Hepatitis C virus (HCV) infection and hepatocellular carcinoma (HCC) are growing health problems around the world. Oxidative stress plays a significant role in the initiation and progression of hepatocellular damage and possibly in the development of HCC in HCV infected patients. In vitro, animal and clinical studies suggest that lycopene, a nonprovitamin A carotenoid and a potent antioxidant, may attenuate the liver injury and possibly prevent the development of HCC. In this article, we discuss the relationship between HCV infection and oxidative stress and review the potential role of lycopene in the treatment of HCV and prevention of HCC.

INTRODUCTION

Hepatitis C virus (HCV) infection is a global public health problem. It is estimated that approximately 3.1% of the world population are chronically infected with HCV. In the United States, at least 2.7 million people are believed to be chronically HCV infected. End stage liver disease due to chronic HCV infection is the most common indication for liver transplantation, and HCV is one of the most common causes of hepatitis, cirrhosis, and hepatocellular carcinoma (HCC) in the United States (1).

In contrast to acute hepatitis B infection in which spontaneous resolution of infection can occur in a proportion of patients throughout the course of the disease, it appears that spontaneous resolution of well-established chronic hepatitis C (CHC) is unusual after the first year or two of HCV infection. Overall, about 75–85% of acute HCV infections become chronic infections. It has been estimated that approximately 30% of patients with chronic HCV infection are at risk for developing long-term complications such as cirrhosis and HCC (2).

It has been estimated that within the next 10 yr, over 800,000 Americans will develop advanced HCV-related liver disease due to progression of disease, and the number of liver disease related deaths in the United States is likely to exceed 40,000/year by 2040 (3). Thus, there is an urgent need for continued optimization of anti-HCV therapies to reduce HCV-associated mortality and morbidity in the coming decades.

In this article, we review the association of oxidative stress with HCV infection, and we discuss the potential role of lycopene in the treatment of CHC and in the prevention of its progression to cirrhosis and HCC.
Lycopene

Lycopene is an open-chain, unsaturated carotenoid with a molecular formula C_{40}H_{56} (Fig. 1). It is responsible for the red color in fruits and vegetables like tomatoes, watermelon, grapefruit, guava, rosehip, and red chilies. Its name is derived from the tomato’s species classification Solanum lycopersicum. Almost all dietary lycopene comes from tomato and tomato product consumption (4). Following absorption into enterocytes, all carotenoids including lycopene are incorporated into triglyceride-rich chylomicron particles for transport to the liver, the major storage site. In the body, lycopene is also deposited in the lungs, prostate gland, testes, colon, and skin. Secondary organs are supplied lycopene from the liver lycopene pool (5). Its concentration in body tissue tends to be higher than all other carotenoids.

Lycopene, a nonprovitamin A carotenoid, is the most efficient singlet-oxygen (a reactive oxygen species) quencher among the natural carotenoids. In healthy human subjects, lycopene- or tomato-free diets result in loss of lycopene and an increase in lipid oxidation. The plasma depletion half-life of lycopene was estimated to be between 12 to 33 days. Moxley et al. (6) showed that healthy individuals consuming a lycopene free diet exhibit a decrease in total serum lycopene of 49% in 14 days. One study has shown that lycopene may have cholesterol lowering properties similar to statins (7).

Although best known as an antioxidant, both oxidative and nonoxidative mechanisms may be involved in lycopene’s biological activity. The biological activities of certain carotenoids such as β-carotene are related to their ability to form vitamin A within the body. Because lycopene lacks a β-ionone ring structure, it cannot form vitamin A, and its biological effects in humans has been attributed to mechanisms other than vitamin A. Lycopene’s configuration enables it to inactivate free radicals and to interfere with free-radical-initiated reactions, particularly lipid peroxidation, thereby preventing tissue injury (8). Because free radicals are electrochemically imbalanced molecules, they are highly reactive and capable of reacting with cell components and causing permanent damage. These toxic chemicals are formed naturally as by-products during oxidative cellular metabolism, infections, and inflammation. Lycopene has singlet-oxygen-quenching ability twice that of β-carotene and 10 times higher than that of α-tocopherol (vitamin E). Its oxygen-quenching ability protects against oxidative DNA damage in vitro and in vivo, thereby preventing potential mutations that may be associated with cancer initiation and progression (9,10). In an organic solution, lycopene was the most rapidly destroyed carotenoid on reaction with peroxyl radicals, indicating its presence in the first line of defense (11). However, studies have shown that the antioxidant effects of lycopene are not powerful enough by themselves to protect liver tissue in the event of acute injury due to oxidative stress (12).

Oxidative Stress and Hepatitis C Viral Infection

The involvement of oxidative stress in the pathogenesis of hepatitis and HCC has been strongly suggested (13). Increased oxidative DNA damage was found not only in hepatitis C but also in transgenic mice expressing hepatitis B virus (14). However, the role of oxidative stress in the progression of chronic hepatitis and hepatocarcinogenesis may be greater in hepatitis C than in other types of hepatitis such as hepatitis B or autoimmune hepatitis (15). Oxidative stress refers to an imbalance between the oxidation reaction caused by reactive oxygen species (ROS) and the antioxidation reaction caused by antioxidant molecules such as lycopene.

The main source of ROS in hepatocytes is the mitochondria. Hepatocytes contain many mitochondria and thus have high production of ROS. In HCV core gene transgenic mice, the malfunction of the electron transfer system of mitochondria has been suggested as an origin of ROS overproduction (13). Additionally, on viral infection, there is a greater degree of ROS production in inflammatory cells by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (31) which is induced by HCV proteins, especially NS3 (a nonstructural protein) (14). Normally, superoxide radical produced in mitochondria is converted to water by detoxification enzymes such as superoxide dismutase and glutathione peroxidase (GPX). However, when these enzymes can not convert the superoxide radicals to water fast enough, oxidative damage occurs in mitochondria. In Hepatitis C, increased production of ROS and the accumulation of mitochondrial DNA damage resulting in mitochondrial dysfunction has been shown (15). Also, HCV RNA was reported to induce nitric oxide synthase (iNOS), which results in increased nitric oxide, a strong oxidant. Indeed, iNOS synthesis was found to be correlated with the intrahepatic viral load in CHC (16). Moreover, cytokines released from inflammatory cells may further induce ROS production from hepatocytes. Another important mechanism of oxidative stress was postulated to be the excessively high iron content in the liver of CHC patients (17). Free iron has been shown to catalyze reactions producing highly reactive HO· (hydroxyl radical), leading to more cellular membrane oxidation and lipid peroxidation (18). The fact that iron removal therapy significantly improves the serum ALT levels in CHC patients supports the close relationship between oxidative stress and liver damage in CHC (19).
Antioxidant Depletion in Chronic Hepatitis C

In previous clinical studies, it has been shown that antioxidants (AO) are severely depleted in serum and liver tissue of patients with chronic hepatitis C infection (20). The inverse relationship between serum antioxidants and viral disease progression has been demonstrated in several studies. In 1 of these, Yadav et al. (21) analyzed serum and liver levels of retinol, tocopherols, lutein, lycopene, α-carotene and β-carotene, and the lipid peroxidation product malondialdehyde (MDA) in 20 patients with CHC and compared them to the 22 healthy individuals who were not on any vitamin supplements. Patients with CHC had lower serum and liver AO levels and higher serum MDA levels. Both MDA and AO levels were correlated with the degree of fibrosis and inflammation in the liver. This study suggests that increasing fibrosis associated with decreased liver antioxidant levels may be a consequence of AO depletion due to oxidative stress or decreased AO storage due to fibrosis. Antioxidant depletion in the liver is postulated to play a role even at the early stages of liver injury until subsequent cirrhosis and HCC develops. Yamamoto et al. (22) measured the oxidized form of ubiquinol-10 (lipid-soluble antioxidant, ubiquinone-10) in plasma of patients with chronic active hepatitis, cirrhosis, and HCC. All groups had a significant increase in ubiquinone-10 percentage, with highest levels observed in patients with HCC. Additionally, there may be a functional defect in the mobilization of AO from the liver in patients with CHC. Rocchi et al. (23) showed decreased serum levels of AO in patients with CHC.

Oxidative Stress and Hepatic Fibrosis

Oxidative stress leads to hepatic fibrosis, which is now believed to be the most important prognostic factor determining the progression of liver disease in CHC (24). Currently, one of the more common causes of hepatic fibrosis is CHC infection in which hepatic steatosis is frequently observed. It has been suggested that HCV nonstructural core protein plays a crucial role in hepatic steatosis (25). Hepatic steatosis leads to an increase in lipid peroxidation and oxidative stress in hepatocytes, which stimulates further lipid peroxidation and hepatic steatosis leading to necroinflammation. The lipid peroxides formed can be chemotactic for neutrophils, causing increased inflammation, which further drives oxidant-mediated injury in the liver (26). Enhanced oxidative stress also initiates a fibrogenesis cascade in the liver of patients with CHC infection and mediates activation of collagen type I gene expression from hepatic stellate cells (HSC) (27,28). HSCs are regarded as the primary target cells for inflammatory stimuli and produce extracellular components like collagen, which leads to fibrosis.

Antioxidants in the Treatment of Chronic Hepatitis C

Previous clinical trials have suggested that antioxidant therapy may have a beneficial effect in patients with CHC infection. Although clinical studies with AO have been conducted in patients with CHC, lycopene has not been studied alone or in combination with antiviral treatment in CHC. Melhem et al. (29) conducted a Phase I clinical trial assessing 50 CHC patients treated with 7 antioxidative oral preparations along with 4 different intravenous preparations daily for 10 wk, monitoring HCV-RNA levels, liver enzymes, and liver histology. The results showed normalization of liver enzymes in 44% of patients, a decrease in viral load in 25% of the patients, and histologic improvement (2-point reduction in the hepatic activity index, HAI) score) in 36.1% of the patients. Treatment was well tolerated by all, and no side effects were noted. In another study, 23 CHC patients refractory to α-interferon therapy were treated with high dose vitamin E (α-tocopherol), a lipid-soluble antioxidant, for 12 wk. In 11 of 23 patients, aminotransferases ALT and AST were lowered by 46% and 35%, respectively, but was followed by a rapid relapse on cessation of treatment with vitamin E (30). Similarly Mahmood et al. (31) had demonstrated decreased serum ALT and thioredoxin (TRX, antioxidant) following the administration of vitamin E. It supports the idea that viral hepatitis is associated with inflammatory events known to be directly related to formation of reactive oxygen species capable of inducing oxidative damage to cellular membranes and DNA. There have been mixed results of N-acetyl cysteine administration in patients with CHC. Ideo et al. (32) conducted a randomized clinical trial assessing the efficacy of α-interferon (IFN) in combination with the antioxidants N-acetyl cysteine and vitamin E in patients who had no response to previous course of IFN therapy. Neither end of treatment response (ETR) nor sustained viral response (SVR) was improved by combination therapy. In contrast, Beloqui et al. (33) reported decreased transaminases and viral load with combined N-acetyl cysteine and IFN therapy in IFN-unresponsive patients. Interferon administration alone was demonstrated to decrease levels of hydroxyl-2-nonenal (HNE, a peroxidation reaction product of lipids) markedly in patients with CHC (34). This benefit of current antiviral therapy could be enhanced by the coadministration of antioxidants like lycopene.

As stated above, steatosis or steatohepatitis (SH) is the initial step in the fibrosis cascade, and its significance on response to treatment of CHC has been shown by Harrison et al. (35). The overall SVR in patients with CHC plus steatosis or SH was 28% compared with 44% in patients with CHC without any steatosis or SH. Another significant observation in patients with CHC who respond to antiviral treatment was an increased caspase activity and production of apoptosis-associated proteins (P < 0.05) when compared to nonresponders. This was closely correlated with virus elimination. Lycopene has been shown to activate the caspase system. In addition to lycopene’s antioxidant effects, enhanced caspase activity might play a role in HCV clearance and could also predict the efficacy of antiviral treatment (36).

Although eradication of HCV RNA is the primary goal of treatment, oxidative stress and lipid peroxidation play major roles in the fatty accumulation in the liver and development of
hepatic fibrosis in CHC. These are the most important prognostic factors determining the progression of liver disease. Antioxidants may have a secondary benefit of reducing progression to fibrosis, thereby decreasing the progression to cirrhosis or possibly reversing early cirrhosis. In the long term, there may be a lower frequency of development of HCC (37). Further clinical trials should be conducted to assess the effect of antioxidants in the treatment of CHC and on disease progression. Lycopene, a powerful antioxidant compound, could break this oxidative stress-fibrosis cycle and also decrease hepatic necroinflammation by its anti-inflammatory effects.

**Hepatocarcinogenesis and Oxidative Stress**

Oxidative stress has been postulated to play an important role in hepatocarcinogenesis in CHC (38). As mentioned previously, mitochondrial DNA, the major source of ROS production in the liver, lacks the protective mechanisms, so it is more susceptible to ROS induced mutations. It is believed that viral core protein acts on the function of mitochondria, leading to overproduction of oxidative stress, which yields genetic aberrations in cell-growth-related genes (38). Both malignant and nonneoplastic liver tissues in patients with HCC showed very high indexes of mitochondrial DNA mutations, which can explain the relationship between oxidative stress induced DNA damage and HCC development (39). Both mitochondrial oxidative stress and endoplasmic reticulum (ER) stress has been hypothesized to result in intracellular and extracellular accumulation of DNA-damaging factors that could predispose a cell to mutagenesis (40).

**Hepatocellular Carcinoma**

Hepatocellular carcinoma has become a disease of increased significance in the United States. Surveillance, Epidemiology, and End Results (SEER) data showed a doubling of HCC incidence rates in the United States between 1985 and 1998, reaching 4.1 per 100,000 persons in 2000, with a greater increase in the incidence rates in younger age groups (41,42).

Worldwide, liver cancer is the third most common cause of cancer mortality, with approximately 550,000 annual deaths (43). Liver cancer is the 8th most frequent cause of cancer mortality in U.S. men and the 12th in women (44). The American Cancer Society estimated 14,270 deaths from liver cancer in the United States in 2004, of which 70%–80% were HCC.

Chronic infection with HCV and/or hepatitis B virus (HBV) is associated with the majority of HCC cases. Recent estimates for the United States have attributed 47% of cases to HCV alone, 15% to HBV alone, and 5% to coinfection with both viruses (45). The remaining 33% of cases appear to be due to nonviral causes. Once HCV infection develops into cirrhosis, HCC develops at an annual rate of 5–7% (42).

**Lycopene and HCC**

The unusually high concentration of lycopene in the liver has prompted researchers to explore the association of lycopene and risk of HCC (Table 1). Evolving evidence suggests that carotenoids may modulate processes related to mutagenesis, carcinogenesis, cell differentiation, and proliferation independent of their roles as AOs or precursors of vitamin A (46,47).

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*Abbreviations are as follows: Hep3B, hepatitis 3B; HCC, hepatocellular carcinoma; LEC, Long-Evans Cinnamon.*
In vitro AFB1-DNA adduct, AFB1-N7-guanine, was significantly higher in subjects with HCC than in controls (HCC patients with chronic hepatitis and cirrhosis showed that the subjects with low plasma levels of carotenoids and lycopene compared to those with high levels. However, in a study with Long-Evans Cinnamon rats, lycopene administration did not reduce the risk of spontaneous liver carcinogenesis (63).

To date, there have been few randomized clinical trials testing the chemopreventive effect of antioxidants on hepatocarcinogenesis. In 1 clinical trial, Takagi et al. (64) divided 83 patients with liver cirrhosis and chronic HCV infection into two groups. After a follow-up of 5 yr, cumulative tumor-free survival and cumulative overall survival rates were higher in the vitamin E group compared to the control group, but this was not statistically significant. Neither improvement in liver function, cumulative survival, or suppression of hepatocarcinogenesis could be demonstrated in the vitamin E group. Only 1 case-control study has examined the relationship between lycopene and aflatoxin B1 (AFB1) initiated hepatocellular carcinoma. Lycopene was thought to influence the binding of AFB1 to hepatic DNA. The urine level of the major urinary carcinogen metabolizing enzymes (56) and i) modulation of immune function (57).

Brienholt et al. (58) also found that lycopene significantly induces phase I enzymes, such as cytochrome P450-dependent enzymes, in a dose-dependent manner and hepatic quinone reductase (QR), a phase II enzyme, by twofold. This class of enzymes is important for the removal of the foreign substances and carcinogens from the body. Lycopene is thought to inhibit proliferation of cancerous cells at the G0-G1 cell cycle phase. In a study on Hep3B human hepatoma cell line, lycopene induced G0/G1 arrest, S phase block, and cell growth inhibition in a dose-dependent manner by almost 40% (59). In addition, lycopene’s antimetastatic properties were shown by adhesion and migration assays on SK-Hep1 human hepatoma cell line. Invasiveness of the cells was reduced by 62% after treatment with 10 mM lycopene (60).

Lycopene has been reported to inhibit the hepatotoxic and carcinogenic effects of aflatoxin B1, alcohol, and smoking. Reddy et al. (61) observed that hepatocytes pretreated with lycopene and β-carotene were protected from the effects of the hepatocarcinogen aflatoxin at both cellular and molecular levels. Yu et al. (62) reported that the effect of alcohol consumption and tobacco smoke on HCC was significantly higher among patients with low plasma levels of carotenoids and lycopene compared to those with high levels. However, in a study with Long-Evans Cinnamon rats, lycopene administration did not reduce the risk of spontaneous liver carcinogenesis (63).

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In an animal model of hepatocarcinogenesis in rats, lycopene decreased the size of preneoplastic foci in liver induced by diethylnitrosamine but did not reduce the number of lesions (67). In a separate mouse study, lycopene (0.005% in drinking water) was given to 30 C3H/He male mice for 40 wk. In contrast Gradlet et al. (68) demonstrated that lycopene reduced the number of liver tumor-bearing mice by 49.7% (88.2% for control vs. 38.5% for lycopene-treated mice) and reduced the average number of tumors per mouse by 88% (7.65–0.92).

Because studies have suggested that lycopene has both antiviral and anticarcinogenic effects on virulent strains of some viruses, the potential prevention of liver HCC by administering lycopene along with antiviral treatment in HCV is an attractive concept. Previous studies have shown that increased lycopene levels have been associated with increased clearance of oncogenic human papilloma virus (HPV) infection (69). Lycopene and other AOs have shown similar anticarcinogenic effects. Beck et al. (70) demonstrated that selenium deficient mice are more prone to develop mutations in the viral genome of both the coxsackie and influenza viruses. Selenium deficiency in mice was associated with conversion of nonvirulent strains of virus to virulent strains suggesting that not only lycopene but other antioxidant micronutrients may have a role in mutagenesis.

**CONCLUSION**

There appears to be an impairment of the antioxidant system in CHC, and oxidative stress plays a central role in the pathogenesis and progression of the disease. Lycopene is a powerful antioxidant and may counteract the liver damage in CHC. Accumulating evidence from cell culture, epidemiologic, animal, and human clinical trials suggests a potential role for lycopene in the prevention of HCC. In addition, lycopene may also enhance overall response rate to antiviral therapy and delay the progression of disease. Further clinical trials are needed to further investigate the potential role of lycopene in decreasing the risk of hepatocellular cancer as well as its potential use as an adjunct to antiviral therapy.

**REFERENCES**


