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LONG-TERM IMMUNOGENICITY SAFETY AND EFFICACY OF A RECOMBINANT HEPATITIS B VACCINE IN HEALTHY ADULTS

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Two hundred healthy adults seronegative for HBV markers received three 10 or 20 mcg injections of a vaccine formulated from HBsAg produced by a recombinant strain of the yeast *Saccharomyces cerevisiae*. The vaccine was administered intramuscularly at 0, 1, and 6 months in the deltoid region. The seroconversion rates, expressed in GMT/IU/1 were determined at 1, 2, 6, 7, 12, 24, 36 and 48 months following the initial injection. No severe or serious adverse reactions attributable to the HB vaccines were observed in any subject. The seroconversion rates following the 20 mcg dose of recombinant vaccine were always higher than those observed after the 10 mcg dose, but the differences were not statistically significant. Also the GMT values were lower after the 10 mcg dose of vaccine. Females showed a higher anti-HBs response than males; an age-dependent effect was observed in the anti-HBs response as regards both the percentage of responders and the antibody concentrations in the serum. No adverse reactions to the vaccine were observed. The rDNA vaccine did not induce a response to yeast-derived impurities and did not increase anti-yeast IgE antibody titres. The results of this study have shown that the Amgen rDNA vaccine is safe and clinically well tolerated, and that it provides protection against infection and disease. A vaccination dose of 20 mcg appears more advantageous for healthy adult subjects.

INTRODUCTION

Viral hepatitis B is a major public health problem in Italy and other developed countries where infection is still high among various groups such as family contacts, health care workers, haemodialysis patients, haemophiliacs and thalassaemics, residents of institutions, drug users and the sexually promiscuous (4-7, 11, 13, 15, 16).

In these populations, vaccination repesents an essential means of prevention. A safe, highly immunogenic, lasting and cheap vaccine is therefore needed. The application of r-DNA technology to produce yeast-derived HB vaccine offered a new alternative to plasma-derived vaccines (3, 8-10, 14). AMGEN Inc. (Thousand Oaks) has developed a hepatitis B vaccine from hepatitis B surface antigen (HBsAg) obtained by recombinant technology (2).

This vaccine is composed only of the surface antigen of the hepatitis B virus which is expressed by the yeast.

A clinical study was performed to establish the safety of the recombinant yeast hepatitis B vaccine in a population of normal low risk adults and to establish the rate and extent of seroconversion following vaccination with two different dose levels of the yeast-derived surface antigen protein.

MATERIALS AND METHODS

Two hundred normal healthy adults were selected. The inclusion criteria were age (18-60 years), good general health, lifestyle associated with low risk of hepatitis B infection, normal serum chemistry and

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haematology and negative hepatitis B virus markers.

The exclusion criteria were pregnancy, HIV positive test, alcoholism or suspected intravenous drug abuse, a history of allergy to yeast, recent overt exposure to hepatitis, or previous vaccination for hepatitis B and evidence of other compromising diseases.

Elisa techniques were used to test for HBsAg, anti-HBs, anti-HBc, HBeAg and anti-HBe according to the instructions of the manufacturer (Abbott Laboratories). The subjects were randomly allocated to two equal groups for intramuscular (i.m.) injection of 10 or 20 mcg of yHBsAg in the deltoid region at 1, 30, and 180 days. Local and general clinical symptoms were monitored by the patients in the seven days following the i.m. administration of the vaccine and recorded on suitable individual sheets. Clinical examinations and laboratory tests were performed at 1, 2, 6, 7, 12, 24, 36 and 48 months after the first vaccination.

The titres of anti-HBs in the post-vaccination sera were determined by ELISA (Ausab quantitation panel, Abbott Lab.) and expressed in IU/l. A geometric mean titre (GMT) was calculated each time.

In order to assess whether the repeated vaccinations had induced hypersensitivity to residual yeast contaminants in the vaccine, antibodies to *Candida albicans* and anti-yeast antibodies of the IgE type were tested in paired sera before and after vaccination.

The antibodies against *Candida albicans* were detected by the indirect *Candida* haemoagglutination test (*Candida albicans*, HA Antigen, Roche). Serum titres up to 1:160 were considered normal. Titre variations of at least 3 grades were considered significant. The determination of circulating specific IgE anti-*Saccharomyces* in the sera was detected by an enzymatic *in vitro* test system based on the Radio Allergo Sorbent Test principle (Phadebas Rast, Pharmacia). The response for each unknown sample was classified and reported either in Phadebas Rast Classes (0-4) or in Phadebas Rast Units (PRU/ml). The level of the allergen-specific IgE antibody was absent or undetectable (class 0), low (class 1), moderate (class 2), high (class 3), and/or very high (class 4). PRU/ml values of 0.35 and higher represented a progressive increase in the relative concentration of allergen-specific IgE antibodies. PRU/ml values lower than 0.35 were assumed to indicate either the absence or presnce of undetectable levels of allergen-specific IgE antibodies.

RESULTS

The characteristics of the 200 healthy adults vaccinated with yeast-derived hepatitis B vaccine are reported in Table 1. No statistically significant differences were observed between the two groups. The drop-outs registered during the study were 1, 2, 5, 19, 38 and 54 at 6, 7, 12, 24, 36 and 48 months, respectively.

After the third dose of vaccine the adverse effects were evaluated in 195 subjects; of the remaining 5 subjects two were drop outs and three did not provide sufficient information.

TABLE 1. - Characteristics of 200 healthy adults vaccinated with a yeast-derived hepatitis B vaccine.

Characteristics	10 mcg		20 mcg	
	Male	Female	Male	Female
Subjects	43	57	35	65
Age (years)				
mean	33 ± 8	26 ± 8	31 ± 7	28 ± 8
range	20 - 62	18 - 49	19 - 51	18 - 47

No major adverse effects attributable to the vaccine were reported by any of the subjects. Table 2 summarises all complaints reported by the participants according to the number of injections. The most common symptom was local discomfort at the injection site. The discomforts frequently noted were: itching, redness, pain and redness, swelling and pain. Most subjects complained of pain: 37.5% of subjects after the first dose of vaccine, 38.5% and 41.0% after the second and third doses, respectively. No side-effect lasted more than 72 hours, and most subsided within 24 hrs. No significant differences in the incidence, severity and type of adverse reaction were noted between the two different regimens of vaccine. During the thirty-six months of observation none of the subjects became HBsAg-positive or anti-HBc.

TABLE 2. - Local and general effects after yeast-derived hepatitis B vaccination.

Symptoms	Dose 1	Dose 2	Dose 3
N° Subjects	200	200	195
Itching	5.5%	4.0%	3.6%
Redness	4.0%	3.0%	2.6%
Swelling	1.0%	2.0%	1.5%
Pain	37.5%	38.5%	41.0%
slight	53.3%	54.5%	53.8%
mild	44.0%	44.2%	42.5%
severe	2.7%	1.3%	3.8%
Fever	3.5%	4.5%	5.6%
Headache	3.5%	1.0%	2.5%
Fatigue	1.5%	1.5%	1.0%
Gastrointestinal sym.	2.5%	0.5%	-

The seroconversion rates at various time intervals after the start of immunization are shown in figure 1. One month after the first vaccination dose 6% of subjects who were vaccinated with 10 mcg developed an anti-HBs response. The percentage of seroconversion rose to 21% one month later. By the sixth month the percentage of seroconversion observed was 49% and rose to 87% one month later after the third dose of vaccine. The percentage of responders one year after the beginning of the vaccination course was 84%. At later follow-ups, the seroconversion rates were 86%, 80% and 78% at 24, 36 and 48 months, respectively. The seroconverters who had received the 20 mcg dose were 2%, 28%, 57% and 91% at 1, 2, 6 and 7 months respectively. At the later follow-ups (12, 24, 36 and 48 months) the percentages observed were 90%, 88%, 85% and 84%, respectively.

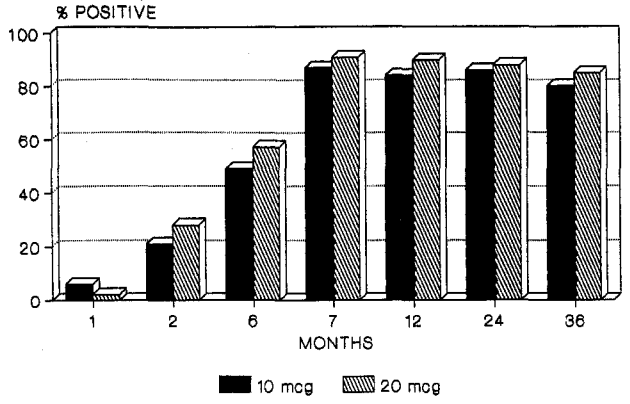

Figure 1. - Anti-HBs antibody responders to rDNA HB vaccine in 200 healthy adults.

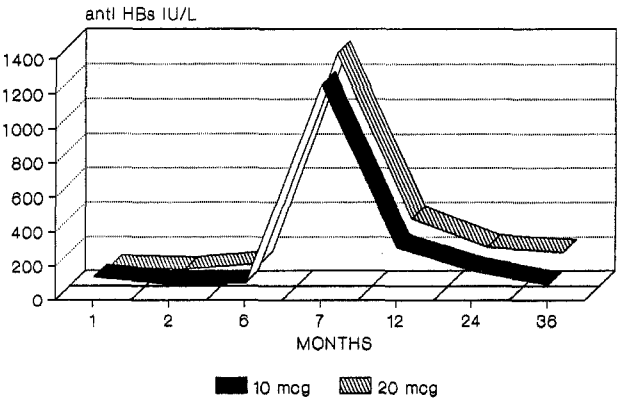

Figure 2. - Anti-HBs antibody response to HB vaccine in 200 healthy adults.

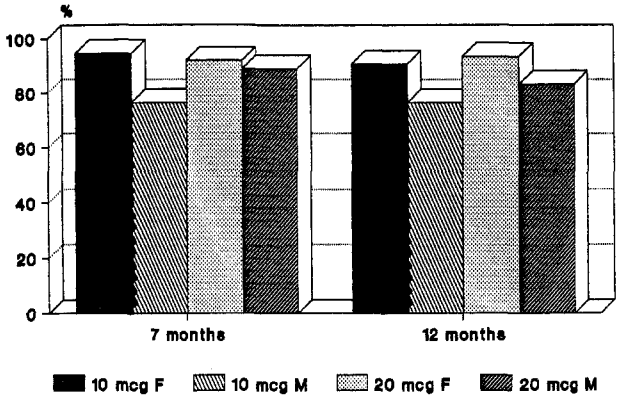

Figure 3. - Percentage of responders by sex and dosage of vaccine.

Figure 2 indicates the anti-HBs geometric mean titres (GMTs) reached at different time intervals according to the vaccine dose. The GMT in recipients of 10 mcg was 131 IU/l one month after the first dose, and 89 IU/l after the second dose. The titre rose from 94 IU/l at six months after the beginning of the vaccination course, to 1252 IU/l at seven months. The GMTs in the follow-up controls were 306 IU/l, 169 IU/l, 95 IU/l and 88 IU/l at 12, 24, 36 and 48 months, respectively. In the recipients of 20 mcg, the GMT increased from 89 IU/l one month after the first dose to 90 IU/l at the second monthly control. Subsequently, the GMTs increased from 124 IU/l to 1340 IU/l, at 6 and 7 months, and decreased from 387 IU/l to 218 IU/l, 199 IU/l and 164 IU/l at 12, 24, 36 and 48 months respectively. The percentage of responders and GMTs were higher in the recipients of the 20 mcg dose of vaccine.

However, no statistically significant differences were noted between the two groups either in terms of percentages of response or in serum antibody titres. The influence of sex on anti-HBs response was analyzed 7 and 12 months after the first injection of vaccine (Fig. 3). The anti-HBs seroconversion rate was always higher in females than in males, using 10 or 20 mcg doses of vaccine, but the difference was not statistically significant. In fact, the percentage of responders at 7 and 12 months among the recipients of the 10 mcg dose of vaccine was 94.6% and 90.7% in females, and 76.7% and 76.7% in males. Among the recipients of the 20 mcg dose of vaccine the percentage at 7 and 12 months was 92% and 93.5% in females, and 88.9% and 83.3% in males.

GMTs were also higher in women than in men (Fig. 4). In fact, at 7 months after the start of the vaccination course 7.5% of the women vaccinated with the 10 mcg dose of vaccine developed an anti-HBs concetration lower than 100 IU/l, 26.4% between 101 and 1000 IU/l and 66% up to 1000 IU/l. The response in men was 12.1%, 39.4% and 48.5%, respectively. Among the recipients of the 20 mcg dose of vaccine, the percentage in the three classes was 3.4%, 22.4% and 74.1% in women, and 21.9%, 40.6% and 37.5% in men, respectively.

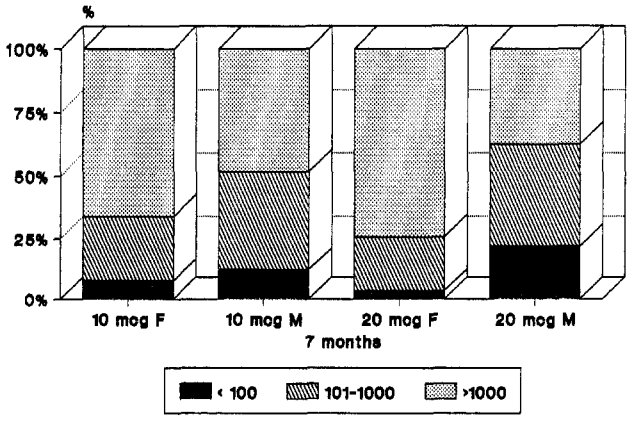

Figure 4. - Levels of anti-HBs concentration by sex and dosage of vaccine.

The relationship between sex, age and doses of vaccine in the non-responder subjects is reported in table 3. The percentage of non-responders was higher in men than in women, both in the recipients of the 10 mcg dose of vaccine and in those of the 20 mcg dose. The highest percentage of non-responders was observed in the 31-50 year age group. In fact, the percentage of non-responders in subjects younger than 30 years was 16.7% in males and 4.9% in females vaccinated with the 10 mcg dose of vaccine.

In the 31-50 year age group the percentages observed were 25% (males) and 6.7% (females). In the subjects who received the 20 mcg dose of vaccine the percentage of non-responders in the age group < 30 years was 10% in males and 4.9% in females while in the 31-50 year age group the percentage was 13.3% and 9.1%, respectively. None of these differences were statistically significant.

In order to evaluate the human response to yeast-derived impurities, the sera obtained prior to (T-0) and after a complete cycle of vaccination (T-7) were analyzed for anti-*Candida* antibodies and *Saccharomyces cerevisiae*. The percentage of subjects positive for anti-*Candida* prior to vaccination was 76.5% and 79.3% after vaccination, but 23.5% were negative before vaccination and 20.7% after (Fig. 5).

Figure 6 shows that the titres of anti-*Candida* antibodies were not modified after a complete course of vaccination.

These data indicate that the yeast-HB vaccine neither induced a reaction to yeast impurities nor raised the titre of anti-*Candida* antibodies in the subjects who resulted positive before the vaccination cycle.

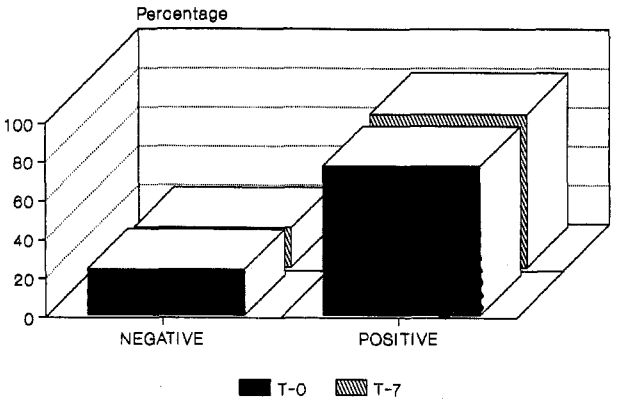

Figure 5. - Anti-*Candida* antibodies in receiving rDNA yeast-HB vaccine.

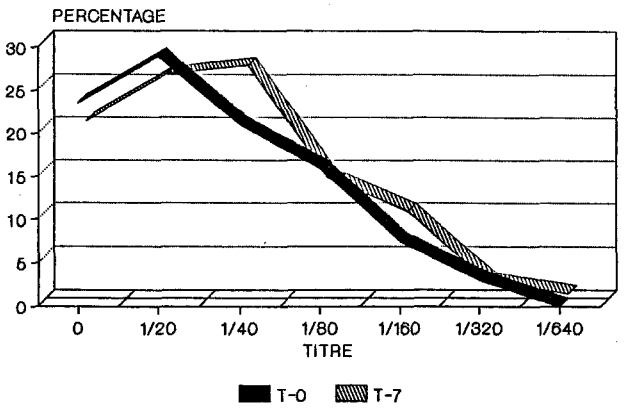

Figure 6. - Anti-*Candida* antibody titres in rDNA yeast-HB recipients.

TABLE 3. - Relationship between sex, age and dose of vaccine in non-responders to the rDNA vaccine at 7 months from the beginning of the vaccination course.

Age groups	10 mcg		20 mcg	
	M	F	M	F
< 30 years	3/18 (16.7%)	2/41 (4.9%)	2/20 (10.0%)	3/41 (4.9%)
31-50	6/24 (25.0%)	1/15 (6.7%)	2/15 (13.3%)	2/22 (9.1%)
> 51 years	1/1 (100 %)		0/1 -	

The *Saccharomyces cerevisiae* IgE antibodies in the sera of 200 subjects before vaccination and in 198 subjects one month after the complete vaccination cycle were negative (Class 0). Positive reaction of IgE (class 1, 2, 3, 4) were not observed at 0 and 7 month controls. Finally, no increase in the IgE antibody percentage and titres of anti-*Saccharomyces cerevisiae* expressed in PRU/ml was observed after the vaccination with yeast-derived vaccine.

These data indicate that the injection of the yeast vaccine did not modify the immunological profile of the anti-yeast specific IgE antibody (Table 4).

TABLE 4. - IgE anti-*Saccharomyces cerevisiae* antibodies in rDNA-HB recipients.

	10 mcg		20 mcg	
	PRE	POST	PRE	POST
Class 0	100/100	99/99	100/100	99/99
Class 1, 2, 3, 4	0/100	0/99	0/100	0/99
IgE PRU/ml				
0	78	68	74	72
0.001-0.090	11	17	19	15
0.091-0.350	11	14	7	12

CONCLUSIONS

The results of this study confirm the protective efficacy of the recombinant DNA yeast-derived hepatitis B vaccine in healthy adults. There were no significant differences in the severity, incidence or type of reaction between the 10 mcg and the 20 mcg doses of the yeast-derived vaccine. The safety of the vaccine is confirmed by the low incidence of adverse reactions which are usually mild, short-lived and similar to those observed with other vaccines. In fact, the general reactions were usually of a subjective type such as fatigue and headache. It was also reported that the incidence of adverse reactions generally decreased with each successive dose of vaccine.

It is difficult to explain the results obtained at the first control of those subjects vaccinated with the 10 mcg dose. In fact, the percentage of responders and antibody titres was higher than in subjects vaccinated with the 20 mcg dose. However, at subsequent controls the percentages and anti-HBs concentrations were always higher in subjects vaccinated with a 20 mcg dose, although no statistically significant differences were noted. The low percentage of responders observed at the first control does not provide reliable information for comparative statistical evaluation. The long-term follow-up study (48 months) revealed evidence of a prolonged protective effect of rDNA hepatitis B vaccine in healthy adults, as reported (1, 12).

The response to the vaccine seems to be related to the age and sex of the recipients as the anti-HBs response is higher in females and decreases with age. However, the differences observed were not statistically significant.

The study of the immune response to yeast-derived impurities, performed by analyzing the antibodies anti-*Candida* and anti-*Saccharomyces cerevisiae* before the start of the vaccination cycle and after the last dose of vaccine, showed that repeated injections did not modify the immunological status observed before the first dose of vaccine when using either 10 or 20 mcg doses. In conclusion, Amgen's rDNA yeast hepatitis B vaccine has been shown to be safe, highly immunogenic and well tolerated. The increased conversion rate and higher titres obtained using the 20 mcg dose of vaccine suggest that this dose of recombinant vaccine should be preferred in the prevention of HBV infection in healthy adults.

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