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Biosynthesis, Physiological Disposition and Biochemical Effects of Nephrotoxic Glutathione and Cysteine S-Conjugates

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The nephrotoxicity of certain halogenated alkanes and alkenes is the result of hepatic glutathione S-conjugate formation, followed by renal metabolism of the conjugates to corresponding cysteine S-conjugates, which then undergo renal bioactivation to the ultimate toxic species. These experiments have used synthetic PCBG as the substrate and hepatic microsomal fractions as the source of enzyme. Results indicate that PCBG is a substrate for microsomal transferases; with the reaction being time-dependent, protein-dependent, PCBG-dependent and dependent upon glutathione concentrations. Methods to study the alkylation of renal DNA have been devised with sulfides I-IV being isolated to allow generation of putative reaction intermediates in high yield. Predictions can be made that the sulfide will be cytotoxic to isolated rat hepatocytes.

PCBC inhibits renal mitochondrial DNA synthesis and the effect is concentration dependent and inhibited by aminooxyacetic acid. The effect on DNA degradation is being investigated.

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SIX-MONTH REPORT

Project title: Biosynthesis, Physiological Disposition, and Biochemical Effects of Nephrotoxic Glutathione and Cysteine S-Conjugates

Project number: AFOSR-86-0302

Principal Investigator: M. W. Anders

Period Covered: 15 August 1986 to 13 February 1987

Biosynthesis of S-(pentachlorobutadienyl)glutathione (PCBG):

Although the synthesis of PCBG by microsomal fractions (Wolf et al., 1984) and in isolated hepatocytes (Jones et al., 1985) has been reported, the enzymology of the reaction has not been investigated. Hence, we have conducted studies with purified hepatic microsomal glutathione S-transferases. The microsomal transferases were isolated and purified by Dr. Yoko Aniya of the the University of the Ryukyus, Okinawa, Japan, according to published procedures (Morgenstern and DePierre, 1983; Morgenstern et al., 1982). The preparation was nearly homogeneous by SDS-PAGE. Hexachloro-1,3-butadiene (HCBD) was a substrate for the purified transferases. The conjugation of glutathione with HCBD was dependent on time, on protein concentration, and on the presence of glutathione. Although the microsomal glutathione S-transferases can be activated by N-ethyl maleimide with chlorodinitrobenzene as the substrate, the conjugation of glutathione with HCBD is not activated by the sulfhydryl reagent. The product of the reaction has been identified conclusively as PCBG by ^{13}C nuclear magnetic resonance spectrometry and by fast atom bombardment mass spectrometry.

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Jones et al. (1985) presented evidence for the formation of a "bis-substituted glutathione conjugate of HCBD, persumably 1,4-bis-(glutathion-S-yl)tetrachloro-1,3-butadiene. We have also investigated the mechanism of diconjugate formation. In these experiments, we have used synthetic PCBG as the substrate and hepatic microsomal fractions as the source of enzyme. These studies indicate that PCBG is a substrate for the microsomal transferases; the reaction is dependent on time, and on protein, PCBG, and glutathione concentrations.

Alkylation of DNA by PCBG Metabolites:

One of the aims of the project is to investigate the alkylation of renal DNA by PCBG metabolites. Such studies are ordinarily hampered by the low level of alkylation, which makes structural identification of the alkylated bases difficult, if not impossible. One solution to this problem is to devise methods to generate the putative reactive intermediates in high yield. Hence, we have undertaken the synthesis of precursors of reactive intermediates of PCBG. Because pentachlorobutadienyl mercaptan I is presumably formed by the action of cysteine conjugate β-lyase on S-(pentachlorobutadienyl)-L-cysteine (PCBC), a metabolite of PCBG, we have attempted to devise synthetic routes to I. The reaction of HCBD with tert-butyl mercaptan or benzyl mercaptan yields tert-butyl pentachlorobutadienyl sulfide II or benzyl pentachlorobutadienyl sulfide III, respectively. The reaction of sulfides II or III with 2-nitrobenzene sulfenyl chloride affords 2-nitrophenyl pentachlorobutadienyl disulfide IV. The reduction of the disulfide IV with sodium borohydride should yield the desired pentachlorobutadienyl mercaptan I, and, if the reduction of the disulfide IV is done in the presence of DNA, one should be able to prepare reasonably large quantities of alkylated DNA. The sulfides II and III have

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been synthesized and fully characterized, and preliminary studies suggest that we also have prepared the disulfide **IV**. These studies are continuing, and we expect positive and interesting results in the near future.

The ability to prepare stable precursors of reactive intermediates offers much potential to understand the events associated with the bioactivation of toxic chemicals. In addition, the availability of benzyl pentachlorobutadienyl sulfide affords another test of the hypothesis that the mercaptan **I** is the toxic intermediate or its immediate precursor: benzyl pentachlorobutadienyl sulfide should be an excellent substrate for cytochrome P-450-dependent monooxygenases, which should metabolize the sulfide to the corresponding hemimercaptal; the hemimercaptal should collapse to yield pentachlorobutadienyl mercaptan. Hence, we predict that the sulfide will be cytotoxic to isolated rat hepatocytes; we will test this prediction in the near future.

Literature cited:

Jones, T. W., Gerdes, R. G., Ormstad, K., and Orrenius, S., Chem.-Biol. Interact. **56**, 251 (1985).

Morgenstern, R., and DePierre, J. W., Eur. J. Biochem. **134**, 591 (1983).

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SIX-MONTH REPORT

Project Title: **Biosynthesis, Physiological Disposition, and Biochemical Effects of Nephrotoxic Glutathione and Cysteine S-Conjugates**

Project Number: **AFOSR-86-0302**

Principal Investigator: **M. W. Anders**

Period Covered: 14 February 1987 to 23 July 1987[*1]

Biosynthesis of S-(pentachlorobutadienyl)glutathione (PCBG):

The studies on the biosynthesis of PCBG, which were described in the first six-month report, are almost complete. The recent studies have focused on the characterization of the conjugates formed by ^{1}H and ^{13}C NMR and by fast-atom bombardment mass spectrometry (FAB-MS). The major product formed by the glutathione S-transferase-catalyzed reaction of glutathione with hexachloro-1,3-butadiene is a mixture (20:1) of E- and Z-1-(glutathion-S-yl)-pentachlorobuta-1,3-diene (PCBG). PCBC is also a substrate for the glutathione S-transferases; the product of the reaction is 1,4-bis(glutathion-S-yl)-1,2,3,4-tetrachlorobuta-1,3-diene (BTCB). Although the microsomal glutathione S-transferases catalyze the formation of PCBG more efficiently than the cytosolic transferases, the cytosolic transferases catalyze the formation of BTCB more efficiently than the microsomal transferases. The toxic effects of BTCB have not been studies, but we may investigate this point, if we can accumulate sufficient BTCB.

Bioactivation of benzyl pentachlorobutadienyl sulfide (BPS):

The toxicity of PCBC and S-(pentachlorobutadienyl)-L-cysteine (PCBC) is thought to be attributable to the cysteine conjugate β-lyse-catalyzed formation of pentachlorobutadienylthiol. As an alternate test of this hypothesis, we prepared BPS the expectation being that cytochromes P-450 would metabolize BPS to the corresponding hemimercaptal, which would eliminate the toxic thiol. (The initial rationale for preparing BPS was given in the first six-month report.) This expectation proved correct: BPS was very cytotoxic in isolated rat hepatocytes and was metabolized to benzaldehyde by purified, reconstituted cytochrome $P\text{-}450_{PBB}$ systems. The analogue tert-butyl pentachlorobutadienyl sulfide, which cannot form the intermediate hemimercaptal, was not, as expected, cytotoxic. BPS may prove useful in our studies of DNA modification during the metabolism of PCBC.

Effect of S-(pentachlorobutadienyl)-L-cysteine (PCBC) on mitochondrial protein and DNA synthesis:

In the original proposal, we planned to examine the alkylation of renal tissue macromolecules by PCBC (Specific Aim 4). As a prelude to these studies, we have studied the effect of PCBC on renal mitochondrial protein and DNA synthesis. PCBC is an effective inhibitor of protein synthesis; the effect is concentration dependent and is inhibited by aminooxyacetic acid, indicating the involvement of β-lyase. PCBC also inhibits renal mitochondrial DNA synthesis and, again, the effect is concentration dependent and is inhibited by aminooxyacetic acid. The effect of PCBC on DNA degradation is presently being investigated.

[1*] The first six-month report covered the period 15 August 1986 to 13 February 1987.