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IMMUNOGENICITY OF AN INACTIVATED HEPATITIS A VACCINE IN DUTCH UNITED NATIONS TROOPS


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Chapter 5

IMMUNOCENICITY OF AN INACTIVATED HEPATIS A VACCINE IN UNITED NATIONS TROOPS

[Text continues]

Chapter 5
Immunogenicity of an inactivated hepatitis A vaccine in Dutch United Nations troops


Limited information existed on the immunogenicity of an inactivated hepatitis A vaccine as part of an extensive vaccination schedule. Dutch marines bound for duty in Cambodia received inactivated hepatitis A vaccine (720 ELISA units of antigen, two intra-gluteal doses at a 2-week interval before departure and an intra-deltoid booster vaccination after 8 months) simultaneously with several other vaccines. Hepatitis A antibodies were determined in blood-samples drawn before and after the booster vaccination, using two laboratory tests (modified HAVAB and SBB-ELISA). At 8 months, before the booster vaccination, 52% (modified HAVAB) and 81% (SBB-ELISA) had seroconverted. Risk factors for non-seroconversion were increasing age and a typhoid vaccination. At 11 months 97.6% (modified HAVAB) and 100% (SBB-ELISA) had seroconverted. Non-seroconversion at 8 months was remarkably high. SBB-ELISA was more sensitive in lower titre ranges.

Keywords: hepatitis A, vaccinations, immunogenicity

Until recently the usual prophylaxis against hepatitis A in the military was immunoglobulin, offering protection for only several months. Therefore, an inactivated vaccine providing safe and long protection was an advantage. It had shown good immunogenicity after simultaneous administration with both hepatitis B and other vaccines, but no information was found on the immunogenicity after extensive simultaneous vaccinations. Dutch marines bound for UN-duty in Cambodia, were the first military group in the Netherlands to receive an inactivated hepatitis A vaccine (HAVRIX 720). Before departure all received extensive vaccinations (total 10–12) administered simultaneously or within a short period. We report on the study objectives: immunogenicity of inactivated hepatitis A vaccine within an extensive vaccination schedule and the comparison of two enzyme-linked immunosassay tests.

STUDY POPULATION AND METHODS

Study population

The retrospectively selected study population consisted of 760 marines (all male, mean age 28.1 years; range 17–52), assigned to UN-duty in Cambodia from June to December 1992. They all underwent the same immunization regime, the only possible difference being in routine vaccinations like typhoid and diphtheria, tetanus and poliomyelitis, due to different booster intervals. All were screened for hepatitis A antibodies (anti-HAV) before the first vaccination: 148/760 (19.5%) persons proved to be anti-HAV positive and were excluded from the study. Only three men needed a booster vaccination for diphtheria, tetanus and poliomyelitis and were also excluded. The remaining 609 anti-HAV negative men, who received hepatitis A vaccination, were divided into two groups: a group who received a typhoid booster vaccination (Ty+: n = 115) and a group who had not (Ty−: n = 494). A random sample of 230 persons (twice the Ty+ group) was selected from the Ty− group. After checking availability of sera and whether participants had received their hepatitis A booster at t = 8, the final study population consisted of 286 persons (Ty+: 96, Ty−: 190). The mean age of the total group was 26.4 years (Ty+: 28.5, Ty−: 25.4).

Written informed consent was obtained from all participants. The study protocol was approved by an
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institutional review board (Dutch Military Medical Committee).

Medical and living conditions

Before departure all subjects were fit for duty. No recent history of alcohol abuse or liver disease could be identified in the medical records. Due to operational restrictions it was not possible to record weight and length at the time of the vaccinations. All troops were instructed verbally and in writing on the risks involved in working in the tropics. Mefloquine (250 mg weekly) was the malaria chemoprophylaxis. In Cambodia troops were accommodated in tents and primitive campsites. Safe food and potable water supplies were available. Dutch military medical facilities supported the troops on site. All consultations, diagnoses and treatments were standardized and entered into a database.

Vaccinations

The vaccination schedule is shown in Table 1. All vaccinations were administered in The Netherlands except for the third hepatitis B vaccination which was given in Cambodia. The formalin-inactivated hepatitis A vaccine contained 720 ELISA units (EU) of hepatitis A antigen in 1 ml and was administered in three doses: the first two doses in the left gluteal muscle with a 14-day interval, a booster dose was given in the right deltoid muscle 8 months after the first dose. All participants were routinely vaccinated against diphtheria, tetanus and poliomyelitis. In Cambodia troops were accommodated in tents and primitive campsites. Safe food and potable water supplies were available. Dutch military medical facilities supported the troops on site. All consultations, diagnoses and treatments were standardized and entered into a database.

Blood sampling and serology

Blood samples (20 ml) were drawn in The Netherlands, before the first vaccination (t = 8); at 8 months (t = 8), before the hepatitis A booster, and at 11 months (t = 11), 3 months after the booster vaccination (Table 1). The samples were directly transported to the Regional Laboratory of Public Health of the Municipal Health Service, Amsterdam, The Netherlands, centrifuged and stored as sera at −20°C. First the samples from t = 0 were screened for anti-HAV using a commercially available enzyme linked immunosassay (HAVAB, Abbott Laboratories, North Chicago, IL, USA). Then anti-HAV was measured according to a modified HAVAB protocol at t = 8 and t = 11 in samples of those who had received the inactivated hepatitis A vaccine. Titres were calculated using the mean values of the test results within the range of the assay and expressed in mIU ml⁻¹ by reference to the WHO standard using logarithmic regression.

Anti-HAV titres of t = 8 and t = 11 were determined in the same samples by SmithKline Beecham Biologicals (SBB), Rixensart, Belgium, using their sensitive enzyme-linked immunosassay test (SBB-ELISA) according to a standardized protocol. In both tests sera with antibody titres ≤20 mIU ml⁻¹ were considered negative (non-seroconversion). The geometric mean titre (GMT) was calculated from seroconverted values only.

Statistical analysis

Univariate analysis was applied using the χ² test. Multivariate logistic regression was used to find independent risk factors and to adjust for possible confounding. P-values of 0.05 or less were considered significant.

RESULTS

Seroconversion

Of the total study population (n = 256) at 8 months (after two hepatitis A vaccinations) 52% (n = 130) using the modified HAVAB and 81% (n = 231) with SBB-ELISA had seroconverted.

Participants were divided in Ty+ (n = 96) and Ty− (n = 190) and in age groups <25 years (n = 116), ≥25 <35 years (n = 123), and ≥35 years (n = 47). Seroconversion rates in the different groups are shown in Table 2. Both laboratory tests showed increasing age and the typhoid booster vaccination to be univariate risk factors for non-seroconversion. After multivariate analysis, adjusting for typhoid vaccination, age remained independently associated with non-seroconversion. The typhoid vaccination, corrected

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Table 1: Vaccination and blood sample schedule in Dutch marines for the Cambodia deployment in 1992

<table>
<thead>
<tr>
<th>Vaccination</th>
<th>Time intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>t = 0</td>
</tr>
<tr>
<td>Hepatitis A</td>
<td>+</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>+</td>
</tr>
<tr>
<td>Rabies</td>
<td>+</td>
</tr>
<tr>
<td>Meningitis A + C</td>
<td>+</td>
</tr>
<tr>
<td>Japanese encephalitis</td>
<td>+</td>
</tr>
<tr>
<td>Typhoid*</td>
<td>+</td>
</tr>
<tr>
<td>DTP*</td>
<td>+</td>
</tr>
<tr>
<td>Blood sample</td>
<td>+</td>
</tr>
</tbody>
</table>

*Booster vaccination when necessary

**DTP: diphtheria, tetanus and poliomyelitis, booster vaccination when necessary.
for age, remained an independent risk factor for non-seroconversion using modified HAVAB (OR 2.45; 95% CI 1.43-4.18) and just lost significance for SBB-ELISA (OR 1.73; 95% CI 0.91-3.29). At 11 months, 3 months after the booster vaccination, the percentages of seroconversion for modified HAVAB and SBB-ELISA were 97.6 (% = 279) and 100%, respectively.

During deployment and a 3-month period after repatriation no clinical cases of hepatitis nor jaundice were diagnosed either in the study population nor in the total battalion.

**Antibody titres**

Geometric mean titres of the seroconverted persons are shown in Table 3, suggesting great differences at both t = 8 and t = 11 for the tests in the various groups. In contrast to the association between seroconversion and a typhoid vaccination, there was, among seroconverted persons, no difference in GMT between the Ty+ and Ty− groups. To compare the results of both tests, the anti-HAV titres of all available samples (n = 618) were divided into seven strata (mIU ml⁻¹): 1–19, 20–99, 100–199, 200–499, 500–999, 1000–1999, ≥2000. Then, the mean of the titres within each stratum was calculated. In lower anti-HAV concentrations the SBB-ELISA was usually higher than modified HAVAB but this reversed in higher concentrations (Table 4).

**DISCUSSION**

The main findings of this study were the low seroconversion rates after two hepatitis A vaccinations at 8 months and the different results obtained by the laboratory tests. Neither low anti-HAV titres nor seroconversion rates had been observed in other

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### Table 2 Predictors for anti-HAV seroconversion in 286 Dutch marines at 8 months

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total number</th>
<th>Modified HAVAB</th>
<th>SBB-ELISA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sero-conversion (%)</td>
<td>OR</td>
</tr>
<tr>
<td>Total study population</td>
<td>286</td>
<td>52.4</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td>Adjusted for age</td>
</tr>
<tr>
<td>&lt; 25</td>
<td>110</td>
<td>62.9</td>
<td>1</td>
</tr>
<tr>
<td>≥25–&lt;35</td>
<td>123</td>
<td>52.0</td>
<td>0.64</td>
</tr>
<tr>
<td>≥35</td>
<td>47</td>
<td>27.7</td>
<td>0.23</td>
</tr>
<tr>
<td>Typhoid vaccination</td>
<td>Yes</td>
<td>98</td>
<td>35.5</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>190</td>
<td>60.5</td>
</tr>
</tbody>
</table>

*Adjusted for typhoid
*A*Adjusted for age

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### Table 3 Anti-HAV GMT in seroconverted Dutch marines

<table>
<thead>
<tr>
<th></th>
<th>GMT mod HAVAB (n = 149)</th>
<th>Range</th>
<th>GMT SBB-ELISA (n = 149)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total study population</td>
<td>56 (150)</td>
<td>20–745</td>
<td>114 (231)</td>
<td>26–908</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>55 (73)</td>
<td>21–745</td>
<td>124 (100)</td>
<td>27–871</td>
</tr>
<tr>
<td>≥25–&lt;35</td>
<td>56 (73)</td>
<td>20–537</td>
<td>111 (103)</td>
<td>25–908</td>
</tr>
<tr>
<td>≥35</td>
<td>67 (13)</td>
<td>22–468</td>
<td>57 (28)</td>
<td>31–712</td>
</tr>
<tr>
<td>Typhoid</td>
<td>Yes</td>
<td>66 (28)</td>
<td>22–537</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>54 (115)</td>
<td>20–745</td>
<td>114 (187)</td>
</tr>
</tbody>
</table>

*aNumber of seroconverted persons over whom GMT was calculated

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### Table 4 Comparison of SBB-ELISA anti-HAV titres to corresponding modified HAVAB titres

<table>
<thead>
<tr>
<th>Concentration stratum (mIU ml⁻¹)</th>
<th>0–19</th>
<th>20–99</th>
<th>100–199</th>
<th>200–499</th>
<th>500–999</th>
<th>1000–1999</th>
<th>≥2000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of SBB-ELISA results within the stratum</td>
<td>69</td>
<td>120</td>
<td>114</td>
<td>72</td>
<td>83</td>
<td>58</td>
<td>92</td>
</tr>
<tr>
<td>SBB-ELISA mean titre</td>
<td>66</td>
<td>140</td>
<td>318</td>
<td>737</td>
<td>1425</td>
<td>3706</td>
<td>6203</td>
</tr>
<tr>
<td>Corresponding modified HAVAB mean titre</td>
<td>2</td>
<td>18</td>
<td>48</td>
<td>199</td>
<td>877</td>
<td>1853</td>
<td>6203</td>
</tr>
</tbody>
</table>

*All available serum samples (n = 618, modified HAVAB+SBB-ELISA at t = 8 and t = 11) were included, exceeding the number of included participants (n = 286).

*All* SBB-ELISA results < 20 mIU ml⁻¹ were given 0 as outcome by the laboratory.
studies. Given the operational military circumstances we were limited in the number of variables.

Apart from the typhoid vaccination, we could not study the influence of other vaccinations. We identified increasing age and concomitant typhoid vaccination as risk factors for non-seroconversion. Slower immunogenicity with increasing age has been observed but this was found in an older age group (40–62 years) than in our study. This in contrast to Bienzle et al., who found higher GMTs in older age groups. Good immunogenicity for hepatitis A after simultaneous hepatitis B vaccination has been reported and similar results were reported after simultaneous administration with limited other vaccines (including typhoid, rabies and Japanese encephalitis). After two vaccinations 100% anti-HAV seroconversion (GMT 147) was found in Norwegian troops before a hepatitis A booster vaccination at 9 months. They also received diphtheria, tetanus, polio, typhoid, cholera and BCG vaccinations simultaneously; some received hepatitis B vaccinations. However, the routes of administration were not mentioned and SBB-ELISA was used, a more sensitive test than modified HAVAB. Looking at our SBB-ELISA results we still consider an adjusted OR of 1.73 relevant for non-seroconversion after a typhoid vaccination.

There may be other explanations for non-seroconversion in our population. First, the injection site could be a reason for non-seroconversion. We were not able to choose the preferred administration in the deltoid muscle because of "competition" with other simultaneous vaccinations. After hepatitis B vaccination better immunogenicity was found via the deltoid muscle than via the gluteal muscle. The explanation was that in adipose persons in particular, the gluteal muscle was not reached due to s.c. fat. Consequently we used long needles (6 cm) for the intra-gluteal vaccination, but the effective antibody levels after the intra-deltoid route of administration support a negative influence of the off-schedule timing of the first two vaccinations. Secondly, we used an accelerated 14-day interval between the first two doses of hepatitis A vaccine. However, several studies showed good results with an accelerated schedule and, indeed, a single dose vaccine containing 1440 ELU is now the preferred choice. Thirdly, an important limitation was the impossibility to collect blood in Cambodia. Hence the level of protection in Cambodia remained unknown, although no clinical cases of hepatitis occurred during or after the stay in this endemic area. These three factors restrict practical consequences. Finally, participants took mefloquine as malaria chemoprophylaxis. Poor antibody response to rabies vaccination has been associated with chloroquine chemoprophyaxis. In our study mefloquine, chemically related to chloroquine, was an unlikely interfering agent. The marines received the second hepatitis A vaccination on 8 weeks before starting chemoprophylaxis and a booster vaccination while still taking mefloquine.

The other main finding of this study was the large difference between the results with modified HAVAB and SBB-ELISA. Both laboratories were well experienced and the tests were performed according to good laboratory practice guidelines. Different results between both tests have been described. Delem compared modified HAVAB with SBB-ELISA titres. In the protocol of the modified HAVAB 100 μl serum was used instead of 200 μl in our study. A good correlation between both assays was found, but the GMT of SBB-ELISA titres in serum samples collected at months 1, 2 and 6 were higher than the GMT determined for modified HAVAB. At month 7, however, the GMT of SBB-ELISA titres was 4180 versus the modified HAVAB GMT of 4223. In our study an explanation for the different titres might be the different affinity of the antibodies against the different antigens used in the two tests. The amount of serum used in both tests could be another explanation.

In conclusion, it was surprising that two doses of inactivated hepatitis A vaccine despite the limitations of this study resulted in such low anti-HAV seroconversion rates at 8 months. The different laboratory test results should also be recognized.

ACKNOWLEDGEMENTS

We thank SmithKline Beecham for financing the laboratory tests, M. de Ridder (Municipal Health Service Laboratory, Amsterdam) and A. Delem (SBB, Rixensart) for their laboratory support. Furthermore we thank J. Couvee (SBB) for statistical advice and the medical personnel of the Marine Barracks Doorn, The Netherlands, for logistical support.

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