TCDD Localization in Centrilobular and Periportal Hepatocytes

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Introduction Immunolocalization of P450 gene expression has revealed that distribution of CYP1A1, CYP1B1 and CYP1A2 proteins is not uniformly expressed across the liver lobule after exposure to TCDD(1-4). These studies raise the possibility that all hepatocytes do not exhibit the same dose-response relationships for P450 gene expression. In addition, recent studies suggest that hepatic AhR protein expression is down-regulated by TCDD with no change in Arnt protein expression (5). The mechanism for the regio-specific induction of P450s mediated by TCDD is unknown, but may involve the different capacity of the specific hepatocyte populations to express P450s regulated by the nonuniform distribution of TCDD. The current study examined dose-dependent localization of TCDD and effects on AhR-mediated responses in centrilobular and periportal hepatocytes after acute exposure of female Sprague-Dawley rats to TCDD. This study demonstrates that a dose-dependent difference in distribution of TCDD exists between centrilobular and periportal cells, which may be related to regional differences in P450 induction resulting in a sequestration of TCDD in centrilobular hepatocytes at low doses.

Methods Chemicals, animals, and treatment 2,3,7,8-Tetrachloro[1,6-^{3}H]dibenzo-*p*-dioxin was purchased from Chemsyn Science Laboratory (Lenexa, KS). Radiochemical purity (≥99%) was verified as described (6). Dosing solutions were prepared as described (7). Female Sprague-Dawley rats (8 weeks; 250 g), purchased from Charles River Laboratories (Raleigh, NC), were allowed free access to food and water. Rats received a single oral dose of 0.0 (corn oil), 0.01, 0.1, 0.3, 1.0 or 10.0 μg [^{3}H]TCDD/kg at 5 ml/kg bw, and centrilobular or periportal hepatocytes (8) were prepared from individual animals three days post-treatment.

Hepatocyte isolation, characterization and concentration of TCDD Centrilobular and periportal hepatocytes from control- and TCDD-treated female Sprague-Dawley rats (n=4-7 animals/dose group) were isolated by a dual pulse digitonin-collagenase perfusion system (8). Cell viability, specificity and yield of hepatocytes were determined (8). Alanine aminotransferase (ALT) activity, a marker of gene expression in periportal hepatocytes (3), was determined in the S9 fraction obtained from centrilobular or periportal cells. Triplicate samples of freshly isolated centrilobular and periportal hepatocytes (200 mg) were combusted (7). TCDD concentration in centrilobular and periportal cells is expressed as attograms (ag) TCDD/viable hepatocyte.

P450 expression EROD and MROD activities were quantitated in centrilobular and periportal hepatocytes (7). mRNA expression was determined using a real time RT-PCR method (9).

Results and Discussion Isolation and characterization of hepatocytes Periportal cells showed an approximate 3-fold elevation in ALT activity as compared to centrilobular cells, which was unaffected by TCDD (data not shown). As with previous studies (3), the periportal dominance of ALT activity was observed indicating a zone selectivity of isolated cells. Centrilobular and periportal hepatocytes prepared from control- and TCDD-treated animals had an average cell viability of ≈70% and yield of ≈$2.0x10^6$ viable hepatocytes/ml. There was no effect of TCDD on cell viability or yield in centrilobular or periportal hepatocytes (data not shown).

Localization of TCDD Table 1 demonstrates a dose-dependent localization of TCDD within both centrilobular and periportal cells. Dose-dependent differences in localization of TCDD were observed within individual hepatocyte populations. An increase in the concentration of TCDD was observed in centrilobular cells as compared to periportal hepatocytes at doses up to 0.3 μg TCDD/kg. A similar TCDD concentration was found within both hepatocytes prepared from animals treated with 1.0 or 10.0 μg TCDD/kg. This is the first study to demonstrate that a dose-dependent difference in regional distribution of TCDD exists within the liver lobule after acute exposure to TCDD.

Effects of TCDD on P450 mRNA expression and associated enzymes in isolated liver cells Table 1 shows a comparison of the dose-dependent effects of TCDD on CYP1A1/1A2 mRNA expression in centrilobular and periportal hepatocytes. Elevated basal CYP1A1/1A2 mRNA expression were observed within centrilobular cells as compared to periportal hepatocytes. TCDD-induced CYP1A1 mRNA expression was elevated in centrilobular cells as compared to periportal hepatocytes prepared from animals treated with 0.01 μg TCDD/kg. An even zonation of TCDD-induced CYP1A1 mRNA expression was found in centrilobular and periportal hepatocytes exposed to higher doses. In contrast, TCDD-induced CYP1A2 mRNA expression was elevated in centrilobular cells as compared to periportal hepatocytes prepared from animals treated with 0.01 and 0.3 μg TCDD/kg. A uniform zonation of TCDD-induced CYP1A2 mRNA expression exists in centrilobular and periportal hepatocytes isolated from female Sprague-Dawley rats treated with 10.0 μg TCDD/kg. Examination of enzymatic activities associated with P450 mRNA expression showed higher basal EROD (CYP1A1) and MROD (CYP1A2) activities in centrilobular cells. A dose-dependent increase in TCDD-induced EROD and MROD activities exists within both centrilobular and periportal hepatocytes obtained from female Sprague-Dawley rats acutely exposed to TCDD. However, some dose-dependent differences in TCDD-induced enzymatic activities were observed within specific cell populations. For example, an increase in TCDD-induced EROD and MROD activities were observed in centrilobular cells as compared to periportal hepatocytes at doses up to 0.3 μg TCDD/kg. However, TCDD-induced EROD and MROD activities were similar in centrilobular and periportal hepatocytes prepared from animals treated with 1.0 or 10.0 μg TCDD/kg.

These data correlate with previous studies showing that TCDD-induced P450 expression is not uniformly expressed across the liver lobule after TCDD exposure (1,4). Previous studies have also shown that exposure of female Sprague-Dawley rats to TCDD results in a down-regulation of AhR protein expression in multiple tissues with no accompanied change in Arnt protein expression (5). In this study, a slight increase in AhR mRNA expression was observed in both centrilobular and periportal cells obtained from female Sprague-Dawley rats exposed to 10.0 μg TCDD/kg. Arnt mRNA expression was unaffected by TCDD-treatment in both cell types. The

slight increase in AhR mRNA expression in animals exposed to 10.0 μg TCDD/kg may compensate for the down-regulation in AhR protein expression observed in previous studies (5).

Figure 1 shows a correlation of the dose-dependent localization of TCDD and TCDD-induced effects on MROD activities in centrilobular and periportal hepatocytes. At doses up to 0.3 μg TCDD/kg, the higher concentration of TCDD within centrilobular hepatocytes resulted in an elevation of TCDD-induced MROD activity in centrilobular hepatocytes as compared to periportal cells. A similar concentration of TCDD within both centrilobular and periportal hepatocytes results in similar effects on TCDD-induced MROD activity within both cell types (Figure 1). These data support the hypothesis, used in the geometric liver model for TCDD (10-11), that regional hepatic differences in P450 induction exist across the liver lobule are mediated by the concentration of TCDD.

Summary These data show that centrilobular and periportal hepatocytes exhibit differences in TCDD localization and the induction of cytochrome P450 gene expression. In addition, this is the first study to demonstrate that the dose-dependent increase in the concentration of TCDD within a specific hepatocyte population correlates with CYP1A2 mRNA expression and MROD activity implying that hepatic regional differences in localization of TCDD may be due to an interaction of TCDD with CYP1A2 resulting in a sequestration of TCDD in centrilobular hepatocytes at low doses.

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TABLE 1. Localization of TCDD and Effects on P450 mRNA Expression and Associated Enzymatic Activities in Centrilobular and Periportal Hepatocytes

Dose[a] (Cells)	TCDD Conc.[b]	CYP1A1[c]	CYP1A2[c]	AhR[c]	Arnt[c]	EROD[d]	MROD[d]
0.0 (Cent.)	ND	4±4	1±.3	1±0.4	1±.3	134±33	66±18
0.01 (Cent.)	2±1	62±54	1±.3	1±0.3	1±.4	118±29	77±10
0.1 (Cent.)	25±8	ND	ND	ND	ND	352±70	118±13
0.3 (Cent.)	123±36	6560±2775	7±3	2±1	1±.3	1111±412	209±74
1.0 (Cent.)	360±106	ND	ND	ND	ND	1613±281	280±116
10.0 (Cent.)	2775±253	24093±5545	19±8	6±4	1±.4	3679±1518	355±105
0.0 (Peri.)	ND	0.8±0.1	.5±.1	.7±.4	.9±.6	47±18	31±9
0.01 (Peri.)	0.4±0.1	7±5	.5±.1	.6±.2	.7±.1	65±31	27±10
0.1 (Peri.)	9±1	ND	ND	ND	ND	144±55	72±17
0.3 (Peri.)	32±17	877±141	2±.2	.5±.1	.6±.2	405±187	98±22
1.0 (Peri.)	206±61	ND	ND	ND	ND	1718±293	276±92
10.0 (Peri.)	2766±1750	19216±1639	15±5	3±.2	.9±.1	2881±1015	342±116

[a]=μg/TCDD/kg; [b]=ag TCDD/viable hepatocyte; [c]=cycle threshold; [d]=pmoles/min/mg microsomal protein. ND=not determined. Cent=Centrilobular cells; Peri=Periportal cells. Data is represented as means±standard deviation.

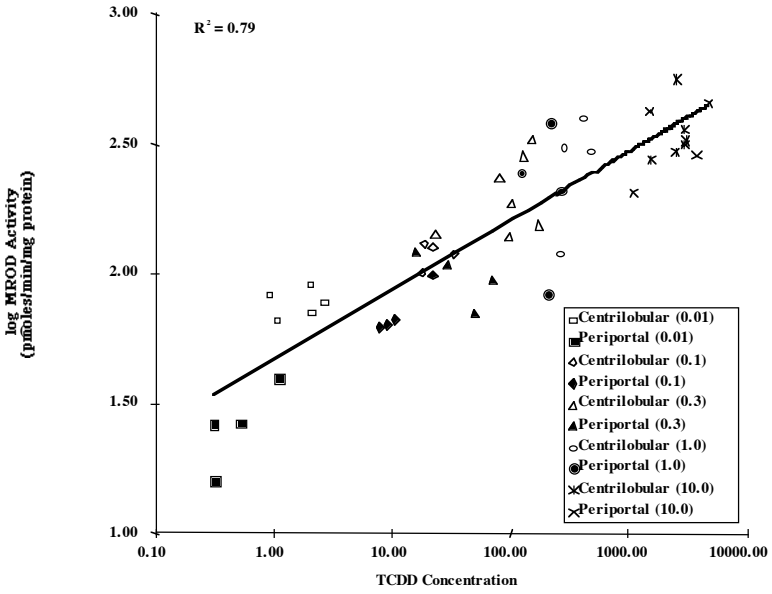

Figure 1. Localization of TCDD and effects on MROD activity in centrilobular and periportal cells obtained from rats treated with 0.01-10.0 μg TCDD/kg. The concentration of [^{3}H]TCDD in each liver cell population was determined by sample combustion (Santostefano *et al*., 1996) and is expressed as ag [^{3}H]TCDD/viable hepatocyte. Data are presented for hepatic cell populations obtained from individual animals (n=4-7 animals/dose group). Also shown is the logarithmic fit of the relationship of MROD activity with TCDD concentration ($y=0.17\text{Ln}(x)+1.67$; $r^2=0.79$).