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SHORT COMMUNICATIONS

Total cytochrome P-450, but not the major phenobarbitone or 3-methylcholanthrene induced isoenzyme, is differentially induced in the lobes of the rat liver

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The mammalian liver is central to xenobiotic metabolism, a function dependent on cytochrome P-450 a family of haemoproteins with distinct but overlapping activity profiles [1]. In recent years attention has focused on the heterogeneous distribution, and differential induction response, of these enzymes across the hepatic acinus. A characteristic they share with a number of other hepatic enzymes [2].

In other respects the liver is considered to be a homogeneous organ, and induction studies usually involve the use of microsomes prepared from whole livers. It has become apparent, however, that a marked inter-lobe heterogeneity also exists. Carbon tetrachloride induced liver damage, for example, predominates in the median lobe [3], while hexachlorobenzene-induced porphyria produces a differential response in hepatic lobes [4]. Additionally Matsubara *et al.* [5] have shown that a number of enzymes, including cytochrome P-450, are distributed asymmetrically in the right, median and left lobes. Particularly interesting was their finding that PB* and β-NF produced different effects on these lobes.

Cytochrome P-450 linked studies have to date relied on the use of tissue homogenates [3, 5]. This study was undertaken to characterise the distribution and induction of cytochrome P-450 in microsomes prepared from multiple locations of the liver, with particular emphasis as to the distribution of the major PB and 3MC induced subforms.

Materials and methods

Reagents were purchased from the Sigma Chemical Co (Poole, U.K.) and were of the highest grade available.

Animals. Male rats (CD1 strain), from a single litter, were obtained from the animal house of the Biological Laboratory. They were maintained under a constant regime of heating and lighting (12 hr light, 12 hr dark) and allowed access to food and water *ad libitum*.

Induction of cytochrome P-450 and preparation of microsomes. PB was administered as a 0.1% (w/v) solution in the drinking water for 8 days. Over this period the average intake of water was 20 ml/day per rat. 3MC, suspended in Mazola Corn Oil, was injected i.p. into animals each day for 3 days (15 mg/kg: approximately 0.2 ml/rat), animals were killed on the fourth day. They were taken to the laboratory between 9.00 and 9.30 a.m., and killed by cervical dislocation between 10.00 and 10.30 a.m. Livers were recovered and each anatomically distinct lobe separated weighed and microsomes prepared as described by De Pierre and Dallner [6].

The nomenclature we use is described by Hebel and Stromberg [7]; we sampled the right (lobus dexter), median (lobus sinister medialls), left (lobus sinister) and caudate lobe (proc. caudatus) and separated the papillary process (proc. papillaris) into its dorsal and ventral components.

Purification of cytochrome P-450 isoenzymes and production of antibodies. The major PB and 3MC induced isoenzymes of cytochrome P-450 were purified from rat liver as described by Guengerich and Martin [8]. Antibodies to each isoenzyme were developed in Balb/c mice as described by Johnstone and Thorpe [9].

Measurement of cytochrome P-450 concentration. The total spectrophotometric content of cytochrome P-450 in microsomes was determined according to Omura and Sato [10]. The concentration of individual isoenzymes was determined by ELISA [9] using the purified protein as the standard.

Protein assay. Protein concentrations were determined using the method of Lowry *et al.* [11] and bovine serum albumin as the standard.

Statistical analysis. Differences between means were evaluated using one way analysis of variance. Significance was ascribed at $P \leq 0.01$.

Results and discussion

The major isoenzymes of cytochrome P-450 induced by PB and 3MC were purified to apparent homogeneity as judged by a single band on SDS-polyacrylamide gel analysis. Their specific cytochrome content was 13.5 and 12.3 nmoles cytochrome P-450 per mg protein respectively. Mouse polyclonal antibodies raised to these proteins reacted only with their specific antigen in the ELISA assay and Western blots of microsomes prepared from PB- or 3MC-induced animals (data not shown).

As a preliminary to this study the total content of cytochrome P-450 in microsomes prepared from complete livers was determined for control (0.69 ± 0.06 nmoles $\cdot$ mg^{-1}) and induced animals. Measurement of the contribution made by cytochrome P-450PB and P-450MC showed that in microsomes from control animals the PB-isoenzyme accounted for only 8.8% of the total (0.06 ± 0.01 nmole $\cdot$ mg^{-1}). The content of the 3MC isoenzyme was below the limit of detection of the assay (≤15 pmoles). Treatment of animals with PB resulted in an induction of spectrophotometrically measurable cytochrome P-450 (to 1.3 ± 0.1 nmoles $\cdot$ mg^{-1}): under these conditions the PB-isoenzyme accounted for 92% of the total (1.2 ± 0.1 nmole $\cdot$ mg^{-1}) while the concentration of the 3MC-isoenzyme remained below the limit of detection. Treatment with 3MC produced a 1.5-fold induction of total cytochrome P-450 (to 1.0 ± 0.14 nmole $\cdot$ mg^{-1}), with cytochrome P-450 MC accounting for 92% of the total cytochrome P-450 present (0.92 ± 0.1 nmole $\cdot$ mg^{-1}); the PB isoenzyme was undetectable. Cytochrome P-450 PB induction data for whole liver microsomes prepared from control and PB are in agreement with Phillips *et al.* [12].

The specific content of cytochrome P-450 in microsomes prepared from individual lobes is given in Table 1. Microsomes prepared from the lobes of control animals returned values within the range of those obtained for whole liver

* Abbreviations used: PB, Phenobarbitone; 3 MC, 3-methylcholanthrene; β-NF, β-naphthoflavone; cytochrome P-450PB, the major PB induced isoenzyme of cytochrome P-450; cytochrome P-450 MC, the major 3 MC induced isoenzyme; ELISA: enzyme linked immunosorbent assay.

Table 1. Distribution of cytochrome P-450 in microsomes prepared from control, PB and 3 MC induced animals

	Cytochrome P-450 (nmoles · mg protein^{-1})					
	Control		PB-induced		MC-induced	
	1	2	1	2	1	3
Right lobe	0.78(0.1)	0.06(0.01)	1.6(0.2)	1.4(0.1)	0.8(0.1)	0.7(0.1)
Median lobe	0.74(0.1)	0.07(0.01)	1.2(0.1)	1.1(0.1)	0.8(0.1)	0.7(0.1)
Left lobe	0.71(0.1)	0.06(0.01)	1.1(0.1)	0.9(0.1)	1.1(0.1)	0.9(0.1)
Dorsal papillary process	0.64(0.1)	0.04(0.01)	1.0(0.1)	1.0(0.2)	0.9(0.1)	0.7(0.1)
Caudate lobe	0.62(0.1)	0.05(0.01)	1.0(0.1)	0.9(0.1)	1.1(0.1)	1.0(0.1)
Ventral papillary process	0.64(0.1)	0.06(0.01)	0.9(0.1)	0.9(0.1)	1.4(0.1)	1.3(0.1)
F-ratio	34.1		36		340	
v_1	5		5		5	
v_2	30		30		30	
P	<0.1%		<0.1%		<0.1%	

Microsomes were isolated as described in the text and the total cytochrome P-450 content determined according to Omura and Sato [10]. The isoenzyme content was determined by ELISA [9]. Each value is the mean of duplicate determinations on microsomes prepared from three animals, the numbers in parenthesis are the standard deviations: (1) total cytochrome P-450; (2) cytochrome P-450 PB; (3) cytochrome P-450 MC.

microsomes. A significant ($P < 0.01$) distribution asymmetry was apparent, however. The ratio of total cytochrome P-450 content in microsomes from the right lobe to that in microsomes from the caudate lobe (which returned the highest and lowest specific content respectively) was 1:0.80. A direct comparison of our data with that of Matsubara *et al.* [5] is not possible given that we used microsomes whereas they used whole tissue homogenates from sites on the right, median and caudate lobes. Nevertheless they report that the specific cytochrome P-450 content of homogenates of the right and median lobes are higher than those from the left lobe. Our data confirms the inter-lobe heterogeneity they report and extends their observations to show that the concentration of this enzyme is further reduced in other lobes. The proportion of cytochrome P-450PB was constant, however; for each set of microsomes it accounted for between 6 and 10% of the total cytochrome P-450, the concentration of the P-450 MC isoenzyme was below the limit of detection. To our knowledge inter-lobe isoenzyme distribution data have to date not been reported.

Treatment of animals with PB had a marked effect on the lobular content of cytochrome P-450. Three effects were observed: firstly, an increase in the specific content of total cytochrome P-450 in microsomes prepared from each lobe; secondly, the asymmetry noted in microsomes from hepatic lobes of control animals became more marked ($P < 0.01$); the ratio of the specific content of cytochrome P-450 in microsomes prepared from the right and caudate lobes decreased to 1:0.6; thirdly the proportion of cytochrome P-450PB was constant for each set of microsomes; in the range 90–100% with the exception of those from the left lobe where it accounted for only 81% of the total (Table 1). Treatment of animals with 3 MC produced qualitatively similar results (Table 1). There was a marked lobular asymmetry with respect to the induction of total cytochrome P-450 but with a gradient opposite to that produced by PB the ratio of the specific activity of microsomes prepared from the right and caudate lobes increased to 1:1.4. There was no asymmetry with respect to the percentage contribution of cytochrome P-450 MC; it accounted for 80–90% of the cytochrome P-450 content for each set of microsomes (Table 1).

The data we obtained on treatment of animals with PB confirms and extends the observations reported by Matsubara *et al.* [5]. Induction studies with 3 MC produced results in partial agreement with that reported in [5], which was obtained using β-NF, a compound which elicits a response similar to that produced by 3 MC in rats. They found an increase in the specific content of cytochrome P-450 in homogenates prepared from individual lobes, but the enzyme asymmetry observed in controls was abolished. This is in direct contradiction to our findings (Table 1); although we found an increase in total haemoprotein content the distribution asymmetry was reversed rather than lost.

Perhaps the most interesting feature of this study, and one which to our knowledge has yet to be reported, is the finding that while there is inter-lobe heterogeneity with respect to total cytochrome P-450 content and induction response there is no asymmetry in terms of contribution of the major induced isoenzyme. A number of reports have suggested that inter-lobe variation may be due to differences in the metabolic capacity of cells, portal streaming and differential uptake of xenobiotics [4, 5, 13, 14]. The data we report are consistent with the view. Furthermore for PB and 3 MC induction it appears that there is no difference in the response of lobes, based on isoenzyme contribution, only on the size and gradient with respect to the total content of cytochrome P-450. We cannot exclude the possibility that there may have been differences in the distribution of other isoenzymes in microsomes prepared from different lobes. It should be noted, however, that epoxide hydrolase, another enzyme involved in xenobiotic metabolism, is reported not to be asymmetrically distributed between the right, median, left and caudate lobes [15]; a finding confirmed by our own preliminary studies (data not shown).

Inter-lobe variability may thus be a parallel of intra-lobe heterogeneity, that is the asymmetric distribution of enzyme activities across the hepatic acinus [2], although due to the limited number of animals which have been used in this and previous studies [3, 5], further work is required to confirm this possibility. It is interesting to speculate that differences in the rate of cytochrome P-450 linked transformation of xenobiotics in lobes could result in a gradient of exposure of lobes to metabolites and hence determine toxicity. This could provide a rationale for the lobular heterogeneity of carbon tetrachloride mediated liver damage [5], the differential susceptibility of lobes to

hexachlorobenzene induced porphyria [4] and as to why there are inter-lobe differences in susceptibility to cell transformation and tumour production, as has been recently demonstrated for diethylnitrosamine-induced tumours [16].

In summary we have determined the concentration of total cytochrome P-450 and the major PB and 3 MC induced isoenzymes in microsomes prepared from the lobes of control and induced rat liver. We have found an asymmetry with respect to the distribution of total cytochrome P-450 in control animals. Treatment of animals with PB enhanced the asymmetry with respect to total cytochrome P-450 while MC treatment provided a reversed gradient. The contribution of the PB and MC induced isoenzymes remained a constant proportion of the total cytochrome P-450 within the lobes. Our data support the current concept of intra-lobular heterogeneity in the liver and may provide a rational for the differences in lobe susceptibility to xenobiotic induced damage.

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The effect of electron-affinic radiosensitizers on ATP levels in V79 379A Chinese hamster cells

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The full potential of nitroaryl radiosensitizers to improve the efficiency of clinical radiotherapy in killing relatively radio-resistant hypoxic tumour cells [1] has not been realized because the toxicity of, for example, nitroimidazoles *in vivo* limits the maximum clinical concentration of misonidazole that can be achieved to ~0.1 mmol/dm^3 in a multifraction radiotherapy regime [2]. The 2-nitroimidazole, misonidazole, has many effects on cellular metabolism (especially at higher concentrations) including depletion of non-protein thiols (NPSH*) and protein thiols [3], stimulation of oxygen consumption [4–6], inhibition of glycolysis [7] and perturbation of purine metabolism [8].

The importance of ATP in repair processes [9–11] and other metabolic pathways means that large changes in cellular ATP levels may affect the ability of cells to survive stresses such as radiation or drug toxicity. Slight radioprotection has been observed with aerobic cells depleted of ATP by treatment with 2-deoxy-D-glucose and rotenone in growth medium with serum [11] or by post-irradiation treatment with 2,4-dinitrophenol in phosphate-buffered saline (PBS) [12, 13]; a more severe post-irradiation treatment with 2,4-dinitrophenol which greatly delayed recovery of ATP levels increased the cellular radiosensitivity [13]. In contrast, uncouplers including 2,4-dinitrophenol radiosensitize aerobic bacteria [14] and mammalian cells (Hodgkiss, unpublished data) when present during, but not after, irradiation.

Previous reports have indicated that misonidazole reduces the amount of ATP in mammalian cells during short (30 min) incubations [8]. In this paper we present data on the ATP content of mammalian cells incubated under a range of conditions commonly used in radiobiology and drug toxicity experiments, and the effect of electron-affinic radiosensitizers on the ATP and non-protein thiol content of mammalian cells.

Materials and methods

V79 379A Chinese hamster cells were maintained as exponentially growing suspension cultures in Eagle's Minimum Essential medium (MEM) modified for suspension culture, with 7.5% foetal calf serum (FCS). ATP monitoring kits were obtained from LKB Instruments Ltd. Misonidazole and Ro 05-9963 were obtained from Dr C. E. Smithen, Roche Products Ltd.; MOA-16 was obtained from Dr J. Parrick and Mr M. Moazzam, Brunel University, Uxbridge; metronidazole and nitrofurantoin were obtained from Sigma Chemical Co.; CMNI was obtained from Aldrich Chemical Co. and 2,4-dinitrophenol was obtained from BDH and purified by recrystallization from hot methanol. PBS was prepared from Dulbecco "A" tablets, Oxoid Ltd. Other reagents were BDH "AnalaR" grade.

* Abbreviations: ATP, adenosine 5'-triphosphate; MEM, Eagle's minimal essential medium; FCS, foetal calf serum; PBS, phosphate-buffered saline; EDTA, ethylene diamine tetra-acetic acid; NPSH, non-protein thiol.