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Adenine arabinoside 5′-monophosphate in patients with chronic hepatitis B: comparison of the efficacy in patients with high and low viral replication

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Abstract

This study compared the response to adenine arabinoside 5′-monophosphate (ARA AMP) in 60 patients with chronic hepatitis B according to the pretreatment serum hepatitis B virus DNA concentration. The level of hepatitis B virus replication was defined as low (30 patients) or high (30 patients) when serum hepatitis B virus DNA concentration was below or above 100 pg/ml, respectively. Patients received a 28 day course of ARA AMP and a second course of ARA AMP was given six months later to patients with persistent hepatitis B virus replication. At the end of the first course of ARA AMP, 11 of the patients (37%) with low replication and one of the patients (3%) with high replication became negative for hepatitis B virus DNA ($p=0{\cdot}0012$); five of the patients (17%) with low replication and none of the patients with high replication had HBe seroconversion ($p=0{\cdot}06$). Two of these five patients lost HBsAg. Kinetics of serum hepatitis B virus DNA during treatment showed a considerable but transient antiviral effect of ARA AMP. Three of 32 retreated patients became negative for hepatitis B virus DNA and one patient had HBe seroconversion. In conclusion, ARA AMP exerts a considerable but transient antiviral effect on hepatitis B virus. Complete and sustained inhibition of hepatitis B virus replication was only obtained in the patients with low hepatitis B virus replication.

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Keywords: hepatitis B virus, purine nucleoside.

Adenine arabinoside is a synthetic purine nucleoside that has antiviral activity against hepatitis B virus. Adenine arabinoside 5′-monophosphate (ARA AMP), the monophosphate ester of adenine arabinoside, is 400 times more water soluble and is therefore suitable for intramuscular administration. In three controlled trials from Europe, ARA AMP administration was followed by sustained interruption of hepatitis B virus replication in 40 to 55% of patients.[1–3] ARA AMP administration did not, however, have a lasting effect on hepatitis B virus replication in patients of three other controlled trials from the United States.[4–6] Differences in the populations studied could account for these discrepancies. In a previous study, we found that anti-HIV negative homosexuals with chronic hepatitis B seemed to respond as frequently as heterosexuals to ARA AMP treatment.[3] In the same study, we found retrospectively that a low level of hepatitis B virus replication, as reflected by low serum hepatitis B virus DNA concentrations, was a factor for predicting response to ARA AMP.

The aims of this study were to assess, in patients with chronic hepatitis B: (a) the response to ARA AMP according to the pretreatment concentration of serum hepatitis B virus DNA, (b) the kinetics of the antiviral effect of ARA AMP as assessed by a quantitative method, and (c) the efficacy of a second course of ARA AMP given six months after a first ineffective course.

Methods

PATIENTS

Sixty patients (aged 18–75) with chronic hepatitis B were enrolled in this multicentre trial. Entry criteria were (a) presence of HBsAg in serum for more than one year; (b) presence of HBeAg and absence of antiHBe antibodies; (c) increased activities of serum alanine aminotransferase (ALT) with stable values assessed three times within the six months before inclusion (less than 50% change); (d) presence of serum hepatitis B virus DNA with stable values assessed three times within the six months before inclusion (less than 50% change); (e) absence of antibodies against hepatitis D virus and HIV; (f) no antiviral treatment in the year before entry; (g) absence of any signs of symptoms of neuropathy (diabetes or chronic alcoholism); (h) histologically confirmed chronic active hepatitis. The criteria for exclusion included (a) a course of antiviral or immunosuppressive treatment with the previous six months or a previous course of interferon; (b) history of decompensated cirrhosis (ascites, bleeding oesophageal varices, or hepatic encephalopathy); (c) a platelet count of less than 50×10^9/l, a serum creatinine concentration of more than 150 μmol/l; (d) patients who were unreliable for longterm follow up.

Thirty patients with low hepatitis B virus replication and 30 patients with high hepatitis B virus replication who fulfilled the criteria were included in this study. The level of

TABLE I *Clinical and biochemical characteristics of the 60 patients with chronic hepatitis B before ARA AMP administration*

	Patients	
	High HBV replication (HBV DNA >100 pg/ml)	*Low HBV replication (HBV DNA ≤100 pg/ml)*
Patients (n)	30	30
Age (y)	39 (12)	47 (18)
Males (%)	77	70
Homo/heterosexual (n)	12/11	7/14
Weight (kg)	66 (13)	68 (12)
Known duration of HBV infection (month)	36 (41)	56 (47)
Serum values*		
Alanine aminotransferase (IU/l)	159 (109)	202 (176)
Albumin (g/l)	47 (4)	45 (7)
Prothrombin (% of normal)	85 (10)	79 (13)
Serum HBV DNA (pg/ml)	227 (182)	42 (26)
Patients with cirrhosis (%)	7	17

*The normal reference range for alanine aminotransferase is 0 to 40 U per litre; for albumin, 35 to 50 g per litre. Data shown as mean (SD). HBV=hepatitis B virus.

hepatitis B virus replication was arbitrarily defined as high when the mean pretreatment hepatitis B virus DNA concentration was above 100 pg/ml or as low when the mean hepatitis B virus DNA concentration was less than or equal to 100 pg/ml. The mean hepatitis B virus DNA concentration was calculated based on three values determined before the start of ARA AMP administration. More patients with high concentrations than patients with low concentrations of serum hepatitis B virus DNA were seen; therefore, inclusions in the first group were stopped while inclusions in the second group necessitated a more prolonged time.

This study was approved by the ethics committee of the Faculté de Médecine Xavier Bichat. Informed consent was obtained from all patients.

TREATMENT

All patients received a 28 day course of ARA AMP (Parke Davis, Courbevoie, France) by intramuscular injection at 12 hourly intervals at a dose of 10 mg/kg/day for the first five days and then 5 mg/kg/day for the remaining 23 days. A second course of ARA AMP with the same schedule, was given six months after the first course to patients who did not respond or experienced side effects and who accepted a second course. Patients were seen weekly during treatment, then every other month for the duration of the 12 month follow up.

STUDY DESIGN

Serum ALT activities, HBeAg, and antiHBe antibodies (RIA, Abbott Laboratories, N Chicago, USA) were assessed before and at the end (28 days) of ARA AMP administration, then every month for six months. Serum HBsAg and antiHBs were assessed before and every two months after ARA AMP administration. In patients who received a second course of ARA AMP, additional measurements were performed at the end of the second course of ARA AMP administration and then every month for six months. Serum hepatitis B virus DNA concentrations were measured using a liquid hybridisation assay (Genostics, Abbott Laboratories, France)[7] before, at the end of ARA AMP administration, and every two months throughout follow up.

Thirty of 60 patients were randomly chosen (15 in each group), and serum hepatitis B virus DNA concentrations were retrospectively measured by liquid hybridisation assay (Genostics, Abbott Laboratories) on frozen serum aliquots collected every week during and one month after the first course of ARA AMP.

The response to ARA AMP was analysed at the end of treatment and during the six month post-treatment follow up. A response was defined as negativation of serum hepatitis B virus DNA concentrations; a complete response as negativation of serum hepatitis B virus DNA concentrations with HBeAg to antiHBe seroconversion.

STATISTICAL ANALYSIS

Values are expressed as mean (SD). Proportions were compared using Fisher's exact test or the χ^2 test. Means were compared using Student's test or variance analysis (with Scheffe's F test and Dunnett's t test).

Results

Nineteen of 60 patients (32%) included in our study were homosexuals. Before ARA AMP administration, 10 patients (17%) had serum hepatitis B virus DNA concentrations higher than 200 pg/ml; 26 patients (43%) had serum ALT activities below three times the upper limit of normal and seven patients (12%) had cirrhosis.

There were no significant differences between low and high replication groups for age, weight, sex ratio, proportion of homosexuals, known duration of hepatitis B virus infection, serum ALT activities, serum albumin, plasma prothrombin, and presence of cirrhosis (Table I). Mean serum hepatitis B virus DNA concentrations were 42 (26) pg/ml and 227 (182) pg/ml in patients with low and high replication, respectively. During the study, one patient dropped out seven days after the start of ARA AMP and treatment was withdrawn in nine patients because of side effects (eight for myalgia and one for vomiting). These 10 patients (five in the high replication group and five in the low replication group) were considered non-responders.

EFFECT ON HEPATITIS B VIRUS REPLICATION

Serum hepatitis B virus DNA became undetectable during ARA AMP administration in 11 of 30 patients (37%) with low replication and in one of 30 patients (3%) with high replication ($p=0{\cdot}0012$) (Table II). Serum hepatitis B virus DNA concentrations remained undetectable during the six month post-treatment follow up in seven of 11 patients with low replication and remained detectable in all the patients with high replication. The seven patients who became negative for hepatitis B virus DNA, had the lowest pretreatment serum hepatitis B virus DNA

TABLE II *Negativation of serum HBV DNA, HBe seroconversion, and HBs seroconversion in patients with chronic hepatitis B after ARA AMP administration*

Patients	Negativation of serum HBV DNA		Seroconversion	
	At the end of ARA AMP	*During 6 month of follow up**	*HBeAg to antiHBe*	*HBsAg to antiHBs*
Low HBV replication (n=30)	11 (37%)†	7 (23%)‡	5 (17%)§	2 (7%)
High HBV replication (n=30)	1 (3%)†	0‡	0§	0

*With three normal values. †p=0·0012; ‡p=0·015; §p=0·06.

concentrations (below 50 pg/ml). Among these seven patients, five had HBeAg to antiHBe seroconversion three or four months after treatment. In two of them, HBsAg to antiHBs seroconversion occurred two months later (Fig 1). No HBe seroconversion occurred in the 30 patients with high replication.

EVOLUTION OF SERUM ALT

There was no significant change of mean serum ALT activities in the 60 treated patients during ARA AMP administration. Moderate increase of serum ALT activities was seen in two of 60 patients during treatment (from 255 IU/l to 466 IU/l and 145 IU/l to 309 IU/l, respectively). These two patients had HBeAg to antiHBe seroconversion and one of them lost HBsAg (Fig 1).

Pretreatment serum ALT activities were higher in the five patients with HBeAg to antiHBe seroconversion after ARA AMP administration than in the other patients (321 (234) IU/ml *v* 173 (124) IU/ml, $p<0·05$).

KINETICS STUDY

The kinetics study of serum hepatitis B virus DNA was performed in 30 patients, and showed a significant decrease during ARA AMP administration (Fig 2). The decrease in serum hepatitis B virus DNA was maximal three weeks after the initiation of ARA AMP, then serum hepatitis B virus DNA concentrations increased again. The kinetics and the magnitude of the decrease in serum hepatitis B virus DNA was not different in patients with low replication and in patients with high replication (Fig 3).

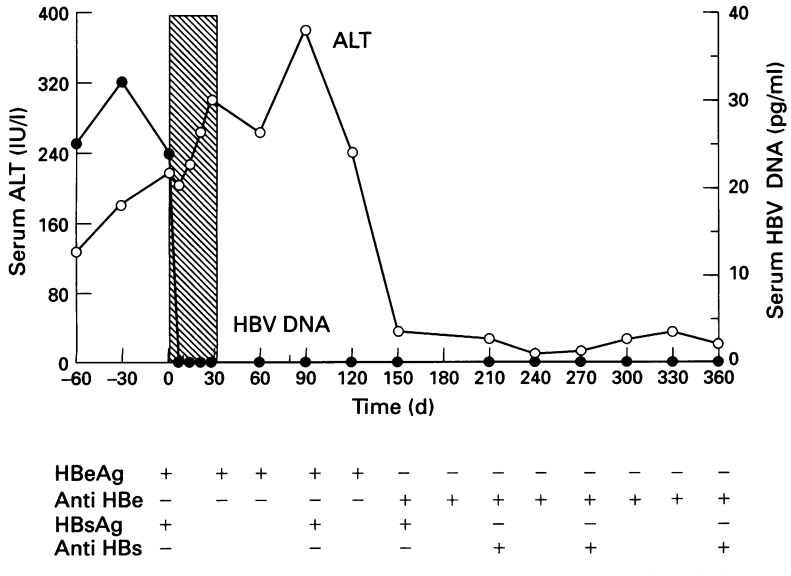

Figure 1: Time course of serum ALT and serum hepatitis B virus DNA before, during, and after ARA AMP in a patient with chronic hepatitis B who had HBs seroconversion.

SIDE EFFECTS OF ARA AMP

Mild side effects were seen in patients during ARA AMP administration: myalgia occurred in 20 patients during the fourth week of treatment, vomiting occurred in six patients at the start of treatment, nausea in two patients, fatigue in 18 patients, diarrhoea in one patient, and abdominal pain in four patients; however, treatment was completed in all these patients. Moderate side effects (vomiting) occurred in two patients requiring a half dose of ARA AMP. Severe side effects affected 10 patients: myalgia occurred in eight patients and required ARA AMP withdrawal 16 to 25 days after the start of treatment; vomiting in one patient required ARA AMP withdrawal. In an obese female patient, severe peripheral neuropathy was diagnosed at the end of treatment and was attributed to an over estimation of ARA AMP dose because of overweight.

EFFECT OF A SECOND COURSE OF ARA AMP

Among the 32 patients who received a second course of ARA AMP, serum hepatitis B virus DNA became negative in three patients. All three patients had serum hepatitis B virus DNA concentrations below 50 pg/ml before the second course of ARA AMP. HBeAg to antiHBe seroconversion occurred in one of them. These three patients had low serum hepatitis B virus DNA concentrations before the first course (19 to 55 pg/ml) and the first course resulted in a sustained lowering of hepatitis B virus DNA in one of three cases. The second course of ARA AMP was well tolerated in all patients.

Discussion

In the patients with chronic hepatitis B in this study, a complete response to ARA AMP administration was only seen in the group of patients with low hepatitis B virus replication. Indeed, all five responders had very low pretreatment serum hepatitis B virus DNA concentrations (below 50 pg/ml). This result shows that a complete antiviral effect of ARA AMP is only achieved in patients with a comparatively low baseline level of hepatitis B virus replication. Moreover, the five responders had higher pretreatment serum ALT activities than non-responders thus showing that an active immune response is also necessary to obtain a complete and sustained response.

The low rate of HBeAg to antiHBe seroconversion seen in this study (8%) does not support the results of other studies, in particular our previous study[1–3] but is in agreement with three other studies.[4–6] This low rate of sustained response may be related to a different patient selection in this study compared with our previous study. An increased number of patients in whom HBsAg was detected by systematic screening, were referred for antiviral treatment. Such patients will probably have

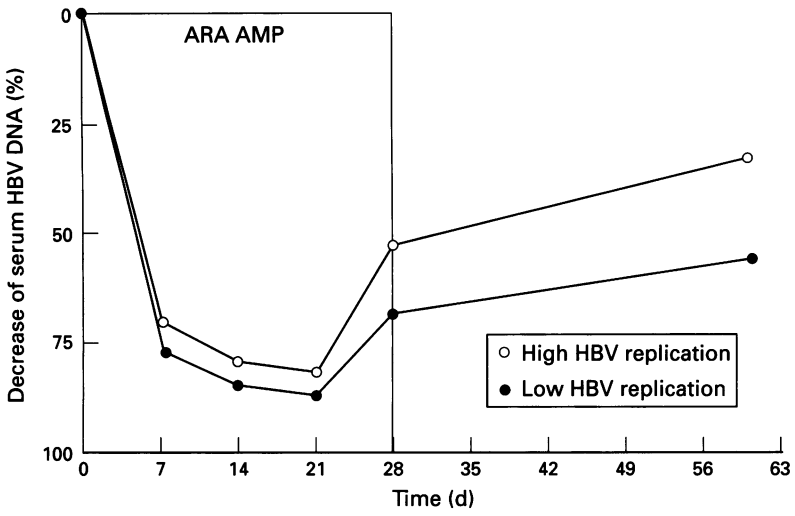

Figure 2: Percentage of decrease of serum hepatitis B virus DNA in patients with chronic hepatitis B according to pretreatment level of hepatitis B virus replication during and one month after ARA AMP administration.

higher hepatitis B virus DNA concentrations and lower serum ALT activities than patients previously referred to our centre. Indeed, in our previous study, we found that patients with high hepatitis B virus DNA concentrations and low serum ALT activity had a lower rate of response than other patients.[3] In a recent trial with recombinant alpha interferon performed after this ARA AMP trial, we also saw a comparatively low rate of HBeAg to antiHBe seroconversion (23%) (unpublished data). Although this study showed a lower rate of complete response with ARA AMP treatment than is usually seen with alpha interferon, ARA AMP should be considered in the treatment of chronic hepatitis B in patients who do not respond to, who develop side effects to, or who have contraindications to, alpha interferon. In particular, ARA AMP might be useful in the treatment of patients who have had a liver or kidney transplant in whom alpha interferon might increase the risk of rejection.[8 9]

The kinetics and the relative magnitude of the decrease of serum hepatitis B virus DNA was similar in patients with low and high replication (Fig 3). A sustained effect, however, was only obtained in patients with a low pretreatment value of serum hepatitis B virus DNA concentration (in whom a more complete viral inhibition was probably reached).

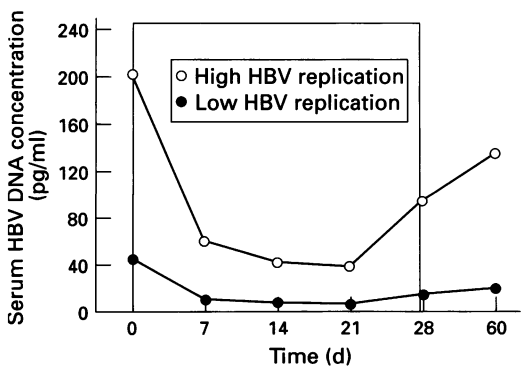

Figure 3: Time course of serum hepatitis B virus DNA in patients with chronic hepatitis B according to pretreatment level of hepatitis B virus replication during and one month after ARA AMP administration.

The low rate of response in our study is not different from the spontaneous rate of HBeAg seroconversion seen in control groups in previous studies[4–6] showing the small sustained effect of ARA AMP administration in chronic hepatitis B. The higher rate of response in patients with a low initial hepatitis B virus DNA value can be matched with a higher spontaneous rate of clearance of hepatitis B virus DNA in patients with low initial hepatitis B virus DNA values. The assumption, however, that the 8% HBe seroconversion rate seen in this study might be spontaneous and not related to ARA AMP administration is unlikely as (a) all patients included had stable values of serum hepatitis B virus DNA and ALT within the pretreatment period and (b) hepatitis B virus DNA disappearance and HBeAg to antiHBe seroconversion were chronologically clearly related to ARA AMP administration.

It is important to note that HBsAg to antiHBs seroconversion occurred in two patients in this study (3·3%). In these two patients, complete inhibition of hepatitis B virus replication was confirmed by disappearance of serum hepatitis B virus DNA, as assessed by polymerase chain reaction.[10] This result is not different from the 3–7% rate of HBsAg to antiHBs seroconversion seen in some alpha interferon studies.[11 12] This result is lower, however, than that reported by Alexander *et al*[13] who showed the disappearance of HBsAg in 22% and the appearance of HBs antibody in 13% of patients. This result shows that ARA AMP, like alpha interferon, may result in a complete inhibition of hepatitis B virus replication.

No significant increase of serum ALT activity was seen in most patients during or after ARA AMP administration. A mild increase of serum ALT was seen in two of five responders but no appreciable increase of serum ALT was seen in any patient with HBe seroconversion as usually seen in patients responding to alpha interferon. This finding illustrates that ARA AMP acts in chronic hepatitis B by an exclusive antiviral effect and that, in contrast with alpha interferon, an increase of hepatocytic necrosis is rarely seen.

The kinetics of serum hepatitis B virus DNA in our patients with chronic hepatitis B confirms an inhibition of hepatitis B virus replication, which is rapid and considerable but usually transient. The maximal decrease of serum hepatitis B virus DNA concentrations was seen three weeks after the start of ARA AMP, followed by an increase in these concentrations during the last week of treatment. This finding suggests that the fourth week of ARA AMP administration does not improve the antiviral effect. In addition, neurotoxicity associated with ARA AMP administration is the main limiting adverse effect for the use of this substance in the treatment of chronic hepatitis B.[14 15] In our study, many patients complained of myalgia (47%) during the fourth week of treatment, requiring withdrawal of treatment in 29% of cases. Among these patients only one developed severe peripheral neuropathy related to an accidental overdose

of ARA AMP administration as previously described.[16] As neurotoxicity depends on the length of the treatment[14] and as the fourth week of treatment does not improve the antiviral effect, a three week course should be considered.

A second course of ARA AMP induced seroconversion from HBeAg to antiHBe in only one of the 32 retreated patients. This result is not in agreement with the 67% rate of HBe seroconversion seen in another study.[16] This discrepancy might be related to the level of hepatitis B virus replication before treatment, which might have been lower in the patients in the study by Trepo *et al.* Indeed, the only responder to a second course of ARA AMP had a sustained lowering of hepatitis B virus DNA after the first course and had a low serum hepatitis B virus DNA concentration (below 50 pg/ml) before the second course of ARA AMP. Our results show that a second course does not seem to improve the rate of response to ARA AMP.

In conclusion, ARA AMP induces a considerable but transient antiviral effect on hepatitis B virus. A complete and sustained inhibition of hepatitis B virus replication with this substance is rare, and only obtained in patients with low hepatitis B virus replication.

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