Dose-dependent acinar induction of cytochromes *P*450 in rat liver

Evidence for a differential mechanism of induction of *P*450IA1 by β -naphthoflavone and dioxin

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Rats received various doses of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), Aroclor 1254 (ARO), β -naphthoflavone (BNF) or phenobarbital (PB), and the hepatic expression of cytochromes *P*450IA1 and/or *P*450IIB1/IIB2 was analysed by immunohistochemistry and Western blotting. A clear heterogeneous acinar induction of IA1 was detected when a low dose of TCDD, ARO or BNF was administered. When a low dose of TCDD or ARO was administered, IA1 was found to be induced primarily in hepatocytes located in acinus zone 3, whereas when a low dose of BNF was administered, IA1 was found to be preferentially induced in hepatocytes located in acinus zone 1. A clear zonal induction of IIB1/IIB2 was also observed when a low dose of PB or ARO was administered. Both compounds induced IIB1/IIB2 preferentially in hepatocytes located in acinus zone 3. When rats were administered high doses of TCDD, ARO, BNF or PB there was no zonal pattern of induction of IA1 or IIB1/IIB2; instead, a pan-acinar induction of these enzymes was observed. These results indicate that the overall hepatic concentration of IA1 or IIB1/IIB2 is merely dependent on the proportion of 'induced hepatocytes' within the acinus, which in turn depends on the dose of the inducer.

INTRODUCTION

The mammalian cytochrome *P*450 superfamily plays an important role in hepatic toxicity and carcinogenicity, since many of its members can activate a wide range of chemicals into highly reactive toxins and carcinogens [1,2]. The *P*450 enzymes are unevenly distributed and induced within the liver acinus [3–5]. The most striking example is the selective induction of *P*450IIB1/IIB2 in hepatocytes located in the area close to the hepatic terminal venule (zones 2 and 3) [3,6–8]. This heterogeneous *P*450 distribution and induction is thought to have important toxicological implications, since activation of chemicals is likely to occur preferentially in the hepatocyte population having the highest levels of *P*450s. The reasons and factors responsible for this heterogeneous distribution and induction of *P*450s in the liver acinus are currently unknown.

Recent immunocytochemical studies have shown a heterogeneous induction of cytochromes *P*450 from different families in primary cultures of rat hepatocytes [9]. Furthermore, the heterogeneity in *P*450 induction could be modulated by the concentration of the inducer in the culture medium in such a way that increasing concentrations of inducer led to an increasing proportion of hepatocytes containing specific *P*450s.

In order to ascertain if the primary culture hepatocyte system was mimicking a dose-dependent phenomenon which occurs *in vivo*, we have examined in the present study how increasing target organ doses of inducer affect the intra-acinar distribution of specific inducible *P*450s in the intact animal.

MATERIALS AND METHODS

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) and β -naphthoflavone (BNF) were obtained from Aldrich, Gillingham, Dorset, U.K. Phenobarbitone (PB) sodium was obtained from BDH, Liverpool, U.K. Aroclor 1254 (ARO) was a gift from Dr. J. Ashby, I.C.I. Central Toxicology Laboratory, Alderley Park, Cheshire, U.K. Peroxidase-labelled goat anti-rabbit IgG was obtained from ICN Biomedicals Ltd., Buckingham, U.K.

Male Alderley Park rats (Alpk:AP,SD) weighing 180–220 g were used. Pairs of animals per treatment group received

vehicle alone (saline or corn oil; 5 ml/kg), PB (15 or 100 mg/kg), ARO (50 or 1000 mg/kg), TCDD (0.05, 0.1, 0.27 or 10 μ g/kg) or BNF (15 or 100 mg/kg). PB was dissolved in 0.9% NaCl, the other inducers in corn oil. All of the inducers were administered by intraperitoneal injection once daily for 4 days (except for TCDD and ARO at 1 g/kg). TCDD was administered only once on day 0 and ARO (1 g/kg) was administered twice, on days 0 and 2. All the animals were killed by an overdose of Halothane 4 days after receiving their first intraperitoneal injection. Microsomal preparations, immunohistochemistry and Western immunoblotting with antibodies reacting specifically with *P*450 families IIB1/IIB2 or IA1 were performed as described previously [9,10].

RESULTS

Western immunoblotting

Polyclonal antibodies raised against *P*450IA1 were used to detect the presence of this enzyme in rat liver microsomes from control animals and animals given various concentrations of TCDD or ARO (Fig. 1). Anti-IA1 reacted essentially with enzyme IA1, and to a much lesser extent with enzyme IA2. IA1 was not detected in control liver microsomes, but was clearly detected in microsomes from rats treated with TCDD or ARO. The intensity of the detected bands corresponding to IA1 was increased when the animals were treated with increasing concentrations of TCDD or ARO. A band corresponding to IA2 was also slightly detected in microsomes from animals given TCDD (10 μ g/kg) or ARO (50 or 1000 mg/kg). Western blot analysis of microsomes from PB- or ARO-treated animals revealed reactivity with anti-IIB1/IIB2 (results not shown; [9]).

Immunohistochemistry

Immunohistochemistry was performed to examine the acinar distribution of *P*450IA1 and/or *P*450IIB1/IIB2 on liver sections from untreated animals or animals treated with submaximal or maximal inducing doses of TCDD, BNF, ARO or PB.

Distribution of *P*450IA1. Using anti-*P*450IA1 antibodies on

Abbreviations used: ARO, Aroclor 1254; PB, phenobarbital; BNF, β -naphthoflavone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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Fig. 1. Immunoblot of rat liver microsomes incubated with anti-P450IA1

Lanes 1 and 2, purified P450-IA2 and -IA1 respectively; lanes 3–9, liver microsomes from rats given corn oil, TCDD (0.05 $\mu\text{g}/\text{kg}$), TCDD (0.1 $\mu\text{g}/\text{kg}$), TCDD (0.27 $\mu\text{g}/\text{kg}$), TCDD (10 $\mu\text{g}/\text{kg}$), ARO (50 mg/kg) and ARO (1 g/kg) respectively. Lanes 1 and 2 contain 0.8 pmol of purified P450 enzymes; lanes 3–9 contain 3 μg of microsomal proteins. The treatment of the animals was as described in the Materials and methods section.

control liver, a very weak immunostaining was detected throughout the liver acinus (Figs. 2a and 2e). When animals were treated with the low doses of TCDD (0.1 or 0.27 $\mu\text{g}/\text{kg}$) or ARO (50 mg/kg), a strong immunostaining was found essentially in hepatocytes located in zones 3 and 3/2 respectively (Figs. 2b, 2c and 2g respectively). A clear-cut boundary was noticeable between the positively stained hepatocytes (zones 3 and 2) and the negatively stained hepatocytes (zone 1). In striking contrast, when animals were treated with the low dose of BNF (15 mg/kg), strong immunostaining was found essentially in zones 1 and 2 (Fig. 2f). The staining intensity faded gradually through zone 2 to become almost undetectable in zone 3. Therefore, when low doses of inducers were administered to animals, there was a clear differential acinar induction of IA1 by TCDD (or ARO) and BNF. However, when animals were given high doses of BNF (100 mg/kg), TCDD (10 $\mu\text{g}/\text{kg}$) or ARO (1 g/kg), the whole hepatocyte population throughout the liver acinus showed strong and homogeneous positive staining (Figs. 2d and 2h).

Distribution of P450IIB1/IIB2. Using anti-P450IIB1/IIB2 antibodies, we were able to detect a few rows of slightly positively stained hepatocytes close to the terminal hepatic venule in control liver. The rest of the parenchymal cells were devoid of positive staining (Figs. 2i and 2l). Following treatment with the low dose of PB (15 mg/kg) or ARO (50 mg/kg), strong immunostaining was detected exclusively in hepatocytes located in zone 3 (Figs. 2j and 2m). Thus a clear heterogeneous induction of IIB1/IIB2 within the liver acinus was observed when PB or ARO was administered to animals at submaximal inducing concentrations. When animals were treated with the high dose of PB (100 mg/kg) or ARO (1 g/kg), strong and homogeneous staining spread to zones 2 and 1 (Figs. 2k and 2n). However, the first few rows of hepatocytes around the terminal portal venules were immunostained less strongly when compared with the rest of the hepatocytes.

DISCUSSION

Early investigations on P450 induction using enzymic assays have shown a clear dose-response phenomenon [11,12]. However, when examining the acinar distribution of the induced P450s using immunocytochemical techniques, most investigators have

used a single dose of inducer, frequently at a maximal inducing concentration [3,13]. Heterogeneous or homogeneous induction of a particular P450 such as IIB or IA1 have both been reported in the literature [3,13,14], and these contradictory reports may have resulted from incomplete analysis of the dose-response of induction.

Our results clearly showed that when a low dose of inducer was administered to animals, a specific zone induction of IA1 and/or IIB1/IIB2 was observed within the liver acinus. High doses of inducer caused the enzyme response to spread to the whole acinus, and a pan-acinar pattern of induction was observed. Therefore, whatever the chemical inducer used in this study, there was a clear dose-dependent intra-acinar induction of IA1 or IIB1/IIB2. As shown by Western blot data, the dose-related increase in P450IA1 acinar induction was paralleled by a dose-related increase in the overall hepatic concentration of this enzyme. This result indicates that, at a defined dose of inducer, the hepatic concentration of P450IA1 is highly dependent on the proportion of responding hepatocytes. However, even when a very high dose of PB or ARO was administered to animals, some hepatocytes immediately surrounding the terminal portal venule appeared to show slightly less induction of IIB1/IIB2 than the rest of the hepatocyte population.

It is relevant to note that a dose-dependent acinar induction has also been observed for other inducible hepatic enzymes such as P450IVA and peroxisomal enzymes such as acyl-CoA oxidase [10]. Moreover, zonal induction of another P450 form (IIE1) has also been observed following chronic treatment with ethanol [14,15]. It is quite possible that the acinar pattern of IIE1 induction is also dependent on the dose of inducer administered to the animals.

Although a clear dose-dependent acinar induction of IA1 by BNF, ARO or TCDD was found in this study, it was intriguing to observe with a low dose of BNF a preferential induction of IA1 in zone 1 and with a low dose of ARO or TCDD a preferential induction in zone 3. The acinar induction of IA1 by BNF has been previously reported to be either preferentially located in zone 1 [3] or evenly distributed within the whole acinus [13]. In the light of our study, these contradictory observations are likely to result from differences in the overall concentration of BNF reaching the liver. The differential zonal induction of IA1 by a low dose of TCDD or BNF could be explained by differences in the delivery of these chemicals within the liver acinus. However, because of the high lipophilicity of these chemicals, it is difficult to conceive that TCDD or BNF would be selectively taken up by hepatocytes in zones 3 and 1 respectively. An alternative explanation would be the existence of variation between cells in the concentration of, or in the affinity of, specific receptors towards types of IA1 inducers such as BNF or TCDD. Indeed, the induction of IA1 is thought to be mediated via such intracellular receptors [e.g. the aryl hydrocarbon (Ah) receptor] [16]. It may be relevant to note here a certain similarity to the heterogeneous induction of other hepatic proteins (e.g. very-low-density apolipoprotein) which are inducible by oestrogens through a receptor-mediated mechanism [17].

Although it appears that the administered dose of inducers can modulate the distribution of P450s, the factors which are responsible for the heterogeneous induction of P450s in hepatocytes are currently unknown. It has been suggested that the heterogeneous induction is due to variation in the sinusoidal microenvironment in different acinar zones resulting from the unidirectional perfusion of the cords of hepatocytes in the acinus [18]. However, it has also been suggested that the differential induction of P450s within the liver acinus is due to differences in hepatocyte phenotypes based on differences in intrinsic cellular

Fig. 2. Sections of rat liver showing immunohistochemical localizations of *P*450IA1 (a–h) or *P*450IIB1/IIB2 (i–n)

Liver sections from animals given vehicle only (a, e, i, l); TCDD, 0.1 $\mu\text{g/kg}$ (b); TCDD, 0.27 $\mu\text{g/kg}$ (c); TCDD, 10 $\mu\text{g/kg}$ (d); BNF, 15 mg/kg (f); BNF, 100 mg/kg (h); ARO, 50 mg/kg (g, m); PB, 15 mg/kg (j); PB, 100 mg/kg (k); ARO, 1 g/kg (n). 1, Acinar zone 1; 3, acinar zone 3. (a)–(d) were taken using a blue filter.

factors [19]. The latter hypothesis is supported by the following observations. Firstly, a clear heterogeneous induction of several *P*450 members (IA1, IIB, IIIA) has been reported in primary culture of hepatocytes following exposure to submaximal

inducing concentrations of BNF, PB and dexamethasone respectively [9]. In this *in vitro* system all of the hepatocyte population was exposed to the same chemical environment. Secondly, heterogeneous induction of IIB1/IIB2 after PB or

ARO administration has also been reported in hepatocytes which have been transplanted for 1 month into the spleens of syngeneic rats [20]. Thirdly, following an exposure to PB *in vitro*, cultured hepatocytes selectively isolated from acinar zone 3 showed a much stronger induction of IIB1/IIB2 (assessed by Western blotting and immunocytochemistry) when compared with cultured hepatocytes selectively isolated from acinar zone 1 (R. G. Bars, K. Lindros & C. R. Elcombe, unpublished work). In conclusion, the present study, in conjunction with the *in vitro* studies cited above, strongly suggest that the heterogeneous induction of cytochrome P450 in hepatocytes is dependent on intrinsic cellular factors which are retained in these parenchymal cells in the absence of the integrated liver architecture.

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