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Interferon-Alpha-2a and Zinc Combination Therapy in Children with Chronic Hepatitis B Infection

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Abstract Zinc has been reported to enhance the response to interferon (IFN) or PEG-IFN plus ribavirin therapy, improve liver function, and ameliorate hematologic side effects in patients with chronic hepatitis C. However, the role of zinc supplementation during IFN therapy in chronic hepatitis B infection (CHB) remains unclear. We therefore aimed to report the results of zinc and IFN-alpha-2a therapy in children with CHB. Twenty-two naive, HBeAg-positive children (mean age 10.4±4.4 years) received IFN-α2a (9 MU/m^2 sc) for 6 months plus peroral zinc (7.5 mg/day for <10 years and 10 mg/day for >10 years) for 12 months. Serum zinc, alanine aminotransferase (ALT), complete blood count, hepatitis B virus DNA (HBV DNA), and serological markers were measured. Histological (HR) and sustained response (SR) were evaluated at 6 months after completion of therapy. Normalization of ALT, HBeAg seroconversion, and HBV DNA<10,000 copies/ml were considered as SR. HR was defined as decrease in Knodell histological activity index (HAI) score by at least 2 points compared to baseline. End of therapy ALT level and log HBV DNA were significantly lower than pretherapy levels (p=0.001 and p=0.001, respectively), while zinc level was not different. Portal inflammation score significantly decreased after therapy (p=0.043), however, total HAI and other HAI components were not different. SR and HR were 25% and 52.9%. In conclusion as a first study investigating the effect of zinc and IFN combination therapy in children with CHB, SR and HR rates were not better than previously reported monotherapy or combination therapies.

Keywords Children · Chronic Hepatitis B infection · Interferon · Zinc

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Introduction

At present, there are only a few therapeutic options available for chronic hepatitis B infection (CHB) in children, such as standard interferon-alpha (IFN-α) and lamivudine and most recently adefovir [1]. Beneficial effects of these drugs have been reported, however, there are still a number of non-responders to these therapies. To improve the response to IFN therapy in patients with CHB, several studies have examined the effects of various modifications of therapeutic regimens such as high-dose IFN [2], combining IFN with lamivudine [3], combining IFN with hepatitis B vaccination [4] and N-acetyl-L-cysteine [5] without a significant succes.

Patients with chronic liver disease show impaired trace element metabolism such as high levels of iron and copper, and low levels of zinc, selenium, phosphorus, calcium, and magnesium [6]. Of these trace elements, zinc is a constituent of a number of enzymes involved in a large number of metabolic process. In addition to the effects of zinc on immune function, oxidative stress, and antiviral defense, it has also anti-inflammatory effect [7, 8]. Owing to these pharmacological mechanisms, zinc supply may have beneficial effects in the treatment of chronic viral hepatitis. The benefical effect of IFN combined with zinc supplementation on chronic hepatitis C has been shown [9, 10]. Morever, effect of zinc level on response to IFN therapy in children with CHB is previously reported [11, 12]. However, there has been no report to date about IFN and zinc combination therapy for CHB. We aimed to report prospectively our experiences with zinc and IFN combination therapy in children with CHB.

Material and Methods

Patients and Study Design

Patients positive for hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg) for at least 6 months and increased serum alanine aminotransferase (ALT) levels between 1.5 and 10 times the upper limit of normal and hepatitis B virus DNA (HBV DNA) levels greater than 10,000 copies/ml and Knodell histological activity index (HAI) scores of 4 or above and normal serum zinc level were included in the study. Patients were excluded from the study if they had serum zinc level of lower or higher than upper normal limit, clinical or biochemical evidence of decompansetad liver disease, hepatitis delta, hepatitis C, or HIV coinfection and contrindication for IFN therapy. Patients were also excluded if they had serious medical illness such as malnutrition and metabolic diseases or if they had history of treatment for HBV infection previously.

In this open-label, prospective study, all patients received IFN-α2a, subcutaneously 9 megaunits/m^2 (maximum 9 MU), 3 days a week, for 6 months and zinc in a daily dosage of 7.5 mg for children less than 10 years or 10 mg for children over 10 years for 12 months. Zinc sulfate was adminitrated orally once a day, 1 h before or 2 to 3 h after food or beverages. Two drugs were initiated simultaneously.

Patients were followed up monthly for routine physical examination, complete blood count, and ALT levels test while receiving IFN-α therapy. Thereafter, until the 6 months after completion therapy, they were followed every 6 months for the same parameters and serum zinc level, HBV DNA, and serological markers including HBeAg antiHBe, HBsAg, and anti-HBs. At every visit, parents and children were questioned for any adverse effect and compliance to therapy.

Liver biopsy was performed at baseline and the 6 months after completion of therapy. The liver biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. The HAI was scored according to the Knodell method on a scale from 0 to 22, with higher scores indicating more severe abnormalities [13]. The overall Knodell score is the sum of scores for periportal±bridging necrosis (0–10), intralobular degeneration and focal necrosis (0–4), portal inflammation (0–4), and fibrosis (0–4). The necroinflammatory activity index is the sum of the first three components. The biopsy specimens were examined by a single pathologist.

This study was approved by the local ethics committee of Ankara University, School of Medicine and informed consent was obtained from the all parents of patients before the study.

Serologic, virologic, and biochemical response ratios were evaluated at the end of therapy and 6 months after completion of therapy. Normalization of ALT was defined as the decline of serum ALT levels to normal range. HBV DNA clearance was defined as HBV DNA<10,000 copies/ml. HBeAg negativity was defined as loss of HBeAg. HBeAg seroconversion was defined as loss of HBeAg and development of anti-HBe. HBsAg negativity was defined as loss of HBsAg. HBsAg seroconversion was defined as loss of HBsAg and development of anti-HBs. Sustained response was defined as normalization of ALT, clearance of HBV DNA, and HBeAg seroconversion at 6 months after completion therapy. Histological response was defined as decrease in HAI score by at least 2 points; worsening as an increase by at least 2 points compared to baseline. If the difference was between 1 and −1 points, then it was defined as unchanged.

Laboratory Analysis

Serum ALT was measured by convention methods (reference value 10–31 U/L). Serum zinc was measured by the atomic absorption spectrophotometry (reference value 71–105.9 mcg/dl). HBs Ag, HBe Ag, anti-HBe, and anti-HBs were detected using commercially available enzyme-linked immunosorbent assays (Abbot Laboratories, North Chicago, IL). Serum HBV DNA levels were evaluated with a fully automated system which combines automated extraction of DNA on the COBAS AmpliPrep Instrument, coupled with a real-time PCR on the COBAS TaqMan Analyzer using COBAS TaqMan HBV 48 test kit (Roche Diagnostics, Mannheim, Germany)

Statistical Analysis

Statistical analysis was done with the SPSS 11.0 computer program. The results were expressed as “mean±standard deviation”. Statistical significance was assessed by Chi-square, Student's *t* test, Wilcoxon signed-Rank, and McNemar tests. $p<0.05$ was considered significant.

Results

Twenty-two children, 13 boys (59%), were enrolled. The mean age was 10.4±4.4 years (2 to 16 years). All patients were naive. Transmission route of infection was vertical in 13 patients (59%), horizontal in 5 patients (22.7%), and unknown in 4 patients (18.8%). Two patients were lost to follow-up after 6 and 12 months of therapy. Finally, 20 patients were analyzed at the end of therapy and 6 months after completion of therapy. Pretreatment mean ALT, log HBV DNA, HAI (Knodell), and serum zinc level were

126.6±83.2 U/L, 8.6±0.5 copy/ml, 7.8±2.4 mcg/dl, and 87.8±10.5 mcg/dl, respectively. Serum ALT, zinc level, and log HBV DNA (copy per milliliter) in each patient during therapy and 6 months after completion of therapy are presented in Fig.1a–c. Evaluation of biochemical and virologic markers of infection are shown in Tables 1 and 2. At the end of therapy, ALT level and log HBV DNA were significantly lower than pretherapy (p=0.001 and p=0.001, respectively), while zinc level was not different. Furthermore, 6 months after completion of therapy, ALT level and log HBV DNA were significantly lower than pretherapy (p=0.02 and p=0.001, respectively), whereas zinc level was not different. At the end of therapy, serum zinc level was increased to above upper limit of normal in three patients, however, after 6 months after completion of therapy, zinc level was decreased to normal levels in all patients except for one. Sustained response was 25%.

Seventeen of 20 patients had paired liver biopsies at baseline and after therapy, because approval for liver biopsy could not be gained in three patients. Before and after therapy Knodell HAI scores were not statistically different (Table 3). We observed mild

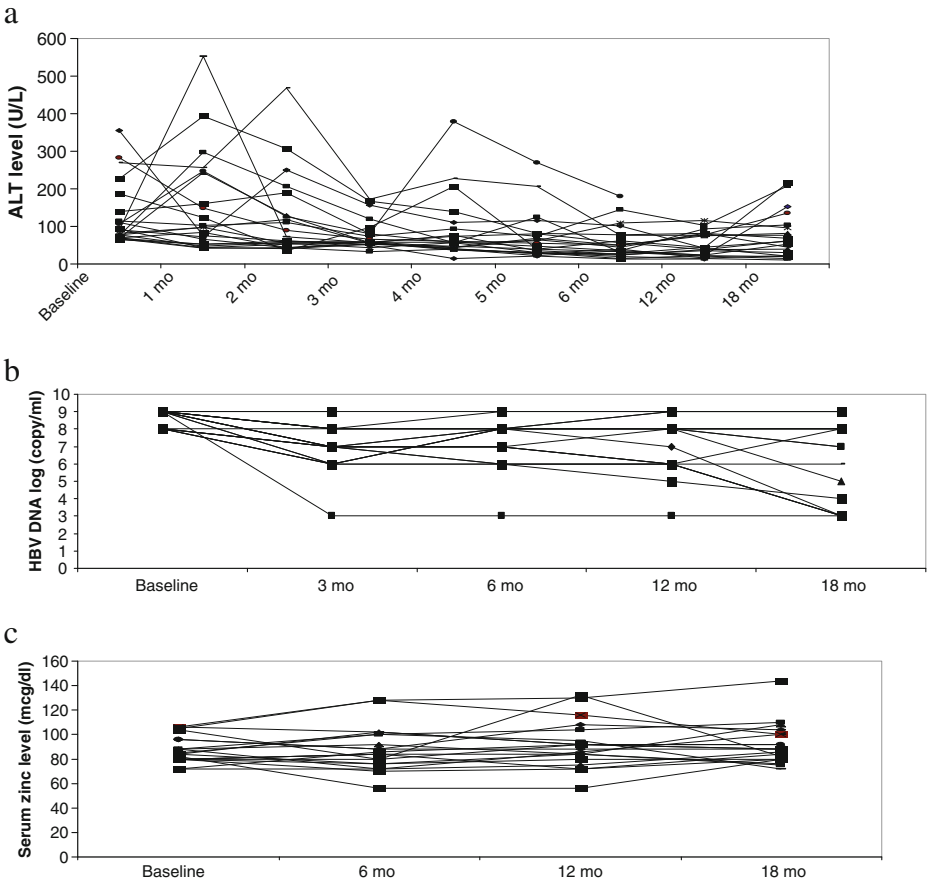

Fig. 1 Changes in serum ALT (**a**), serum logHBV DNA (**b**), and zinc level (**c**) at baseline, during therapy and 6 months after completion of therapy. Serum ALT and HBV DNA were significantly decreased compared with values before therapy whereas serum zinc level did not change

Table 1 Evaluation of biochemical and virologic markers of infection during therapy and 6 months after completion of therapy

	Before therapy (n=22)	6 months (n=22)	12 months (n=21)	18 months (n=20)
ALT (U/L)	126.6±83.2	51.1±34.1	51.4±32.1	75.9±60.6
Log HBV DNA (copy/ml)	8.6±0.5	7.2±1.3	7.1±1.5	6.4±2.3
Serum zinc level (mcg/dl)	87.8±10.5	88.0±17.6	91.7±18.9	91.9±18.2

significant decrease in the scores of portal inflammation after therapy (p=0.043), however, no significant change in other HAI components was observed (Table 3). Histologic response was observed in nine cases (52.9%), worsening in two cases (11.7%) and unchanged in six cases (35.2%). After therapy, Knodell HAI was less than 4 in six of 17 patients (35.2%).

Although baseline ALT levels and HAI score were higher in sustained responders than non-responders, baseline age, sex, route of transmission, HAI scores, ALT, and HBV DNA log levels were not statistically different (Table 4). After therapy, the Knodell HAI scores were not different in responders and non-responders (6.0±4.2 vs 6.3±3.7). Among responders, four patients had paired liver biopsies at baseline and after therapy, three of them (75%) had histologic response and, worsening was observed in the other (25%). Among non-responders, 13 patients had paired liver biopsies, histologic response was observed in 6 of them (46.1%), unhanged in 6 cases (46.1%) and worsening in 1 case (7.7%).

Patients tolerated the treatment well. Minor side effects such as flu-like symptoms (15/22; 68.1%), anorexia (3/22; 13.6%), hair loss (1/22, 4.5%), and vomiting (1/22; 4.5%) were reported. However, recovery was noted in each of these patients soon after cessation of IFN therapy. Severe side effect was observed in only one patient. Generalized seziure developed during a febrile episode in third month of IFN therapy in a 6-year-old boy. His medical and family history were unremarkable. His EEG and cranial CT were normal, medication was not started but IFN dosage was reduced. Seziure did not recur during the follow-up. Patients also tolerated zinc therapy well.

Table 2 Response to therapy

	6 months (n=22)		12 months (n=21)		18 months *(n=20)*	
	n	%	n	%	n	%
ALT normalization	8	36.3	8	38.1	6	30
HBVDNA clearence	1	4.5	1	4.7	5	25
HBeAg negativity	1	4.5	3	14.2	5	25
HBsAg negativity	1	4.5	1	4.7	1	5
Seroconversion	1	4.5	3	14.2	5	25
Sustained response					5	25
Histologic response					9/17	52.9

Table 3 Changes in Knodell HAI components before and after therapy

	Before therapy	After therapy	*p*
Periportal±bridging necrose	1.8±1.1	1.4±0.9	n.s
Intralobular degeneration and focal necrosis	2.1±0.9	2.0±1.2	n.s
Portal inflammation	2.8±0.7	2.1±1.2	0.043
Fibrosis	1.0±0.7	0.7±0.7	n.s
Necroinflammatory activity index	6.6±1.6	5.7±3.1	n.s
HAI	7.8±2.4	6.2±3.7	n.s

Discussion

To our knowledge, the effect of zinc supplementation to IFN therapy in chronic hepatitis C or B infection in childhood hasn't been explored before. We used zinc sulfate at a fixed amount for age and to avoid exceeding the Dietary Reference Intakes, zinc was administered 7.5 to 10 mg, once a day. Our results showed that sustained response rate was 25% and histologic response was 52.9%.

Earlier in vitro studies have shown that antiviral effect of zinc ions was found to be as effective as that of interferon [14]. Because of the effects of zinc on immune function, antiviral and antioxidant defense, zinc may play an important role in achieving sustained response to IFN therapy in CHB. However, contraversial results about the impact of zinc levels on response to IFN therapy in children with CHB have been rarely addressed [11, 12, 15]. It is reported that in complete responders to IFN-α therapy, serum zinc level was significantly higher than in non-responders [11, 12] whereas contradictory to this, pretreatment zinc level was found to have no effect on the outcome of IFN-α treatment [15]. A negative corrrelation with HAI and portal inflammation scores and serum zinc level has also been shown [11].

Studies investigating the effect of zinc supplementation on PEG-IFN or IFN-α/ribavirin therapy for chronic hepatitis C in adults reported conflicting results. Some studies suggested that zinc supplementation enhanced the response [10] and reduced the side effects [16, 17]; whereas others showed no beneficial effect on virologic responses [16–18].

Table 4 Baseline features of sustained responders and non-responders

	Responders (*n*=5)	Non-responders (*n*=15)	*p*
Age (years)	11.2±3.6	10.4±4.4	n.s
Sex (M/F)	4/1	8/7	n.s
Transmisson route			
Vertically (*n*)	2	9	n.s
Horizontaly (*n*)	1	4	
Unknown (*n*)	2	2	
ALT (U/L)	128.1±76.6	80.6±9.3	n.s
Log HBV DNA (copy/ml)	8.6±0.4	8.6±0.5	n.s
Serum zinc level (mcg/dl)	89.1±11.3	84.0±8.9	n.s
HAI (Knodell)	9.6±2.9	7.5±2.1	n.s

The exact mechanism of the beneficial role of zinc on IFN therapy is not fully elucidated. Clearance of viral infections requires cytotoxic T lymphocytes which are highly dependent on zinc. In vitro, zinc induces the production of antiviral IFN-α and IFN-γ [19, 20] and it also potentiates the antiviral action of IFN-α [21]. Also, zinc mediates the interaction between IFN dimers which is required for the activation of the IFN recoptors [22]. Zinc has also anti-inflammatory and antioxidant effect through inducing metal-lothionein which function as a free radical scavenger [23–26]. Through these mechanisms, zinc supplementation may decrease the inflammation in the liver and reduce side effects of IFN.

A multinational, randomized, controlled trial of interferon in children with CHB resulted in 26% hepatitis e seroconversion rate, compared to 11% of controls [27]. In our previous studies, complete response rate was 31.2% with standard-dose IFN-α [28], 37.5% with high-dose IFN and 40% with high-dose IFN and lamivudine combination therapy [29]. The lack of a placebo-control group and small number of patients in this study makes difficult to asses the contribution of zinc supplementation. Sustained response rate in this study is compatible with those of previous reports, however, lower than we expected. This may be due to the low dose of zinc we administered. For another possible explanation, high rate of vertical transmission and high viral load in our patients might have adversely affected the response.

Oral administration of zinc significantly decreased serum levels of aminotransferese in patients with chronic hepatitis C by antioxidant effect [30]. Similarly, in our study, ALT levels significantly decreased at 6 months after beginning of therapy compared to baseline, and ALT levels remained unchanged untill the end of therapy, after which a mild elevation was observed at 6 months after completion of therapy.

Improvement of liver histological findings, especially of liver fibrosis is the most important endpoints in therapy. Only a few studies have analyzed the effect of IFN-α therapy on the histopathology in children with CHB [29, 31, 32]. It has been reported that IFN-α therapy showed a histological benefit in 50% of children. Our results are compatible with those of previous reports, however, additive effect of zinc on IFN therapy was not observed. One possible reason for this result may be the low dose of zinc we administered as discussed above. We propose, therefore, to increase the dosage of zinc in future trials.

Both drugs were well tolerated by our patients. The observed adverse effects seemed to be related to IFN therapy and these did not necessitate interrupting the therapy.

In conclusion, as a first study investigating the effect of IFN and zinc combination therapy in children with CHB, sustained and histologic response rates were not different than previously reported monotherapy or combination therapies. However, further large scale randomized controlled clinical studies with higher doses of zinc will be needed to clarify this effect.

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