Serum hyaluronic acid during lamivudine treatment in chronic hepatitis B

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Abstract

Purpose: We address the question whether lamivudine treatment modulates serum concentrations of hyaluronic acid and whether the pre-treatment HA level can give the information about the presumptive result of treatment and whether HA level evaluation can be useful in monitoring the antiviral therapy in chronic hepatitis B.

Material and methods: Forty-nine patients, 31 man, aged 40 ± 2.5 years were treated with 100 mg lamivudnie per day for 48 weeks. Serum hyaluronic acid level was determined using enzyme-linked binding protein commercial assay (Corgenix Inc., USA).

Results: The mean HA pre-treatment levels were higher than among controls (69.6 ± 11.6 ng/ml vs 36.5 ± 7.6 ng/ml, mean ± SEM) and correlated with AST activity, $p = 0.002$; GGT activity, $p = 0.006$; ALP activity $p < 0.001$; prothrombin time, $p = 0.01$; and peripheral blood platelets count, $p = 0.001$ but did not correlated with ALT activity. The pre-treatment HA concentration correlated also with interlobular necroinflammatory activity score, $p = 0.049$ and with fibrosis score, $p = 0.026$, according to Scheuer classification. The mean HA levels decreased gradually during lamivudine treatment, up to levels lover than among controls (26.3 ± 5.7 ng/ml). There were not significant differences in pre-treatment levels observed between patients neither with HBs seroconversion versus those without it, nor between patients with HBe seroconversion versus those without it and among patients with normalization of ALT activity versus ones without it.

Conclusions: Serum hyaluronic acid level decreases during lamivudine treatment both in patients with HBeAg seroconversion and without it; serum hyaluronic acid pre-therapy levels correlate with necroinflammatory lobular activity score and with liver fibrosis score; serum hyaluronic acid is of no predictive value for lamivudine therapy response; serum hyaluronan may be valuable complementary marker in chronically HBV infected patients.

Key words: hyaluronan, chronic hepatitis B, lamivudine.

Introduction

Effective treatment of chronic hepatitis B, despite the progress of recent years, remains one of the major world health problems [1].

Lamivudine, an oral nucleoside analogue, has an established role for treatment of patients with chronic hepatitis B [1-4]. Nevertheless the sustained response is achieved in limited number of patients and several factors, as low ALT activity, HBeAg presence, high viral load, liver necroinflammatory activity and liver HBcAg has been associated with low response to the treatment [1,5]. Even in cases of lack of the virological response the improvement in hepatic necroinflammatory activity can be seen after treatment [4]. Non-invasive markers of the liver fibrogenesis and fibrolysis could become clues to predict the subsequent clinical course of the patient, to estimate the likely and to determine the actual response to antifibrotic therapy [6]. Although liver biopsy is still regarded as the gold standard for the stage of liver fibrosis assessment it provides static information about fibrotic process and cannot be performed on a day-to-day basis [6]. Serum biochemical markers are ideal candidates for this purpose. The favored tests should be safe and easy to perform, to be done repeatedly and at short

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time intervals. Among many candidates evaluated hyaluronic acid (hyaluronan, HA) seems to be a promising one [6-9].

HA is a ubiquitous glycosaminoglycan of extra cellular matrix, originating from repeating disaccharide units ([D-glucuronic acid (1-β-3) N-acety-D-glucosamine (1-β-4)]n, [10,11].

The increase of serum hyaluronic acid concentration has been shown in various chronic liver diseases, suggesting that progressive liver damage can be identified early on and monitored by serum hyaluronan evaluation [12-14].

In our previous study we have demonstrated the positive correlation of HA level and the presence of liver steatosis [15], which occurence in chronic hepatitis C predispose to disease progression [16].

There were no studies on HA in chronic hepatitis B patients treated with lamivudine so far. In this paper we address the question whether lamivudine treatment modulates serum concentrations of hyaluronic acid and whether the pre-treatment HA level can give the information about the presumptive result of treatment and whether HA level evaluation can be useful in monitoring the antiviral therapy.

Table 1. **Baseline characteristics of the patients**

Age (years)	
Median	41
Range	18-70
Male sex n.(%)	31 (63)
Histological activity score	
Portal/periportal necroinflammatory activity	
Median	3
Range	0-4
Lobular necroinflammatory activity	
Median	2
Range	1-4
Fibrosis score	
Median	1
Range	0-3
Serum alanine aminotransferase (IU/l)	
Median	75
Range	7-350
Serum bilirubin (mg%)	
Median	0.93
Range	0.4-3.2
Serum albumin	
Median	3.92
Range	2.8-4.92

Material and methods

Patients

Patients with detectable hepatitis B surface antigen (HBsAg) and HBeAg in serum at the time of screening and for at least the previous 6 months, and with ALT activities that were 1.3 to 10 times the upper limit of normal for at least previous 6 months were eligible for the study.

The antigens HBs, HBe and antibodies HBc were detected by micro-enzyme immunological method (MEIA, ABBOTT, Germany).

Patients were excluded if they were younger than 18 years old, if they had hepatitis C or D or human immunodeficiency virus infection, if they had decompensated liver disease (defined by serum bilirubin level more than 2.5 times the upper limit of normal, a prothrombin time prolonged by more than 3 seconds and a serum albumin level lower then 3 g/dl or a history of ascites, variceal hemorrhage or hepatic encephalopathy); if they had evidence of autoimmune hepatitis or metabolic liver disease, if they received corticosteroids within 6 months before enrolment.

The study was carried out in accordance with the Helsinki Declaration and all patients enrolled gave informed consent. Local Ethical Committee approved the study.

Evaluation

Patients were evaluated at baseline, than at the beginning of treatment and at weeks 4, 12, 24, 48. Patients received 100 mg/day of lamivudine (Glaxo-SmithKline) for 48 weeks.

In 39 patients liver biopsy was preformed within 6 months before the enrolment.

Assays

The standard liver function tests, including total serum bilirubin, alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, gamma-glutamyl transpeptidase (GGT) activity, alkaline phosphatase activity (ALP), serum total proteins and albumin, platelet count were measured using Cobas Mira instrument (Roche). Prothrombin time and prothrombin index (PI) was determined using Kselmed K–3002 (Poland). Clinical data of the patients are presented in *Tab. 1*.

The fasting serum hyaluronic acid concentration was assessed with an enzyme-linked binding protein assay (commercially available kit, Corgenix Inc.,USA) using a 'capture molecule' known as the hyaluronic acid binding protein, according to the producer.

Serum HA normal levels determined in the laboratory from 24 age and sex matched healthy subjects were 36.5 ng/ml ± 7.6 ng/ml (mean ± SEM).

The HBV-DNA was isolated from 200 μl of serum with commercial reagents for DNA isolating (GenElute Blood Genomic DNA Kit, Sigma). The HBV-DNA in serum was measured in the Clinical Molecular Biology Department of the Medical University of Białystok in Poland. The amplification products of conserve part of HBV genome were stained with brome ethydyne and than visualized and analyzed in the computer system UVI-KS400i/Image of PC (Syngen Biotech, USA).

Histologic score

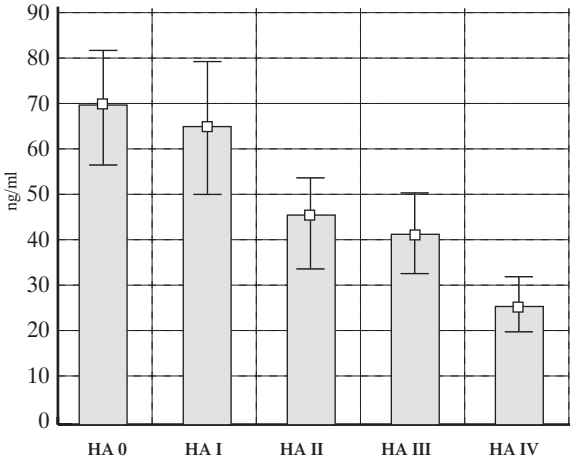
Liver biopsy was performed with the Menghini technique applying the 1,4 mm Hepafix needles (Braun, Germany). Biopsy specimens were fixed in 10% buffered formalin and embedded in paraffin. Serial sections (thickness 4-5 μm) were cut and stained with hematoxilin-eosin staining and picric acid staining for fibrosis evaluation.

Table 2. **Median and range of hyaluronic acid concentrations (ng/ml) in patients with chronic hepatitis B during 54 weeks lamivudine treatment; HA 0 – the level of hyalurinic acid assessed before treatment, HA I – after 4 weeks of lamivudine treatment, HA II – after 12 weeks, HA III – after 24 weeks, HA IV – after 48 weeks**

	Range (ng/ml)	Median (ng/ml)	p between HA 0 and HA I, HA II, HA III, HA IV
HA 0	1.0 – 326.5	37.5	
HA I	1.0 – 506.8	31.1	$p < 0.01$
HA II	1.0 – 319.8	20.1	$p < 0.001$
HA III	1.0 – 231.5	14.9	$p < 0.001$
HA IV	1.0 – 189.0	12.5*	$p < 0.001$

* p with norm

Figure 1. **The mean ± SD concentrations of hyaluronic acid (HA), in patients with chronic hepatitis B infection treated with lamivudine; HA 0 – the level of hyalurinic acid assessed before treatment, HA I – after 4 weeks of lamivudine treatment, HA II – after 12 weeks, HA III – after 24 weeks, HA IV – after 48 weeks**



The scoring system (0-4) of portal/periportal and lobular necroinflammatory activity and fibrosis was applied according to Scheuer [17].

Clinical endpoints

The endpoints included the loss of detectable levels of HBeAg and HBV DNA in serum and the appearance of antibody to HBeAg (referred to as HBeAg seroconversion) at the end of treatment; loss of detectable levels of HBsAg and appearance of antibody to HBsAg (referred to as HBsAg seroconversion) at the end of treatment; return of serum ALT levels to normal (≤40 IU/l).

Statistical analysis

Descriptive data are given as means ± SEM. The Mann-Whitney U test was used for statistical comparisons of quantitative variables as the distribution of hyaluronan values in our study group was not normal. The Pearson correlation coefficient and Spaermans's correlation coefficient were used to assess the degree of correlation of HA concentrations and other liver function tests and histology indexes, respectively. A two-tailed *p* value ≤0.05 was considered statistically significant.

Results

Forty-nine patients, 31 male and 18 female, aged 40.0 ± 2.5 years (mean ± SEM) accomplished study follow-up.

The mean serum HA pre-treatment level (69.6 ± 11.6 ng/ml) was higher than mean levels detected in healthy age matched controls (36.5 ng/ml ± 7.6 ng/ml; mean ± SEM).

The mean HA level 4 weeks after the treatment initiation decreased slightly up to 64.9 ± 14.5 ng/ml and than the mean HA levels decreased gradually during lamivudine treatment and the mean level detected at the end of the therapy was even lower than among controls (26.3 ± 5.7 ng/ml vs 36.5 ng/ml ± 7.6 ng/ml $p = 0.149$), *Tab. 2*, *Fig. 1*.

The pre-treatment HA levels correlated with AST activity, $p = 0.002$; GGT activity, $p = 0.006$, ALP activity, $p < 0.001$; prothrombin time, $p = 0.01$, and peripheral blood platelets, $p = 0.001$ but did not correlate with ALT activity, $p = 0.397$.

Virologic and biochemical response

Elimination of HBsAg was observed in two from 49 subjects (4.0%). The HA pre-treatment level did not differ significantly between those who eliminated HBsAg and those who did not.

The HBeAg seroconversion was detected in 17 from 49 individuals (34.6%). Similarly to the HBsAg seroconversion, the HA levels there were not statistically significant difference detected between those two groups of patients. The HA pre-treatment levels did not differ significantly between the responders to treatment and no responders.

The normalization of ALT activity was observed in 17 of 49 patients (34.6%). There were no differences in HA pre-treatment levels between subjects with ALT activity normalization and those with persistent elevated ALT activity, $p = 0.76$.

Histologic score and HA

The liver biopsy was performed in 39 patients. Serum pre-treatment level correlated with interlobular necroinflammatory activity score, $p = 0.049$ and with fibrosis score, $p = 0.026$, *Tab. 2*.

Discussion

The clinical course of HBV infection varies according to the phase of viral replication and the risk factors to which the patient is exposed, particularly superinfection with another hepatitis viruses [17]. Thus the histological diagnosis, not free from sampling errors, reflex only current status of the disease and every individual case should be carefully regularly monitored. So the non-invasive markers of prognostic value are needed. Hyaluronic acid fulfill those criteria, at least in some clinical conditions, e.g. among cirrhotic patients infected with HCV [14].

Table 3. **Correlation expressed through r-value and it's significance (* $p < 0.05$;** $p < 0.001$) between analysed biochemical indices and hyaluronic acid in patients with chronic hepatitis B before treatment**

	r-value in respect to HA (ng/ml)	
	mean ± SEM	r
Bilirubin (mg%)	1.1 ± 0.1	0.18
ALT (U/L)	119.0 ± 13.2	0.13
AST (U/L)	81.3 ± 9.1	0.42*
ALP (U/L)	103.8 ± 7.1	0.69**
GGT (U/L)	63.8 ± 10.3	0.4*
Prothrombin time	13.7 ± 0.2	0.37*
PI (%)	89.9 ± 1.5	-0.29
Total protein (g%)	7.1 ± 0.1	-0.15
Albumin (g%)	3.9 ± 0.1	-0.53*
Gamma-globulin (g%)	1.5 ± 0.1	0.54*
PLT ($x10^3/\mu L$)	161.8 ± 7.1	-0.5**
HGB (g/L)	14.6 ± 0.2	-0.07
Histological scores		
Lobular inflammation	2.72 ± 0.16	0.36*
Periportal inflammation	1.69 ± 0.13	0.30
Fibrosis	1.48 ± 0.13	0.40*

The histological activity index improved after one year lamivudine treatment in majority of patients regardless the HBeAg seroconversion [3,4]. Although in our study the end-point liver biopsy was not performed serum HA levels decreased significantly in all patients observed, both those who eliminated HBeAg and those who did not.

We have shown the positive correlation of serum HA concentration and lobular inflammation index and fibrosis index. The correlation of HA with fibrosis score was demonstrated in asymptomatic chronic HBV carriers [8] and in patients chronically infected with HBV and HCV viruses [18-20].

The rise of serum HA concentrations is believed to be caused both by increased synthesis and decreased removal by sinusoidal endothelial cells.

There is no available literature data on HA during lamivudine treatment. However HA levels during interferon treatment were in disagreement with ours where the increase of HA level was observed [21]. Although Zohren's et al. [21] follow-up included also patients with active cirrhosis among whom the increase was higher than among individuals with chronic persistent hepatitis B.

In our work we have demonstrated the positive correlation of serum concentrations HA and other biochemical parameters: AST, ALP, GGT, gammaglobulin, and negative correlation with serum albumin concentrations and platelets count. It is in line with observation among cirrhotic patients were the correlation of HA with albumin concentrations and platelets count was found but not with ALT activity [22].

There is several works on HA value as a prognostic marker in chronic hepatitis C patients treated with interferon [19,20]. Ueno et al. [19] hypothesized that serum HA levels, reflecting also hepatic sinusoidal capillarisation may explain the worse response to INF. The occurrence of hepatic capillarisation impair the movement of inflammatory cells and cytokines, including heterogeneous INF from the hepatic sinusoids to the space of Disse.

In our study the initial HA level was relatively low in comparison with chronic hepatitis C patients [19,20] 69.6 ± 11.6 (mean ± SEM) which may explain the lack of significant differences between responder and non-responders observed.

Although we did not demonstrate the prognostic HA value for lamivudine treatment response, HA seems to be a valuable complementary test in monitoring chronically HBV infected patients.

Conclusions

Serum hyaluronic acid level decreases during lamivudine treatment both in patients with and without HBeAg seroconversion.

Serum hyaluronic acid is of no predictive value for lamivudine therapy response.

Serum hyaluronan may be valuable complementary marker of liver fibrosis and necroinflammatory activity in chronically HBV infected patients.

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