Relationship of Oxygen and Glutathione in Protection against Carbon Tetrachloride-induced Hepatic Microsomal Lipid Peroxidation and Covalent Binding in the Rat

Rationale for the Use of Hyperbaric Oxygen to Treat Carbon Tetrachloride Ingestion

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Abstract. CCl_4 exerts its toxicity through its metabolites, including the free radicals $CCl_3^\cdot$ and $CCl_3OO^\cdot$. Oxygen strongly inhibits the hepatic cytochrome P-450-mediated formation of $CCl_3^\cdot$ from CCl_4 and promotes the conversion of $CCl_3^\cdot$ to $CCl_3OO^\cdot$. Both these free radicals injure the hepatocyte by causing lipid peroxidation and binding covalently to cell structures. A reduced glutathione (GSH)-dependent mechanism can protect the liver microsomal membrane against CCl_4-induced damage under aerobic conditions but not under anaerobic conditions (Burk, R. F., K. Patel, and J. M. Lane, 1983, *Biochem. J.*, 215:441–445). Experiments were carried out using rat liver microsomes to examine the effect of O_2 tensions found in the liver and of GSH on CCl_4-induced covalent binding and lipid peroxidation. An NADPH-supplemented microsomal system was used. CCl_4 or $^{14}CCl_4$ was added to the sealed flask that contained the system, and after 20 min $CHCl_3$ production, thiobarbituric acid-reactive substances (an index of lipid peroxidation), and covalent binding of ^{14}C were measured. O_2 tensions of 0, 1, 3, 5, and 21% were studied. Increases in O_2 tension caused a fall in $CHCl_3$ production, which indicated that it decreased $CCl_3^\cdot$. GSH had no significant effect on $CHCl_3$ production at any O_2 tension. Lipid peroxidation and covalent binding of ^{14}C fell progressively as O_2 tension was increased from 1 to 21%. The addition of GSH decreased both lipid peroxidation and covalent binding, but did so better at the higher O_2 tensions than at the lower ones. These results indicate that low O_2 tensions such as are found in the centrilobular areas of the liver favor conversion of CCl_4 to free radical products which cannot be detoxified by the GSH-dependent mechanism. They suggest that hyperbaric O_2 might decrease free radical formation in the liver in vivo and promote formation of $CCl_3OO^\cdot$ from $CCl_3^\cdot$. This should result in diminished CCl_4-induced lipid peroxidation and liver damage. Rats given CCl_4 (2.5 ml/kg) were studied in metabolic chambers. Production of $CHCl_3$ and ethane, the latter an index of lipid peroxidation, were measured. Rats in two atmospheres of 100% O_2 produced much less $CHCl_3$ and ethane than rats in air. This strongly suggests that hyperbaric O_2 is decreasing free radical formation from CCl_4 and/or promoting the formation of $CCl_3OO^\cdot$ from $CCl_3^\cdot$. These results provide the rationale for the use of hyperbaric O_2 in the treatment of CCl_4 ingestion.

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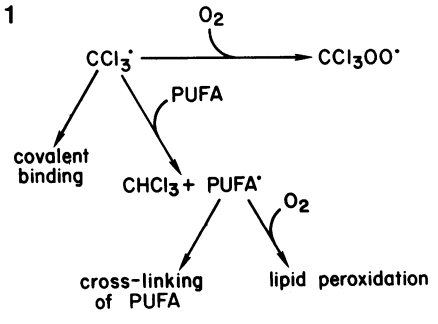
Introduction

The hepatotoxicity of CCl_4 results from its metabolic conversion to free radical products (1). Evidence of $CCl_3^\cdot$ formation from CCl_4 was provided in the 1960's by the identification of $CHCl_3$ (2) and C_2Cl_6 (3) in tissues of animals that were given CCl_4. More recently, spin-trapping experiments have verified directly

Scheme 1



the production of $CCl_3^{\cdot}$ from CCl_4 by the rat liver microsomal cytochrome P-450 system (4).

The major reactions of $CCl_3^{\cdot}$ with liver microsomes (Scheme 1) appear to be covalent binding (5) and $H^{\cdot}$ abstraction from polyunsaturated fatty acids (PUFA).[1] The $H^{\cdot}$ abstraction results in $CHCl_3$ and $PUFA^{\cdot}$ formation. $PUFA^{\cdot}$ can enter into cross-linking reactions or lipid peroxidation, depending on the O_2 tension.

In 1978, Packer et al. (6) reported that O_2 reacts very rapidly with $CCl_3^{\cdot}$ to yield $CCl_3OO^{\cdot}$. Thus, the presence of O_2 when $CCl_3^{\cdot}$ is formed results in the formation of a second radical species as shown in Scheme 1. Because this reaction is so rapid, O_2 can be expected to compete with microsomal constituents for $CCl_3^{\cdot}$.

$CCl_3OO^{\cdot}$ is many orders of magnitude more reactive with PUFA than is $CCl_3^{\cdot}$ (7), and therefore is a potent initiator of lipid peroxidation. It or some of its breakdown products can bind covalently to microsomes as well (8). $CCl_3OO^{\cdot}$ seems unlikely to serve as a source of $CHCl_3$ (9). Therefore, although both $CCl_3^{\cdot}$ and $CCl_3OO^{\cdot}$ can cause membrane injury through covalent binding and lipid peroxidation, $CHCl_3$ arises only from $CCl_3^{\cdot}$ and may serve as an index of $CCl_3^{\cdot}$ concentration.

O_2 inhibits the cytochrome P-450-mediated formation of $CCl_3^{\cdot}$ from CCl_4. This inhibition is profound, but is not complete even under 21% O_2 (10, 11). Thus, O_2 diminishes $CCl_3^{\cdot}$ concentration by (1) inhibiting its formation and (2) promoting its conversion to $CCl_3OO^{\cdot}$. The highest concentration of $CCl_3^{\cdot}$ in a microsomal system will occur then under anaerobic conditions, and its concentration should fall as O_2 tension increases. The effect of O_2 on $CCl_3OO^{\cdot}$ concentration will be more complex. As O_2 tension rises, a greater fraction of the $CCl_3^{\cdot}$ that is present will be converted to $CCl_3OO^{\cdot}$, but a smaller amount of $CCl_3^{\cdot}$ will be formed. Thus, the highest $CCl_3OO^{\cdot}$ concentration should be achieved at an intermediate O_2 tension. At higher O_2 tensions, virtually all the CCl_4-derived free radicals will be $CCl_3OO^{\cdot}$, but the total conversion of CCl_4 to radicals can be expected to be small, so $CCl_3OO^{\cdot}$ concentration will decline.

In an earlier study with NADPH-supplemented rat liver microsomes (11), we found that substantial amounts of CCl_4 disappeared from the flask under anaerobic conditions. $CHCl_3$ appeared and covalent binding occurred. When the atmosphere in the flask was 21% O_2, no disappearance of CCl_4 could be detected by the method used. No $CHCl_3$ production was detected. A small amount of CCl_4 metabolism was occurring, however, because covalent binding and lipid peroxidation were detected.

It seems likely that nearly all the CCl_4-derived free radicals present under the anaerobic conditions (see Scheme 1) were $CCl_3^{\cdot}$ and nearly all those present under 21% O_2 were $CCl_3OO^{\cdot}$. This has potential importance because reduced glutathione (GSH) inhibited covalent binding and lipid peroxidation under 21% O_2, but had no effect on covalent binding or $CHCl_3$ formation under anaerobic conditions, which suggests that a GSH-dependent mechanism can protect the membrane against $CCl_3OO^{\cdot}$ but not against $CCl_3^{\cdot}$.

We undertook the present study to investigate the relevance of these observations to the hepatotoxicity of CCl_4. Experiments were designed to examine the effect of O_2 tensions present in the liver and the effect of GSH on CCl_4 metabolism and the injurious processes of lipid peroxidation and covalent binding. The effect of hyperbaric O_2 in vivo was also assessed as a potential treatment for CCl_4 ingestion.

Methods

Animals. Male Sprague-Dawley strain rats were purchased from Timco Laboratories, Houston, TX. They were given food and water ad libitum and were housed in rooms with alternating 12-h light and dark cycles. Rats used in the experiments depicted in Figs. 3 and 4 were fed standard laboratory rations and rats from which microsomes were prepared (Figs. 1 and 2) were fed a nutritionally adequate semisynthetic diet (12). Experiments were begun between 8 and 10 a.m.

Rats were killed by cervical dislocation and exsanguination. Hepatic microsomes were prepared as described before (13) and were used for experiment on the day of preparation.

Microsomal incubations. The microsomal incubations were carried out in a volume of 5 ml in 25-ml Erlenmeyer flasks that were sealed with rubber septums. They were shaken in a water bath at 37°C, and the buffer used (microsomal incubation buffer) was 50 mM Tris–HCl, pH 7.5, with 140 mM NaCl and 50 μM EDTA. When used, GSH was added to a concentration of 1 mM.

The incubations reported in Figs. 1 and 2 were carried out under O_2–N_2 mixtures. O_2 concentration is indicated on the figures. The gas mixtures were made with a three-tube flowmeter (Matheson Gas Products, Newark, NJ) and solutions and buffers used were equilibrated by bubbling the buffer and solutions to be added with the gas mixture for 15 min. Then the flask that contained the incubation mixture without CCl_4 and NADPH was flushed with the gas mixture for 5 min at 37°C before the flask was sealed and CCl_4 (10 μl vol in ethanol) was injected. After a further 5 min equilibration, the incubation was started by injection of NADPH. Flask protein concentrations varied from 1.14 to 1.60 mg/ml. NADPH concentration was 400 μM. After 20 min of incubation, flask atmosphere was sampled for $CHCl_3$ analysis. Then the sealed flask was chilled on ice for several minutes before it was opened. A sample was taken immediately for assay of thiobarbituric acid-reactive substances when the flask was opened.

For the experiment in Fig. 1, 1 mM CCl_4 was used. For the one in Fig. 2, 72 μM ^{14}C-labeled CCl_4 (2.8 Ci/mol, sp act) was used. To determine covalent binding to microsomes, 1 ml of 10% trichloroacetic acid was added to each flask after cooling and the microsomes were pelleted by centrifugation. The pellet was washed three times with 5 ml ethyl acetate to remove ^{14}C-labeled material that was not covalently bound. Then the pellet was digested in a scintillation vial by 0.5 ml Protosol and ^{14}C was determined after addition of 10 ml of Ready-Solv EP (Beckman Instruments, Palo Alto, CA).

In vivo studies. The in vivo studies were carried out using airtight plexiglas metabolism chambers (14). Each chamber contained soda lime to remove carbon dioxide and anhydrous $CaSO_4$ to remove water vapor. Hyperbaric conditions were maintained by pressurization of the O_2-filled chambers to two atmospheres using compressed 100% O_2. An air atmosphere was maintained by attachment of the chambers, sealed with air inside, to a respirometer that contained 100% O_2. As

1. *Abbreviations used in this paper:* PUFA, polyunsaturated fatty acids; GSH, reduced glutathione.

O_2 was consumed by the rat, it was replaced by O_2 from the respirometer, which maintained the original O_2 concentration. 3 h after the chamber measurements were begun, the chambers were flushed with room air for 3 min and then resealed for a subsequent 3-h experimental period under an air atmosphere. Chamber atmosphere was sampled through a rubber septum with a gas-tight syringe and needle and was injected directly into the gas chromatograph for analysis.

Rats given phorone (2,6-dimethyl-2,5-heptadien-4-one) received 250 mg/kg i.p. 2 h before CCl_4 was given. The phorone was dissolved in corn oil and 2 ml of the mixture per kilogram was injected. Controls received corn oil injections. Liver glutathione content was reduced to 4% of control 2 h after this phorone administration, as determined in a separate experiment (data not shown).

CCl_4 was mixed with an equal volume of mineral oil and 0.5 ml of the mixture was given by stomach tube per 100 g body weight immediately before the rat was put into the metabolism chamber. $CHCl_3$ in chamber atmosphere was used to determine its production from CCl_4. Because $CHCl_3$ can be metabolized by rats, it was necessary to determine the extent to which metabolism would influence chamber $CHCl_3$ levels. Separate experiments were performed (results not shown). Injection of $CHCl_3$ into a chamber that contained a rat resulted in rapid disappearance of the $CHCl_3$ from the chamber atmosphere. $CHCl_3$ did not disappear, however, when the rat in the chamber was treated with CCl_4 simultaneously. Therefore, $CHCl_3$ in chamber atmosphere represented production under these conditions. The absence of metabolism was probably due to CCl_4-induced destruction of cytochrome P-450.

Assays. Liver glutathione was determined by the recirculating assay of Tietze (15) as modified by Griffith (16). Thiobarbituric acid-reactive substances were measured as described previously (17). Ethane was determined in chamber atmosphere as described previously (14). $CHCl_3$ was measured using a Porasil C (80:100) column in a Hewlett-Packard 5880A gas chromatograph with a flame-ionization detector. Oven temperature was 80°C. Chamber atmosphere (1 ml) was injected directly into the instrument. $CHCl_3$ in flask atmosphere was measured by its direct injection into the gas chromatograph. Standard curves for the chamber studies were made by dilution of $CHCl_3$ in glass gas vessels. Standard curves for the microsomal experiments were made by injecting $CHCl_3$ into 25-ml Erlenmeyer flasks that contained 5 ml of buffer.

Materials. CCl_4 and phorone were purchased from Aldrich Chemical Co., Milwaukee, WI and $CHCl_3$ from American Scientific Products, Houston, TX; Protosol and $^{14}CCl_4$ were supplied by New England Nuclear, Boston, MA; NADPH was purchased from Boehringer-Mannheim Biochemicals, Indianapolis, IN; GSH, disodium EDTA, and Tris were from Sigma Chemical Co., St. Louis, MO.

Statistics. Statistical significance was determined with *t* test.

Results

The O_2 tension in the rat liver varies from the equivalent of 7% of an atmosphere of O_2 to almost 0% (18). The regions around the central veins have the lowest O_2 tensions. For this study of CCl_4 metabolism by liver microsomes, we chose three different O_2 concentrations in the range found in the liver, as well as 0 and 21% O_2 (Figs. 1 and 2).

Figure 1 *A* demonstrates the effect of O_2 tension on $CHCl_3$ formation by microsomes. As O_2 tension increased, $CHCl_3$ formation decreased. Under 21% O_2, no $CHCl_3$ production was detected. Because $CHCl_3$ is derived from $CCl_3^{\cdot}$, these results indicate that increasing the O_2 tension decreases $CCl_3^{\cdot}$ concentration. GSH had no detectable effect on $CHCl_3$ formation. These results support our earlier suggestion that GSH has little or no effect on $CCl_3^{\cdot}$ metabolism (11).

Fig. 1 *B* shows the effect of O_2 and GSH on CCl_4-induced lipid peroxidation. The greatest amount occurred under 1% O_2. Further increments in O_2 were associated with decrements in lipid peroxidation. No significant lipid peroxidation occurred under anaerobic conditions because the process is O_2-dependent. $CHCl_3$ production (Fig. 1 *A*) and lipid peroxidation (Fig. 1 *B*) were both diminished by increases in O_2 tension above 1%,

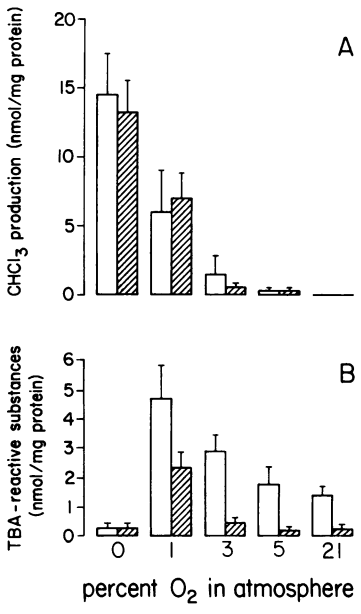


Figure 1. Effect of O_2 concentration and GSH on $CHCl_3$ production from CCl_4 (*A*) and CCl_4-induced lipid peroxidation (*B*) in rat liver microsomes. Open bars indicate that no GSH was present; shaded bars indicate that 1 mM GSH was present. Incubations were carried out as described in Methods. Values are mean±1 SD (n = 4 animals).

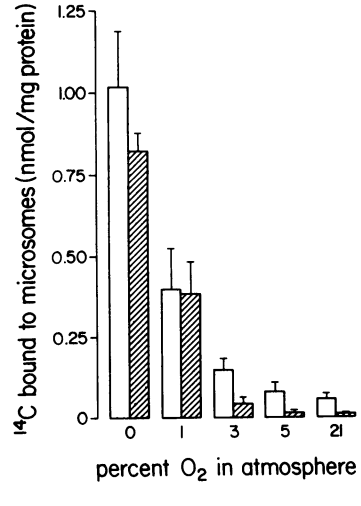


Figure 2. Effect of O_2 concentration and GSH on covalent binding of ^{14}C derived from ^{14}C-labeled CCl_4. Open bars indicate that no GSH was present; shaded bars indicate that 1 mM GSH was present. Incubations were carried out as described in Methods. Values are mean±1 SD (n = 4 animals).

but the diminution in $CHCl_3$ production was the greater. At 21% O_2, no $CHCl_3$ production could be detected, yet significant lipid peroxidation occurred.

GSH protected against lipid peroxidation but did so best at the higher O_2 tensions. Lipid peroxidation was inhibited 83–88% by GSH under 3–21% O_2, but under 1% O_2 the inhibition was only 50%. This result is consistent with the hypothesis that the GSH-dependent protection is effective against $CCl_3OO^{\cdot}$ but not against $CCl_3^{\cdot}$.

Fig. 2 demonstrates that covalent binding decreases as O_2 tension increases. It, like lipid peroxidation, did not fall as profoundly as did $CHCl_3$ production, particularly at the higher O_2 concentrations. This is illustrated in Fig. 3, which shows that the respective decreases in $CHCl_3$ production and in covalent binding were the same when O_2 tension rose from 0 to 1%. Successive increases in O_2 tension led to progressively more profound drops in $CHCl_3$ production without comparable drops in covalent binding. As O_2 tension rose from 5 to 21%, $CHCl_3$ production ceased completely, but covalent binding fell only by 33%. GSH inhibited covalent binding under 3–21% O_2 but had no effect on it at 0 or 1% O_2 (Fig. 2). These results are consistent with the GSH-dependent protection, being effective against $CCl_3OO^{\cdot}$ but not against $CCl_3^{\cdot}$.

Based on these results, raising hepatic O_2 tensions could be expected to lessen CCl_4 injury. The higher O_2 tension would inhibit the formation of $CCl_3^{\cdot}$ by the cytochrome P-450 system and promote the conversion of $CCl_3^{\cdot}$ formed to $CCl_3OO^{\cdot}$, which can be detoxified by a GSH-dependent mechanism. If this supposition is correct, raising hepatic O_2 tension with hyperbaric O_2 might constitute effective treatment for CCl_4 poisoning.

Using metabolic chambers, we studied the effect of hyperbaric O_2 on $CHCl_3$ production from CCl_4 in rats, and also measured ethane production as an index of lipid peroxidation. The effect of GSH depletion was examined as well.

Fig. 4 shows that hyperbaric O_2 sharply depressed $CHCl_3$ production by the rats. GSH depletion had no statistically significant effect on $CHCl_3$ production, although it appeared to augment the hyperbaric O_2 effect at 2 and 3 h. The inhibition was relieved when hyperbaric O_2 was replaced by air after 3 h. All rats produced the same amount of $CHCl_3$

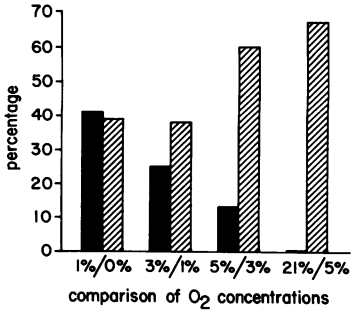


Figure 3. Comparison of the O_2 effect on $CHCl_3$ production and covalent binding. Values used here were calculated from the data presented in Figs. 1 *A* and 2 without GSH addition. These values are ratios and indicate the effect of the indicated increment in O_2 concentration on $CHCl_3$ production (solid bars) and on covalent binding (shaded bars).

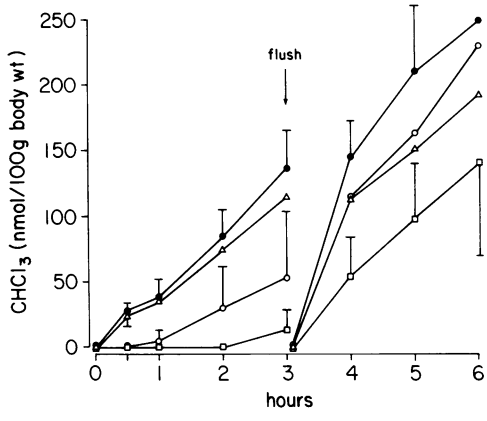


Figure 4. Effect of hyperbaric O_2 and GSH depletion on CCl_4 metabolism to $CHCl_3$ in the rat. The experiment was carried out as described in Methods. All rats received 2.5 ml CCl_4/kg at zero time and were put into the metabolism chambers. Treatments were as follows: •, air atmosphere; ○, hyperbaric O_2 atmosphere from 0–3 h; △, air atmosphere and depleted of GSH; □, hyperbaric O_2 atmosphere from 0–3 h and depleted of GSH. All rats were in a normobaric air atmosphere from 3 to 6 h. Values shown are averages of four rats. Brackets indicate 1 SD.

from 4 to 6 h after CCl_4 administration while breathing air. The apparent differences in $CHCl_3$ production in the hour after the chambers were flushed were likely due to redistribution of $CHCl_3$ from the tissues of the animals into the chamber atmosphere. These results strongly suggest that hyperbaric O_2 diminishes $CCl_3^{\cdot}$ concentration in rat liver after CCl_4 administration. This finding correlates well with the results that were obtained using the microsomal system (Fig. 1 *A*).

Fig. 5 shows that hyperbaric O_2 sharply depressed ethane production after CCl_4 administration, which indicated that it prevented CCl_4-induced lipid peroxidation. GSH depletion had a striking effect on lipid peroxidation. It doubled ethane production by animals breathing air. The ethane production by animals in hyperbaric O_2 was so low, however, that no effect of GSH depletion could be seen. The hyperbaric O_2 inhibition of ethane production was not reversed when the animals were returned to an air atmosphere (Fig. 5). These in vivo experiments lend support to the hypothesis that O_2 and GSH are protective against the hepatotoxicity of CCl_4.

Discussion

Fig. 6 is a proposed scheme of the early free radical portion of CCl_4 metabolism. It serves as a basis for discussing the present results. The conversion of CCl_4 to $CCl_3^{\cdot}$ is catalyzed by cytochrome P-450 (19). O_2 inhibits this reaction. Once $CCl_3^{\cdot}$ has been formed, it reacts very rapidly with O_2 to give $CCl_3OO^{\cdot}$ a much more reactive radical than $CCl_3^{\cdot}$ (6, 7). If little or no O_2 is present, $CCl_3^{\cdot}$ can bind covalently to lipids and proteins or abstract an $H^{\cdot}$, usually from a PUFA. The PUFA$^{\cdot}$

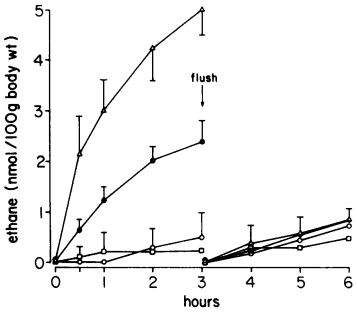


Figure 5. Effect of hyperbaric O_2 and GSH depletion on lipid peroxidation as measured by ethane production in the rat. These data are from the same experiment shown in Fig. 3 and the symbols used are the same.

can then react with other free radicals, which leads to polymerization, or, if any O_2 is present, PUFA$^\cdot$ can undergo lipid peroxidation.

$CCl_3OO^\cdot$ is very reactive and is a good initiator of lipid peroxidation (7). It, or compounds derived from it, can bind covalently also (8). Our earlier work indicated that GSH can affect the metabolism of $CCl_3OO^\cdot$, preventing membrane damage by it, but that GSH could not protect against $CCl_3^\cdot$ (11). Those conclusions are supported by the experiments shown in Figs. 1 and 2. GSH was more efficient in blocking CCl_4-induced lipid peroxidation and covalent binding at the higher O_2 tensions, where $CCl_3OO^\cdot$ formation would be favored, than at the lower ones, where $CCl_3^\cdot$ concentration would be higher.

Mico and Pohl (8) have recently presented evidence that further reactions of $CCl_3OO^\cdot$ yield an electrophilic chlorine and phosgene, both of which are potentially toxic metabolites. The present studies were undertaken before that report and did not measure production of those metabolites. Consequently, determination of the role of GSH in their formation and detoxification will require further studies.

This scheme (Fig. 6) can potentially be used to understand the pattern of liver injury by CCl_4. In the well-oxygenated periportal areas, cytochrome P-450 produces a small amount of $CCl_3^\cdot$, which is rapidly converted to $CCl_3OO^\cdot$. $CCl_3OO^\cdot$ is very

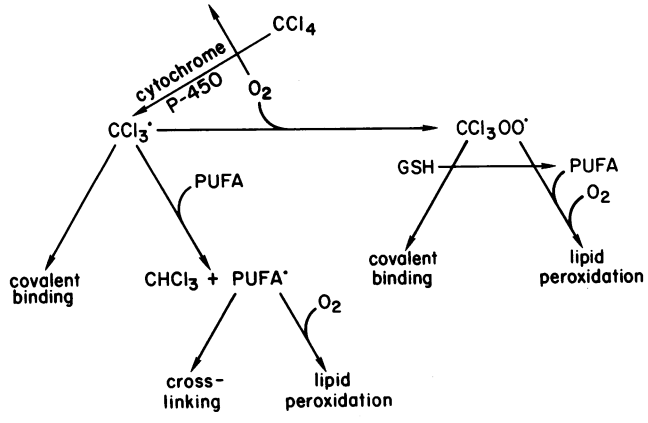


Figure 6. Scheme to explain O_2 and GSH effects on CCl_4 metabolism by rat liver microsomes.

reactive and may destroy the activating cytochrome P-450 by direct attack or by damage to the adjacent membrane (11, 19, 20). GSH protects the rest of the cell against $CCl_3OO^\cdot$.

In the poorly oxygenated centrilobular regions of the liver, $CCl_3^\cdot$ is produced in large quantities by cytochrome P-450. Because O_2 tension is low, little $CCl_3^\cdot$ is converted to $CCl_3OO^\cdot$. $CCl_3^\cdot$, being less reactive than $CCl_3OO^\cdot$, can diffuse farther before reacting and therefore should not destroy the cytochrome P-450 which activates CCl_4 to the extent that $CCl_3OO^\cdot$ does. Thus, there is much greater metabolism of CCl_4 under these conditions (11). $CCl_3^\cdot$ can bind covalently and can cause lipid peroxidation (if some O_2 is present). GSH does not appear to inhibit these events. Because there is an O_2 gradient between the periportal and the centrilobular regions, some areas will have O_2 tensions in which both $CCl_3^\cdot$ and $CCl_3OO^\cdot$ are present. Since the O_2 tension is relatively low, $CCl_3^\cdot$ may diffuse away from the activating cytochrome P-450 before reacting with O_2, to produce the highly reactive $CCl_3OO^\cdot$. This may protect the activating cytochrome P-450 and allow more CCl_4 metabolism. In those areas, perhaps analogous to the 1 and 3% O_2 in Figs. 1 and 2, GSH would be expected to exhibit significant protection. This may explain the protective effect of GSH against lipid peroxidation that was observed in air-breathing animals (Fig. 5).

The entire hepatic lobule would be well oxygenated under hyperbaric O_2. GSH protects the membrane remote from the activating cytochrome P-450 species from $CCl_3OO^\cdot$, but under well-oxygenated conditions it should have a small effect because of the small $CCl_3OO^\cdot$ production. This is a potential explanation for the failure of GSH depletion to cause increased ethane production in CCl_4-treated rats under hyperbaric O_2 (Fig. 4). Also contributing to the lowering of ethane production by hyperbaric O_2 could be reaction of O_2 with the ethyl radical, as suggested by Cohen (21).

This scheme (Fig. 6) can explain why hypoxic conditions are associated with increased lipid peroxidation and hepatotoxicity from CCl_4 (22, 23). Likewise, it provides the rationale for the use of hyperbaric O_2 in the treatment of CCl_4 poisoning. European workers have shown that prolonged treatment with hyperbaric O_2 protects rats against CCl_4-induced liver damage (24, 25), and there have been case reports of its efficacy in human CCl_4 poisoning (26). Hyperbaric O_2 should inhibit the metabolism of CCl_4 to free radicals and promote the formation of $CCl_3OO^\cdot$ in all parts of the liver lobule. That should result in decreased lipid peroxidation and covalent binding, but still allow destruction of the activating cytochrome P-450 with cessation of CCl_4 metabolism (11). Further studies are needed to determine the significance of CCl_4 metabolism to $CHCl_3$, which takes place under normoxic conditions even after a period of hyperbaric O_2 exposure (Fig. 4).

Hyperbaric O_2 would appear to be a logical treatment for CCl_4 poisoning. Available data (24–26) indicate it should be instituted as soon after ingestion as possible and that exposure should be maintained for as long as possible. This will allow

excretion of CCl_4 while inhibiting its conversion to injurious free radical intermediates. Conversely, hypoxia should be avoided.

These findings may have relevance to the hepatotoxicity of other agents. Halothane metabolism has recently been shown to yield a peroxy radical under aerobic conditions (7), and the GSH-dependent microsomal free radical scavenging mechanism might protect against it as well as against $CCl_3OO^{\cdot}$.

Acknowledgments

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