

Prevention of hepatocellular carcinoma: Focusing on antioxidant therapy

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Abstract

Oxidative stress has been investigated in the context of alcoholic liver injury for many years and shown to be a causal factor of chronic hepatitis C (CHC), nonalcoholic steatohepatitis (NASH), drug-induced liver injury, Wilson's disease, and hemochromatosis. In CHC, it has been demonstrated that oxidative stress plays an important role in hepatocarcinogenesis. In cases with persistent hepatitis due to failure of hepatitis C virus eradication,

or chronic liver disease, such as NASH, the treatment of which remains unestablished, it is important to reduce serum alanine aminotransferase levels and prevent liver fibrosis and development of hepatocellular carcinoma. This also suggests the importance of antioxidant therapy. Among treatment options where it would be expected that anti-inflammatory activity plays a role in their confirmed efficacy for chronic hepatitis, iron depletion therapy, glycyrrhizin, ursodeoxycholic acid, Sho-Saiko-To, and vitamin E can all be considered antioxidant therapies. To date, however, the ability of these treatments to prevent cancer has been confirmed only in CHC. Nevertheless, anti-inflammatory and anti-fibrotic effects have been demonstrated in other liver diseases and these therapies may potentially be effective for cancer prevention.

Key words: Chronic hepatitis; Antioxidant therapy; Hepatocellular carcinoma; Prevention; Iron depletion therapy

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Core tip: Among treatment options where it would be expected that anti-inflammatory activity plays a role in their confirmed efficacy for chronic hepatitis, iron depletion therapy, glycyrrhizin, ursodeoxycholic acid, Sho-Saiko-To, and vitamin E can all be considered antioxidant therapies. In chronic liver diseases, it has been demonstrated that antioxidant therapy may potentially be effective for suppressing inflammation and liver fibrosis and expected to prevent carcinogenesis.

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INTRODUCTION

Oxidative stress has been investigated for many years as a possible cause of alcoholic liver injury. Recently, it has attracted attention as one of the causal factors for a variety of liver diseases, such as chronic hepatitis C (CHC), nonalcoholic steatohepatitis (NASH), drug-induced liver injury, Wilson's disease, and hemochromatosis. Furthermore, it has been demonstrated that oxidative stress plays an important role in hepatocarcinogenesis in CHC.

Recent studies have shown that excess hepatic iron accumulation in CHC patients contributes to liver injury^[1-3]. It is believed that free iron in the liver facilitates the formation of reactive oxygen species (ROS), including hydroxyl radicals ($\bullet\text{OH}$), which cause oxidative damage to numerous cellular components, including lipids, proteins and nucleic acids, and also cause an up-regulation of collagen synthesis^[4]. Further, $\bullet\text{OH}$ is known to generate promutagenic bases such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), which has been implicated in spontaneous DNA mutagenesis and carcinogenesis^[5,6]. Although the mechanism of hepatocarcinogenesis due to hepatitis C virus (HCV) infection remains unclear, long-term follow-up studies indicate that most patients with progressive liver disease who develop cirrhosis and/or hepatocellular carcinoma (HCC) have persistently elevated or fluctuating serum alanine aminotransferase (ALT) levels, suggesting that they have a background of chronic active inflammation and regeneration of the liver^[7]. Further, we have demonstrated in a 6-year follow-up study of CHC patients that iron depletion therapy, consisting of intermittent phlebotomies and a low-iron diet, significantly reduced serum ALT levels, the histological hepatic fibrosis grade, and hepatic 8-OHdG levels^[3].

In cases with persistent hepatitis due to failure of HCV eradication and chronic liver disease, such as NASH, the treatment of which remains unestablished, it is important to reduce serum ALT levels and prevent liver fibrosis and development of HCC. This also suggests the importance of antioxidant therapy. To date, reported effective treatment options expected to exert anti-inflammatory activity for chronic hepatitis include iron depletion therapy, glycyrrhizin, ursodeoxycholic acid, and Sho-Saiko-To, which can be considered as antioxidant therapies. Cancer prevention by antioxidants such as vitamin E has also been investigated (Table 1). Here, we review iron depletion as an antioxidant therapy for the treatment of the inflammatory effects of chronic hepatitis to reduce fibrosis and prevent cancer, as illustrated in this paper by an analysis of own cases.

IRON DEPLETION THERAPY

The liver is the major iron storage organ in the body; thus, it is not surprising that disorders of iron

metabolism are involved in chronic liver diseases. We have shown previously that in Long-Evans cinnamon rats, an abrupt accumulation of iron in the liver causes spontaneous hepatitis and subsequent development of HCC^[8]. Free iron in the liver is believed to catalyze the formation of ROS^[4]. In particular, the Fenton reaction, in which Fe^{3+} , $\bullet\text{OH}$ and OH^- are produced in the presence of Fe^{2+} and H_2O_2 , generates large amounts of highly toxic promutagenic ROS hydroxyl radicals ($\bullet\text{OH}$)^[9,10].

Iron overload in the setting of hereditary hemochromatosis has long been known to be associated with an increased risk for HCC^[11]. Standard of care is phlebotomy to reduce total body iron levels and achieve normal ferritin levels. Although for ethical reasons the beneficial effect of phlebotomy has never been formally demonstrated in controlled trials, Bomford *et al.*^[12] reported that the percentage survival 5 years after diagnosis was 66% in 85 patients treated by phlebotomy, and 18% in 26 untreated patients who died before phlebotomy had become widely accepted. Hepatic iron accumulation has also been reported in patients with CHC^[13] but the mechanisms responsible for this have not been fully elucidated. Possibly, inflammatory cytokines stimulate iron uptake *via* up-regulation of transferrin receptor expression in hepatocytes, as described previously^[14]. Nishina *et al.*^[15] demonstrated in mice that HCV-induced reactive oxygen species may down-regulate hepcidin transcription which leads to increased duodenal iron transport and macrophage iron release, causing hepatic iron accumulation. We have reported previously that iron depletion improves serum ALT levels as well as hepatic oxidative DNA damage in patients with CHC, and that long-term phlebotomy together with a low-iron diet lowers the risk of developing HCC^[3,16]. In this cohort study, we undertook weekly phlebotomy (200 g) until the patients achieved a state of mild iron deficiency, and we followed this by monthly maintenance phlebotomy for 107 mo (median). Patients were advised to consume a low-iron diet (5-7 mg iron/d). We have continuously followed these patients, with the result shown in Figure 1. If dietary iron intake is not restricted, phlebotomy may lead to enhanced iron absorption; therefore, a low-iron diet is essential for a successful outcome of this treatment.

It was recently reported that a high frequency of patients with NASH develop HCC. NASH is a severe form of nonalcoholic fatty liver disease (NAFLD)^[17] suggested by Day *et al.*^[18] to require two hits for its development, (1) excess accumulation of triglyceride in the hepatocyte; and (2) factors such as free radicals capable of inducing oxidative stress. Slight increases of hepatic iron concentration have been reported in NAFLD/NASH patients^[19]. Although the exact mechanisms involved in iron overload remain to be clarified, it can be hypothesized that insulin plays a role by stimulating cellular iron uptake through

Table 1 Clinical trials of chemoprevention effects in hepatocarcinogenesis

Therapy	Ref.	Year	Study design	Treated patients/control	Disease	Combined medication	Hepatocarcinogenesis rate
Phlebotomy	Kato <i>et al</i> ^[16]	2007	Open labeled	35/40	Chronic hepatitis C	None	Hepatocarcinogenesis rates in iron depletion and control were 5.7% and 17.5% at the end of the fifth year, and 8.6% and 39% in the tenth year, respectively ($P = 0.018$)
Glycyrrhizin	Ikeda ^[29]	2007	Case-control	244/102	Chronic hepatitis C	None	Crude carcinogenesis rates in the treated and untreated group were 13.3%, 26.0% at the fifth year, and 21.5% and 35.5% at the 10 th year, respectively ($P = 0.021$)
Glycyrrhizin	Arase <i>et al</i> ^[32]	1997	Case-control	84/109	Chronic hepatitis C	None	The 10 th -year rates of cumulative HCC incidence for the treated and untreated group were 7% and 12%, and the 15 th -yr rates were 12% and 25%, respectively ($P = 0.032$)
Ursodeoxycholic Acid	Tarao <i>et al</i> ^[44]	2005	Case-control	56/46	Hepatitis C virus -associated liver cirrhosis	Sho-saiko-to, Ursodeoxycholic acid	The cumulative 5-yr incidence of HCC in the patients treated with UDCA was 17.9% and was significantly lower than that in patients not treated with UDCA (39.1%; $P = 0.025$)
Vitamin E	Kakizaki <i>et al</i> ^[48]	2001	Randomized controlled	44/39	Chronic hepatitis C	None	Cumulative tumor-free survival tended to be higher in the Vit E group than in controls, albeit statistically insignificant
Sho-saiko-to	Oka <i>et al</i> ^[64]	1995	Randomized open controlled	130/130	Cirrhosis from chronic liver disease	None	The cumulative incidence curve for 5 yr of the trial group was lower than that of the control group ($P = 0.071$), albeit statistically insignificant

HCC: Hepatocellular carcinoma; UDCA: Ursodeoxycholic acid.

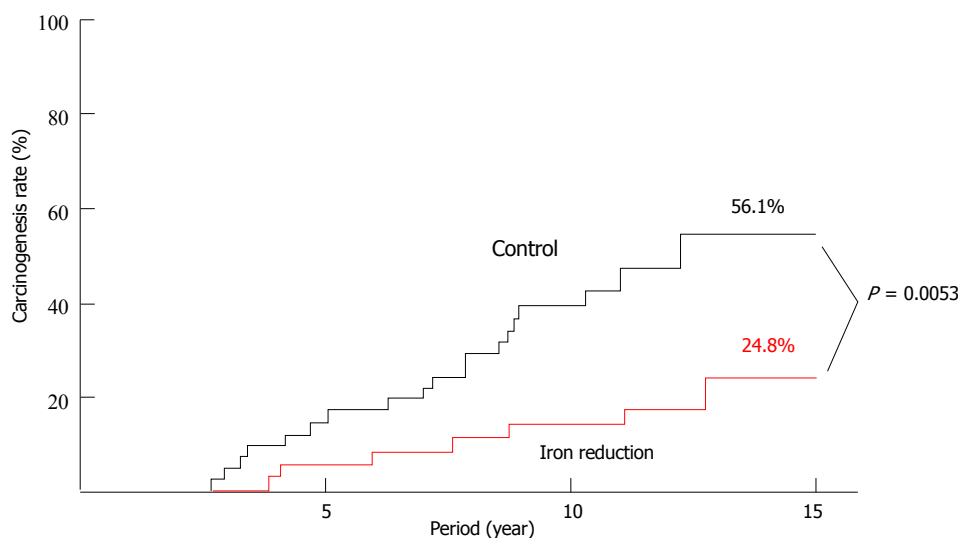


Figure 1 Crude hepatocarcinogenesis rate in iron reduction and control groups.

increased transferrin receptor expression^[20]. Facchini *et al*^[21] reported an improvement in ALT levels and plasma insulin concentrations following phlebotomy in 17 NAFLD patients with impaired glucose tolerance. Riquelme *et al*^[22] reported histological resolution of NASH after iron depletion therapy in a case report. According to Fargion *et al*^[23], HOMA-IR and ALT were significantly reduced after phlebotomy in 42 patients with NAFLD. Sumida *et al*^[24] also reported that aspartate aminotransferase (AST) and ALT were reduced by phlebotomy in 9 Japanese patients with NASH. Valenti *et al*^[25] reported that 64 NAFLD patients

treated by phlebotomy achieved significant reduction in insulin resistance compared with 64 NAFLD patients who underwent lifestyle modifications only. Fujita *et al*^[26] showed that iron reduction resulting from a combination of phlebotomy and a low iron diet resulted not only in improvement of ALT levels but also normalization of hepatic levels of 8-OHdG in 11 NASH patients. In a phase II trial on 31 patients with NAFLD, phlebotomy resulted in a significant improvement in the NAFLD activity score (NAS), AST and ALT^[27]. In a phase III trial, Valenti *et al*^[28] studied 38 NAFLD patients randomized to phlebotomy ($n = 21$) or

lifestyle changes alone ($n = 17$). It was concluded that phlebotomy was associated with improvement in NAS, AST, ALT and γ GT without adverse events.

Because it has been reported that iron depletion therapy has anti-inflammatory effects in NASH, it may also contribute to the prevention of hepatocarcinogenesis in these patients. However, it has been reported that it is not effective in all cases^[25]. It would therefore be valuable to establish a method for selecting those NASH patients most likely to benefit clinically from iron depletion therapy.

GLYCYRRHIZIN (GLYCYRRHIZIC ACID), STRONGER NEO-MINOPHAGEN C

Glycyrrhizin is a triterpene glycoside from licorice root (*Glycyrrhiza glabra*) and consists of one molecule of glycyrrhetic acid (GA) and two molecules of glucuronic acid. Glycyrrhizin is widely used in patients with chronic viral hepatitis because of its anti-inflammatory action and beneficial effects on ALT levels and histology^[29]. The anti-inflammatory action of glycyrrhizin is believed to be due to its protective effect on the hepatic cellular membrane, which may explain its ability to lower the serum transaminase level in patients with chronic hepatitis. Kiso *et al.*^[30] demonstrated that GA inhibited free radical generation and lipid peroxidation *in vitro*. Stronger neo-minophagen C (SNMC, Minophagen Pharmaceutical, Tokyo, Japan), was first reported by Yamamoto *et al.* in 1958, and has now been used in the treatment of chronic liver disease for more than 50 years. SNMC is a compound GA tablet that includes GA (2 mg) together with glycine acid (20 mg) and L-cysteine hydrochloride (1 mg)^[31]. In 1977, Arase *et al.*^[32] confirmed its ability to reduced aminotransferase levels in patients with histologically-documented chronic hepatitis in a double-blind randomized controlled trial using a dose of 40 mL daily for a month. According to a retrospective study of 84 patients (Group A) who had been treated with SNMC at a dose of 100 mL daily and 109 patients (Group B) who could not be treated with SNMC or interferon, 36% of Group A achieved ALT normalization. The 10-year HCC rates in Groups A and B were 7% and 12%, respectively, and the 15-year rates 12% and 25%^[32]. van Rossum *et al.*^[33] performed a double-blind randomized placebo-controlled trial in which glycyrrhizin was administered three times per week for 4 wk, but reported that only 10% of the European patients so treated normalized their ALT levels. These investigators also performed an open study in which SNMC was administered six times per week at a dose of 100 mg for 4 wk. At the end of treatment, 20% (3 of 15) of the patients achieved normal ALT levels^[34]. Shiota *et al.*^[35] demonstrated in mice treated with diethylnitrosamine as a model of hepatocarcinogenesis due to viral hepatitis that AST and albumin values were significantly improved and the occurrence of HCC decreased in the glycyrrhizin group. A long-term prospective randomized

trial in humans is actually difficult from both ethical and medical viewpoints. Therefore, Ikeda^[29] retrospectively analyzed 346 patients with chronic hepatitis with high alanine transaminase, 244 of whom had received glycyrrhizin injections. Carcinogenesis rates in the treated and untreated groups were 13.3% and 26.0% at the fifth year, and 21.5% and 35.5% at the 10th year, respectively.

URSODEOXYCHOLIC ACID

Ursodeoxycholic acid (UDCA) is a hydrophilic bile acid which has cytoprotective effects not only in chronic cholestatic liver disease, but also in various other liver diseases. The therapeutic properties of UDCA include hypercholeresis, protection of cell membranes by replacing hydrophobic bile acids, and immunomodulation^[36-38]. Ljubuncic *et al.*^[39] reported that UDCA can act as an antioxidant blocking hydrophobic bile acids which otherwise oxidatively activate Kupffer cells to generate reactive oxygen species *in vitro*. Mitsuyoshi *et al.*^[40] also proposed the antioxidant effect of UDCA in cultured rat hepatocytes. They demonstrated that UDCA increased thiol-containing proteins such as metallothionein, and activated γ -glutamylcysteine synthetase, which regulates glutathione.

A decrease of serum transaminase levels in patients with chronic hepatitis after UDCA administration was first reported from a pilot study by Leuschner *et al.*^[41]. The effect was confirmed in a double-blind study by Crosignani *et al.*^[42] and Bellentani *et al.*^[43], who also established efficacy in long-term treatment. A retrospective study by Tarao *et al.*^[44] implied an association of UDCA use with lower incidence of hepatocellular carcinoma in hepatitis C virus-associated liver cirrhosis.

Crosignani *et al.*^[42] reported that 250 mg/d of UDCA was effective in improving biochemical markers of liver function, but no further improvement could be attained with higher doses in patients with chronic hepatitis. By contrast, according to a large multicenter randomized controlled dose study of UDCA for chronic hepatitis C, Takano *et al.*^[45] suggested that UDCA at a dose of 600 mg/d was optimal.

VITAMIN E (α -TOCOPHEROL)

Vitamin E, an essential lipid-soluble nutrient, is a potent peroxy radical scavenger that prevents the propagation of free radicals in membranes and in plasma lipoproteins^[46]. Vitamin E has been shown to protect against liver damage induced by oxidative stress in animal experiments^[47,48]. In 1997, von Herbay *et al.*^[49] treated 23 chronic hepatitis C patients refractory to interferon therapy with high doses of vitamin E (2 x 400 IU α -tocopherol/d) for 12 wk. In 11 of these patients, ALT and AST levels improved during treatment. Mahmood *et al.*^[50] also suggested that Vitamin E can act as a supportive therapy to

protect the liver from damage caused by oxidative stress. In their study, 17 CHC patients, receiving anti-inflammatory drug therapy at least 6 mo prior to Vitamin E administration, were given α -tocopherol 500 mg/d, orally, for a period of 3 mo. The ALT level was lowered in those patients initially with high levels (ALT > 70 IU/L). The thioredoxin (TRX) level was reduced in all patients. Houglum *et al*^[51] showed that treatment of 6 interferon-refractory patients with α -tocopherol (1200 IU/d for 8 wk) decreased the level of carbonyl modification of plasma proteins, a sensitive index of oxidative stress, but it did not significantly affect serum ALT levels, hepatitis C virus titers, or the histologically-determined degree of hepatocellular inflammation or fibrosis. These results suggest that the treatment may need to be prolonged. In 2000, the effect of vitamin E against NASH/NAFLD was first reported in a cohort^[52]. Eleven children < 16 years old with NAFLD were prescribed oral vitamin E (400-1200 IU/d for 4-10 mo) with the result that serum ALT, AST and alkaline phosphatase decreased significantly during treatment. However, liver histology was not assessed. In a small, uncontrolled pilot trial, Hasegawa *et al*^[53] demonstrated improvement in fibrosis in 66% of NASH patients who took vitamin E in doses of 300 mg/d for 1 year. In 2003, according to a prospective, double-blind, randomized, placebo-controlled trial with 45 NASH patients, combination vitamin E and C (1000 IU and 1000 mg per day, respectively) was well tolerated and effective in improving fibrosis scores^[54]. Nonetheless, no improvement in inflammatory activity or ALT was seen with this combination. Two subsequent studies by Kugelmas *et al*^[55] in 16 NASH patients and Vajro *et al*^[56] in 28 NAFLD children also showed no improvement in ALT levels^[55,56]. Dufour *et al*^[57] showed that two years of treatment with UDCA (12-15 mg/kg per day) and vitamin E (400 IU twice a day) improved laboratory values (AST, ALT) and hepatic steatosis of NASH patients compared with UDCA alone or placebo. Sanyal *et al*^[58] concluded that vitamin E therapy was associated with a significantly higher rate of improvement in hepatic steatosis, lobular inflammation, ALT and AST.

SHO-SAIKO-TO (TJ-9, XIAO-CHAI-HU-TANG)

Sho-saiko-to is a traditional Chinese herbal medicine derived from seven species of medicinal plants^[59]. Although the mechanism by which Sho-saiko-to protects hepatocytes against liver disease is not fully elucidated, clinical trials have shown its efficacy in patients with chronic hepatitis and liver cirrhosis in Japan, Korea and China. Some plausible actions as an antioxidant have been reported, for example, scavenging free radicals^[60,61] and reducing the hepatic level of malondialdehyde, a product of lipid peroxidation^[62]. Shiota *et al*^[61] demonstrated that TJ-9 lowered

diethylnitrosamine-induced ROS, resulting in reduction of 8-OHdG formation and hepatocarcinogenesis in rats. A double-blind multicenter trial reported an improvement in AST and ALT values in 116 chronic hepatitis patients treated with TJ-9 for 12 wk^[63]. Oka *et al*^[64] showed a weak not statistically significant benefit of TJ-9 treatment at a daily dose of 7.5 g and decreased hepatic carcinogenesis rate in a randomized study of patients with cirrhotic chronic liver disease.

CONCLUSION

In CHC cases with persistent inflammation where HCV eradication is difficult, it has been reported that a combination of antioxidant therapies is an effective method to prevent the onset of liver cirrhosis and HCC. In other chronic liver diseases, in particular NASH, it has been demonstrated that antioxidant therapy may potentially be effective for suppressing inflammation and liver fibrosis and expected to prevent carcinogenesis.

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