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Immunogenicity and safety of combined intradermal recombinant Hepatitis B with BCG vaccines at birth☆,☆☆

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Summary This randomized, prospective, non-inferiority study aimed to quantify anti-HBs titers induced by recombinant Hepatitis B vaccine from healthy infants vaccinated with combined Hepatitis B and Bacillus Calmette-Guérin (BCG) vaccines (HbsAg 10 μg plus BCG suspension 0.1 mg) and compare them to titers obtained with separated vaccines.

☆ Preliminary results of these findings were presented at the New Approaches to Vaccine Development—from bench to the field, Berlin, Germany, 8–10 September 2005.

☆☆ All of the authors declare that the paper ''Immunogenicity and Safety of Combined Intradermal Recombinant Hepatitis B with BCG Vaccines at birth.'' was not submitted for consideration elsewhere.

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Infants were immunized at birth either with combined intradermal (ID) BCG and Hepatitis B or ID BCG alone and intramuscular (IM) Hepatitis B. Both groups received IM Hepatitis B at 1 and 6 months of age. After the third dose anti-HBs titers $\geq$10 IU/mL were observed in 99% of vaccinees and $\geq$1000 IU/mL in 71%. There were no adverse events in both groups. Combination of HbsAg with BCG as first dose did not modify the profile of the humoral immune response for Hepatitis B indicating safety and immunogenicity of this vaccine in newborn.

Introduction

The high prevalence of Hepatitis B virus (HBV) infection in Brazil (7.9%) has been associated with lower socioeconomic class, with rates of 21% in Manaus (Amazon Basin, north), 7.5% in Porto Alegre (south), 5.5% in Rio de Janeiro (southeast). Lower seroprevalence rates were reported in northeast urban areas, as in the city of Fortaleza (1.2%). The anti-HBc antibody has been detected in children younger than 1 year, suggesting vertical transmission. Also, anti-HBc detection was significantly higher in adult men than in adult women and children suggesting sexual and transmission through intravenous drug use [1,2]. Since 1998 the Brazilian National Immunization Program (NIP) has incorporated neonatal Hepatitis B vaccination into the routine infant immunization schedule as recommended by World Health Organization in 1991 [3]. This program was expanded in 2001 to include adolescents up to 19 years as routine and catch-up immunization. This strategy was proved economically feasible with the recombinant yeast-derived Hepatitis B vaccine Butang® manufactured by Instituto Butantan, São Paulo, Brazil, which possesses widely confirmed immunogenicity when applied at birth, one and six months of age [4,5].

Hepatitis B vaccines are composed of highly purified preparations of recombinant Hepatitis B surface antigen (HbsAg) obtained from yeast cells and induce a vigorous antibody response in early life. This response correlates with the protective efficacy of Hepatitis B vaccine against virus vertical transmission [6]. Although vaccine-induced neutralizing antibodies play an important role in protecting young infants from infection, the control of viral replication also involves helper T lymphocytes [7]. In adults and older children, Hepatitis B vaccine activates HbsAg-specific T cells producing both type 1 and 2 cytokines [8—10].

BCG vaccine is recommended at birth in Brazil and remains the only tuberculosis vaccine available for field usage. Evaluation of BCG vaccine immunogenicity demonstrated that human newborns make strong Th1 responses to BCG detectable as long as one year suggesting immunological memory. Moreover, it has been shown that BCG may be a useful Th1 inducing adjuvant at birth in humans and mice [11—13]. It markedly increases the primary immune response to Hepatitis B vaccine in newborns and may have influence on infant memory responses [14]. This could be related to the potent antigen presenting cell-activating properties of BCG and/or to its persistence during the maturation of the immune system.

Because immunization at birth generally primes to subsequent vaccine doses, new combinations of vaccines are an important public health endeavor. Recombinant Hepatitis B (Butang®) combined with BCG was recently developed by Instituto Butantan of São Paulo, Brazil, and its evaluation in newborn infants has become necessary, in order to check the effects on immunogenicity and safety of these antigen mixtures. In this study Hepatitis B was administered in three doses and only the first was intradermal and combined. Our main goal was to examine whether a Hepatitis B and BCG combined vaccine could prime similar antibody responses to Hepatitis B vaccine injected alone.

Materials and methods

Study participants and vaccination

This randomized controlled clinical trial was conducted between October 2004 and December 2005. A total of 548 healthy newborns from Campinas Maternity were included. The preterm (gestational age $\leq$37 weeks) and the low birth weight newborns (weight <2500 g), with congenital or genetic defects or whose mothers were younger than 18 years or had Hepatitis B carrier status, infants with family history of tuberculosis, syphilis or human immunodeficiency virus infection were excluded. Infants were randomized into two groups.

The type of vaccine administered was revealed to the entire research team only at the preparation of this report, but vaccinators and vaccine recipients' parents were aware of which vaccine was being injected since the beginning of the study.

Within 24 h after birth, infants from Group I received combined intradermal BCG and Hepatitis B, which was prepared as follows: a solution of purified recombinant HBsAG protein (100 μg) and BCG Moreau Rio de Janeiro strain suspension (1 mg) were mixed in 4 mL vials containing 2% sodium glutamate as stabilizer and lyophilized. Immediately before administration the vaccine was reconstituted in 1 mL of 0.85% NaCl solution, without preservative. Each 0.1 mL dose contained 0.1 mg of BCG and 10 μg of HbsAg. The combined vaccine was available in amber glass vials with 10 lyophilized doses, 0.1 mL each. Within 24 h after birth, children from Group II received intradermal BCG (0.1 mg lyophilized BCG Moreau strain in 0.85% NaCl with 1.1 mg sodium glutamate), stored in amber glass vials with 10 doses of 0.1 mL each, and intramuscular Hepatitis B vaccine (Butang®, 10 μg HbsAg with 0.625 mg aluminum hydroxide and 0.05 mg thimerosal), available in glass vials of 10-dose liquid solution, 0.5 mL each. Both groups received the second and third doses of intramuscular Hepatitis B vaccine (Butang®) into the anterolateral area of the thigh, at one and six months of age, respectively. All vaccines were produced by Instituto Butan-