Antioxidant Maintenance of Hepatic Triglyceride Secretory Activity And Hepatic Function in Carbon Tetrachloride Poisoned Rats.* (31997)

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The administration of carbon tetrachloride (CCl_4) has been amply demonstrated to produce a profound increase in hepatic triglyceride content, impairment in hepatic function, and centrolobular necrosis(1-5). The increased hepatic triglyceride concentration is associated with a significant fall in plasma lipid and lipoprotein fractions due to either an inhibition of: a) lipoprotein secretion(5), or b) lipoprotein formation(6-8).

The early impairment in lipoprotein secretion after CCl_4 administration was initially demonstrated by the employment of Triton, a non-ionic detergent which inhibits the removal of lipoprotein from the plasma compartment(4,5). The validity of the Triton experiment, relative to the concept of a failure in the triglyceride secretory mechanism in CCl_4-treated animals, was established by isotopic and other studies on intact rats(7,9) and isolated perfused rat livers(8).

Recently, it was reported that the oral (10), intravenous(11) or intraperitoneal(10-12) administration of a variety of lipid antioxidants inhibited liver triglyceride accumulation, hepatic necrosis, and lethality following CCl_4 administration and prevented ethanol-induced lipid derangements(11-15). These studies resulted in the formulation of an hypothesis that lipid peroxidation is a possible factor in the pathogenesis of acute ethanol and CCl_4-induced liver injury(10-15). This concept was extended by the observations of Comporti *et al*(16,17) and Recknagel and Ghoshal(18,19).

The present studies were designed to evaluate whether the release of plasma triglycerides, as assessed by the use of Triton, was maintained in CCl_4-treated rats which received antioxidants. Additional data relative to hepatic functional activity in control and antioxidant-treated rats were obtained by means of the clearance of sulfobromophthalein sodium (BSP).

Methods. Male Sprague-Dawley rats, weighing from 125 to 225 g and previously maintained on Wayne Lab Blox (Allied Mills, Inc., Chicago, Ill.), received 2 intraperitoneal injections of a corn oil suspension of the antioxidant, N, N'-diphenyl-p-phenylenediamine (DPPD), in the amount of 60 mg per 100 g body weight. Control rats received 2 injections of an equivalent volume of corn oil. These injections were given 48 and 24 hours before the oral intubation of either undiluted CCl_4 or isotonic saline in a dose of 0.25 ml per 100 g body weight. The rats were fasted 16 hours prior to oral intubation and received no food after treatment.

Three hours after CCl_4 administration, p-iso-octyl polyoxyethylene phenol polymer (Triton, WR-1339, Winthrop Labs, New York) was administered *via* tail vein in a dose of 50 mg per 100 g body weight as a 20% solution (w/v) in isotonic saline; control rats received isovolumetric saline. Ninety minutes after Triton or saline administration, the rats were lightly anesthetized with ether and a terminal blood sample was taken from the inferior vena cava. Plasma samples were analyzed for triglycerides(20).

In other experimental and control groups, the concentration of BSP in plasma was measured 10 minutes following intravenous injection of 50 mg BSP per kg body weight (2). The BSP was administered 24 hours after CCl_4. All analyses were conducted in duplicate and the data analyzed for difference between means with a 95% confidence level.

Results. As has been previously demonstrated(3,5,9), oral administration of CCl_4

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TABLE I. Plasma Triglyceride Alterations in Antioxidant-Treated Control and Carbon Tetrachloride-Poisoned Rats.*

Group	Number	Triton treatment	Plasma triglyceride, mg %
Corn oil + saline	9	—	20.7 ± 3
" + CCl_4	9	—	7.4 ± 1
" + saline	9	+	255.0 ±56
" + CCl_4	8	+	50.6 ±11
DPPD + saline	6	—	25.0 ± 6
" + CCl_4	9	—	17.4 ± 3
" + saline	9	+	198.5 ±27
" + CCl_4	9	+	155.2 ±26

* Values are expressed as means ± standard error.

to normal rats resulted in a significant decrease in plasma triglyceride concentration (Table I). Administration of Triton to the control rats resulted in a pronounced plasma lactesence due to a 12-fold increase in triglyceride levels. In accordance with previous observations(5), the Triton response in the CCl_4-treated group was significantly inhibited, as the increment in plasma triglyceride concentration was but 43 mg%, in contrast to 234 mg% in the saline-treated group which received Triton.

Administration of DPPD did not significantly alter plasma triglyceride concentration in the saline group. The characteristic decline in plasma triglyceride following CCl_4 poisoning was prevented in the antioxidant-treated CCl_4 group. The plasma triglyceride concentration of the latter group was comparable to the plasma triglyceride concentration in the DPPD or non-DPPD, saline treated control rats.

The hypertriglyceridemic response following Triton was manifested to a comparable degree in the saline and CCl_4-treated rats which received DPPD. The latter group had a triglyceride concentration which was increased three-fold over the control CCl_4 group which received Triton, denoting the essential maintenance of lipoprotein secretion.

The removal of BSP from plasma was significantly impaired 24 hours after oral administration of CCl_4, as indicated by the 225% increase in the plasma BSP concentration (Table II). Administration of DPPD did not alter the plasma BSP concentration in the saline group. The plasma BSP concentration in the DPPD group which received CCl_4, while significantly increased in contrast to the corn oil-saline group, was not significantly altered from the DPPD-saline group. The plasma BSP in the DPPD-CCl_4 group was significantly decreased 40%, relative to the CCl_4 group indicating a reduced BSP retention.

Discussion. The present studies confirm previous observations of functional hepatic derangements of rats exposed to CCl_4 as reflected in: a) decreased plasma triglyceride concentration(3,5,9), b) inhibition of the post-Triton hypertriglyceridemia(5), and c) impaired vascular clearance of BSP(1,3). Administration of antioxidants, prior to CCl_4 exposure, effectively inhibited the CCl_4-induced hepatic accumulation of triglyceride (11), hepatic necrosis(11), and mortality (13). The present findings demonstrate that the antioxidant, DPPD, prevented the CCl_4-induced depression in plasma triglyceride concentration, and maintained at essentially normal values the hepatic release of lipoprotein triglyceride into the plasma compartment as assessed by the Triton response.

Seakins and Robinson(6) demonstrated that CCl_4 induces a profound impairment in the synthesis of the protein moiety of plasma lipoproteins which is followed by the development of the fatty liver and in reduction of plasma lipids. It appears that antioxidants, such as DPPD, which can effectively inhibit lipoperoxidation(15,16) can negate the CCl_4-induced defect in lipoprotein formation.

Reynolds has demonstrated that poisoning with CCl_4 results in early morphologic, functional, and compositional changes of the parenchymal cell among which are striking

TABLE II. Plasma Bromsulphalein Concentrations in Antioxidant-Treated Carbon Tetrachloride-Poisoned Rats.*

Group	Treatment	Plasma BSP, mg %
Corn oil	Saline	9.5 ± 1.6
"	CCl_4	30.9 ± 1.6
DPPD	Saline	12.5 ± 1.5
"	CCl_4	18.7 ± 2.4

* Values expressed as means ± standard error are derived from 8 rats per group.

alterations in the endoplasmic reticulum, plasma membrane, mitochondria, and Golgi apparatus(21). The selective permeability of the parenchymal cell is also altered as reflected in the rise in intracellular calcium and the loss of intracellular enzymes(21,22). The influence of antioxidant administration on these various CCl_4-induced cellular derangements remains to be established.

Since it has been demonstrated that administration of CCl_4 induces profound structural and functional alterations in the membranous components of the endoplasmic reticulum of the liver cell(21,23), a site at which protein synthesis, hepatic triglyceride or lipoprotein synthesis, and lipoprotein secretory mechanisms exist(4), it can be postulated that prior administration of antioxidants results in preservation of functional activity of the endoplasmic reticulum, as reflected by the maintenance of triglyceride secretion. This concept is supported by the ability of antioxidants to prevent the mitochondria and endoplasmic reticulum ultrastructural changes in acute ethanol-treated rats(24). Antioxidants, such as DPPD, also prevented *in vivo* and *in vitro* ethanol-induced enhancement in hepatic lipoperoxidation(16). These observations further validate the hypothesis of enhanced peroxidation of cellular lipids as a factor in the pathogenesis of CCl_4 and ethanol-induced liver injury(10-19).

The present findings demonstrate that it is possible to maintain functional integrity of the hepatic cell in CCl_4 poisoned animals by antioxidant administration(16), suggesting that the antioxidant potential may be an important factor in determining the degree and nature of hepatic cell injury.

Summary. Prior administration of the lipid antioxidant, DPPD, to rats which subsequently received CCl_4 orally prevented the CCl_4-induced depression of plasma triglyceride concentration and maintained a normal hepatic release of triglyceride into the plasma compartment, as assessed by the post-Triton hypertriglyceridemic response. Administration of DPPD also prevented the CCl_4-induced impairment in the hepatic removal of BSP. These findings, coupled with prior observation of inhibition of CCl_4-induced hepatic fatty infiltration, hepatic necrosis, and lethality, indicate that antioxidants can modify the degree and nature of hepatic cell injury resulting from exposure to certain toxic substances.

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