Intradermal hepatitis B vaccine in thalassaemia and sickle cell disease

Q MOK,* G UNDERHILL,† B WONKE,* M ALDOURI,‡ M KELSEY,* AND D JEFFERIES†

*Whittington, †St Mary's, and ‡the Royal Free Hospitals, London

SUMMARY Thirty two patients with β thalassaemia and sickle cell disease who were having regular blood transfusions were selected to test the efficacy and immunogenicity of low dose (2 μ g or 0.1 ml) intradermal hepatitis B vaccine compared with the standard (20 μ g or 1 ml) intramuscular dose. There was no significant difference in the rates of seroconversion, seroconversion had occurred in all patients by seven months. There were no significant differences in antibody titres between the intramuscular and intradermal groups at 1, 2, and 6 months. Although the titres were significantly higher in the intramuscular group at seven months and at 12–18 months, the antibody titre in the intradermal group did not fall below 10 IU/l. The results of this study suggest that low dose intradermal hepatitis B vaccination is an effective and economical way of stimulating an immune response in patients with β thalassaemia and sickle cell disease.

Blood transfusion is still a common mode of transmission of viral hepatitis, and so patients with β thalassaemia and homozygous sickle cell disease who require regular blood transfusions constitute a high risk group. This risk is accentuated in affected children as their need for transfusions begins early in their first year of life and persists into adulthood. Although a group in the United States reported a low incidence of hepatitis B after blood transfusions in patients with thalassaemia,¹ a study in London and Athens² has indicated that it remains a serious problem. Vaccination against hepatitis B is a safe and effective way of preventing hepatitis B virus infection.^{3–9} The recommended method of immunisation is by a course of three intramuscular injections into the deltoid muscle of 0.5–1.0 ml vaccine at 0, one, and six months. The high cost of the vaccine (£27.15 to £36.21 in 1988), however, has restricted its use, especially in developing countries where hepatitis is endemic and thalassaemia and sickle cell disease are common.

Low dose intradermal administration has been successfully assessed in healthy adult volunteers and health workers,^{10–15} but we know of few reports of this method of vaccination against hepatitis B being used in children and young adults. No study on low dose vaccination of patients with β thalassaemia or sickle cell disease has been reported to our knowledge. The immunological response in these patients

may be different, as shown by their increased susceptibility to infections especially after splenectomy (either from autoinfarction in patients with sickle cell disease or surgical splenectomy in patients with thalassaemia).^{16–20}

The aim of this study was to investigate whether low dose intradermal hepatitis B vaccination was as effective as vaccination by the standard intramuscular route in provoking an immune response against the hepatitis B virus in patients with thalassaemia and sickle cell disease.

Patients and methods

PATIENTS AND STUDY DESIGN

Thirty two patients who required transfusions of packed cells every two to four weeks were selected for this study. They were all negative for hepatitis B surface antigen (HBsAg), antibody to surface antigen (anti-HBs), and antibody to core antigen (anti-HBc). Twenty eight patients had β thalassaemia (26 β thalassaemia major, and two sickle thalassaemia) and four had homozygous sickle cell disease. There were 16 boys and 16 girls of Greek, Turkish, Indian, or West Indian origin, but they were all born in Britain and received all their transfusions in this country. To prevent and treat transfusional iron overload all the patients received regular subcutaneous desferrioxamine infusions for a period of one

to nine years. The iron concentration was monitored at three monthly intervals by measuring the serum ferritin concentration. Liver function tests were also assayed at six monthly intervals for the period of the study.

The study was prospective and the 32 patients were paired for diagnosis, sex (except for pairs 14 and 16), age, serum ferritin concentration, amount of blood transfused, and whether they had had a splenectomy (except for pairs 3 and 11) (table 1).

One member of each pair was given the standard 0.5–1.0 ml (10–20 µg) of plasma derived hepatitis B vaccine (H-B-Vax) intramuscularly into the deltoid muscle. The other member of the pair was given 0.1 ml (2 µg) of the vaccine intradermally into the flexor surface of the forearm. A visible cutaneous bleb indicated a successful intradermal injection. All the vaccinations were performed by the same nursing sister or one of the authors (QM or BW) using a 1 ml tuberculin syringe and a 27 gauge needle. The regimen comprised three doses at 0, one, and six months. Blood was taken for serological assay at 0, one, two, six, seven, and 12–18 months after the initial vaccination. The Wilcoxon signed rank test was used to calculate the significance of differences in the antibody titres between the two groups. The study was approved by the hospital medical ethical committee, and signed consent was obtained.

LABORATORY TESTS

All serum samples for serological examination were stored at –20°C. Samples taken at time 0 were tested for HBsAg, anti-HBc and anti-HBs to give base line values using commercially available enzyme linked immunosorbent assays (Auszyme, Corzyme, and Ausab, respectively). Samples taken at one, two, six, seven, and 12–18 months were tested for anti-HBs, and the amount of surface antibody was determined by diluting them in negative control serum (Abbott Laboratories) until a titre was obtained using the Abbott semiquantitative assay. Results are expressed in IU/l. Seroconversion was taken to have occurred when anti-HBs reached a titre of more than 10 IU/l. Samples taken at the end

of the study were also tested for HBsAg and anti-HBc to see if infection with hepatitis B virus had occurred during the study.

Serum ferritin concentration was measured by immunoradiometric assay²¹; the normal ranges for our laboratory are 39–340 µg/l for boys and 14–148 µg/l for girls.

Liver function tests were measured by the standard method of analysis. The normal value for aspartate transaminase is 40 IU/l and for γ-glutamyl transpeptidase 8–50 IU/l.

Six of the patients had persistently raised aspartate transaminase or γ-glutamyl transpeptidase concentrations at the start of the study; four were in the intradermal and two in the intramuscular group. They were seronegative for HBsAg, anti-HBs, and anti-HBc. Liver biopsy specimens were available for these patients,²² but no hepatitis B surface or core antigen were detected, and the abnormal liver function was thought to be due to non-A non-B hepatitis or to iron overload. The remaining patients had normal liver function tests throughout the study.

Results

The only side effects after intradermal injection were transient induration, and in negroid patients hyperpigmentation at the site of injection that faded within a few months.

Seroconversion had occurred in every patient by seven months. The seroconversion rates after the first, second, and third doses are shown in fig 1. Seroconversion had occurred in all the patients in the intramuscular group by six months, before the third dose was given. Titres of anti-HBs are shown in fig 2. At one, two, and six months there were no

Table 1 Details of patients at start of study

	Intramuscular group (n=16) Median (range)	Intradermal group (n=16) Median (range)
Age (years)	16.9 (1.4–25.4)	16.0 (1.2–23.8)
No of units of blood transfused	295 (15–420)	275 (17–573)
Serum ferritin concentration (µg/l)	800 (380–2160)	850 (180–3100)

Fig 1 Seroconversion rates in intramuscular and intradermal groups after vaccination with H-B-Vax.

significant differences between the two groups, but by seven months the titres were higher in the intramuscular group ($p < 0.01$). At 12–18 months after the initial vaccination the antibody titres had fallen in both groups, but were significantly lower in the intradermal group ($p < 0.01$). No patient had a titre below 10 IU/l, however, that would have been considered seronegative.

Table 2 shows some of the details about the patients and the antibody titres achieved. There were seven patients with high serum ferritin concentrations (greater than 1000 $\mu\text{g/l}$); these were the less compliant patients who used desferrioxamine less regularly than the other patients in the study. Of these patients, four also had abnormal liver function tests.

Within the low dose intradermal group of patients, four had abnormal liver function tests. These patients achieved significantly lower antibody titres (indicated by arrows in fig 2) than the patients with normal liver function tests within the group. The Wilcoxon

rank sum test showed significant differences at two months ($p < 0.05$), six months ($p < 0.01$), seven months ($p < 0.05$), and 12–18 months ($p < 0.005$). There did not appear to be a correlation between splenectomy and degree of antibody response.

Of the 14 patients who had been followed up for two years at the time of writing, 12 continue to show titres ranging from 51 to 21 540 IU/l. In only one patient (from the intramuscular group) was the titre undetectable, and one from the intradermal group had a titre of 12 IU/l. Both these patients had poor antibody responses during the study, their highest titres being 191 and 116 IU/l, respectively.

All patients remained seronegative for anti-HBc and HBsAg at the end of the study, confirming that none had contracted natural hepatitis B infection.

Discussion

Hepatitis is a common complication of blood transfusions,^{23 24} although most of the cases are non-A, non-B hepatitis. World wide hepatitis B remains the most important cause, as it may lead to chronic hepatitis and cirrhosis. There is also a strong association between long term carriage of hepatitis B virus and hepatocellular carcinoma.^{25–30}

The incidence of hepatitis B virus infection is increasing in Britain³¹ where 2–4% of the normal population have serological evidence of exposure to hepatitis B virus, and at least 0.1% remain carriers.³² Routine screening for hepatitis B surface antigen in donated blood was introduced in Britain in 1971. Nevertheless, 1–3% of donors have antibody to the core antigen as their sole marker of hepatitis B virus infection³³ and may therefore transmit the virus by blood transfusion.^{34 35}

The results of this study have shown that a low dose (2 μg) intradermal hepatitis B vaccination can stimulate a humoral immune response comparable with the standard (20 μg) intramuscular dose in patients with β thalassaemia and homozygous sickle cell disease whose immunological responses are thought to be compromised.^{16–20} Seroconversion had occurred in all patients by the end of the course of three injections, which is comparable with the results of other studies of healthy volunteers with intramuscular or intradermal vaccine. In the original trials that tested the efficacy of intramuscular hepatitis vaccination, seroconversion occurred in 85 to 100%.^{4–8} A few trials of the intradermal vaccination were conducted in healthy adults with similar seroconversion rates (83 to 100%).^{10–15} Miller *et al* reported that seroconversion occurred in 83% of 14 healthy adult volunteers,¹⁰ and Zoulek *et al* reported that seroconversion occurred in all of a smaller group of nine healthy adults.^{11 12}

Fig 2 Titres of antibody to hepatitis B surface antigen as a function of time after initial vaccination. Arrows indicate patients with abnormal liver function. Only titres above 10 IU/l are plotted as this is the minimum activity indicating positive seroconversion.

Table 2 Details of patients and titres of antibody to hepatitis B surface antigen

Details of patients						Titres of antibody to hepatitis B surface antigen (IU/l) at time intervals (months) initial vaccination				
Pair No	Route	Diagnosis	Ferritin (µg/l)	Liver function tests	Splenectomy	1	2	6	7	12-18
1	Intramuscular	Thalassaemia	950	Normal	Yes	0	43	34	6740	2470
	Intradermal	Thalassaemia	900	Abnormal	Yes	0	67	14	116	17
2	Intramuscular	Thalassaemia	660	Normal	No	56	92	840	19 500	3600
	Intradermal	Thalassaemia	580	Normal	No	0	59	115	92	2400
3	Intramuscular	Thalassaemia	640	Normal	No	38	37	412	688 000	37 800
	Intradermal	Thalassaemia	640	Normal	Yes	0	33	10	1870	235
4	Intramuscular	Thalassaemia	640	Normal	Yes	0	0	42	292	77
	Intradermal	Thalassaemia	610	Normal	Yes	7	1796	137	4020	1600
5	Intramuscular	Thalassaemia	740	Normal	Yes	0	5	91	2140	345
	Intradermal	Thalassaemia	1800	Abnormal	Yes	0	11	70	1160	50
6	Intramuscular	Thalassaemia	840	Normal	No	38	38	1410	1 619 200	37 500
	Intradermal	Thalassaemia	800	Normal	No	32	630	1200	460	481
7	Intramuscular	Thalassaemia	2160	Abnormal	Yes	0	80	240	10 800	5350
	Intradermal	Thalassaemia	1040	Normal	yes	0	12	208	900	73
8	Intramuscular	Thalassaemia	920	Normal	yes	42	65	1540	11 280	9300
	Intradermal	Thalassaemia	1400	Normal	Yes	21	106	205	2650	240
9	Intramuscular	Thalassaemia	600	Normal	Yes	0	60	298	440 800	42 200
	Intradermal	Thalassaemia	650	Normal	Yes	5	105	24 320	520	291
10	Intramuscular	Thalassaemia	900	Normal	Yes	0	0	191	174	46
	Intradermal	Thalassaemia	680	Normal	Yes	8	213	206	7740	500
11	Intramuscular	Thalassaemia	380	Normal	No	3	558	555	337 200	11 100
	Intradermal	Thalassaemia	180	Normal	Yes	5	104	337	4820	940
12	Intramuscular	Thalassaemia	760	Abnormal	Yes	0	0	20	2050	160
	Intradermal	Thalassaemia	2100	Normal	Yes	11	608	920	550	145
13	Intramuscular	Thalassaemia	920	Normal	Yes	0	37	134	8040	33 600
	Intradermal	Thalassaemia	3100	Abnormal	Yes	0	0	5	155	68
14	Intramuscular	Sickle thalassaemia	520	Normal	Yes	3	425	267	151	59 000
	Intradermal	Sickle thalassaemia	1000	Normal	Yes	0	88	180	582	74
15	Intramuscular	Sickle cell disease	880	Normal	Autosplenectomy	52	925	1390	0	2950
	Intradermal	Sickle cell disease	600	Normal	Autosplenectomy	0	0	0	37 680	300
16	Intramuscular	Sickle cell disease	1000	Normal	Autosplenectomy	0	20	17	1314	184
	Intradermal	Sickle cell disease	1420	Abnormal	Autosplenectomy	28	0	52	0	156

Redfield *et al* carried out a trial in which they compared the vaccine given intramuscularly and intradermally to a group of 50 healthy army volunteers; seroconversion occurred in 96% in the intradermal group compared with 100% in the intramuscular group.^{13 14} A preliminary report on the use of intradermal vaccine in 604 hospital personnel in Indonesia by Prasetya *et al* showed that seroconversion occurred in 90%.¹⁵ In the present study good seroconversion rates with both the intramuscular and intradermal methods were achieved in a group of patients whose B cell function and immune state were in question. It is likely that this higher rate of seroconversion is the result of the lower age of our groups, as children have better antigenic responses to immunisation than adults.

The anti-HBs titres obtained were not significantly different at one, two, and six months, but were significantly lower at seven and 12-18 months in the intradermal group. Several studies have shown that the risk of hepatitis B virus infection increases as antibody titres decline, and it is closely associated with the persistence of minimum titres of antibody.³⁶⁻³⁹ This implies that the protective effect after intradermal vaccination may not last as long as that after intramuscular vaccination. At 12-18 months none of the patients had anti-HBs titres below 10 IU/l, which is the currently accepted minimal protective titre. It has therefore been suggested that patients who have had the intradermal vaccine should be revaccinated earlier than the currently recommended five year interval. We are continuing

to monitor antibody titres among these patients. Those with abnormal liver function tests may need to be monitored more closely if they are given the low dose intradermal vaccine, as our data show that they had a significantly lower antibody response.

The objection that may be raised against using intradermal hepatitis vaccination on a large scale is the skill that is required to avoid administering the injection subcutaneously; the results of a recent study among haemophiliacs, however, showed effective rates of seroconversion even with vaccine administered subcutaneously (Miller EJ, Lee CA, Karayiannis P, Holmes S, Thomas HC, Kernoff PBA. Evaluation of subcutaneous and intradermal hepatitis B vaccination in patients with haemophilia. Presented at the British Society for Haematology annual meeting, 1987).

The more widespread use of hepatitis B vaccine is restricted by the current high cost of a course of intramuscular injections. The 10 fold reduction in cost and the proved immunogenicity of the intradermal route of administration of vaccine in patients with β thalassaemia and homozygous sickle cell disease may encourage its more widespread use in the developing world.

This was only a small pilot study of 32 patients, and a much more extensive study is required to confirm the efficacy in patients with β thalassaemia and sickle cell disease.

We thank Mrs S Ahmet for a grant towards this project. We are also grateful to Miss M Laulich (department of haematology, Royal Free Hospital) for help with measuring the ferritin concentrations, Miss N Shah (department of virology, St Mary's Hospital) for technical help with the immunoassays, and Sister J Kavanagh for carrying out the vaccinations.

References

- Pearson HA, Wood C, Andiman W, Bore J, Rink L. Low risk of hepatitis B from blood transfusions in thalassaemic patients in Connecticut. *J Pediatr* 1986;**108**:252-3.
- Mieli-Vergani G, Vergani D, White Y, et al. Hepatitis B virus infection in thalassaemia major treated in London and Athens. *Br Med J* 1984;**228**:1804.
- Blumberg BS, Millman I. *Vaccine against viral hepatitis and process*. Patent No 36 36 191. Washington DC: United States Patent Office, 1972.
- Szmunn W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high risk population in the United States. *N Engl J Med* 1980;**303**:833-41.
- Szmunn W, Stevens CE, Harley EJ, et al. The immune response of healthy adults to a reduced dose of hepatitis B vaccine. *J Med Virol* 1981;**8**:123-9.
- Krugman S, Holley HP Jr, Davidson M, Simberkoff MS, Matsaniatis N. Immunogenic effect of inactivated hepatitis B vaccine: comparison of 20 μ g and 40 μ g doses. *J Med Virol* 1981;**8**:119-21.
- Francis DP, Hadler SC, Thompson SE, et al. The prevention of

- hepatitis B with vaccine: report of Centers for Disease Control multicentre efficacy trial among homosexual men. *Ann Intern Med* 1982;**97**:362-6.
- Papaevangelou G, Roumeliitou-Karayannis A, Vissoulis CH, et al. Reduction of the dose of hepatitis B vaccine. *J Infect* 1983;**7**(suppl):53-5.
- Hepatitis B virus vaccine safety: report of an interagency group. *MMWR* 1982;**31**:465-7.
- Miller KD, Gibbs RD, Mulligan MM, Nutman TB, Francis D. Intradermal hepatitis B virus vaccine: immunogenicity and side effects in adults. *Lancet* 1983;**ii**:1454-6.
- Zoulek G, Lorbeer B, Jilg W, Deinhardt F. Antibody responses and skin reactivity after intradermal hepatitis B virus vaccine. *Lancet* 1984;**ii**:568.
- Zoulek G, Lorbeer B, Jilg W, Deinhardt F. Evaluation of a reduced dose of hepatitis B vaccine administered intradermally. *J Med Virol* 1984;**14**:27-32.
- Redfield RR, Innis BL, Cannon HG, McNair-Scott R, Bancroft WH. Immunogenicity and reactogenicity of low-dose intradermally administered heptavax-B-vaccine in man. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. *Viral hepatitis and liver disease*. Orlando: Grune and Stratton, 1984:694-5.
- Redfield RR, Innis BL, McNair-Scott R, Cannon HG, Bancroft WH. Clinical evaluation of low dose intradermally administered hepatitis B virus vaccine. A cost reduction strategy. *JAMA* 1985;**254**:3203-6.
- Prasetya E, Linggadja W, Syambali R, Kusumo S, Dharmesetyawan DF, Rahardja U. Intradermal vaccination against hepatitis B in Immanuel Hospital personnel—a preliminary report. *Ann Acad Med Singapore* 1986;**15**:153-7.
- Constantoulakis M, Trichopoulos D, Argoustaki O, Economidou J. Serum immunoglobulin concentrations before and after splenectomy in patients with homozygous β thalassaemia. *J Clin Pathol* 1978;**31**:546-50.
- Millard RE, Banarjee DK. Changes in T and B blood lymphocytes after splenectomy. *J Clin Pathol* 1979;**32**:1045-9.
- Khan AJ, Lee CK, Wolff JA, Chang H, Khan P, Evans HE. Defects of neutrophil chemotaxis and random migration in thalassaemia major. *Pediatrics* 1977;**60**:349-51.
- Giuntoli JMT, Estevez ME, Sen L, Penalver JA. Defective function of the peripheral blood neutrophils in thalassaemia major. *Am J Pediatr Hematol Oncol* 1984;**6**:215-16.
- Di Padova F, Durig M, Harder F, Di Padova C, Zanussi C. Impaired antipneumococcal antibody production in patients without spleens. *Br Med J* 1985;**290**:14-6.
- Addison GM, Beamish MR, Hales CN, Hodgkins M, Jacobs A, Llewellyn P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *J Clin Pathol* 1972;**25**:326-9.
- Aldouri MA, Wonke B, Hoffbrand V, et al. Hepatitis pathology in regularly transfused β thalassaemia major patients on long term subcutaneous desferrioxamine therapy: the role of parentally transmitted hepatitis. *J Clin Pathol* 1987;**40**:1353-9.
- Aach RD, Lander JJ, Sherman LA, et al. Transfusion-transmitted viruses: interim analysis of hepatitis among transfused and non-transfused patients. In: Vyas GN, Cohen SN, Schmid R, eds. *Viral hepatitis*. San Francisco: Franklin Institute Press, 1978:383-96.
- Barbara JAJ, Briggs M. Post-transfusion hepatitis in north London in 1981: a review including a case of hepatitis A. In: Hopkins R, Field SE, eds. *Proceedings of hepatitis workshop*, Stirling. Edinburgh: Nuclear Enterprises, 1982:31-5.
- Szmunn W. Hepatocellular carcinoma and the hepatitis B virus: evidence for a causal association. *Prog Med Virol* 1978;**24**:40-69.
- Beasley RP, Hwang LY, Lin CC, Chien CS. Hepatocellular carcinoma and hepatitis B virus. *Lancet* 1981;**ii**:1129-33.
- Beasley RP. Hepatitis B virus as the etiological agent in hepatocellular carcinoma—epidemiological considerations. *Hepatology* 1982;**2**:21-68.

- ²⁸ Arthur MJP, Hall AJ, Wright R. Hepatitis B, hepatocellular carcinoma, and strategies for prevention. *Lancet* 1984;i:607-10.
- ²⁹ Beasley RP, Hwang LY. Epidemiology of hepatocellular carcinoma. In: Vyas GN, Dienstag JL, Hoofnagle JH, eds. *Viral hepatitis and liver disease*. Orlando: Grune and Stratton, 1984:209-24.
- ³⁰ Hall AJ, Winter PD, Wright R. Mortality of hepatitis B positive blood donors in England and Wales. *Lancet* 1985;i:91-3.
- ³¹ Polakoff S. Acute viral hepatitis B. Laboratory reports 1980-84. *Br Med J* 1986;293:37-8.
- ³² Finch R. Time for action on hepatitis B immunisation. *Br Med J* 1987;294:197-8.
- ³³ Tedder RS, Cameron CH, Wilson-Croome R, Howell DR, Colgrove A, Barbara JAJ. Contrasting patterns and frequency of antibodies to the surface, core and envelope antigens of hepatitis B virus in blood donors and in homosexual patients. *J Med Virol* 1980;6:323-32.
- ³⁴ Hoofnagle JH, Seeff LB, Bales ZB, et al. Type B hepatitis after transfusion with blood containing antibody to hepatitis B core antigen. *N Engl J Med* 1978;298:1379-83.
- ³⁵ Katchaki JN, Bronwer R, Siem TH. Anti-HBc and blood infectivity. *N Engl J Med* 1978;298:1422.
- ³⁶ Hadler SC, Francis DP, Maynard JF, et al. Long term immunogenicity and efficacy of hepatitis B vaccine in homosexual men. *N Engl J Med* 1986;315:209-14.
- ³⁷ Zachoval R, Jilg W, Lorbeer B, Schmidt M, Deinhardt F. Passive active immunisation against hepatitis B. *J Infect Dis* 1984;150:112-7.
- ³⁸ Jilg W, Schmidt M, Deinhardt F, Zachoral R. Hepatitis B vaccination: how long does protection last? *Lancet* 1984;ii:458.
- ³⁹ Zoulek G, Lorbeer B, Jilg W, Deinhardt F. Persistence of anti-HBs after intradermal inoculation of reduced doses of hepatitis B vaccine. *Lancet* 1984;ii:983-4.

Correspondence to Dr B Wonke, Department of Haematology, Whittington Hospital, Highgate Hill, London N19 5NF.

Accepted 17 October 1988