MONOOXYGENASE AND GLUCURONYLTRANSFERASE ACTIVITIES IN SHORT TERM CULTURES OF ISOLATED RAT HEPATOCYTES

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1. Introduction

Isolated hepatocytes in culture are useful for the study of many parameters of drug metabolism and action which can not be examined in vivo. However, the applicability of the culture system may be severely limited by changes in the level of drug-metabolizing enzymes during culture. A number of studies have shown that cytochrome *P*-450-containing monooxygenases which play a crucial role in the activation of xenobiotics to their cytotoxic, mutagenic or carcinogeneic form [1,2] were reduced by ⩾66% after 24 h in culture [3–5]. Little is known about the potential loss of monooxygenase activities during the early hours of hepatocyte culture [6] and the specificity of their decline.

Another important factor in the overall metabolism of xenobiotics is the activity of conjugating enzymes. A differential loss or increase in microsomal monooxygenase and conjugase activities might readily alter the steady state level of the reactive intermediates and change the metabolite pattern. Thus we examined the stability of two glucuronyl-transferase activities during short term hepatocyte cultures.

In this study we observed that the *in vitro* activities of two monooxygenases, aldrin epoxidation and benzo[a]pyrene hydroxylation*, and the glucuronidation of morphine decreased by 50–60% in hepatocytes from adult rats cultured for 10 h. In contrast, the glucuronidation of naphthol declined by ⩾25% during this period. The data indicate that the absolute and relative activities of drug-metabolizing enzymes change rapidly during early adaptation of hepatocytes to culture conditions, and underline the need to use caution in extrapolating observations in hepatocytes cultured for more than a few hours to hepatic drug metabolism in vivo.

* This monooxygenase function is also known as aryl hydrocarbon hydroxylase (cf. [2])

2. Materials and methods

2.1. *Isolation and viability of hepatocytes*

Hepatocytes were isolated from male Sprague Dawley rats (~200 g body wt) following established procedures [7,8] except that hyaluronidase was omitted from the perfusion medium. Some animals were pretreated by intraperitoneal injection of 16 mg 5,6-benzoflavone in 1.5 ml corn oil 24 h before isolation of hepatocytes. As described previously, cell preparations were considered satisfactory for experimental use when:

(i) Trypan-blue exclusion was >92%;
(ii) The respiratory control quotient was >2.0 after addition of carbonylcyanide-*m*-chlorophenylhydrazone (2 μM);
(iii) Stimulation of oxygen consumption was ⩾20% after 1 mM succinate [7,8].

After 10 h incubation ~90% of the hepatocytes formed aggregates of more than 50 cells. Trypan-blue was taken up almost exclusively by single cells.

2.2. *Incubation of hepatocytes*

Freshly-isolated hepatocytes (1.2×10^6 cells/ml, equiv. 2.4 mg protein) were incubated in spinner

flasks under a 95% O_2, 5% CO_2 atmosphere in 100 ml Waymouth MB 752/1 medium containing 2 mM glutamine, 5 mM L-lactate, 100 μg/ml streptomycin, and 100 units/ml penicillin [9].

2.3. *Enzyme assays*

Aliquots of 1–3 ml were removed from the incubation mixture and frozen at –70°C to be used for enzyme assays within 3 weeks. Aldrin epoxidation was determined by gas chromatography according to [10] with minor modifications [11]; benzo[a]pyrene hydroxylation was determined by spectrofluorimetry as in [12]. Glucuronidation was naphthol and morphine was assayed according to [13] and [14] respectively, with the addition of 0.1% Brij 58 (Atlas Chemie, Essen) to activate the glucuronyltransferases [15]. Protein was determined by a modified biuret method [16].

3. Results and discussion

As shown in fig.1,2, the two monooxygenase activities, aldrin epoxidation and benzo[a]pyrene-hydroxylation, declined by ~60% during the first 10 h of hepatocyte culture. The decrease of enzyme activities was similar in hepatocytes from untreated rats in which cytochrome *P*-450-dependent monooxygenases predominate [17], or rats pretreated with 5,6-benzoflavone* (5,6-BF) which strongly induces a different, cytochrome *P*-448-dependent benzo[a]-pyrene monooxygenase [18] (fig.1,2B). The rates of decline are compatible with the low activities of aminopyrene-*N*-demethylation, or aniline- and benzo[a]pyrene monoxygenase in 24 h old hepatocyte cultures observed [3]. It is interesting to note that polycyclic hydrocarbon-induced benzo[a]pyrene hydroxylation in hamster embryo cells decreased at a comparable rate when protein synthesis was inhibited or when the inducer was removed from the medium [2].

In order to examine whether the two forms of benzo[a]pyrene monooxygenases lose their activities at different rates during hepatocyte culture we studied the effects of the modifier 7,8-benzoflavone. This

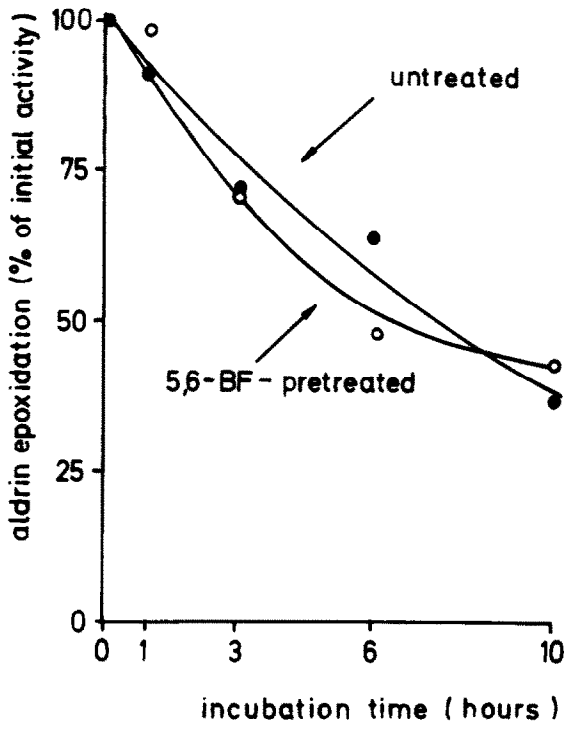

Fig.1. In vitro activity of aldrin epoxidation of cultured hepatocytes. Hepatocytes were isolated from untreated rats or rats treated with 5,6-benzoflavone. Aldrin was 0.1 mM, and protein 0.5 mg/ml. Values represent the % of initial enzyme activities immediately after isolation of the hepatocytes. The initial activities were 0.35–1.0 (untreated) and 0.09 (5,6-BF-treated) nmol dieldrin . min^{-1} . mg $protein^{-1}$. The data show a typical experiment of 3 (untreated) and 2 (5,6-BF-treated) determinations. Duplicates varied by ⩾10% of the mean.

compound strongly inhibits the monooxygenase induced by polycyclic hydrocarbon-type inducers but does not affect or stimulate the constitutive monooxygenase in hepatic microsomes [19]. As shown in fig.2A,2B, the specific response of benzo[a]pyrene monooxygenase to 7,8-benzoflavone observed in hepatic microsomes was retained in cultured hepatocytes. Furthermore, the degree of stimulation or inhibition of benzo[a]pyrene hydroxylation by the modifier did not appreciably change over the 10 h period of hepatocyte culture suggesting that the ratio of the two monooxygenase forms remains unaltered under these conditions.

These results are in agreement with the parallel

* 5,6-Benzoflavone belongs to the class of polycyclic hydrocarbon inducers typified by 3-methylcholanthrene [18]

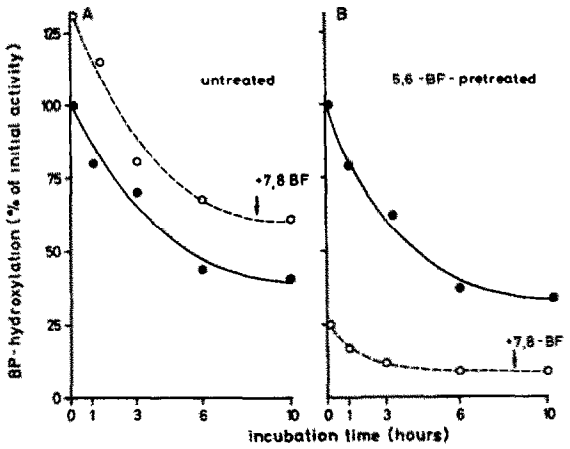

Fig.2. In vitro benzo[a]pyrene monooxygenase activity of cultured hepatocytes. Hepatocytes were isolated from (A) untreated rats or (B) rats treated with 5,6-benzoflavone. In vitro 7,8-benzoflavone was added together with the substrate in 50 μl acetone to give 50 μM final conc. Values represent the % of initial enzyme activities which were determined immediately after isolation of the hepatocytes. Initial specific activities in cell preparations (A) and (B) were 15 and 70 pmol . min^{-1} . mg $protein^{-1}$, respectively. (A) and (B) show typical results of 3 and 2 experiments, respectively. Triplicate determinations varied by >10% of the mean.

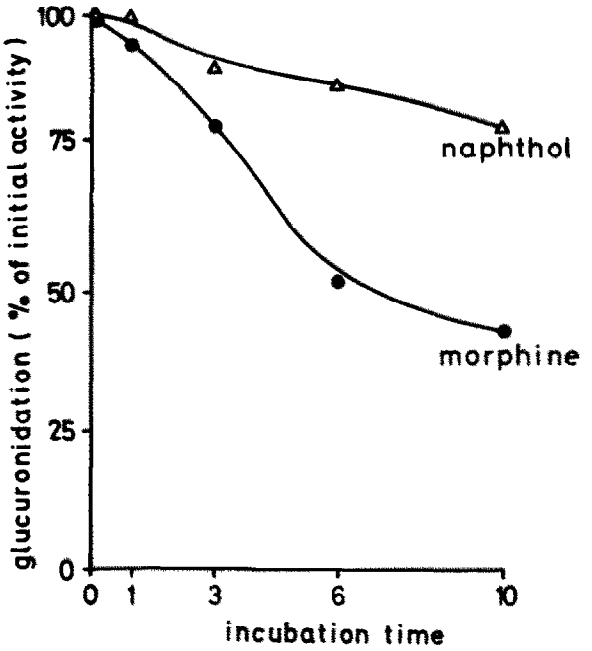

Fig.3. In vitro activity of morphine and napthol glucuronidation of cultured hepatocytes. Morphine and naphtol were used at 1.3 mM and 0.45 mM final conc. Values represent the % of initial enzyme activities immediately after isolation of the hepatocytes. Initial specific activities of the glucuronidation of morphine and naphthol were 1.4 and 9 nmol . min^{-1} . mg $protein^{-1}$, respectively. The data show typical results of 4 experiments which varied by >10%.

decline in the activities of induced and constitutive benzo[a]pyrene monooxygenase. It should be noted that aldrin epoxidation which showed a similar loss of activity represents a cytochrome *P*-450-dependent form different from that oxidizing benzo[a]pyrene (Th.W., unpublished results). In conclusion, our data give no indication of a selective decrease in any of the monooxygenases tested in our hepatocyte cultures.

This is different for the two forms of glucuronyltransferases studied (fig.3). The glucuronidation of morphine declined at the same rate as the monooxygenase activities, i.e., ~55% during the 10 h culture period. However, the glucuronidation of naphthol appeared to be much more stable and was reduced by >25% over this time period. Thus, a different lability response to the culture conditions might afford another distinction of the two forms of glucuronyl transferases which are already characterized by their physico-chemical properties, their substrate and inducer specificities [20]. Alterations of culture conditions suggested by others, such as addition of serum [6,9] or of a mixture of various hormones [5,21], did not significantly affect the decline in glucuronyltransferase and monooxygenase activities (data not shown).

The present data show that the activity of microsomal enzymes involved in the activation and inactivation of xenobiotics differentially decrease during the first 10 h of hepatocyte culture suggesting that drug metabolism in these early hepatocyte cultures may not truly reflect hepatic metabolism in vivo.

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