

Effects of prednisolone/azathioprine in chronic hepatitis B viral infection

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SUMMARY Changes in markers of hepatitis B viral replication and standard liver function tests were studied in 30 patients with HBsAg positive chronic liver disease starting or stopping prednisolone/azathioprine therapy, and compared with those occurring in 15 patients who did not receive therapy. On stopping prednisolone/azathioprine, 10 out of 11 HBeAg positive patients and one out of three patients negative for HBeAg and anti-HBe, lost HBV-DNA polymerase activity ($p < 0.01$), five lost HBeAg, three developed anti-HBe and HBsAg concentration decreased ($p < 0.01$). Only one out of seven untreated HBeAg positive patients lost HBeAg and there were no significant changes in DNA polymerase activity. In the anti-HBe positive patients, 14 starting therapy and eight untreated, there were no significant changes in the markers of viral replication – although two patients developed DNA polymerase activity on high maintenance doses of prednisolone – but a significant decrease ($p < 0.05$) in aspartate transaminase in the treated group. It is concluded that the cessation of prednisolone/azathioprine therapy in HBeAg positive patients will result in a reduction in viral replication. In anti-HBe positive patients such therapy may be beneficial.

Prednisolone with or without azathioprine is widely used in treating chronic active hepatitis and prospective controlled trials have shown benefit in HBsAg negative patients, particularly those with the 'autoimmune' type of disease.¹⁻³ Further analysis of one of these trials⁴ suggested that HBsAg positive patients failed to respond to conventional doses of prednisolone. More recent evidence indicates that such therapy may potentiate viral replication^{5,6} and have a deleterious effect.⁷

There are two phases of chronic HBV infection. In the early years the virus can be detected readily in the serum by electron microscopy⁸ and by measuring the HBV-specific DNA polymerase activity (DNAP).⁹ These patients are HBeAg positive. After a varying period of time the virus

disappears from the blood, HBsAg concentrations fall, and anti-HBe develops.¹⁰

The aim of this study was to determine whether starting or stopping prednisolone/azathioprine in patients with chronic HBeAg or anti-HBe positive HBV induced liver disease, altered the markers of viral replication (HBsAg, HBeAg/anti-HBe and DNA polymerase activity) and biochemical tests of liver function, and to identify those who might benefit from this therapy.

Methods

PATIENTS

Forty-five patients (26 Southern European, eight British, eight Middle Eastern, one Asian, and one South American) presenting between 1976 and 1981 with HBsAg positive chronic liver disease were studied. The diagnosis was established histologically in all patients except four in whom abnormal clotting prevented liver biopsy (one chronic persistent hepatitis, 10 chronic active hepatitis, 28 active cirrhosis, and two inactive cirrhosis). Thirty patients started or stopped therapy (21 prednisolone, three azathioprine, six prednisolone and

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azathioprine) and 15 received no treatment. Paired serum samples taken from patients on the day or day before and then a variable time after beginning or end of therapy and paired samples a similar interval apart taken from untreated patients were analysed. Sera was stored at -20°C . HBsAg was quantified by rocket immunoelectrophoresis¹¹ (data expressed as a percentage of a laboratory standard) and, in patients with low concentrations, by passive haemagglutination (Hepatest). HBeAg and anti-HBe were detected by radiimmunoassay (Abbott Laboratories). HBV-DNA polymerase activity (DNAP) was measured by the method of Robinson,¹² which was modified by initially filtering serum samples through $0.22\ \mu\text{m}$ Millex GS filters (Millipore)¹³ and layering $200\ \mu\text{l}$ serum over $600\ \mu\text{l}$ 30% (W/V) sucrose containing TNEM-BSA. Methyl thymidine 5'-triphosphate (Radiochemicals, Amersham) was used as the tritiated base. Paired serum samples were analysed within the same assay. The upper limit of the normal range was 850 dpm/ $200\ \mu\text{l}$ (2 SD above the mean of 50 HBsAg negative controls). Serum aspartate transaminase, bilirubin, alkaline phosphatase and albumin were estimated in the routine laboratory. The differences between paired samples for each variable were analysed statistically when the number of pairs was

greater than five, using the two-tailed Wilcoxon matched pairs signed rank test. Variables in the text are expressed as median with range.

Results

HBEAG POSITIVE PATIENTS

Serology (Fig. 1)

One out of seven HBeAg positive patients (four British, two Southern European, and one Asian) untreated for 10 months (three to 24 months), lost HBeAg. The group showed no significant change in DNAP and HBsAg concentration.

Therapy was stopped in 11 HBeAg positive patients and one patient negative for HBeAg and anti-HBe (two British, eight Southern European, and one South American). Five had received prednisolone and azathioprine, four prednisolone, and three azathioprine for 16.5 months (two to 48 months). After 8.5 months (1.2-19 months), HBeAg became negative in six, three developing anti-HBe. DNAP became negative in 10 ($p < 0.01$) and HBsAg concentration decreased significantly ($p < 0.01$).

Therapy was started in two HBeAg positive patients and two negative for HBeAg and anti-HBe (one Southern European and three Middle

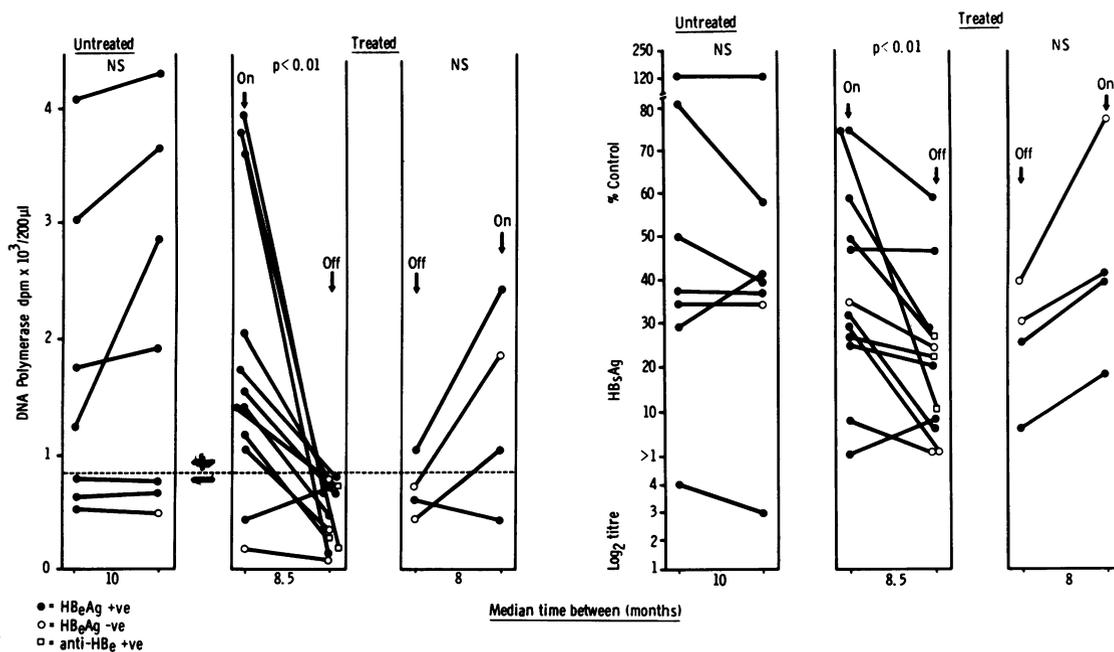


Fig. 1 HBeAg positive patients: changes in HBeAg, DNAP, and HBsAg concentrations.

Eastern). One patient who received 60 mg prednisolone, reducing by 15 mg on alternate days for only seven days became DNAP positive. The other three patients were treated with prednisolone 10 mg daily for seven to 13 months and in one HBeAg and in two DNAP became positive. HBsAg concentrations increased in all four patients but numbers were too small for statistical analysis.

Liver function tests

Biochemical tests of liver function in the HBeAg positive patients and those who were HBeAg and anti-HBe negative did not change significantly in the untreated or treated groups (Table). On stopping therapy, however, a transient rise in aspartate transaminase was seen in the one patient followed closely (Fig. 2).

ANTI-HBE POSITIVE PATIENTS

Serology (Fig. 3)

Eight anti-HBe positive, DNAP negative patients (four Southern European and four Middle Eastern) were observed without treatment for 10.5 months (three to 24 months). All remained anti-HBe positive and DNAP negative and HBsAg concentration did not change significantly.

Therapy was started in 14 anti-HBe positive, DNAP negative patients (two British, 11 Southern European, and one Middle Eastern). Thirteen received prednisolone and one prednisolone and azathioprine. All remained anti-HBe positive and all except two were DNAP negative. The two patients who became DNAP positive received prednisolone 20 and 30 mg daily compared with a daily maintenance dose of 10–15 mg in the other patients. Overall, the changes in DNAP and HBsAg concentration were not significant, although HBsAg concentration increased in both patients in whom DNAP became positive.

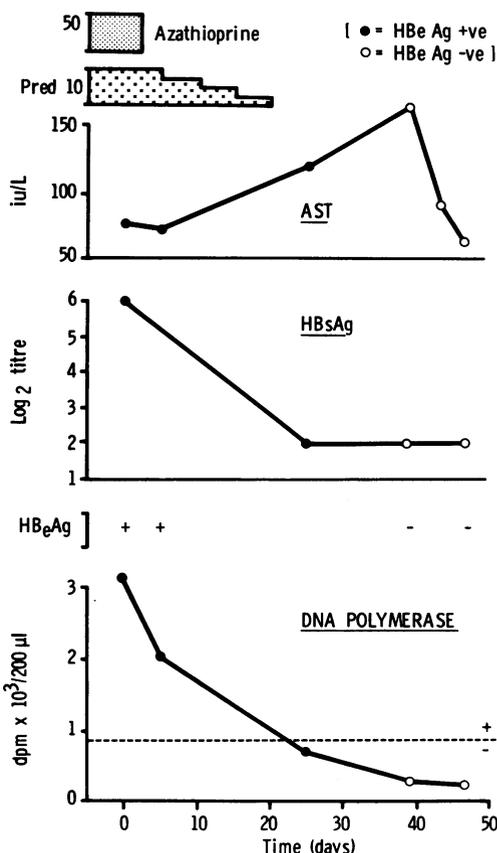


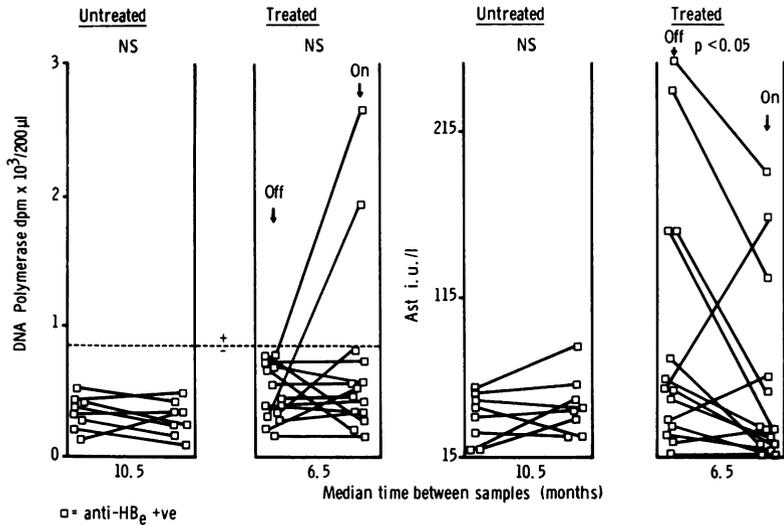
Fig. 2 Sequential changes in HBV markers in one patient.

Table Liver function tests on paired samples (values expressed as median with range)

	Treatment	Sample no.	AST (IU/l)	Bilirubin (mmol/l)	Alk phos (KA units)	Albumin (g/l)	
HBeAg +ve (and HBeAg -ve anti-HBe -ve) (no.)	None	1	32 (22-69)	14 (7-30)	8 (4-12)	46 (42-48)	
		2	41 (17-59)	12 (4-41)	8 (5-25)	44 (30-48)	
	On	1	33 (20-88)	12 (8-41)	8 (2-23)	39.5 (28-50)	
		2	35 (13-78)	14 (6-56)	9.5 (3-17)	42 (29-50)	
	4	Off	1	49 (36-89)	23.5 (13-42)	16.5 (13-22)	38.5 (36-43)
		On	2	35 (27-36)	27 (18-49)	17.5 (7-21)	38.5 (35-44)
Anti-HBe +ve (no.)	None	1	43.5 (20-59)	19.5 (5-65)	9.5 (6-19)	44 (28-46)	
		2	45.5 (30-84)	20 (8-56)	9.5 (5-18)	41.5 (26-48)	
	14	Off	1	55.5* (16-260)	23.5 (9-416)	14 (6-80)	39 (27-45)
		On	2	32* (12-190)	35 (8-238)	11.5 (5-21)	40.5 (28-46)

* $P < 0.05$, other differences not significant.

Fig. 3 Anti-HBe positive patients: changes in DNAP and aspartate transaminase.



Liver function tests (Fig. 3)

Biochemical tests of liver function in the eight untreated anti-HBe positive patients did not change significantly. There was a significant decrease in aspartate transaminase in the 14 anti-HBe positive patients starting therapy ($p < 0.05$), but no significant changes in other tests (Table). In the two patients who developed DNAP, aspartate transaminase decreased. It should be noted that the treated patients had higher initial enzyme levels compared with untreated patients, suggesting greater inflammatory activity.

Discussion

Stopping prednisolone/azathioprine in HBeAg positive patients led in 10 out of 11 cases to disappearance of circulating HBV particles (measured by determination of HBV-DNAP), loss of HBeAg in six, seroconversion to anti-HBe in three, and a decrease in HBsAg concentration. In contrast only one out of seven untreated patients became HBeAg negative and there were no significant changes in DNAP and HBsAg concentration over a similar period of time. These data confirm earlier uncontrolled reports^{5,14} that withdrawal of prednisolone/azathioprine leads to a decrease in the level of viral replication. In our study, starting prednisolone in HBeAg positive patients and in those without HBeAg and anti-HBe caused an increase in the markers of viral replication. These changes strongly suggest that drugs with immunosuppressant properties

potentiate viral replication. There are a number of possible explanations for the decrease in the level of viral replication seen in HBeAg positive patients on withdrawal of prednisolone/azathioprine. Spontaneous annual seroconversion rates from HBeAg to anti-HBe can be calculated from a few published studies and vary from less than 5% in untreated British patients¹⁵ to 15% in untreated Italians.¹⁰ A high annual rate of 25% was seen in an American study¹⁶ but seven of the 25 patients studied received steroids and, in two (8%), the seroconversion was related to steroid withdrawal. Our patients were of mixed origin and, although Southern Europeans predominated, it is unlikely that the loss of DNAP in 10 and HBeAg in six out of 11 patients in 8.5 months (1.2–19 months) could be explained by spontaneous seroconversion. It is more likely that immunosuppression delayed spontaneous seroconversion with the loss of DNAP and HBeAg and development of anti-HBe occurring when therapy ended. Alternatively, the withdrawal of an immunosuppressive effect may have actively precipitated seroconversion in patients who would not have undergone such changes spontaneously.

On stopping prednisolone/azathioprine loss of DNAP with continuing HBe antigenaemia was observed in some patients. This state is also seen in HBeAg positive patients who have received antiviral therapy and usually precedes loss of HBeAg and development of anti-HBe.¹⁶ In one HBeAg positive patient in whom serial observations were available, stopping therapy was followed by a transient rise in aspartate transaminase, coincident

with loss of DNAP and HBeAg and a decrease in HBsAg concentration. This sequence has also been described in patients who lose DNAP and HBeAg with antiviral therapy and intradermal BCG¹⁸ and is ultimately followed by a fall in transaminase to below pretreatment levels. These events may represent development of a state of enhanced immunity to the virus or virally determined antigens, with lysis of hepatocytes, in this study after the withdrawal of an immunosuppressive effect.

There are important practical implications of our observations in HBeAg positive patients. There is a strong association between HBe antigenaemia, the presence of Dane particles, DNAP, high concentrations of HBsAg, and a high level of infectivity.¹⁹⁻²¹ Thus drugs with immunosuppressant properties used for long periods may delay the spontaneous seroconversion from HBeAg to anti-HBe, prolonging the state of high infectivity and in this respect are undesirable. If the withdrawal of an immunosuppressant effect, however, actively precipitates seroconversion in those who would not have undergone such changes spontaneously, then it is possible that a short course of prednisolone followed by rapid withdrawal may precipitate seroconversion in some HBeAg positive patients. The loss of HBeAg, DNAP and seroconversion to anti-HBe is used as an index of successful treatment with antiviral drugs. As can be seen from our study, this response can be provoked by withdrawal of prednisolone/azathioprine. Starting prednisolone/azathioprine in anti-HBe positive patients produced a significant fall in aspartate transaminase levels with no significant changes in the markers of viral replication. It should be noted that the two patients who received the highest maintenance doses of prednisolone did develop DNAP in the continuing presence of anti-HBe. This suggests that more profound immunosuppression will result in reactivation and that antibody to HBeAg may not alone be responsible for neutralisation of the virus.²² Nevertheless, conventional doses of prednisolone may produce a reduction in inflammatory activity without increasing viral replication in anti-HBe positive patients.

It has been suggested that the presence of HBeAg is associated with a failure to respond to corticosteroids.²³ In a single-blind, randomised controlled trial,⁷ although treatment with prednisolone produced a decrease in serum bilirubin and globulin, there was no change in transaminase levels and the complication and death rates were increased. In that study there was no attempt to differentiate between HBeAg and anti-HBe positive patients. Our study shows that in HBeAg positive patients prednisolone/azathioprine therapy potentiates viral replication

and long-term therapy may delay the spontaneous HBeAg to anti-HBe seroconversion and prolong the high infectivity state. Stopping such therapy in the majority of our patients led to a decrease in the level of viral replication. In anti-HBe positive patients starting such therapy with conventional maintenance doses led, however, to a decrease in aspartate transaminase, suggesting decreased inflammatory activity, without a significant alteration in the level of viral replication.

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