Clinical trial

Randomised controlled trial of adenine arabinoside 5'-monophosphate (ARA-AMP) in chronic hepatitis B virus infection

I V D WELLER, ANNA S F LOK, A MINDEL, P KARAYIANNIS, SARAH GALPIN, J MONJARDINO, SHEILA SHERLOCK, AND H C THOMAS

From the Departments of Medicine and Physiology, Royal Free Hospital and School of Medicine and Academic Department of Genito Urinary Medicine, Middlesex Hospital Medical School

SUMMARY A randomised controlled trial was conducted in 29 HBV carriers who had been HBs and HBe antigen positive for more than six months. Fifteen patients were treated with ARA-AMP 10 mg/kg/day given as intramuscular injections 12 hours apart for five days followed by 5 mg/kg/day for 23 days. The 14 controls received no treatment. Serum HBV-DNA polymerase, and HBV-DNA decreased in all patients during therapy. Six treated patients lost serum HBV-DNA polymerase, HBV-DNA and HBeAg, HBsAg concentrations decreased, and five developed anti-HBe. One of these six patients lost HBsAg and developed anti-HBs. No such changes were observed in the control group over a similar 18 month period of observation. A four week course of ARA-AMP inhibits HBV replication and in a significant minority of patients this is long lasting and is associated with a reduced level of inflammatory activity in the liver.

Adenine arabinoside (ARA-A) is a synthetic purine nucleoside with anti-viral activity against a large number of DNA viruses.¹ It has been used, largely in uncontrolled studies, in patients with chronic liver disease caused by hepatitis B virus (HBV) infection. In the majority of those treated, a transient decrease in HBV-DNA polymerase (HBV-DNAp) has been observed with no change in HBsAg concentration, HBeAg status, liver function tests or histology. In a minority of patients, however, inhibition of HBV-DNAp is prolonged beyond the treatment period with a decrease in HBsAg concentration, loss of HBeAg and the development of anti-HBe.²⁻⁵ Similarly, in uncontrolled studies with ARA-A and/or human leucocyte interferon, 37% of treated patients have shown this prolonged inhibition of HBV replication with HBeAg to anti-HBe seroconversion, accompanied by an improvement in liver function tests and histology.^{6,7} Spontaneous HBeAg to anti-HBe seroconversion does occur, however, with a variable frequency in different populations and may occur following withdrawal of immuno-

suppressant therapy.⁸⁻¹² Controlled trials are therefore required in chronic HBV infection to show with certainty the long term effects of any drug. ARA-A alone, in a randomised controlled study, induced HBe antigen to anti-HBe seroconversion with loss of HBV-DNAp, a decrease in HBsAg concentration and aspartate transaminase in three out of seven treated patients and no such changes occurred in the control group.¹³

Adenine arabinoside 5'-monophosphate (ARA-AMP) is the synthetic monophosphate ester of ARA-A, is at least 400 times more water soluble and therefore suitable for intramuscular administration. A three to five week course of ARA-AMP, given twice daily intramuscularly, to three consecutive HBeAg positive patients was associated with loss of HBV-DNAp, serum HBV-DNA, HBeAg to anti-HBe seroconversion and a decrease in HBsAg concentration.¹⁴

The aim of this study was to determine, in a randomised controlled trial, whether ARA-AMP had a permanent effect on the level of viral replication in patients with HBsAg/HBeAg positive chronic liver disease and if so to assess the effects of this on liver function and histology.

Address for correspondence: Prof H C Thomas, Academic Department of Medicine, Royal Free Hospital, Pond Street, London NW3 2QG.

Received for publication 24 August 1984.

Methods

PATIENTS

Between August 1980 and November 1981, all patients who had been HBsAg and HBeAg positive for more than six months attending the Department of Medicine at the Royal Free Hospital and the Department of Genito Urinary Medicine at the Middlesex Hospital, were considered for entry. The following were excluded: those under 18, women of childbearing potential, patients with clotting abnormalities preventing liver biopsy, renal failure or decompensated cirrhosis, those receiving immunosuppressant or anti-viral therapy less than six months before entry, those with an alcohol consumption great than 80 g per day and those patients from overseas who would not be available for long term follow up. Fifteen patients were excluded on these grounds. Twenty nine patients were randomised using sealed envelopes¹⁵ to receive no treatment (n=14) or ARA-AMP (n=15) given intramuscularly twice daily for 28 days; 10 mg/kg/day for five days and then 5 mg/kg/day for 23 days. The majority of patients were British and white other than two Asians in the control group and in the treatment group; one Asian, two Spanish, one Italian, one North American and one South African, both white. Treated patients were seen weekly during therapy and for four weeks afterwards and thereafter monthly for 18 months. Control patients were followed up monthly for a similar period. Blood was taken at each visit for full blood counts, prothrombin time, liver function tests (aspartate transaminase, bilirubin, alkaline phosphatase and albumin), urea, creatinine and electrolytes and a sample of serum stored at -20°C for viral markers. Patient characteristics are shown in the Table. Only three patients had ever received antiviral therapy. Two patients in the control group had received ARA-AMP for 10-15 days, 15, and 17 months respectively before entry into the trial. One patient in the treatment group had received ARA-A for 10 days two years before entry.

HBsAg was detected and quantified using a monoclonal antibody based radioimmunoassay (RIA) which has been shown to be as sensitive as the Ausria-II (Abbott).¹⁶ Serial duplicate, 10-fold dilutions of HBsAg positive specimens were used. A standard curve was plotted using a pooled serum standard of mixed subtype which was standardised against the British standard and contained 85 µg/ml of HBsAg. All serial samples on each patient were run within the same assay. HBeAg and anti-HBe were detected by RIA (Abbott Laboratories).

HBV-DNAp was measured by using 200 µl of serum layered over 600 µl of 30% (w/v) sucrose

Table Patient characteristics on entry - (all male)

	Treatment	Control
Patients (no)	15	14
Age	30 (22-46)	32 (20-46)
Homosexual	7	7
Intravenous drug abuse	2	0
Others	6	7
Total	15	14
HBsAg (µg/ml)	240 (30-3800)	260 (50-1000)
HBeAg	+	+
HBV-DNA polymerase (dpm/200 µl)	3614 (1108-10 158)	3281 (204-6166)
HBV-DNA (cpm/200 µl)	1668 (32-2556)	1236 (28-2133)
Aspartate transaminase (IU/l)	67 (36-179)	59 (34-95)
Histology		
CPH	2	3
CPH/CAH	1	1
CAH	12	9
Cirrhosis	0	1

CPH=Chronic persistent hepatitis; CAH=Chronic active hepatitis.

Values expressed as median with range.

containing TNEM-BSA.¹⁷ Methyl thymidine 5'-triphosphate was used as the tritiated base. After the DNAp reaction, samples were TCA-precipitated and washed individually in petri dishes. The upper limit of the normal background count was 568 dpm/200 µl of serum (mean of 47 negative controls + 2SD). HBV-DNAp positive samples from 22 HBeAg positive patients were analysed and then stored at -20°C for a mean of 10 months (range 4-16 months) and retested. There was no significant difference between the values before and after storage at -20°C for this period of time (Wilcoxon's matched pairs signed ranks test). Serial samples from the trial patients were run within the same assay. Within assay variance was studied by analysing 41 duplicates of different samples (five to seven duplicates in each of eight assays) and the overall coefficient of variation for a single sample found to be +16%.

Serum HBV-DNA was measured by molecular hybridisation using ³²P-labelled cloned HBV-DNA using a method previously described.¹⁸ This was modified to improve the non-uniformity of spots. Phenol extraction was carried out in microfuge-centrifuge reaction vials (LIP Ltd) and the aqueous layer separated by centrifugation in an eppendorf centrifuge (Anderman). The HBV-DNA in 50 µl of the aqueous phase was denatured by adding it to 100 µl of 0.15 M NaOH/1.5 M NaCl. The mixture was vortexed and left for five minutes at room temperature and then neutralised by adding 100 µl of 0.15 N HCl/2.1 M NaCl and vortexing. These 250 µl

samples were then pipetted into the wells of the BRL 'hybridot' manifold containing a nitrocellulose filter.¹⁹ Hybridisation was carried out as previously described. The HBV-DNA positive spots were quantified after 18 hours autoradiography by punching out the corresponding spots on the nitrocellulose filter with a 6 mm diameter cork-borer. These discs were then counted in 4 ml scintillation fluid in a Philips liquid scintillation counter. The amount of HBV-DNA in the samples was then expressed as counts per minute (cpm) per 200 µl of serum minus background. The highest negative control on the filter was usually 2–3 cpm.

The possibility of intercurrent infection during the study period with either hepatitis A virus (HAV) or delta agent was studied. All patients' sera were examined for anti-HAV by radioimmunoassay (HAVAB, Abbott) on entry and at the end of the study period. All positive sera were examined for IgM anti-HAV (HAVAB-M, Abbott). Similarly paired sera spanning the study period were examined for antibodies to delta agent detected by a solid-phase blocking RIA by Dr M Rizzetto, Turin.²⁰

Liver specimens were obtained by percutaneous liver biopsy within six months of entry into the trial and after 12 months of post-treatment follow up in the treated group and at the same time in the control group. Assessment of portal and periportal inflammatory activity and lobular inflammation and spotty necrosis was made.

Statistical evaluation was carried out using Wilcoxon's rank sum test or a two-tailed Fischer's exact test.

Results

On entry to the trial, there were no statistically significant differences between patients in the treatment and control group in terms of age, proportion of homosexuals, levels of markers of HBV replication (HBsAg, HBV-DNAp and HBV-DNA), aspartate transaminase and the histological spectrum of liver disease on the initial biopsy (Table). Two patients in the treatment group were identical twins, abused intravenous drugs and shared needles. Of the 13 non-homosexual, non-intravenous drug abusers, five were health care workers and three lived or had lived in parts of the world with a high prevalence of HBV infection. In the remaining five patients risk factors for hepatitis B were not identified. The most frequent histological finding was chronic active hepatitis.

HBV REPLICATION

Two patterns of response to ARA-AMP were seen (Fig. 1). During the treatment period all patients

showed a decrease in HBV-DNAp and HBV-DNA. In 11 patients these returned immediately to pre-treatment levels and HBeAg remained positive. Four patients showed a prolonged inhibition of HBV-DNAp and HBV-DNA after treatment and this was associated with the development of anti-HBe six to eight months after therapy. A further two patients lost HBV-DNA, and DNA polymerase and HBe between 12 and 18 months after treatment and one of these seroconverted to anti-HBe. There were no consistent changes in HBV-DNAp or HBV-DNA in the control group and all patients remained HBeAg positive up to 18 months post treatment.

LIVER FUNCTION TEST

Four of the patients in the treatment group who developed anti-HBe showed three to nine-fold increases in aspartate transaminase five to seven months after treatment. In one homosexual, this occurred three weeks after a trip to Amsterdam. He developed nausea, loss of appetite and jaundice. Serum IgM anti-HAV was positive (Fig. 2). This acute hepatitis A was associated with a rise in alkaline phosphatase and bilirubin as well as transaminase, loss of HBV-DNAp, HBV-DNA, HBeAg and seroconversion to anti-HBe. Furthermore, HBsAg disappeared from the serum followed by the development of anti-HBs.

Both the identical twin intravenous drug abusers developed an asymptomatic rise in transaminase five months after therapy (Fig. 3). Inhibition of HBV-DNAp and HBV-DNA had persisted for at least three to four months post-treatment in both twins before this rise in aspartate transaminase. Serum antidelta, which was negative in both on entry into the trial, became positive at the time of elevation in aspartate transaminase. Delta antigen was detected by direct immunofluorescence in the nuclei of hepatocytes in both liver biopsies 12 months after therapy.

After loss of HBeAg in the six treated patients, the aspartate transaminase showed improvement. In the patients without delta infection, the transaminase returned to within the normal range. In the two delta infected intravenous drug abusers, aspartate transaminase decreased but remained above the normal range.

Of those who remained HBeAg positive, one patient in the treatment group and one control showed asymptomatic three- to four-fold rises in aspartate transaminase during the follow up period. In neither case was this associated with a decrease in the level of HBV replication, HAV or delta infection. There were no consistent changes in liver function tests in the other patients in either the

Fig. 1 Serum HBV-DNA polymerase, DNA and HBsAg concentration — responders — — — — non-responders. HBV-DNAp is expressed as $\text{dpm} \times 10^3/200 \mu\text{l}$ of serum, HBV-DNA as $\text{cpm} \times 10^3/200 \text{ ml}$ serum and HBsAg as $\mu\text{g/ml}$.

treatment or control groups. Fourteen patients showed evidence of past HAV infection on entry and those negative for anti-HAV remained so throughout the study period. No other patients showed serological evidence of delta infection.

Inflammatory activity and degree of hepatocyte necrosis was reduced in two patients who showed long term inhibition of HBV replication without delta superinfection. In the twins who had superinfection with the delta agent, both spotty necrosis and periportal and portal inflammatory activity were increased. The remaining two patients seroconverted after the follow up biopsy. There were no significant changes in the level of inflammatory

activity in the untreated and treated non-responder groups.

Fourteen of the 15 treated patients complained of increasing fatigue during the treatment period. In four, this was associated with loss of appetite and in two, nausea. Six patients complained of either lower limb pain or muscle cramps. In three patients these symptoms persisted throughout therapy and in one patient they were intermittent. In one case these symptoms appeared during the initial five day period on 10 mg/kg/day of ARA-AMP and in one they developed during the week following therapy. In no patient were these symptoms associated with neurological deficit on clinical examination. None of

Fig. 2 ARA-AMP and acute HAV infection. Aspartate transaminase is expressed as IUI/l (normal range 5–40); HBsAg as ug/ml, HBV-DNAp as dpm $\times 10^3/200 \mu\text{l}$ of serum.

the above side effects necessitated analgesics or the cessation of therapy.

One patient developed acute right loin pain radiating to the groin five days after therapy began and after a day of sunbathing. This was associated with haematuria and crystalluria microscopically and an urgent intravenous urogram (IVU) revealed an obstructed right pelvicalyceal system. Serum calcium and 24 hour urine calcium, phosphate, uric acid and oxalate were all normal. The 24 hour urine xanthine excretion was 235 mg (normal 10–20 mg). A stone was not recovered with sieving of the urine. ARA-AMP was continued at the 5 mg/kg/day dose and a $[^{99}\text{Tc}]\text{-Sn-DTPA}$ scan after 48 hours and a repeat IVU one week later showed normal kidneys with no evidence of obstruction.

Full blood count, urea, creatinine and electrolytes remained normal throughout the treatment period.

Discussion

ARA-AMP given twice daily by intramuscular injection for four weeks inhibited HBV replication during therapy as shown by the decrease in HBV-DNAp and HBV-DNA in treated patients when

Fig. 3 ARA-AMP and delta infection in two patients. Aspartate transaminase is expressed as IUI/l, HBsAg as ug/ml, HBV-DNAp as dpm $\times 10^3/200 \mu\text{l}$ of serum.

compared with controls. Similar changes have been shown in uncontrolled studies.^{14 21} In four of the 15 treated patients this inhibition was prolonged and accompanied by HBeAg to anti-HBe seroconversion six to eight months after therapy. A further two patients lost HBe antigen between 12 and 18 months after therapy. No such changes developed in the control group. This difference in seroconversion from HBeAg to anti-HBe between treated patients and controls was statistically significant using a two tailed Fischer's exact test, $p < 0.03$. In four of these six patients there were three- to nine-fold rises in aspartate transaminase preceding the seroconversion. In two patients intercurrent infection with the delta agent and in one HAV occurred during the follow up period. In the two intravenous drug abusers the delta hepatitis occurred four to five months after HBV-DNAp and HBV-DNA had become negative after ARA-AMP therapy. A mean interval of three months has been previously observed between therapy and loss of HBV-DNAp and HBV-DNA and six months before loss of HBeAg, after ARA-A and ARA-AMP.^{14 22} In HBV carrier chimpanzees it has been shown that inoculation with delta agent is followed by a hepatitis, intrahepatic expression of delta antigen after three weeks and the development of antidelta after eight to nine weeks. This superinfection is

associated with transient inhibition of HBV replication as shown by a decrease in serum HBsAg and HBeAg and HBcAg display in the liver demonstrated by immunofluorescence.²³ Similar decreases in HBsAg have been observed in human HBV carriers superinfected with delta agent but prolonged inhibition with HBeAg to anti-HBe seroconversion has not been described.²⁴ If the delta virus incubation period is similar in the chimpanzee and human HBV carrier it would seem that in the two intravenous drug abusers inhibition of HBV replication was well established after ARA-AMP before delta infection occurred.

In the patient who had acute HAV infection, inhibition of HBV-DNAp and HBV-DNA was prolonged beyond the treatment period but then returned towards pretreatment levels. An acute hepatitis A seven months after therapy was followed by loss of HBV-DNAp and HBV-DNA and HBeAg to anti-HBe seroconversion. It would seem from the temporal relationship of the hepatitis and the loss of HBV markers that in this patient acute HAV infection contributed to the inhibition of HBV replication.²⁵ Similar rises of transaminase associated with loss of HBeAg and development of anti-HBe have been observed in other studies at a similar time after therapy with ARA-A and ARA-AMP and without concurrent hepatitis virus infection.^{14 21 22} In addition, similar changes have been described after therapy with human leucocyte and lymphoblastoid interferon,^{4 6 7} after immunosuppressant withdrawal in HBeAg positive patients and during immunostimulant therapy with BCG.⁵ It has been suggested that this rise in transaminases is mediated by an enhancement of the immune response to the virus or virally determined antigens perhaps facilitated by a preceding lowering of viral load.¹⁴ Because the acute HAV infection in our patient occurred at a time when loss of HBeAg might be expected after antiviral therapy the extent of its role in the inhibition of HBV replication is difficult to evaluate.

Seroconversion from HBeAg to anti-HBe after antiviral therapy and that which occurs spontaneously is often associated with a decrease in transaminases and hepatic inflammatory activity.^{7 8 10 14} Of the five treated patients who developed anti-HBe, transaminases returned to normal in three and in two of these hepatic inflammatory activity was considerably reduced. In the two intravenous drug abusers who had superinfection with the delta agent, transaminases remained raised and inflammatory activity persisted. This observation is consistent with evidence that in chronic HBV carriers with concomitant delta infection there is a more rapid progression of chronic liver disease.^{26 27}

Thus the continuing inflammation in the two delta infected patients was probably attributable to this superinfection and not to continuing HBV infection. Liver histology showed no significant change in the untreated and treated non-responder groups.

Three of the four 'early' responders were heterosexual and the fourth patient, who suffered the acute HAV infection, was homosexual. The two 'late' responders were homosexual. Although this is not significant with such small numbers this data and the observation that the responders in our previous studies¹⁴ were all heterosexual raises the possibility that there may be different responses to treatment in homosexual and heterosexual HBV carriers.²⁸ This may relate to the coexistence of other infective agents and immunodeficiency in the homosexual group.

Mild side effects were seen in the majority of the ARA-AMP treated patients. Fatigue was present in all patients and in a minority, cramps and paraesthesia occurred late in the course of the treatment, and in some cases, after treatment.

ARA-AMP has been shown to be effective in producing inhibition of HBV replication and, in a significant proportion of patients, this effect is long lasting, and associated with a reduced level of inflammatory necrosis of liver cells. In two patients showing long term inhibition of HBV replication, intercurrent delta infection occurred and at this stage, the transaminase and hepatic histology showed an increased level of inflammatory activity. Further controlled studies are in progress to determine whether a longer course of ARA-AMP is associated with a higher rate of seroconversion from HBeAg to anti-HBe.

IVDW was an MRC training fellow, and is now lecturer in the Academic Department of Genitourinary Medicine, Middlesex Hospital Medical School, ASFL was supported by the University of Hong Kong and HCT was a Senior Wellcome Fellow of Clinical Science. ARA-AMP used in this study was supplied by Warner Lambert - Parke-Davis. The authors would like to thank Professor M Adler for his help in the clinical trial, Professor P J Scheuer and Dr I Lindsay for reviewing the liver biopsies, Dr M J F Fowler for his help in the HBV-DNA assays and Mr D Hindley for statistical advice.

References

- 1 Shannon WM. Antiviral activity in vitro. In: Pavan-Langston D, Buchanan RA, Alford CA Jr, eds. *Adenine arabinoside: an antiviral agent*. New York.

- Raven Press, 1975.
- 2 Chadwick RG, Bassendine MF, Crawford EM, Thomas HC, Sherlock S. HBsAg-positive chronic liver disease: inhibition of DNA polymerase activity by vidarabine. *Br Med J* 1978; **2**: 531-3.
 - 3 Pollard RB, Smith JL, Neal A, Gregory PB, Merigan TC, Robinson WS. The effect of vidarabine on chronic hepatitis B virus infection. *JAMA* 1978; **239**: 1648-50.
 - 4 Merigan TC, Robinson WS. Antiviral therapy in HBV infection. In: Vyas GN, Cohen SN, Schmid R eds. *Viral hepatitis*. Philadelphia. Franklin Institute Press, 1978.
 - 5 Thomas HC, Bassendine MF, Weller IVD. Treatment of chronic hepatitis B virus infection. In: Collier LH, Oxford J. eds. *Developments in antiviral therapy*. London, Academic Press, 1980; 88-103.
 - 6 Scullard GH, Pollard RB, Smith JL *et al*. Antiviral treatment of chronic hepatitis B virus infection. Changes in viral markers with interferon combined with adenine arabinoside. *J Infect Dis* 1981; **143**: 772-83.
 - 7 Scullard GH, Andres LL, Greenberg HB *et al*. Antiviral treatment of chronic hepatitis B virus infection: improvement in liver disease with interferon and adenine arabinoside. *Hepatology* 1981; **1**: 228-32.
 - 8 Realdi G, Alberti A, Rugg M. *et al*. Seroconversion from hepatitis B 'e' antigen to anti-HBe in chronic hepatitis B virus infection. *Gastroenterology* 1980; **79**: 195-9.
 - 9 Viola LA, Barrison IG, Coleman JC, Paradinas FJ, Fluker JL, Murray-Lyon IM. Natural history of liver disease in chronic hepatitis B surface antigen carriers: a survey of 100 patients from Great Britain. *Lancet* 1981; **2**: 1156-9.
 - 10 Hoofnagle J, Dusheiko GM, Seef LB *et al*. Seroconversion from hepatitis B 'e' antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981; **94**: 744-8.
 - 11 Weller IVD, Bassendine MF, Murray A, Craxi A, Thomas HC, Sherlock S. The effects of prednisolone/azathioprine in chronic hepatitis B viral infection. *Gut* 1982; **23**: 650-5.
 - 12 Scullard GH, Smith CI, Merigan TC, Robinson WS, Gregory PB. Effects of immunosuppressive therapy on viral markers in chronic active hepatitis B. *Gastroenterology* 1981; **81**: 987-91.
 - 13 Bassendine MF, Chadwick RG, Salmeron J, Shipton U, Thomas HC, Sherlock S. Adenine arabinoside therapy in HBsAg-positive chronic liver disease: a controlled study. *Gastroenterology* 1981; **80**: 1016-21.
 - 14 Weller EVD, Bassendine MF, Craxi A *et al*. Successful treatment of HBs and HBeAg positive chronic liver disease: prolonged inhibition of viral replication by highly soluble adenine arabinoside 5'-monophosphate (ARA-AMP). *Gut* 1982; **23**: 717-23.
 - 15 Peto R, Pike MC, Armitage P *et al*. Design and analysis of randomised clinical trials requiring prolonged observation of each patient. *Br J Cancer* 1976; **34**: 385-612.
 - 16 Goodall AH, Meek FL, Waters JA, Miescher GC, Janossy G, Thomas HC. A rapid one-step radiometric assay for hepatitis B surface antigen utilising monoclonal antibodies. *J Immunol Methods* 1982; **52**: 167-74.
 - 17 Marion PL, Oshiro LS, Regnery DC, Scullard GH, Robinson WS. A virus in Beechey ground squirrels that is related to hepatitis B virus of humans. *Proc Natl Acad Sci USA* 1980; **77**: 2941-5.
 - 18 Weller IVD, Fowler MJF, Monjardino J, Thomas HC. The detection of HBV-DNA in serum by molecular hybridisation - a more sensitive method for the detection of complete HBV particles. *J Med Virol* 1982; **9**: 273-80.
 - 19 Berninger M, Hammer M, Hayer B, Gerin JL. An assay for the detection of the DNA genome of hepatitis B virus in serum. *J Med Virol* 1982; **9**: 57-68.
 - 20 Rizzetto M, Shih JWK, Gerin JL. The hepatitis B virus associated delta antigen: Isolation from liver, development of solid-phase radioimmunoassays for delta antigen and anti-delta and partial characterisation of delta antigen. *J Immunol* 1980; **125**: 318-24.
 - 21 Hoofnagle JH, Minuk GY, Dusheiko GM *et al*. Adenine arabinoside 5'-monophosphate treatment of chronic type B hepatitis. *Hepatology* 1982; **2**: 784-8.
 - 22 Craxi A, Weller IVD, Bassendine MF, *et al*. Relationship between HBV specific DNA polymerase and HBe antigen/antibody system in chronic HBV infection: factors determining selection of patients and outcome of antiviral therapy. *Gut* 1983; **24**: 143-7.
 - 23 Rizzetto M, Canese MG, Gerin JL, London WT, Sly LD, Purcell RH. Transmission of the hepatitis B virus associated delta antigen to chimpanzees. *J Infect Dis* 1980; **141**: 590-602.
 - 24 Frommel D, Allain JP, Courouce AM, *et al*. Long lasting abatement of HBsAg synthesis induced by acute delta infection. *Lancet* 1983; **1**: 656-7.
 - 25 Viola LA, Barrison IG, Coleman JC, Murray-Lyon IM. The clinical course of acute type A hepatitis in chronic HBsAg carriers - a report of 3 cases. *Postgrad Med J* 1982; **58**: 80-1.
 - 26 Rizzetto M, Verme G, Recchia S, *et al*. Chronic hepatitis in carriers of hepatitis B surface antigen, with intrahepatic expression of the delta antigen. An active and progressive disease unresponsive to immunosuppressive treatment. *Ann Intern Med* 1983; **98**: 437-41.
 - 27 Weller IVD, Karayiannis P, Lok ASF. The significance of delta agent infection in chronic hepatitis B virus infection: a study in British carriers. *Gut* 1983; **24**: 1061-3.
 - 28 Thomas HC, Novick DM, Lok ASF, Karayiannis P, Fowler MJF, Monjardino J. Diminished responsiveness of homosexual men to antiviral therapy for HBsAg-positive chronic liver disease. *J Hepatol* 1985; **1**: 29-35.