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ARTICLE

HBV DNA suppression during entecavir treatment in previously treated children with chronic hepatitis B

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Abstract The aim of this study was to assess HBV DNA suppression after 24 weeks of treatment with entecavir in previously treated children with CHB. Thirty children aged 5–17 years (25 males and 5 females) with CHB were treated with entecavir 0.5 or 1 mg daily. Twenty-two children were HBeAg-positive, eight were HBeAg-negative, and in eight HBV polymerase mutations were detected. After 24 weeks of treatment, mean and median HBV DNA levels and ALT activity were lower versus baseline, overall and in both subgroups. The overall median HBV DNA level decreased from 1.2 x 10^7 IU/mL to 3.3 x 10^2 IU/mL ($p < 0.000004$), in HBeAg-positive from 7.8x10^7 IU/mL to 6.3x10^3 IU/mL ($p < 0.00004$), and in HBeAg-negative from 2.5x10^4 IU/mL to 5.01x10^1 IU/mL ($p < 0.03$). The serum HBV DNA disappearance was observed in 7/8 (88%) HBeAg-negative and in 5/22 (23%) HBeAg-positive patients. The overall mean ALT activity decreased from 164+ 290 U/L to 34.1+ 18.9 U/L ($p < 0.000007$), in HBeAg-positive from 214+326 U/L to 38.59+19.2 U/L ($p < 0.000074$), and in HBeAg-negative from 27+14 U/L to 20+8 U/L ($p < 0.03$). Twenty-four weeks of treatment with entecavir results in suppression of HBV DNA in a substantial proportion of children previously treated ineffectively with CHB.

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Introduction

In the time of common anti-HBV vaccination, children at risk for HBV infection include those who were not vaccinated, had an inadequate response to vaccination, were exposed prior to being vaccinated, were born to HBV-infected mothers but did not receive active/passive immunoprophylaxis or this immunoprophylaxis was ineffective [1].

Chronic hepatitis B in children is mostly asymptomatic, but they are at risk for severe complications like liver cirrhosis and hepatocellular carcinoma (HCC).

Individuals who acquire the virus vertically during childbirth or at a young age are the priority for effective treatment. The current goals of treatment are: suppression of viral replication to undetectable HBV DNA levels, ALT level normalisation, HBe/anti HBe seroconversion, an improvement in liver histology and preventing liver complications by reducing the risk of progressive liver disease.

Anti-HBV therapies approved for adults include interferon alpha, an immune modulator and nucleoside/nucleotide analogues that suppress viral replication [2, 3]. Treatment options for children with chronic hepatitis B are limited. In Poland, standard anti-HBV therapy in children now includes only recombinant interferon.

The aim of this study was to assess the HBV DNA suppression after 24 weeks of treatment with entecavir of

Table 1 Baseline characteristics of patients

Characteristic	HBeAg-positive (n=22)	HBeAg-negative (n=8)	All patients (n=30)
Age (years), mean±SD (range)	15.3±2.68	15.3±1.04	15.3±2.34
Male : female	3 : 2	6 : 2	9 : 4
HBV DNA, IU/ml, mean±SD	8.1x10^7±9.7x10^7	3.5x10^4±2.9x10^4	6.1x10^7±9.1x10^7
Serum ALT (IU/l[a]), mean±SD	214±326	27±14.04	164±290
Fibrosis stage, mean (range)	2 (1–2)	1.57 (0–2)	2 (0–2)
Necroinflammatory grade, mean (range)	2 (1–2)	1.86 (1–2)	2 (1–2)

[a] Upper limit of normal: females 31 IU/l; males 40 IU/l

previously treated children and adolescents with chronic hepatitis B (CHB).

Materials and methods

Thirty children with chronic hepatitis B, who did not respond to previous anti-HBV therapy were included in treatment with entecavir. There were 25 males and five females in the group, aged 5–17 years (mean age 15.3± 2.34). Twenty-two children were HBeAg-positive and eight were HBeAg-negative. Seven children had previously been treated with recombinant interferon (IFN), two with lamivudine (LAM), one with adefovir (ADV), 19 with IFN and LAM, and one with IFN, LAM and ADV.

Baseline HBV DNA viral load was >10^7 IU/mL in 15 children, between 10^6 and 10^7 IU/mL in three patients, between 10^5 and 10^6 in three, between 10^4 and 10^5 IU/mL in seven and between 10^3 and 10^4 IU/mL in two children. In eight children, HBV polymerase mutations L180M and M204V were detected, and L80I and M204I in one.

All children had a liver biopsy and liver ultrasound as a prerequisite to enrolment and treatment. Liver biopsy specimens were scored according to the modified Scheuer scale and were assigned a grade for necroinflammation between 0 and 4 and a stage between 0 and 4 for fibrosis [4]. No child had liver disease assessed greater than grade 2, stage 2.

In the examined group there were no patients with histological evidence of hepatocellular carcinoma or chronic liver disease other than CHB. Patients co-infected with hepatitis C virus or human immunodeficiency virus were not eligible for treatment.

Children and adolescents included in the study received entecavir in the dose 0.5 or 1 mg daily.

The protocol was approved by the ethics committee of Collegium Medicum Nicolaus Copernicus University in Bydgoszcz, Poland. Informed consent was provided in writing by the legal guardian of each patient and each child older than 12 years before treatment was initiated.

During treatment patients were required to return to the clinic at regular intervals at which time blood samples were obtained for determination of serum HBV DNA, HBeAg, HBsAg, and ALT activity. A complete blood count was performed at each clinic visit.

Serum HBV DNA was determined at baseline and at week 24 by quantitative polymerase chain reaction assay (COBAS® AmpliPrep/COBAS TaqMan® HBV Test, limit of quantitation=55 IU/mL; limit of detection 12 IU/mL [Roche Diagnostics]).

In 24 patients serum HBV DNA was assessed at weeks 4 and 12 during therapy.

Safety was monitored at each clinic visit by means of laboratory tests, physical examination and adverse events reported by the patient or guardian.

Statistical analysis

Serum HBV DNA levels, ALT activity, grading and staging were analysed by descriptive statistics. Means and standard

Table 2 Serum HBV DNA (IU/mL) level and ALT activity after 24 weeks of treatment vs. baseline in examined patients

Measure	Baseline		After 24 weeks of treatment		p (Wilcoxon's test)
	Mean±SD	Median	Mean±SD	Median	
Serum HBV DNA (IU/mL)	61088601±91363098 Min 1000 Max 410000000	12000000	69547±223304 Min 0.0000 Max 1100000	326	0.000004
ALT activity (U/L)	164±290	60	34.10±18.9	30	0.000007

Table 3 Serum HBV DNA and ALT activity after 24 weeks of treatment vs. baseline in HBeAg- positive patients

Measure	Baseline		After 24 weeks of treatment		*p*
	Mean±SD	Median	Mean±SD	Median	
Serum HBV DNA (IU/mL)	80514591±97425806 Min 1000 Max 410000000	77500000	91654±253703 Min 0.0000 Max 1100000	6325	0.000040
ALT activity (U/L)	214±326	75	38.59±19.2	31	0.000074

deviations and median values and interquartile ranges were calculated for values collected at baseline and in week 24. To compare those results in HBeAg positive and HBeAg negative patients, the U Mann-Whitney's and Wilcoxon's tests were used.

Results

The baseline characteristics of the patients are presented in Table 1.

In all patients we observed the increased ALT activity values during the 2 years prior to treatment, although ALT within normal limits was recorded at the start of entecavir treatment in eight patients. No child had severe liver disease assessed as greater than grade 2, stage 2 on the pretreatment liver biopsy but had at least grade 1 and/or stage 1 at baseline.

Mean baseline serum HBV DNA level and ALT activity were statistically significantly lower in HBeAg-negative patients in comparison to HBeAg-positive ($p<0.0003$ and $p<0.0006$, respectively).

HBV DNA levels and ALT activity decreased in all patients during treatment with entecavir.

After 24 weeks of treatment, mean and median HBV DNA levels and ALT activity were lower than the baseline value, overall and in the HBeAg-positive and HBeAg-negative subgroups. The overall median HBV DNA level decreased from 1.2 x 10^7 IU/mL at baseline to 3.3 x 10^2 IU/mL after 24 weeks of treatment ($p<0.000004$). In HBeAg-positive patients the median HBV DNA level decreased from 7.8x10^7 IU/mL at baseline to 6.3x10^3 IU/mL ($p<0.00004$), and in HBeAg-negative from 2.5x10^4 IU/mL to 5.0^{1}x10 IU/mL, respectively ($p<0.03$) (Tables 2, 3, 4). Mean serum HBV DNA level and ALT activity after 24 weeks of treatment were statistically significantly lower in HBeAg-negative patients in comparison to HBeAg-positive (respectively, $p<0.002$ and $p<0.005$).

After 24 weeks of treatment serum HBV DNA was undetectable in 12 patients: The serum HBV DNA disappearance was observed in seven of the eight (88%) HBeAg-negative patients, and in five of the 22 (23%) HBeAg-positive. Among seven HBeAg negative patients with undetectable HBV DNA after 24 weeks of therapy, six were with normal ALT activity while one patient was with increased ALT activity at baseline. In all five HBe-positive patients with HBV DNA disappearance after 24 weeks of entecavir therapy the baseline ALT activity was increased.

In six patients with HBV DNA disappearance, serum HBV DNA was undetectable from the 5th week of treatment. Among them there were five HBeAg-negative and one HBeAg-positive patients at baseline. Until the 24th week of therapy we did not observe HBe/anti-HBe seroconversion in this and other patients who were HBeAg-positive at baseline.

The overall mean ALT activity decreased from 164±290 U/L at the baseline to 34.1±18.9 U/L after 24 weeks of entecavir therapy ($p<0.000007$). In HBeAg-positive patients the mean ALT activity decreased from 214±326 U/L to 38.59±19.2 U/L ($p<0.000074$) and in HBeAg negative patients from 27±14 U/L to 20±8 U/L ($p<0.03$), respectively.

During the period analyzed we did not observe any adverse events of entecavir therapy.

Discussion

Treatment of children with chronic hepatitis B seems to be controversial. Some authors declare that most HBV infected children will remain in the immune tolerant

Table 4 Serum HBV DNA and ALT activity after 24 weeks of treatment vs. baseline in HBeAg- negative patients

Measure	Baseline		After 24 weeks of treatment		*p*
	Mean±SD	Median	Mean±SD	Median	
Serum HBV DNA (IU/mL)	35491±29886 Min 6840 Max 96200	25000	69±51 Min 50 Max 184	50	0.027709
ALT activity (U/L)	27±14	21	20±8	19	0.027709

phase until late childhood or adolescence and treatment in this phase of HBV infection is not indicated [5]. Although most children with chronic hepatitis B are asymptomatic and severe liver disease during childhood is rare, they are at risk for developing serious complications, including liver cirrhosis and HCC.

The results of this study demonstrate that a 24-week course of entecavir therapy in children and adolescents can produce suppression of HBV DNA replication in HBeAg-positive and HBeAg-negative individuals, previously treated ineffectively with anti-HBV. After 24 weeks of entecavir treatment the serum HBV DNA decreased in all treated patients. The serum HBV DNA disappearance was observed in seven of the eight (88%) HBeAg-negative patients, and in five of the 22 (23%) HBeAg-positive.

These results are clinically significant because HBV DNA levels correlate with an increased risk of liver disease. There is a linear relationship between the serum concentration of HBV DNA and the long-term risk of cirrhosis and hepatocellular carcinoma in patients with CHB [6–8]. Effective treatment is particularly important in children with CHB, because the virus cannot be eradicated and, as a result, complications can evolve over many decades in these individuals.

In five of the six patients with HBV DNA disappearance after 24 weeks of therapy, undetectable levels of HBV DNA were observed after the initial 4 weeks of therapy (RVR). It seems that early disappearance of HBV viremia may by a predictor of beneficial treatment response. Sustained suppression of HBV DNA replication results in histological improvement, normalization of ALT levels and, in some patients with HBeAg-positive disease, seroconversion to an anti-HBe state [9–11]. Treatment with entecavir decreasing HBV DNA replication induced the biochemical and histological improvement of liver disease in adults [12, 13].

In the examined patients, treatment with entecavir was well tolerated. There were no unknown safety issues emerging in this population and the safety profile was similar to that seen in adult populations. We did not observe ALT flares and entecavir resistance mutations.

In conclusion, the results of this pilot study show that 24 weeks of treatment with entecavir results in suppression of HBV DNA in a substantial proportion of children previously treated ineffectively with CHB. Larger and longer trials are now required to better define the magnitude of the benefit in this population.

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