CLINICAL RESEARCH

Passive-active immunisation of neonates of HBsAg positive carrier mothers: preliminary observations

J ADRIAAN MAZEL, SOLKO W SCHALM, BERT C DE GAST, AD S M NUIJTEN, RUDOLF A HEIJTINK, MEINDERT J BOTMAN, JOHN R J BÄNFFER, LEO J GERARDS, JOHN ZWIJNENBERG, JAN METTAU, YURIY W WLADIMIROFF, WILLEM P F FETTER

Abstract

Screening of pregnant women for hepatitis B surface antigen (HBsAg) in three areas of Holland led to the identification of HBsAg carriers, 20 of whom were subsequently delivered. Within two hours after birth all infants received hepatitis B immune globulin (0·5 ml/kg body weight) and, after randomisation, hepatitis B vaccine (10 μg) was given either at 0, 1, and 2 months of age or at 3, 4, and 5 months of age, the latter concomitantly with DPTP vaccination. Eighteen infants complying with the protocol were followed up for at least six months. No side effects were observed after either passive or active immunisation. All infants developed high concentrations of anti-HBs antibodies; no interference of high dose passive immunisation with active immunisation was observed. Concentrations of anti-HBs at three months were significantly lower in infants given delayed active immunisation than in those given early active immunisation.

These data suggest that passive-active immunisation against hepatitis B virus infection is well tolerated by neonates under 3 months of age and that both early and late active immunisation in combination with passive immunisation will result in excellent anti-HBs production.

Academisch Ziekenhuis Dijkzigt, Sophia Kinderziekenhuis, Verloskundige Kliniek, and van Dam-Bethesda Ziekenhuis, Rotterdam; the Streeklaboratorium voor Pathologie en Microbiologie, Enschede; and the Academisch Ziekenhuis and Wilhelmina Kinderziekenhuis, Utrecht, the Netherlands

J ADRIAAN MAZEL, MD
SOLKO W SCHALM, MD
BERT C DE GAST, professor
AD S M NUIJTEN, MD
RUDOLF A HEIJTINK, PHD
MEINDERT J BOTMAN, MD
JOHN R J BÄNFFER, MD
LEO J GERARDS, MD
JOHN ZWIJNENBERG, MD
JAN METTAU, professor
YURIY W WLADIMIROFF, professor
WILLEM P F FETTER, MD

Correspondence to: Dr Solko W Schalm, Department of Internal Medicine II, Academisch Ziekenhuis Dijkzigt, 3015 GD Rotterdam, the Netherlands.

Introduction

Passive immunisation has been shown to be effective in preventing perinatal and postnatal hepatitis B virus infection in infants of mothers who are carriers of hepatitis Be antigen (HBeAg). Multiple injections of hepatitis B immune globulin (HBIg) gave better protection than one single dose directly after birth.[1] Combined passive-active immunisation may be equally effective and possibly superior to repeated passive immunisation. Uncertainty exists about the safety of the vaccine in neonates and about the optimal timing of active immunisation. Protection seems to be most important in the first month after birth, whereas the immune response is thought to be sufficiently developed from three months onwards. In order to test the safety and efficacy of early passive-active immunisation we administered at random to neonates of hepatitis B surface antigen (HBsAg) positive carrier mothers either HBIg at birth and hepatitis B vaccine at 0, 1, and 2 months of age or HBIg at birth and hepatitis B vaccine at 3, 4, and 5 months of age. We present here our preliminary findings.

Patients and methods

In 1982 sera of pregnant women from the predominantly rural area of Twente-Gelderse Achterhoek and from women attending several hospitals in the urban areas of Utrecht and Rotterdam in Holland were tested for HBsAg. Screening was generally done at the first visit to the doctor or midwife; women found to have the antigen were retested at 28 weeks. Women admitted to the delivery room

without prenatal screening for HBsAg were often tested at that time. Of the 6991 women tested, 68 (1·0%; ratio of foreign to Dutch women 9:1) repeatedly gave a positive result and were, for the purpose of this study, considered as HBsAg carriers.

Passive-active immunisation—Irrespective of the mother's HBe state all neonates of mothers who were carriers of HBsAg were given HBIg 0·5 ml/kg body weight (100 IU anti-HBs/ml, Central Laboratory of Netherlands Red Cross Blood Transfusion Service, Amsterdam) intramuscularly within two hours after birth by the physician or midwife in charge of delivery. The newborns were then referred to a paediatrician for administration of the hepatitis B vaccine. They were allocated at random to receive 10 μg hepatitis B vaccine (HB-Vax; Merck, Sharp and Dohme, West Point, USA) intramuscularly either within two days after birth and at 1 and 2 months of age (scheme A) or at 3, 4, and 5 months of age (scheme B) concurrently with the diphtheria, pertussis, tetanus, poliomyelitis (DPTP) vaccination. In the case of delayed active immunisation (scheme B) a second injection of HBIg (1·0 ml) was given at the age of 3 months. A booster dose of hepatitis B vaccine was given to all infants at the age of 11 months.

Follow up—Venous blood from the mother and cord blood from the infant were collected at birth. Blood samples were taken from the infant at 3, 6, 11, and 12 months of age. Serum was stored at −20°C and tested for anti-HBs and anti-HBc.

Laboratory methods—HBsAg was detected in the sera of pregnant women by radioimmunoassay (Ausria II; Abbott), enzyme immunoassay (Auszyme; Abbott), or reversed passive haemagglutination assay (Auscell; Abbott). All positive samples from HBsAg carriers detected by radioimmunoassay were also detected by reversed passive haemagglutination assay in experiments carried out to validate that assay. All sera from infants undergoing passive-active immunisation were tested in the hepatitis laboratory of the Erasmus University of Rotterdam for anti-HBs and anti-HBc; HBIg was also tested for anti-HBs and anti-HBc. Anti-HBs concentrations were expressed in Ausab ratio (RIA) units.

Statistical—Groups were compared by the unpaired Wilcoxon test; for comparisons within groups the test for paired samples was used.

Results

Twenty infants from 20 pregnancies were followed up for more than six months. Five infants were born to HBeAg positive mothers. Two infants (of anti-HBe positive mothers) did not receive HBIg or vaccine and are excluded from further analysis. The figure gives the results for the remaining 18 infants. Anti-HBs was absent in cord blood and detectable in all samples at 3 and 6 months of age. At the age of 3 months anti-HBs concentrations of children in scheme A (early active immunisation) were significantly higher than those of the children who received only HBIg at birth (scheme B; $p < 0·01$). At the age of 6 months anti-HBs concentrations had increased significantly in both groups and the difference in concentrations between the groups (figure) had diminished.

All samples were positive for anti-HBc, presumably owing to passive transfer from the mother; however, HBIg also contains anti-HBc, which may have been partly responsible for that result.

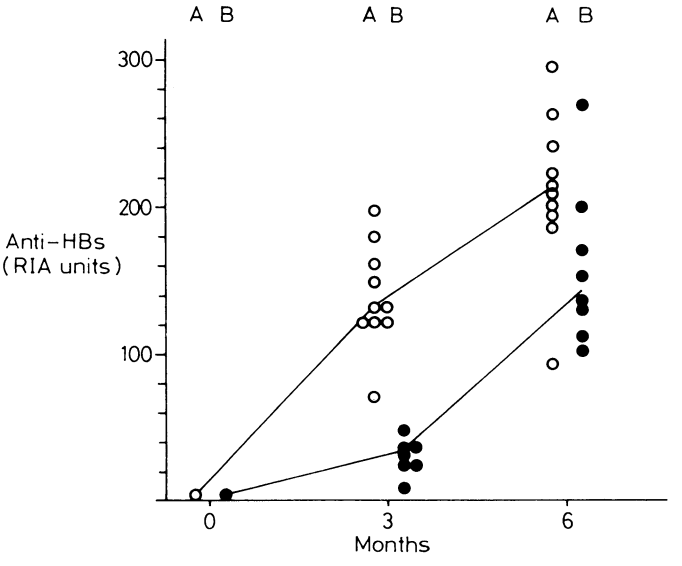
Directly after vaccination the children were observed for 30 minutes, and the mothers were asked about side effects at subsequent visits. No side effects occurred.

Discussion

Intramuscular administration of a single injection of HBIg directly after birth gives roughly 90% protection for three months[1] and is not associated with clinically significant side effects.[1] Since the protective effect of HBIg diminishes after three months, however, additional measures are required.[1] Combined passive-active immunisation has been advocated provided that the vaccine is found to be safe in neonates and that the HBIg does not interfere with active immunisation. Our preliminary observations show that no side effects occur and that the vaccine is highly efficacious in inducing anti-HBs antibodies in neonates treated with large doses of HBIg. We also compared early active immunisation with delayed active immunisation starting at 3 months of age.

Passive-active immunisation starting directly after birth, resulted in high concentrations of anti-HBs in all infants at 3 months of age. The excellent immune response after hepatitis B immunisation in neonates described by others[2] was confirmed even in the presence of passive anti-HBs. We found no evidence of an impaired immune response with regard to HBsAg, the anti-HBs concentrations at 3 months in the group given early active immunisation being comparable to those at 6 months in the group given delayed active immunisation.

Administration of 0·5 ml HBIg/kg body weight gave anti-HBs concentrations of more than 20 RIA units in all infants at 3 months of age. Trials of hepatitis B vaccination[3] suggest that concentrations above 10 RIA units definitely protect against hepatitis B virus infection, while uncertainty exists about the effectiveness of concentrations under 10 RIA units. In the sera of our infants concentrations greatly exceeded this "protective" value, although we do not know whether this would apply for passively administered antibody. Based on the serological data, active immunisation against hepatitis B given concurrently with DPTP vaccination might be an acceptable alternative to immediate vaccination in areas with limited resources for infant care, provided that a high dose of HBIg is administered directly after birth.



Anti-HBs concentrations in neonates after HBIg administration at birth and active vaccination at 0, 1, and 2 months of age (scheme A; ○) or 3, 4, and 5 months of age (scheme B; ●). Infants in scheme B received a second injection of HBIg at age 3 months.

Formal recommendations on the immunisation of infants against hepatitis B virus infection will probably be based on the results of efficacy studies. The incidence of hepatitis B virus infection is low after passive immunisation; to show the superiority of combined passive-active immunisation over passive immunisation or differences between various schemes of passive-active immunisation large numbers of infants will need to be studied. Hence definitive reports from areas of low prevalence such as west European countries may not appear for several years. Randomised investigations, in progress among newborns of HBeAg positive carrier mothers in areas of high prevalence, will probably yield results sooner.

HBsAg positive mothers who are also HBeAg positive carry a more than 90% risk of transmitting hepatitis B virus to their children. Transmission is usually followed by chronic HBs antigenaemia, often without early morbidity. Late morbidity and mortality is to be expected from cirrhosis or hepatocellular carcinoma. In view of these risks there is general agreement that infants of HBeAg positive carrier mothers need to be immunised against hepatitis B. Among HBsAg positive carrier

mothers with anti-HBe the incidence of perinatal transmission of hepatitis B virus is much lower, estimates varying between 10% and 20%.[4-6] The prevalence of HBsAg, anti-HBe positive women, however, is about five times that of HBsAg, HBeAg positive mothers.[5 6] Because of the relatively high proportion of HBsAg, anti-HBe positive mothers the number of children infected with hepatitis B virus from this source should not be neglected. It is, however, important that according to several reports infants of anti-HBe positive mothers develop chronic HBs antigenaemia only in a small minority of cases,[6 7] but the manifestations of the disease vary from usually asymptomatic to fulminant hepatitis.[7 8] In view of the (albeit infrequent) occurrence of severe disease in children of anti-HBe positive mothers we, like others,[9 10] suggest that all neonates of HBsAg positive mothers should be candidates for immunisation against hepatitis B infection. Current costs of active immunisation, however, may prevent general acceptance of this until a cheaper vaccine becomes available.

We thank Mrs H Ribbers, M Bakker, A Vollaard, and M Reynen for excellent administrative help.

The study was supported by a grant from the Praeventiefonds, the Netherlands. The advisory committee was composed of J Huisman, J W Stoop, H Bijkerk, and S Krugman.

ADDENDUM—A recent study has shown the efficacy of passive-active immunisation in neonates of oriental HBeAg positive carrier mothers.[11] The dose of HBIg (145 IU) was similar to that in our study (roughly 150 IU), but doses of vaccine were twice as high (20 μg compared with 10 μg in our study).

References

[1] Palmer Beasley R, Hwang LY, Stevens CE, *et al.* Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of hepatitis B virus carrier-state: final report of a randomized double-blind, placebo-controlled trial. *Hepatology* 1983;**3**:135-41.

[2] Barin F, Goudeau A, Denis F, *et al.* Immune response in neonates to hepatitis B vaccine. *Lancet* 1982;i:251-3.

[3] Francis DP, Hadler SC, Thompson SE, *et al.* The prevention of hepatitis B with vaccine. *Ann Intern Med* 1982;**97**:362-6.

[4] Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJTh, Kalshoven-Benschop J, Brummelhuis HGJ. Prevention of chronic HBsAg carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. *Lancet* 1979;ii:436-7.

[5] Ypma TjD, Kater L, Gerards LJ, Heiden Cvd, Heijtink RA, Haspels AA. Perinataal verworven virushepatitis B. *Ned Tijdschr Geneeskd* 1979; **123**:1820-8.

[6] Rosendahl C, Kochem MM, Kretschemer R, Wegschneider K, Kaiser D. Avoidance of perinatal transmission of hepatitis B virus: is passive immunization always necessary? *Lancet* 1983;i:1127-9.

[7] Shiraki K, Yoshihara N, Sakurai M, Eto T, Kawana T. Acute hepatitis B in infants born to carrier mothers with the antibody to hepatitis Be antigen. *J Pediatr* 1980;**97**:768-70.

[8] Naggan L, Gorodischer R, Bar-Shany S, Sarow B. Clinical hepatitis B in two infants born to asymptomatic HBsAg carrier mothers. *Isr J Med Sci* 1983;**19**:292-3.

[9] Sinatra FR, Shah P, Weissman JY, Thomas DW, Meritt RJ, Tong MJ. Perinatal transmitted acute icteric hepatitis in infants born to hepatitis B surface antigen positive, and antihepatitis Be positive carrier mothers. *Pediatrics* 1982;**70**:557-9.

[10] Delaplane D, Shulman ST, Esteban JI, *et al.* Immunoprophylaxis for infants born to HBsAg-positive mothers. *Lancet* 1983;ii:170-1.

[11] Palmer Beasley R, Hwang Lu-Yu, Chin-Yun Lee George, *et al.* Prevention of perinatally transmitted hepatitis B virus infections with hepatitis B immune globulin and hepatitis B vaccine. *Lancet* 1983;ii: 1099-102.

(*Accepted 18 October 1983*)

Development of pituitary adenoma in women with hyperprolactinaemia: clinical, endocrine, and radiological characteristics

ANTONIO E PONTIROLI, LEOPOLDO FALSETTI

Abstract

Sixty eight women referred for treatment of hyperprolactinaemia entered a three year follow up study to determine the clinical and endocrine course of the disease and its association with microadenoma of the pituitary. Details recorded before treatment included medical history, gonadotrophin and ovarian hormonal concentrations, and release of prolactin in response to protirelin (thyrotrophin releasing hormone), benserazide, cimetidine, and nomifensine. Sellar tomography was then performed yearly for three years in all women, 54 of them also undergoing computed coronal and sagittal tomography. At baseline evaluation 27 women showed radiological evidence of pituitary adenoma; at the end of the follow up period the number had increased to 41. Amenorrhoea, steady and raised serum prolactin concentrations, a low ratio of luteinising hormone to follicle stimulating hormone, a longer duration of disease, and low serum progesterone concentrations were more common in women with a final diagnosis of pituitary adenoma than in those whose sella remained normal. Tests for release of prolactin had yielded abnormal results from the outset in all 41 women with radiological evidence of pituitary adenoma and in about half of those whose sella had remained radiologically normal. Response to medical treatment (metergoline in 20 patients, bromocriptine in 21) was similar and showed no difference between patients with tumorous and non-tumorous hyperprolactinaemia.

These findings suggest that a large proportion of women with hyperprolactinaemia may harbour a prolactin secreting pituitary adenoma which becomes apparent over a relatively short period. Amenorrhoea and steady and raised serum prolactin concentrations are more common in these women. Tests for release of prolactin are of predictive value in identifying women who will develop a pituitary adenoma.

Clinica Medica, Università di Milano, Istituto Scientifico Ospedale San Raffaele, Milan, Italy

ANTONIO E PONTIROLI, MD, established investigator

Clinica Ostetrica, Universitâ di Brescia, Spedali Civili, Brescia

LEOPOLDO FALSETTI, MD, senior registrar

Correspondence to: Dr Antonio E Pontiroli, Ospedale San Raffaele, 20132 Milano, Italy.