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HBV vaccination of HCV-infected patients with occult HBV infection and anti-HBc-positive blood donors

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Abstract

Anti-HBc positivity is a frequent cause of donation rejection at blood banks. Hepatitis B virus (HBV) infection may also occur in HBsAg-negative patients, a situation denoted occult infection. Similarly, very low levels of HBV-DNA have also been found in the sera of patients with chronic hepatitis C virus (HCV) infection, even in the absence of serum HBsAg. Initially we searched for HBV-DNA in serum of 100 blood donors and 50 HCV-infected patients who were HBsAg negative/anti-HBc positive by nested-PCR and by an HBV monitor commercial test for HBV-DNA. Anti-HBs seroconversion rates were measured in 100 blood donors and in 22 patients with chronic HCV infection after HBV vaccination to determine if the HBV vaccination could eliminate an occult HBV infection in these individuals. Occult HBV infection was detected in proportionally fewer blood donors (6/100 = 6%) than chronic hepatitis C patients (12/50 = 24%) ($P < 0.05$). We noted seroconversion in 6/6 (100%) HBV-DNA(+) and in 84/94 (89.4%) HBV-DNA(-) blood donors ($P > 0.05$). All subjects who were HBV-DNA(+) before the first dose of HBV vaccine (D1), became HBV-DNA(-) after D1, D2, and D3. Among 22 HCV-positive patients, 10 HBV-DNA(+) and 12 HBV-DNA(-), seroconversion was observed in 9/10 (90%) HBV-DNA(+) and in 9/12 (75%) HBV-DNA(-) subjects ($P > 0.05$). The disappearance of HBV-DNA in the majority of vaccinated patients suggests that residual HBV can be eliminated in patients with occult infection.

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- HBV vaccination
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Introduction

Infection with hepatitis B virus (HBV) leads to a wide spectrum of clinical presentations, ranging from acute self-limited or, rarely, fulminant hepatitis, to an asymptomatic chronic carrier status, or chronic hepatitis with progression to cirrhosis. During acute and chronic HBV infection, antibodies against the core antigen of HBV (anti-HBc) are found together with the surface antigen of the virus (HBsAg), whereas resolved infection is accompanied only by antibodies against HBsAg (anti-HBs).

Anti-HBc positivity is an important cause of blood rejection at blood banks. Anti-HBc screening continues to be necessary because, in some cases, HBsAg negative/anti-HBc-positive blood donors are able to transmit HBV. In Northeast Brazil, the high prevalence of anti-HBc-positive blood donors has resulted in a very high level of donation rejection, with obvious consequences for blood availability, generating very high costs (1). As the sensitivity of polymerase chain reaction (PCR) method has improved, individuals carrying HBV-DNA as the only marker of infection have been detected more often. In most parts of Europe and the United States, 10-20% of all individuals with hepatitis B have anti-HBc as the only marker of HBV infection (2). In about 10% of these individuals HBV-DNA can be detected by PCR (2,3). HBV infection may also occur in HBsAg-negative patients who have serological markers of previous infection (anti-HBs) (4). These HBV infections in patients who lack detectable HBsAg are called occult infections (4), and their serum HBV-DNA levels are usually less than 10^4 copies/mL (5). Similarly, very low levels of HBV-DNA have also been detected in the sera of patients with chronic hepatitis C virus (HCV) infection, even though there was no demonstrable HBsAg in their serum (4,6-11).

We used PCR to determine the prevalence of occult HBV infection in serum samples from 100 blood donors who were HCV negative/HBsAg negative/anti-HBc positive, with anti-HBc positive being the only marker, and from 50 patients with chronic HCV infection who were HBsAg negative/anti-HBc positive, with anti-HBc being the only marker. HBV-DNA was detected in 6 and 24% of these patient groups, respectively (12).

In the present investigation, we studied the anti-HBs seroconversion rates in anti-HBc-positive blood donors and in patients with chronic HCV infection after HBV vaccination. We also examined the possibility of eliminating an occult HBV infection with HBV vaccination of these individuals.

Material and Methods

Patients

The study was conducted at the Hospital das Clínicas, University of Campinas, Campinas, SP, Brazil, between 2000 and 2003, after approval by the institutional Ethics Committee. We selected 100 HBsAg-negative (Hepanostika Uniform II, Organon Teknika, Boxtel, Netherlands) and anti-HBc-positive (Hepanostika anti-HBc Uniform, Organon Teknika) blood donors. All of these blood donors were negative for HCV (HCV MEIA Abbott/AxSYM, Chicago, IL, USA) and HIV (anti-HIV 1.2.0, Murex, Dartfort, UK) antibodies. These donors were retested for HBsAg (AxSYM HBsAg, Abbott) and anti-HBc (AxSYM Core, Abbott), and were tested for anti-HBs (AxSYM AUSAB, Abbott).

The study included also 50 HCV-RNA-positive patients who were HBsAg negative/anti-HBc positive/anti-HBs negative.

Vaccination protocol

All 100 blood donors and 22 HCV patients, who were anti-HBc positive alone, gave written informed consent to take hepatitis B recombinant vaccine (Engerix B®; group 1). A blood sample was taken 30 days after the first dose of the vaccine (D1) to quantify anti-HBs antibodies. Individuals who seroconverted (anti-HBs >10 mIU/mL) after D1 did not receive additional doses of the vaccine. Patients without seroconversion after D1 received a second dose of vaccine (D2), and a blood sample was collected 30 days later to evaluate anti-HBs seroconversion. In cases without seroconversion after D2, a third dose of vaccine (D3) was given 5 months after D2, and a blood sample was collected after 30 days to

measure anti-HBs levels. Before the administration of each dose of HBV vaccine, a serum sample was collected to check for HBV-DNA by PCR in order to reveal an occult HBV infection if present. All HBV-DNA-positive blood donors and HCV-RNA-positive patients received three doses of vaccine. Non-responders were individuals with <10 mIU/mL anti-HBs after 3 doses of HBV vaccine. All individuals who presented more than 10 mIU/mL anti-HBs after three doses were considered to be seroconverters (Figure 1).

Nested-PCR

The nested-PCR method for the detection of HBV-DNA in the 100 blood donors and the 50 HCV-infected patients was performed essentially as described by Kaneko et al. (13). Carryover contamination was prevented as described by Kwok et al. (14). A 10-µL aliquot of serum was amplified in a 100-µL reaction volume containing 2.5 U Taq polymerase (Bethesda Research Laboratories, Life Technologies Inc., Gaithersburg, MD, USA), 200 µmol each of the four deoxyribonucleic triphosphates, 1 µmol of the primer pair (primers 1763 5'-GCTTTGGGGCATGGACATTGACCCGTATAA-3' and 2032R 5'CTGACTACTAATTCCCTGGATGCTGGGTCT-3'), 50 mM KCl, 10 mM Tris-HCl, pH 8.3, and 1.5 mM $MgCl_2$. For reamplification of the samples, a 10-µL aliquot of the primary PCR was amplified using a primer pair (1778-E 5'-GACGAATTCCATTGACCCGTATAAAGAATT-3' and 2017R-B 5'-ATGGGATCCCTGGATGCTGGGTCTTCCAAA-3'). Water was used as a negative control. Serum from an individual who was HBV positive by EIA, and quantified by an Amplicor HBV Monitor test (Roche Diagnostic Systems, Branchburg, NJ, USA), was used as a positive control. Serial dilutions were made with HBsAg low-titer performance panel (PHA 105; Boston Biomedica, Inc., Boston, MA, USA), which made it possible to establish a limit of detection by PCR of 10^2 copies/mL. Samples considered to be positive by PCR "in house" were subsequently subjected to a commercial test for HBV-DNA (HBV Monitor; Roche) with a higher detection limit of 1,000 copies/mL.

Statistical analysis

Fisher's exact test and the chi-square test, when applicable, were used to compare proportions. All P values were two-tailed, and a P value <0.05 was considered to be significant. Samples were considered to be positive if they yielded at least two positive results after three amplifications, and were considered to be negative when they yielded two negative results in two independent reactions.

Results

Occult infection with HBV was significantly less frequent among blood donors (6/100 = 6%) than among HCV-infected patients (12/50 = 24%; P < 0.05).

Figure 2 is a schematic representation of the anti-HBs seroconversion and the rates of elimination of HBV-DNA in the blood donors and HCV-infected patients. After D1,

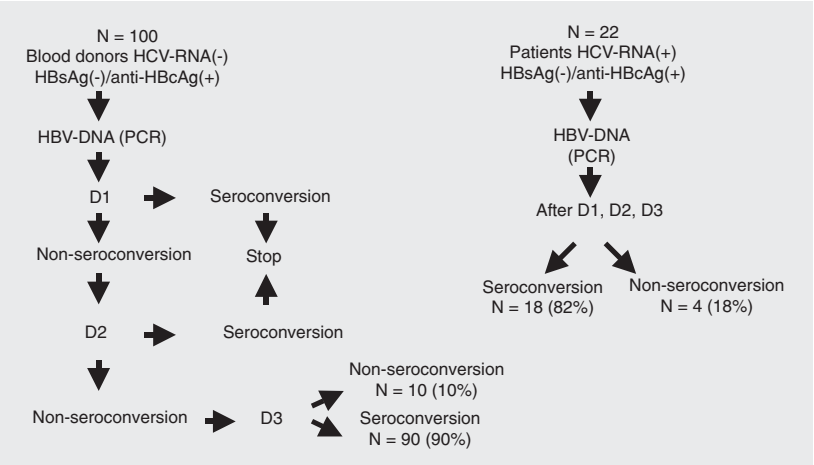

Figure 1. Vaccine procedures and resulting anti-HBs seroconversion.

seroconversion was observed in 3/6 (50%) HBV-DNA(+) subjects and in 56/94 (59.6%) HBV-DNA(-) (P > 0.05). After D2, seroconversion was observed in 4/6 (66.7%) HBV-DNA(+) subjects and in 79/94 (84%) HBV-DNA(-) subjects (P > 0.05). After D3, cumulative seroconversion was observed in 6/6 (100%) HBV-DNA(+) and in 84/94 (89.4%) HBV-DNA(-) (P > 0.05). Only 10/94 (10.6%) HBV-DNA(-) subjects did not seroconvert after three doses of vaccine. All HBV-DNA(+) subjects in group 1 before D1 became HBV-DNA(-) after D1, D2 and D3.

Among the HCV-infected patients, 22 completed the vaccine schedule (10 HBV-DNA-positive subjects and 12 HBV-DNA-negative subjects). After D1, seroconversion was observed in 5/10 (50%) HBV-DNA(+) subjects and in 6/12 (50%) HBV-DNA(-) individuals (P > 0.05). After D2, we observed seroconversion in 7/10 (70%) HBV-DNA(+) subjects and in 8/12 (66.7%) HBV-DNA(-) subjects (P > 0.05). After D3, we observed cumulative seroconversion in 9/10 (90%) HBV-DNA(+) and in 9/12 (75%) HBV-DNA(-) (P > 0.05). Only 1/10 (10%) HBV-DNA(+) and 3/12 (25%) HBV-DNA(-) did not seroconvert after three doses of vaccine. Only 1/10 (10%) of the HBV-DNA(+) subjects continued to be positive after three doses of vaccine

As can be seen, among the blood donors, 10, 50, and 40% had <10 mIU/mL, 10-99 mIU/mL and >100 mIU/mL anti-HBs levels after three doses of vaccine, respectively. These rates were significantly different from those found in HCV-infected patients, which were 36, 27, and 36%, respectively (P < 0.05, Table 1).

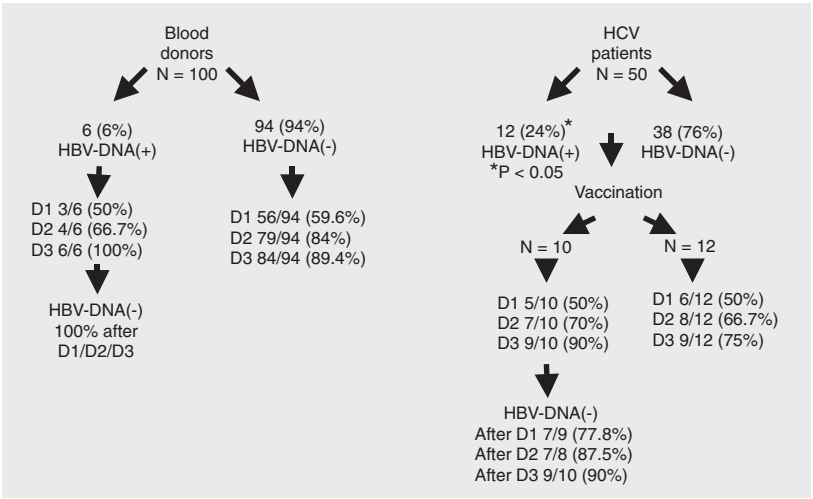

Figure 2. Anti-HBs seroconversion and HBV-DNA elimination after each HBV vaccination dose. *P < 0.05, HCV patients compared to blood donors (Fisher's test).

Table 1. Accumulated anti-HBs seroconversion in response to the vaccine after three doses.

Anti-HBs	Anti-HBc(+)/HCV(-) Group 1 (N = 100)	Anti-HBc(+)/HCV(+) Group 2 (N = 22)
<10 mIU/mL	10 (10%)	8 (36%)
10-99 mIU/mL	50 (50%)	6 (27%)
>100 mIU/mL	40 (40%)	8 (36%)

Data are reported as the number of patients and percentage in parentheses.

Discussion

The serological response to HBV vaccine may help distinguish between the various diagnostic possibilities associated with an isolated anti-HBc pattern (15). It is known that immune individuals show an early, high-titer anti-HBs response after hepatitis B vaccinations (16,17), while this response is unusual in a non-immune group (18). Furthermore, those individuals with chronic hepatitis B infection are serologically unresponsive to vaccination (19).

We initially observed occult HBV infection in 24% of the HCV-infected patients who were anti-HBc reactive. This percentage is about four times higher than the prevalence otherwise encountered in blood donors, which was 6% and could be due to the fact that these blood donors constituted a low risk population. In any case, negativity for HBsAg by the ELISA test does not necessarily mean absence of infection, which can be detected by PCR due to its higher sensitivity compared to an ELISA test. Patients infected with HBV, who are co-in-

fected with HCV, seem to have lower viral replication activity, indicating mutual interference and suggesting that HCV has a suppressive effect on HBV (20-24).

In a recent study, 33 blood donors repetitively found to have anti-HBc alone were vaccinated against HBV infection, and 13 (39%) showed seroconversion 30 days after taking one dose of the HBV vaccine. The other 20 patients received a second dose and 15 (75%) seroconverted. One of 5 patients without anti-HBs seroconversion received a third dose and finally seroconverted, with a total seroconversion rate of 88% (29/33). Next, 24 of 33 blood donors had their blood tested for HBV-DNA by PCR several months later and none was reactive (25). In another study, 120 repeatedly reactive isolated anti-HBc blood donors received a single dose of HBV recombinant vaccine. An HBV-DNA determination by PCR was carried out for those who did not test positive for anti-HBs after vaccination. Thirty-eight donors (34%) showed seroconversion after a single dose of HBV vaccine. Hepatitis DNA was not found in any of the donors (26).

In our study, we vaccinated blood donors and monitored their response to HBV vaccination. We found that 59% responded to the first vaccine dose, 83% had cumulative seroconversion after the second vaccine dose, and 90% had cumulative seroconversion after the third vaccine dose. We also found no significant difference between the seroconversion rates of HBV-DNA-positive versus -negative subjects. This response pattern led us to postulate that these blood donors could be immune to hepatitis B, having low levels of antibodies, undetected by traditional methods. All of the patients who were HBV-DNA positive before vaccination became negative after vaccination. This leads us to think that the vaccine stimulus eliminated the residual HBV or reduced the viral load to the point that it was no longer detectable by PCR. On this basis, we propose that a small quantity of HBV, with residual replication rates, would be incapable of stimulating the immune system and producing high levels of anti-HBs. Inoculation with a vaccine made with HBV surface antigens could have provoked this stimulation and consequently increased the production of anti-HBs.

Stimulating the appearance or the reappearance of anti-HBsAg by vaccination is a way of checking for the development of immunity against HBV and the possible elimination of the infectious agent (25). This could be a means of reducing the transmission of HBV in endemic regions and could also make a rejected donor acceptable, reducing the blood supply problem. The elimination of HBV-DNA demonstrated by our relatively sensitive PCR "in house" test (100 copies/mL) indicates that we may have succeeded in eliminating residual HBV in patients with occult infection. In the studies cited above, HBV-DNA was only tested after vaccination. Additional research should be done to confirm and extend our conclusion that vaccination with seroconversion of anti-HBs is able to eliminate HBV-DNA in patients with occult HBV infection.

Hepatitis B vaccination is recommended for patients with chronic HCV infection (27). Among HCV-infected patients without HBV infection, the main objective is to protect them against HBV infection. In the present study, among anti-HBc reactive patients, with or without hepatitis C, we observed seroconversion rates of 82 and 90%, respectively. This indicates that patients co-infected or not with HCV will seroconvert, independent of the presence or absence of this infectious agent. In other words, HCV did not inhibit the production of anti-HBs in hepatitis C patients who already had anti-HBc antibodies. In this anti-HBc reactive/HCV-positive group, the presence or absence of HBV-DNA had no significant effect on the seroconversion rate, as was also found in the blood donor group. We also analyzed elimination of HBV-DNA after vaccination. Among the patients who had HBV-DNA,

we observed elimination in 77.8, 87.5, and 90%, after D1, D2 and D3, respectively. All samples positive for HBV-DNA by PCR "in house" were negative for HBV-DNA when assayed with an automatic commercial test. This was not the same as we found for the blood donors, 100% of whom seroconverted after three doses of HBV vaccine. Among the patients with HCV, vaccination was not able to reduce or eliminate the virus in 10% of cases. It is known that patients with hepatitis C, co-infected with HBV or HIV, had lower rates of sustained response (elimination of the virus) when treated with interferon, possibly as a consequence of interactions between these viruses. It is also known that there is less seroconversion to anti-HBs in patients with HCV who are vaccinated against HBV (27).

We did not find a significant difference in the percentage of seroconversion to anti-HBs between groups with or without HBV-DNA. However, when we compare the two groups, we note that there are significantly more non-responders among the HCV-positive patients than among those unaffected by HCV.

Additional studies with hepatitis B vaccination are needed to determine its efficacy to protect patients infected with hepatitis C, and to see if it is feasible to eliminate residual HBV in previously infected patients.

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