Chapter 18
Antioxidant Approach to the Therapy of Chronic Liver Diseases

Le Thi Thanh Thuy, Hoang Hai, and Norifumi Kawada

18.1 Introduction

18.1.1 The Antioxidant System

The liver plays a central role in metabolic homeostasis, being responsible for the metabolism, synthesis, storage, and redistribution of nutrients, carbohydrates, fats, and vitamins. The liver is also a significant site of free radical synthesis. Liver enzymes, including diamine oxidase, aldehyde dehydrogenase, tryptophan dual oxidase, liver dehydrogenase, and the cytochrome P450 system, induce oxidation and uncoupling, triggering free radical production [1]. Oxygen-free radicals, more generally termed reactive oxygen species (ROS), and reactive nitrogen species (RNS) are the most important groups of radicals generated in living systems [2].

The body has developed important antioxidant defence mechanisms to protect tissues from free radical-induced damage. An antioxidant is any substance that, when present at low concentrations, as compared to that of an oxidisable substrate, significantly delays or inhibits oxidation of that substrate [3]. Antioxidants can act at several stages of an oxidative sequence; first, by removing oxygen intermediates created during normal oxygen metabolism. Second, antioxidants can remove metal ions required by catalytic proteins (enzymes). Third, antioxidants also remove key ROS including superoxide (O_2^-) and hydrogen peroxide (H_2O_2) that are generated in excess in certain disease states. Fourth, antioxidants scavenge initiator-free radicals including hydroxyl, alkoxyl, and peroxyl species. Fifth, antioxidants can break the chain of an oxidative sequence and quench singlet oxygen [3]. An antioxidant molecule

L.T.T. Thuy • H. Hai • N. Kawada (✉)
Department of Hepatology, Graduate School of Medicine,
Osaka City University, Osaka, Japan
e-mail: thuylt@med.osaka-cu.ac.jp; hhai@med.osaka-cu.ac.jp;
kawadanori@med.osaka-cu.ac.jp

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may have only one of the actions described above. For example, transferrin, lactoferrin, and albumin act as metal-binding proteins. However, many antioxidants have several actions, particularly all of the third, fourth, and the fifth listed above. Thus, the antioxidant enzymes, superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSHPx), for example, exert multiple effects.

Depending on function, antioxidants are located at different sites in cells and can be classified as intracellular, membrane-associated, or extracellular in nature.

18.1.2 Antioxidant Classification

18.1.2.1 Intracellular Antioxidants

Intracellular antioxidant defence systems include SOD, catalase, glutathione (GSH) and an associated family of enzymes, the polypeptide thioredoxin (TRX), and peroxidases of the peroxiredoxin (Prx) family [4].

SOD catalyses the dismutation of superoxide ($O_2^{\bullet -}$) to H_2O_2 and O_2 (18.1):

$$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2 \tag{18.1}$$

A CuZn-SOD is present in the cytosol and in the space between the inner and outer mitochondrial membranes, whereas a manganese-containing SOD is present in the mitochondrial matrix. The actions of both of these enzymes are critical to prevent ROS-induced toxicity [5].

Catalase is found primarily in the peroxisomes of all major organs, especially the liver. The enzyme catalyses a reaction between two H_2O_2 molecules, resulting in formation of water and O_2 [6] (18.2):

$$2H_2O_2 \rightarrow O_2 + 2H_2O \tag{18.2}$$

Catalase action is very important for protecting the cell from oxidative damage caused by ROS; the enzyme can convert millions of molecules of H_2O_2 to water and oxygen each second [7].

GSH and an associated enzyme family (including peroxidases) also play major roles in removing hydrogen peroxide generated by SOD via oxidation of GSH to glutathione disulphide (GSSG) (18.3) [3]:

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O \tag{18.3}$$

GSSH generated, as shown in (18.3), is next re-converted to GSH in a reaction involving nicotinamide adenine dinucleotide phosphate [8]. GSH precursors, including *N*-acetylcysteine (NAC), are widely prescribed commercial antioxidants. The antioxidant effects of NAC are exerted by reducing cystine to cysteine, via

synthesis of reduced GSH; by increasing glutathione-*S*-transferase levels; and by capture and neutralisation of ROS [9].

The Prx family of peroxidases is present in organisms of all kingdoms [10]. Prx enzymes exist in multiple isoforms in all eukaryotic cells that reduce hydrogen peroxide and alkyl hydroperoxides to water and alcohol, respectively, with consumption of reducing equivalents. Such equivalents are specifically derived from thiol-containing donor molecules, including TRX. Prx I and II are located in the cytoplasm and Prx III in mitochondria [11].

The TRX cytoplasmic protein occupies a specific intracellular site under pathophysiological conditions [12]. TRX efficiently donates electrons to human GSHPx and members of the Trx superfamily, as mentioned above. TRX per se also acts as an antioxidant or as a scavenger of singlet oxygen, hydroxyl, and hydrogen peroxide radicals [13].

18.1.2.2 Membrane and Extracellular Antioxidants

The extracellular fluids of the human body, including blood plasma, tissue fluid, cerebrospinal fluid, synovial fluid, and seminal plasma, contain little or no catalase activity and only low levels of SOD and selenium-containing GSHPx [14, 15]. Metal-binding proteins are the principal antioxidants of extracellular fluids. Such proteins can sequester iron and copper ions that, in their free forms, catalyse oxidative reactions. Transferrin, lactoferrin, haemoglobin, and myoglobin bind iron, whereas albumin, metallothionein, and ceruloplasmin bind copper [16]. The iron transport protein transferrin is usually one-third loaded with iron and effectively controls plasma iron concentration [3]. Lactoferrin is a glycoprotein exhibiting a high affinity for iron. The capacity of lactoferrin to bind iron is twice that of transferrin; two ferric ions are bound by one lactoferrin molecule [17].

Haemoglobin (and also myoglobin), in their free forms, can mediate oxidative tissue damage by accelerating lipid peroxidation. Haemoglobin-binding proteins such as haptoglobin and heme-binding proteins including haemopexin are serum proteins that function as antioxidants by virtue of their ability to bind to haemoglobin and haem, respectively, thereby preventing oxidative stress that might be triggered by free haemoglobin [18].

The principal copper-binding protein of human plasma is ceruloplasmin, which carries over 95 % of plasma copper [19]. Ceruloplasmin is also an effective chain-breaking antioxidant for a variety of radicals, including those formed via ferroxidase activity [19].

Other extracellular or exogenous antioxidants are derived from food or synthesized in vivo and include vitamin C, vitamin E, bilirubin, melatonin, lipoic acid, coenzyme Q, uric acid, and the melanins. Ascorbic acid (vitamin C) and α-Tocopherol (vitamin E) are food antioxidants that can prevent accumulation of free radicals and lipid peroxidation, in the human body [20, 21]. Ascorbic acid exhibits multiple antioxidant properties including the ability to scavenge $O_2^{\bullet -}$, $HO_2^{\bullet}$, peroxyl ($RO_2^{\bullet}$), thiyl

and sulphenyl, and nitricoxide radicals [22]. α-Tocopherol, a fat-soluble vitamin, is a poor antioxidant outside the membrane bilayer but very effective when incorporated into the membrane [23] and combats hydrogen peroxide, lipid peroxides, and other oxidants [20].

Another group of antioxidants are natural phenolic compounds and flavonoids found in vegetables, tea, and red wine [24]. These include quercetin, resveratrol, cathechine, and epigarocathechin. For example, quercetin is a strong antioxidant because the compound chelates metals, scavenges oxygen-free radicals [24, 25], and prevents oxidation of low-density lipoprotein (LDL) in vitro [26].

18.1.2.3 Antioxidants That Modulate Nitric Oxide Metabolism

Nitric oxide (NO), a lipophilic, highly diffusible, and short-lived physiological messenger, regulates many physiological processes including vasodilation, respiration, cell migration, the immune response, and apoptosis [27]. Under pathological conditions, NO production is increased via the action of an inducible isoform of NOS (iNOS). This isoform produces NO in large (potentially damaging) amounts, in a calcium-independent manner. NO can react with O_2^- to produce the harmful molecule peroxynitrite ($ONOO^-$), a form of RNS. Materials including polyphenols and other antioxidants that influence the cell redox balance (including SOD activity) may directly affect the iNOS activity level and the expression thereof [28].

A recently discovered vertebrate globin, cytoglobin (CYGB) [29], the molecular characteristics of which are similar to myoglobin, is an antioxidant by virtue of an ability to scavenge NO. CYGB may facilitate oxygen diffusion through tissues, scavenge NO or other ROS, or exert a protective function during oxidative stress [30]. In vitro studies have shown that *CYGB* overexpression rescues the human neuronal cell line TE671 from pro-oxidant Ro19-8022-induced DNA damage [31]. *CYGB* overexpression also protected human neuroblastoma SH-SY5Y cells from H_2O_2-induced cell death [32, 33]. Furthermore, it has been reported that overexpression of CYGB in rat hepatic stellate cells, either in vitro or in vivo, protected such cells against oxidative stress and inhibited the differentiation thereof to the activated form [34]. Such reports suggest that CYGB may act as a cytoprotective and radical-scavenging molecule in addition to playing a role as gas carrier.

18.2 Antioxidant Therapy for Chronic Viral Hepatitis

As mentioned in Chap. 14, oxidative stress is an important contributor to the development of liver damage during chronic viral hepatitis, a disease in which both the virus per se and the host-mounted immune response can trigger oxidative stress [35], as reflected in increased levels of oxidised proteins and nucleic acids and decreases in antioxidant levels [36, 37].

18.2.1 Vitamins Given Singly or in Combination

Antioxidants have been evaluated over the past 20 years in clinical trials involving chronic hepatitis C (CHC) patients (Table 18.1), commencing with a small open-label study featuring vitamin E supplementation [38]. In the cited work, six CHC patients resistant to interferon (IFN) therapy were given vitamin E 1,200 IU/day for 8 weeks [38]. The vitamin delayed fibrosis progression and reduced oxidative stress, as indicated by reduced activation of hepatic stellate cells and decreased malonaldehyde levels, respectively. However, no change in either serum ALT or HCV-RNA levels was evident, and liver histology was not affected. In another study, 17 CHC patients were given vitamin E (500 mg/day) for 3 months and showed modest reductions in serum ALT levels associated with small decreases in oxidative stress. Thus, TRX levels fell from 59 to 40 ng/mL at the end of treatment [39]. Similar results were found in another study in which 23 CHC patients refractory to IFN therapy were treated; a prospective, randomised, double-blind cross-over design was used. Twelve-week supplementation with vitamin E reduced serum ALT levels. However, serum ALT levels became elevated once more within 1 month of discontinuing vitamin E treatment. Re-treatment of responders (exhibiting ALT decreases of at least 35 %) for 3 months reduced ALT levels once more [40]. Thus, supplementary vitamin E alone did not have any notable effect on significant clinical features. Subsequent studies evaluated antioxidant combinations (Table 18.1) [41–43]. Seven oral antioxidative preparations (glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, L-glutathione, and alpha-tocopherol) and four intravenous preparations (glycyrrhizin, ascorbic acid, L-glutathione, and B-complex) were given to 50 CHC patients for 20 weeks [41]. Interestingly, at the end of treatment, normalisation of liver enzyme levels was evident in 44 % of patients; ALT levels remained normal throughout the follow-up period in 72.7 % of patients; and a decrease in viral load (of one log or more) was observed in 25 % of patients. However, such encouraging results were not replicated in a larger study of 100 patients treated with antioxidant cocktails for 24 weeks [42]. Another combination therapy, featuring vitamins E, C, and selenium, was tested in 23 CHC patients over 6 months, but no beneficial effect on either HCV-RNA or ALT level, or liver histology, was evident [43].

Mitochondrial damage is a common feature of CHC pathogenesis, and MitoQ was evaluated as a therapeutic agent in the phase II study of Gane et al. [44]. Thirty patients with contraindications to pegylated interferon (PEGIFN) and/or ribavirin (RBV) were randomised to receive MitoQ (40–80 mg/day), or placebo. At the end of 28 days, compared with baseline levels, 40 mg of MitoQ daily decreased both ALT (from 153 to 110 IU/L; $P=0.002$) and AST levels (from 131 to 95 IU/L; $P=0.003$). The figures after administration of 80 mg of MitoQ daily were 131–95 IU/L, $P=0.024$ and 87–75 IU/L, $P=0.017$, respectively. However, no change in HCV-RNA level was noted, suggesting that antioxidants may mildly decrease ALT levels, but that this alone was inadequate to counter CHC.

Table 18.1 Clinical trials of antioxidant therapies for chronic hepatitis C patients

Study no.	Reference [number]	Sample size	Patient details	Antioxidant strategy	Duration	Response
1	Beloqui et al. [48]	24	CHC patients	Oral NAC (600 mg)	5–6 months	Improved ALT levels
2	Houglum et al. [38]	6	CHC patients refractory to IFN therapy	Vitamin E (1,200 UI/day)	8 weeks	Reductions in oxidative stress marker levels; decreased HSC activation; no change in HCV-RNA status
3	Von Herbay et al. [40]	23	CHC patients refractory to IFN therapy	Vitamin E (400 UI/day)	12 weeks	Decreased ALT levels
4	Ideo et al. [46]	120	IFN-non-responders with HCV infections	NAC (1,200 mg/day) + vitamin E (600 mg/day)	6 months	No benefit
5	Look et al. [45]	24	IFN-naïve CHC patients	NAC (1,800 mg/day) + sodium selenite (400 µg/day) ± vitamin E (544 UI/day)	24 weeks	A 2.4-fold greater chance of a complete response and a significantly greater reduction in viral load in test patients
6	Grant et al. [49]	30	CHC patients	Mitoquinone (40 or 80 mg) or placebo	4 weeks	Decreased ALT and AST levels ($P < 0.005$ for both), but no change in HCV-RNA status
7	Neri et al. [50]	77	CHC patients	IFN ± NAC (2.4 g/day)	6 months	Increased time to relapse compared to IFN alone (22 vs. 31 weeks); decreased oxidative stress levels; but the effects were not of long duration
8	Mahmood et al. [39]	17	CHC patients	Vitamin E (500 mg/day)	3 months	Small decreases in ALT levels; reductions in levels of oxidative stress markers
9	Morisco et al. [115]	92	CHC patients	A functional food with a high level of natural antioxidants and high carotenoid bioavailability	3 months	Lower ribavirin levels and higher haemoglobin levels in test patients than controls
10	Saeian et al. [47]	47	CHC patients	Interferon/ribavirin therapy plus vitamin E 800 IU b.d.	24 weeks	No significant difference in ALT or haemoglobin levels, and no sustained antiviral response

11	Melhem et al. [41]	50	CHC patients	Seven antioxidative oral preparations (glycyrrhizin, schisandra, silymarin, ascorbic acid, lipoic acid, L-glutathione, and alpha-tocopherol); and four different intravenous preparations (glycyrrhizin, ascorbic acid, L-glutathione, and B-complex)	20 weeks	Normalisation of liver enzyme levels occurred in 44 % of patients; ALT levels remained normal throughout follow-up in 72.7 % of patients. Decreases in viral loads (of one log or more) were observed in 25 % of patients
12	Groenbaek et al. [43]	23	CHC patients	Ascorbic acid (500 mg/day), D-alpha-tocopherol (945 IU/day) and selenium (200 µg/day); or placebo tablets	6 months	The antioxidant group exhibited significantly higher levels of plasma ascorbic acid and alpha-tocopherol than did the placebo group, and the activity of erythrocyte glutathione peroxidase significantly increased from baseline to month 6 in test patients. No differences were observed in ALT or HCV-RNA levels
13	Gordon et al. [60]	24	CHC patients	Silymarin 600 or 1,200 mg/day	12 weeks	No change
14	Hino et al. [116]	32	CHC patients	IFN/ribavirin + vitamin E 500 mg/day + vitamin C 750 mg/day	26 weeks	Attenuation of the ribavirin-induced decrease in eicosapentaenoic acid levels in erythrocyte membranes
15	Gabbay et al. [42]	100	IFN-failed CHC patients	Combined intravenous and oral antioxidant or placebo; or oral treatment. Oral formulation: glycyrrhiza, 500 mg bid; schizandra, 500 mg tid; ascorbate, 2,000 mg tid; L-glutathione, 150 mg bid; silymarin, 250 mg tid; lipoic acid, 150 mg bid; D- -TOCOPHEROL, 800 IU/day, once daily for 24 weeks. Intravenous formulation: 120 mg glycyrrhiza; 10 g ascorbate; 750 mg L-glutathione; 1 mL B complex, twice weekly	10 weeks	Modest improvement in ALT levels and combined HAI scores upon both oral and iv antioxidant therapy, measured at treatment end. Benefits not sustained after discontinuation of therapy

(continued)

Table 18.1 (continued)

Study no.	Reference [number]	Sample size	Patient details	Antioxidant strategy	Duration	Response
16	Falasca et al. [117]	40	Caucasian CHC patients	SPV complex	3 months	Hepatoprotective efficacy apparent. Reduction in inflammatory cytokine levels and decreased viral load. Decreases in the levels of all of ALT, AST, GGT, alkaline phosphatase, total cholesterol, fasting glucose, insulinemia, HOMA value, and C-reactive protein level, in hepatic steatotic patients
17	Hawke et al. [56]	32	IFN-failed CHC patients	Silymarin (140, 280, 560, or 700 mg every 8 h)	1 week	No change
18	Feld et al. [118]	21	IFN-non-responders among HCV-infected patients	PEG-IFNα2a and ribavirin for 2 weeks followed by SAMe (1,600 mg/day) for 2 weeks and PEGIFN and ribavirin for 48 weeks	2 weeks	Improved early viral kinetics and enhanced induction of ISGs upon SAMe treatment, associated with enhanced Stat1 methylation
19	Filipowicz et al. [119]	29	CHC patients	SAMe, betaine, PEG-IFNα2b, and ribavirin treatment	6 or 12 months	Improved early virological response
20	Yakoot et al. [120]	66	IFN-naïve CHC patients	Spirulina 1,500 mg/day or silymarin 420 mg/day	3 and 6 months	A significantly greater reduction in serum ALT levels in the spirulina- than the silymarin-treated group
21	Biermer et al. [121]	20	IFN-failed CHC patients	Silibinin 1,400 mg/day	2 days	Reduction in HCV-RNA levels; 3/20 patients achieved SVR
22	Fried et al. [122]	154	IFN-failed CHC patients	Silymarin 1,260 or 2,100 mg/day, or placebo	24 weeks	No significant change in either ALT or HCV-RNA levels
23	Adeyemo et al. [123]	32	IFN-non-responders with HCV infections	Silymarin 1,260 or 2,100 mg/day, or placebo	20 weeks	No change in ALT or HCV RNA levels
24	Grant et al. [49]	147	CHC	3MU IFN-alpha three times weekly plus NAC 1,800 mg daily ($n=73$); IFN alone ($n=74$)	6 months	No obvious benefit upon addition of *N*-acetyl cysteine to conventional therapy featuring interferon-alpha

Several trials have evaluated antioxidants as adjuvants to IFN therapy in the era before RBV was introduced. In one such study, 24 treatment-naïve CHC patients were randomised to receive IFN monotherapy alone, or in combination with NAC + sodium selenite or NAC + sodium selenite + vitamin E. After 6 months of treatment, a higher proportion of patients treated with a regimen including vitamin E achieved negative HCV-RNA status, as compared to those receiving IFN monotherapy or IFN + NAC + sodium selenite (6/8 vs. 3/8 vs. 2/8; $P = 0.11$). Comparisons of vitamin E- ($n = 8$) vs. non-treated ($n = 16$) subjects showed that the odds of achieving negative HCV-RNA status at the end of vitamin E treatment was 2.4-fold higher than otherwise ($P = 0.02$). However, no beneficial effect on the sustained viral response (SVR) was evident (2/8 vs. 1/8 vs. 1/8; $P =$ NS). Surprisingly, no effect on oxidative stress marker levels was noted, as assessed by measurement of trolox-equivalent antioxidant capacity and levels of thiobarbituric acid-reactive substances (TBARSs) [45]. The enhanced end-of-treatment response was not confirmed in a placebo-controlled study of 120 CHC patients who were IFN non-responders, randomised to receive IFN with or without NAC (1,200 mg/day) + vitamin E (600 mg/day) for 6 months. ALT normalisation rates were similar at the end of treatment (10.3 % vs. 9.7 %; $P =$ NS) and 6 months after completing treatment (1.3 % vs. 0 %; $P =$ NS). No patient in either group achieved negative HCV-RNA status by the end of treatment [46]. A similar lack of any benefit afforded by vitamin E on HCV-RNA loss or SVR was noted in another randomised controlled trial (RCT) in 47 CHC patients [47].

Thus, vitamins E and/or C alone, or in combination with anti-HCV therapy, tend to reduce serum ALT levels. However, neither HCV titres, nor the histological extent of inflammation, nor the extent of fibrosis, was influenced by the vitamins. Furthermore, in most studies, the decrease in ALT level was marginal and not sustained after treatment cessation, thereby throwing any clinical significance into doubt. Modern treatment regimens feature viral protease inhibitors and other novel antiviral medications, and it remains to be seen if antioxidants can exert beneficial effects as components of such regimens.

18.2.2 N-*acetylcysteine*

In an open pilot study on 14 CHC patients who were documented IFN non-responders, addition of NAC at 1.8 g/day to IFN improved liver enzyme levels, with a decrease in viral load [48], encouraging testing of the NAC/IFN combination in better-controlled studies [43, 44]. In a placebo-controlled double-blind RCT, addition of 1.8 g/day NAC to IFN did not improve the SVR rate [49]. In another prospective, randomised open-label study, the viral response rates were similar in the test and control groups but the time to relapse after treatment discontinuation was longer after use of NAC (31 vs. 22 weeks; $P < 0.05$). In terms of HCV infection, the goal of treatment (as mentioned earlier) is achievement of SVR, and a 9-week delay in relapse of HCV infection is of minimal clinical significance [50].

18.2.3 Natural Compounds

An oral formulation of extracts from the milk thistle, silymarin, is widely used in the USA for treatment of viral hepatitis. A report by [51] showed that, of 1,145 study participants, 33 % had used silymarin either in the past or at the baseline. In terms of function, in vitro studies have shown that silymarin exerts anti-inflammatory and immunomodulatory functions via inhibition of NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) [52]. Furthermore, silymarin significantly downregulated HCV core mRNA (by 20–36 %) and protein (by 30–60 %) levels in CNS3 cells [53]. Clinical trials showed that silymarin exerted biochemical effects [54], allowing attainment of negative HCV-RNA status [55, 56], and one case report even found an SVR [57]. However, several studies have reported limited effects of, or no consistent benefit afforded by, silymarin in CHC patients [58–61]. Such results may have been compromised by small numbers of patients, short treatment duration, or the low doses used.

In summary, many trials of silymarin have yielded inconsistent results. Silymarin, given as an antioxidant to CHC patients, has been either efficient or inefficient in terms of reducing enzyme levels in vivo and affecting HCV-RNA levels and/or liver histological properties (Table 18.1). Thus, it is difficult to conclude that antioxidants are valuable in CHC patients. Silymarin or NAC may potentially be effective but further longer-term trials with larger numbers of patients are required.

18.3 Antioxidant Therapy for Alcoholic Liver Diseases

Acute or chronic alcohol consumption increases production of both ROS/RNS and other free radical species (e.g., the 1-hydroxyethyl radical), as has been shown in both animal models and patients with alcoholic liver diseases (ALD), as described in Chap. 13. The high-level increase in oxidative stress mediated by alcohol consumption suggests that it is appropriate to use antioxidants to protect against liver damage. Several antioxidants, including vitamin E, *S*-adenosylmethionine (SAMe), polyenylphosphatidylcholine (PPC), and silymarin, have been trialed to this end.

18.3.1 Vitamins E and C

A randomised study featuring administration of tocopherol 500 mg daily for 1 year found that no benefit was afforded to ALD patients in terms of either clinical or biochemical function, hospitalisation rate, or mortality [62]. Another double-blind, placebo-controlled randomised trial of vitamin E at 1,000 UI/day was performed in 25 patients with alcoholic hepatitis [63]. Significant decreases in the serum levels of the fibrogenesis marker, hyaluronic acid, were observed in treated patients, suggesting that further investigations should be performed in large patient cohorts.

18.3.2 Polyenylphosphatidylcholine

PPC prevents excess collagen accumulation by enhancing collagenase activity both in cell cultures and animal models [64] and decreases oxidative stress via reduction in, or normalisation of, the levels of 4-hydroxinonenal, isoprostanes, and GSH [65, 66]. PPC (4.5 g/day given orally as 1.5 g tablets three times daily) was tested in 789 veterans (97 % male; mean age 49 years) with biopsy-proven alcoholic cirrhosis in a randomised, double-blind, placebo-controlled multicentre study [67]. The average alcohol intake was comparable in both the control and treatment groups, being about 225 g/day for 19 years before the start of treatment, but was unexpectedly reduced to about 35 g/day during the study period. Liver biopsy was repeated at 24 months, and the main outcome measure was the fibrosis stage, as compared to that at baseline. Two-year biopsies were performed on 412 patients. PPC did not differ from placebo in terms of any effect on the principal outcome. However, a trend toward a reduction in the development of ascites was evident in the PPC group (9 % vs. 14 %; $P<0.057$). Fibrosis progression was more common in those with concomitant HCV infection (32 % vs. 17 %; $P<0.001$). In this latter subgroup, PPC treatment improved liver function, as reflected by the serum levels of liver enzymes and bilirubin [67].

18.3.3 Silymarin

A total of six RCTs have explored the utility of silymarin in the management of ALD patients. Of these, three found that silymarin was effective. In a study of 91 patients with alcoholic cirrhosis, those given silymarin (520 mg/day) on a long-term basis exhibited improvements in 4-year survival, as compared to placebo-treated patients (58 % vs. 39 %; $P=0.03$) [55]. Another study, in which silymarin was given at 420 mg/day, revealed no effect on survival but histological and biochemical parameters improved. However, silymarin was given for only 4 weeks [68]. Lirussi et al. [69] performed an RCT on 60 outpatients; the test group received a combination of silymarin and beta-cyclodextrin (silybin-beta-cyclodextrin) for 6 months. Significant reductions in serum glucose and triglyceride levels were evident, as was a decrease in the levels of an oxidative stress marker, malondialdehyde (MDA), in the test, as compared to the placebo-treated group. Such effects may be attributable to recovery of the levels of energy substrates, consistent with reduced lipid peroxidation and improved insulin activity. No clinically relevant side-effect was observed in either group. Three RCTs ($n=60$–97 subjects; silymarin dose: 280–450 mg/day; duration: 6–24 months) failed to show any biochemical, histological, or survival benefit [70–72], although one study found a significant decrease in the levels of oxidative stress markers [70].

18.3.4 S-*adenosylmethionine*

SAMe is a promising antioxidant for treatment of ALD patients. SAMe is a precursor of key metabolites including glutathione and polyamines. Oral administration of SAMe (1,200 mg/day for 6 months) significantly increased hepatic glutathione levels in subjects both with and without ALD [73]. More importantly, a long-term, randomised, placebo-controlled, double-blind, multicenter clinical trial of SAMe in 123 patients with alcoholic cirrhosis found that oral SAMe (1,200 mg/day) improved survival compared with placebo [74].

In general, antioxidant therapy for ALD using SAMe or silymarin potentially improves survival if high doses are given long-term. Both agents are well-tolerated; no significant adverse effects were noted [69, 74].

18.4 Antioxidant Therapy for Non-alcoholic Fatty Liver Disease and Non-alcoholic Steatohepatitis

The associations between non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) and biomarkers of oxidative stress and lipid oxidation have been assessed in many human and animal studies [75–78]. To date, no established pharmacological treatment for NAFLD/NASH has been reported. Depletion of antioxidants within hepatocytes, thus impairing ROS inactivation, forms the basis of antioxidant supplementation to potentially treat NASH. Several agents have yielded encouraging results; these include vitamin E, vitamin C, betaine, ursodeoxycholic acid (UDCA), pentoxifylline, metformin, and NAC (Table 18.2).

18.4.1 Vitamins E and C

Hasegawa et al. found that the serum alanine transaminase level decreased in NAFLD patients, but not NASH patients, after 6 months of vitamin E supplementation [79]. However, histological findings, including those of steatosis, inflammation, and fibrosis, improved after alpha-tocopherol treatment of NASH patients. Similarly, Kawanaka et al. showed that serum transaminase activities, and the levels of oxidative stress markers including TRX and TBARS, decreased significantly after 6 months of vitamin E therapy [80]. Interestingly, in obese children with NASH, daily oral vitamin E administration for 4–10 months normalised serum aminotransferase and alkaline phosphatase levels [81], although only 11 subjects were involved in the cited study. A large-scale trial involved 247 adults with NASH (but without diabetes) given pioglitazone at 30 mg daily (80 subjects), vitamin E at 800 IU daily (84 subjects), or placebo (83 subjects) over a long duration (96 weeks) [82]. The results clearly showed that, as compared to placebo, vitamin E therapy

Table 18.2 Clinical trials of antioxidant therapies for NALFD/NASH patients

Study no.	Reference [number]	Sample size	Patients	Antioxidant strategy	Duration	Response
1	Hasegawa et al. [79]	22	Non-alcoholic fatty liver ($n=12$); non-alcoholic steatohepatitis ($n=10$)	Dietary instructions for 6 months, following with vitamin E 300 mg/day for 1 year	6 months to 1 year	ALT level decreases in non-alcoholic fatty liver patients, but not non-alcoholic steatohepatitis patients. Improvement of steatosis, inflammation and fibrosis
2	Kawanaka et al. [80]	10	NASH	Vitamin E	6 months	Significant drops in serum transaminase activity, and levels of oxidative stress markers including thioredoxin and thiobarbituric acid-reactive substance (TBARS)
3	Lavine et al. [81]	11	Obese children with NASH	Vitamin E 400–1,200 IU daily	4–10 months	Normalisation of serum aminotransferase and alkaline phosphatase levels
4	Sanyal et al. [82]	247	Non-alcoholic steatohepatitis patients without diabetes	Pioglitazone 30 mg daily ($n=80$); vitamin E 800 IU daily ($n=84$), or placebo ($n=83$)	96 weeks	Compared to placebo, vitamin E therapy was associated with significant improvement in NASH activity scores, with no worsening of fibrosis (43 % vs. 19 %, $P=0.001$). Both drugs reduced liver enzyme levels, local inflammation, and hepatic steatosis
5	Harrison et al. [83]	49	NASH	Combination of alpha-tocopherol (vitamin E) 1,000 IU and vitamin C 1,000 mg	6 months	Significant improvement in fibrosis scores. However, no improvement in necroinflammatory activity or ALT level

(continued)

Table 18.2 (continued)

Study no.	Reference [number]	Sample size	Patients	Antioxidant strategy	Duration	Response
6	Nobili et al. [84]	90	NAFLD children	Combination of vitamins E (600 IU/day) and C (500 mg/day), plus nutritional programming	12 months	Diet and physical exercise afforded significant improvements in both liver function and glucose metabolism
7	Abdelmalek et al. [85]	10	NASH	Betaine (anhydrous) as an oral solution (Cystadane) given in two divided doses daily	12 months	Significant improvements in the serum levels of aspartate aminotransferase ($P=0.02$); evidently extents of fibrosis and steatosis, and necroinflammatory grade
8	Abdelmalek et al. [86]	55	NASH	Oral betaine (20 g daily) or placebo	12 months	Compared to placebo, neither intra- or inter-group differences nor any changes in either non-alcoholic fatty liver disease activity scores or fibrosis stage were noted. Betaine did not improve hepatic steatosis but may protect against worsening of steatosis
9	Adams et al. [88]	20	NASH	Pentoxifylline (1,600 mg/day)	12 months	Alanine and aspartate aminotransferase levels significantly reduced, as compared to baseline (84 ± 64 vs. 138 ± 76; $P=0.002$; and 58+/37 vs 102+/62; $P=0.003$, respectively)
10	Laurin et al. [89]	24	NASH	Ursodeoxycholic acid (UDCA)	12 months	Significant improvement in alkaline phosphatase, ALT, GGT, and hepatic steatosis

11	Lindor et al. [90]	166	NASH	UDCA	2 years	Decreased liver enzyme levels but no change in extent of steatosis, necroinflammation, or fibrosis
12	Dufour et al. [91]	48	NASH	UDCA + vitamin E; UDCA + placebo	2 years	UDCA with vitamin E improved laboratory test values and hepatic steatosis
13	Haukeland et al. [95]	48	NAFLD	Metformin ($n=24$) or placebo ($n=24$)	6 months	Effects of metformin were observed on changes in body-weight ($P<0.001$), serum levels of cholesterol ($P=0.004$), LDL-cholesterol ($P<0.001$), glucose ($P=0.032$) and on HbA1c ($P=0.020$) but not in hepatosteatosis and NAS score
14	Pamuk et al. [99]	35	NASH	NAC 600 mg/day	4 weeks	Improvements in liver enzyme levels
15	de Oliveira et al. [94]	20	NASH	NAC 1.2 g/day and metformin 500 mg/day	12 months	Improvements in liver enzyme levels, insulin resistance, body mass index, and liver histological findings (including those indicating steatosis and fibrosis)

was associated with significant improvement in NASH, with no worsening of fibrosis (43 % vs. 19 %, $P=0.001$). Both compounds reduced liver enzyme levels, local inflammation, and hepatic steatosis [82].

A combination of daily alpha-tocopherol (vitamin E 1,000 IU) and vitamin C (1,000 mg) given for 6 months in a prospective, double-blind, randomised, placebo-controlled trial significantly improved fibrosis scores in 49 patients; 45 completed the study [83]. However, no improvement in necroinflammatory activity or ALT level was seen using this combination therapy. Not only combinations of vitamin E and C, but also a nutritional programme improved outcomes. Nobili et al. found that both diet and physical exercise in NAFLD children seemed to significantly improve both liver function and glucose metabolism, even in the absence of any antioxidant therapy [84] (Table 18.2).

18.4.2 Betaine

Betaine, the donor of methyl groups for remethylation of homocysteine, may be a therapeutic agent in the context of NASH. Abdelmalek et al. conducted a pilot study on ten patients with NASH who were given an oral solution of anhydrous betaine (Cystadane) in two divided doses daily for 12 months [85]. Significant improvements in the serum levels of aspartate aminotransferase ($P=0.02$), the extent of steatosis, the necroinflammatory grade, and the fibrosis stage were evident during treatment. Eight years later, the cited authors conducted a much larger-scale, randomised placebo-controlled study of 55 patients with biopsy-proven NASH given either oral betaine (20 g daily) or placebo for 12 months [86]. However, as compared to placebo, no intra- or inter-group differences in any of the non-alcoholic fatty liver disease activity scores, or fibrosis stage, were noted. Betaine did not improve hepatic steatosis, but may protect against worsening steatosis [86].

18.4.3 Pentoxifylline

A newer antioxidant drug, pentoxifylline, is a methylxanthine compound inhibiting production of tumor necrosis factor alpha (TNF-α) and has yielded promising results when used to treat NASH patients [87]. Adams et al. performed a 12-month pilot-scale trial to assess the efficacy and safety of pentoxifylline (1,600 mg/day) in 20 patients with NASH. Alanine and aspartate aminotransferase levels were significantly reduced, as compared to those at baseline (84 ± 64 vs. 138 ± 76, $P=0.002$ and 58 ± 37 vs. 102 ± 62, $P=0.003$, respectively) [88]. However, nine patients withdrew from the study, primarily because of nausea, although no serious adverse event occurred [88].

18.4.4 Ursodeoxycholic Acid

UDCA is a naturally occurring bile acid with many hepatoprotective activities and was suggested to benefit 24 NASH patients given the compound for 12 months in the open-label study of Laurin et al. [89]. Later, the same authors conducted a large-scale randomised trial on 166 patients with liver biopsy-proven NASH given UDCA for 2 years. Liver enzyme levels decreased, but no change in the extent of steatosis, necroinflammation, or fibrosis was evident [90]. In contrast, UDCA given in combination with Vitamin E for 2 years yielded results differing from those afforded by UDCA + placebo in that both liver enzyme levels and histology improved in NASH patients [91].

18.4.5 Metformin

As insulin resistance plays roles in the pathogenesis of NAFLD and NASH, insulin-sensitising drugs including metformin have been tested in NASH patients [92–94]. Several open-label, RCTs [95–98] have shown that metformin reduced liver enzyme levels in, and improved histology of, NALFD/NASH patients, as also shown in other studies [92, 94, 96, 98].

18.4.6 N*-acetylcysteine*

NAC has been tested in two studies on NASH patients. Prescription of 600 mg/day NAC given in an open-label, prospective randomised study that ran for 4 weeks improved liver enzyme levels [99]. Another open-label prospective trial included 20 NASH patients given NAC 1.2 g/day and metformin 500 mg/day for 12 months. All of liver enzyme levels, the extent of insulin-resistance, body mass index, and liver histological findings (including steatosis and fibrosis), improved, although NAC had no effect on ballooning or inflammation [94].

In general, oxidative stress and antioxidant defences are both complicated networks of enzymatic and non-enzymatic processes that are not in balance as NALFD/NASH progresses. The effects of antioxidant therapy on such patients may depend on disease stage and severity. As the results of many antioxidant trials are contradictory, the utility of antioxidant therapy in those with NAFLD/NASH requires further evaluation, including the performance of large-scale studies using combinations of two or more antioxidants. Moreover, both lifestyle modifications and pharmaceutical interventions specifically targeting the principal signaling pathways involved not only in oxidative stress but also in inflammation, lipid peroxidation, and fibrosis should be tested.

18.5 Antioxidant Therapy for Liver Fibrosis and Cirrhosis

Cirrhosis, an advanced stage of liver fibrosis, may be reversible not only histologically [100], but also in terms of clinical outcomes [101]. Therefore, therapies that prevent or reverse cirrhosis are in great demand. Oxidative stress is pathogenetically associated with fibrosis development and progression via ROS-induced cellular injury and RNS-induced dysregulation of the hepatic microcirculation. Thus, antioxidants appear to afford great therapeutic potential as treatments of fibrosis/cirrhosis, provided that sufficient levels of antioxidant activity can be delivered to sites of injury within the liver. Experimental models of liver fibrosis/cirrhosis have been used to evaluate antioxidant compounds including food supplements and drugs including polyunsaturated phosphatidylcholine (PPC) [102], peroxisome proliferator-activated receptor (PPAR) α ligand [103], UDCA [104], and resveratrol [105–108].

SAMe, silymarin, and vitamin E have been tested in liver fibrosis/cirrhosis patients. SAMe is required for methylation of many substrates (DNA, proteins, lipids, and many small molecules) and polyamine synthesis. Thus, if the SAMe concentration falls below a certain level, or rises excessively, normal liver function will be compromised [109]. SAMe has exhibited beneficial effects when used to treat alcoholic liver cirrhosis, as discussed in Sect. 18.3.2 [74]. The results of the study cited therein indicated that long-term treatment with SAMe may improve survival or delay the need for liver transplantation in patients with alcoholic liver cirrhosis, especially those with less advanced liver disease. Moreover, treatment with SAMe seemed to be safe and free of side-effects [74]. In a double-blind, placebo-controlled, multicenter clinical trial performed on 220 patients with chronic liver disease (chronic active hepatitis and cirrhosis), SAMe significantly improved serum marker levels (bilirubin and alkaline phosphatase) and subjective symptoms (pruritus and fatigue) associated with cholestasis [110].

Treatment of hepatic cirrhosis with silibinin, the major active constituent of silymarin, improved antioxidant status, enhanced cytoprotection, reversed fibrosis, and stimulated regeneration. Dose-dependent decreases in hepatic enzyme activity after silibinin treatment have been reported [111]. In a placebo-controlled trial, patients with cirrhosis taking silibinin had higher total glutathione concentrations and exhibited concurrent decreases in the level of the N-terminal propeptide of type III collagen, a biomarker of hepatic fibrosis [70]. In RCTs, lower mortality rates have been documented when silibinin was given to patients with cirrhosis [112]. All-cause mortality of cirrhotic patients decreased by 4.4 % and mortality from liver disease by 7.3 % [113]. Although evidence supporting efficacy is, therefore, good, silibinin is not considered suitable for use as a sole treatment but, rather, as an adjunct drug indicated for treatment of a variety of acute and chronic diseases affecting liver function. The available information on silibinin disposition and pharmacodynamics in both small domestic animals and large animal herbivores is limited [114]. Additional pharmacokinetic and pharmacodynamic studies of potentially valuable agents in healthy animals are essential to accumulate evidence-based clinical data.

The effects of vitamin E in alcoholic or NASH patients with fibrosis or cirrhosis have been discussed in Sects. 18.4 and 18.5 above. The most promising results were afforded by the PIVEN trial [82] involving 247 patients treated for 96 months with vitamin E. Histological regression was evident, and fibrosis did not progress.

In summary, although antioxidant treatments may be used as adjunct therapies, prevention or reversion of liver fibrosis/cirrhosis requires elimination of the relevant etiologic agent or disruption of the pathogenic processes causing liver injury. There is as yet but slim evidence that antioxidant treatment alone can achieve these goals.

18.6 Conclusion

The antioxidant defence system is complex, involving the actions of intracellular enzymes, non-enzymatic substances that serve as scavengers, and dietary components. The defence system normally controls production of both ROS and RNS. Oxidative stress occurs when a significant imbalance develops between ROS and RNS production and removal. Thus, antioxidant therapy targets (1) recovery of antioxidant enzyme/compound levels, and (2) reduction in ROS and RNS production. Although antioxidant therapies have yielded valuable results in animal models, the results from human trials remain inconsistent. Today, only silymarin is recommended as a therapy for patients with ALD and vitamin E as an antioxidant for patients with NASH. The discrepancies in treatment outcomes after delivery of antioxidant therapies might be less were several deficiencies of most trials need to be overcome. These include small sample size, short treatment duration, and difficulties in defining clinical endpoints. Moreover, variations in the antioxidants used and the doses thereof fundamentally influence biological outcomes. Although it is difficult to design studies that overcome all of these limitations, especially when funding for such work is sparse, antioxidant therapies remain attractive and promising approaches for the treatment of liver diseases.

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