

# Induction of drug metabolizing enzymes in human liver cell line Hep G2

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Human cytochrome P-450, UDP-glucuronosyltransferase and sulphotransferase activities have been measured in the cell line Hep G2 following treatment of cells with 3-methylcholanthrene or phenobarbital. 3-Methylcholanthrene treatment caused a 20–30-fold increase in the O-deethylation of 7-ethoxycoumarin. The glucuronidation and sulphation of the product 7-hydroxycoumarin were increased 36 and 7 fold, respectively. In comparison, phenobarbital treatment did not increase these activities significantly. However, phenobarbital-inducible proteins were identified on 'Western blots' using antibodies to a rat liver phenobarbital inducible P-450 form. The molecular masses of the proteins did not coincide with those expected for cytochromes P-450. However, characteristic of P-450 forms, the synthesis of these proteins was suppressed by 3-methylcholanthrene treatment. The Hep G2 cell line represents a potentially useful model for studying the regulation of human P-450 genes.

Cytochrome P-450    Hep G2    Cell line    Drug metabolism    Enzyme induction    UDP-glucuronosyltransferase

## 1. INTRODUCTION

The ability to metabolise xenobiotics is a function normally lost by liver cells in culture [1–3]. The activity drops to approximately 25% of the in vivo level within the first 24 h (e.g., [2,3]). This loss of drug metabolising activity coincides with the loss of a number of other specialised functions and appears to be a form of de-differentiation of the cells [3]. The Hep G2 cell line is an immortalised liver cell line of human origin [4]. These cells have been found to retain many of the specialised functions which are usually lost upon culturing. These functions include synthesis and secretion of major plasma proteins including  $\alpha$ 2-plasmin inhibitor, apolipoproteins and insulin-like growth factor carrier protein [5–9]; synthesis of hepatitis

B surface antigen [4,7] and metabolic activation of benzo(a)pyrene [10]. The purpose of the present investigation was to evaluate whether this cell line could be used as a model for the study of the regulation of human drug metabolizing enzymes.

## 2. MATERIALS AND METHODS

Umbelliferone, 7-ethoxycoumarin, sulphatase (type H-5) and  $\beta$ -glucuronidase solution (glucurase) were purchased from Sigma, Poole, Dorset. All other chemicals were of Analar reagent grade, purchased through local chemical suppliers.

Hep G2 cells were kindly donated by Dr B. Knowles of the Wistar Institute, Philadelphia.

The cells were grown in 75 cm<sup>2</sup> flasks in RPMI 1640 containing 5% fetal calf serum and 0.03% glutamine at 37°C in an atmosphere of humidified air containing 5% CO<sub>2</sub> until there were approximately 10<sup>7</sup> cells per flask. To each flask 3-methylcholanthrene (5  $\mu$ M) (3-MC) as a solution in dimethyl sulphoxide, or sodium phenobarbital

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(1 mM) was added. No additions were made to control flasks. Three or four days after addition of inducing agents, the cells were harvested by addition of trypsin, counted and washed repeatedly with fresh cell culture medium to remove the trypsin.

For drug metabolism studies the cells were resuspended in cell culture medium, to which 7-ethoxycoumarin (250  $\mu$ M) had been added, at a concentration of approx.  $10^6$  cells/ml. The cells were incubated in 25 ml conical flasks in a shaking water bath at 37°C. At the relevant times, an aliquot (2 ml) was withdrawn from each flask and placed in a screw-capped tube on ice. To the tube was added 7 ml ice-cold ether/isoamyl alcohol (1.5% v/v) and the tubes extracted for 10 min using a tube rotator. A portion (5 ml) of the ether layer was then back extracted into 3.5 ml glycine/NaOH buffer (0.2 M, pH 10.4) and the fluorescence of the aqueous layer was determined at 370/450 nm using a Perkin Elmer spectrofluorimeter (see [11] for method). Acetate/acetic acid buffer (0.5 ml, 0.1 M, pH 4.8) and glucurase (0.5 ml) was added to the aqueous layer remaining after the ether extraction. The tubes were incubated overnight at 37°C and the free 7-hydroxycoumarin released was extracted and assayed as described above. To the aqueous layer remaining after this second ether extraction, sulphatase in acetate/acetic acid buffer (30 units/tube) was added and the tubes incubated and extracted as above. A set of standard and control tubes was also taken through the extraction procedure. Standards of 0.1 nmol 7-hydroxycoumarin per ml were used.

Western blots were carried out using antibodies to purified forms of rat liver cytochromes P-450, PB<sub>1</sub>, PB<sub>2</sub>, MC<sub>1</sub> and MC<sub>2</sub> [12,13]. These antibodies have previously been shown to react with P-450 isozymes present in human liver [14]. The method has been described previously [14] except that whole cells instead of microsomal fractions were used with 30  $\mu$ g of cellular protein per lane. Protein determinations were by the method of Lowry et al. [15].

### 3. RESULTS

Metabolism of 7-ethoxycoumarin by untreated and phenobarbital-treated Hep G2 cells was

characterised by a marked lag period of approx. 1–2 h (fig.1a,b); the metabolism was then essentially linear. No lag period was measured with

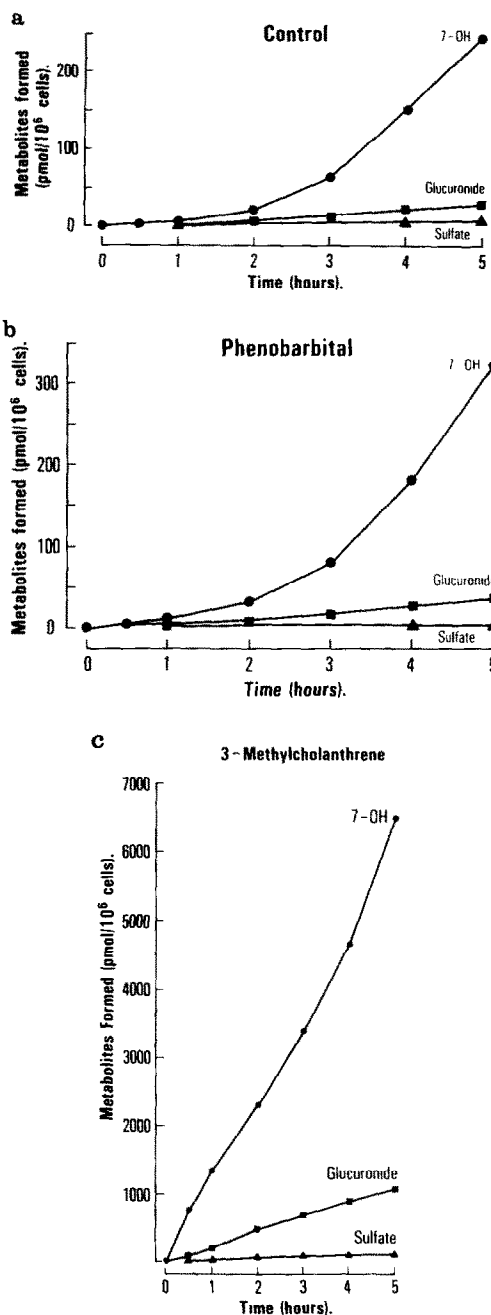


Fig.1. 7-Ethoxycoumarin metabolism in untreated Hep G2 cells (a) or cells following treatment with phenobarbital (b) or 3-MC (c). Experimental details are given in section 2.

3-MC-treated cells and the O-deethylation of 7-ethoxycoumarin was linear with time (fig.1c). Glucuronidation of the 7-hydroxycoumarin released by the P-450-mediated O-deethylation was the dominant conjugation pathway, sulphation being virtually undetectable (fig.1). Note that the P-450 metabolism was much more active than conjugation in all cell samples.

Treatment of the Hep G2 cells with phenobarbital for 4 days prior to the 7-ethoxycoumarin assay resulted in a small increase in P-450 activity (124% of control), but this increase was not significant. Glucuronidation by phenobarbital-induced cells was also slightly increased. However, treatment of the Hep G2 cells with 3-MC (5  $\mu$ M)

resulted in a very marked, 20–30-fold, increase in P-450-mediated metabolism (fig.1c). There was also a 38-fold increase in glucuronide conjugation and a 9-fold increase in sulphate conjugation in 3-MC-treated cells (fig.1c). The use of 3-MC at a concentration of 15  $\mu$ M was cytotoxic to the Hep G2 cells (not shown). In Western blots of Hep G2 cells with antibodies to 4 rat liver cytochrome P-450 isozymes, two induced by phenobarbital (PB<sub>1</sub> and PB<sub>2</sub>) [13] and two induced by 3-MC (MC<sub>1</sub> and MC<sub>2</sub>) [12], only the antibody to PB<sub>2</sub> gave positive results (fig.2). Two proteins were identified, with molecular masses of 64 and 63 kDa. Both proteins were slightly induced by phenobarbital and suppressed by treatment with 3-MC.

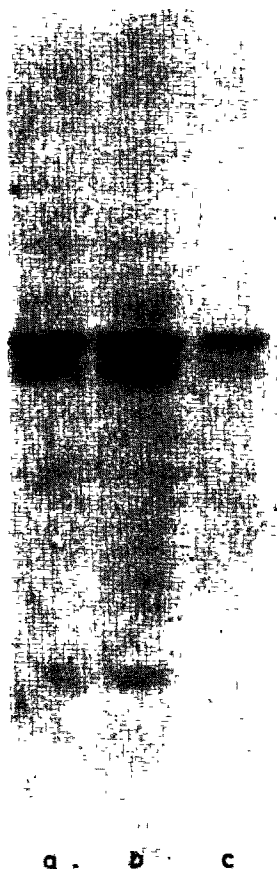


Fig.2. Western blot of Hep G2 cells (30  $\mu$ g/track) using an antibody to rat liver P-450 PB<sub>2</sub>. (a) Control cells, (b) cells treated with phenobarbital, (c) cells treated with 3-methylcholanthrene. Other details are given in section 2.

#### 4. DISCUSSION

Although there have been reports of the induction of drug metabolizing enzymes in cell lines from other species [10], this is the first report of induction in cell lines of human origin. Both cytochrome P-450-dependent monooxygenase activity as well as UDP-glucuronosyltransferase activity were significantly increased by treatment of the Hep G2 cells with 3-methylcholanthrene.

In untreated Hep G2 cells, conjugating activity was low with glucuronidation more active than sulphation (fig.1). In hepatocytes, sulphate conjugation represents a major pathway at low substrate concentrations (similar to those used here) [16]. It has been reported that normal human lung tissue more readily sulphates than glucuronidates phenol substrates. However, in cancerous tissue, glucuronidation is the major pathway [17,18]. The Hep G2 cell line originates from a biopsy sample from a primary hepatoblastoma, so the lack of sulphate conjugation by these cells could be associated with their cancerous origin [1].

Attempts to identify the P-450 isozymes present in these cells have not proved very successful. We have recently demonstrated that the antibodies to the rat liver P-450 isozymes used here will react with human liver microsomal samples, the strongest reactivity being with the antibodies to the phenobarbital-inducible proteins PB<sub>1</sub> and PB<sub>2</sub> [14]. The only antibody to react with the Hep G2 cells was the antibody to PB<sub>2</sub>. However, the pro-

teins identified had higher molecular masses than those normally associated with P-450 forms. Consistent with P-450 forms, however, these protein bands were induced by phenobarbital and suppressed by 3-MC [14]. Unusual glycosylation of proteins in Hep 3B cells (another human hepatoma cell line) has been reported [19] which could explain the high  $M_r$  bands observed here. However, this possibility remains to be clarified.

The regulation of cytochromes P-450 and UDP-glucuronosyltransferases in an immortalized cell line of human origin, such as Hep G2, is of considerable potential value both in the study of the properties of human drug metabolizing enzyme systems as well as in the testing of potential carcinogens, for mutagenic activity, where a more accurate estimate of risk to man could be obtained.

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