Ann Natl Acad Med Sci (India), **44(1):** 1-10, 2008

Oxidative Stress in the Development and Complications of Liver Cirrhosis

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Abstract

End stage-liver cirrhosis is associated with complications such as spontaneous bacterial peritonitis and hepatorenal syndrome. The generation of free radicals and biochemical alterations at the cellular and subcellular level in the intestine and kidney has been suggested to play an important role in these complications of cirrhosis. In addition, oxidative stress induced alterations in the intestinal cell surface glycosylation and qualitative, quantitative changes in the luminal bacterial flora might result in damage to the intestinal barrier and enhance bacterial adherence, resulting in translocation of bacteria into ascitic fluid leading to bacterial peritonitis. This review highlights the important role of oxygen free radicals involved in the different organ damage during and after development of experimental model of liver cirrhosis.

Key words: Spontaneous bacterial peritonitis, oxidative stress, ascites, carbon tetrachloride and thioacetamide

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Introduction

Oxidative stress elicited by reactive oxygen species has been suggested to play a role in the development of human liver disorders such as chronic viral hepatitis, alcoholic liver disease and primary biliary cirrhosis (1). Oxidative stress arises when there is an imbalance between radical-generating and radical–scavenging activity (2). Various studies have shown an increase in oxidative damage to lipids and proteins in serum and liver tissue as assessed by increased malondialdehyde and protein carbonyls content in patients with liver cirrhosis, which correlated with decreased antioxidant defense mechanisms seen in these patients (2-4). In addition, many etiological agents of fibrogenesis stimulate free radical generations (5), implicating these active species in the process. At the cellular level, it has also been shown that reactive oxygen species generated by the mitochondrial electron transport chain during experimental liver cirrhosis can damage subcellular compartments such as microsomes and mitochondria (6,7).

Liver cirrhosis is an advanced stage of progressive fibrosis due to chronic ongoing liver injury and is associated with two important cellular events: continuous hepatocyte loss and activation of hepatic stellate cells. Activation of hepatic stellate cells (HSC) is associated with increased extra cellular matrix production, which surrounds the regenerating hepatocytes (8,9). The development of liver fibrosis or cirrhosis is associated with increased morbidity and mortality(10). Most common etiologies of liver cirrhosis include alcoholism, chronic viral hepatitis, bile duct obstruction and autoimmune hepatitis. Cirrhosis can be induced in rats by administering various hepatotoxins such as carbon tetrachloride (CCl_4), thioacetamide (TAA), ethionine, dimethyl nitrosamine or through common bile duct ligation. Studies have suggested that histological and biochemical changes that develop in animals chronically treated with hepatotoxins like CCl_4 and TAA are comparable to human cirrhosis with different etiology.

Comparison of two different experimental models of liver cirrhosis:

Our studies using CCl_4 and TAA in experimental animals have shown that liver cirrhosis developed by 3 months of treatment and by 5 months, both the models were similar, where micro nodular cirrhosis was seen in CCl_4 and macro nodular form in TAA. Increased lipid and protein oxidation were

observed in mitochondria, peroxisomes and microsomes from the liver after carbon tetrachloride or thioacetamide treatment at different stages of cirrhosis development. Oxidative stress was more severe in animals treated with CCL_4 than thioacetamide. Mild oxidative stress was evident at 1 and 2 months of treatment and a significant increase was seen by 3 months of treatment with either compound, when compared to controls. This was accompanied by a decrease in antioxidant enzymes suggesting a role of oxygen free radicals in the early development of fibrosis and cirrhosis in both the models. The gradual increase in oxidative stress in the subcellular organelles such as mitochondria, peroxisomes and microsomes suggests that ROS are generated early during development of liver cirrhosis, though it is not yet clear if these active species are a result or cause of liver injury (11).

Complications of liver cirrhosis:

Mortality due to liver cirrhosis is predominantly due to development of complications of the disease. These include portal hypertension, spontaneous bacterial peritonitis and hepatorenal syndrome, which are major end-stage complications of patients with liver cirrhosis (12). Bacterial infection is responsible for up to one quarter of the deaths of patients with chronic liver disease (13). Spontaneous bacterial peritonitis (SBP) is a common and serious infection developing in cirrhotic patients, which appears as a consequence of impaired defense mechanisms against infection (14). SBP is defined as an abrupt onset of acute bacterial peritonitis without any apparent external or intra-abdominal focus of infection in patients with ascites caused by liver disease (15). Translocation of bacteria from the intestinal lumen to blood stream has been suggested to be involved in the pathogenesis of SBP (16). End-stage liver cirrhosis also results in marked alterations in systemic circulation and renal function (17). The progressive reduction in the renal blood flow and glomerular filtration rate along with impaired ability to excrete sodium and water lead to ascites in cirrhosis (18, 19). The worsening of renal function and sodium and water retention in cirrhosis not only correlates with the elevation in plasma vasopressin, renin, aldosterone, and nor epinephrine but also with the degree of portal hypertension (20, 21).

Small intestinal alterations in animal model of liver cirrhosis:

Intestinal bacterial overgrowth, altered permeability of the mucosa and

deficiencies in host immune defenses has been implicated in the development of SBP. Clinical and experimental evidence indicate that translocation of bacteria from the intestinal lumen into mesenteric lymph node and to the blood stream is directly involved in the pathogenesis of SBP (16, 22). The gastrointestinal tract is affected during cirrhosis and mucosal abnormalities secondary to portal hypertension may exist (23). Our studies have demonstrated oxidative stress in the intestinal mucosa after CCl_4 induced-liver cirrhosis, where the activity of xanthine oxidase, an important source of free radicals in the small intestine is elevated (24,25). Mucosal alterations attributed to oxidative stress, including disturbed enterocyte mitochondrial function and increased lipid peroxidation of mitochondria and brush border membranes were also evident

Bacterial adherence is accomplished by specific adhesins on the outer surface of bacteria that attach to receptors containing sugars such as sialic acid, hexose, fucose and amino sugars on the surface of the epithelial cells (26,27). We showed that liver cirrhosis results in increased sugar content of both intestinal brush border membrane and surfactant layers. These changes could be the result of oxidative stress, since free radicals are known to modulate the glycosyl transferase or glycosidases which might in turn alter the glycosylation pattern (28,29). This was accompanied by changes in bacterial flora in the gut, which showed increased bacterial hydrophobicity and adherence onto the epithelial cells. This might facilitate translocation across the mucosa, resulting in complications such as SBP. The increased adherence of bacteria from cirrhotic rats was sugar specific, since prior addition of sugars such as galactose, fucose and mannose in the *in vitro* system, inhibits and reverses the adherence property of the bacteria. The role of oxidative stress was further confirmed by the inhibition of xanthine oxidase using sodium tungstate and antioxidant supplementation using vitamin E, which offered significant protection against the oxidative stress, changes in brush border membrane sugar content and bacterial adherence (30).

Spontaneous bacterial peritonitis (SBP) results in oxidative and nitrosative stress in ascitic fluid:

As an extension of the animal studies, we also carried out experiments on ascitic fluid from patients with SBP. Ascitic fluid from cirrhotic patients with and without SBP were examined for oxidative and nitrosative stress. The

first line defenses against infection in the peritoneal cavity are neutrophils and macrophages (31). Human neutrophils contain inducible nitric oxide synthase which produces nitric oxide (NO). Myeloperoxidase, an enzyme present in the neutrophils can produce oxygen free radicals. Simultaneous presence of both NO and ROS can result in the formation of reactive nitrogen species such as peroxynitrite (32), which is a highly reactive anion and contributes to microbial killing (33). In patients with SBP, increased nitrate and increased oxidative stress parameters such as malondialdehyde, protein carbonyls were evident in ascitic fluid as compared to cirrhotic controls without SBP. Treatment of these patients with the antibiotic cefotaxime for 48 hours reversed the oxidative stress and decreased the nitrate levels in ascitic fluid. Thus the measurement of oxidative stress markers and nitrate levels in the ascitic fluid would probably be useful in the diagnosis of SBP and in the follow-up after antibiotic treatment (34).

Renal damage in animal models of liver cirrhosis:

As mentioned earlier, the hepatorenal syndrome is a complication of cirrhosis, and it has been demonstrated that cirrhosis with ascites is associated with impaired renal function accompanied by sodium and water retention. Inhibition of nitric oxide synthases significantly improves renal function in cirrhotic animals, suggesting a role for nitric oxide in renal pathophysiological events induced by decompensated cirrhosis (35). Our ultra structural studies demonstrated mitochondrial dilation and glomerular epithelial swelling in the kidneys of cirrhotic animals. Platelets and cytoplasmic blebs were also present in the kidneys of rats treated with both carbon tetrachloride and thioacetamide for a period of 3 months. It was observed that cirrhosis results in oxidative stress in the kidney as seen by increased lipid peroxidation and protein oxidation parameters, accompanied by decreased anti-oxidant status. Liver cirrhosis also affected the function of renal mitochondria, as seen by decreased respiratory control ratio, swelling of mitochondria and altered calcium flux across the mitochondrial membranes. Increased lipid peroxidation and changes in lipid composition were also evident in the renal brush border membranes, with compromised transport across these membranes. What could be the sources for these oxygen free radicals? The uncoupling of

mitochondrial respiration and oxidative phosphorylation in the kidney, indicated by our experimental evidence, might result in enhanced generation of super oxide anions which may be involved in the damage. In addition, free radicals generated from infiltrating neutrophils have been proposed to be a major cause for cellular damage associated with many chronic inflammatory diseases (36). We observed an increased activity of myeloperoxidase in the kidney of cirrhotic animals indicating neutrophil infiltration, suggesting that this could be an additional source for reactive oxygen species in this context. The changes seen in the kidney *per se*, as well as in renal mitochondria and renal brush border membrane were minimal after the first and second months of treatment with these hepatotoxins and were prominent only after full development of liver cirrhosis, which occurred after 3 months of treatment (37). These ultrastructural and biochemical changes seen in the kidney might play an important role in the development of complications of cirrhosis such as hepatorenal syndrome.

Conclusion

The studies presented here have demonstrated the involvement of oxygen free radicals in the development of liver cirrhosis and its complications in experimental animals. Oxidative stress was seen in the liver of rats treated with carbon tetrachloride and thioacetamide for a period of 3 months which continued to increase till five months of treatment. Liver cirrhosis also resulted in oxidative stress in the intestine due to the generation of super oxide from xanthine oxidase and mitochondrial dysfunction resulting in protein and lipid oxidation. Development of liver cirrhosis resulted in alterations in the intestinal cell surface glycosylation and qualitative and quantitative changes in the luminal bacteria. These alterations in the glycocalyx on the surface of the intestinal epithelium may result in damage to the intestinal barrier and enhance bacterial adherence, resulting in translocation of bacteria into ascitic fluid leading to bacterial peritonitis. In addition, patients with spontaneous bacterial peritonitis showed evidence of oxidative stress and increased nitrate level in the ascitic fluid which was decreased with antibiotic treatment. Oxidative stress was also observed in the kidney in experimental liver cirrhosis which was evident at 3 months of treatment and continued to increase till 5 months, suggesting a role for liver cirrhosis induced oxidative stress in the renal damage seen in cirrhosis (Fig 1).

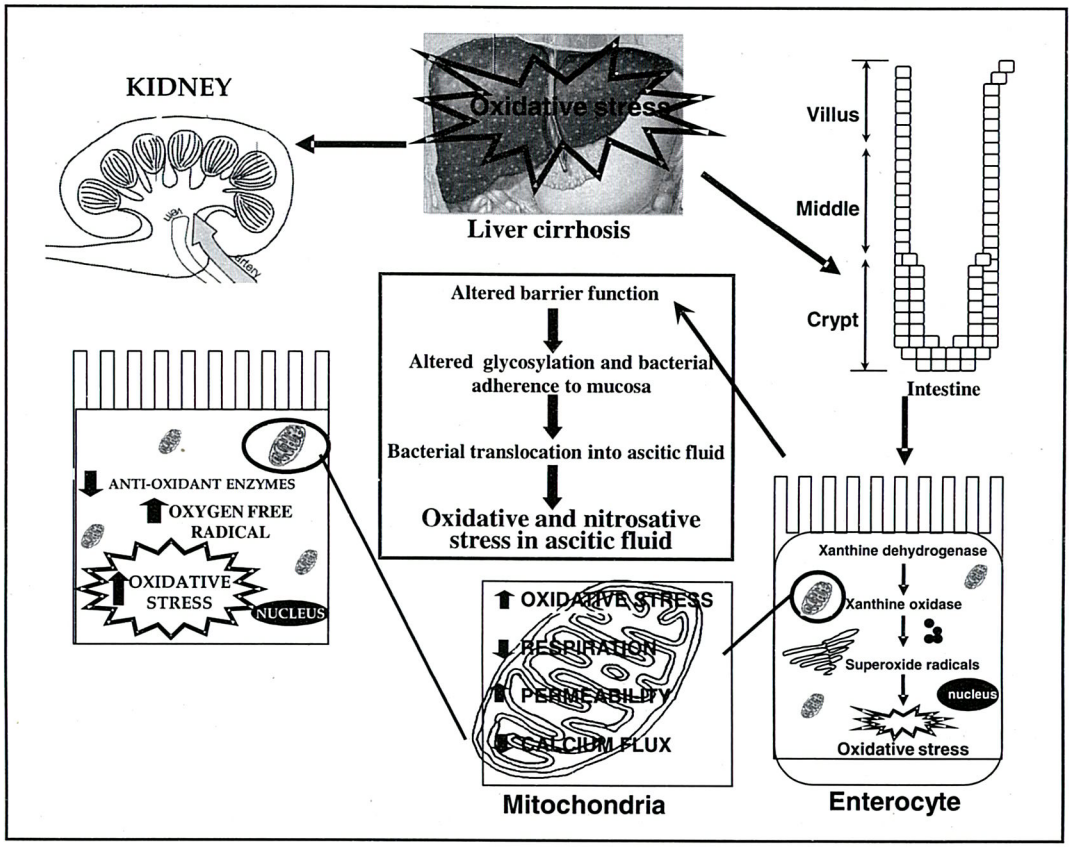

Figure 1: Events in the complications of liver cirrhosis. Oxidative stress in the intestine following liver cirrhos is due to the possible conversion of xanthine dehydrogenase into xanthine oxidase leading to increased super oxide generation and brush border membrane damage. These changes together result in altered gut barrier function followed by increased glycosylation and increased bacterial adherence into mucosa, and thereby translocation of bacteria into ascitic fluid. On the other hand, increased oxygen free radicals and decreased antioxidant enzymes were also observed in the kidney. In addition to this, mitochondrial dysfunction was evident in both intestine and kidney following liver cirrhosis.

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