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Chapter 6

VITAMIN E AND LIVER DISEASES

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Abstract

Oxidative stress is one of the major pathogenetic events of liver disorders such as metabolic to proliferative ones. The main sources of reactive oxygen species (ROS) are represented by mitochondria and cytocrome P450 enzymes in the hepatocyte, by Kupffer cells and by neutrophils. Vitamin E, an antioxidant has been found to protect the organs against oxidative stresses. It is well known that serum concentration of vitamin E decreased in accordance with the progression of the liver diseases. Because of its antioxidant effect, vitamin E has been used for liver disease for long time. In this issue, we described about the antioxidant, anti-inflammatory, anti-carcinogenic effect of vitamin E on liver diseases including viral hepatitis, non-alcoholic steatohepatitis (NASH), and hepatocellular carcinoma. In addition, vitamin E has recently been reported to act as a ligand for pregnane X receptor (PXR), an orphan nuclear receptor regulating xenobiotic metabolizing enzymes. The roles of vitamin E binding to nuclear receptors and regulating enzymes were also discussed in addition to its antioxidant activity.

Keywords: Vitamin E, Liver disease, Oxidative stress, Antioxidant, Hepatitis, Hepatocellular carcinoma, Pregnane X receptor.

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Introduction

As causes of liver injury, many factors such as apoptosis, necrosis, inflammation, immune response are concerned. Liver diseases varied from acute hepatitis to hepatocellular carcinoma (HCC). Reactive oxygen species (ROS) involve in these many steps of liver diseases (1). The main sources of ROS are represented by mitochondria and cytocrome P450 (CYP) enzymes in the hepatocyte, by Kupffer cells and by neutrophils (1). ROS reduce the antioxidant and energy in the liver, and cause the liver injury. In deed, the concentration of lipid peroxidase elevated in serum of patients with liver diseases and those of vitamin E decreased. Vitamin E (alpha-tocopherol) is an essential vitamin with antioxidant properties (2). Vitamin E acts as an antioxidant and is able to terminate the chain reactions that generate free radicals through a scavenging effect on peroxyl radicals that are formed from unsaturated fatty acids by oxygen-derived free radicals. Therefore, it is likely that vitamin E is important in the prevention of lipid peroxidation of polyunsaturated fatty acids of membrane phospholipids (3, 4). In this sight, vitamin E is using for long time as antioxidant for the treatment of liver diseases. In addition, vitamin E has recently been reported to act as a ligand for pregnane X receptor (PXR), an orphan nuclear receptor regulating xenobiotic metabolizing enzymes. In this issue, we described the relation between vitamin E and liver diseases. The roles of vitamin E binding to nuclear receptors and regulating enzymes were also discussed.

1 Oxidative Stress in the Liver

Liver is a main organ concerning the metabolism in the body. Hepatocytes contain much mitochondria system and have important roles in metabolism. Mitochondria produce adenosine triphosphate (ATP) through beta-oxidation of fatty acid and provide the energy for metabolism processes. Mitochondria and cytochrome P450 (CYP) enzymes are the main sources of ROS in hepatocytes acutely and/or chronically exposed to toxic injuries such as alcohol, therapeutical drugs, and viruses (1). ROS is also derived from Kupffer and inflammatory cells, in particular neutrophils (1). In hepatocytes, ROS play a role in a very large cascade of reactions, such as Ca^{++} accumulation, circulatory status and transport function, nitric oxide (NO) synthesis and metabolism, and cytokine gene expression and so on (1). Table 1 shows radical scavenging system for ROS in the body.

Hepatocytes related to many these scavenging systems for ROS and contain about 10% of total body pool of glutathione (GSH), an antioxidant (5). A normal homeostasis of GSH is necessary for the activity of the CYP enzyme system and of mitochondria for the maintenance of a normal cellular redox status. By inducing GSH depletion, ROS induce oxidative stress and reduce the antioxidant capability of other antioxidants (6-9). The decrease of GSH in liver and circulation in patients with liver diseases such as alcoholic and viral cirrhosis was reported (1). Not only antioxidants but also antioxidative enzymes, superoxide dismutase (SOD), catalase, and glutathione S-transferase (GST) have roles in defense mechanisms against the damaging effect of ROS (10-12). The induction of a SOD-catalase insensitive free radical species facilitates liver damage (11, 12). Antioxidative proteins such as metallothionein, thioredoxin (TRX) also have roles in defensive mechanisms against for ROS.

Table 1

Scavenging system for reactive oxygen species (ROS) in the body.

Antioxidants	Vitamin C, Vitamin E, Glutathione (GSH), Cysteine, Carotenoid, Coenzyme Q, etc
Antioxidative enzymes	Superoxide dismutase (SOD), Catalase, Glutathione peroxidase Glutathione S-transferase (GST), etc
Antioxidative proteins	Metallothionein, Thioredoxin (TRX), Albumin, Ferritin, Transferrin, Ceruloplasmin, etc

Oxidative and nitrosative stress initiate and regulate the transcription and activation of a large series of other mediators in all liver cells, which culminate in common mechanisms of liver damage: apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression, and regeneration (1). The induction of the CYP enzyme system, the endotoxin-induced cytokine expression in Kupffer cells and the neutrophil infiltration further enhance the production of ROS and deplete ATP reserves in hepatocytes. In fact, ROS generated from neutrophils, migrate into the hepatocytes and potentiate the shift of apoptosis to necrosis (13).

It has been well known for many years that oxidative damage is a substrate for fibrogenesis (14). During chronic liver injuries, hepatic stellate cells are activated and assume the typology of active fibroblasts, particularly those next to necrotic areas (14). ROS, transforming growth factor beta (TGF-beta) activation, 4-hydroxynonenal (4-HNE), cytokines, GSH depletion, directly and/or indirectly increase procollagen type 1 gene expression and synthesis (1). The activation of several CYP forms directly takes part in the carcinogenesis process because of the metabolic activation of procarcinogens to reactive, DNA-binding intermediates, and the quenching or inhibition of oxidative stress-related pathways may be suggested as a mechanism to arrest cancer cell proliferation (15).

2 Hepatic Vitamin E Concentration and Liver Disease

It was well known that hepatic level of vitamin E decreased in patients with liver cirrhosis (16). Portal hypertension and absorption disorder was supposed to be the mechanism of low level in vitamin E concentration (16). The plasma concentration of vitamin E correlated with liver function according to Child-Pugh classification (17) and also reported to decrease in patients with severe viral hepatitis (18). The decrease of hepatic vitamin E content did not depend on the causes of liver cirrhosis (19). Rocchi et al. (19) reported that vitamin E content in liver tissue is significantly decreased in cirrhosis (0.26 ± 0.03 μmol/g prot), with respect to its content in liver specimens of healthy controls (0.46 ± 0.03 μmol/g prot). Vitamin E concentration is further reduced by 50% in malignant liver nodules of HCC, with respect to

surrounding cirrhotic tissue (19). Not only hepatic but also serum vitamin E concentrations were significantly decreased to 40% of those in the controls at the early stages rat hepatic carcinogenesis (20).

Alpha-tocopherol transfer protein (α-TTP) is regulating the transport of vitamin E. A rodent α-TTP exhibited markedly lower messenger RNA (mRNA) amounts in rat HCC than in healthy controls (20). α-TTP mRNA of the rats were decreased at the early stage of hepatocarcinogenesis, and remained 3-5-fold reduced as the tumor progressed (20). A decrease of α-TTP mRNA was preferentially localized in the tumor nodules of rats and humans with HCC revealed with in situ hybridization (20). Repressed transcription of α-TTP is supposed to be associated with a decrease of serum vitamin E and with hepatic carcinogenesis (20).

3 Hepatitis and Vitamin E

3.1 Chronic Hepatitis C

Treatment for viral hepatitis is principlely anti-viral therapy such as interferon (IFN) therapy (21, 22, 23). Currently recommended treatment for previously untreated and relapsed patients is a combination of pegylated IFN and ribavirin, resulting in a sustained virological response in approximately 50–60% of patients (21, 22, 23). The cases not being available for IFN treatment for the reason such as adverse effects, complication, and old age are the candidates for antioxidant therapy. Vitamin E supplementation was reported to improve immune responsiveness in healthy elderly individuals (24). This effect appears to be mediated by a decrease in prostaglandin E2 and/or other lipid-peroxidation products (24). The pathological mechanisms of disease progression of chronic hepatitis C are unclear but oxidant stress may play a role in progression of this disease. The lipid peroxidation marker 8-isoprostane and the ratio of oxidized to reduced GSH were significantly elevated in the patients with chronic hepatitis C (25). The antioxidants, GSH, selenium and vitamins A, C and E were significantly decreased in the patients with chronic hepatitis C (25). Abnormal values were more marked in liver cirrhosis (25). Thus, oxidative stress is a significant feature of hepatitis C infection and oxidative stress in liver supposed to be up-regulated in liver infected with hepatitis C virus (HCV). Antioxidant therapy may therefore have a role in slowing disease progression to cirrhosis (25).

Some recent preliminary trials showed its possible beneficial role in the treatment of both chronic hepatitis B (26) and chronic hepatitis C (27-30). Houglum et al. (27) reported that treatment with vitamin E (1200 IU/day for 8 weeks) in the patients, who were refractory to IFN therapy, prevented the fibrogenesis cascade observed before antioxidant treatment. In addition, vitamin E treatment significantly decreased the carbonyl modifications of plasma proteins, a sensitive index of oxidative stress (27). However, 8 weeks of vitamin E treatment did not significantly affect serum alanine aminotransferase (ALT) levels, HCV titers, or histological degree of hepatocellular inflammation or fibrosis (27). Mahmood et al (28). also reported that oxidative stress induced liver damage is reduced by vitamin E in patients with hepatitis C, particularly those with initial ALT levels more than 70 IU/l. Vitamin E treatment causes reduction of oxidative stress markers as thioredoxin (TRX) and ALT in sera (28). TRX is a stress inducible, multifunctional protein, secreted during oxidative stress. Therefore,

vitamin E can act as a supportive therapy to combat liver damage caused by oxidative stress, in such patients with continuously high levels of ALT even after anti-viral and anti-inflammatory drug therapy. (29)

In combination with IFN, the effects of antioxidative co-therapy were analyzed (30, 31). IFN alpha-naive patients with chronic hepatitis C who were randomized to either receive IFN monotherapy, or IFN and N-acetylcysteine (NAC, 1.800 mg/day) plus sodium selenite (400 μg/day) supplementation, or treatment as in NAC, sodium selenite, and vitamin E (544 IU/day), over 24 weeks (30). Vitamin E treated patients had a 2.4 greater chance (95% CI: 1.05-5.5) of obtaining a biochemical response and had significantly greater reduction in viral load than patients without vitamin E at the end of treatment. However, relapses, i.e. re-appearance of detectable HCV RNA and/or re-elevation of ALT-activity occurred in 7 out of the 11 responders within 6 months after termination of therapy (30). Thus, no overall beneficial effect of antioxidant/IFN therapy was detected by their studies (30). The randomized study carried out by Ideo et al. (31) on 120 patients with chronic hepatitis C not responsive to alpha-IFN, oral supplementation with NAC and vitamin E did not improve the poor efficacy of retreatment with alpha-IFN alone. Thus, no additional effect to IFN was obtained by vitamin E in patients with hepatitis C.

3.2 Chronic Hepatitis B

The main strategies to treat chronic hepatitis B virus (HBV) infection rely on the stimulation of the specific anti-viral immune response and on the inhibition of viral replication. The IFN therapy is moderately effective and often limited by dose-dependent side effects. Recently, new nucleoside analogues, such as lamivudine, adefovil have shown very promising results in terms of anti-viral effect and tolerance. The prolonged administration of lamivudine is most often associated with a control of viral replication rather than eradication, and may induce the resistant mutants.

Andreone et al. (26) evaluated vitamin E supplementation as therapy for chronic hepatitis B. Twenty-four patients with elevated serum ALT levels and serum HBV DNA were randomly assigned to receive vitamin E, 300 mg twice daily for 3 months, or no treatment. Surprisingly, the HBeAg-positive patients seroconverted to HBeAb during the study period, whereas none of the controls seroconverted from HBeAg to HBeAb (26). Three of 5 patients with complete response had not responded to previous IFN-alpha treatment (26). Because treatment with vitamin E had no obvious side effects, they suggest that vitamin E is a safe and useful treatment for chronic hepatitis B (26). Because of preliminary data due to small numbers of patients, further study is needed to confirm the effect of vitamin E on chronic hepatitis B.

HBV transgenic mice with chronic active hepatitis display greatly increased hepatic oxidative DNA damage (32). Moreover, the DNA damage occurs in the presence of heightened hepatocellular proliferation, increasing the probability of fixation of the genetic and chromosomal abnormalities and the development of hepatocellular carcinoma (32). In this sight, antioxidant agents including vitamin E are promising agent for prevention of hepatocarcinogenesis due to HBV infection.

3.3 Non Alcoholic Steatohepatitis (NASH)

Non-alcoholic steatohepatitis (NASH) is pathologically mimicking to alcoholic hepatitis, characterized by fatty change, lobular inflammation and fibrosis of the liver, in the absence of alcohol abuse (33). Some cases of NASH progress to cirrhosis, although its pathogenesis is not fully understood (34-36). The oxidative stresses have important roles in pathogenesis of this disease (Figure 1). Established therapy for NASH does not exist. Because NASH mostly complicated with metabolic disorders, principle therapy is dietary therapy, weight loss, treatment for diabetes mellitus or hyperlipidemia. As a drug therapy for NASH, ursodeoxycholic acid (UDCA), troglitazone, clofibrate, vitamin E are supposed to be effective (34-36).

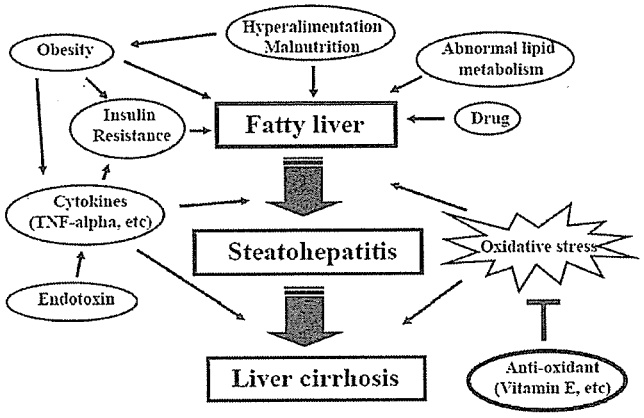


Figure 1 Mechanisms of liver injury in non alcoholic steatohepatitis (NASH).

Daily oral vitamin E administration normalized serum ALT and alkaline phosphatase levels in children with NASH according to Lavine et al (37). They supposed that obese children with NASH should be encouraged to lose weight as part of a comprehensive weight reduction program and to consider taking supplemental vitamin E. Hasegawa et al. (38) reported the study of vitamin E for the adult patients with NASH and non-alcoholic fatty liver disease (NAFLD). Patients were given dietary instruction for 6 months, and then vitamin E (300 mg/day) was given for 1 year (38). The serum ALT level decreased in NAFLD patients, but not in NASH patients, after 6 months of dietary therapy (38). The serum ALT level in NASH patients was reduced during the 1-year vitamin E treatment. The histological findings, such as steatosis, inflammation and fibrosis, of the NASH patients were improved after vitamin E treatment (38). They concluded that long-term vitamin E treatment may be safe and effective for NASH (38, 39). Harrison et al. (40) reported the efficacy of combination vitamin E (1000IU/day) and vitamin C (1000 mg/day) in improvement in fibrotic score with prospective, double-blind, randomized, placebo-controlled trial in NASH patients. Sanyal et al. (41) performed a randomized prospective trial to compare the efficacy and safety of vitamin E alone (400 IU/day) vs. vitamin E (400 IU/day) and insulin sensitizer (pioglitazone, 30 mg/day) in nondiabetic, noncirrhotic subjects with NASH. Treatment with vitamin E only produced a significant decrease in steatosis. Compared with baseline, combination therapy

produced a significant decrease not only in steatosis but also in cytologic ballooning, Mallory's hyaline, and pericellular fibrosis (41). Combination therapy with vitamin E and pioglitazone produced a significant increase in metabolic clearance of glucose and a decrease in fasting free fatty acid (FFA) and insulin (41). A combination of vitamin E and pioglitazone produces a greater improvement in NASH histology than vitamin E alone (41).

Figure 2 shows a case of NASH successfully treated with vitamin E. Patients were 57 years old man and diagnosed as NASH. Figure 3 shows liver biopsy specimens before treatment. He was treated with oral vitamin E and ALT decreased after administration.

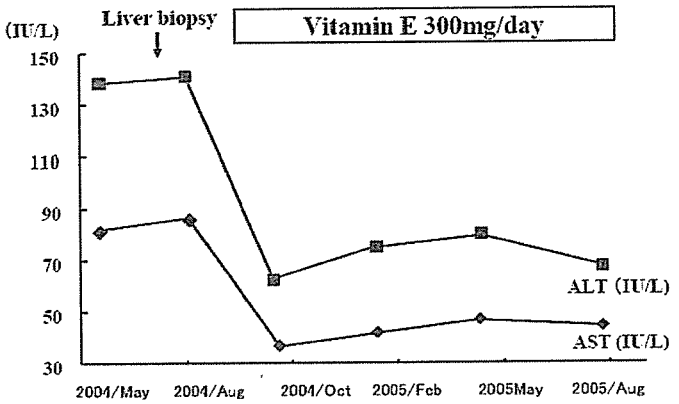


Figure 2 Clinical course of NASH patient successfully treated with vitamin E.

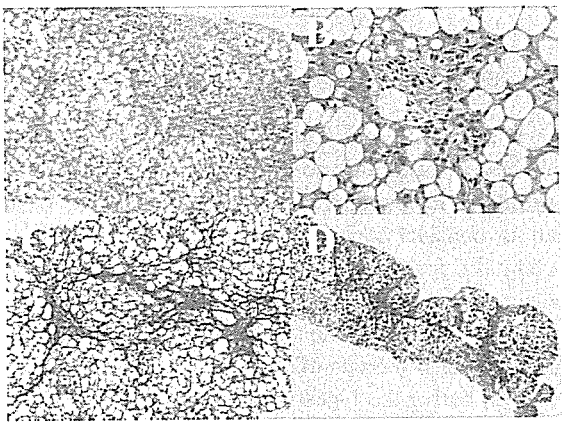


Figure 3 Histological findings of liver biopsy specimen with NASH patient. A, B; Steatosis, ballooning of hepatocytes, inflammatory cell infiltration and fibrosis were observed (Hematoxylin and Eosin staining). C; Silver staining. D; Azan-Mallory staining.

About the effect on NASH of other antioxidant agents such as betaine (a methyl donor in an alternative pathway for remethylation of homocysteine to methionine) (42), and N-acetylcysteine (43), betaine has shown encouraging results. Silymarin (44), a popular milk

thistle extract, is used commonly by patients with liver disease but there were no published studies in NASH (45).

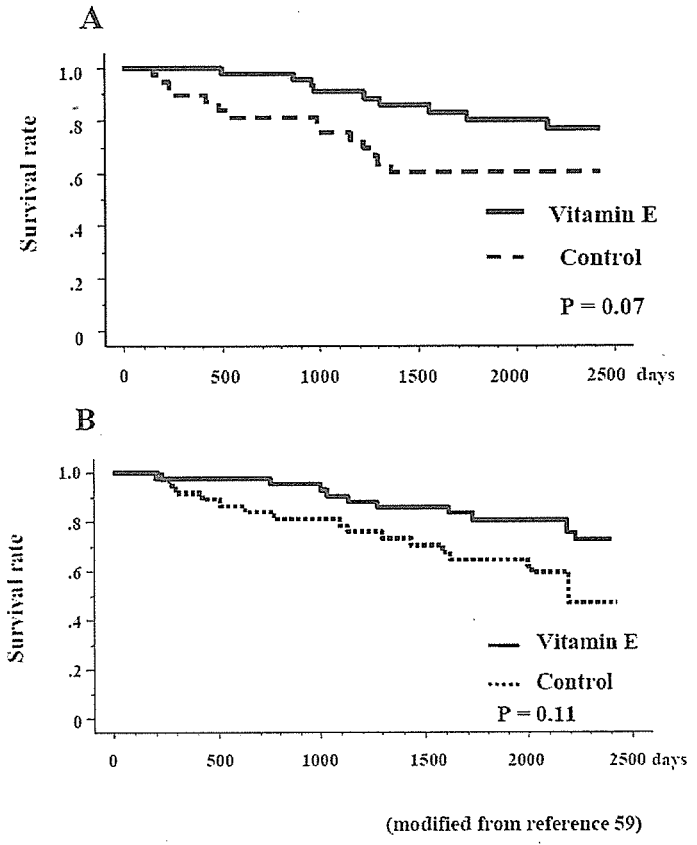
4 Anti-carcinogenic Effect of Vitamin E

Hepatocellular carcinoma (HCC) is one of the most intractable malignancies in the world. Interferon therapy against viral hepatitis could eventually reduce the incidence of HCC by the eradication of HBV or HCV and result in a kind of chemoprevention of hepatocarcinogenesis in response to the hepatitis virus (46, 47). However, IFN has many adverse effects and sometimes is not suitable for patients with liver cirrhosis. Vitamin E has been reported as having a chemopreventive effect on hepatocarcinogenesis in some experimental models (48-51). Antioxidants are known to induce glutathione S-transferase (GST) and UDP-glucuronyl transferase (UGT) activity in liver cells in vitro and in vivo, and this action correlates with their chemopreventive characteristics (52). Vitamin E has been recently proved as an activator for nuclear receptor PXR (pregnane X receptor) (53). Inducibility for GST and UGT activity by vitamin E may be regulated by nuclear receptors. The effects of vitamin E on PXR regulation are described in next chapter. Vitamin E also supposed to prevent tumor formation by stimulating a potent immune response to selectively destroy tumor cells as they began to develop into recognizable microscopic foci of carcinoma (50). It is known that with dietary supplementation, vitamin E accumulates in the hepatocytes and not in nonparenchymal cells (54). Vitamin E is distributed in the hepatic nuclear fraction in a dose-dependent manner, but this phenomenon is not observed in cytoplasmic fractions (48), suggesting that vitamin E is an effective antioxidant for preventing oxidative damage to DNA.

Vitamin E-deficient diet accelerated the hepatocarcinogenesis of transforming growth factor-alpha (TGF-alpha) transgenic mice treated with diethylnitrosamine (DEN) (55). Further, co-expression of TGF-alpha and c-myc transgenes in mouse liver promotes overproduction of ROS and creates an oxidative stress environment (56). Increased ROS generation might be responsible for the extensive chromosomal damage and acceleration of hepatocarcinogenesis characteristic for TGF-alpha/c-myc mice (56). Dietary supplementation of vitamin E can effectively inhibit liver cancer development in TGF-alpha/c-myc mice (57). Vitamin E decreased ROS generation coincident with a marked inhibition of hepatocyte proliferation while increasing the chromosomal as well as mitochondrial DNA stability in the liver (57). Similarly, dietary vitamin E reduced liver dysplasia and increased viability of hepatocytes (57). At 6 mo of age, vitamin E treatment decreased the incidence of adenomas by 65% and prevented malignant conversion (57). Thus, dietary supplementation of vitamin E can effectively inhibit liver cancer development in animal model (57). Oxidants play a role in several stages of carcinogenesis. A high antioxidant capacity is expected to protect initiated cells from excessive oxidant toxicity (58). Vitamin E was reported to have chemopreventive effect during the initiation stage of carcinogenesis (58).

In human, we evaluated the chemopreventive effect of vitamin E on hepatocarcinogenesis in patients with liver cirrhosis caused by HCV infection (59). The serum level of vitamin E was low in enrolled patients with liver cirrhosis as previously reported (16, 17, 19). Cumulative tumor-free survival (Figure 4A) and cumulative survival rate (Figure 4B) tended to be higher in the vitamin E group than in controls however it did not reach the statistical significance (59). The chemopreventive effect of single and combination doses of radical

scavengers has been controversial in human (60, 61, 62). Meta-analysis of antioxidant supplement for prevention of liver cancer failed to certify the effect of antioxidant (63). Inversely, the supplement with beta-carotene, vitamin C and vitamin E increased relative risk for HCC (63). Further studies are needed to confirm the anti-carcinogenic effect of vitamin E for HCC.



(modified from reference 59)

Figure 4 A; Comparison of cumulative tumor-free survival rate between the two groups of liver cirrhosis patients with and without vitamin E. B; Comparison of cumulative survival rate between the two groups of liver cirrhosis patients with and without vitamin E.

Concerning the treatment for HCC, Clerici et al. (64) reported a pilot study performed to evaluate the effect of all-trans retinoic acid associated with tamoxifen and vitamin E on patients with advanced HCC. Fifteen consecutive patients with advanced HCC were included in their study. A combination therapy of all-trans retinoic acid, tamoxifen and vitamin E increases the survival rate and ameliorates the clinical outcome in patients with inoperable HCC (64).

Further study is needed to confirm the effect of vitamin E on chemoprevention or therapeutic efficacy for HCC.

5 Nuclear Receptors and Vitamin E

Although vitamin E has long been considered just as an antioxidant, it has now become clear that vitamin E has functions far exceeding that as an antioxidant (53, 65, 66). These include regulation of cellular signaling processes and gene expression (53, 65, 66). Vitamin E recently had been identified as an inducer of nuclear receptor PXR (53, 65, 66) regulating drug or xenobiotic-metabolizing enzymes including CYPs. CYPs degrade various endogenous and exogenous compounds and many of them are induced by their substrates. Vitamin E is also metabolized with CYPs and induces the CYPs via PXR. CYPs are induced via the activation of the nuclear receptors including PXR, constitutive androstane receptor (CAR), and peroxisome proliferator-activated receptors (PPAR). PXR and CAR are activated by a large number of lipophilic xenobiotics and regulate the drug or xenobiotic-metabolizing enzymes. Vitamin E has effects on genes modulated by PPAR or CAR, as well as retinoic acid-related orphan receptor (ROR) (67).

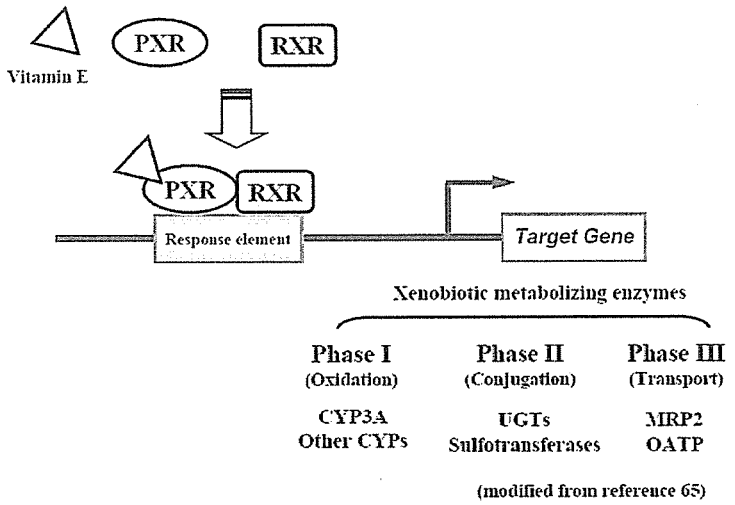


Figure 5 Schematic representation of PXR-mediated regulation with xenobiotic metabolizing enzymes.

Nuclear receptors comprise a superfamily of ligand-activated transcription factors. The activated nuclear receptor stimulates expression of genes by binding to the response elements located in the promoter regions of target genes. PXR is a broad-specificity nuclear receptor that recognizes xenobiotics. On ligand binding, PXR, as a heterodimer with the retinoid X receptor (RXR), binds to the promoter region of genes (figure 5) and induces oxidation systems (phase I), conjugation systems (phase II), and transporters (phase III) that effectively clear xenobiotics from the liver (65, 68). PXR-regulated genes are CYPs, UDP glucuronosyl transferases (UGT), sulfotransferases, glutathione *S*-transferases (GST), and transporters such as multidrug resistance-associated protein (MRP) and organic anion transporter peptide (OATP) (69). Thus, PXR-related genes may not be the only ones or even the primary ones involved in vitamin E metabolism. Vitamin E has the potential to regulate a variety of cellular

responses. Research is urgently needed into the interactions between vitamin E, nuclear receptors and xenobiotic metabolism.

Conclusion

Vitamin E regulates oxidative stress related many aspects of liver diseases. Antioxidant property, vitamin E may contribute the treatment of liver diseases. However, the precise roles of vitamin E are further needed to investigate on many liver diseases. In addition, vitamin E has recently been found as a ligand for PXR, an orphan nuclear receptor regulating xenobiotic metabolizing enzymes. More research is mandatory to clarify the interactions among vitamin E, nuclear receptors and xenobiotic metabolism.

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CLINICAL RESEARCH

Efficacy of balloon-occluded retrograde transvenous obliteration, percutaneous transhepatic obliteration and combined techniques for the management of gastric fundal varices

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Abstract

AIM: To evaluate the effect of three interventional treatments involving transvenous obliteration for the treatment of gastric varices, and to compare the efficacy and adverse effects of these methods.

METHODS: From 1995 to 2004, 93 patients with gastric fundal varices underwent interventional radiologic embolotherapy at our hospital. Of the 93 patients, 75 were treated with the balloon-occluded retrograde transvenous obliteration (BRTO) procedure; 8 were with the percutaneous transhepatic obliteration (PTO) procedure; and 10 were with the combined BRTO and PTO therapy. A follow-up evaluation examined the rates of survival, recurrence and rebleeding of the gastric varices, worsening of esophageal varices and complications in each group.

RESULTS: The BRTO, PTO, and combined therapy were technically successful in 81% (75/93), 44% (8/18), and 100% (10/10) patients, respectively. Recurrence of gastric varices was found in 3 patients in the BRTO group and in 3 patients in the PTO group. Rebleeding was observed in 1 patient in the BRTO group and in 1 patient in the PTO group. The 1- and 3-year survival rates were 98% and 87% in the patients without hepatocellular carcinoma (HCC) in the BRTO group, 100% and 100% in the PTO group, and 90% and 75% in the combined therapy group, respectively.

CONCLUSION: Combined BRTO and PTO therapy may rescue cases with uncontrollable gastric fundal varices that remained even after treatment with BRTO and/or PTO, though there were limitations of our study, including retrospective nature and discrepancy in sample size between the BRTO, PTO and combined therapy groups.

Key words: Gastric varices; Balloon-occluded retrograde transvenous obliteration; Percutaneous transhepatic obliteration; Combined therapy

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INTRODUCTION

Currently, various types of therapeutic procedures, including surgery, endoscopic procedures, and direct therapeutic intervention, are available for the treatment of gastric varices. Interventional therapy for gastric varices is classified as transjugular intrahepatic portosystemic shunt (TIPS) and transcatheter embolotherapy. Many studies have reported the use of TIPS in patients with gastric varices[1-5]. Although interventional embolotherapy has been applied for the treatment of gastric varices and a recent study has shown that embolotherapy may control gastric varices better than TIPS, treatment indications and a recommended embolotherapeutic strategy for the eradication of gastric fundal varices have not yet been strictly established[5-10]. In this study, we evaluated the effect of three interventional treatments involving transvenous obliteration for the treatment of gastric varices; the efficacy and adverse effects of these methods were compared. The present study involved 93 patients in whom interventional embolotherapy was performed as a treatment for gastric fundal varices. In addition, 10 of the 93 patients were treated with a combined balloon-occluded retrograde

Table 1 Main clinical and biochemical characteristics of 93 patients treated with interventional radiology for gastric varices *n* (%)

Clinical characteristics	BRTO	PTO	Com-T	Total
Sex (M/F)	37/38	5/3	5/5	47/46
Age (mean ± SD)	63.4 ± 10.0	62.9 ± 8.4	68.9 ± 12.8	63.9 ± 10.2
Cause of portal hypertension				
Alcoholic liver cirrhosis	10 (13)	1 (13)	3 (30)	14 (15)
HBV (+) liver cirrhosis	3 (4)	0 (0)	1 (10)	4 (4)
HCV (+) liver cirrhosis	52 (69)	6 (75)	5 (50)	63 (68)
HBV (-) HCV (-) liver cirrhosis	7 (9)	0 (0)	1 (10)	8 (9)
Idiopathic portal hypertension	1 (1)	1 (13)	0 (0)	2 (2)
Primary biliary cirrhosis	1 (1)	0 (0)	0 (0)	1 (1)
Traumatic	1 (1)	0 (0)	0 (0)	1 (1)
Presence of Hepatocellular carcinoma	21 (28)	0 (0)	0 (0)	21 (23)
Child-Pugh class				
A	42 (56)	3 (38)	7 (70)	52 (56)
B	29 (39)	4 (50)	2 (20)	35 (38)
C	2 (3)	0 (0)	1 (10)	3 (3)
Location of gastric varices				
IGV1	56 (75)	2 (25)	9 (90)	67 (72)
GOV2	19 (25)	6 (75)	1 (10)	26 (28)
Form of gastric varices				
Tumorous	26 (35)	1 (13)	2 (20)	29 (31)
Nodular	49 (65)	7 (88)	8 (80)	64 (69)
Previous gastric variceal bleeding				
Present	36 (48)	4 (50)	2 (20)	42 (45)
Absent	39 (52)	4 (50)	8 (80)	51 (55)
Temporary hemostasis				
Clipping	3 (4)	1 (13)	2 (20)	6 (7)
EVL	5 (7)	1 (13)	0 (0)	6 (7)
Histacryl	3 (4)	1 (13)	0 (0)	4 (4)
Balloon tamponade	2 (3)	0 (0)	0 (0)	2 (2)
Spontaneous	23 (31)	1 (13)	0 (0)	24 (26)
Duration of hospitalization (mean ± SD, d)	17.9 ± 23.4	14.6 ± 12.8	10.4 ± 4.0	16.8 ± 21.4
Follow-up (mean ± SD, d)	1040 ± 713	600 ± 381	704 ± 389	966 ± 677
Range (d)	35 - 2805	1134 - 150	83 - 1183	35 - 2805

BRTO : Balloon occluded retrograde transvenous obliteration; PTO : Percutaneous transhepatic obliteration; Com-T : Combined therapy; EVL : Endoscopic variceal ligation; IGV1: Isolated gastric varices; GOV2 : Gastroesophageal varices.

transvenous obliteration (BRTO) and percutaneous transhepatic obliteration (PTO) therapy, and the clinical efficacy, the complications and outcomes of this therapy were investigated. The aim of this retrospective study was to evaluate the effect of interventional embolotherapy, in particular the clinical efficacy of combined therapy, for the treatment of patients with gastric fundal varices.

MATERIALS AND METHODS

Patients

From 1995 to 2004, 93 patients with gastric fundal varices underwent interventional radiologic embolotherapy at our hospital. The algorithmic strategy used for the treatment of gastric fundal varices in our hospital is shown in Figure 1. According to the treatment algorithm, all the patients were first treated with the BRTO procedure. When the BRTO procedure could not obliterate the gastric varices because of collateral venous drainage, the PTO procedure was chosen as the second line of treatment. When neither the BRTO nor the PTO procedure could obliterate the gastric varices, the patients were treated with a combination of BRTO and PTO as the third line of treatment. Of the 93 patients, 75 were treated with the BRTO procedure; 8 were with the PTO procedure; and 10 were with the combined therapy. The characteristics of the 93 patients are shown in Table 1. The study included 47 men and 46 women with a mean age of 63 years. Table 1 shows the underlying cause of portal hypertension, the presence of serum hepatitis virus markers, and modified Child's classification of the patients[11]. Left-side portal hypertension due to splenic vein obstruction after traumatic pancreatitis was observed in 1 patient. Patients with Child's class C cirrhosis with ascites were excluded from this study because interventional radiologic embolotherapy may increase the portal pressure[12]. Of the 93 patients, 37 (40%) underwent treatment of esophageal varices prior to treatment of gastric varices. 21 (23%) had complications due to hepatocellular carcinoma (HCC). Computed tomography (CT) or ultrasonography could be used to exclude the presence of portal tumor thrombus in all patients who had HCC. Patients with portal vein thrombus were excluded.

According to the classification system originally proposed by Sarin *et al*[13,14], gastric fundal varices are classified as type 2 gastroesophageal varices (GOV2; often long and tortuous, extending from the esophagus below the gastroesophageal junction toward the fundus) or type 1 isolated gastric varices (IGV1; varices located in the fundus that are often tortuous and complex in shape). In our study, 67(72%) patients showed IGV1 and 26 (28%) showed GOV2. The form of fundal varices was massively tumorous in 29 (31%) patients and nodular in 64 (69%).

Gastric fundal varices with active bleeding (spurting or oozing) or signs of recent bleeding or those that were in danger of rupturing were identified for treatment. Variceal bleeding was endoscopically confirmed in all patients immediately after their hematemesis. The bleeding episodes were attributed to ruptured gastric varices based on one of the following criteria: actively bleeding varices at the time of endoscopic examination, presence of adherent clots on varices, or presence of blood in the stomach with gastric varices as the only possible cause of the bleeding. The indications for gastric varices embolization in patients who have not previously had gastric variceal bleeding are as follows: gastric varices with red spots observed during upper intestinal endoscopy or growing gastric varices were considered to be in danger of rupturing.

Bleeding gastric varices were observed in 42 patients, and the remaining 51 patients were prophylactic nonbleeding cases that were in danger of rupturing. An emergency procedure was performed in 14 patients who had active bleeding during urgent endoscopy. To achieve temporary hemostasis, gastric variceal bleeding was treated in the following manner: endoscopic variceal ligation (EVL) in 6 patients, endoscopic injection sclerotherapy (EIS) using cyanoacrylate with lipiodol in 4 patients, clipping treatment in 6 patients, and balloon tamponade in 2 patients. Spontaneous hemostasis was achieved in the remaining 24 patients. Written informed consent was obtained from all patients.

BRTO procedure

Under local anesthesia, a 6.5-French occlusive balloon catheter with a diameter of 20 mm (Create Medic, Yokohama, Japan) was inserted through the femoral or internal jugular vein into the gastrorenal shunt, gastrocaval shunt, or both. Results of balloon-occluded retrograde transvenous varicerography (BRTV) were obtained in advance. Following identification of the gastric varices and their associated feeding and draining veins, the balloon was inflated with CO_2. When the gastric varices were visualized and retention of the contrast medium in the gastric varices was identified, a 50 g/L ethanolamine oleate (EO) solution with iopamidol (50 g/L EOI), which contained equal amounts of 100 g/L EO (Grelan, Tokyo, Japan) and iopamidol 300 (Schering, Berlin, Germany), was used as a sclerosant; the solution was slowly infused through the catheter into the gastric varices and their feeding veins in a retrograde manner during balloon occlusion under fluoroscopy. When varices and feeding veins could be shown in their entirety, injection was suspended. If draining minor collaterals were visualized by retrograde transvenous varicerography, microcoils were used in addition to 50 g/L EOI to embolize the collateral veins in order to prevent leakage of the sclerosing agent into the systemic circulation. After the sclerosant had remained in place for 1 h to allow thrombi formation, hemolyzed blood and excess uncoagulated sclerosant were collected through the catheter; subsequently, the balloon was deflated. Immediately prior to the treatment, 4000 U of haptoglobin (Welfide, Osaka, Japan) was administered by drop infusion to prevent renal damage induced by hemolysis due to the use of EO. The PTO procedure was chosen another day later on when retrograde venography showed that retention of the contrast medium was insufficient even though the collateral vessels had been embolized using metallic coils. (Figure 2A)

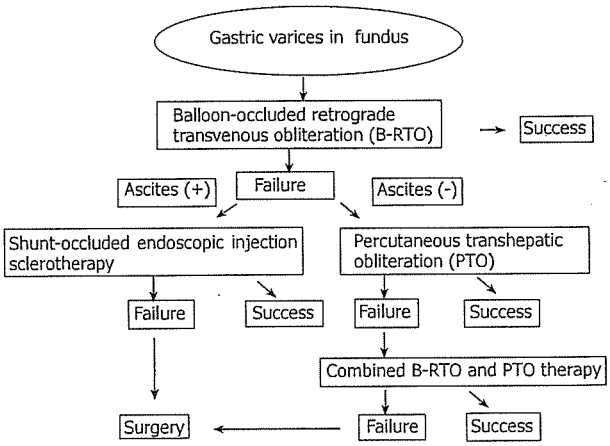

Figure 1 The algorithmic strategy for the treatment of gastric varices.

PTO procedure

We believe that the basic goal of embolotherapy in the treatment of gastric varices is to obliterate the variceal lumen and induce submucosal sclerosis. Therefore, in our hospital, PTO is used to obliterate gastric varices by using a sclerosing agent (50 g/L EOI); this procedure differs from the PTO procedure used previously[15]. Percutaneous transhepatic portography (PTP) using a 5-French catheter with a diameter of 11 mm was performed to determine the hemodynamics of the feeding and draining veins of the gastric varices. Due to the presence of multiple feeding veins, a coaxial catheter was inserted into these feeding veins. Some metallic coils were placed in these feeding veins with the exception of one feeding vein. The balloon catheter was selectively inserted into the remaining feeding vein. Through the balloon catheter, antegrade venography was performed with the balloon inflated. When the gastric varices were visualized and retention of the contrast medium in the gastric varices was identified, 50 g/L EOI, as a sclerosant, was slowly injected in the antegrade direction into the gastric varices under fluoroscopy. When varices could be shown in their entirety, injection was suspended. After the sclerosant had remained in place for 1 h to allow thrombi formation, the hemolyzed blood and excess uncoagulated sclerosant were collected through the catheter. Then, the microcoils were used to embolize the remaining feeding vein and to stabilize the thrombus

in the gastric varices. Portography was repeated to assess obliteration of the feeding veins and the absence of flow to the gastric varices. Immediately prior to the treatment, 4000 U of haptoglobin was administered by drop infusion. When antegrade venography showed that retention of the contrast medium in the gastric varices was insufficient, combined therapy was chosen (Figure 2B).

Combined BRTO and PTO therapy

The BRTO and PTO procedures were performed simultaneously. In other words, the feeding and the draining veins of gastric varices were simultaneously obliterated by the balloon catheters. Through the balloon catheter in the feeding vein, antegrade venography was performed with the double balloon inflated. When the gastric varices were visualized and retention of the contrast medium in the gastric varices was identified, 50 g/L EOI, as a sclerosant, was slowly injected in the antegrade direction into the gastric varices under fluoroscopy. When varices could be shown in their entirety, injection was suspended. After the sclerosant had remained in place for 1 h to allow thrombi formation, the hemolyzed blood and excess uncoagulated sclerosant were collected through the catheter. Immediately prior to the treatment, 4000 U of haptoglobin was administered by drop infusion (Figure 2C).

Evaluation of efficacy of the procedure and follow-up

A follow-up evaluation was performed to check for recurrence and rebleeding of the gastric varices, the aggravation of esophageal varices, complications, and rates of survival in each procedure group. CT was performed 1 wk after the procedure to evaluate obliteration of the gastric varices. Technical success was defined as complete clotting of the gastric varices that were observed during contrast-enhanced CT scanning 1 wk after the treatment. For patients in whom considerable blood flow remained, interventional embolotherapy was repeated until disappearance of blood flow within the gastric varices was confirmed. Initially, endoscopic examination was performed after 1 wk, 3 and 6 mo of the procedure to evaluate the obliteration of the gastric varices. Later, the examination was performed after every 6 mo or whenever clinically required. The laboratory data, including hepatic and renal function tests, were analyzed after 1 d, 1 wk, 1 and 6 mo of the procedure. When red spots on the esophageal varices were detected during endoscopy, the varices were considered to have aggravated and were treated endoscopically as soon as possible. The survival follow-up period was measured as the number of days from the date when interventional embolotherapy was performed until the date of the patient's death or the most recent clinical visit.

Statistical analysis

The cumulative survival rate and the nontreatment rate of esophageal varices were calculated using the Kaplan-Meier method, and the values were compared by means of the Wilcoxon signed rank test. Statistical software (Statview version 5.0; SAS Institute, North Carolina, USA) was used for statistical analysis. $P < 0.05$ was considered statistically significant.

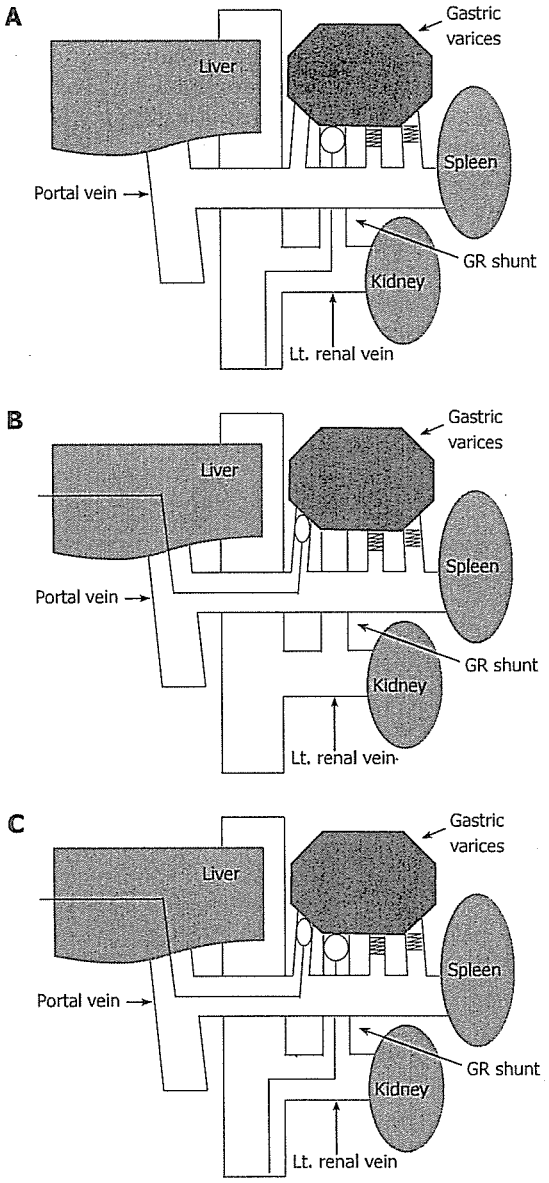

Figure 2 Illustration of radiologic embolotherapy for gastric varices. **A**: Balloon-occluded retrograde transvenous obliteration (BRTO); **B**: percutaneous transhepatic obliteration (PTO); **C**: combined BRTO and PTO therapy; GR = gastrorenal.

RESULTS

The mean observation period was 966 ± 677 d. The mean duration of hospitalization for the treatment of the gastric varices was 17 d. According to the treatment algorithm, the BRTO, PTO, and combined therapy were angiographically successful in 81% (75/93), 44% (8/18), and 100% (10/10) patients, respectively. The three-tiered treatment strategy used in this study enabled successful treatment of all the gastric fundal varices. In 68 of the 75 (91%) patients in the BRTO group, the gastric varices were completely thrombosed; this was shown by the CT that was performed 1 wk after the initial BRTO. The remaining