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SIMULTANEOUS VACCINATION AGAINST HEPATITIS A AND B: RESULTS OF AN OPEN, RANDOMIZED STUDY FROM THE OCCUPATIONAL HEALTH POINT OF VIEW

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Abstract.
Objectives: The primary objective of the study was to evaluate the immunogenicity of a combined hepatitis A and hepatitis B vaccine compared to the effect of the administration of two monovalent hepatitis A and B vaccines by quantitative measurements of the anti-HBs antibody levels at month 2 of the vaccination course. Secondary objectives were to assess immunogenicity of the vaccines investigated at other time points in the vaccination course (months 1, 6 and 7), including the comparison of quantitative measurements results as well as to evaluate seroconversion and seroprotection rates. **Materials and Methods:** The study was designed as open, controlled, randomized, monocentric study with two parallel groups. A total of 304 subjects, aged 18–45 years, were enrolled in the study. Group 1 received a combined hepatitis A and hepatitis B vaccine, group 2 was vaccinated concomitantly with a monovalent hepatitis A and hepatitis B vaccine. Seroprotection against hepatitis B was defined as anti-HBs antibody concentration ≥10 IU/l, and longterm seroprotection as ≥100 IU/l. **Results:** In all, 288 subjects completed the study. One month after the second vaccine dose, the percentage of subjects with anti-HBs antibody concentrations ≥100 IU/l as well as the seroconversion rate were significantly higher in group 1 than in group 2. Similarly, anti-HBs GMC was higher after combined vaccination one month after the third vaccine dose (1.684 IU/l vs. 528 IU/l; $p < 0{,}0001$). After the vaccination course, all individuals were anti-HAV positive. The overall incidence of symptoms (solicited/unsolicited, local/general) tended to be similar in each of the two groups. **Conclusions:** If health care personnel are exposed to hepatitis A and B virus, the combined vaccination should be preferred to the concomitant one, as this vaccination scheme induced earlier seroprotection against hepatitis B virus infection (≥10 IU/l and ≥100 IU/l).

Key words:
Combined hepatitis A and B vaccination, Health care professionals, Seroprotection, Immunogenicity

INTRODUCTION

Despite the availability of effective vaccines, hepatitis B virus infection is still one of the most frequent work-related infectious diseases in German health care occupational group. In the 2000 report of the German workers´ compensation board for health care professionals [1], hepatitis B virus infections accounted for 255 cases of notified work-related infectious diseases (total number, 1.080).

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A similar number (252; 23.3%) was due to hepatitis C virus (HCV) infection, whereas the figures for work-related hepatitis A virus (HAV) infections are generally very low in Germany (in 1999; 29 cases, 3%). However the figures of notified diseases varied widely, simultaneous exposure to hepatitis A and B virus has to be assumed for health care personnel, especially in departments of pediatric or infectious diseases and for laboratory workers [2]. Similar exposure to infectious agents may also occur in other occupational groups such as rescue forces or sewage and waste disposal workers.

In Germany, following Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work [3], vaccination has to be provided to exposed workers on a voluntary basis. In cases, where simultaneous exposure to hepatitis A and B virus may occur, vaccination against both infectious agents should be recommended. Several vaccines against hepatitis A and B are commercially available in Germany. Besides monovalent vaccines against hepatitis A or B, which have been proven to be safe and efficacious [4–7], a combined hepatitis A and B vaccine, shown to be highly immunogenic and safe, has been available for several years [8,9].

The study focused on the immunogenicity, safety and reactogenicity of the combined hepatitis A and B vaccine compared to the effect of the concomitant administration of a monovalent hepatitis A and monovalent hepatitis B vaccine. Antigen concentration per dose (HAV and HBsAg) differed in both vaccination schemes. Being aware how it is important that workers at a high occupational exposure be reliably protected against hepatitis B as soon as possible after vaccination, the primary focus of our investigation was to assess which hepatitis B vaccine induced higher seroprotection rates one month after the second vaccination.

Table 1. Inclusion and exclusion criteria for enrolment

Inclusion criteria	Exclusion criteria*
Age 18–45 years	Positive results for anti-HAV, anti-HBs or anti-HBc antibodies at screening
Good physical condition (clinical examination and history)	History of any vaccination against hepatitis A and/or hepatitis B
Avoidance of becoming pregnant: female participants: contraceptive program for at least 2 months before entry	Elevated serum liver enzymes: ALT, AST, GT (>2 fold the upper normal laboratory values)
	History of significant and/or persisting hematologic, hepatic, renal, cardiac, respiratory or neurological/psychiatric disease
Written informed consent	Any acute disease at the moment of entry
	Chronic alcohol or drug consumption
	Hepatomegaly, right upper quadrant abdominal pain or tenderness
	Any chronic drug treatment, including any treatment with immunosuppressive drugs, which in the investigator's opinion precludes inclusion into the study
	History of allergic disease to be stimulated by any component of the vaccine
	Positive pregnancy test
	Simultaneous participation in any other clinical trial 30 days before entering the study and during study participation
	Simultaneous administration of any other vaccine within 4 weeks before study start up to month 1 four weeks pre- and post booster vaccination
	Administration of immunglobulins in the preceding 3 months within the first 3 months of the study four weeks pre- and post booster vaccination

* During the study applicable as elimination criteria.

MATERIALS AND METHODS

The study was designed as open, controlled, randomized, monocentric study with two parallel groups. Healthy male and female subjects, aged 18–45 years, were enrolled in the study if they satisfied the inclusion and exclusion criteria (Table 1). All vaccinees had to be seronegative for anti-HBc, anti-HBs, and anti-HAV.

Group 1 received a combined hepatitis A and hepatitis B vaccine (TWINRIX® ADULT, GlaxoSmithKline), and is

Table 2. Composition of vaccines

	Combined vaccination group (group 1)	Concomitant vaccination group (group 2)	
	Hepatitis A/Hepatitis B	Hepatitis A	Hepatitis B
Volume in each dose	1 ml	1 ml	1 ml
Inactivated hepatitis A virus	At least 720 ELISA units	50 units	–
Hepatitis B surface antigen Recombinant HBsAg	20 µg	–	At least 10 µg
Aluminium as salt	0.5 mg	0.45 mg	Present

Note: Hepatitis A antigen units are not comparable between manufacturers.

referred to as the "combined vaccination group". Group 2 received a monovalent hepatitis A (VAQTA®, Aventis Pasteur MSD) and hepatitis B vaccine (GEN H-B-VAX®, Chiron Behring) and is concomitantly referred to as the "concomitant vaccination group". All vaccines were commercially available in Germany at the time of the study. The composition of vaccines is shown in Table 2.

The combined hepatitis A and hepatitis B vaccine and the monovalent hepatitis B vaccine were supplied as monodose vials. The hepatitis A vaccine was supplied as a monodose pre-filled syringe. Table 3 summarizes the presentation of vaccines, sites of their administration and the vaccination schedules for the two groups.

The protocol defined the intervals between study visits. Besides vaccination visits at month 0, 1 and 6, additional blood sampling visits were dated with the study participants (Table 4).

All serum samples were stored at –20°C until analysis. Serological testing for specific antibodies was performed using ELISA technique by working with an automatic system (ETILAB, DiaSorin). The following serological analysis were done, using assays manufactured by DiaSorin: anti-HBs (ETI-AB-AUK-3, cut-off: 1 IU/), anti-HBc (ETI-AB-COREK-2) and anti-HAV (ETI-AB-HAVK-3, cut-off: 20 IU/l). Biochemical assays were performed in a contractor's

Table 3. Vaccine presentation, sites of its administration and vaccination schedule

Group	Vaccine	Presentation	Site of administration	Schedule
1	Combined hepatitis A and hepatitis B vaccine	Monodose vial	Intramuscular injection in the deltoid region of the left arm	0, 1, 6 months
2	Hepatitis A vaccine	Pre-filled syringe	Intramuscular injection in the deltoid region of the right arm	0, 6 months
	Hepatitis B vaccine	Monodose vial	Intramuscular injection in the deltoid region of the left arm	0, 1, 6 months

Table 4. Protocol-defined study visits and serology plan

Vaccination		Blood sampling time point	Immunological and biochemical assays
	Screening	Day – 14 to day 0	Anti-HBs, anti-HBc, anti-HAV, ALT, AST, γGT
Vaccination 1	Pre-vaccination 1	Day 0	Anti-HBs, anti-HAV, ALT, AST, γGT
Vaccination 2	Post-vaccination 1	Month 1	Anti-HBs, anti-HAV, ALT, AST, γGT
	Post-vaccination 2	Month 2	Anti-HBs, anti-HAV, ALT, AST, γGT
Vaccination 3	Pre-vaccination 3	Month 6	Anti-HBs, anti-HAV, ALT, AST, γGT
	Post-vaccination 3	Month 7	Anti-HBs, anti-HBc, anti-HAV, ALT, AST, γGT

laboratory, using standardized, validated procedures with adequate controls (ALT, AST: German Association for Clinical Chemistry, γGT: Szasz).

Reactogenicity variables were assessed using diary cards, distributed among the subjects to record local and general signs and symptoms (both solicited and unsolicited) or illnesses occurring during a 4-day (day 0 to day 3) follow-up period. Solicited symptoms were defined in the study protocol and were actively asked for. Unsolicited symptoms comprised any adverse event reported in addition to those solicited during the clinical study. Diary cards were checked by the investigator at the subsequent visit. The intensity of all symptoms was scored. All solicited local (injection site) reactions were considered to have a causal relationship to vaccination. General adverse events were assessed whether they were causally related to vaccination. A serious adverse effect was defined as any untoward medical occurrence that resulted in death or was life threatening, induced persisting or severe disability/incapacity, required hospitalization or prolongation of existing hospitalization, or was manifested by a congenital anomaly/birth defect of the offspring of the study subject. Subjects who became pregnant during the study period did not receive additional doses of vaccine.

The study was conducted according to Good Clinical Practice (GCP) and in accordance with the Declaration of Helsinki as amended in Somerset West, Republic of South Africa, October 1996. The protocol and statement of informed consent were approved by the responsible ethics review board. Written informed consent was obtained from the subjects prior to entry into the study.

Sample size

The objective of the study was to assess the immunogenicity (primarily one month after second vaccination), safety, and reactogenicity of the combined hepatitis A and hepatitis B vaccine compared to the effect of the concomitant administration of two monovalent vaccines. For assessing immunogenicity, the percentage of subjects with anti-HBs antibody concentration ≥10 IU/l (seroprotection, SP) and ≥100 IU/l (long-term seroprotection, LT-SP) was calculated besides the quantitative measurement of anti-HBs antibody levels. In accordance with a former study [10], sample size estimation was based on a detectable difference of 15% between both treatment groups (combined vaccination: 55%; concomitant vaccination: 40%) with respect to the primary objective when using a one-sided Chi-square test with $\alpha = 5\%$ and $\beta = 20\%$. Thus, for detecting differences in LT-SP, a sample of 136 participants per group was appropriate (program NQuery 2.0). Since a drop-out rate of 10% was assumed, the number of subjects enrolled per group was 152. Female participants had to agree to avoid becoming pregnant during the study period and had to be on a contraceptive program for at least two months before entry. Good physical condition of the subjects was established by clinical examination and medical history taken at the time of entry. Screening investigations, including blood analysis, were performed on 377 subjects. Due to exclusion criteria, 73 subjects (40 males and 33 females) could not be enrolled in the study. The included participants were allocated to one of the two treatment groups by blockwise-randomization. Randomization was performed by PROC PLAN of the SAS® program, Version 6.12, running on Windows NT platform.

Statistical analysis

The primary endpoint of the statistical analysis was the anti-HBs antibody concentration at month 2 (one month after the second vaccine dose) and the LT-SP rate at the same time point. LT-SP was defined as the percentage of subjects with anti-HBs antibody concentration ≥100 IU/l. Secondary endpoints were anti-HAV and anti-HBs antibody concentrations at any other observation time, especially before and after the third vaccine dose. Analysis was done by calculating the following derived variables: seroconversion (SC) rate (percentage of subjects with anti-HBs concentration ≥1 IU/l or anti-HAV concentration ≥20 IU/l), SP rate (percentage of subjects with anti-HBs concentration ≥10 IU/l) and geometric mean concentration (GMC). The GMC calculations were performed by taking the anti-log concentration transformations of the mean of the log-transformed anti-HBs and anti-HAV antibody concentrations. The antibody concentrations below the assay cut-off value were arbitrarily given half of the cut-off value.

The variation of anti-HBs and anti-HAV antibody concentrations within each group was summarized by geometric coefficient of variation (GCV). At each time point and for each treatment group, anti-HBs and anti-HAV antibody SC rates and anti-HBs antibody SP rates were tabulated with the 95% confidence interval (CI). Antibody concentrations were summarized by GMCs with the 95% CI. Reverse cumulative curves (RCCs) were plotted for anti-HBs antibody concentrations at months 1, 2, 6, and 7 and anti-HAV antibody concentrations at months 1 and 7.

SP rates for anti-HBs antibodies one month after the second vaccination (month 2) were calculated for both groups and compared using one-sided Chi-square test with $\alpha = 5\%$ to assess whether group 1 was superior to group 2. Additional descriptive analyses were performed on the age (subjects aged <40 years and aged ≥40 years), gender, body mass index (BMI) (four ranges: 18.5–24.9, 25–29.9, 30–39.9, and ≥40) [11], and smoking habit status (smokers vs. past- and non-smokers) in both groups. The effects of demographic variables (age, gender, BMI, smoking habit status) on the immune response were determined using regression analysis with log concentration as a dependent variable. Treatment effect was included as a dependent variable in the regression analysis, resulting in the model of treatment effect with adjustments for covariates. In order to reconfirm the results of the regression analysis, formal inference-based recursive modeling (FIRM) tool was used.

Two cohorts were defined: the total cohort and according-to-protocol (ATP) cohort. The total cohort included all vaccinated subjects for whom demographic and reactogenicity data were available. The ATP cohort for the immunogenicity analysis included all the subjects who met all eligibility criteria, complied with the procedures defined in the protocol and for whom data concerning immunogenicity endpoint measures were available. An analysis of reactogenicity for the total cohort was performed for solicited (local/general) and unsolicited symptoms. The incidence of symptoms was calculated according to per-dose and per-subject analyses. The overall incidence of local, general and both local and general symptoms were calculated. The percentage, with exact 95% CI of subjects reporting each individual solicited symptom during a 4-day follow-up period after vaccination (day 0 to day 3), was tabulated in addition to intensity and relationship. The incidence, intensity and relationship of individual unsolicited symptoms during a 30-day (day 0 to day 29) follow-up period after vaccination were tabulated.

RESULTS

Characteristics of the study population

A total of 304 subjects (152 in each group) were enrolled as planned in the protocol. As 16 subjects (8 per group) dropped out from the study, only 288 completed the study. Further 32 subjects had to be eliminated from the ATP cohort for different reasons (e.g., receiving vaccines forbidden in the protocol, protocol violations with regard to vaccination or blood-sampling schedule, pregnancy). Thus, the number of subjects in the ATP cohort for immunogenicity was 256, including 131 in the combined vaccination group (group 1) and 125 in the concomitant vaccination group (group 2). All the subjects were caucasian. There was no significant difference in demographic characteristics (age, gender, BMI, smoking habit status) between the total cohort and the ATP cohort as well as between both treatment groups (Table 5).

The mean age in the ATP cohort was 28.5 years with a standard deviation of 7.72 years. The male/female ratio was 1:5 (154/102) in the ATP cohort, 1:3 in group 1 and 1:7 in group 2 (no significant difference). The proportion of current smokers and past or non-smokers was 38.9% in group 1 and 39.2% in group 2.

Immunogenicity analysis – anti-HBs antibody response

At all time points investigated, SC (anti-HBs ≥1 IU/l), SP (anti-HBs ≥10 IU/l) and LT-SP (anti-HBs ≥100 IU/l) rates were higher in group 1 than in group 2. The same was true for GMC and GCV (Table 6).

One month after the administration of the second vaccine dose (month 2), the SC rates for anti-HBs antibodies were significantly higher in the combined vaccination group than in group 2 (85.2% vs. 70.1%; $p < 0.01$, one-sided Chi-square test).

Table 5. Characteristics of the study population

Characteristics	Parameters or categories	Combined vaccination group (1) (N = 131)		Concomitant vaccination group (2) (N = 125)		ATP cohort (N = 256)		Total cohort (N = 304)	
		n	%	n	%	n	%	n	%
Age (years)	Mean	28.7	–	28.4	–	28.5	–	29.0	–
	SD	7.66	–	7.81	–	7.72		7.84	–
	Median	27	–	26	–	26		27	–
	Minimum	18	–	18	–	18		18	–
	Maximum	45	–	45	–	45		45	–
Gender	Male	75	57.3	79	63.2	154	60.2	180	59.2
	Female	56	42.7	46	36.8	102	39.8	124	40.8
Height (cm)	Mean	175.4	–	176.3	–	175.8	–	175.5	–
	SD	9.49	–	9.05	–	9.30	–	9.20	–
	Median	176	–	176	–	176	–	176	–
Weight (kg)	Mean	77.0	–	77.5	–	77.3	–	76.7	–
	SD	14.72	–	15.15	–	14.90	–	15.20	–
	Median	75	–	75	–	75	–	75	–
BMI (kg/m^2)	Mean	25.0	–	24.8	–	24.9	–	24.8	–
	SD	4.01	–	3.89	–	3.9	–	4.0	–
	Median	24.9	–	24.62	–	24.7	–	24.6	–
Smoking status	Current smokers	51	38.9	49	39.2	100	39.6	115	37.8
	Past smokers	16	12.2	12	9.6	28	10.9	34	11.2
	Non-smokers	64	48.9	64	51.2	128	50.0	155	51.0

SP rates were higher in group 1 than in group 2 (61.2% vs. 53.2%). However, the one-sided Chi-square test did not reveal a statistically significant difference ($p = 0.0988$). The percentage of subjects with anti-HBs antibody concentrations ≥100 IU/l (LT-SP) was significantly higher in group 1 (13.2%) than in the concomitant vaccination group (4.8%) ($p < 0.05$, one-sided Chi-square test). The results of the inferential analysis indicating the treatment differences between the two groups are summarized in Table 7. With respect to the level of anti-HBs antibody

Table 6. Anti-HBs antibody response in both groups

Group	Timing	No. of subjects	SC		SC 95% CI		SP		GMC (IU/l)	GMC 95% CI		GCV	LT-SP	
			n	%	LL	UL	n	%		LL	UL		n	%
Combined vaccination group (1)	Post-dose 1 (month 1)	130	45	34.6	26.4	100.0	31	23.8	1.72	1.2	2.4	6.30	4	3.1
	Post-dose 2 (month 2)	129	110	85.2	77.9	100.0	79	61.2	14.17	9.9	20.1	7.73	17	13.2
	Pre-dose 3 (month 6)	131	127	96.9	92.3	100.0	124	94.7	106.8	81.0	140.6	3.41	74	56.5
	Post-dose 3 (month 7)	126	124	98.4	94.3	100.0	123	97.6	1683.95	1215.5	2332.8	5.43	120	95.2
Concomitant vaccination group (2)	Post-dose 1 (month 1)	125	25	20.0	13.3	100.0	10	8.0	0.83	0.6	1.0	1.51	0	0.0
	Post-dose 2 (month 2)	124	87	70.1	61.2	100.0	66	53.2	6.56	4.6	9.2	6.28	6	4.8
	Pre-dose 3 (month 6)	124	112	90.3	83.7	100.0	98	79.0	27.22	20.2	36.5	3.86	27	21.8
	Post-dose 3 (month 7)	122	121	99.1	95.5	100.0	120	98.4	528.18	398.1	700.7	3.32	108	88.5

Table 7. Inferential analysis: difference between the two groups at month 2

Group	No. of subjects (%)	Group	No. of subjects (%)	Inference		
				Difference	Value	95% CI
Seroconversion rate (anti-HBs ≥1 IU/l)						
Group 1	129 (85.3)	Group 2	124 (70.2)	Group 2 – Group 1	-15.1	-26.9 -3.7
Seroprotection rate (anti-HBs ≥10 IU/l)						
Group 1	129 (61.2)	Group 2	124 (53.2)	Group 2 – Group 1	-8.0	-21.0 4.6
Longterm-seroprotection rate (anti-HBs ≥100 IU/l)						
Group 1	129 (13.2)	Group 2	124 (4.8)	Group 2 – Group 1	-8.3	-18.1 0.2

concentration, anti-HBs GMC in group 1 was two times higher than in group 2 (14.17 IU/l vs. 6.56 IU/l; $p < 0.01$) (Fig. 1). With regard to the influence of age, gender, BMI and smoking status on immunogenicity at month 2, there was a significant effect of age ($p < 0.0001$) and gender ($p < 0.01$) on anti-HBs GMC; GMC was decreasing with increasing age, and in females it was higher than in males, also individuals with higher BMI showed lower GMC. Similar differences were revealed with regard to SC and SP rates in both groups.

In contrast to the findings after the second vaccine dose, SC and SP rates for anti-HBs antibodies were higher in group 2 than in group 1 at month 7 (one month after the third vaccine dose) (SC: 99.1% vs. 98.4%, SP: 98.4% vs. 97.6%). Yet, LT-SP rates were higher in group 1 than in group 2 at that time point (LT-SC: 95.2% vs. 88.5%), which was reflected by a lower proportion of "low-responders" (subjects with anti-HBs antibody concentration of 10–99 IU/l) (group 1: 2.4%, group 2: 9.8%) ($p < 0.05$) and higher

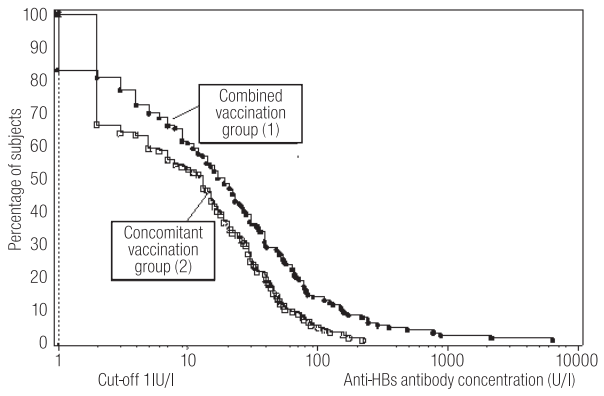

Fig. 1. Reverse cumulative curves (RCC) for anti-HBs antibody concentration at month 2, the combined vaccination group (n = 129 subjects) and the concomitant vaccination group (n = 124 subjects)

GMC in group 1 than in group 2 (1683.95 IU/l vs. 528.18 IU/l) ($p < 0.0001$). Like at month 2, there was a significant effect of age ($p < 0.01$) and BMI ($p < 0.001$) on anti-HBs GMC. GMC was decreasing with increasing age and BMI. There was no significant effect of smoking status on the anti-HBs antibody GMCs at either time point, month 2 and month 7.

Immunogenicity analysis – anti-HAV antibody response

Anti-HAV antibody response was analyzed with regard to seroconversion rates (SC, anti-HAV ≥20 IU/l), GMC and GCV. Whereas one month after the first vaccine dose, SC was lower in the combined vaccination group than in the concomitant vaccination group (90.0% vs. 97.6%, $p < 0.05$, two-sided Chi-square test), this value was slightly higher at month 2 (98.4% vs. 97.5%, no significant difference). At that time point, anti-HAV GMC for group 1 and group 2 were 270.75 IU/l and 142.76 IU/l, respectively. At month 7 all subjects were seroconverted for anti-HAV antibodies. One month after the last vaccine dose, there was a significant effect of age ($p < 0.0001$), BMI ($p < 0.01$) and smoking status ($p < 0.05$) in terms of anti-HAV antibody GMC. GMC was decreasing with increasing age and BMI, and non-smokers showed higher anti-HAV GMC than smokers. There was also a significant difference in terms of treatment groups ($p < 0.05$), wherein the combined vaccination group showed higher GMC than the concomitant vaccination group (Table 8).

An analysis of the total cohort (including p-values obtained from one-sided Chi-square test) revealed similar results to those obtained for the ATP cohort.

Table 8. Seroconversion rates and GMCs for anti-HAV antibodies by age ranges

Group	Age range	Timing	No. of subjects	SC	SC 95% CI		GMC (IU/l)	GMC 95% CI	
				No. of subjects (%)	LL	UL		LL	UL
Combined vaccination group (1)	< 40 years	Post-dose 1 (month 1)	113	103 (91.2)	84.3	95.7	91.32	75.1	110.9
		Post-dose 2 (month 2)	112	110 (98.2)	93.7	99.8	281.58	239.0	331.6
		Pre-dose 3 (month 6)	114	113 (99.1)	95.2	100.0	184.00	151.8	222.9
		Post-dose 3 (month 7)	109	109 (100.0)	96.7	100.0	2940.53	2497.1	3462.6
	≥ 40 years	Post-dose 1 (month 1)	17	14 (82.4)	56.5	96.2	68.80	38.0	124.3
		Post-dose 2 (month 2)	17	17 (100.0)	80.5	100.0	209.09	145.3	300.8
		Pre-dose 3 (month 6)	17	17 (100.0)	80.5	100.0	151.38	107.8	212.4
		Post-dose 3 (month 7)	17	17 (100.0)	80.5	100.0	1617.10	1256.1	2081.8
Concomitant vaccination group (2)	< 40 years	Post-dose 1 (month 1)	106	104 (98.1)	93.4	99.8	173.94	142.6	212.0
		Post-dose 2 (month 2)	105	104 (99.0)	94.8	100.9	157.40	130.4	189.9
		Pre-dose 3 (month 6)	105	102 (97.1)	91.9	99.4	152.31	125.9	184.1
		Post-dose 3 (month 7)	103	103 (100.0)	96.5	100.0	2500.29	2154.2	2901.9
	≥ 40 years	Post-dose 1 (month 1)	19	18 (94.7)	74.0	99.9	108.22	65.7	178.0
		Post-dose 2 (month 2)	19	17 (89.5)	66.9	98.7	83.24	51.0	135.7
		Pre-dose 3 (month 6)	19	17 (89.5)	66.9	98.7	74.51	46.8	118.6
		Post-dose 3 (month 7)	19	19 (100.0)	82.4	100.0	1275.59	889.6	1828.8

Safety and reactogenicity analyses

Data on solicited signs and symptoms reported following 904 doses (451 in group 1 and 453 in group 2) were documented on diary cards. An analysis of the overall incidence of symptoms was done in the total cohort. According to the per-dose analysis, the incidence of any symptom (solicited/unsolicited, local/general) was similar for both groups. This was evident from the results of Fisher´s exact test, which did not reveal any statistically significant difference at a 0.05 significance level. All solicited local symptoms were considered to have a causal relationship to vaccination. Fisher´s exact test revealed that there was no statistically significant difference in the percentage of subjects reporting local symptoms between the two groups (a 0.05 significance level). Pain at the injection site was the most frequently reported solicited local symptom in both

Table 9. Incidence of solicited local symptoms (total and grade "3") reported during a 4-day follow-up period after vaccination (total cohort)

		Group 1			Group 2					
					Hepatitis A vaccine			Hepatitis B vaccine		
		N (%)	95% CI		N (%)	95% CI		N (%)	95% CI	
Per-dose analysis		n = 441			n = 445					
Pain	Total	182 (41.3)	36.6	46.0	82 (18.4)	14.9	22.3	122 (27.4)	23.3	31.8
	Grade "3"	2 (0.5)	0.1	1.6	3 (0.7)	0.1	2.0	4 (0.9)	0.2	2.3
Redness	Total	53 (12.0)	9.1	15.4	39 (8.8)	6.3	11.8	54 (12.1)	9.2	15.5
	Grade "3"	1 (0.2)	0.0	1.3	2 (0.4)	0.1	1.6	3 (0.7)	0.1	2.0
Swelling	Total	24 (5.4)	3.5	8.0	14 (3.1)	1.7	5.2	25 (5.6)	3.7	8.2
	Grade "3"	1 (0.2)	0.0	1.3	1 (0.2)	0.0	1.2	1 (0.2)	0.0	1.2
Per-subject analysis		n = 150			n = 152					
Pain	Total	94 (62.7)	54.4	70.4	63 (41.4)	33.5	49.7	78 (51.3)	43.1	59.5
	Grade "3"	2 (1.3)	0.2	4.7	3 (2.0)	0.4	5.7	4 (2.6)	0.7	6.6
Redness	Total	39 (26.0)	19.2	33.8	30 (19.7)	13.7	27.0	34 (22.4)	16.0	29.8
	Grade "3"	1 (0.7)	0.0	3.7	1 (0.7)	0.0	3.6	2 (1.3)	0.2	4.7
Swelling	Total	21 (14.0)	8.9	20.6	10 (6.6)	3.2	11.8	20 (13.2)	8.2	19.6
	Grade "3"	1 (0.7)	0.0	3.7	1 (0.7)	0.0	3.6	1 (0.7)	0.0	3.6

groups. The majority of the symptoms resolved within a 4-day follow-up period after vaccination. The Wilcoxon rank-sum test revealed that there was no statistically significant difference between the two groups with respect to incidence of the solicited local symptoms (Table 9).

The solicited general symptoms reported by the subjects of the two groups were similar; Fisher's exact test revealed that there was no statistically significant difference between them. Solicited general vaccination-related symptoms were reported as follows (per-dose analysis): fatigue ≈10%, gastrointestinal symptoms ≈2.5%, headache ≈6%, and fever ≈0.5%. With respect to unsolicited symptoms, the safety profile for both groups was similar (no statistically significant difference in Fisher's exact test). A total of seven subjects (3 in group 1 and 4 in group 2) reported serious adverse events during the study period, but none of them were considered to have a causal relationship to vaccination.

With regard to biochemical analysis, ALT, AST and γGT concentrations were tabulated for the ATP cohort. There was no statistically significant difference between both treatment groups at the time points investigated (two-sided Mann-Whitney test). Comparing the pre- and post-vaccine concentrations at single time points, the Wilcoxon rank-sum test (two-sided) revealed significant differences in the enzyme concentrations only for AST (pre-vaccine 3 > post-vaccine 3) and γGT (pre-vaccine 1 > post-vaccine 1; pre-vaccine 2 < post-vaccine 2) without any difference between the groups. There was no evidence to prove a systematic increase in liver enzymes due to vaccination. High fluctuations of the enzyme concentrations were documented, especially for γGT (Table 10, Fig. 2).

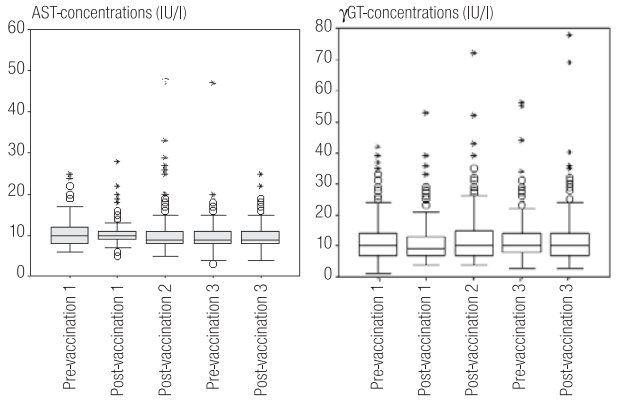

Fig. 2. AST- and γGT-concentrations in the ATP cohort during vaccination course in both groups.

DISCUSSION

In the present study, the immunogenicity, safety, and reactogenicity of a combined hepatitis A and hepatitis B vaccine (TWINRIX® ADULT, GlaxoSmithKline) compared to the effect of the concomitant administration of a hepatitis A vaccine (VAQTA®, Aventis Pasteur MSD) and a hepatitis B vaccine (GEN H-B-VAX®, Chiron Behring) were evaluated in healthy volunteers, aged 18–45 years.

Consistent with the results of another investigation in young adults [12], one month after the second vaccine dose, the

Table 10. Biochemical analyses: concentration of enzymes in both treatment groups

Group	Timing	No. of subjects	ALT (IU/l)				AST (IU/l)				γGT (IU/l)			
			Mean	SD	95% CI		Mean	SD	95% CI		Mean	SD	95% CI	
					LL	UL			LL	UL			LL	UL
Combined vaccination group (1)	Pre-dose 1	131	12.30	6.26	11.22	13.39	10.04	2.56	9.59	10.48	11.97	7.35	10.70	13.24
	Post-dose 1	130	12.15	6.16	11.08	13.22	10.09	2.68	9.63	10.56	11.32	6.14	10.25	12.38
	Post-dose 2	129	12.95	8.09	11.54	14.35	10.26	3.97	9.56	10.95	12.01	7.44	10.71	13.30
	Pre-dose 3	131	11.77	7.30	10.51	13.03	10.59	10.8	8.73	12.46	11.79	7.43	10.51	13.08
	Post-dose 3	126	10.97	6.04	9.90	12.03	9.26	2.56	8.81	9.71	12.14	8.28	10.68	13.60
Concomitant vaccination group (2)	Pre-dose 1	125	12.90	6.39	11.80	14.07	10.59	3.04	10.05	11.13	11.83	6.97	10.60	13.07
	Post-dose 1	125	12.65	7.11	11.39	13.91	10.38	2.97	9.86	10.91	11.61	7.17	10.34	12.88
	Post-dose 2	124	14.10	9.44	12.43	15.78	10.73	3.63	10.09	11.38	12.77	8.73	11.22	14.33
	Pre-dose 3	124	12.68	12.9	10.37	14.99	10.24	4.38	8.73	12.46	12.00	7.49	10.67	13.33
	Post-dose 3	122	11.61	7.54	10.26	12.97	9.87	3.50	9.24	10.50	12.14	8.76	10.57	13.71

anti-HBs seroconversion and seroprotection rates (≥10 IU/l) were higher in the combined vaccination group than in the concomitant vaccination group. An 8% difference in terms of SP between the two treatment groups was observed (61.2% vs. 53.2%). A similar difference could be documented for the longterm-seroprotection rate (≥100 IU/l) (13.2% vs. 4.8%; $p < 0.05$). This corresponded with the higher SP (94.7% vs. 79.0%) and LT-SP rates (56.5% vs. 21.8%) on the day of the third vaccine dose (month 6) in group 1. Similarly, one month after the third vaccine dose, the LT-SP rate was higher in the combined vaccination group (95.2% vs. 88.5%), whereas SC (99.1% vs. 98.4%) and SP rates (98.4% vs. 97.6%) were higher in the concomitant vaccination group. Anti-HBs antibody concentration was higher in the combined vaccination group than in group 2 at all time points investigated, resulting in a difference of more than 1.000 IU/l (1683.95 IU/l vs. 528.18 IU/l; $p < 0.0001$) one month after the third vaccine dose. The differences observed could well be explained by the doses of vaccine antigen administered in both vaccination schemes (combined vaccine: 20 μg HBsAg, monovalent vaccine: 10 μg HBsAg). In earlier studies of the effect of various antigen concentrations (administration of different vaccine formulations [13–17] or different concentrations of the same vaccine [17–22]), a higher dose of HBsAg showed a positive and partly significant effect on SC, SP and/or antibody GMC with the highest impact on GMC. This correlation seemed to be less pronounced comparing different concentrations of the same vaccine: differences in seroconversion rates and antibody concentration could be observed during (months 1, 2 or 3) but not after completing the vaccination course [22–24].

When analyzing the level of antibody concentration after the primary course of vaccination, it has to be borne in mind that vaccination experts in several countries have decided to only observe the seroconversion and seroprotection criteria, but not the antibody concentration level above 100 IU/l with regard to the evaluation of protective efficacy of the primary vaccination course. This may be explained by considerable discrepancy between antibody concentrations measured by different laboratories or available tests. Yet, several investigations have revealed that the persistence of anti-HBs was closely related to the peak anti-HBs response of the vaccine [22,25–27] as well as to the time elapsed since vaccination [22,28–30].

The criteria for seroprotection generally consider the level above 10 IU/l to be protective [31]. Some countries have adopted a higher reference level (e.g., 20 IU/l in Austria [32] or 100 IU/l in the UK [33], Germany [34,35], and Switzerland [36]). To confirm these differences in the present study, two levels of anti-HBs concentration were investigated: the concentration of anti-HBs ≥10 IU/l was referred to as seroprotection, the concentration of ≥100 IU/l as longterm-seroprotection. In Germany, serological testing is recommended after primary vaccination course for all individuals at an increased risk of infection (e.g., health care personnel). Similarly, serological control has to be provided for individuals reporting contamination with infectious materials, as it may occur for example in needlestick injuries [37–39]. In all these cases, booster vaccination is recommended if the anti-HBs concentration is below 100 IU/l [34], thus enlarging the recommendations of the European Consensus Group on Hepatitis B Vaccination [40]. HBV infection is still considered to be the most important occupational disease affecting health service workers in Germany [41] and in many other countries [28,42]. In order to prevent occupationally acquired HBV infections as well as their nosocomial spread to patients [31,43,44], hepatitis B vaccination in health care personnel should be performed with the aim to induce effective seroprotection as early as possible. As exposure to HBV coincides with the risk of occupational HAV infection in many fields of health care [45], simultaneous vaccination against both kinds of viral hepatitis will be offered to those exposed. Against the background of the presented results, the combined vaccine should be preferred to the concomitant vaccination scheme for this occupational health indication as it induced higher anti-HBs seroconversion, seroprotection and longterm-seroprotection rates than those produced by the concomitant vaccination. This will lead the an additional advantage, namely a wider acceptance of only three injections with the combined vaccine in total as compared to five injections in concomitant vaccination scheme.

With regard to early protection against hepatitis A virus infection, the concomitant vaccination showed advantages one month after the first vaccine dose (97.6% vs. 90.0%, $p < 0.05$). This was attributed to the fact that the antigen concentration in the monovalent hepatitis A vaccine was higher than in the combined vaccine. After the second dose of the combined vaccine, anti-HAV antibody seroconversion rates were similar in both groups. In contrast to the occupational health point of view outlined above, it may be efficient in travel medicine to offer the concomitant vaccination in all cases, where protection against hepatitis A has to be ensured as early as possible. In case of utilization of the combined vaccine, at least two vaccine doses should be administered before departure.

At the end of the vaccination course, there was no significant difference with regard to LT-SP against hepatitis B. Especially, the rate of so called anti-HBs non-responders (anti-HBs antibody concentration <10 IU/l) did not differ significantly (group 1, 1.6%, group 2, 1%). Yet, anti-HBs antibody and anti-HAV antibody GMCs were higher and the proportion of "low-responders" (anti-HBs antibody concentration 10–99 IU/l) was lower in the combined vaccination group (group 1, 2.3%, group 2, 9.8%). The respective figures of low- and non-responders and anti-HBs GMCs corresponded well with the results of a variety of studies on immunogenicity of monovalent hepatitis B vaccines and the simultaneous application of a hepatitis A vaccine as either combined or concomitant vaccine [13–24,46–55]. In contrast, evaluation of another combined vaccine on the basis of VAQTA® and Recombivax HB/H-B-Vax II®, administered in a two-dose scheme, showed insufficient production of anti-HBs antibodies four weeks after the second injection [56].

The finding on significant effect of age, gender, BMI, and smoking status on both anti-HBs and anti-HAV GMCs, reported in this study, correspond with the results of similar investigations on immunogenicity of monovalent hepatitis A and B or the combined vaccine [57–60]. There is no evidence that immunogenicity with regard to anti-HAV antibodies was reduced in elder subjects (aged 40–45 years) as discussed in some papers on rare cases of hepatitis A despite vaccination [61,62].

The overall incidence of symptoms (solicited/unsolicited, local/general) tended to be similar in each of the two groups. No systematic increase in liver enzymes due to vaccination was observed. The vaccines investigated proved to be safe, well tolerated and highly immunogenic.

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