Biochemical Pharmacology, Vol. 26, pp. 1521–1524. Pergamon Press, 1977. Printed in Great Britain.

DELETERIOUS EFFECTS OF SUBACUTE CARBON DISULPHIDE EXPOSURE ON MOUSE LIVER

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(*Received* 7 *December* 1976; *accepted* 24 *February* 1977)

Abstract—Female mice, fed *ad lib.*, were exposed to 1.5 mg of carbon disulphide per liter of air for 4 hr a day, 5 days a week, for approximately 3.5 weeks. During the first week of the experiment the liver changes were similar to those found after acute carbon disulphide intoxication, i.e microsomal cytochrome P-450 content was lowered, the activities of NADPH cytochrome *c*-reductase and 7-ethoxycoumarin deethylase were decreased and the measurable UDP-glucuronosyltransferase activity was increased. The copper and phospholipid contents of the liver were also increased somewhat. Later on the activities of the microsomal enzymes were either partially or totally restored in spite of continuing exposure. The liver phospholipid content was also restored. The activity of UDP-glucuronosyltransferase was decreased significantly below the control level. The diene conjugation of liver phospholipids was increased, and this finding indicates that the exposure was able to damage the membrane lipids of the liver continuously. It is suggested that the partial or total restoration of the enzyme activities resulted either from their decreased sensitivity to the toxic metabolites of carbon disulphide or from their stimulated synthesis. The increased diene conjugation of the liver phospholipids indicates that the lipid environment of membrane bound enzymes changes during subacute exposure to carbon disulphide.

Carbon disulphide (CS_2) poisoning is known to depress microsomal drug hydroxylation in the liver, especially in animals pretreated with phenobarbitone [1–4]. In addition CS_2 also increases the activity of microsomal UDP-glucuronosyltransferase *in vivo* in a manner resembling the effect of the related drug disulfiram [5, 6]. In a previous article it was suggested that one reason for the stimulation of the transferase activity could be the stimulation of microsomal lipid peroxidation by the metabolism of CS_2 [7].

The mechanism by which CS_2 is able to enhance microsomal lipid peroxidation is obscure. It has, however, been reported that CS_2 exposure causes cholestasis in perfused rat liver [8], and cholestasis itself is known to affect microsomal drug metabolism [9, 10]. Cholestasis is also known to change the metal metabolism of liver, because some metals, e.g. copper, are mainly excreted from the liver in the bile [11, 12].

The changes in liver microsomes that CS_2 induces seem to be reversible in 2–3 days [1, 6], and, if so, recovery after an acute CS_2 intoxication is probably achieved through the synthesis of new proteins [1]. Accordingly, it seems possible that in the course of subacute CS_2 exposure the toxic effects of CS_2 on the liver could be cumulative. In order to elucidate this possibility, we exposed mice subacutely to CS_2. We also measured the amount of copper in the liver and liver lipid peroxidation in order to explore the basis of CS_2 stimulated microsomal UDP-glucuronosyltransferase.

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MATERIALS AND METHODS

Twenty-eight adult female mice of the CB-20 strain (age 15–19 weeks, weight 23–30 g) were exposed to 1.5 mg CS_2/l of air for 4 hr a day, 5 days a week. Nine animals were used as controls. The mice were fasted during the daily exposure but were otherwise allowed to eat *ad lib.* On day 2, 3, 5, 9, 12, 16 and 23 of the experiment four exposed animals were killed 3 hr after the daily exposure. The livers of the animals were removed in an ice bath, they were weighed, and about 300 mg of each liver was used for the copper determinations.

The liver microsomes were prepared by the conventional procedure [13]. The microsomes were analyzed for their cytochrome P-450 content according to the method of Omura and Sato [14], and for their NADPH cytochrome *c*-reductase activity according to Phillips and Langdon [15]. The 7-ethoxycoumarin deethylase activity was measured by the method of Ullrich and Weber [16]. The excitation wavelength was 340 nm, and that of emission 440 nm. The activity of UDP-glucuronosyltransferase was measured according to a method described earlier and *p*-nitrophenol was used as the aglycone [17, 18]. Protein determinations were performed [19], bovine serum albumin being used as the standard.

Liver lipids were extracted, and the diene conjugation of the lipid extracts was measured essentially as described by Recknagel and Ghoshal [20]. Butylated hydroxytoluene (50 mg/ml) was, however, added to all the solvents used to diminish lipid peroxidation during the procedure. The cholesterol content of the extracts was measured by the Liebermann–Burchard reaction [21], and the phospholipids according to the method of Naito [22].

To determine the liver copper content, about 300 mg of liver was solubilized with concentrated HCl. The copper in the solubilate was chelated with diethylammoniumdiethyldithio-carbamate and extracted into methylisobutylketone. The copper in the organic phase was measured with a flame atomic absorption spectrophotometer (Perkin Elmer 400). The precision and accuracy of the method was tested with SRM 1577 bovine liver (National Bureau of Standards, Washington, U.S.A.), the result being $199 \pm 10\,\mu g/g$ (S.D., $n = 8$). The certified value for copper in this control sample was $193 \pm 10\,\mu g/g$ of liver.

The 7-ethoxycoumarin was synthesized as described by Ullrich and Weber [16], and the purity of the compound was tested with infrared spectrometry and thin layer chromatography.

The statistical significance were evaluated with Student's *t*-test.

RESULTS

The mice tolerated the subacute CS_2-exposure well. No clinically observable neurological signs developed during the experiment.

Microsomal enzyme activities. In the beginning of the experiment microsomal P-450 content decreased remarkably (Table 1). In spite of the continuing exposure, the measurable P-450 content of the microsomes increased later on, and it reached a new, stable level during the last part of the experiment.

The activity of microsomal NADPH cytochrome *c*-reductase was affected by CS_2 exposure in a manner similar to the effect on cytochrome P-450 content (Table 1). At the beginning of the experiment the activity was low, but it increased later on to the control level and was even slightly above it by the end of the experiment.

As found earlier [6], the activity of microsomal 7-ethoxy-coumarin deethylase of liver was affected more by CS_2 exposure than the microsomal cytochrome P-450 content was (Table 1). At days 9 and 12 the 7-ethoxycoumarin deethylase had reached its lowest levels (20–25% of controls). In contrast, at the same time points the cytochrome P-450 contents had almost and the NADPH cytochrome *c*-reductase activities had fully reached control levels again (Table 1).

CS_2 exposure enhanced slowly microsomal UDP-glucuronosyltransferase activity (Table 1). The increase became evident after five daily exposures of the mice to CS_2. Unexpectedly, the phase of stimulation of microsomal UDP-glucuronosyltransferase after day 9 was followed by a decrease in the measurable enzyme activity, and the activity continued to decrease up until the end of the experiment, when the activity of UDP-glucuronosyltransferase of the liver microsomes in the exposed animals was significantly lower than the corresponding activity of the controls (Table 1).

Effect of CS_2 exposure on liver copper content. The liver copper content seemed to follow the changes of liver microsomal UDP-glucuronosyltransferase; the extent of the changes was, however, much smaller. The liver copper content increased to 105.7% ($2\ P < 0.05$) of the control value ($8.26 \pm 0.43\,\mu g/g$ wet wt.) during first week of exposure and stayed on this level until day 12 (106.2%; $2\ P < 0.05$). Thereafter the content dropped to a new level 6–7% below control, which was 92.3% ($2\ P < 0.01$) on day 23.

Effect of CS_2 exposure on liver lipids. CS_2 exposure initially increased the liver phospholipid content and liver phospholipid-cholesterol ratio (Table 1). No changes were found in liver cholesterol content. Later on, the content of phospholipids in the livers of the exposed animals decreased to the level of the controls (Table 1).

CS_2 exposure doubled the diene conjugation of liver lipid extracts during the first 16 days of the experiment (Table 2). The CS_2 induced level of lipid peroxidation remained high still at the end of the experiment (Table 1).

DISCUSSION

The present study clearly indicates that the subacute CS_2 poisoning of mouse liver differs from the acute one. During the beginning of the experiment the changes found in mouse liver were similar to those reported after acute intoxications [1–4, 7] in that the components of microsomal mixed-function oxidase as well as overall drug hydroxylation reactions were inhibited, UDP-glucuronosyltransferase activity was enhanced, and liver phospholipid content was increased. Later on, all the mentioned changes either partially or totally disappeared.

The metabolites of CS_2 seem to attack microsomal P-450 molecules in a way which inevitably leads to the degradation of the cytochrome molecules [23–25]. In accordance with earlier reports, we found diminished cytochrome P-450 content in the microsomes during the first days of the study (Table 1). From the end of the first exposure week, however, microsomal P-450 content increased to a value nearer that of the control animals. Even if the mechanism of this partial restoration of cytochrome P-450 activity remains obscure, it seems possible that the newly synthetized molecules of cytochrome are less vulnerable to the attack of the toxic metabolites of CS_2.

CS_2 exposure affected microsomal NADPH cytochrome *c*-reductase activity in a manner similar to the effect on the cytochrome P-450 content of the microsomes in that during the first days of the experiment the activity was below the level of the controls. But later it increased to a level higher than the control value [26]. It therefore seems possible that the toxic metabolites of CS_2 are also able to reach the molecules of NADPH cytochrome *c*-reductase, the result being the enzyme inhibition which was seen during the first days of the experiment. The later increase in the reductase activity is in accordance with the earlier report of Sokal [26], and it may result from the compensatorily increased synthesis of the enzyme. It is a general feature of microsomal-bound drug-metabolizing enzymes that at the same time when the measurable UDP-glucuronosyltransferase activity is increased as a result of treatment with membrane perturbants, the component reactions of microsomal mixed-function oxidase, e.g. NADPH cytochrome *c*-reductase, as well as the overall drug oxidation reactions, are decreased [27].

Table 1. The effect of CS_2 exposure on microsomal drug-metabolizing enzymes and phospholipids of mouse liver*

Time (experimental days)	Cytochrome P-450 (nmol/mg microsomal protein)	NADPH cytochrome *c*-reductase (nmol cytochrome c reduced/mg × min)	7-ethoxycoumarin deethylase (nmol/mg × min)	UDP-glucuronosyl-transferase (nmol/mg × h)	Phospholipids (mg P/g wet wt.)	Lipid peroxidation ($DA_{240}/\mu g$ phospholipid)
Control	0.39 ± 0.04	341.0 ± 77.3	0.46 ± 0.04	131.8 ± 35.1	0.57 ± 0.11	0.39 ± 0.12
2	0.19 ± 0.01†	173.5 ± 12.6†	0.35 ± 0.04‡	120.9 ± 8.2	0.91 ± 0.11†	0.40 ± 0.05
3	0.21 ± 0.03†	118.3 ± 23.6†	0.24 ± 0.07†	127.8 ± 17.4	0.89 ± 0.23‡	0.42 ± 0.16
5	0.34 ± 0.02†	N.D.	N.D.	174.0 ± 7.4§	0.73 ± 0.09§	0.45 ± 0.10
9	0.33 ± 0.03§	N.D.	0.10 ± 0.04†	196.8 ± 33.6§	0.64 ± 0.09	0.57 ± 0.05‡
12	0.28 ± 0.01†	327.8 ± 26.2	0.11 ± 0.02†	119.5 ± 8.0	0.69 ± 0.06§	N.D.
16	0.32 ± 0.04§	434.2 ± 28.7§	0.30 ± 0.06‡	70.5 ± 12.5§	0.52 ± 0.02	0.80 ± 0.08†
23	0.30 ± 0.02‡	383.0 ± 18.3	0.30 ± 0.04†	63.2 ± 10.5†	0.51 ± 0.02	0.77 ± 0.06†

* The values are means ± S.D. In the control, $n = 9$; in the others, $n = 4$.
† The value differs from the control, 2 $P < 0.001$.
‡ The value differs from the control, 2 $P < 0.01$.
§ The value differs from the control, 2 $P < 0.05$.
N.D. = not determined.

In our experimental conditions the activity of microsomal ethoxycoumarin deethylase was not restored as early as was the cytochrome P-450 content of the microsomes. This phenomenon may indicate that the restorations of the two activities are not coupled. One reason for the difference could be a difference in the turnover rates of the various components of the microsomal mixed-function oxidase, but it is also possible that newly synthetized cytochrome P-450 molecules are not as effective in catalyzing deethylation as the cytochrome P-450 molecules in control livers are.

CS_2 has been shown to cause cholestasis in perfused rat liver [8]. The slight increase in liver copper found in the beginning of our experiment could have resulted from decreased bile flow. The changes measured for the liver copper content are, however, much less than those found after rats have been treated with disulfiram [5]. Whether the enhanced lipid peroxidation is due to cholestasis requires more specific further studies, and the increased activity of UDP-glucuronosyltransferase found in CS_2 treated animals but that some other mechanism is responsible for these changes.

In accordance with earlier findings we found that the liver phospholipid content increased in CS_2 exposed animals, but later on returned to normal. In addition, the diene conjugation of liver phospholipids increased, and this result suggests that continuous CS_2 exposure is able to damage microsomal membranes in spite of the partial or total restoration of various enzyme activities. In fact, it is possible that the activity of microsomal enzymes that are dependent on phospholipids, such as cytochrome P-450 or UDP-glucuronosyltransferase, may become affected when the function of microsomal phospholipids becomes deteriorated by lipid peroxidation. The initial increase in microsomal UDP-glucuronosyltransferase activity is most probably due to an activation caused by CS_2 exposure [6, 7]. The decline of the enzyme activity later on may be explained by a decreased stability of the enzyme in the activated form, or to the renewed constraint probably due to liver regeneration despite continuing lipid peroxidation.

In conclusion, the effects of subacute CS_2 exposure on liver microsomes cannot be regarded as a cumulation of acute intoxication only, but rather they result from the compensative adaptation of liver metabolism to a new level at which microsomal enzyme activities are partially or totally restored but liver phospholipids are increasingly degraded by lipid peroxidation.

Acknowledgements—The authors wish to thank Mrs. Anja Sarasjoki and Miss Tuula Korhonen for their skillful technical assistance.

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