Enhanced hepatotoxicity induced by repeated exposure to polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin in combination in male rats

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Abstract

2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin (TCDD) and polychlorinated biphenyls (PCBs) are among persistent polyhalogenated aromatic hydrocarbons that exist as complex mixtures in the environment worldwide. The present study was attempted to investigate the hepatotoxicity following repeated exposure to TCDD and PCBs in combination in male rats, and to reveal the involvement of potential mechanisms. Male Sprague-Dawley rats were exposed to TCDD (10 \(\mu\)g/kg) and Aroclor 1254 (10 mg/kg, a representative mixture of PCBs) alone or in combination by intragastric administration. After 12-day exposure, all treatments produced significant hepatotoxicity as characterized by changes of plasma biochemistry and histopathological changes. These effects were more prominent in the combined group. Furthermore, all treatments induced hepatic cytochrome P450 1A1 (CYP1A1) expression, and the maximal level of CYP1A1 expression was observed in the combined group, as in the case of the most severe hepatotoxicity evoked by the combined exposure. These findings indicated that the hepatotoxicity induced by TCDD and Aroclor 1254 might be ascribed to the high expression of hepatic CYP1A1. The present study demonstrates the enhanced hepatotoxicity after exposure to TCDD and PCBs in combination in rats.

Key words: 2,3,7,8-tetrachlorodibenzo-\(p\)-dioxin; polychlorinated biphenyls; combined exposure; hepatotoxicity

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Introduction

A variety of synthetic polyhalogenated aromatic hydrocarbons (PHAHs), such as polychlorinated dibenzo-\(p\)-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), have been released into the environment through human activities worldwide. On a daily basis humans interact with their environments and, as a consequence, are exposed to these pollutants present in the food they eat, the air they breathe, and the water they drink. The risk to human health posed by exposure to these persistent organic pollutants has been one of worldwide concerns (Arisawa et al., 2005; Humblet et al., 2008; Larsen, 2006).

Dioxins and dioxin-like compounds (including 7 of PCDDs, 10 of PCDFs and 12 of PCBs), which share a similar chemical structure and a common mechanism of toxic effects, have long been recognized as a class of highly toxic, persistent and bioaccumulative environmental contaminants (Schecter et al., 2006; van den Berg et al., 2006). It has been widely accepted that the toxicities of dioxins and dioxin-like compounds are primarily mediated through specific binding to and activation of cytosolic aryl hydrocarbon receptor (AhR) and the subsequent induction of gene expression (Mandal, 2005). 2,3,7,8-Tetrachlorodibenzo-\(p\)-dioxin (TCDD) is the most potent dioxins and the prototypical compound for the study of AhR-mediated toxicity. It displays a broad spectrum of toxicological effects such as immunosuppression, neurotoxicity, endocrine disruption, teratogenicity and carcinogenicity (Cole et al., 2003; Nagayama et al., 2007). PCBs, consisting of 209 theoretically possible congeners with different numbers and positions of chlorines around the biphenyl ring, exist as complex mixtures in environmental and human matrices including blood, adipose tissues, milk and fetal tissues. Dioxin-like PCBs mainly include the coplanar PCB congeners which operate through the AhR signal transduction pathway. The mono-
ortho coplanar PCBs have both dioxin-like effects via the AhR and other mechanisms of action, for example, a phenobarbital-like spectrum of enzyme induction (Safe, 1994). Non-dioxin-like PCBs, i.e., ortho-substituted PCBs do not bind to the AhR, eliciting a different pattern of toxicity (Zoeller et al., 2000). Aroclors are commercially used mixtures with variable percentages of chlorine, of which Aroclor 1254 is a representative mixture of more than 60 PCB congeners, consisting of both dioxin-like and non-dioxin-like congeners.

In environmental matrices and biota PHAHs such as PCBs, PCDDs and PCDFs are generally present as complex mixtures, to which humans are always simultaneously exposed. However, current understandings of the toxicity profiles of these environmental contaminants are primarily based on the toxicity studies performed on laboratory animals exposed to individual compounds, thereby, effects of any interaction between such compounds on their toxicity are virtually unknown. Toxicity studies of individual compounds are important for the acquisition of basic toxicological information, yet results from individual compound studies are considered limited value for predicting human health hazards caused by exposure to complex mixtures of environmental contaminants. Furthermore, assessment of hazards is usually assumed to be a summation of effects from each individual compound that acts on target organs. However, this assumption may result in under- or over-estimation of the toxic effects of the mixtures to which humans are exposed.

Although some studies have been performed on the effects of complex mixtures of PHAHs including dioxins and PCB congeners (Li and Hansen, 1996; van der Plas et al., 2001; Wade et al., 2002; Walker et al., 2005), there are few reports concerning the combined effects of TCDD and PCBs on the liver. Since the liver has been long considered the primary target organ of both TCDD and PCBs, we attempted to reveal their potential combined hepatotoxicity. Aroclor 1254 was used in the present study as a cost-effective representative PCBs, because its composition is representative of PCBs environmental pollution and comprises a significant proportion of the ingested and accumulated body burden of PCBs (Stack et al., 1999). In addition, the assessment of risks to humans from environmental PCBs toxicity has been based on the reference doses derived from animal studies with Aroclor 1254 and other commercial PCB mixtures (Cogliano, 1998). The possible interactions between the two PHAHs and the involvement of possible mechanisms were also discussed.

1 Materials and methods

1.1 Chemicals

TCDD (purity 99%) was obtained from Cerilliant Corporation (Round Rock, USA; Lot no. ER011005-01). Aroclor 1254 was obtained from AccuStandard, Inc. (USA; Lot no. 124-191-B). The rabbit anti-CYP1A1 polyclonal antibody, rabbit anti-β-actin antibody and goat anti-rabbit IgG-horseradish peroxidase were provided by Santa Cruz Biotechnology (USA). All other chemicals were of analytical grade commercially available.

1.2 Animals and treatments

Male Sprague-Dawley rats, 6–8 weeks of age (180–200 g), were obtained from Beijing Vital River Laboratory Animal Co. (China) and allowed to acclimatize for one week prior to the dosing. The laboratory conditions were maintained on a 12-h light/12-h dark cycle at (22 ± 2)°C and (50 ± 10)% relative humidity. The rats were given free access to standard commercial rodent feed and drinking water. After acclimatization, the animals were assigned randomly into four groups of five animals each, including a control group and three treatment groups. Rats of the TCDD group received 10 µg/kg of TCDD; PCBs group received 10 mg/kg of Aroclor 1254; the combined group received the combination of TCDD (10 µg/kg) and Aroclor 1254 (10 mg/kg). Control rats received an equivalent volume of the vehicle olive oil only. Animals were treated by intragastric administration (2.5 mL/kg) daily for 12 consecutive days. TCDD and Aroclor 1254 were initially dissolved in DMSO and then diluted into olive oil. All animal procedures were approved by the Institutional Animal Care and Use Committee of Academy of Military Medical Sciences.

The animals were fasted overnight, weighed and subjected to anesthesia by intraperitoneal injection of sodium pentobarbital (1.5 mg/kg) on the day following the last administration. Blood was withdrawn from the abdominal aorta for plasma biochemical assay. Livers of all rats were then immediately isolated and washed in ice-cold physiological saline. Portions of the livers were collected and quickly frozen in liquid nitrogen for western blot analysis, and a portion of left lobe of each liver was fixed in formalin immediately for histopathological examination.

1.3 Plasma biochemistry analysis

Blood collected was allowed to clot at room temperature and then centrifuged at 1200 × g for 15 min, and the resulting plasma was removed for biochemical assay within 2 hr. Typical parameters which are indicative of hepatic injury were determined: activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

1.4 Histopathological examination

Formalin-fixed livers were processed according to the routine procedure, and 4 µm of thickness was sectioned. The sections were stained with haematoxylin and eosin (H&E), and the slides were examined by an expert in histopathological evaluation.

1.5 Western blot analysis

Frozen portions of the livers were homogenized in liquid nitrogen and then lysed for 30 min on ice, and the extracts were centrifuged at 14,000 × g for 25 min at 4°C. Nuclei and unlysed cellular debris were removed by centrifugation and the proteins were contained in
the supernatant. The protein concentration was determined by bicinchoninic acid assay kit (Sigma Corporation, USA). Protein (12 µg) was separated on a 12% SDS-polyacrylamide gel and electrophoretically transferred onto polyvinylidene difluoride membranes. After blocking, the membrane was incubated with primary antibody (rabbit anti-CPY1A1 polyclonal antibody) and then with horseradish peroxidase-conjugated secondary antibody. Immunoreactive bands were detected using enhanced chemiluminescence and visualized with radiographic film. The membrane was stripped and successively reprobed with an anti-rabbit β-actin antibody and later with a corresponding secondary antibody. The intensities of bands were measured using Bio-Rad Quantity One software. β-Actin was used as an internal standard and the results of CYP1A1 expression were normalized to β-actin expression.

1.6 Statistical analysis

Data were presented as mean ± SD. Data analysis was performed using a two-factor analysis of variance (ANOVA) followed by a post hoc test. Values were considered significant at \( P < 0.05 \).

2 Results

2.1 Effects of combined exposure to TCDD and Aroclor 1254 on plasma biochemistry

As presented in Table 1, there were almost no significant alterations in the biochemical parameters tested following repeated exposure to TCDD or Aroclor 1254 alone. In contrast, combined exposure produced remarkable increases in the activities of ALT, AST and ALP as compared to the control group, indicating the occurrence of hepatic injuries. Furthermore, factorial analysis revealed a significant interaction between TCDD and Aroclor 1254 on ALP activity \( (F_{1,16} = 14.74, P < 0.01) \), which was believed to be synergistic. Although there was no significant interaction on AST activity \( (F_{1,16} = 1.42, P > 0.05) \), the finding that TCDD or Aroclor 1254 alone produced insignificant changes but their combination caused a marked increase indicated the combined effect might be additive.

2.2 Liver histopathological changes by combined exposure to TCDD and Aroclor 1254

Histopathological examination on the livers was performed to further reveal the hepatotoxicity following exposure to TCDD and Aroclor 1254 alone or in combination. As shown in Fig. 1, treatment-related histopathological changes in livers were observed in all the exposed-groups. In control group, there was no evidence of hepatic abnormality except for minimal inflammation in only a few livers (Fig. 1a). In Aroclor 1254 group, a visible increase in the severity of lymphocyte infiltration was noted in all livers as compared to that of controls, and several rats also showed mild hepatocellular vacuolation (Fig. 1b). Rats in TCDD group exhibited moderate diffuse hepatocellular vacuolation; focal necrosis, dissociation of hepatic cords and hepatocellular hypertrophy in the centriacinar regions were also observed (Fig. 1c, d). In the combined group, mild to moderate hepatocellular vacuolation was more frequent and the inflammatory infiltration was more severe.

**Table 1** Effects of combined exposure to TCDD and PCBs on plasma biochemistry

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>ALP (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>51.0 ± 5.2</td>
<td>236.0 ± 13.2</td>
<td>375.2 ± 53.7</td>
</tr>
<tr>
<td>PCBs</td>
<td>65.8 ± 8.1*</td>
<td>298.0 ± 52.2#</td>
<td>362.2 ± 61.1##</td>
</tr>
<tr>
<td>TCDD</td>
<td>50.2 ± 8.3a</td>
<td>351.4 ± 67.6##</td>
<td>402.2 ± 53.2##</td>
</tr>
<tr>
<td>TCDD + PCBs</td>
<td>63.4 ± 10.5*</td>
<td>568.4 ± 89.1**</td>
<td>626.6 ± 98.4**</td>
</tr>
</tbody>
</table>

ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase.

Data are presented as the mean ± SD (\( n = 5 \)).

* \( P < 0.05 \), ** \( P < 0.01 \), significantly different from control group.

# \( P < 0.05 \), ## \( P < 0.01 \), significantly different from the combined group.

Fig. 1 Liver histopathological changes induced by combined exposure to TCDD and PCBs. Representative photographs are shown. (a) vehicle control liver section; (b) PCBs-treated liver section; (c) and (d) TCDD-treated liver sections; (e) and (f) combination-treated liver sections. H&E staining, a, c, d, e: 10x objective; b and f: 20x objective.
Moreover, there were significant differences in CYP1A1 expression induced by co-administration of TCDD and PCBs was the most remarkable, which was well consistent with the finding that the most severe liver injuries were observed in the combined group. The enhanced hepatotoxicity as evidenced by more severe biochemical changes and histopathological injuries in the liver than individual exposure.

The present study revealed exposure to TCDD and Aroclor 1254 in combination induced more severe liver injuries than individual exposure. Further analyses indicated that there were interactive effects between TCDD and Aroclor 1254 on the parameters measured, and the combined effects may be additive or synergistic. In the current study, TCDD and PCBs possibly produced additive toxic effects on AST activity when co-administered. This is probably attributable to the fact that TCDD and dioxin-like PCB congeners who were full agonists of AhR may share a common AhR-mediated pathway and elicit similar biological responses, which are common for dioxin-like compounds (Chen and Bunce, 2004; Chu et al., 2001). Combination of TCDD and PCBs produced synergistic effects on ALP level and CYP1A1 expression. Similar synergistic effects of PCBs and TCDD were also reported previously. Bannister and Safe (1987) reported that PCB 153 (a non-dioxin like PCB congener) and TCDD produced a remarkable synergism on hepatic ethoxyresorufin-O-deethylase (EROD) in C57BL/6j mice. van Birgelen et al. (1996) revealed that PCB 153 alone had no effect on the hepatic porphyrin level, however, it produced a strong synergistic effect when combined with TCDD. Crofton et al. (2005) examined the effects of a mixture of 18 polyhalogenated aromatic hydrocarbons (dioxins, dibenzofurans, and dioxin-like and non-dioxin-like PCB congeners) on thyroid homeostasis in female Long-Evans rats. Dose-dependent synergism of decrease in serum total thyroxine concentrations was found at relative high doses. Since the mixture of TCDD and Aroclor 1254 used in the present study consists of both dioxin-like and non-dioxin-like compounds, it is presumed that the synergism is probably due to multiple mechanisms other than the AhR activation that can result in a common phenotype, producing a greater effect.

It is widely accepted that the majority of toxic effects of dioxins and dioxin-like PCB congeners are mainly mediated by AhR. Upon binding a ligand such as TCDD, AhR translocates to the nucleus, where it binds to the aryl hydrocarbon receptor nuclear translocator, intranuclear events leading to the regulation of target gene expression is then initiated. The most thoroughly studied AhR-mediated gene expression, in this regard, is that of CYP1A (Fujii-Kuriyama and Mimura, 2005; Knerr et al., 2006). A number of studies have suggested that chronic expressions of CYP1A particularly CYP1A1 contribute to TCDD- or PCBs-induced toxicity (Chubb et al., 2004; Leung et al., 2005). In the present study, the expression of hepatic CYP1A1 was all pronouncedly upregulated in the three treatment groups, it is reasonable to presume that the observed hepatic lesions may also be ascribed to the high expression of hepatic CYP1A1. Furthermore, the hepatic CYP1A1 expression induced by co-administration of TCDD and PCBs was the most remarkable, which was well consistent with the finding that the most severe liver injuries were observed in the combined group. The
synergistic combined effect of TCDD and PCBs on the hepatic CYP1A1 expression might explain the markedly enhanced hepatotoxicity provoked by exposure to TCDD and PCBs in combination.

Previous findings obtained from investigators indicated the combined effects of mixtures of PHAHs are complicated, depending on many factors including the composition of the mixture, dose levels of individual compound, exposure duration and routines, animal species, and even the toxicity endpoints (Chu et al., 2001; Li and Hansen, 1996; van der Plas et al., 2001; Wade et al., 2002). Our present study suggests potentials of synergistic and antagonistic interactions, although these potentials have not been fully explored, taking account of the experimental design, i.e., only one control, one high dose of PCBs or TCDD alone and in combination. Nevertheless, this study implies when non-dioxin-like PCBs are present, evaluation of the combined effects of TCDD and PCBs should be used with caution. In order to accurately estimate and predict the combined effects of TCDD and PCBs, combinations of more dose ratios of TCDD and PCBs and reasonable endpoints should be considered, and other information such as the mechanisms of action, toxicokinetic profile and tissue distribution of TCDD and PCBs should also be taken into account.

In summary, the present study has demonstrated that combined exposure to TCDD and PCBs produced an enhanced hepatotoxicity in rats as compared to the individual exposure, which may be attributable to the remarkable expression of hepatic CYP1A1.

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References


