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Intradermal hepatitis B vaccination in non-responders after topical application of imiquimod (Aldara®)

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ABSTRACT

Trial registration: NTR1043 (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=1043).
Background: Five to ten percent of immunocompetent persons fail to develop a protective immune response to hepatitis B vaccination, and are defined non-responders (NR). We investigated the immune response to intradermal hepatitis B vaccination after pre-treatment of the skin with the TLR7 agonist imiquimod.
Methods: Twenty-one non-responders (anti-HBs <10 IU/l after at least 6 intramuscular hepatitis B vaccinations) were randomly assigned to the control group ($N = 11$) or the experimental group ($N = 10$). Participants in both groups received 3 intradermal (ID) vaccinations with 5 μg HBsAg (0.125 mL) at 0, 1 and 6 months. In the experimental group, the dermal site of injection was pre-treated with 250 mg imiquimod ointment. Anti-HBs antibodies were determined at 0, 1, 2, 6 and 7 months.
Results: In both study groups, 70% of the participants developed a protective immune response (anti-HBs ≥10 IU/l), after the 3rd intradermal vaccination.
Conclusion: The application of imiquimod on the skin prior to intradermal vaccination did not enhance the humoral response to hepatitis B vaccine. However, irrespective of imiquimod application, 70% of the NR who had not responded to 6 previous intramuscular vaccinations, developed a protective immune response with high affinity antibodies after 3 ID hepatitis B vaccinations with 5 μg HBsAg.

1. Introduction

The immune response to the injected hepatitis B surface antigen (HBsAg) can vary greatly in healthy subjects [1]. Whereas most healthy vaccinees develop an adequate antibody response, defined as an anti-hepatitis B surface antigen (anti-HBs) titer of >100 IU/l, 5–10% of immunocompetent persons fail to develop a protective immune response and never reach an anti-HBs titre exceeding 10 IU/l; these are defined as non-responders (NR) [2]. The most commonly chosen strategy is to give an additional series (1–3 vaccinations) of conventional intramuscular vaccinations, leading to seroconversion in 61% of the revaccinated [3]. True NR, defined as NR to 2 series of intramuscular hepatitis B vaccinations are presumed unlikely to develop adequate anti-HBs titers with further vaccine doses, although no thorough research has been performed to confirm this [4].

The protective efficacy of hepatitis B vaccination is directly related to the induction of anti-HBs antibodies [5–7]. An antibody titre of ≥10 IU/l measured 1–3 months after the administration of the last dose of the primary vaccination series is considered to be a reliable marker of immediate and long-term protection against infection, and those who have an anti-HBs titer of ≥10 IU/l are considered to have protective immunity.

Non-responsiveness to the vaccine has major implications for health care workers and sexual partners of HBV (hepatitis B virus). In terms of biological mechanisms, non-responsiveness to hepatitis B vaccination has been associated with the presence or absence of specific of MHC alleles. The most pronounced associations with non-responsiveness were with excess of HLA-DR3, -DR7, -DQ2 and -DP11 and with absence of HLA-DR1, -DR5, -DR2, -DQ5 and -DP4 [8,9]. Other characteristics correlated with an inadequate anti-HBsAg response are; older age, obesity, male gender, and cigarette smoking [10–12].

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Several strategies to increase the immune response to the hepatitis B vaccine in NR, besides additional series of standard vaccinations, have been investigated such as vaccination with HBsAg combined with other antigens or additional adjuvants [13–15], or alternative routes of administration.

Another alternative to enhance immunogenicity in NR would be to administer hepatitis B vaccine in the dermal layer of the skin, instead of injecting intramuscularly. Although the intradermal (ID) vaccination route has shown to elicit slightly lower antibody responses to hepatitis B vaccine in healthy subjects [16–23], in low responders (anti-HBs of 10–100 IU/l) and NR, ID vaccination yielded slightly higher antibody titers compared to the intramuscular (IM) route during the first 6 months after vaccination [15,24,25].

In this study, we combined the ID vaccination route with local stimulation of dermal antigen presenting cells as a new approach to obtain a protective antibody response in true hepatitis B vaccine NR. Imiquimod (Aldara®) activates antigen presenting cells (APCs) through the toll-like receptor 7 (TLR7) and is registered for the treatment of (genital) warts and basal cell carcinoma.

2. Methods

2.1. Objectives

This study was conducted to determine whether in NR to hepatitis B vaccination, pre-treatment of the injected skin with a TLR stimulant (Aldara®, one sachet (250 mg) applied on 20 cm^2 skin) before ID hepatitis B vaccination (5 μg; 0.125 mL) would elicit a higher antibody response compared to ID vaccination (5 μg; 0.125 mL) without pre-treatment of the skin. Efficacy of vaccination was determined by serum anti-HBs antibody measurement.

2.2. Study design and participants

The protocol and consent forms were approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC, The Netherlands; protocol P05.187), and registered in the Dutch Trial Register (#NTR1043). Written informed consent was obtained from each participant.

Healthy volunteers of 18 years and older with a history of at least 2 series of hepatitis B vaccination (one series comprises 3 vaccinations of at least 10 μg HBsAg per vaccination) and no post-vaccination antibody titer of ≥10 IU/l, were eligible for inclusion. Participants were recruited via the University Medical Centers of Leiden and Utrecht (The Netherlands). These were health care workers and their sexual partners. Records of previous hepatitis B vaccinations and antibody responses were obtained. We excluded volunteers with a compromised immunity due to underlying illness or immunosuppressive medication, pregnant volunteers and those with (possible) autoimmune disorders. The study was carried out between May 2007 and October 2008. Subjects were randomly assigned to the experimental (with imiquimod pre-treatment of the injected skin) or control (without pre-treatment of the injected skin) group. Randomisation was performed with the use of sealed envelopes containing the vaccination code balanced through in permuted blocks of 4.

2.3. Hepatitis B vaccine

The hepatitis B vaccine used in this study, HBVAXPRO® 40 μg HBsAg/mL, is a recombinant vaccine with alum adjuvant, manufactured by Sanofi Pasteur MSD (Lot no. ND37720) and stored according to manufacturer's guidelines. Multiple dosages (maximally 4) were obtained from one vial for ID vaccination. One ID vaccination of 0.125 mL contained 5 μg HBsAg.

3. Procedures

3.1. Vaccination and data collection

At the time of inclusion, data on demographic and clinical characteristics of the participants were obtained. Participants received 0.125 mL hepatitis B vaccine (equivalent to 5 μg HBsAg) intradermally on the dorsal side of the right forearm at 0, 1 and 6 months. This vaccination site enables careful monitoring of possible adverse events. The quality of the ID injection was defined by the diameter of the arisen cutaneous wheal (adapted from the tuberculin skin test) [http://www.cdc.gov/tb/pubs/Mantoux/part1.htm (accessed 27th of March 2009)], with 6 mm being the lowest acceptable diameter. In the experimental group, a square surrounding 20 cm^2 (equal of 7.9 $in.^2$) was marked on the dosal side of the forearm. The participant applied the content of one sachet of Aldara® (5 g, 50 mg/g) to the marked surface on the skin. This is the advised dosage per application for the treatment of skin lesions. After the ointment was taken up by the skin (in approximately 3 min), the vaccine was injected in the center of the marked area. The ointment was removed by the participant by washing after 6 h. In the control group, the vaccine was administered without pre-treatment of the skin.

Blood samples were collected before vaccination (time point 0), and at 1, 2, 6 and 7 months. In the first blood sample (at time point 0) HBsAg and anti-HBcore antibodies were measured as control for infection with HBV.

Participants were asked to document clinical symptoms (local and systemic) after vaccination in a 4-week diary. Solicited symptoms were; erythema, pain and swelling at the site of injection, fever and myalgia. Severity of adverse events was documented as −(absent), ±(mild), +(moderate) or ++(severe).

3.2. Anti-HBs detection

Anti-HBs titers were assessed by the ARCHITECT Anti-HBs assay (Abbott Laboratories, Chicago, IL, USA) according to the manufacturer's instructions, and were expressed in International Units (IU)/l.

3.3. Anti-HBs avidity determination

Avidity of anti-HBs antibodies was measured in duplicate by adding 0 M PBS, 2, 4 and 6 M urea to the serum of non-responders who mounted an antibody concentration ≥30 IU/l after three intradermal vaccinations (with or without imiquimod). The avidity index was calculated as the ratio of anti-HBs with 6 M urea (dilution 1:1) to anti-HBs in PBS (dilution 1:1). As a control, the avidity index of healthy responders (anti-HBs ≥50 IU/l after 3 hepatitis B vaccinations) was measured. The antibody concentration had no effect on the avidity index, measured by diluting serum (2-, 5- and 10-fold) in the presence of 6 M urea (data not shown). In five study participants who mounted a protective response anti-HBs avidity was determined longitudinally throughout the course of the 3 intradermal vaccinations, to envisage the process of avidity maturation.

3.4. HLA allele determination

Study participants were typed for HLA-DRB1, -DQB1 en DPB1 as described previously [26], in the European Foundation of Immunogenetics (EFI)-accredited HLA laboratory of the Department of Immunology and Haematology, LUMC (Leiden University Medical Centre), the Netherlands. Briefly, DNA was isolated using a commercially semi-automated beads based assay (Chemagen, Baesweiler, Germany). The HLA-DRB and DQB typing was performed with a reversed approach of the PCR/SSOP technique and