

Successful treatment of HBs and HBeAg positive chronic liver disease: prolonged inhibition of viral replication by highly soluble adenine arabinoside 5'-monophosphate (ARA-AMP)*

I V D WELLER,† MARGARET F BASSENDINE,‡ A CRAXI,§ M J F FOWLER, J MONJARDINO, H C THOMAS,¶ and SHEILA SHERLOCK

From the Departments of Medicine and Physiology, Royal Free Hospital and School of Medicine, London

SUMMARY In eight HBs and HBe antigen positive patients with chronic active liver disease, adenine arabinoside 5'-monophosphate (ARA-AMP) given, intravenously or intramuscularly, six or 12 hourly, produced inhibition of viral replication. In five patients given a short course of therapy with 10 or 15 mg/kg/day this effect was transient and in two thrombocytopenia occurred. In three further consecutive cases given a longer course with 5 mg/kg/day after five days of the high dose, thrombocytopenia was not seen and inhibition of viral replication for up to 13 months occurred. These patients lost HBV-DNA polymerase activity, serum viral DNA and HBeAg, developed anti-HBe, and HBsAg concentrations decreased. A course of twice daily intramuscular ARA-AMP given for three to five weeks as an outpatient may be expected to produce a long-term reduction in infectivity.

Adenine arabinoside (ARA-A) is a synthetic purine nucleoside with a broad spectrum of antiviral activity against DNA viruses.¹ Uncontrolled studies in HBsAg-positive chronic liver disease²⁻⁵ indicated that ARA-A can inhibit hepatitis B virus (HBV) replication transiently with no change in HBsAg concentration, HBeAg status, liver function tests, or histology. In a few patients, inhibition of DNA polymerase activity has been permanent with a later decrease in HBsAg concentration and loss of HBeAg. Recent uncontrolled studies with ARA-A and/or human leucocyte interferon have shown similar changes in 37% of treated patients.⁶ In a randomised controlled study,⁷ in three out of seven HBeAg positive patients, ARA-A alone produced permanent inhibition of HBV DNA polymerase activity with a loss of HBeAg and a decrease in

HBsAg concentrations and aspartate transaminase. No such changes occurred in the control groups.

Although ARA-A has a low toxicity, relative to other antiviral agents,⁸ its usefulness is limited by insolubility and the need for continuous intravenous administration. Adenine arabinoside 5'-monophosphate (ARA-AMP), the synthetic monophosphate ester of ARA-A, is at least 400 times more water soluble. It is therefore suitable for intramuscular or intravenous administration. The aim of this study was to determine whether ARA-AMP would also inhibit HBV replication and, if so, to determine an effective and convenient dosage regime.

Methods

PATIENTS

Initially five HBsAg positive males, age range 29-56 years, were treated with ARA-AMP 10 or 15 mg/kg/day intravenously or intramuscularly for nine to 14 days. All were serum HBeAg and HBV-DNA polymerase positive. Each had chronic active liver disease proven by biopsy and had been chronically infected and untreated for one to four years. In particular they had never received immuno-

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† MRC Training Fellow.

‡ HAM Thompson Fellow of the Royal College of Physicians. Present address: Department of Medicine, University of Newcastle upon Tyne.

§ NATO scholar from the Department of Medicine, University of Palermo.

¶ Senior Wellcome Fellow.

Address for reprint requests: Dr I V D Weller, Academic Department of Medicine, Royal Free Hospital, Pond Street, London NW3 2QG.

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suppressant therapy (Table 1). Case 1, an intravenous drug abuser, defaulted on the ninth day of treatment. Case 4 had a second course of treatment at higher dosage two months after the first. Case 5 was given a lower dosage (5 mg/kg) of ARA-AMP at the end of the initial treatment period to assess the effectiveness of the reduced dose in maintaining inhibition of HBV-DNA polymerase activity.

Subsequently and consecutively three HBsAg/HBeAg/HBV-DNA polymerase positive males, age range 28–36 years, with histologically proven chronic active liver disease for one to three years, who also had not received antiviral or immunosuppressant therapy, were treated with ARA-AMP at the lower dose of 5 mg/kg/day given 12 hourly intramuscularly. In two cases it was given for 16 and 19 days after five days of ARA-AMP at 10 mg/kg/day and, in one case, the low dose was given throughout for 34 days. Two cases were treated as outpatients.

Serum samples were taken before, during, and after therapy and stored at -20°C . HBsAg was detected by radioimmunoassay (Ausria-II 125, Abbott Laboratories) and quantified by rocket immunoelectrophoresis (data expressed as a percentage of a laboratory standard).⁹ HBeAg and antibody (anti-HBe) were detected by radioimmunoassay (Abbott Laboratories) and HBeAg quantified by serial dilution, the titre expressed as the first dilution at which the ratio between sample and mean negative control cpm dropped below 2.1. HBV-DNA polymerase activity was measured by the method of Robinson,¹⁰ which was modified by using 200 μl of serum layered over 600 μl of 30% (w/v) sucrose containing TNEM-BSA. Methyl thymidine 5'-triphosphate was used as the tritiated base. The 95% confidence limit of the normal range

was 850 dpm/200 μl (2 SD above the mean of 50 negative controls) and levels above this are regarded as positive. Serum HBV-DNA in case 5 was measured by Dr J Summers (Institute for Cancer Research, Philadelphia) by hybridisation with ³²P cloned HBV-DNA. The autoradiograph spots were quantified photometrically and HBV-DNA expressed as area in cm^2 under the peak at 17 hours. Serum HBV-DNA in cases 6, 7, and 8 were measured using the same method in our own laboratory¹¹ and the spots analysed by scanning densitometry (Quick Scan Jr, Helena Labs), HBV-DNA expressed as area in arbitrary units under the peak after 11 days' exposure. Liver specimens were obtained by percutaneous needle biopsy. HBsAg was detected by indirect immunofluorescence using an IgG1 monoclonal antibody and tetraethylrhodamine isothiocyanate-conjugated goat anti-mouse IgG1 as a second layer. HBeAg was detected by direct immunofluorescence using a human antiserum to HBe. Clinical condition, full blood count, liver function tests, plasma urea and electrolytes, serum creatinine, and urinalysis were also monitored throughout the study.

Results

CASES 1–5 (Short course ARA-AMP)

All the patients showed inhibition of DNA polymerase activity during treatment. In five cases (nos 1–5) this was transient and followed by a rebound in DNA polymerase activity on stopping treatment (Fig. 1). In case 5 the changes in DNA polymerase were accompanied by parallel changes in serum HBV-DNA. In case 1, the drug abuser, inhibition was sustained during the treatment period but there was no follow up beyond the ninth day until one year later when he was still HBsAg, HBeAg, and DNA polymerase positive. In cases 2–5 there was no consistent change in HBeAg titre (remaining 1/2560), HBsAg concentration, or AST level for a period of five to eight months after treatment and all remained DNA polymerase positive. Two of the five patients were biopsied again after this time and there was no change in the histological diagnosis, both continuing to have mild to moderate chronic active hepatitis displaying both HBs and HBeAg in the hepatocytes.

Intramuscular injection appeared to be as effective as intravenous. Twelve hourly injections were as effective as six hourly, but in four cases (2, 3, 4i, and 5) (Fig. 1) daily injections were not as effective in maintaining inhibition of DNA polymerase activity.

In cases 4ii and 5, when treatment was prolonged beyond 10 days, there was a greater than 50%

Table 1 Case and treatment details

Case no.	Histology	Route	Dose (mg/kg/day)	Frequency of administration	Duration (days)
1	AC	IM	10	6 hrly for 4 d	9
2	AC	IV	10	12 hrly for 3 d	10
3	CAH	IV	10	daily for 3 d	10
4	CAH	IM	10		10
5	CAH	IM	10	12 hrly for 7 d daily for 3 d	13
4*	CAH	IM	then 5 15	12 hourly	14
6	CAH	IM	10 then 5	12 hourly	5 19
7	CAH	IM	10 then 5	12 hourly	5 16
8	CAH	IM	5	12 hourly	34

* Second course of treatment.

AC=active cirrhosis. CAH=chronic active hepatitis.

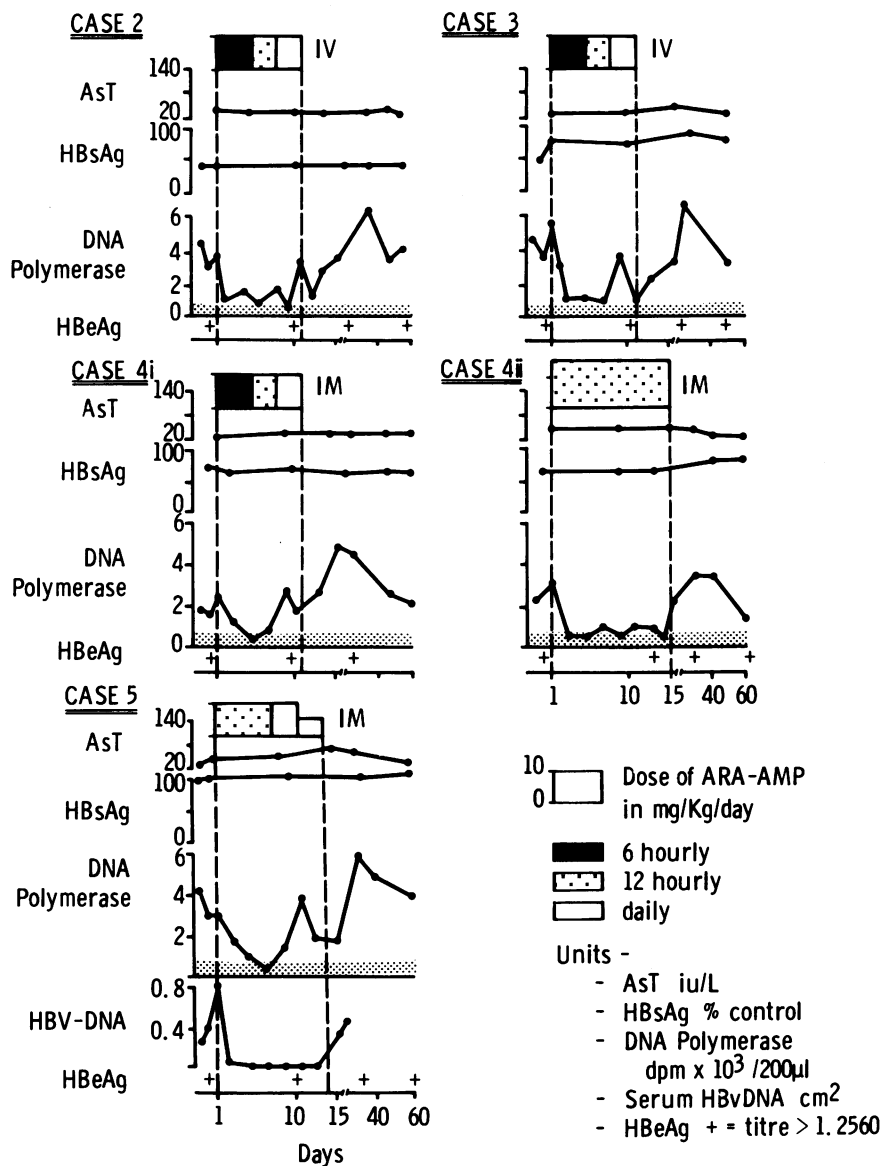


Fig. 1 Transient inhibition of viral replication (duration of treatment 10–14 days).

reduction in platelet count accompanied by a less marked reduction in total white cell count, necessitating stopping treatment. The thrombocytopenia corrected spontaneously within 10 days. There were no changes in urea and electrolytes or serum creatinine during or after treatment. Urinalysis was normal, except in case 3 who

developed macroscopic haematuria on treatment. He had a previous history of this on two occasions. On microscopy there were no red cell casts and the haematuria disappeared spontaneously while treatment continued. Intravenous pyelography and cystoscopy were normal. All patients complained of lethargy during treatment. Two patients experi-

enced transient nausea during the first two days of treatment. One patient had diarrhoea for two days after the treatment stopped.

CASES 6-8 (Long course ARA-AMP)

All three cases showed inhibition of DNA polymerase activity (Fig. 2). In cases 6 and 7 there was a rebound on stopping therapy but then a fall into the negative range. In each case inhibition of DNA polymerase activity was prolonged and associated with a fall in HBeAg titre. In three to seven months after treatment HBeAg became undetectable, with the development of anti-HBe and a decrease in HBsAg concentration in all three

cases. Serum viral DNA in cases 7 and 8 became undetectable during treatment (Fig. 3). In case 7 low amounts were detectable at the time of appearance of anti-HBe. There was a 73 to 96% reduction during treatment in case 6 with a rebound but then viral DNA became undetectable with the appearance of anti-HBe. The patients have remained anti-HBe positive for eight to 13 months after treatment. There was a marked rise in AST after therapy but in each case this has returned to below pre-treatment levels (Table 2). The pattern of response in terms of viral markers and AST and their temporal relationship were similar in each case. The histological and immunofluorescence data

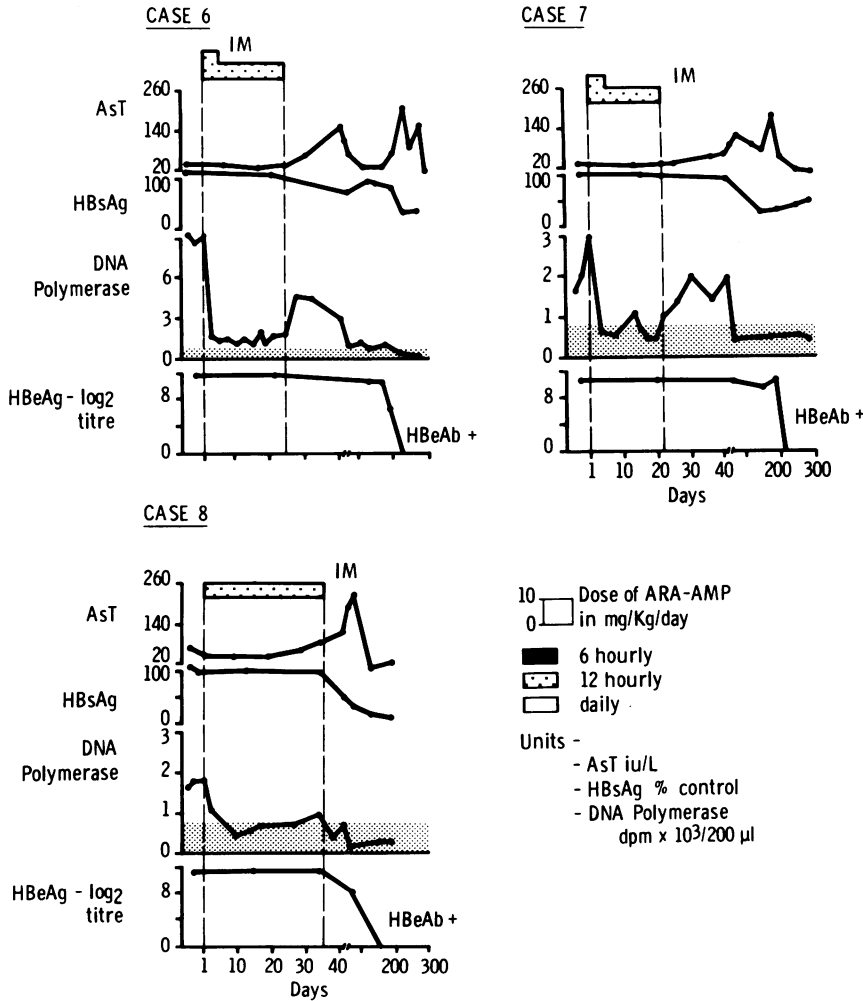
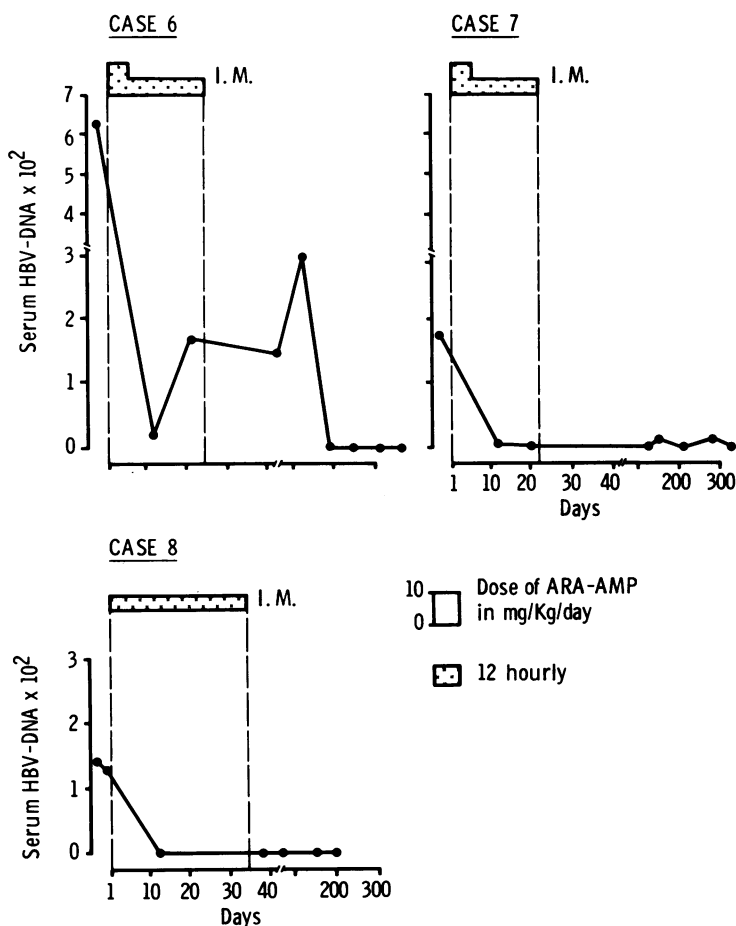


Fig. 2 Prolonged inhibition of viral replication (duration of treatment 21-34 days).

Fig. 3 Serum HBV-DNA in cases 6, 7, and 8 (DNA expressed as area under peak in arbitrary units).



are shown in Table 2. Case 6 lost HBcAg from the liver and showed no piecemeal necrosis in the post-treatment biopsy. In case 7 HBcAg was still detectable in the liver and histologically there was no change. Case 8 refused repeat biopsy. Significant thrombocytopenia (platelet count <100,000) did not occur. Lethargy was experienced by each patient during treatment but there were no other side-effects. Renal function and urinalysis remained normal.

Discussion

ARA-AMP given as intermittent bolus injections intravenously or intramuscularly inhibits HBV-DNA polymerase activity and causes a parallel decrease in serum HBV-DNA. Six hourly and 12 hourly injections are effective but daily administration seemed less useful in maintaining inhibition.

In five cases receiving a short course of ARA-AMP (10 days) this inhibition of viral replication was only a transient phenomenon and, furthermore, was not accompanied by changes in HBsAg or HBeAg concentrations. Reversible thrombocytopenia prevented prolongation of therapy at the 10 and 15 mg/kg/day dose levels.

Reducing the dosage after five days, to 5 mg/kg/day, or treating with this lower dose throughout, allowed longer-term therapy without significant thrombocytopenia or other serious side-effects. In three consecutive patients who received this longer course, long-term inhibition of viral replication (loss of DNA polymerase activity, serum viral DNA and HBeAg) occurred and all have developed anti-HBe in the period of follow-up. These changes in viral markers were associated with a transient rise in AST after therapy but then a fall to below pretreatment levels.

Table 2 Biochemical, histological, and immunofluorescence data in cases 6, 7, and 8 before and after therapy

Case no.	Aspartate transaminase (IU/l)		Light microscopy		Histology				Timing of biopsy (months after treatment)
	Before	After (months after treatment)			IF HBsAg		IF HBcAg		
			Before	After	Before	After	Before	After	
6	34 (27-37)	17 (12-19) [11-14]	CAH	CPH	+	+	+	-	12
7	37 (27-58)	20 (16-24) [12-15]	CAH	CAH	+	+	+	±	13
8	46 (45-76)	32 (19-35) [4-8]	CAH	ND	+	ND	+	ND	ND

AST: Normal range (4-15 IU-l); data expressed as median with range of observations made before therapy and late in course of follow-up. IF: Immunofluorescence.

Spontaneous seroconversion from HBeAg to anti-HBe does occur and may be associated with improvement in liver function tests and histology. The annual rates of seroconversion vary from <5% in a population of London patients¹² to approximately 15% in a series of untreated Italian patients.¹³ Taking the highest recorded spontaneous annual seroconversion rate, there is a low probability ($p < 0.005$: using binomial test) that the observed responses to the long course of therapy occurred by chance. The constant pattern of response and temporal relationship of the changes in viral markers and AST, to treatment in the three consecutive cases, are further support for the view that these seroconversions were drug induced.

The rebound in DNA polymerase activity after therapy and delay in loss of HBeAg suggests that the direct antiviral activity of ARA-AMP may only be part of the mechanism involved in producing prolonged inhibition of viral replication. The associated rise in AST after treatment in those that later seroconverted suggests that hepatocyte lysis is also involved in the process. This raises the possibility that enhancement of the immune response to the virus or virally determined antigens, perhaps facilitated by a preceding lowering of viral load, is an important component.

The aim of antiviral therapy in patients with chronic HBV infection is to reduce infectivity and to reduce hepatic inflammatory activity. As it is well established that blood from anti-HBe positive patients is relatively less infectious than blood from HBeAg positive individuals,¹⁴ it is highly probable that our patients who have developed anti-HBe after therapy have undergone a substantial reduction in infectivity. This contention is further supported by the disappearance from the serum of the HBV-DNA, a more sensitive direct assay for the presence of HBV particles. As far as improvement in liver disease is concerned the controlled trial of ARA-A⁷ showed a significant decrease in AST, particularly in those patients who developed anti-HBe. Uncontrolled studies with ARA-A and/or

human leucocyte interferon have also shown improvement in symptoms, biochemistry, and histology as seroconversion from HBeAg to anti-HBe occurred.⁶ The AST in our three patients eventually fell to below pre-treatment levels. In addition in one there was histological improvement. In the other patient whose liver biopsy remained unchanged, serum viral DNA persisted during the initial period of development of anti-HBe and 13 months after treatment this patient still had HBcAg in the liver. This suggests that viral replication may continue in the liver in the absence of markers in the serum¹⁵ and that longer periods of histological follow-up may be required.

These observations indicate that a long course of ARA-AMP is successful in reducing the level of viral particles in the blood and therefore in reducing the level of infectivity of HBeAg positive patients. More extensive controlled trials are necessary to determine the effect of this induced seroconversion (HBeAg to anti-HBe) on the level of hepatic inflammatory activity and on the long-term prognosis of these patients.

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