EFFICACY OF VACCINE AGAINST HEPATITIS B
EPIDEMIOLOGICAL DATA

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Introduction

The risk of getting hepatitis B infection is a serious risk in haemodialysis units. In Tours, the annual incidence is as high as 40% with a prevalence of 50% amongst the patients. Hepatitis B infection also affects staff members, but in micro-outbreaks.

In view of this situation we attempted to reduce the incidence of hepatitis B by the usual methods. Strict hygienic measures were unfortunately insufficient to decrease appreciably the rate of infection. Passive immunisation with specific anti-HBs gammaglobulins has been shown to be very effective1,2 but it was impossible to include every staff member and patient because of shortage of anti-HBs antibody. Moreover the long-term safety of repeated injections of homologous proteins remains unknown.

Several attempts at active immunisation by means of heat inactivated HBs Ag positive sera had been carried out in man, but further trials were impeded by the lack of suitable cellular and animal models3,4. More recently specific active immunisation of chimpanzees with purified hepatitis B virus envelope (HBsAg) followed by virulent challenge, has been performed successfully5,6. Considering these promising initial results, we felt justified in attempting to induce active protection at our centre by means of a vaccine prepared from purified and inactivated HBsAg.

Safety and potency tests were carried out on five chimpanzees. The preparation was then offered to the patients and staff members of the Tours haemodialysis unit.

Efficacy of immunisation was assessed by the study of humoral immunity to HBsAg among vaccinated people and by a statistical survey of hepatitis B infection in groups of vaccinated and non-vaccinated subjects.

Methods

The vaccine was prepared from HBs antigen obtained from healthy blood donors.
The HB₅Ag was purified to eliminate most of the contaminating proteins. The purified envelope is not infectious, but 1% formalin was added to eliminate any remaining viruses which could contaminate the preparation (Table I).

**TABLE I. Characteristics of the Hepatitis B Vaccine**

<table>
<thead>
<tr>
<th>Morphological</th>
<th>Immunological</th>
<th>Biochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spherical forms</td>
<td>- HB₅Ag titre:</td>
<td>Protein content</td>
</tr>
<tr>
<td>Some tubular forms</td>
<td>1/2 CEP</td>
<td>100 µg (Lowry)</td>
</tr>
<tr>
<td>No Dane particles</td>
<td>1/520 PHA</td>
<td>Isotonic tris buffered</td>
</tr>
<tr>
<td>No other viruses</td>
<td>1/1000 RIA</td>
<td>Saline, pH : 7.4</td>
</tr>
<tr>
<td></td>
<td>- Subtype ayw₃</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- 'e' Ag negative</td>
<td></td>
</tr>
</tbody>
</table>

One dose of vaccine: one millilitre
Formalin: 1%

Being aware of the high risk of infection by hepatitis B, volunteers from the haemodialysis unit accepted injection of the vaccine but not that of a placebo. Therefore we were not able to conduct a classical double-blind experiment; the control group being patients and staff members who did not wish to be vaccinated.

The HB₅Ag carriers were not vaccinated because of the hypothetical cytotoxic potential of anti-HB₅ for hepatocyte membranes bearing HB₅ antigenic sites.

Since October 1975, 246 people have been vaccinated by two subcutaneous injections of one millilitre of vaccine at an interval of one month, and a booster injection after one year.

The results of the first 126 vaccinated volunteers who have been followed for at least six months (94 staff members and 32 haemodialysis patients) are presented. In this population 20 staff members and 6 patients had a low level of anti-HB₅ and were vaccinated to assess the antigenic potency of the vaccine.

**Results**

**Safety**

There was no local or general reaction to the immunisation. A monthly biological and clinical survey of the vaccinated subjects showed no evidence of complications due to vaccination, especially no sign of autoimmunity.

**Potency**

*Humoral response to HB₅Ag* Anti-HB₅ antibody was detected by radio-immunoassay AUSAB° (Abbott), cut-off value: R ≥ 2.1.

Twenty-six subjects (6 patients and 20 staff members) who had a low level
TABLE II. Anti-HB$_5$ Humoral Response in Staff Members

<table>
<thead>
<tr>
<th>Staff Members</th>
<th>Anti-HB$_5$ Response</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Anamnestic</td>
</tr>
<tr>
<td>Primary contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with HB virus</td>
<td>No: 20</td>
<td>0</td>
</tr>
<tr>
<td>Free of any contact</td>
<td>No: 74</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>93%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Number of response/number vaccinated: 95%

TABLE III. Anti-HB$_5$ Humoral Response in Haemodialysed Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Anti-HB$_5$ Response</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Anamnestic</td>
</tr>
<tr>
<td>Primary contact</td>
<td></td>
<td></td>
</tr>
<tr>
<td>with HB virus</td>
<td>No: 6</td>
<td>0</td>
</tr>
<tr>
<td>Free of any contact</td>
<td>No: 26</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>70%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Number of response/number vaccinated: 75%

of anti-HB$_5$ before vaccination responded to immunisation by a quick and abrupt rise of antibody (anamnestic response).

Of the 74 staff members who had no serological sign of previous contact with HB virus, 69 (93%) developed a specific anti-HB$_5$ immunity (Table II). Twenty-six patients were free from previous contact with HBV. Eighteen of them (70%) responded to vaccination (Table III). The humoral response of the patients is significantly different from that of the nursing staff members (p = 0.001).

Kinetics of anti-HB$_5$ response Three distinct profiles of anti-HB$_5$ were seen (Figure 1). Type I corresponds to an early response within the 15th day after the first injection. Type II is more common; the antibodies appear one month after the second injection. Type III is a delayed response, beginning 3 to 4 months after the initial vaccination.

Despite the variety of anti-HB$_5$ titres, there was a progressive decline in antibody levels between the sixth and the ninth month after the first injection. A booster injection was then performed which was followed by an anamnestic rise of anti-HB$_5$ antibody.
Figure 1. Kinetics of anti-HBs primary humoral response. Continuous line: Early response (1) Dashes: 'Standard' response (2); Dots and dashes: Delayed response (3)

**Efficacy**

**Ward staff members** None of the 94 vaccinated staff members developed jaundice. Hepatitis B infection is endemic in the centre and accidental inoculations have occurred, resembling thereby true 'virulent challenge'. Thus, in seven vaccinated staff members we noticed a sudden rise of anti-HBs antibody and transient appearance of anti-HBs antigens without clinical or biological signs of illness.

One of the vaccinated residents whose antibody titre was very low (R = 2.5) developed a sub-clinical HBsAg positive hepatitis. The anti-HBs rose to a high level shortly after this infection with an anamnestic type of response. Anti-HBc appeared and lasted for two months.

During the same period, we observed six cases of acute hepatitis and four of transient HBs antigenaemia among the non-vaccinated ward staff members.

**Patients** The response of the patients to immunisation is significantly lower compared with that of ward staff. Thus we performed a statistical study of HBs antigenaemia in a restricted population of vaccinated (N = 16) and non-vaccinated (N = 22) patients who were free of previous contact with HBV when they were first dialysed in Tours (Figure 2). They were immunised when they entered the unit.

Since October, 1975 the risk of infection by HBV is similar to that observed in the three preceding years, when we compare 65 patients who joined the Unit between April, 1972 and October, 1975 and the 22 non-vaccinated patients who entered the unit since October, 1975 (p = 0.4).

On the contrary, vaccinated patients have been protected from infection...
(p = 0.008). A patient who was in the unit several months before being vaccinated and did not develop anti-HBs antibody, became HBsAg positive one year after immunisation.

**Discussion**

The formalin-treated hepatitis vaccine has been used as prophylaxis against infection in patients and staff members of the Tours Haemodialysis Centre for more than 18 months. According to all serological, epidemiological and clinical data, its safety and efficiency are very satisfactory.

The potency of the vaccine was demonstrated by the appearance of anti-HBs antibody. Ninety-five per cent of the staff members responded to immunisation, but only 75% of patients developed a humoral response. The anti-HBs response of patients was very often of the delayed type. HBs antigenaemia occurred in one of the 8 vaccinated patients who did not develop anti-HBs. It is difficult to conclude definitely whether this group is exposed to the same risk as the non-vaccinated patients, or is partially protected, for instance, by cellular immunity.

Eight out of 22 non-vaccinated patients and 10 non-vaccinated staff members became HBsAg positive. The staff members were mostly contaminated during an epidemic of hepatitis B which developed in the unit in May–June, 1976.

During this time, infections by HBV have occurred in the vaccinated subjects
(8 staff members and 2 patients).

A physician had a borderline level of anti-HB$_s$ antibody (R = 2.5) when he was contaminated. He became HB$_s$Ag positive (by RIA) for one month and presented as anicteric hepatitis with asthenia and transient increase of the SGPT (one week, 180 IU). In the others, antibody titres were between 3 and 20, and neither patients nor staff members showed any biological or clinical signs of hepatitis. In these ten subjects contamination by hepatitis B virus was demonstrated by a significant anamnestic anti-HB$_s$ response and by a transient (two months) appearance of anti-HB$_c$ antibody. According to these observations, the threshold value of protection afforded by the immunisation could be estimated as an anti-HB$_s$ ratio by RIA equal to or greater than 3.

After one year, the antibody titre decreases noticeably. A booster injection was always followed by an anamnestic rise of the specific antibody.

For further clinical trials, the protocol of immunisation could be fixed to three injections at intervals of one month and a booster injection after a year (1 ml); this is similar to other inactivated antigen vaccines.

In the past six months, a vaccine prepared with the two main HB$_s$Ag subtypes 'ad', 'ay' and one ml aluminium hydroxide as adjuvant has been prepared. It conferred a quicker and higher antibody response.

The industrial adaptation of this vaccine has been carried out in collaboration with the Pasteur Institute (Paris). The usual biological and chemical controls for the issue of a licence are now being performed before the beginning of wider clinical trials.

References

7 Houwen, B, Goudeau, A and Dankert, J (1975) J. Immunol. Methods, 8, 185
8 Maupas, Ph., Goudeau, A, Coursaget, P et al (1976) Lancet, i, 1367

Open Discussion

CAMBI (Italy) Is your vaccine commercially available?

PENGLOAT An industrial form of the vaccine is now being prepared by the Institute Pasteur of Paris. The chemical tests are finished, a trial in chimpanzees is just beginning and human trials will begin in October with the industrial drug.

PAPADIMITRIOU (Thessaloniki) What is the percentage of hepatitis in your dialysis patients before and after vaccination?

PENGLOAT In the patients the hepatitis is generally quite different from what was found in staff members. In staff members we had, usually, typical jaundice,
but patients seldom have jaundice, most often they only have antigenaemia and a rise in transaminases. That is what we saw in the non-vaccinated patients of the control group and that is what we saw too, in the one vaccinated patient who developed antigenaemia; he had no jaundice. This case is very recent. The last results we received showed a disappearance of HBs antigen and so we hope that he is not becoming a chronic carrier.

DRUEKE (Paris) Did you check whether there was a relationship between antibody formation to hepatitis antigen and the HLA-system in your patients?

PENGLOAN No, we have begun to do it, but we have not enough results yet.

DRUEKE Do you envisage vaccination against different subtypes of hepatitis antigen?

PENGLOAN Of course, the new vaccine is against the two subtypes.

ZIMMERMANN (Vienna) Will there be any interaction between vaccination and transplantation?

PENGLOAN We have a few transplantations now but I cannot give a useful answer.

LULU (Kuwait) How soon is the vaccine effective, when someone is exposed to infection?

PENGLOAN Normally the vaccine is effective when the anti-HBs antibodies rise, so in the staff members it is normally one and a half months after beginning vaccination. In patients it may be later; but we have seen an anamnestic rise of anti-HBs antibodies suddenly appearing after contact with the virus in patients who did not develop a detectable level of HBs antibodies after vaccination, and vaccination had seemed to be a failure. So, I think that the protection is maybe earlier. We also had evidence of that with one student who joined the unit and had only one injection of vaccine. He was not considered fully vaccinated, and he travelled elsewhere. When he came back we told him that he had transient antigenaemia, but he had not noticed it. It seems that he had an abortive viral infection because of the anamnestic anti-HBs response.