ま と め

界面活性剤を加えた仔牛血清 albumin と MMC の混合液を加熱攪拌することにより，45±8 μm の直径を有する小球体 (microsphere) を作製し，その in vivo, in vitro における MMC の徐放性を確め，次いで臨床例に投与し市販 MMC では得られない抗腫瘍効果を認めた。

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4. Trial prevention of hepatitis B virus (HBV) infection in the field of obstetrics and gynecology

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As demonstrated by many investigators[1-3], vertical transmission of hepatitis B virus (HBV) from carrier women to their offspring appears to be a principal source of hepatitis B surface antigen (HBsAg) carriers in Japan. HBsAg carriers may potentially run the risk of developing chronic active hepatitis, liver cirrhosis or hepatoma[4]. At present, we have no means of removing HBV from carriers successfully even with interferon[5] or adenine arabinoside[6]. Accordingly it is quite important to prevent the development of carrier state in the infants born from the carrier women.

Materials and Methods

Hepatitis B vaccine (HB vaccine) was administered to 43 volunteers, 7 males and 36 females, who worked for Chiba University Hospital. Their Ages varied from 21 to 59 years old. All of them were free from HBsAg, hepatitis B surface antibody (HBsAb) and hepatitis B core antibody (HBcAb). With liver function tests, they stayed all within normal limits prior to the first HB vaccination. During the period from April 1981 to April 1983, thirty two infants born from the carrier women with hepatitis B e antigen (HBeAg) have received hepatitis B immunoglobulin (HBIG) and HB vaccine to prevent the development of HBV carrier state. Their umbilical cord sera were confirmed to be negative for HBsAg and HBsAb by reverse passive hemagglutination (R-PHA) and passive hemagglutination (PHA), respectively. All newborns apparently seemed normal at birth.

Used HBIG was donated by Green Cross Ltd. (Lot. 107GS, Osaka) and The Chemo-sero-therapeutic Research Institute (Lot. 12, 14, 16 and 17, Kumamoto). These products were purified

from human HBsAb-positive sera and lyophilized for storage. They carried basically over 32000 HBsAb-titers by PHA. HB vaccination was performed with 2 kinds of HB vaccine offered by Kitasato Institute (Lot. 001GD and TV-1, Tokyo) and The Chemo-sero-therapeutic Research Institute (Lot. KB-1). They were purified from human HBsAg- and HBeAb-positive as well as deoxyribonucleic acid (DNA) polymerase activity-negative sera. These products contained 40 μg/ml of HBsAg as protein and 200 μg/ml of Al $(OH)_3$ as an adjuvant, and were exhaustively free from Dane particles. In addition, they were inactivated at 60°C for 10 hours and then with formalin diluted 1:2000 at 37°C for 96 hours.

Twenty one of the 43 volunteers were vaccinated with 40 μg HBsAg and the remainders, with 20 μg HBsAg. An additional vaccination was performed within one month after the first vaccination. In the latter, the third vaccination was carried out around 9–10 months after the first vaccination. All the 32 infants except one received the first HBIG within 24 hours after birth. Thereafter, they received combined passive and active immunization with 1 ml of HBIG and 4 μg of HB vaccine intramascularly and subcutaneously, respectively, according to the protocol (Table 1). These 43 volunteers and 32 infants underwent liver function checks and testing for HBsAg, HBsAb and HBcAb at approximately one and 2 weeks, and every one month after the immunization. Besides, these 32 infants underwent urinalysis occasionally.

Testing for HBsAg was carried out by R-PHA (Yamanouchi Ltd., Osaka), enzyme immunoassay (EIA : Behringwerke AG, Marburg/FRG) and radioimmunoassay (RIA : Abbott, Chicago/U. S. A.). HBsAb was determined by PHA (Eisai Ltd., Tokyo) and RIA (Abbott). HBeAg and hepatitis B e antibody (HBeAb)were measured by RIA (Abbott) and EIA (Behringwerke AG). HBcAb was determined by RIA (Abbott).

Results

The cumulative detection rates of HBsAb in the 43 volunteers vaccinated with 20 μg (defined group 20) and 40 μg (defined group 40) of HBsAg are summarized in Table 2 by periods after

Table 1. Protocol of combined passive and active immunization

	No. of cases	HBIG administration	HB vaccination
Group A	8	0, 4, 8, 12M	12, 13M and additional vaccination, if necessary
Group B	5	0, 3, 6, 9, 12M	12, 13M and additional vaccination, if necessary
Group C	13	0, 3M	4, 5M and additional vaccination, if necessary
Group D	1	3, 6, 10, 13M	14, 15M
Group E	5	0, 3M	2, 3M and additional vaccination, if necessary

M : Months

Table 2. Cumulative detection rates of HBsAb after HB vaccination

HBsAg dose	Detection methods	2W	1M	2M	3M	4M	5M	6M	7M	8M	9M	10M	11M	12M
20 μg (n : 22)	R-PHA	0 (0)	0 (0)	4 (18.1)	4 (18.1)	9 (40.9)	11 (50.0)	11 (50.0)	11 (50.0)	11 (50.0)	12* (54.5)	21 (95.5)	21 (95.5)	
	RIA	0 (0)	1 (4.5)	7 (31.8)	12 (54.5)	14 (63.6)	15 (68.1)	16 (72.7)	16 (72.7)	16 (72.7)	17 77.3)	— —	— —	
40 μg (n : 21)	R-PHA	1 (4.8)	1 (4.8)	3 (14.3)	12 (57.1)	12 (57.1)	13 (61.9)	13 (61.9)	13 (61.9)	13 (61.9)	13 (66.7)	14 (66.7)	—	14 (66.7)
	RIA	— —	— —	— —	— —	15 (71.4)	16 (76.2)	16 (76.2)	16 (76.2)	16 (76.2)	16 (76.2)	16 (76.2)	—	16 (76.2)

* : The third booster vaccination was added. W : Weeks M : Months

vaccination. In group 40, HBsAb was first detected in a female within 2 weeks after vaccination. The detection rates of HBsAb by R-PHA increased slowly in the period from 2 to 8 weeks, and rapidly during the period from 2 to 3 months, attaining approximately 57%. The final detection rates amounted to 66.7% by R-PHA and 76.2% by RIA. In group 20, the detection rate of HBsAb revealed a more slowly increasing curve by R-PHA, attaining 54.5%. However,within 4 months the detection rates by RIA were founed to be as high as those in group 40. In group 20, the marked increase of HBsAb detection rates was found after the third vaccination. Of 22 volunteers, twenty one (95.5%) represented HBs-Ab-positive by R-PHA one month after the third vaccination.

While 26 of 36 females (72.2%) developed HBs Ab-positive by the second vaccination, all seven males, who were younger than 40 years old, acquired immunity from HBV. Twenty three of 27 females (85.2%) who were younger than 40 years old became HBsAb-positive by the second vaccination. On the other hand, only three of 9 females (33.3%) who were older than 40 years old developed HBsAb-positive.

No uncomfortable symptoms such as pyrexia, arthrodynia and anaphylactic shock were complained of.

As summarized in Table 3, fifteen of the 32 infants born from HBeAg-positive carrier women had acquired immunity from HBV by combined passive and active immunization, while mere 2 of the infants (6.3%) developed carrier state. These 2 carrier infants were treated according to the protocol of group A. On the other hand, no carriers had taken place in the other groups.

In group A, B and D where active immunization was carried out beyond 12 months after birth, eight of 14 infants (57.1%) represented persistent HBsAb-positive. Similarly 7 of 13 infants (53.8%) had acquired immunity from HBV in group C where the first active immunization was performed at the age of 4 months. In group E, the outcome of 5 infants remains still undetermined.

To elucidate possible effects of HBIG on the liver function, serum glutamic pyruvic transaminase (S-GTP) was repeatedly measured for one year in 20 infants who received HBIG and 18 control ones born from HBeAg-positive carrier women and followed up without any medication. In the 18 control infants, S-GPT values ranged from 18 to 331 mu/ml (mean value: 69 mu/ml), while S-GPT values varied from 8 to 113 mu/ml (mean value: 32 mu/ml) in the 20 ones who received HBIG. Besides, no side effects of HBIG and HB vaccine had been appreciated both locally and systemically. Furthermore, HBcAb was not detected in the 43 volunteers with HB vaccination.

Discussion

Clinical utility of HB vaccine in preventing

Table 3. Outcome of 32 infants with combined passive and active immunization

Groups	No of cases	Undetermined	Carrier state	Persistent HBsAb
Group A	8	1	2	5
Group B	5	3	0	2
Group C	13	6	0	7
Group D	1	0	0	1
Group E	5	5	0	0
Total	32	15	2(6.3%)	15(46.9%)*
Control	78	6**	57(73.1%)	15(19.2%)

Control : 78 infants born to HBeAg-positive carrier women (without immunization)

* : Percentage (%)

** : Six infants stayed free from HBV infection at age 24 months.

HBV horizontal infection was first described by Krugman et al.[7]. To prevent HBV vertical transmission, Kohler et al.[8] applied HBIG to 3 neonates successfully. For the last decade, passive and/or active immunization with HBIG and HB vaccine has been theoretically accepted as one of rational means for preventing HBV infection. However, practical details of these therapeutic methods are still under investigation.

To elucidate fundamental efficacy and vaccination method of HB vaccine, two different doses of HBsAg, i. e. 20 and 40 μg, were applied to 43 volunteers. Their immune responses were quicker in group 40 than in the other, supporting the results of Szmuness et al.[9]. However, there were no significant differences in the final rate of HBsAb occurrence between the two groups, suggesting sufficiency of 20 μg HBsAg for raising HBsAb in adult cases. This study also demonstrated that the first and second booster vaccination could immunize approximately 80% of cases successfully in accord with the report of Yano et al.[10]. Besides, the third booster vaccination revealed significantly high efficacy ($p<0.005$) in raising HBsAb in the remaining non-responders, attaining 96% in rate of HBsAb occurrence.

With their immune responses to HB vaccine, there were no marked differences by sex. On the other hand, the volunteers under 40 years old achieved significantly higher acquistion of HBsAb ($p<0.005$), as compared with that of the volunteers over 40 years old.

Inaba et al.[2,11] have already demonstrated that 73.1% of the 78 infants born from HBeAg-positive carrier women developed carrier state within 4 months postnatally, 19.2% acquired persistent HBsAb during the period of 2–8 months and mere 7.7% remained free from HBV infection for 24 months (control in Table 3). On the other hand, only 6.3% of the 32 infants who were born from HBeAg-positive carrier women and received combined passive and active immunization developed carrier state, indicating remarkable efficacy of HBIG in the prevention of infantile development of carrier state. These two carrier infants in group A received HBIG every 4 months, while no children had developed carrier state in the other groups where HBIG was administered every 3 months. These data suggest that 4 month-interval of HBIG administration may be too long and 3 month-interval, adequate length of period to mainatin circulating HBIG in refering to the decreasing curve of administered HBIG[12]. In addition, HBsAg was first detected at the age of 5 months in the 2 carrier infants, while all carrier infants became HBsAg-positive within 4 months neonatally in the control. HBIG might delay the infantile development of carrier state. As demonstrated by Yano et al.[13], seven of 32 infants (21.9 %) who received only HBIG acquired persistent HBsAb within 12 months after birth. This spontaneous immunization rate is not significantly higher than that of the control (19.2 %). Supposedly passive immunization with HBIG might not enhance the postnatal spontaneous acquisition of HBs Ab.

The active immunization rate with HB vaccine has reached 46.7% of the infants at present and may be expected to increase further in the near future, indicating promising practical utility of HB vaccine in preventing vertical and/or horizontal infantile infection of HBV.

As to infantile immune responses to HB vaccine, there were no significant differences between the two groups, i. e. group A, B and D where the first vaccination was carried out beyond 12 months, and group C, at the age of 4 months. Accordingly, HB vaccination could be started at the age of 4 months at latest as successfully as, beyond the age of 12 months. Further clinical trials in HB vaccination might enable us to start the first HB vaccination much earlier, as represented by group E. Early infantile HB vaccination carries certain advantages such as saving on HBIG and all accompanied efforts and troubles of medical staffs, patients and their families, as well.

In summary, this study demonstrates and suggests:

1) Approximately 76% of the volunteers deve-

loped persistent HBsAb-positive response without any side effect by the first and second booster vaccination. The third booster vaccination revealed prominent immunizing effect, attaining 96% in rate of HBsAb occurrence.

2)Of the 32 infants, two (6.3%) developed carrier state, fifteen (46.9%) acquired persistent HBsAb and the remaining 15 stayed undetermined, suggesting promising efficacy of combined passive and active immunization therapy.

3)The two carrier infants received HBIG every 4 months, while no infants who received HBIG every 3 months developed carrier state, indicating proper interval of HBIG administration.

4)As to infantile immune responses to HB vaccine, there were no significant differences between the two groups, 14 infants vaccinated beyond 12 months and 13 ones done at the age of 4 months. This finding suggests the possibility of early successful HB vaccination for preventing HBV vertical transmission.

5)No serious side effects were found.

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