MECHANISM OF THE NEUROTOXIC AND HEPATOTOXIC EFFECTS OF CARBON DISULFIDE

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SUMMARY

The mechanisms of carbon disulfide toxicity can be divided into two categories; nonmicrosomal and microsomal. The nonmicrosomal pathway involves nonenzymatic spontaneous reaction of carbon disulfide with amino or thiol groups that leads to formation of dithiocarbamates or GSH conjugates as well as inhibition of certain enzymes such as dopamine β-hydroxylase. These reactions primarily lead to neurotoxic effects. The second mechanism of carbon disulfide toxicity involves its metabolism by hepatic microsomal enzymes to two reactive sulfur atoms that bind covalently to cell macromolecules causing hepatotoxicity. This oxidative metabolism of carbon disulfide has been suggested to be responsible for much of the liver pathology and impairment of liver metabolism of other endogenous substrates as well as exogenous compounds entering the body.

I. INTRODUCTION

Carbon disulfide, which is extensively used in the rubber and rayon industries, is an important industrial solvent and therefore its inhalation certainly becomes a major route of occupational exposure to this compound /1-2/. Besides this route, its formation in the body via degradation of other sulfur xenobiotics such as disulfiram presents another likelihood of a minor exposure to carbon disulfide /3/.

Two major types of toxic manifestations resulting from exposure to carbon disulfide include neurotoxicity and hepatotoxicity. Neurotoxicity is developed as a result of direct, spontaneous reaction of carbon disulfide with amino, thiol and hydroxyl groups of cellular components /4/. The major metabolic products thus formed are represented primarily by dithiocarbamates capable of inactivating metalloenzymes by chelation of essential metal ions required for enzyme activity. Secondly, direct reaction of carbon disulfide with the aforementioned functional groups on proteins can also contribute to CS2-induced neurotoxicity /5/. The second type of CS2 toxicity is related to its oxidative metabolism that occurs predominantly in the liver by microsomal mixed-function oxidases leading primarily to liver injury. In recent years, a number of studies have focussed on

the liver metabolism of carbon disulfide and its hepatotoxic implications /6-8/. The experimental material that will be used to explain the mechanism of liver toxicity of CS2 is taken from our previously published work.

II. NEUROTOXICITY OF CARBON DISULFIDE AND ITS MECHANISM

Typically, various neurological effects of CS2 toxicity include tremors, dyspnea, cyanosis, delirium, peripheral vascular collapse, hypothermia, convulsions, coma and death from respiratory paralysis /9/. Chronic exposure to CS2 also results in neurological lesions characterized by degenerative changes of neurons of the brain, spinal cord, and motor and sensory nerves including those of the eyes /10-15/. Several mechanisms for CS2-induced neurotoxicity have been proposed. Carbon disulfide is a small molecule, soluble in lipids and therefore can dissolve in the lipids of the nervous system and physically block nerve impulses. Biochemically, CS2 has been reported to decrease membrane bound ATPase activity in the brain and inhibit brain dopamine β-hydroxylase and monoamine oxidase /9-10/. The latter two enzymes contain copper and utilize pyridoxal phosphate as a coenzyme for their full activity. A proposed mechanism suggests that CS2-derived dithiocarbamates chelate copper and zinc, and also react with pyridoxine, thus causing their deficiency in the nervous system /4/. Consequently, vital enzymes such as monoamine oxidase and dopamine β-hydroxylase are inactivated and neurological symptoms are produced following exposure to CS2. Depletion of copper and zinc by CS2-derived dithiocarbamates would also interfere with other enzymatic functions (Table 1). Thus copper is an essential cofactor of cytochrome oxidase, tyrosinase and other tissue oxidases. Zinc is similarly essential for the activity of lactate dehydrogenase, carbonic anhydrase, glutamic dehydrogenase, alcohol dehydrogenase and other enzymes. Therefore, inhibition of the activity of these enzymes should also account, at least in part, for the neuropathy observed in CS2 intoxication.

Carbon disulfide also reacts with certain proteins and peptides. It has been shown that CS2 reacts directly with albumin, pepsin, trypsin, chymotrypsin, haemoglobin and other plasma proteins

TABLE 1

Enzymes Affected by Carbon Disulfide

Membrane bound ATPase	Dopamine β-hydroxylase
Monoamine oxidase	Cytochrome oxidase
Tyrosinase	Lactate dehydrogenase
Carbonic anhydrase	Glutamic dehydrogenase
Alkaline phosphatase	Alcohol dehydrogenase
Aldehyde dehydrogenase	Proteolytic enzymes
Xanthine oxidase	(pepsin, trypsin and
Liver microsomal enzymes	chymotrypsin)

/5,16/. This and its binding to brain proteins and spinal neurofilaments are likely to be responsible for many neurological manifestations /17-18/ such as axonopathy of the peripheral and central nervous system /13-15/.

Since heart is an excitable tissue it is not inconceivable to expect the same neurotoxic mechanisms of carbon disulfide to be operative in the induction of cardiovascular lesions. It is known that occupational exposure to CS2 enhances the development of atherosclerosis. Although some of the mechanisms of carbon disulfide neurotoxicity such as pyridoxine deficiency may be important in the production of CS2-derived cardiopathy, the exact mechanism by which CS2 produces cardiovascular toxicity is unclear. Several experimental studies in laboratory animals have shown that exposure to CS2 results in elevated serum cholesterol, phospholipids and triglycerides /19-20/, which may play an important role in the genesis of atherosclerotic disease connected with occupational exposure to CS2.

III. MECHANISM OF LIVER TOXICITY OF CARBON DISULFIDE

Toxicity of carbon disulfide to liver was first reported by Lewey /21/. Autopsy findings and other observations such as an increase of serum cholesterol and a decrease of prothrombin levels in workers chronically exposed to CS2 suggested to him and his coworkers that CS2 was responsible for the dysfunction of liver. Furthermore, the

affected livers were also found to have fatty degeneration and hemorrhage. However, at that time the exact mechanism by which CS2 produced hepatic injury was not known. Subsequent studies on the liver metabolism of CS2 performed by Bond et al. /22/ revealed that the liver damage attributed to CS2 was dependent on CS2 metabolism in the liver. They showed that the oral administration of carbon disulfide to normal rats at a level as small as 1 mg/kg produced only an increase in fat in the liver. However, when the same dose of CS2 was administered to rats pretreated with phenobarbital, an inducer of liver microsomal enzymes, extensive centrilobular hepatic necrosis occurred in the rats. As a result of this observation, it was postulated that the hepatotoxicity of carbon disulfide was dependent on the activity of the hepatic drug metabolizing enzyme system and on the rate of metabolism of CS2 by the microsomal enzymes to a more toxic reactive metabolite rather than carbon disulfide itself /23/.

TABLE 2

Binding of ^{35}S from ^{35}S-Carbon disulfide to hepatic microsomes from phenobarbital-pretreated (PB) and normal (N) rats

NADPH	Source of microsomes	^{35}S bound (nmoles/lO min/mg protein)
+	Pb	7.28 ± 0.40
-	Pb	1.10 ± 0.03
+	N	2.42 ± 0.23
-	N	1.18 ± 0.08

The results are expressed as means ± S.D.M. Data from Dalvi et al. /24/.

The *in vitro* and *in vivo* work on CS2 metabolism reported previously by us has further provided an evidence to support the above hypothesis /7,24-25/. Table 2 shows the results of an experiment in which ^{35}S-CS2 was incubated with microsomes isolated from normal and phenobarbital-pretreated rats. It can be seen that the NADPH-stimulated binding of sulfur to the hepatic

TABLE 3

Effect of carbon monoxide on ^{35}S binding to microsomes incubated with ^{35}S-carbon disulfide

Atmosphere	^{35}S bound (nmoles/10 min/mg protein)
CO:Air (9:1)	2.50 ± 0.17
N2:Air (9:1)	6.07 ± 0.36

The results are expressed as means ± S.D.M.
Data from Dalvi et al. /24/.

microsomes from phenobarbital-pretreated rats was about 5 times more than that with the microsomes from the normal rats. These results suggested that CS2 was a substrate for the hepatic mixed function oxidase enzyme system. In order to confirm this, an experiment was performed to examine the effect of carbon monoxide (CO), an inhibitor of the microsomal enzymes, on the binding of sulfur to liver microsomes from phenobarbital-pretreated rats. The results of this experiment (Table 3) showed that the amount of sulfur bound to the microsomes in the incubations carried out in an atmosphere containing 9:1 CO:air was markedly less than that bound in the incubations carried out in an atmosphere of 9:1 N2:air, thus indicating that the reaction was catalyzed by the microsomal mixed function oxidases. Results of an additional experiment in which liver microsomes were incubated separately with ^{14}C-CS2 and ^{35}S-CS2 demonstrated that the sulfur bound to the microsomes was in the atomic form and free of the carbon atom of CS2. This led to the hypothesis that CS2 may be metabolized by microsomal enzymes to carbonyl sulfide (COS) with the release of a reactive atom of sulfur binding covalently to the microsomes. Subsequently, using mass spectrometry and gas chromatography it was shown that COS is indeed formed in the incubations containing rat liver microsomes, NADPH and CS2 /24/.

Additional studies indicated that COS is metabolized *in vitro* to C02 in a reaction catalyzed by the hepatic mixed function oxidases

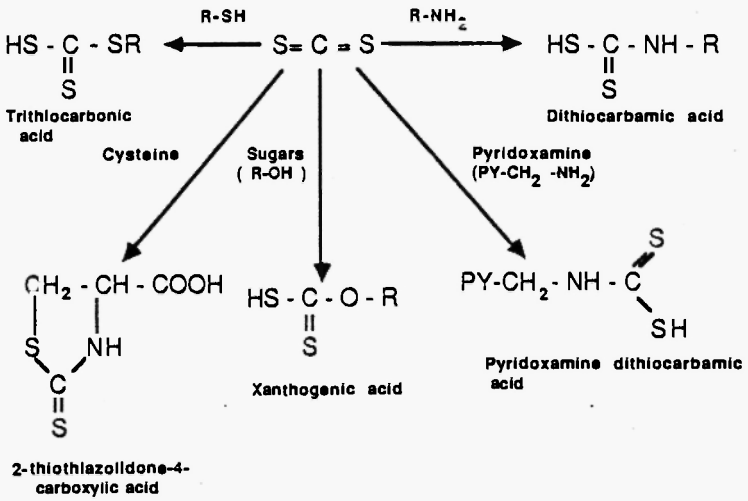

Fig. 1: Nonmicrosomal metabolism of carbon disulfide involving neurotoxicity.

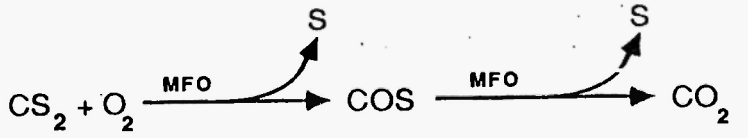

Fig. 2. Microsomal metabolism of carbon disulfide leading to hepatotoxicity.

/7/. That the sequence of these *in vitro* reactions is also operative in the intact rat was subsequently shown by administering ^{14}C-CS2 to rats and detecting COS and CO2 in their breath /26/. These investigations have helped propose a mechanism for the mixed function oxidase catalyzed metabolism of CS2 to carbonyl sulfide and carbon dioxide (Figure 2). It has been proposed that the two sulfur atoms released from CS2 exist as highly reactive electrophilic species and as such would readily react with nucleophiles on the microsomal membranes. Therefore it is possible that the binding of this reactive species of sulfur to microsomal membranes coupled with the inhibition of other cellular enzymes listed above may be

responsible for the hepatic necrosis seen on administration of CS2 *in vivo* to experimental animals. Since the hepatotoxicity is proportional to the rate of CS2 metabolism by the microsomal enzymes, the liver injury would be severe in animals pretreated with microsomal enzyme inducers such as phenobarbital.

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