THE EFFECT OF PREINDUCTION WITH NON-TOXIC DIOXINS ON THE METABOLISM OF THE TOXIC 2378-TCDD

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Introduction

The metabolism of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2378-TCDD) is a detoxification process, but proceeds at an extremely slow rate in all species studied. Metabolism of halogenated aromatic compounds proceeds via monooxygenase activities, i.e. the cytochrome P450 family of microsomal enzymes. Cytochromes P4501A1 (CYP1A1) and 1A2 (CYP1A2) are known to be inducible by 2378-TCDD, however, CYP1A2 serves as a specific binding protein for 2378-TCDD in the liver, rather than a metabolizing enzyme. 2378-TCDD may be able to induce its own metabolism, but conflicting data exists in the literature.

Additional information on the mechanism of 2378-TCDD metabolism would be desirable so that strategies could be designed to either prevent its bioaccumulation or permit remediation of exposed populations. Non-toxic tetrachlorodibenzo-*p*-dioxin congeners are extensively metabolized in mammals, although the specific cytochrome isozymes involved are not known[1, 2, 3]. The purpose of this study was to investigate the effect of non-toxic dioxin pretreatment on the metabolism of 2378-TCDD as measured by biliary elimination of ^{14}C derived from 2378-TCDD.

Materials and Methods

Chemicals: [UL-^{14}C] 2378-TCDD was obtained from Chemsyn Laboratory (Lenexa, KS), and radiochemical purity of >98% was determined by silica TLC (50:50 hexane:methylene chloride). Unlabeled 2378-TCDD was synthesized in-house[4] and used to dilute the radiolabel to the desired specific activity. Unlabeled 1278-, 1378-, and 1478-TCDD were synthesized in-house as described above. Minor amounts of 2378-TCDD contamination in 1278-TCDD and 1378-TCDD were removed by reversed phase HPLC[5]. 2378-TCDD was less than 0.05% in 1278-and 1378-TCDD by HR-GC/MS according to EPA Method 1613. Solutions of each non-toxic dioxin congener in peanut oil were prepared for preinduction.

Animals: The rats used in this study were male Sprague-Dawley (255 ± 14g; Taconic Labs, Germantown, PA), and were divided into groups of five. All groups of rats were housed in a controlled environment of 20°C, 12h light/dark cycle, and a relative humidity of 25% ± 5%. Following surgery, the rats were housed in stainless steel metabolism cages that allowed for the separate collection of urine, bile and feces, and were allowed free access to water and feed.

Treatment and Surgery: Groups of rats were pretreated with a non-toxic dioxin congener (1.0 μmol/kg in 0.5 ml peanut oil) 5.5 days prior to dosing with [^{14}C] 2378-TCDD (0.07 μmol/kg in 0.5 ml peanut oil; 1.0 μCi /rat). Control rats received vehicle alone. One day prior to 2378-TCDD treatment the rats were anaesthetized with halothane for surgery. A ventral midline incision was made, and the bile duct was cannulated with medical polyethylene tubing 1.5 cm

from the base of the liver. The cannula was fastened with 4-0 silk ligature, and exited near the base of the tail. The radiolabeled 2378-TCDD was administered as a single oral dose. Bile collection was immediately initiated, and continued for 48h. Rats were anaesthetized with CO_2, and killed by dorsal vein exsanguinations. Liver, muscle, skin and epididymal fat were removed and stored at -40° C until combustion analysis could be performed.

Sample Analysis: Aliquots of bile for each time interval (50 _l) were added to Ecolite scintillator (ICN, Costa Mesa, CA) and measured for radioactivity in a liquid scintillation counter (LSC). Aliquots of urine ^{14}C were measured daily, and blood was measured at 48h on the LSC. Tissues were homogenized, lyophilized, and aliquots combusted on a Packard Model 307 sample oxidizer. Pooled 0-4h bile samples were deproteinated with methanol, centrifuged, and volumes reduced with a rotary evaporator. The samples were analyzed by reversed phase HPLC using a C18 DeltaPak column (Waters Assocates, Milford, MA) with a 60 min linear gradient from 95:5 water (0.1% TFA):isopropanol (0.05% TFA) to 100% isopropanol (0.05% TFA). The data are presented as means ± standard deviation. Statistical analysis was the Students t-test with a predetermined level of uncertainty of 5% ($P < 0.05$).

Results and Discussion

The effect of non-toxic dioxin pretreatment on the short-term biliary elimination of [^{14}C] 2378-TCDD is presented in Figure 1. Biliary elimination of 2378-TCDD derived ^{14}C was low in male Sprague-Dawley rats, i.e. <0.5% of the dose was eliminated in 8h, and was linear in control and pretreated rats. A 5.5 day pretreatment with 1278- or 1478-TCDD did not result in a statistically significant increase in the biliary elimination of 2378-TCDD; however, a 5.5 day pretreatment with 1378-TCDD did result in a slight increase ($P < 0.05$) in ^{14}C elimination (Figure 1). At a shorter, two day pretreatment time, the ability of 1378-TCDD to increase 2378-TCDD derived ^{14}C biliary elimination was no longer observed (data not shown). Biliary elimination is the primary means of 2378-TCDD metabolite clearance, and is used as an indirect measure of metabolism of 2378-TCDD[6]. It is not known what isozymes may be induced by the non-toxic dioxins, however, based on the present data, the putative induction was not able to increase the overall metabolism of 2378-TCDD.

The majority of the previous work has demonstrated that 2378-TCDD does not induce its own metabolism in the rat, since no increase in biliary elimination was observed regardless of pretreatment conditions and despite a marked increase in CYP1A1 and CYP1A2 induction[7]. 2378-TCDF was also unable to induce metabolism of 2378-TCDD in vivo[8]. However, under certain conditions 2378-TCDD metabolism could be induced in the rat. A small, yet significant increase in rat 2378-TCDD metabolism was observed over 72h when 2378-TCDD pretreatment occurred 8 days before a challenge dose was administered[9]. The present results indicated an effect using a 5.5 day preinduction with 1378-TCDD that was not observed when a 2 day preinduction period was used. Therefore, the length of induction prior to administration of a challenge dose may be important in activating other metabolizing enzyme systems. Rats pretreated with ABT (a P450 inhibitor) demonstrated a significant, but not complete, decrease in biliary elimination of a radiolabelled 2378-TCDD dose[10]. Therefore, metabolism of 2378-TCDD may not be completely dependant on hepatic cytochrome P450s. Minor qualitative differences in metabolite profiles were observed in HPLC chromatograms of 0-4h bile from each treatment group when compared to controls. Figure 2 shows the presence of possibly three new metabolites in the bile of the 1478-TCDD pretreated rat. This may indicate a slightly different route of 2378-TCDD metabolism in response to different enzymes induced with the 1478-TCDD pretreatment.

At 48h, the liver contained the highest concentrations of 2378-TCDD (Table 1). However, none of the pretreatments resulted in a statistically significant increase in hepatic ^{14}C concentration when compared with the control. CYP1A2 is a specific, inducible hepatic binding species for 2378-TCDD[11]. Apparently CYP1A2 was not significantly induced by the non-toxic dioxin pretreatments. Total hepatic cytochrome P450 was not measured. Therefore, the overall effects of non-toxic dioxins on hepatic cytochromes are not known, but studies currently underway in our lab are attempting to characterize the hepatic enzyme(s) induced and responsible for the non-toxic dioxin metabolism.

Table 1. Effect of pretreatment on tissue concentration (% dose/g) of a dose of 2378-TCDD on male rats.

	Control	1278	1378	1478
Liver	1.62 ± 0.49	1.68 ± 0.43	1.84 ± 0.25	1.26 ± 0.36
Muscle	0.004 ± 0.002	0.017 ± 0.019	0.007 ± 0.003	0.003 ± 0.001
Fat	0.189 ± 0.112	0.186 ± 0.118	0.118 ± 0.092	0.092 ± 0.059
Blood	0.0004 ± 0.003	0.008 ± 0.012	0.0005 ± 0.005	0.0002 ± 0.00009
Skin	0.037 ± 0.008	0.144 ± 0.194	0.030 ± 0.015	0.036 ± 0.016
Liver/Fat	8.6	9.0	15.6	13.7

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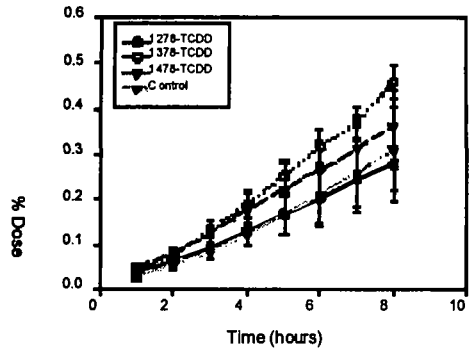

Figure 1. Cumulative 0-8h biliary elimination of 2378-TCDD derived ^{14}C following a pretreatment period of 5.5 days with various non-toxic dioxin congeners.

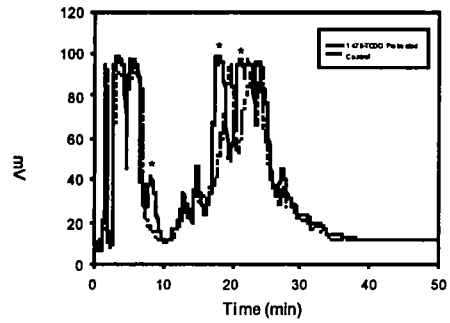

Figure 2. Reversed phase HPLC chromatograms of 2378-TCDD derived ^{14}C from pooled 0-4h bile of a control (·····) and 1478-TCDD pretreated male rat (——). The * indicates possible new metabolites present in 1478-TCDD pretreated rat bile.