal cytochrome P450, 7-ethoxyresorufin (IA1-IA2 specific), 4-nitrophenol hydroxylase and dimethylnitrosamine N-demethylase (IIE1 specific) activities in HepG2 cells

Treatment	N	P450[1]	3-Cys-acet-aminophen[2]	7-ethoxyresorufin-O-deethylase[3]	4-nitrophenol hydroxylase[4]	dimethylnitrosamine N-demethylase[5]
Control	6	0.43±0.04a	0.10±0.04a	3.2±0.2a	8.0±0.8a	5.9±0.8a
Acetone	6	0.45±0.02a	0.73±0.12b	3.2±0.2a	16.8±1.2b	13.7±1.0b

[1] P450 expressed as pmol cytochrome P450/mg microsomal protein, mean ± S.D. Means followed by different letters are significantly different by Fisher's Least Significant Difference Test, $p \leq 0.05$.
[2-5] Activities are expressed as pmol product/min/mg microsomal protein, mean ± S.D.

HepG2 cells cultured in medium containing 0.1% acetone were found to have a 2-3 fold increase of microsomal P450 IIE1-dependent activity compared to controls ($P \geq 0.05$, $n = 6$). Previous investigations have shown that ethanol and acetone are P450 IIE1-specific inducers (5,6). Of these two inducers, acetone seems to increase the level of P450 IIE1 protein to a greater extent than ethanol. Ding and Coon (23) were able to induce P450 IIE1 in rabbit olfactory mucosa three times greater with acetone than with ethanol. Yang and coworkers have reported that induction of P450 IIE1 by ethanol and acetone may be a result of increased translation or protein stabilization as opposed to transcriptional mechanisms (24).

These results indicate that HepG2 cells can bioactivate acetaminophen and contain constitutive P450 IIE1. The failure of earlier studies to demonstrate IIE1 activity in HepG2 cells may be the result of using an insufficient number of cells as a source of microsomes, or the common practice of cryopreserving microsomes in buffer containing glycerol (25). The activities reported here were obtained using freshly prepared microsomes.

The data presented here indicate that HepG2 cells may provide an *in vitro* model for assessing acetaminophen toxicity, and demonstrate that treatment of HepG2 cells with acetone increases P450 IIE1-dependent activity. The potential for induction of P450 IIE1 in HepG2 cells suggest this cell model may be useful for evaluating other small molecules bioactivated by IIE1.

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