

PRELIMINARY REPORT

Hepatic Paraoxonase Activity Alterations and Free Radical Production in Rats With Experimental Cirrhosis

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Relationships between hepatic antioxidant paraoxonase (PON1) activity, lipid peroxidation, and liver injury were investigated in rats with CCl₄-induced cirrhosis. The study was performed in 60 CCl₄-treated rats and 60 control animals receiving a standard diet or one supplemented with zinc. Subsets of 10 animals each were killed at weeks 1, 5, and 7 of the study. Results showed that PON1 significantly decreased in rats given CCl₄ alone compared with control animals. This effect was partially reversed in animals receiving zinc. Conversely, lipid peroxides were significantly increased in rats given CCl₄ alone and returned to approximately normal values in animals receiving zinc supplement. PON1 was inversely correlated with lipid peroxidation in all the animals studied. These alterations coincided with changes in serum alanine aminotransferase activity. *In vitro* incubation of isolated microsomes with CCl₄ or malondialdehyde did not produce any significant changes in PON1, indicating that the decrease in PON1 in CCl₄-treated animals was not secondary to a direct inhibitory effect of lipid peroxidation products. These data show a time course and quantitative relationship between PON1 activity and lipid peroxidation in rats with CCl₄-induced cirrhosis and suggest that this enzyme plays a significant role within the antioxidant systems in liver microsomes.

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PARAOXONASE (PON1; arylalkylphosphatase E.C. 3.1.8.1) is an ester hydrolase that catalyzes the hydrolysis of organophosphorates and other xenobiotics.¹ The enzyme is found in several tissues, predominantly in the liver,^{2,3} and also in serum, in which it is bound to high-density lipoproteins (HDL).⁴ Its physiologic role is still unclear, although evidence exists for a protective effect of PON1 against oxidative stress.⁵ Being tightly associated with HDL, PON1 may be involved in the protection conferred by HDL on low-density lipoprotein (LDL) oxidation⁶ and, as such, with aspects of cardiovascular disease.

In contrast, there is a paucity of data on the hepatic enzyme. Rat and human liver PON1 are essentially microsomal enzymes associated with vesicles derived from the endoplasmic reticulum.^{7,8} An antioxidant hepatoprotective role may be readily hypothesized for hepatic PON1 because liver microsomes are the major sites for the catabolism of xenobiotic compounds, reactions in which increases in free radical species are observed. Several studies have suggested that oxidative stress plays an important role in the pathogenesis of liver diseases.^{9,10} The effects of antioxidants on the production of lipid peroxides and fibrogenesis have been investigated in models of experimental cirrhosis. For example, addition of zinc, a metal known to regulate several intracellular antioxidant systems,¹¹ to the diet has been associated with decreases in lipid peroxidation products and collagen synthesis, as well as with hepatic histologic and functional amelioration in rats treated with CCl₄.¹⁰

In view of these observations and the absence of data on the possible alterations of liver PON1 in cirrhosis, we decided to investigate the relationships between hepatic microsomal PON1 activity, lipid peroxidation and the progress of the disease in rats with CCl₄-induced cirrhosis. Further, we assessed the modulation of these processes by the dietary administration of zinc.

MATERIALS AND METHODS

In Vivo Experiment

The study was performed in 60 male Wistar rats submitted to a CCl₄ cirrhosis-induction program¹² and in 60 control animals. At the start of

the experiment, control and CCl₄-treated rats were divided into 4 groups (n = 30): group A, control rats fed with a standard diet (Panlab, Barcelona, Spain); group B, control rats fed with standard diet plus a supplement of zinc (227 mg/L of ZnSO₄ dissolved in the drinking water); group C, CCl₄-treated rats fed with a standard diet; group D, CCl₄-treated rats fed with a standard diet plus the supplement of zinc. Subsets of 10 animals from each group were sacrificed at weeks 1, 5, and 7 of the study. All animals received humane care in compliance with our institution's criteria for the care and use of laboratory animals.

Analytical Measurements

Livers were processed to obtain whole homogenates and microsome fractions as previously described.¹³ The determination of PON1 activity in rat liver microsomes was analyzed by measuring the rate of hydrolysis of paraoxon.¹⁴ Arylesterase activity was analyzed, as an indirect estimation of PON1 protein concentration,¹⁵ by measuring the rate of hydrolysis of phenylacetate.¹⁴ Units of PON1 and arylesterase activity were defined as in the report of Hasselwander et al.¹⁶ The production of lipid peroxides was measured in rat whole-liver homogenates by the thiobarbituric acid-reactive substances (TBARS) method.¹⁷ Serum alanine aminotransferase (ALT) was analyzed, as an index of hepatic necrosis, by the International Federation of Clinical Chemistry (IFCC) method, which uses L-alanine and oxoglutarate as substrates (ITC Diagnostics, Barcelona, Spain). Protein concentration in liver fractions was determined by the colorimetric technique of pyrogallol red (ITC Diagnostics). Sections of liver specimens were

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stained with hematoxylin, reticulin, and Masson trichrome stains for histologic examination.

In Vitro Study

The possible effects of CCl_4 and of malondialdehyde (MDA) on microsomal PON1 activity were investigated by incubating these compounds at 37°C for 30 minutes with isolated microsomes obtained from normal rats in an incubation medium containing ATP (0.1 mmol/L), NADPH (0.1 mmol/L), and an NADPH regenerating system.¹⁸ CCl_4 was added to the incubation mixture at concentrations of 9, 27, and 81 mmol/L. MDA was added at 15, 45, and 135 $\mu\text{mol/L}$. The concentrations of these 2 compounds in this experiment were similar to those previously used in studies on free radical generation in isolated liver microsomes.¹⁹ Experiments were performed in triplicate.

RESULTS AND DISCUSSION

Histopathologic assessment of the hepatic tissue indicated a normal architecture in all of the control animals (groups A and B). Rats given CCl_4 alone (group C) had normal livers at week one; at week 5, 6 of the 10 animals' livers were fibrotic and 4 were cirrhotic. By week 7, most of the rats (8 of 10) in this group were diagnosed as cirrhotic. The rate of progress of the disease was delayed by zinc administration (group D). In rats given CCl_4 and zinc, fibrosis was observed in all the rats at week 5 and cirrhosis was observed at week 7 in only 2 of 10 animals.

The microsomal concentration of proteins in CCl_4 -treated rats decreased at week 1 (1.86 ± 0.26 v 2.54 ± 0.15 g/L; $P < .001$) and remained low for the rest of the study (week 5, 1.63 ± 0.24 v 2.49 ± 0.15 g/L; week 7, 1.19 ± 0.27 v 2.00 ± 0.29 g/L; $P < .001$). Similar results were obtained in rats given

CCl_4 and zinc at weeks 1 (1.35 ± 0.39 v 2.56 ± 0.13 g/L) and 5 (1.53 ± 0.34 v 2.58 ± 0.28 g/L), but at week 7 this parameter increased to values similar to those of control animals (2.17 ± 0.17 v 2.10 ± 0.21 g/L).

Results of the PON1 activity measurement are shown in Fig 1A. PON1 activity was significantly decreased from the first week of treatment in the rats administered CCl_4 alone. This effect was partially reversed in animals receiving a zinc-supplemented diet. Similar results were obtained for the ratio of PON1 to arylesterase (Fig 1B). The reverse situation applied in TBARS concentrations (Fig 1C), which were significantly increased in rats treated with CCl_4 and returned to normal values at week 7 in rats given CCl_4 and zinc. Serum ALT activity (Fig 1D) progressively increased in rats treated with CCl_4 alone; this increase was lower in the animals given CCl_4 and zinc. PON1 activity was inversely correlated with the concentrations of TBARS ($r = .56$; $P < .001$) and with ALT ($r = .45$; $P < .001$) in all the animals studied. These results establish a chronologic sequence and quantitative relationship between the decrease in hepatic microsomal PON1 activity and the generation of lipid peroxidation products in CCl_4 -treated rats. Further, clear relationships between the biochemical and histologic improvement after zinc administration together with a partial recovery of PON1 activity are observed. These data suggest that hepatic PON1 may play a protective role against free radical production in the hepatic organelles.

Liver production of PON1 may be reduced as a part of a general sign of hepatic toxicity. This concept is supported by the observation that changes of the microsomal PON1 activity parallel those of the microsomal concentrations of total pro-

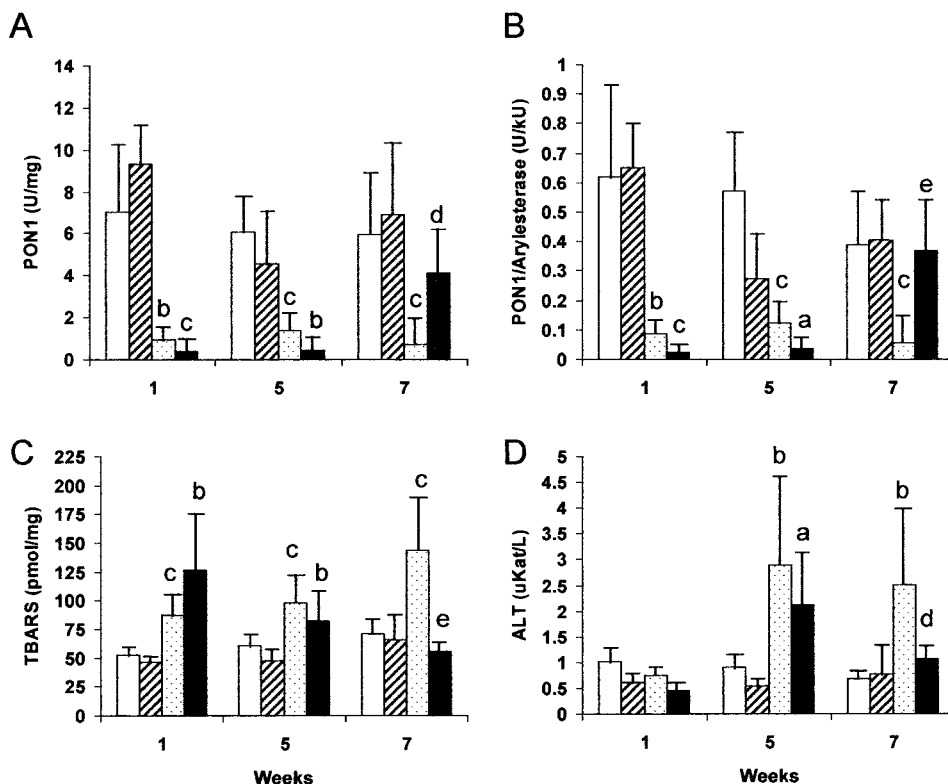


Fig 1. Results (means and SD) of the in vivo experiment in control rats (□), control rats given zinc (▨), CCl_4 -treated rats (▤), and rats treated with CCl_4 and zinc (■). ^a $P < .05$; ^b $P < .01$; ^c $P < .001$ compared with control rats. ^d $P < .01$; ^e $P < .001$ compared with rats treated with CCl_4 . Results of PON1 are expressed as units per milligram of microsomal protein. Results of TBARS are shown as picomoles per milligram of homogenate protein.

teins. However, our study shows alterations of the enzymatic specific activity (PON1 activity/mg protein) and of the ratio of PON1 to arylesterase activity, indicating that a more specific inhibition of the enzyme activity takes place as well.

The onset and development of cirrhosis have previously been related to an increase in hepatic free radical generation in this experimental model, and the hepatoprotective effects of zinc have been shown to be associated with an inhibition of free radical production.²⁰ Serum PON1, which is carried in circulation bound to HDL particles, protects LDL from peroxidation. This protection may be related to the ability of PON1 to hydrolyze some oxidized phospholipids⁴ and cholesteryl hydroperoxides²¹ present in oxidized LDL. However, the protective effect of serum PON1 on LDL has been shown to be accompanied by some inactivation of the enzyme by oxidized lipids.²² To investigate whether lipid peroxidation products inhibited hepatic PON1 activity, an *in vitro* experiment was performed using hepatic microsomes. The results showed that microsomal PON1 activity was not affected either by CCl₄ or by MDA (Table 1). The reasons for this discrepancy from human serum PON1 inactivation by oxidized lipids cannot be ascertained from the present investigation. It could be related to the differences in organ and/or species investigated or, more likely, to the compositional differences of the oxidized lipids produced under different conditions. For example, the compounds involved in PON1 inactivation by LDL are oxidized arachidonate derivatives²³ that are likely to be in higher concentrations in LDL than in liver microsomes. These results suggest that the PON1 decrease in CCl₄-treated rats is not the

Table 1. PON Activity in Isolated Microsomes

	PON (U/mg)
Basal*	4.7 ± 0.5
CCl ₄	
9 mmol/L	4.3 ± 0.3
27 mmol/L	4.5 ± 1.0
81 mmol/L	5.1 ± 0.7
MDA	
15 μmol/L	4.9 ± 1.1
45 μmol/L	5.2 ± 0.3
135 μmol/L	4.6 ± 0.8

NOTE. Results are expressed as means ± SD.

*No xenobiotics added.

consequence of direct inhibition of lipid peroxidation products on PON1 activity.

In summary, the present investigation shows for the first time that a decrease in liver microsomal PON1 activity is an early biochemical change related to lipid peroxidation and liver injury observed in rats with CCl₄-induced cirrhosis. As such, this enzyme may have a role within the antioxidant systems of liver microsomes. Further studies are needed to fully define this role and to establish the relationships between hepatic PON1 alterations and the pathophysiology of chronic liver disease.

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