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Antiviral Research 43 (1999) 113–122

Antiviral Research

www.elsevier.com/locate/antiviral

Interferon/antioxidant combination therapy for chronic hepatitis C—a controlled pilot trial

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Received 30 November 1998; accepted 28 April 1999

Abstract

The effects of two forms of antioxidative co-therapy were analyzed in 24 interferon-alpha (IFN)-naive patients with chronic hepatitis C who were randomized to either receive IFN monotherapy (3×4.5 million units IFN-α 2a per week), (group A), or IFN and *N*-acetylcysteine (*N*-acetylcysteine (NAC) 1.800 mg/day) plus sodium selenite (400 μg/day) supplementation (group B), or treatment as in group B plus vitamin E (544 IU/day) (group C), over 24 weeks. Changes in histology, normalization of ALT, reduction of viral RNA, serum levels of glutathione, selenium, vitamin E, erythrocyte glutathione peroxidase, trolox equivalent antioxidative capacity (TEAC), thiobarbituric acid reactive substances (TBARS) and protein carbonyl groups were measured. Low baseline TEAC and elevated TBARS indicated increased oxidative stress before therapy, which was not affected by antioxidant supplementation. At the end of treatment complete responses were found in 3/8, 2/8 and 6/8 patients in groups A, B and C, respectively, but liver histology had not significantly improved. Vitamin E treated patients had a 2.4 greater chance (95% CI: 1.05–5.5) of obtaining a complete response and had significantly greater reduction in viral load ($P = 0.028$) than patients without vitamin E. Relapses, i.e. re-appearance of detectable hepatitis C virus (HCV) RNA and/or re-elevation of ALT-activity occurred in 7 out of the 11 responders within 6 months after termination of therapy (group A: 2/3, group B: 1/2 and group C: 4/6). Thus, no overall beneficial effect of antioxidant/IFN therapy was detected. However, the apparent trend towards a more favorable outcome with vitamin E supplementation warrants to further study this substance as an adjuvant to IFN therapy in chronic hepatitis C.

Keywords: Hepatitis C; IFN-α; TBARS

1. Introduction

Chronic infection with the hepatitis C virus (HCV) frequently leads to liver cirrhosis and is an

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0166-3542/99/$ - see front matter
PII: S0166-3542(99)00041-8

important risk factor for hepatocellular carcinoma (Sharara et al., 1996). Treatment with interferon-alpha (IFN-α) alone or in combination with ribavirin is currently the only therapeutic option with proven efficacy. However, sustained virus elimination and normalization of serum aminotransferases is achieved in only ~20% of treated individuals. To improve the success rate of IFN therapy, combination with further agents has been attempted (i.e. acyclovir, IFN-γ). Antioxidant supplementation, with vitamin E and *N*-acetylcysteine (NAC) has been considered as an attractive option for combination with IFN-α (Beloqui et al., 1993; Barbaro et al., 1996; Houglum et al., 1997a,b). It is hypothesized that during HCV infection NAC and vitamin E counteract oxidative stress in the hepatic microenvironment caused by activated Kupffer cells and infiltrating mononuclear cells. Surrogate markers of oxidative stress, i.e. serum thiobarbituric acid-reactive substances (TBARS) (Farinati et al., 1995; De Maria et al., 1996), protein carbonyl groups (PCGs) (De Maria et al., 1996) and hepatic 8-hydroxydeoxyguanosine (Shimoda et al., 1994) levels were found to be elevated in HCV-infected individuals. Although not confirmed in all studies (Lim et al., 1995; Bernhard et al., 1998), hepatic and systemic levels of the endogenous antioxidant glutathione (GSH), which may be reduced in chronic hepatitis C, were correlated with serum ALT activity, viral load and the grade of hepatic inflammation (Beloqui et al., 1993; Suarez et al., 1993; Barbaro et al., 1996). It is now accepted, that a constant pro-oxidative milieu, due to chronic viral infection, alcohol toxicity or transition metal overload, accelerates hepatic fibrosclerosis (Hernández-Muñoz et al., 1997; Houglum et al., 1997a,b; Poli and Parola, 1997).

A key-event in hepatocellular damage is the decline in cellular GSH concentrations, particularly in the mitochondrial 'slow-turnover'-GSH-pool (Reed, 1995). GSH is one of the key constituents of the host's antioxidative capacity. It contributes to an effective immune surveillance (Liang et al., 1989) and to the metabolism of xenobiotics (Meister and Anderson, 1983). Since GSH itself is not transported over biomembranes and cannot be substituted directly, its repletion might be possible by providing precursors such as *N*-acetylcysteine (NAC) or GSH esters. In this respect, an uncontrolled study suggested that HCV clearance might be improved by oral NAC treatment in combination with standard α-IFN application (Beloqui et al., 1993).

The essential trace element selenium, acts as an integral constituent of the antioxidative enzyme glutathione peroxidase (GSH-Px, EC 1.11.1.9), which detoxifies hydrogen peroxide and organic lipid peroxides at the expense of reduced glutathione (GSH). The oxidized form, glutathionedisulfide (GSSG), is in turn reverted to GSH by glutathione reductase (Rotruck et al., 1973). Thus, GSH-Px activity is an important constituent of the host's antioxidative defence mechanisms and the addition of selenium may synergistically enhance the action of NAC. Furthermore, several favorable effects on the immune system have been attributed to selenium supplementation (Kiremdjian-Schumacher and Stotzky, 1986; Kiremdijan-Schumacher et al., 1994; Roy et al., 1994).

Whereas NAC and Se act mainly within the hydrophilic compartment, the lipid soluble antioxidant vitamin E acts within the lipophilic compartments (i.e. biomembranes), thereby reducing hepatocyte lipid peroxidation. Vitamin E has been shown to reduce oxidative stress in IFN-α nonresponders with chronic hepatitis C (Houglum et al., 1997a,b). This effect was accompanied by a decreased transcription-rate of fibrosis genes and lower serum levels of oxidative stress markers (i.e. TBARS). Another double-blind study showed that vitamin E monotherapy leads to a reduction of serum ALT activity in HCV-infected IFN-nonresponders (Von Herbay et al., 1997). These findings prompted us to perform a prospective and controlled pilot trial in 24 IFN-α-naive patients with chronic HCV infection. Our main objective was to verify, whether promising results from previous uncontrolled observations on antioxidative supplementation during IFN therapy would hold true under controlled conditions (Beloqui et al., 1993). If so, this would warrant to perform a subsequent large-scale trial. A further aim was to investigate whether changes in surrogate markers for oxidative stress, such as the serum levels of

TBARS and PCGs, glutathione and the trolox equivalent antioxidative capacity (TEAC) (Miller et al., 1993), could be detected when a standard IFN-α-therapy was combined with adjuvant NAC/Se vitamin E combinations.

2. Methods

2.1. Patients and study design

All patients enrolled in the pilot trial had a diagnosis of chronic hepatitis C based on a positive HCV-PCR and elevated aminotransferases (> 24 U/l). Subjects with cirrhosis, prior IFN treatment, renal disorders, coinfection with other viruses (HBV, HIV), other causes of chronic liver disease, or contraindications against IFN-therapy were excluded. The patients were then randomized into three groups (A, B and C) of eight patients each by using blinded envelopes. Group A received standard interferon-α 2a mono-therapy (Roferon®, Hoffman LaRoche) 4.5 million units (MU) three times weekly for 24 weeks as subcutaneous injections. Group B received the same treatment in combination with NAC, (1800 mg/day; effervescent tablets; Fluimucil long®, Zambon GmbH, Gräfelfing, Germany) and sodium selenite (400 μg/day Na_3SeO_4; drinking vials; Selenase GN-Pharm, Fellbach, Germany). The patients in group C received the same treatment as in group B plus vitamin E 544 IE/day (D-α-tocopherol acetate soft gel capsules, Spondyvit®, Brenner-Efeka, Münster, Germany) once daily. The baseline characteristics of patients according to the stratification into the groups are given in Table 1. All patients had given written consent, and the study was in accordance with the guidelines of the declaration of Helsinki, as approved by the Ethics Committee of the University of Bonn.

Blood was drawn at baseline and after 24 weeks for determination of erythrocyte GSH-Px, plasma GSH, serum vitamin E, selenium, ferritin, TBARS, PCGs and TEAC (Miller et al., 1993). The primary objective was complete response, i.e. normalization of serum ALT and negative HCV-RNA at the end of the treatment (week 24). In addition, decreases in HCV-RNA and ALT activity as well as changes in liver histology and changes in surrogate markers of oxidative stress were analyzed separately. Liver biopsies were performed prior to enrolment and within four months after termination of therapy.

Table 1
Patients' characteristics at baseline

Parameter	Group A	Group B	Group C
Age (years) (mean, range)	38.7 (26–45)	35.7 (24–51)	34 (26–45)
Sex: m = male; f = female	3 m/5 f	6 m/2 f	5 m/3 f
HCV genotype 1a:	2	3	1
1b:	2	1	4
1a/1b:	2	1	3
3a:	1	3	–
5a:	1	–	–
Knodell score (median, rage)	7 (2–13)	5 (3–8)	4 (2–8)
A prior likelihood for sustained response according to Noventa et al. (1997), (P_E: median, range)	0.15 (0.09–0.54)	0.19 (0.07–0.79)	0.12 (0.06–0.31)
Viral load log 10 (median, range)	4.78 (3.95–5.41)	5.23 (3.77–6.41)	5.83* (6.32–5.46)
Serum ALT (U/l) (95% CI)	73.9 (28.7–119)	68.6 (23.9–118.3)	54.4 (24.2–84.5)
Serum ferritin (ng/ml) (95% CI)	106.6 (63–150.2)	131.6 (78.5–184.7)	89.2 (42.8–135.7)

* $P < 0.05$ versus group A.

Semiquantitative determination of HCV viral load—Qualitative analysis of HCV RNA in sera was performed with the Amplicor™ kit and viral load was assessed semiquantitatively by the HCV Monitor™ according to the instructions of the Manufacturer (i.e. Roche Diagnostic Systems, Inc. Branchburg, NJ, USA).

Determination of HCV genotypes—All patients were characterized with respect to the infecting HCV subtype by the INNOLIPA test (Innogenetics, Zwijndrecht, Belgium).

Histological evaluation—Biopsy specimens were graded according to the hepatic activity index (Knodell score) by the same pathologist who was not aware of the allocation to the different treatment groups (Knodell et al., 1981).

Determination of selenium—Selenium concentrations in serum were determined by atom absorption spectrometry (AAS) using a 1100 Perkin Elmer atomic absorption spectrometer with a mercury hydride system (Look et al., 1997).

Determination of vitamin E—Vitamin E concentration in serum was determined by reverse phase high performance liquid chromatography (HPLC) with a Nucleosil C18 column on a Millipore-Waters-equipment. Elution was by methanol:water (96/04). Detection was by UV-absorption at 294 nm. Results were determined quantitatively by a standard curve of peak height ratios with retinyl acetate as the internal standard (Look et al., 1999).

Determination of GSH-Px in erythrocytes hemolysate and of GSH in plasma—Plasma GSH and GSH-Px activity were determined enzymatically and spectrophotometrically according to established protocols (Tietze, 1969, Wendel, 1981; Look et al., 1997).

Determination of thiobarbituric acid reactive substances (TBARS) in serum—TBARS were determined according to the method of Yagi (Ohkawa et al., 1978) with the modification that the absorbance of the thiobarbituric acid-malondialdehyde chromogen was read in the aqueous supernatant without butanol extraction.

Determination of protein carbonyl groups (PCGs) in serum—PCGs were determined spectrophotometrically according to the method of De Maria et al. (1996).

Determination of the trolox equivalent antioxidant capacity (TEAC) in serum—TEAC was determined in the serum according to the method of Miller et al. (1993). In the presence of endogenous antioxidants (reductants or hydrogen donors) the absorbance of the long-lived radical cation 2,2′ azinobis-(3-ethylbenzothiazoline-6-sulphonic acid, $ABTS^{\circ+}$) is quenched to an extent related to the antioxidant capacity of the added fluid, e.g. serum or plasma. The TEAC measures this inhibition as a function of the α-tocopherol (vitamin E) analogue Trolox, purchased from Sigma, Weinheim, Germany.

Statistical analyses—The data are given as mean (95% CI), or median and range as indicated. The values for the HCV RNA load were log-transformed. One factorial ANOVA and a Student–Newman–Keuls post-hoc adjustment were performed to compare the mean changes in HCV RNA, plasma GSH, erythrocyte-GSH-Px-activity, serum ferritin, selenium and vitamin E between the groups A, B and C. Changes within groups (compared to baseline) were evaluated by the Student *t*-test for dependent samples and by the Wilcoxon matched-pairs signed-ranks test for the changes in serum ALT activity. The Student *t*-test for independent samples was used to compare the changes in serum HCV RNA between patients treated with vitamin E and those not treated with vitamin E. For estimation of the primary treatment effects, i.e. achievement of complete response in vitamin E-treated versus non vitamin E-treated patients, X^2-statistics and risc ratios were computed.

For all calculations the SPSS PC^+ software package version 6.1.3., SPSS Chicago, IL was used. Differences with $P < 0.05$ were considered to be statistically significant and differences with $0.05 \leq P \leq 0.1$ were considered to indicate a significant trend.

Normal ranges for selenium, vitamin E, TBARS, PCGs, TEAC, GSH and E-GSH-Px—The normal range for serum selenium (90.5 μg/l, 95% CI: 84.3–94.2) concentrations had been obtained from 72 healthy individuals with a mean age of 45.7 years (range: 23–66; 44 male; 28 female) (Look et al., 1997). Reference values for serum vitamin E concentrations (10.2 μg/ml, 95%

CI: 9.3–11) were obtained from 49 healthy subjects, with a mean age of 43.3 years (range: 24–64; 33 male; 15 female) (Look et al., 1999). Serum TBARS, PCGs and the TEAC were determined in 28 healthy control subjects with a mean age of 34.7 years (range: 23–45; 15 male; 13 female) without HCV infection (TBARS: 2.34 μmol/l, 95% CI: 2.0–2.7, PCGs: 0.92 nmol/mg protein 95% CI: 0.89–0.95, TEAC: 1.20 mmol/l, 95% CI: 1.17–1.24).

3. Results

The medication was well tolerated by all patients and no patient stopped therapy due to side effects.

3.1. Baseline values

Baseline values of ALT activity, serum ferritin, HCV genotype, serum selenium, vitamin E, TBARS and PCG concentrations, Knodell score, serum TEAC as well as erythrocyte GSH-Px activity did not differ significantly between groups A, B and C (Tables 1 and 2). In addition, a comparison of the three treatment groups according to the prognostic score published by Alberti's group (Noventa et al., 1997) did not reveal significant differences between groups A, B and C. However, the mean serum HCV RNA level in group C was significantly higher than in group A ($P < 0.01$, Table 1) and mean baseline plasma GSH level in group B was significantly higher than in group A. ($P < 0.05$, Table 2).

3.2. Response-rates in groups A, B and C

Complete responses (normalization of ALT and negative HCV RNA) at the end of treatment were achieved in 3/8 patients with IFN monotherapy (Group A), 2/8 patients with IFN/NAC/Se (Group B) and 6/8 patients with IFN/NAC/Se/vitamin E (Group C), ($P = 0.11$, X^2-test). The combined complete response analysis of vitamin-E-treated (six responders out of eight) versus non-vitamin-E-treated subjects (five responders out of 16) revealed a risk ratio of 2.4 (95%-CI: 1.05; 5.50) in favor of vitamin-E-supplemented patients. Due to the limited number of subjects, however, Fisher's exact test did not reach the predefined significance in a two-tailed analysis ($P = 0.082$)

Figs. 1 and 2 show HCV RNA levels and ALT activity in groups A, B and C at baseline and after 24 weeks of treatment. When groups A and B, who did not receive vitamin E, were analyzed together versus group C, the decrease in HCV viral load was significantly greater in group C ($P = 0.028$, groups A + B versus group C). The mean ALT-activity decreased in all three groups ($P = 0.036$, 0.021 and 0.06 in groups A, B and C, respectively). However, the extent of the decreases was not different between the three groups. No significant changes in histology were observed in the groups at the end of treatment (Knodell-score, mean ± S.D. at baseline versus end of treatment: Group A: 7.2 ± 4.9 versus 4.8 ± 3.8, group B: 5.8 ± 3.2 versus 4.5 ± 3.6 and group C, 5.3 ± 2 versus 4.71 ± 2.5, P = n.s.). Relapses, i.e. re-appearance of detectable HCV RNA and/or re-elevation of ALT-activity occurred in 7 of the 11 responders within 6 months after termination of therapy (Group A: 2/3, group B: 1/2 and group C: 4/6).

3.3. Serum selenium, vitamin E concentrations, plasma glutathione levels and erythrocyte-glutathione-peroxidase activities

Serum selenium, vitamin E, ferritin levels, plasma GSH and erythrocyte-GSH-Px activities at baseline and after 24 weeks treatment are shown in Table 2. Serum vitamin E levels in the patients were significantly lower than in healthy controls (8.2 ng/ml 95% CI: 7.3–9.3, $n = 24$ versus 10.2 ng/ml 95% CI: 9.3–11, $n = 49$, $P = 0.01$), whereas serum selenium levels were not significantly different from healthy controls. Serum selenium in groups B and C and vitamin E concentrations in group C increased significantly under treatment, whereas no significant changes from baseline values were observed in the group with IFN monotherapy (group A). In all three groups, a significant increase in erythrocyte-GSH-Px activity, compared to baseline values was ob-

Table 2
Plasma/serum antioxidants and oxidative stress markers at baseline and after 24 weeks treatment in groups A–C

Parameter	Group A			Group B			Group C		
	Baseline	24 weeks	Change (median)	Baseline	24 weeks	Change (median)	Baseline	24 weeks	Change (median)
Selenium (μg/l)	81.2 (75.3–87)	85.6 (80.4–90.7)	−0.05	84 (73.2–94.7)	123** (107.8–138.2)	32.6†	87.8 (72.9–102.6)	129.2** (109.3–149.1)	54.1†
Vitamin E (μg/ml)	8.8 (6.7–10.8)	8.8 (6.1–11.5)	0.1	7 (5.5–8.4)	7.8 (6–9.7)	0.2	9.1 (7.1–11.1)	13.3* (8.9–17.7)	4.2#
P-GSH (mmol/l)	16.1## (14.1–18.1)	16.9 (11.1–22.6)	0.75	12.9 (10.8–15)	16.8* (13.2–20.3)	3.5	14.6 (12.8–16.5)	15.8 (13–18.5)	2
Erythrocyte-GSH-Px (U/g Hgb)	14.4 (11.8–17.1)	18.4** (15.2–21.7)	3.9	16.1 (11.4–20.7)	23.3*** (17.8–28.9)	8.3†	12.9 (11.7–14.2)	20.7*** (17.2–24.1)	7.2†
TEAC (mmol/l)	1.13 (1.03–1.2)	1.08 (1.02–1.24)	–6.8	1.15 (1.1–1.9)	1.07 (1.0–1.14)	–8.8	1.12 (1.01–1.24)	1.12 (1.01–1.22)	−0.55
TBARS (μmol/l)	3.4 (2.5–4.3)	3.5 (2.3–4.7)	–0.3	3.6 (2.5–4.6)	2.8 (2.4–3.5)	–0.1	2.9 (1.1–4.8)	3.0 (2.1–4)	−0.1
PCGs (nmol/mg protein)	0.82 (0.58–1.1)	0.88 (0.58–1.1)	0.04	1.07 (0.68–1.45)	1.0 (0.79–1.22)	–0.04	0.98 (0.71–1.25)	0.88 (0.66–1.1)	−0.07

* $P<0.05$.
** $P<0.01$.
*** $P<0.001$ vs. baseline.
† $P<0.05$ vs. IFN mono.
# $P<0.05$ vs. IFN mono and IFN/NAC/Se.
$P<0.05$ vs. IFN/NAC/Se.

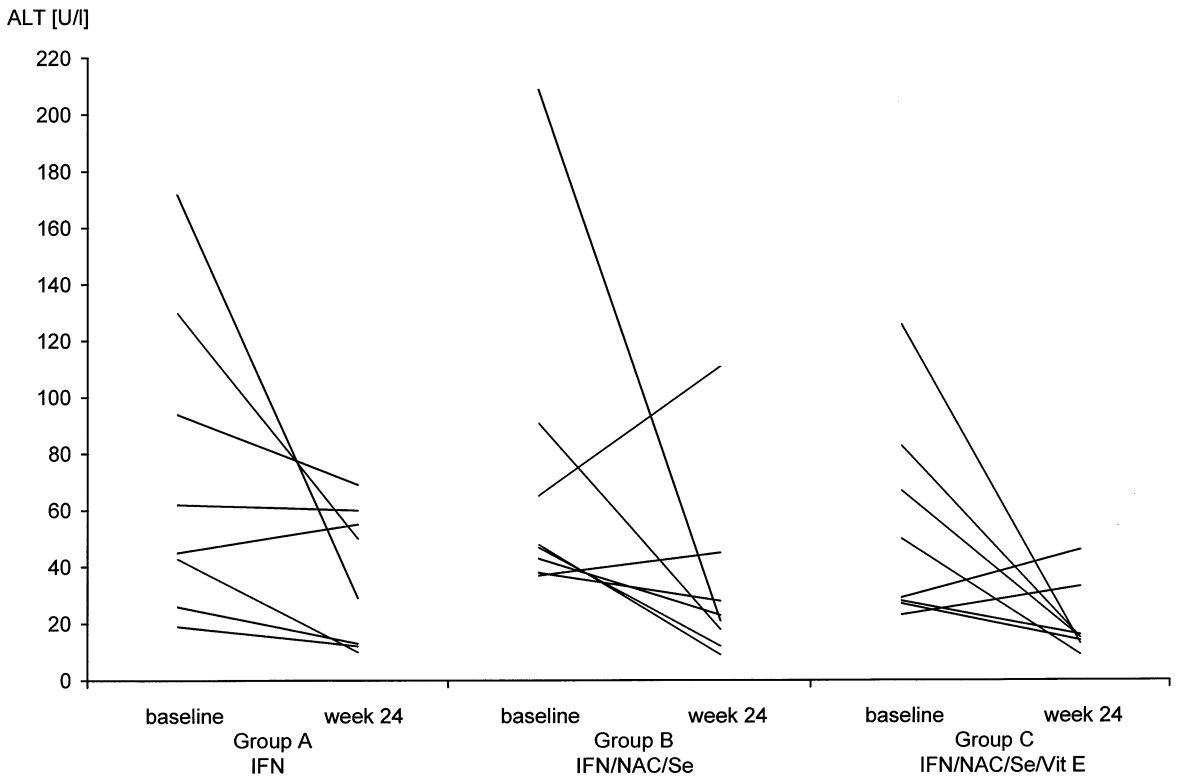

Fig. 1. Changes in serum ALT activity in groups A ($P = 0.036$), B ($P = 0.021$) and C ($P = 0.06$) from baseline to week 24.

served. However, it is noteworthy that in a multivariate analysis the increase in erythrocyte-GSH-Px activity was significantly greater in the two selenium-supplemented groups B and C than in group A with IFN monotherapy ($P < 0.05$, both).

3.4. Markers of oxidative stress (protein carbonyl groups, thiobarbituric acid reactive substances and trolox equivalent antioxidative capacity)

Baseline serum TBARS concentrations of our hepatitis C patients were significantly higher that those of healthy age-matched controls (3.31 μmol/l 95% CI 2.73–3.9 versus 1.81 μmol/l 95% CI 1.5–2.06, $P = 0.0025$), whereas patients and controls did not differ in serum PCGs (0.96 μmol/mg protein 95% CI: 0.8–1.1 versus 0.92 μmol/mg protein 95% CI: 0.88–0.95, $P = 0.56$). TEAC-values were lower in the patients as compared to healthy controls (1.13 trolox-95% CI: 1.01–1.17 versus 1.21 trolox-eq 95% CI: 1.17–1.24, $P = 0.0044$), indicating a diminished antioxidant capacity in the patients. After IFN treatment there were no differences in PCGs, TBARS or TEAC-values between group A, which received IFN monotherapy, and groups B and C, which were treated with IFN supplemented by antioxidants. Furthermore, there were no significant correlations between plasma GSH, TBARS, PCGs or TEAC and ALT activity, virus type or viral load in a combined analysis of the baseline values of the three groups.

4. Discussion

In this controlled randomized pilot trial, no significant overall advantage of antioxidant/IFN combination therapy in terms of frequency of

complete primary response after 6 months was found as compared to IFN monotherapy. Seen in perspective, our data are in line with another recent study of Bernhard et al. (1998) who also did not find increased response rates when IFN was combined exclusively with NAC. It should, however, be mentioned that the current consensus duration for IFN monotherapy of one year was not completed in our study. This fact can explain the relatively high rate of relapses seen within six months after termination of therapy and probably also the lack of significant histological improvement. However, the data suggest a potential beneficial effect for combining IFN treatment with vitamin E supplementation (group C) indicating a 2.4-fold favorable risk ratio (95% CI: 1.05; 5.50) for complete response. It is particularly noteworthy that a greater decline in serum viral load was achieved under vitamin E supplementation. This may indicate that either vitamin E alone or the combination of hydrophilic (NAC/Se) and lipophilic (vitamin E) antioxidants may enhance the antiviral efficacy of IFN-α. This finding is in contrast with results from Bellobuono's group who reported that a combination of IFN-α with NAC and vitamin E was not effective in increasing overall response rates. However, these investigators had studied patients who had failed a previous course of IFN monotherapy (Bellobuono et al., 1997), whereas we studied IFN-naive patients.

This study was designed as a pilot trial, comprising a relatively small number of patients. However, the results allow to draw several important conclusions: (1) Previous uncontrolled observations on successful NAC/IFN combination

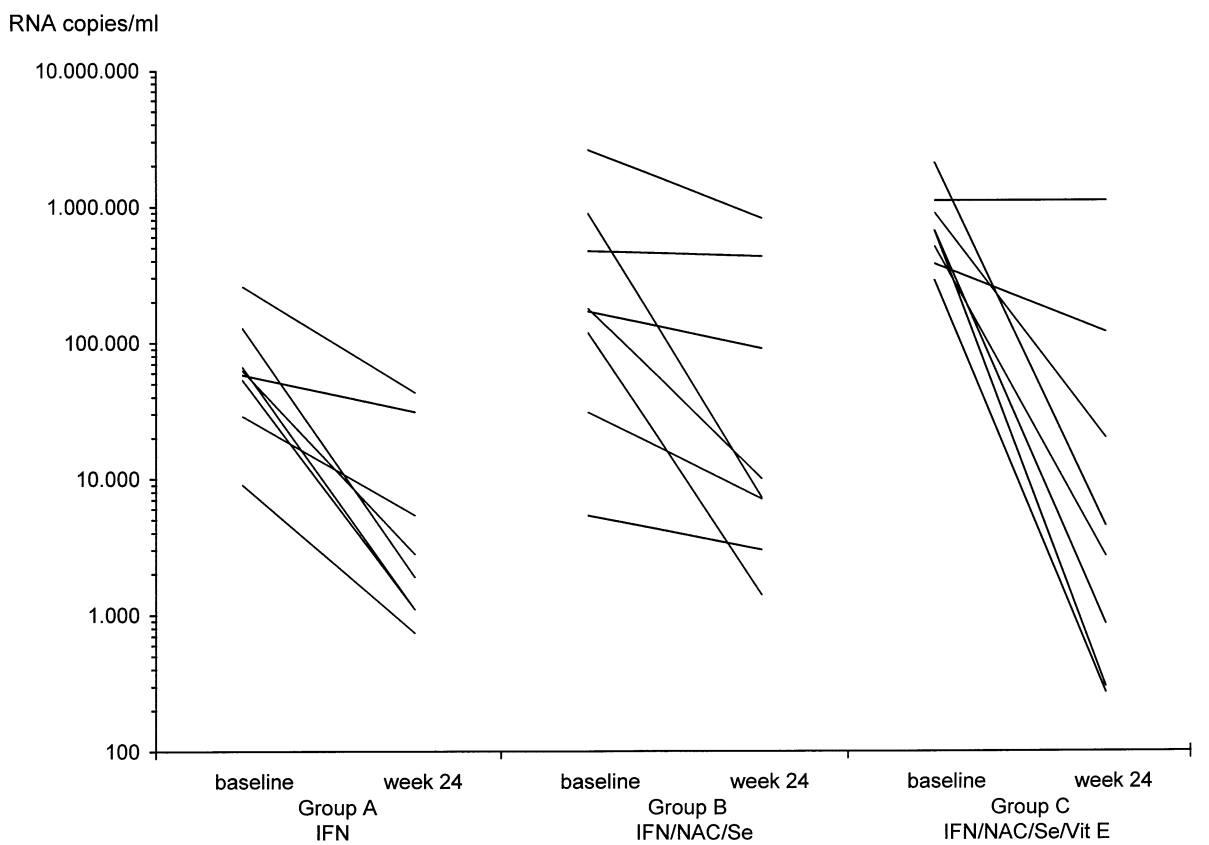

Fig. 2. Changes of the log-transformed quantitative HCV RNA levels in serum in groups A, B and C from baseline to week 24. The decrease in HCV RNA was significantly greater in group C than group A ($P = 0.028$).

therapy (Beloqui et al., 1993) could not be confirmed by using a prospective and controlled study design; (2) We found evidence for increased oxidative stress during chronic HCV infection reflected by increased serum TBARS levels and decreased TEAC. However, antioxidant supplementation had no detectable impact on altered surrogate markers of oxidative stress or histopathology.

The lower TEAC in our patients probably corresponds to the fact that serum vitamin E levels in the patients were significantly lower than in healthy controls. Yet, in contrast to the findings of De Maria et al. (1996) we did not find a significant elevation of serum PCGs in patients with HCV infection. Increases in erythrocyte-GSH-Px activity were seen after 24 weeks of treatment in all three groups of this study. This observation may indicate that IFN-α therapy upregulated synthesis or the activity of this antioxidative enzyme, probably indicating an adaptive response mechanism to the pro-inflammatory actions of IFN-α. The significantly greater increase of erythrocyte-GSH-Px activity in the two selenium-supplemented groups B and C as compared to group A, however, is in line with the fact that selenium administration per se can increase the activity of erythrocyte-GSH-Px until a saturation level has been reached (Clausen and Nielsen, 1988). It also indicates, that the enzyme-dependent antioxidant capacity can be augmented in individuals with chronic HCV infection. This is a particularly important finding considering the fact that antioxidant therapy might be able to reduce hepatofibrosis at the level of gene transcription (Houglum et al., 1997a,b).

We have no conclusive explanation for the mechanism contributing to better IFN-induced clearance of HCV during vitamin E supplementation. A recent study in healthy elderly people showed improvements in clinically relevant indexes of cell-mediated -and humoral immunity through vitamin E supplementation (Meydani et al., 1997) suggesting that pharmacological doses of vitamin E can strengthen the efficacy of the host's immune system. This hypothesis is in line with the observation that vitamin E administration alone was also effective in normalizing serum ALT levels and in clearing HBV DNA in patients with chronic Hepatitis B (Andreone et al., 1998). Possible greater antiviral activity of IFN during vitamin E supplementation despite the lack of detectable improvements in serum surrogate markers for oxidative stress (TBARS and PCGs) or antioxidant defence mechanisms (TEAC) in our study could therefore indicate that vitamin E might act via these immunostimulatory effects. However, the exact connections between vitamin E and immune function have not yet been established.

Although our study supports the concept of increased oxidative stress in chronic hepatitis C, adjuvant antioxidative therapy by NAC/Se co-supplementation did not increase the antiviral response to six month IFN-α monotherapy. However, a potential benefit of combination of IFN treatment with vitamin E substitution could not be ruled out. Therefore, it is warranted to further study vitamin E supplementation as an adjuvant to IFN-α-, or IFN-α/ribavirin combination therapies. Such studies should also clarify whether higher doses (800–1000 IU, or more) or mixed tocopherols (i.e. α-, β-, δ-, γ-tocopherol and tocotrienols) exert even greater effects and whether addition of hydrophilic antioxidants and trace elements (e.g. lipoic acid, NAC, selenium, zinc) is required.

Acknowledgements

We acknowledge the excellent technical assistance of Helmut Lemoch and Bianca Arendt.

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