A New Mechanism of Toxicity for Polychlorinated Biphenyls (PCBs): Redox Cycling and Superoxide Generation

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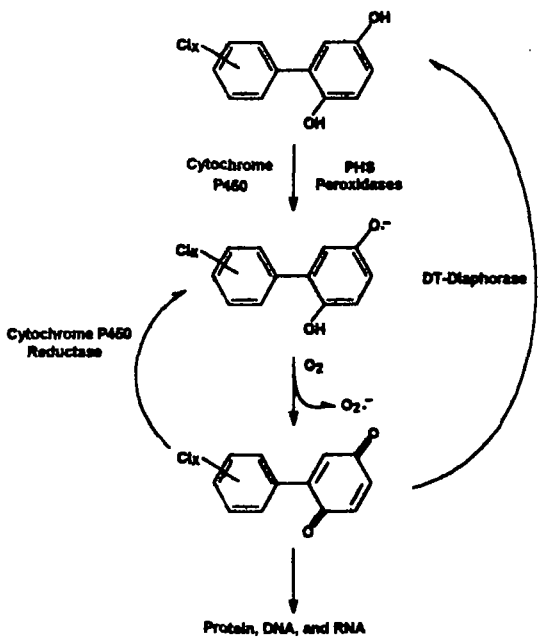
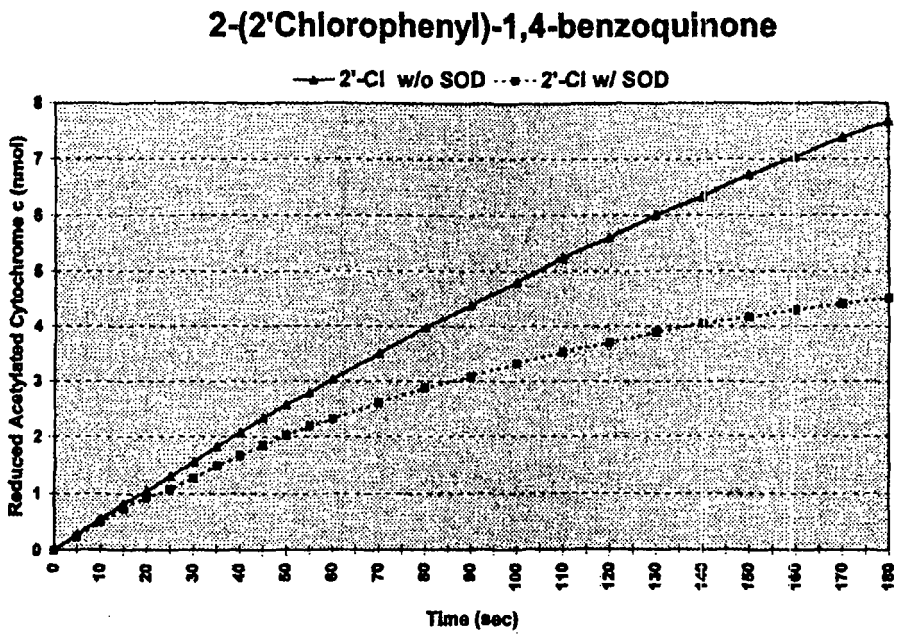
Introduction

Commercial PCB preparations are complete liver carcinogens in rodents and are efficacious promotors in two-stage hepatocarcinogenesis. Numerous congeneric PCBs also promote rat two-stage hepatocarcinogenesis, but the question of their initiating properties has not been resolved. We are currently investigating the activation of congeneric PCBs, principally resulting from their metabolism to dihydroxy metabolites. The ortho- and para-dihydroxy metabolites may subsequently undergo either a one or two electron oxidation to semiquinone and quinone, respectively. Although both oxidation products react with nucleophiles, such as amino acids, thiol compounds, and DNA, we are also quite interested in the ability of the quinoid-like PCB metabolites to form reactive oxygen species (ROS) during their metabolism and in their ability to redox cycle, as a part of an overall effort to evaluate the importance of this pathway in PCBs toxicity. We have shown that microsomal incubations of 4-chlorobiphenyl (and other lower chlorinated biphenyls) lead to the formation of 2,3-, 2,5-, and 3,4-dihydroxy-metabolites, potential precursors for ortho- and para-quinones [1]. We have further shown that these catechols and hydroquinones may be oxidized to the corresponding (semi) quinones, and that a number of peroxidases and prostaglandin synthase will catalyze these oxidation reactions [2]. The PCB



results, namely 3.2 nmols, is the quantity of acetylated cytochrome c reduced via superoxide in 180 seconds. This basic pattern was found for all of the compounds tested, except 2-(4'-chlorophenyl)-1,4-benzoquinone which had the same rate of acetylated cytochrome c reduction in both the presence and absence of SOD. However, this interpretation assumes that the presence of SOD does not alter the equilibria of the reactions. It is known that SOD not only decreases superoxide reduction of acetylated cytochrome c, but also indirectly decreases the background level of acetylated cytochrome c reduction due to the semiquinone radical [7,8]. Nevertheless, since we found no reduction of acetylated cytochrome c in the presence of a quinone with 1) NADPH alone and 2) microsomes (reductase) alone in either the absence or presence of SOD, these data demonstrate that the PCB-like quinones will stimulate electron flow from NADPH. Presumably, within an intact cellular environment, this may contribute to a pro-oxidant environment via depletion of cellular reducing equivalents.



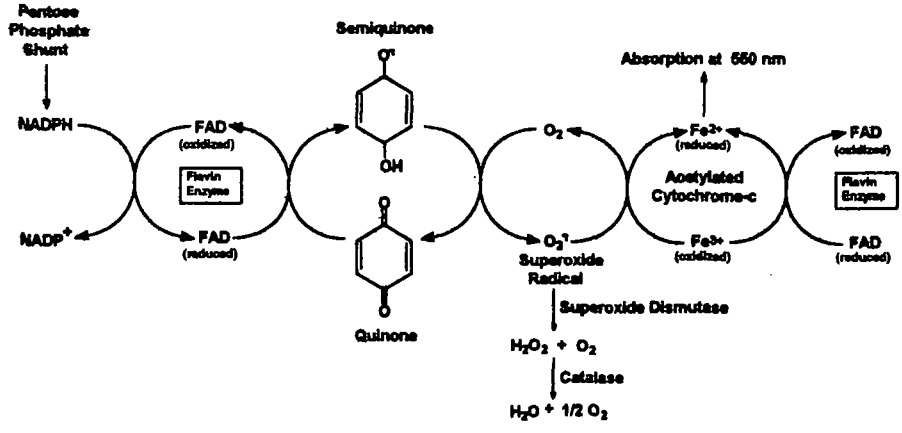
The quantity of cytochrome c reduced for each test quinone in 180 seconds, in the presence and absence of SOD, is presented below:

Compound:	"Total"	-	"SOD"	=	Difference
1,4-benzoquinone	6.3		3.8		2.5
2-phenyl-1,4-benzoquinone	8.4		4.7		3.7
2-(2'-chlorophenyl)-1,4-benzoquinone	7.7		4.5		3.2
2-(3'-chlorophenyl)-1,4-benzoquinone	6.4		4.2		2.2
2-(4'-chlorophenyl)-1,4-benzoquinone	2.4		2.4		0.0
2-(3',4'-dichlorophenyl)-1,4-benzoquinone	4.4		2.9		1.5
2-(3',5'-dichlorophenyl)-1,4-benzoquinone	3.6		2.5		1.1
2-(3',4',5'-trichlorophenyl)-1,4-benzoquinone	5.4		4.0		1.4

derived quinones are strongly electrophilic and will react slowly with N nucleophiles, like the amino acid glycine, but react instaneously with the S nucleophiles, such as N-acetyl-cysteine and glutathione [3]. PCB-derived quinones are reactive with nucleotides [4] and DNA [2], producing adducts detectable with sensitive ^{32}P-postlabeling methods. Our *in vitro* studies have also shown that, during the oxidation of PCB catechols and hydroquinones, increased levels of 8-oxodeoxyguanosine are produced [5]. Recently, our attention has focused on the production of ROS during enzymatic oxidation/reduction of these metabolites and the potential of these compounds to redox cycle, producing superoxide. Evidence for the formation of superoxide (detectable by NBT reduction) and other ROS that cause DNA strand breaks is presented in the accompanying abstract by Dr. Ludewig. The present study focuses on the question of whether hydroquinone- or quinone-like PCB metabolites redox cycle with the generation of superoxide or other free radicals.

Materials and Methods

The synthesis of the PCB quinones has been described [3]. The redox-cycling assay [6] was performed by incubating PCB quinoid metabolites with NADPH-cytochrome P-450 reductase (rat liver microsomes) in the presence of oxidized acetylated cytochrome c, 100 µM EDTA, 2 mM $MgCl_2$, 500 µM desferroxamine, 5.2 units catalase and a NADPH-regenerating system. Radical production was monitored by the reduction of acetylated cytochrome c at 550 nm. The determination of the apparent kinetic constants Km, Vmax, *kcat*, *kcat*/Km was carried out with each PCB quinone. The reduction of acetylated cytochrome c was monitored over a 3 minute time course for each quinone, at 10 fold higher concentration than its apparent Km. The incubations were carried out both with and without superoxide dismutase (SOD). Major components and their interactions are pictured below:



Results and Discussion

The time course of the incubation of 2-(2'-chlorophenyl)-1,4-benzoquinone in this system is shown below. In the absence of SOD approximately 7.7 nmols of oxidized acetylated cytochrome c was reduced in 180 seconds. When 150 units of SOD were present, only 4.5 nmols were reduced in 180 seconds. In the classical interpretation (6), the difference of these two

The following general conclusions can be drawn from these studies:

1. Semiquinone was the primary initial product, since the rate of acetylated cytochrome c reduction (at early time points) was identical in the presence or absence of SOD.

2. At later time points (45 seconds or longer) the presence of SOD dramatically diminished the rate of reduction of acetylated cytochrome c.

3. The rank order of activities for a given benzoquinone was practically unchanged at both the early and later time points, with non-/ mono-chloro substituted > meta-para > 4 chloro quinones.

4. Aside from the expected influences of SOD on acetylated cytochrome c reduction, studies are underway to investigate whether the relative concentrations of several free radical species may be influenced by SOD.

Acknowledgements
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References

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