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Original Contribution

Oxidative DNA adducts after Cu^{2+}-mediated activation of dihydroxy PCBs: Role of reactive oxygen species

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ABSTRACT

Polychlorinated biphenyls (PCBs) are toxic industrial chemicals, complete carcinogens, and efficacious tumor promoters. However, the mechanism(s) of PCB-mediated carcinogenicity remains largely undefined. One likely pathway by which these agents may play a role in carcinogenesis is the generation of oxidative DNA damage by redox cycling of dihydroxylated PCB metabolites. We have now employed a new ^{32}P-postlabeling system to examine novel oxidative DNA lesions induced by Cu^{2+}-mediated activation of PCB metabolites. ^{32}P postlabeling of DNA incubated with various PCB metabolites resulted in over a dozen novel polar oxidative DNA adducts that were chromatographically similar for all active agents. The most potent metabolites tested were the hydroquinones (hydroxyl groups arranged *para* to each other), yielding polar oxidative adduct levels ranging from 55 to 142 adducts/10^6 nucleotides. PCB catechols, or *ortho*-dihydroxy metabolites, were up to 40% less active than their corresponding hydroquinone congeners, whereas monohydroxylated and quinone metabolites did not produce detectable oxidative damage over that of vehicle. With the exception of 2,4,5-Cl-2′,5′-dihydroxybiphenyl, this oxidative DNA damage seemed to be inversely related to chlorine content: no chlorine ≈ mono->di->trichlorinated metabolites. Importantly, copper, but not iron, was essential for activation of the PCB metabolites to these polar oxidative DNA adducts, because in its absence or in the presence of the Cu^{+}-specific scavenger bathocuproine, no adducts were detected. Intervention studies with known reactive oxygen species (ROS) modifiers suggested that H_2O_2, singlet oxygen, hydroxyl radical, and superoxide may also be involved in this PCB-mediated oxidative DNA damage. These data indicate a mechanistic role for several ROS, in addition to copper, in PCB-induced DNA damage and provide further support for oxidative DNA damage in PCB-mediated carcinogenesis.

Polychlorinated biphenyls (PCBs)[1] (Fig. 1) are widespread environmental contaminants once produced commercially for a variety of diverse applications, including dielectric, heat transfer, and hydraulic fluids; flame retardants; lubricating and cutting oils; and additives in paints, adhesives, sealants, plastic, and pesticides [1]. PCBs were mass produced from 1929 up until the early 1980s, resulting in an estimated worldwide production of 1.5 million tons [2]. Relatively large amounts, estimated to be as high as 31% of the total production, of these stable compounds have been released into the environment owing to inappropriate disposal practices, accidents, and leakages from industrial facilities. Sixty-five percent of the total environmental burden is still in use or in storage, whereas only 4% is believed to have been destroyed [2,3]. Environmental sampling has shown that PCBs are almost always present as complex mixtures and found in just about every environmental compartment or matrix including human and animal tissues [4]. The environmental impact of PCBs is further confounded by their ability to bioaccumulate and biomagnify in higher trophic organisms owing to their high stability and lipophilicity.

Concerns regarding the potential health detriments of PCBs arose in the early 1970s after the Yusho incident of 1968, during which approximately 1800 Japanese men and women ingested relatively large amounts of a complex mixture of PCBs and polychlorinated dibenzofurans from accidental contamination of rice oil [5]. Reports of chloracne, follicular accentuation and pigmentation, eye discharge and swelling, and peripheral neuropathy soon followed this disaster. A second contamination of rice oil occurred in Taiwan in 1978 (Yucheng incident). Similar effects of PCB toxicity were observed in the more than 2000 people affected by this incident. In a 40-year follow-up study of the Yusho accident examining the mortality of 1664 patients, men showed an elevated mortality, compared to the general population, from all types of cancer, particularly lung and liver, whereas in women the mortality ratio was increased only for liver cancer [6]. A similar follow-up study of the Yucheng accident did not find any increase in cancer mortality in either sex, compared to the general Taiwanese population, but did find an early increase in liver mortality in men, and a delayed increase in systemic lupus erythe-

Abbreviations: PCB, polychlorinated biphenyl; ROS, reactive oxygen species; 8-oxodG, 8-hydroxydeoxyguanosine; M1dG, 3-(2′-deoxy-β-D-erythropentofuranosyl) pyrimido[1,2-a]purin-10(3*H*)-one; PEI, polyethyleneimine; RAL, relative adduct level.

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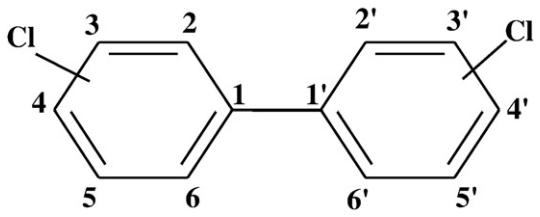

Fig. 1. General structure and numbering system of polychlorinated biphenyls (PCBs).

matosus mortality was found in women [7]. In addition to these accidental poisonings by PCBs and dibenzofurans, epidemiologic, wildlife, and experimental studies of the effects of PCBs have revealed reproductive toxicity, birth defects, abnormal growth and development in children of exposed mothers, dermatological toxicity, neurocognitive deficiencies, and increased risk to immunological and infectious diseases [8]. Evidence has also linked PCB exposure to cardiovascular disease [9] and a variety of cancers [10–12].

Several routes of exposure to PCBs have been identified; however, ingestion and dermal are believed to be the most common in humans. Owing to their high lipophilicity, PCBs are easily absorbed through these avenues [13]. Although many congeners of PCBs have been found to be quite stable in the body and tend to accumulate in fatty tissues, metabolism and excretion do occur to some extent, albeit at a slower rate than would be expected for less lipophilic compounds. In the first step of metabolism, P450 enzymes catalyze epoxidation or hydroxylation of the PCB. Subsequent glucuronidation, sulfation, or binding to glutathione may then result in excretion of these metabolites. If not excreted, the hydroxylated and epoxide metabolites can be further metabolized by P450 or epoxide hydrolase to dihydroxylated compounds or dihydrodiols, respectively. The dihydrodiols, through the action of dihydrodiol dehydrogenase, can also be metabolized to dihydroxylated species [13]. Several studies have suggested that formation of dihydroxylated metabolites in the form of catechols (hydroxyl groups are *ortho*) and hydroquinones (hydroxyl groups are *para*) may play a significant role in PCB toxicity and carcinogenicity owing to their redox cycling capacity [10,14,15].

Studies from our laboratory as well as others have shown that catechols and hydroquinones can be oxidized to semiquinones and ultimately quinones both nonenzymatically and enzymatically [14,16–18]. The resulting (semi)quinones can bind to DNA and protein, producing DNA [16,17,19–21] and protein [22] adducts. Studies from this laboratory on lower chlorinated PCB congeners found that both human and rodent hepatic enzymes were able to metabolize these compounds to quinone metabolites, resulting in direct DNA adduction [19]. Subsequent studies suggested the involvement of intermediary semiquinone radicals in the binding of benzoquinones and hydroquinones to DNA [20]. Further, Lin et al. [22] provided some of the earliest evidence that quinone metabolites are a reactive species formed during PCB metabolism in vivo. Using human hepatocytes, Borlak et al. [21] were able to establish a link between PCB exposure and DNA damage. In recent reports from our laboratory and others, redox cycling of the PCB (semi)quinones has also been shown to increase oxidative DNA damage as measured by DNA strand breaks and 8-oxodeoxyguanosine (8-oxodG) and 3-(2′-deoxy-β-D-erythropentofuranosyl)pyrimido[1,2-a]purin-10(3*H*)-one (M1dG) adducts [15,23–25].

In this study we have employed a new ^{32}P-postlabeling system [26] coupled with Cu^{2+}-mediated activation to evaluate the oxidative DNA-damaging potential of several dihydroxylated PCBs and PCB quinones. By using this model system we were able to detect numerous PCB catechol- and hydroquinone-induced novel polar oxidative DNA adducts. To study the reactive oxygen species (ROS) that may mediate the formation of these DNA adducts we have also employed several ROS modifiers.

Experimental procedures

Chemicals

Hydroxylated PCBs and PCB quinones were synthesized and characterized as previously described [15,27,28]. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA) unless stated otherwise. Sources of chemicals used for ^{32}P-postlabeling analysis were as described previously [29].

DNA adduction

Salmon testis DNA (300 μg/ml), freed from RNA by treatment with RNases and solvent extractions [29], was incubated (37°C, 1 h) with either vehicle or various PCB metabolites (100 μM) in the presence or absence of $CuCl_2$ (100 μM) in 10 mM Tris–HCl, pH 7.4. Intervention with ROS scavengers was carried out as described above in the presence of 600 U/ml superoxide dismutase (SOD), 200 U/ml catalase, 100 mM sodium azide, 10 mM tiron, 10 mM bathocuproine, 10 mM sodium benzoate, 100 mM mannitol, or 10% dimethyl sulfoxide. Modified DNA was purified by solvent extractions and ethanol precipitation of DNA as previously described [29]. Concentrations of reactants and ROS scavengers were chosen based on earlier studies from this laboratory in which oxidation of PCB dihydroxy metabolites, in the presence of $CuCl_2$, increased 8-oxodG levels [23].

^{32}P-postlabeling analysis

Analysis of polar oxidative DNA adducts was as described elsewhere [26]. Briefly, DNA (6 μg) was hydrolyzed to 3′-monophosphates with micrococcal nuclease and spleen phosphodiesterase and adducts were enriched by treatment with nuclease P1. Adducts were 5′ ^{32}P labeled in the presence of a molar excess of [γ-^{32}P]ATP (<3 μM; 550 Ci/mmol) and T4 polynucleotide kinase. The labeled nucleotides were treated with a mixture of nuclease P1 and potato apyrase to convert labeled bisphosphates of the residual normal nucleotides to monophosphates and unused [γ-^{32}P]ATP to $^{32}P_i$, respectively. Adducts were resolved by two-directional polyethyleneimine (PEI)–cellulose thin-layer chromatography (TLC) (D1, 50 mM sodium phosphate, pH 5.8/1 M formic acid onto a 6-cm Whatman No. 17 paper wick; D2, 80 mM sodium phosphate, pH 6.0/10% acetonitrile (v/v)).

Total nucleotides were analyzed by labeling a dilute DNA digest (2 ng) in parallel with adducts followed by one-directional PEI–cellulose TLC in 0.5 M acetic acid/2 M formic acid [30]. Adducts were visualized and quantified by a Packard InstantImager. Adduct levels were determined by relative adduct labeling (RAL), i.e., RAL = [cpm of adducts/cpm of normal nucleotides] × 1/dilution factor. Levels are expressed as adducts/10^6 nucleotides.

Statistical analysis

Statistical comparisons were made using the two-way ANOVA followed by Fisher's least significant difference procedure. Values were considered significantly different when $p \leq 0.05$.

Results

Detection of novel oxidative DNA adducts

Cu^{2+} (100 μM)-mediated activation of PCB catechols and hydroquinones (100 μM), but not PCB quinones or monohydroxylated metabolites, in the presence of salmon testis DNA (300 μg/ml) resulted in the formation of nearly a dozen novel polar oxidative DNA adducts (Fig. 2). Trace amounts of adduct 11 were also detected in several vehicle-treated samples (data not shown). The characterization of