Review Article

Vitamins in the treatment of chronic viral hepatitis

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Abstract

Hepatitis B virus (HBV)- and hepatitis C virus (HCV)-related chronic infections represent a major health problem worldwide. Although the efficacy of HBV and HCV treatment has improved, several important problems remain. Current recommended antiviral treatments are associated with considerable expense, adverse effects and poor efficacy in some patients. Thus, several alternative approaches have been attempted. To review the clinical experiences investigating the use of lipid- and water-soluble vitamins in the treatment of HBV- and HCV-related chronic infections, PubMed, the Cochrane Library, MEDLINE and EMBASE were searched for clinical studies on the use of vitamins in the treatment of HBV- and HCV-related hepatitis, alone or in combination with other antiviral options. Different randomised clinical trials and small case series have evaluated the potential virological and/or biochemical effects of several vitamins. The heterogeneous study designs and populations, the small number of patients enrolled, the weakness of endpoints and the different treatment schedules and follow-up periods make the results largely inconclusive. Only well-designed randomised controlled trials with well-selected endpoints will ascertain whether vitamins have any role in chronic viral hepatitis. Until such time, the use of vitamins cannot be recommended as a therapy for patients with chronic hepatitis B or C.

Key words: Liver: Clinical pharmacology: Viral hepatitis: Inflammation: Vitamins

Hepatitis B virus (HBV)- and hepatitis C virus (HCV)-related chronic infections represent a worldwide public health problem, with more than 300 and 170 million people being infected, respectively[1–4]. HBV, a DNA virus, and HCV, an RNA virus, are both hepatotropic, and both lead to chronic hepatitis in many patients with potentially fatal complications including decompensated cirrhosis and hepatocellular carcinoma.

The current treatment of chronic HBV infection includes immunomodulating agents (pegylated interferon-α (PEG-IFN-α)) and several antiviral molecules specifically targeting the virus polymerase[4,5]. Interferon-based therapies have a fixed duration and are able to induce a sustained suppression of HBV viraemia but carry a high probability of relapse after the end of the treatment. Moreover, they can give rise to adverse events, so that their use in advanced liver disease is not recommended. Antiviral drugs, such as nucleos(t)ide analogues, need to be continued for a very long time or even indefinitely, and their efficacy is seriously hampered by the onset of drug-resistant strains.

A combination therapy with PEG-IFNα and ribavirin is currently the treatment of choice for chronic HCV infection. However, this dosage regimen is effective in approximately 50–60 % of patients and can induce severe side effects[6–8]. So far, several HCV protease and polymerase inhibitors have been developed, but they are still under investigation[9,10].

Despite remarkable virological differences, HBV and HCV seem to share common behaviours, such as the induction of long-term chronic liver disease, and similar pathogenetic mechanisms of liver damage[11]. In both infections, inflammatory liver injury is largely mediated by the host's cellular immune response to infected hepatocytes[11–13]. Conversely, a defective or weak specific immune response has been postulated as the main factor

leading to chronic evolution of the infection and an unfavourable disease outcome[13,14].

Several data suggest that HBV and HCV favour the generation of reactive oxygen species within the infected cells. This contributes to liver damage and carcinogenesis via oxidative stress, as extensively shown by several studies[15–25]. In addition, reactive oxygen species are produced by both neutrophils and macrophages during the immune response against pathogens[26] and during the accumulation of viral components in infected hepatocytes[27–30].

On this basis, compounds exerting immune-stimulatory and antioxidant properties could be effective in reducing liver damage[31,32]. It is well known that several vitamins have an essential role in neutralising reactive oxygen species activity[26–30] and in regulating both innate and adaptive immune response[26,33], so that their deficiency is associated with an increased susceptibly to infections. The immunomodulation of vitamins is driven through multiple pathways, such as promoting the differentiation into Th1 and Th2 subsets and enhancing lymphocyte proliferation and cytokine production.

This background represents the rationale to evaluate the effectiveness of vitamins in attenuating liver damage in chronic viral hepatitis. Interestingly, although not limited to hepatic disease of viral aetiology, significant reductions in serum levels of vitamins such as β-carotene, vitamin C, vitamin D and vitamin E in patients with chronic viral hepatitis have been reported[34–40], but the underlying mechanisms have not been elucidated.

The aim of the present study was to review clinical studies investigating the efficacy of vitamins, alone or in addition to standard therapy, in the treatment of HBV- and HCV-related chronic hepatitis.

Materials and methods

In order to identify clinical studies involving vitamins in the treatment of chronic hepatitis B and C, a literature search was performed in the following electronic databases: PubMed, MEDLINE, the Cochrane Library and EMBASE. No *a priori* selection regarding a specific vitamin was performed so as to extract all the available experience in this context. The search terms used were chronic hepatitis, chronic hepatitis B, chronic hepatitis C, vitamins, vitamin A, retinol, vitamin B_1, thiamine, vitamin B_2, riboflavin, vitamin B_3, niacin, vitamin B_5, pantothenic acid, vitamin B_6, pyridoxine, vitamin B_7, biotin, vitamin B_9, folic acid, folate, vitamin B_{12}, cobalamin, vitamin C, ascorbic acid, vitamin D, cholecalciferol, vitamin E, alpha toco- pherol, vitamin K and antiviral treatment. The search was carried out in February 2009 without a lower limit on the search and was restricted to peer-reviewed, full-text English language publications.

Only studies evaluating the effects of vitamins alone or in combination with standard antiviral treatment on viral replication and/or serum transaminases or liver histology parameters were included. Both controlled and uncont- rolled clinical trials, pilot studies and case series in adult and paediatric patients with different clinical and serovirological features were evaluated. *In vitro* studies, or studies with other major aims such as the role of vitamins in counteracting the standard antiviral treatment side effects, were excluded.

Results

Hepatitis B virus infection

Vitamin B_1 (thiamine) supplementation. An association between high serum and/or hepatic iron level and either an increased risk of disease progression or a reduced response to IFN therapy has been reported in patients with chronic hepatitis B[41]. Thiamine is required for the formation of dihydrolipoate, a complex able to remove ferritin-bound Fe[42]. Thus, it was hypothesised that thiamine might slow or reverse liver damage in chronic HBV infection (Table 1) via the reduction of Fe overload.

On this topic, the search strategy generated three studies eligible for inclusion. The first study randomised twenty- four Chinese children with hepatitis B e antigen (HBeAg)- positive chronic hepatitis to receive either recombinant IFNα-2a or thiamine-enriched syrup as placebo for 12 weeks[43]. The two groups underwent the same rate of sus- tained HBV-DNA and HBeAg loss (17 and 8 %, respectively). The second study randomised ninety HBeAg-positive Chinese children to be treated with recombinant IFNα-2a, either with or without prednisone, or vitamin B complex, as placebo. In contrast with the results of the previous trial[44], none of the children who had received vitamin B underwent HBeAg seroconversion, which occurred in 3 and 13 % of cases treated with IFN alone or IFN plus steroid, respectively. Considering the controversial results of these two early studies, no benefit of thiamine can be demonstrated in children with chronic hepatitis B.

In a more recent study, three adult patients with HBV- related chronic hepatitis who had failed to respond to or did not tolerate IFN were given thiamine at 100 mg/d, in different time schedules[45]. Thiamine administration was stopped whenever transaminases normalised and was resumed when they increased again. During thiamine administration, alanine aminotransferase (ALT) became normal, and HBV-DNA fell to undetectable levels in each patient. In addition, the two HBeAg-positive patients sero- converted to hepatitis B e antibody (HBeAb). However, the extremely small cohort, the descriptive design of the study and the possibility that transaminase fluctuations and spon- taneous HBe seroconversion can occur during the natural history of chronic hepatitis B make this result largely inconclusive.

Vitamin E (α-tocopherol) supplementation. Antioxidant activity is one of the most important biological properties

Table 1. Summary of clinical studies of vitamin use in the treatment of chronic hepatitis B

Reference	Study design (n)	Patient characteristics	Treatment	Treatment duration and F-up	Endpoint	Main findings	Compliance data	Tolerability data
Thiamine								
Lai et al.[43]	RCT (n 24)	HBeAg-positive children	IFNα-2a 10 × 10⁶ Iu/m² tiw Thiamine-enriched syrup (15 ml twice a day) as placebo	Treatment: 3 months F-up: 15 months	Not specified	IFN group and thiamine/placebo group showed the same rate of both sustained HBV-DNA (17 %) and HBeAg seroconversion (8 %)	Not reported	Not reported
Lai et al.[44]	RCT (n 90)	HBeAg-positive children	IFNα-2b 5 × 10⁶ Iu/m² tiw Prednisone for 6 weeks + IFNα-2b Thiamine-enriched syrup placebo	Treatment: 4 months F-up: 24 months	Not specified	HBeAg seroconversion was obtained in 13 % of the prednisone + IFN group v. 3 % of the IFN group v. 0 % of the placebo group	Not reported	Not reported
Wallace & Weeks[45]	CS (n 3)	Adults: two HBeAg-positive, one HBeAg-negative	Thiamine 100 mg/d with a demand schedule	Treatment: variable F-up: not specified	ALT decrease	During thiamine treatment, ALT normalised and serum HBV-DNA tested negative in all three patients	Not reported	No side effects
α-Tocopherol								
Andreone et al.[49]	RCT (n 32)	Adults: twelve HBeAg-positive, twenty HBeAg-negative	Vitamin E 600 mg/d No treatment	Treatment: 3 months F-up: 12 months	HBV-DNA clearance and ALT normalisation	Endpoint obtained in 47 % of the vitamin E group and in 0 % of the untreated group ($P=0.0019$)	Not reported	No side effects
Dikici et al.[50]	RCT (n 58)	Immunotolerant HBeAg-positive children	Vitamin E 100 mg/d No treatment	Treatment: 3 months F-up: 6 months	HBV-DNA clearance and HBeAg seroconversion	No beneficial effect of vitamin E (no one obtained the endpoint)	Not reported	No side effects
Gerner et al.[51]	RCT (n 92)	Children HBeAg-positive	Vitamin E from 200 to 600 IU/d according to body wt Placebo	Treatment: 6 months F-up: 12 months	HBV-DNA clearance, HBeAg seroconversion and HBeAg loss	HBeAg seroconversion was obtained in 23 % of the vitamin E group and in 9 % of the placebo group ($P=$NS)	7·6 % of non-compliance	Essentially well tolerated: three cases of self-limited gastroenteritis

F-up, post-treatment follow-up; RCT, randomised clinical trial; HBeAg, hepatitis B e antigen; IFN, interferon; tiw, three times a week; HBV, hepatitis B virus; CS, case series; ALT, alanine aminotransferase.

of vitamin E (α-tocopherol), a liposoluble vitamin widely available in nature[46]. Vitamin E acts as a free radical 'scavenger' and effectively contributes to neutralise free radicals and reactive oxygen species. Therefore, it plays an important role in stabilising and protecting cellular structures from oxidative damage[47,48]. Vitamin E also exerts immunomodulant properties, as it can enhance cell-mediated immunity[46]. Three studies evaluated the effect of vitamin E on chronic hepatitis B.

A small clinical trial randomised thirty-two patients with chronic hepatitis B to receive either vitamin E or no treatment[49]. The patient population included both HBeAg- and HBeAb-positive adult subjects, as well as naive patients and non-responders to previous IFN treatment. At the end of the study period, seven patients (47 %) in the vitamin E group and none of the controls ($P = 0.0019$) achieved a complete response, defined as ALT normalisation and undetectable serum HBV-DNA.

In a more recent study, fifty-eight HBeAg-positive children with normal ALT levels and a high viral load received either vitamin E or no treatment[50]. Vitamin E failed to either reduce the viral load or promote HBeAg seroconversion. Divergent results seem to emerge from another study involving ninety-two children randomised in a 3:1 ratio to receive either vitamin E according to body weight or placebo for 6 months. The intention-to-treat analysis showed that HBeAg seroconversion was obtained in sixteen of sixty-nine (23 %) vitamin E-treated children and in two of twenty-three (9 %) placebo-treated children. Although such a difference was not statistically significant, this study suggests that vitamin E could favour HBeAg seroconversion[51].

These studies in children and adults have major limitations represented not only by the small size but also by the serological and clinical heterogeneity of patients enrolled, making the results inconclusive. However, the use of vitamin E in the treatment of chronic hepatitis B provides some interesting cues. Only one study reported a complete clinical failure, but it was conducted on immunotolerant children treated with a lower vitamin E dosage than that administered in the other paediatric study[50,51]. This may suggest that 'successful' stimulation of the immune system by vitamin E could be obtained mainly in the immunoclearance phase, since the immune system is too silent and weak in immunotolerance to be reinforced.

Hepatitis C virus infection

Vitamin A (retinoic acid) supplementation. An HCV replicon model has recently shown that HCV inhibits the activity of gastrointestinal glutathione peroxidase[52], a potent antioxidant enzyme expressed by epithelial cells of the gastrointestinal tract and liver, which contains retinoic acid response elements[53] (Table 2). The same

study reported an effect of all-*trans* retinoic acid (ATRA) in inducing a down-regulation of the HCV replicon[52]. The addition of ATRA to HCV-transfected liver tumour cell lines also up-regulated type-I IFN receptors, which in turn enhanced the antiviral effects of IFNα *in vitro*[54]. These experiments provided the rationale for evaluating the effectiveness of ATRA in patients with chronic hepatitis C. So far, the only available study[55] randomly assigned twenty so-called difficult-to-treat patients defined by HCV genotype 1 infection and/or non-response to previous antiviral treatments to receive either ATRA plus sodium selenite or a combination of ATRA, sodium selenite and PEG-IFNα-2a. An end-of-treatment virological response, defined as a drop ≥ 2 log of serum HCV-RNA, was obtained in one (10 %) patient in the ATRA group and in four (40 %) patients in the ATRA plus PEG-IFNα group. During the subsequent follow-up period, this response was only maintained in the patient from the ATRA group. Adverse events were reported in detail (Table 2). In particular, no evidence for liver toxicity was observed, but typical retinoid-associated toxicity (skin dryness, pruritus, headache, asthenia and hypertriacyl-glycerolaemia) occurred in the majority of patients requiring three ATRA dose reductions and four pre-term withdrawals. Based on these results, the authors argued that ATRA has an *in vivo* anti-HCV activity and suggested a potential additive or synergistic effect of ATRA and PEG-IFNα, warranting larger controlled clinical trials. This conclusion is questionable because the endpoint is not correct as it does not refer to sustained virological response defined as HCV-RNA negativisation, and the authors emphasised an event that occurred in just a single patient. Furthermore, a major concern in the use of vitamin A is its tolerability profile and well-known hepatotoxicity, which can limit the potential benefits.

Vitamin E supplementation. The safety and potential efficacy of vitamin E in patients with chronic hepatitis C have been evaluated in several trials.

A double-blind randomised placebo-controlled trial with a cross-over design administered vitamin E to twenty-three HCV patients not responding to previous IFN treatment[56]. At the end of the study, even though eleven patients (48 %) were classified as responders because ALT was reduced by > 35 %, none normalised ALT or obtained HCV-RNA negativisation.

Another study blindly randomised twenty-four naive chronic hepatitis C patients to receive IFNα-2a or IFNα plus *N*-acetylcysteine (NAC) and sodium selenite or IFNα plus NAC, sodium selenite and vitamin E[57]. At the end of the treatment, a complete response (normalisation of ALT and negativisation of HCV-RNA) was obtained in three of eight (37·5 %) patients treated with IFN monotherapy, two of eight (25 %) patients treated with IFN plus NAC and sodium selenite and six of eight (75 %) patients treated with IFN plus NAC, sodium selenite and vitamin E. However, IFN plus antioxidant therapy was

Table 2. Summary of clinical studies of vitamin use in the treatment of chronic hepatitis C

Reference	Study design (n)	Patient characteristics	Treatment	Treatment duration and F-up	Endpoint	Main findings	Compliance data	Tolerability data
Retinoic acid								
Böcher et al.[55]	RCT (n 20)	Difficult-to-treat patients	ATRA 45 mg/m^2 twice a day + sodium selenite (30 μg qd) ATRA + sodium selenite + PEG-IFNα-2a 180 μg/week	Treatment: 3 months F-up: 3 months	≥2 log drop of HCV-RNA in serum at the end of the treatment	Endpoint obtained in 10% of the ATRA group and in 50% of the ATRA + PEG-IFN group. At the end of the F-up, the endpoint was maintained only in one patient (10%) of the ATRA group	Not reported	Hypertriacylglycerolaemia Dry mucous Transient headache Tympanic pressure Fatigue Minor depression Myalgia, arthralgia
α-Tocopherol								
von Herbay et al.[56]	RCT (n 23)	IFN refractory patients	Vitamin E (800 IU/d) Placebo	Treatment: 3 months of vitamin E and 3 months of placebo with a cross-over design F-up: not specified	ALT decrease >35% of the basal value	Endpoint obtained in 48% of patients only during vitamin E treatment. No one normalised ALT or cleared HCV-RNA	Not reported	No side effects
Look et al.[57]	RCT (n 24)	Naive patients	Group A: IFNα-2a 4·5 MU tiw Group B: IFN + NAC (1800 mg/d) + sodium selenite (400 μg/d) Group C: IFN + NAC + sodium selenite + vitamin E (544 IU/d)	Treatment: 6 months F-up: 6 months	ALT normalisation HCV-RNA clearance at the end of the treatment	Endpoint obtained in 37·5% of patients of group A, 25% of group B and 75% of group C. Overall, 63% relapsed within the end of the F-up period (group A: 2/3; group B: 1/2; group C: 4/6)	Not reported	No side effects
Idéo et al.[58]	RCT (n 120)	Non-responder patients	IFNα-n₃ 6−9 MU tiw according to body wt IFN + NAC (1200 mg/d) + vitamin E (600 mg/d)	Treatment: 6 months F-up: 6 months	ALT normalisation and HCV-RNA clearance at the end of both the treatments and the F-up period	ALT normalisation did not differ between the two groups both at the end of the treatment and at the follow-up. None obtained HCV-RNA clearance	Not reported	Not reported
Houglum et al.[59]	CS (n 6)	Non-responder patients	Vitamin E 1200 IU/d	Treatment: 2 months No F-up	Histological evaluation	Vitamin E was able to inhibit hepatic stellate cell activation decreasing liver fibrogenesis No clinical effect	Not reported	Not reported
Groenbaek et al.[60]	RCT (n 23)	Naive and non-responder patients	Vitamin E (945 IU/d) + ascorbic acid (500 mg/d) + Se (200 mg/d) Placebo	Treatment: 6 months F-up: 3 months	Not specified	No effect on serum ALT or HCV load	Complete compliance	Not reported
Falasca et al.[61]	RCT (n 40)	Naive patients	SPV complex four pills/d No treatment	Treatment: 3 months No F-up	Not specified	Treated patients obtained only a reduction in ALT, with no change in viral load	Complete compliance	No side effects

RCT, randomised clinical trial; F-up, post-treatment follow-up; ATRA, all-*trans* retinoic acid; qd, once daily; PEG-IFNα, pegylated interferon-α; HCV, hepatitis C virus; tiw, three times a week; CS, case series; ALT, alanine aminotransferase; NAC, *N*-acetylcysteine; SPV complex, one pill contains 15 mg vitamin E and 47 mg silybin.

not effective, since a virological and/or biochemical relapse occurred in seven of the eleven (63%) responders within 6 months after the end of the treatment[57].

In an attempt to demonstrate the efficacy of the association between IFNα and antioxidant drugs in chronic hepatitis C, 120 patients not responding to a previous treatment with IFN were randomised to receive natural IFNα or IFNα plus NAC and vitamin E for 6 months. This study also disclosed no difference in the biochemical or virological responses observed in the two groups[58].

Houglum et al.[59], in a series of six patients with chronic hepatitis C refractory to IFN therapy, showed that treatment with vitamin E inhibited important steps in hepatic fibrogenesis such as stellate cell activation and collagen-α1(I) gene expression, suggesting a potential role of this vitamin in preventing hepatic fibrosis, which is a critical step in the progression of HCV-related liver disease. However, no change in either serum ALT and HCV-RNA levels or histological degree of inflammation and fibrosis was observed throughout the study period.

In addition, a subsequent small double-blind, placebo-controlled trial of supplementation with antioxidant compounds such as vitamin C, vitamin E and Se did not produce any clinical effect in well-compensated patients with chronic hepatitis C and did not affect the erythrocyte activities of antioxidative enzymes or the plasma levels of oxidative markers[60].

In a more recent study, naive patients with chronic hepatitis C were divided into two groups: thirty received silybin-phospholipids and vitamin E complex for 3 months and ten did not receive any treatment[61]. The aim of this observational study was to evaluate the liver anti-inflammatory effect of this antioxidant compound. Treated patients only obtained a significant reduction in ALT levels, while the viral load did not significantly change. However, in the silybin-phospholipids and vitamin E complex group, a significant increase in serum IL-2 and a significant reduction in IL-6 were observed. This finding, suggesting a preferential shift towards the Th1 profile, led the authors to conclude that the silybin-phospholipids and vitamin E complex exert liver anti-inflammatory effects, even though data on liver histology were not reported.

In summary, there is no evidence for a direct anti-HCV effect by vitamin E or C. However, two studies[56,61] described a transient improvement in liver necroinflammatory activity. This finding deserves further investigation to establish the potential of these vitamins as a supportive treatment in patients who cannot undergo standard therapy for HCV.

Discussion

The available clinical experiences on the use of vitamins to treat chronic viral hepatitis, either alone or in combination with IFN-based therapies, are inconsistent and hampered by major methodological drawbacks, such as small sample size, the lack of double-blinding or placebo controls, the heterogeneous features of patients and the use of surrogate endpoints. The different dosage regimens, treatment durations and endpoints also make it difficult to formulate a patient-relevant clinical message. Furthermore, a number of studies have reported the administration of multi-complex formulations, including more vitamins or vitamins in addition to other antioxidant and mineral compounds[55,57,58,60,61]. This does not allow the role of a single vitamin to be established even when an appropriate randomisation has been performed.

Disappointingly, almost all the reviewed studies lack some critical information. With very few exceptions (Tables 1 and 2), no data on compliance with vitamin therapy were found, making it difficult to extrapolate the real effectiveness of experimental treatment. Similarly, data on side effects are not available or fairly elusive, providing a misleading message on a universal vitamin good safety profile. Indeed, vitamins have been demonstrated to induce a wide range of toxic effects albeit at higher dosages than those commonly used[62,63].

Although the antiviral activity of vitamins in chronic hepatitis B or C is not demonstrated, some data seem to show that vitamin E alone or in combination with a silybin–phosphatidylcholine complex is able to reduce inflammation-induced oxidative stress and to restore the immune response. Thus, vitamin E could have a role in improving the necroinflammatory state once the principal aim of the treatment, that is viral eradication and/or viral suppression, cannot be achieved. On the other hand, viral eradication often does not prevent the progression of liver disease, mainly when obtained after a long course of inflammation leading to fibrosis. So, achieving a suppression of necroinflammatory activity could play a role in the prevention of the long-term sequelae of chronic viral infection.

Based on the studies reviewed here, no vitamin can be recommended for the treatment of chronic viral hepatitis.

Clinical trials must compare the therapeutic efficacy of vitamins, if any, to standard state-of-the-art treatment measured using hard endpoints as established by international guidelines[4,64]. Future research directions should include well-designed randomised clinical studies evaluating whether vitamins in combination with standard antiviral treatment increase the current response rate, or whether they serve as a monotherapy for patients with contraindications or intolerance to standard therapy to decrease the incidence of cirrhosis and its complications.

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