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Influence of the Co-Administration of Heptavalent Conjugate Vaccine PCV7-TT on the Immunological Response Elicited by VA-MENGOC-BC® and Heberpenta®-L in Rabbits

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ABSTRACT

Finlay Vaccine Institute is developing a new heptavalent conjugate vaccine against *Streptococcus pneumoniae*. As infants are the target population, PCV7-TT will be necessarily co-administered with other vaccines, and then, the interactions represent a concern. The aim of this work is to evaluate the possible immunological interferences in rabbits as animal experimental model. Rabbits were immunized with Heberpenta®-L, VA-MENGOC-BC®, and PCV7-TT. Blood samples were taken fourteen days after final immunization for obtaining sera. Antibody responses to all antigens were evaluated by indirect ELISA. Functional responses against diphtheria and tetanus toxoid were done by *in vivo* seroneutralization assay. No interference was observed by PCV7-TT over the humoral response against diphtheria toxoid and meningococcal antigens ($p > 0.05$). A nonstatistically significant reduction ($p > 0.05$) was observed in the case of the humoral response against *Haemophilus influenzae* type b oligosaccharide. Concomitant administration of Heberpenta®-L and PCV7-TT increased twice the antibody titers as well as the protective activity against tetanus toxoid, but no statistical differences were found. The co-administration did not induce a reduction in the percent of responders against pneumococcal polysaccharides contained in PCV7-TT vaccine. Concomitant administration of PCV7-TT did not induce interferences over the evaluated antigens of Heberpenta®-L and VA-MENGOC-BC®. Also, no interference was observed on the immune response elicited by PCV7-TT. These preclinical results suggest that PCV7-TT will not result in a serious problem over the immune response elicited by the licensed vaccines Heberpenta®-L and VA-MENGOC-BC®. However, the clinical interference could be strictly studied during clinical trials in infants.

KEYWORDS

Concomitance; immune response; interference; nonclinical evaluation; pneumococcal vaccine

Introduction

Vaccination is considered the most cost–benefit strategy for controlling pathogen-associated infectious diseases. Vaccines are also one of the most important victories of medicine in the 20th century and had a tremendous impact in the incidence reduction, prevention, and control of some infectious diseases (Ehreth, 2003; Ochoa, 2008). This

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health intervention prevents more than 3 million of deaths each year, and around 750,000 infants are saved from discapacity (Ochoa, 2008).

Nowadays, the number of vaccines included in infant vaccination programs is growing, as much in conventional (e.g., whole cell inactivated and purified toxoid vaccines) as new generation vaccines (e.g., recombinant and conjugate vaccines) included in health programs around the world (Fletcher et al., 2004; Sesardic et al., 1999). Even so, the combined formulation of different antigens in the same vaccine is a well-established practice (Pöllabauer et al., 2009).

In current immunization programs, vaccines are usually administered concomitantly or in the same schedule with very short temporal separation (Dagan et al., 1998; Fletcher et al., 2004). Recently, different studies have shown evidences of immunological interactions between vaccine antigens that were immunized in the same schedule. This complex phenomenon is known as interference (López and Montané, 2010; Pöllabauer et al., 2009).

These interactions can cause an increased or, in the worst of the cases, a reduction in the immunogenicity or biological activity of one or more vaccine components (Fletcher et al., 2004). Such a problem, it may impact in the efficacy or effectiveness of vaccines. It had seen these interactions could be antigen or vaccine-specific and have a certain degree of unpredictability (Pöllabauer et al., 2009).

The Cuban immunization program is based on World Health Organization (WHO) and Cuban Public Health Ministry (MINSAP) recommendations and epidemiological criteria (Ochoa, 2008). This program includes vaccines characterized by its well-established efficacy and high coverage. Some of these vaccines are produced in Cuba, like Heberpenta®-L, VA-MENGOC-BC® and Vax-TET®, and QUIMI-Hib®. Heberpenta®-L is a combined vaccine constituted by diphtheria (DT) and tetanus toxoids (TT), pertussis inactivated cells, recombinant Hepatitis B (HB) protein and *H. influenzae* type b (Hib) synthetic oligosaccharide conjugated to TT. This vaccine is administered at 2, 4, and 6 months in infants. VA-MENGOC-BC®, which is immunized at 3 and 5 months, contains outer membrane vesicles from *Neisseria meningitidis* serogroup B (MenB) and capsular polysaccharide from serogroup C (MenC).

Finlay Vaccine Institute has been working in the research and development of a pneumococcal conjugate heptavalent vaccine (PCV7-TT). This vaccine is composed by polysaccharides 1, 5, 6B, 14, 18C, 19F, and 23F conjugated to TT as carrier protein. Recently, PCV7-TT finished phase I clinical trials in adults and children with excellent safety and preliminary immunological results (Dotres et al., 2014; González et al., 2015). The inclusion of PCV7-TT in the Cuban immunization program will be an important step in prevention of pneumonia and other pneumococcal-associated diseases, as well as a promising victory to the Cuban biotechnological industry. Nevertheless, as PCV7-TT will be introduced in infants, possible interactions between PCV7-TT immunogens over Heberpenta®-L and/or VA-MENGOC-BC® immunogens could occur. Therefore, the aim of this study is to evaluate the immunological response against the antigens contained in Heberpenta®-L and VA-MENGOC-BC® when they are co-administered with PCV7-TT in a rabbit model.

Materials and methods

Vaccines

Heberpenta®-L was kindly provided by the Production Direction of Center for Genetic Engineering and Biotechnology. Commercial lot 1AA0402/0 was used. Each dose of 0.5 ml

contains TT (10 Lf, level of flocculation determined by the Ramon's flocculation method), DT (25 Lf), *Bordetella pertussis* inactivated whole cells (16 OU), recombinant Hepatitis B surface protein (10 μg), and *H. influenzae* type b synthetic capsular oligosaccharide conjugated to TT (10 μg related to saccharide). VA-MENGOC-BC® was produced by Finlay Vaccine Institute, Cuba. Each dose of 0.5 ml contains *N. meningitidis* serogroup B outer membrane vesicles and *N. meningitidis* serogroup C purified polysaccharide. PCV7-TT was produced in Development Direction of Finlay Vaccine Institute following the Good Manufacturing Practices. Each human dose of 0.5 ml contains 2 μg of 1, 5, 14, 18C, 19F, and 23F capsular polysaccharide of *S. pneumoniae* and 4 μg of serogroup 6B capsular polysaccharide, all individually conjugated to tetanus toxoid and adsorbed on aluminum phosphate as adjuvant (Dotres et al., 2014).

Experimental animals

Experimental animals were purchased from National Center for Laboratory Animals Breeding (CENPALAB). New Zealand White female rabbits were used for immunogenicity evaluation. For challenge potency test against tetanus and diphtheria toxin, Balb/C female mice (16–18 g) and Duncan-Hartley guinea pigs (500 g) were employed, respectively. Experimental procedures were in accordance with institutional and international protocols and directives. Experimental protocols were approved by the Institutional Ethic Committee.

Immunogenicity schedule

New Zealand female rabbits were distributed in six groups of five individuals each one (Table 1). Animals were immunized intramuscularly with Heberpenta-L, VA-MENGOC-BC®, and/or PCV7-TT as explained in Table 1. In the corresponding cases, Heberpenta-L and PCV7-TT were co-injected in different legs. Each vaccine was administered at half of single human dose (0.25 ml) following the schedule of three doses for Heberpenta®-L and PCV7-TT and two doses for VA-MENGOC-BC®. Fourteen days after final administrations, blood was extracted. Sera were obtained by centrifugation and then were frozen until use.

Antibody response determination

IgG antibodies were evaluated by indirect quantification ELISA. In all evaluations, in-house standard sera were used for the construction of calibration curves.

Table 1. Vaccination schedule.

Group	Time 0 days	Time 14 days	Time 28 days	Time 42 days	Time 56 days	Time 70 days
1	H+P	V	H+P	V	H+P	-
2	H+P	-	H+P	-	H+P	-
3	H	-	H	-	H	-
4	-	V	-	V	-	-
5	P	-	P	-	P	-
6	C	-	C	-	C	-

Groups of five rabbits were immunized with the studied vaccines. H, Heberpenta-L; V, VA-MENGOC-BC; P, heptavalent pneumococcal vaccine; C, alum negative control. Heberpenta-L and PCV7-TT, when administered in the same day, were immunized in different legs.

Anti-TT and anti-DT IgG response

ELISA microwell MAXISORP plates (Nunc, Denmark) were sensitized with 4 μg/ml of TT. Plates were incubated at 4°C overnight. The unbound material in all steps was removed by three washes with 0.05% Tween 20 in phosphate buffer solution (PBS) pH 7.2. In-house standard serum was used with arbitrary activity of 1000 arbitrary units per milliliter (AU/ml). Twofold dilutions of sample sera, starting in 1/200, were done. Horseradish peroxidase-conjugated anti-rabbit monoclonal antibodies (SIGMA) were applied in 1/10,000 dilution. Tetramethylbenzidine substrate solution was applied, and plates were incubated 20 min in darkness. Reaction was stopped with 2 M sulfuric acid solution. Plates were read at 450 nm in ELISA plate reader (Tecan).

In the case of the ELISA for determining anti-DT IgG response, plates were coated with 3.5 Lf/ml DT in carbonate-bicarbonate buffer pH 9.6. The assay was performed following the same protocol described for TT.

In vivo *protection test against tetanus toxin*

Anti-tetanus toxin elicited protection was determined by a modification of Food and Drug Administration *in vivo* test for potency evaluation of TT-containing vaccines (US National Institute of Health, 1952). Five dilutions of rabbit sera samples were incubated with a fixed amount of tetanus toxin, calibrated in the L+/10/50 level. A standard antitoxin serum, calibrated with the international standard sera, was assayed in parallel. Each dilution, after 1-h incubation in darkness, was immunized into mice. Animals were observed each 24 h up to 120 h, and animal deaths were registered. Specific activity was determined using the Spearman and Kärber method and reported in international units per milliliter (IU/ml).

Sera obtained after the third dose from groups 1, 2, and 3 were evaluated, and group 6 sera were used as negative control.

In vivo *protection test against diphtheria toxin*

The anti-diphtheria activity of rabbit sera was determined by seroneutralization assay (US National Institute of Health, 1947). Diphtheria toxin calibrated at L_R/100 level was obtained from Reference Material Laboratory of Finlay Institute. As previously described for anti-tetanus activity, sera dilutions were mixed with a fixed amount of diphtheria toxin and incubated in darkness for 1 h. In parallel, a standard reference serum was evaluated. The backs of two guinea pigs were depilated, and toxin-serum dilutions were administered intradermically. Animals were observed up to 72-h post-administration, and formed erythemas were measured. Antitoxin activity was expressed in IU/ml. The sera evaluations were done in pools per group.

Anti-MenB IgG response

Immunological response determinations against outer membrane vesicles (OMV) obtained from MenB were performed by indirect ELISA. Maxisorp plates were coated with 20 μg/ml OMV (Finlay Vaccine Institute) in carbonate-bicarbonate buffer and incubated overnight at 4 °C. Standard curve was constructed using an "in-house" hyperimmune serum (500 AU/ml, working dilution 1/200). ELISA was performed as previously described for TT antibody determination protocol.

Anti-HB IgG response

Purified recombinant HB protein was diluted in carbonate-bicarbonate buffer at 5 μg/ml and applied in ELISA plates for coating. The procedure was the same described for tetanus quantitative ELISA.

Anti-Hib IgG response

Synthetic Hib capsular oligosaccharide-human serum albumin conjugate was used as coating material, obtained from Laboratory of Glycoconjugation of Finlay Vaccine Institute. Coating conjugate oligosaccharide was diluted in PBS (1 μg/ml, 100 μL per well) and applied in Maxisorp plates. After overnight incubation at 4°C, plates were washed and sera were applied. A calibration curve was constructed using a standard serum with 100 AU/ml antibody concentration. The rest of procedure was the same described for anti-TT IgG determination.

Anti-pneumococcal polysaccharide response

Immunological response determinations against pneumococcal polysaccharides were done by indirect ELISA. Briefly, plates were coated with the specific pneumococcal polysaccharide (10 μg/ml in PBS) and incubated overnight. An "in-house" standard curve (100 AU/ml) and positive controls were included. Protocol was the same previously described for anti-TT quantitative ELISA. Animals were considered as responders when reaching 20 AU/ml or higher.

Statistical analysis

Statistical analysis was carried out by nonparametric test of Kruskal Wallis ($\alpha = 0.05$) with GraphPad Prism 4 program. Multiple comparisons Dunns test was employed to evaluate statistical differences between experimental treatments *a posteriori*. Tables and graphics were constructed in Windows Microsoft Office Excel 2007 and GraphPad Prism 4 softwares.

Results

Anti-tetanus humoral response

The immune response against TT elicited by vaccines was determined by indirect quantitative ELISA. Antibody concentrations determined after complete doses schedule are presented in Figure 1A. Heberpenta®-L induced anti-TT IgG concentrations greater than to 270 AU/ml (GMC = 1050 AU/ml). The co-administration of Heberpenta®-L and PCV7-TT elicited higher, but no statistically different, antibodies titers (Group 2) than Heberpenta®-L alone. No statistical differences were observed between the groups 1, 2, and 3. Protective activity of antibodies against tetanus toxin was also determined by seroneutralization assay. Heberpenta®-L induced, as expected, a mean protective activity superior to the cutoff established for this assay (2 IU/ml) (Figure 1B). Co-administration of Heberpenta®-L and PCV7-TT elicited protective activity superior (GMC = 19.18 IU/ml), but nonstatistically different, than Heberpenta®-L alone. The rabbits received concomitantly three doses of Heberpenta®-L and PCV7-TT alternating with 2 doses of VA-MENGOC-BC® (Group 1) showed protective capacity against the TT similar to the other groups (GMC 15.22 IU/ml).

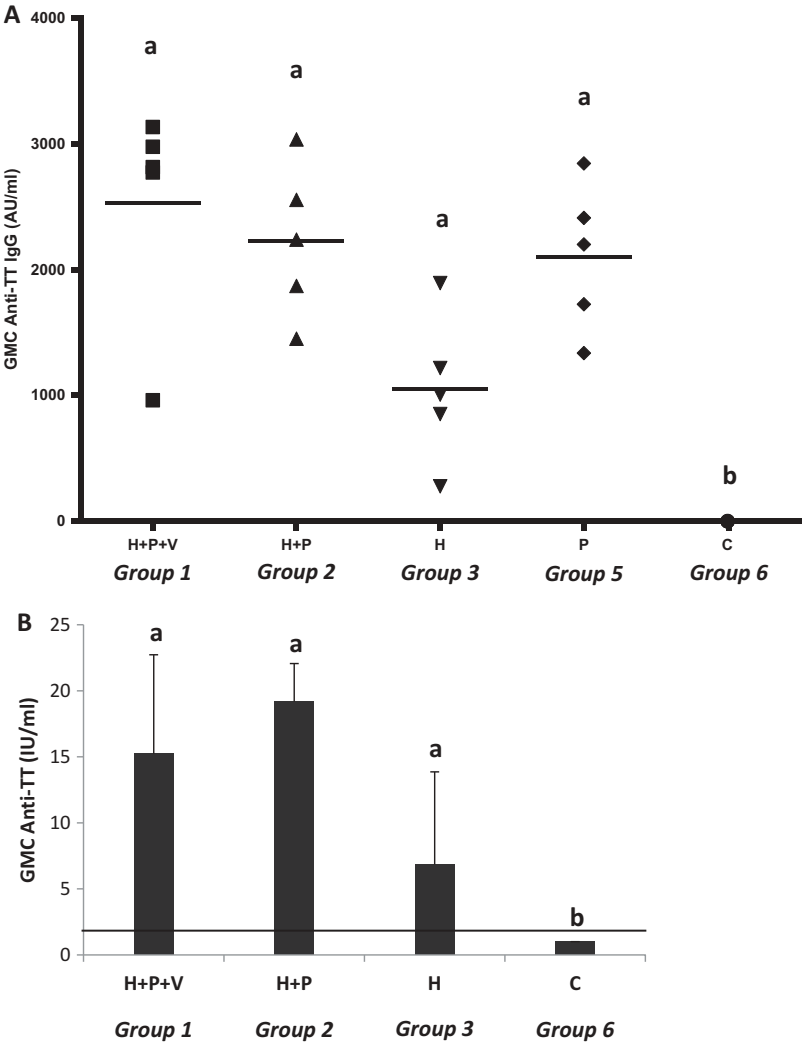

Figure 1. IgG elicited against tetanus toxoid in rabbits. A. Immunogenicity of vaccines against tetanus toxoid. Graphic represents the geometrical mean concentration in arbitrary units per milliliter of IgG antibodies (AU/ml). Individual concentrations are showed by geometric figures. Geometric means are presented as horizontal lines. B. Protective activity against tetanus toxin determined by *in vivo* seroneutralization assay at level L+/10/50. Results correspond to final extraction. Geometrical mean concentrations are presented by vertical bars. Error bars showed the standard deviations. The cut-off for seroneutralization assay was 2 IU/ml. H, Heberpenta-L; P, heptavalent pneumococcal vaccine; V, VA-MENGOC-BC; C, alum negative control. Different letters mean statistical differences (Kruskal-Wallis test, $p < 0.05$). Detailed experimental schedule is described in the text.

Anti-diphtheria humoral response

The immune response against DT was determined by indirect ELISA using an "in-house" standard curve. Results are presented in Figure 2A. Co-administration of Heberpenta®-L and PCV7-TT did not affect the humoral response elicited by Heberpenta®-L against DT. Heberpenta®-L induced antibodies concentrations of 4803, 3518, and 5792 AU/ml in group 1, 2, and 3, respectively. No statistical differences were found.

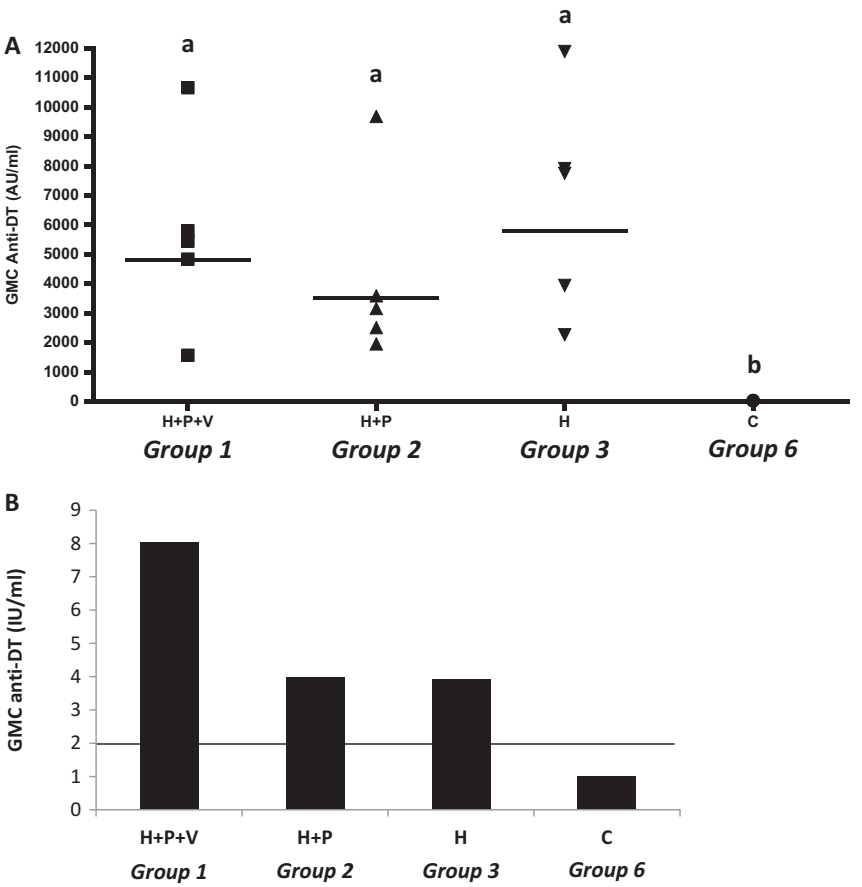

Figure 2. IgG antibody response against diphtheria toxoid. A. Immunogenicity of vaccines elicited against diphtheria toxoid. Individual serum concentrations and the geometrical mean concentrations are presented as geometrical figures and horizontal lines, respectively. B. Protective activity against diphtheria toxin determined by seroneutralization assay in a L_R/100 level. Bars represent the concentration in international units per milliliter (IU/ml) for each group. Sera were evaluated in pool per group. Horizontal line represents the cutoff established for the potency assay. H, Heberpenta-L; P, heptavalent pneumococcal vaccine; V, VA-MENGOC-BC; C, alum negative control. Different letters mean statistical differences (Kruskal-Wallis test, $p < 0.05$).

A little different scenario was observed in protection activity results (Figure 2B). Heberpenta®-L induced antibodies concentrations of 3.91 and 3.98 IU/ml at final dose when it was administered alone or with PCV7-TT. Nevertheless, the administration of VA-MENGOC-BC® in the same immunization schedule favored the protective response against diphtheria toxin. In the seroneutralization assay, the administration of the three vaccines induced an activity of 8.03 IU/ml.

Anti-MenB humoral response

In Figure 3, the results of antibodies against outer membrane vesicles of MenB are presented. The administration of the three vaccines in the same immunization schedule

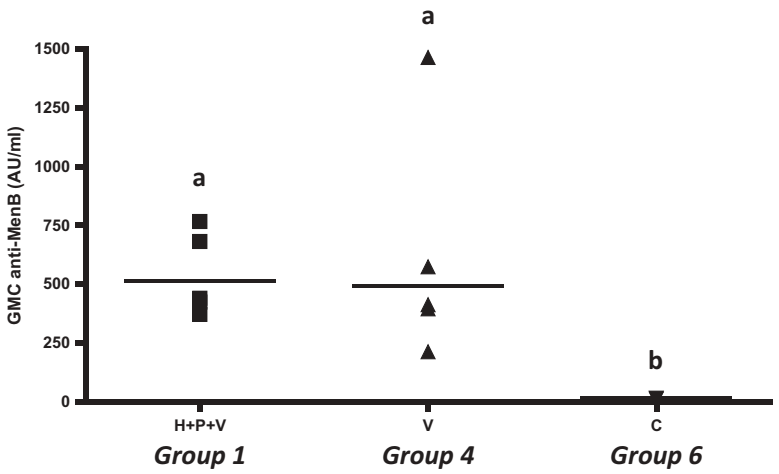

Figure 3. Specific IgG response elicited by VA-MENGOC-BC against meningococcal B outer membrane vesicle. Sera concentrations are showed as figures and the geometric mean concentrations, as horizontal lines. H, Heberpenta-L; P, heptavalent pneumococcal vaccine; V, VA-MENGOC-BC; C, alum negative control. Statistical differences are illustrated by different letters (Kruskal Wallis test, $p < 0.05$).

did not interfere with the response elicited by VA-MENGOC-BC® as the results suggest. VA-MENGOC-BC® induced concentrations of 493.7 AU/ml (Group 4), similar to the same vaccine administered with Heberpenta®-L and PCV7-TT (515.2 AU/ml). Control group showed low antibodies levels, as expected (13.83 AU/ml).

Anti-Hib humoral response

The active pharmaceutical ingredient, contained in Heberpenta®-L, which induces response against Hib oligosaccharide, is a synthetic saccharide conjugated to TT (Hib-TT). As PCV7-TT and Hib-TT share the same carrier protein, possible interferences can be expected. In Figure 4, humoral responses of the groups 1, 2, 3, and 6 against Hib are presented. Heberpenta®-L elicited antibodies titers around 222.90 AU/ml. This value is a little higher than the results showed by treatments groups 1 and 2 (113.70 and 133.21 AU/ml respectively). However, statistical analysis did not show any difference between these results.

Anti-HB humoral response

The humoral response against the recombinant protein of HB virus was measured by indirect quantitative ELISA. The results are present in the Figure 5 as a dot plot graphic. Heberpenta®-L induces a geometric mean concentration of 196.0 AU/ml, slightly but nonstatistically superior to the response elicited by concomitant administration of Heberpenta®-L and PCV7-TT (157.8 AU/ml). The administration of the three vaccines induced a lower response (98.0 AU/ml), but no statistical difference between this and the experimental groups 2 and 3 was observed. Even though the statistical

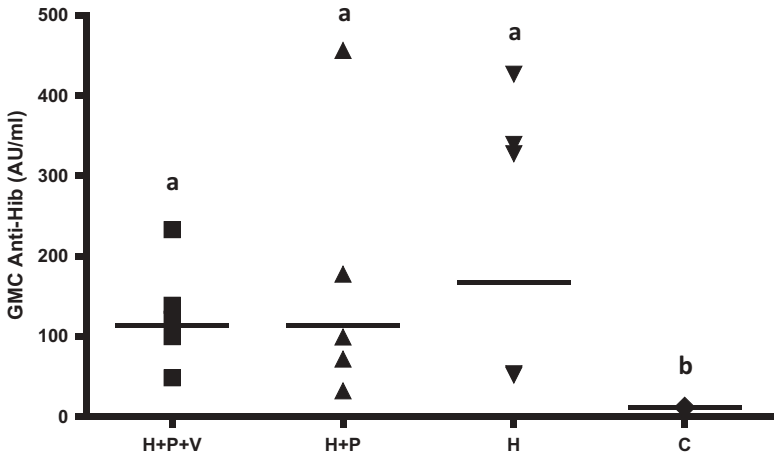

Figure 4. Specific IgG response against Hib polysaccharide. Sera concentrations are represented by figures and the geometrical mean concentrations, by horizontal lines. H, Heberpenta-L; P, heptavalent pneumococcal vaccines; V, VA-MENGOC-BC; C, alum negative control. Statistical differences are presented as different letters (Kruskal-Wallis test, $p < 0.05$).

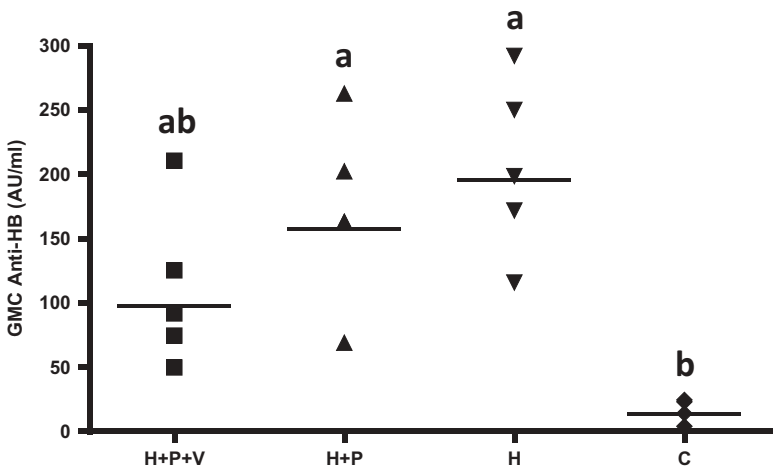

Figure 5. Specific IgG response anti-HB induced by Heberpenta-L. Sera concentrations are presented as figures and the geometric mean concentrations, as horizontal lines. H, Heberpenta-L; P, heptavalent pneumococcal vaccines; V, VA-MENGOC-BC; C, alum negative control. Different letters illustrate statistical differences (Kruskal-Wallis test, $p < 0.05$).

analysis did not find differences between group 1 and the negative control (13.5 AU/ml), the lower value in group 1 was 50 AU/ml, and the upper concentration in group 6 was 25.5 AU/ml.

Anti-pneumococcal polysaccharide response

In Figure 6, the results of IgG antibodies against pneumococcal polysaccharides are presented as percentage of seroconverted animals. The positivity criterion was IgG concentrations equal or superior to 20 AU/ml. In all cases, the percentages were equal to 100% for all experimental treatments.

Discussion

Cuba, despite being a developing country, has an advanced immunization program, which includes vaccines against 13 infectious diseases. This is a result of many years of medical and Public Health experience and political will. As occur in many countries, during the infant's life, some vaccines are administered concomitantly. In Cuba case, in the first months of life, children receive Heberpenta®-L and VA-MENGOC-BC®. Heberpenta®-L is administered at 2, 4, and 6 months, while VA-MENGOC-BC® is immunized at 3 and 5 months of life.

PCV7-TT is a heptavalent conjugate vaccine that shall be administered in infants in the same immunization schedule that the mentioned vaccines. Therefore, it is possible that PCV7-TT could induce interference over the immune response elicited by Heberpenta®-L and VA-MENGOC-BC®, or vice versa. To answer this scientific question, we evaluated the immune response induced by these vaccines in rabbits as experimental model. There are several studies for evaluating the interferences between vaccines in clinical reports. However, it is not the same scenario in preclinical evaluation. Sadly, we have not found enough preclinical studies evaluating the immunological interferences between the study antigens for establish adequate comparison. That is why we used the clinical findings and reports as comparing studies.

One of the most important variables to analyze in the study was the effect of the increased amount of tetanus toxoid over the immune response against this antigen. PCV7-TT contains around 20 μg of tetanus toxoid as carrier protein and Heberpenta®-L, 10 Lf (approximately 33 μg) of unconjugated and about 30 μg of tetanus toxoid conjugated to Hib. When these

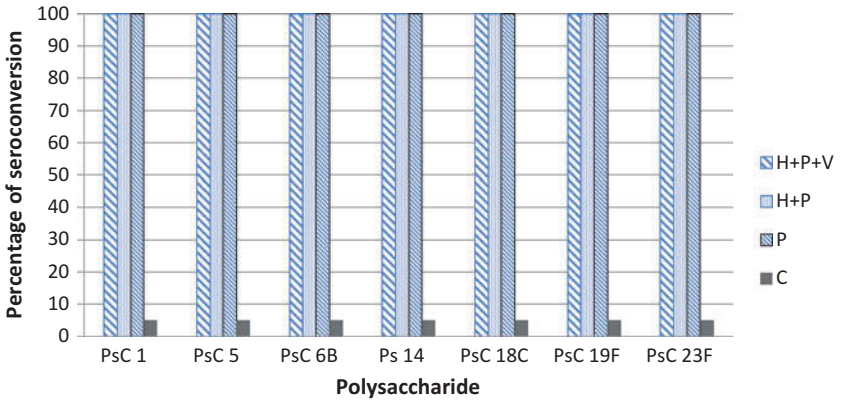

Figure 6. IgG response against pneumococcal polysaccharides. Vertical bars represent the percentage of seroconverted animals (IgG concentration ≥20 AU/ml). H, Heberpenta-L; P, heptavalent pneumococcal vaccines; V, VA-MENGOC-BC; C, alum negative control. Negative control bars show values only for visualization, and actual percentages are 0.

vaccines are co-administered, the immune system is stimulated with around 83 μg of tetanus toxoid at the same time. After completing the entire schedule, the individual has received 249 μg of tetanus toxoid. This quantity does not include the booster doses.

The augmented amount of tetanus toxoid could be then an excessive quantity, resulting in negative effects. The continue stimulation of the immune system with the same antigen or epitopes could induce the development of the tolerance, with the expression of regulatory and suppressor patterns (Dagan et al., 2010). Other option is the exhaustion of carrier-specific T cell populations against the carrier protein for the continued stimulation, competition, and the epitopic suppression (Dagan et al., 2010; Pobre et al., 2014). In this case, the immunization effect could be completely counteractive. That's why it should exist a compromise between the dose, the temporal separation (timing) and number of administration of each vaccine.

The humoral response against TT was not affected by the co-administration of PCV7-TT and Heberpenta®-L. Certainly, no statistical differences were found, but a twofold increasing in the geometrical means of concentration was observed in the experimental groups where the vaccines were co-administered. VA-MENGOC-BC®, in the other side, did not interfere in any degree.

However, not all immunoglobulin isotypes prevent the tetanus (Borrow et al., 2006); antibodies can be specific but not protective against tetanus toxin, and it was suggested that the antibodies glycosilation symmetry plays an important role in protective activity (Dokmetjian et al., 2000). To find out whether the immune responses elicited by Heberpenta®-L co-administered or not with PCV7-TT are compromised or remain protective, rabbit sera were evaluated by seroneutralization assay in mice. The results obtained confirm that PCV7-TT does not affect the antibody and protective responses elicited by Heberpenta®-L. Nevertheless, other studies must be conducted to corroborate and strongly demonstrate this observation, especially at the cellular response level. These results suggest that the increased dose of tetanus toxoid at level of the combination of Heberpenta®-L co-administered with PCV7-TT does not represent a risk to the adequate immune response against tetanus.

As expected, no interference was observed when the three vaccines were administered over the response against meningococcus.

Regarding the antibody concentration against diphtheria toxoid, no interference was observed when administering the three vaccines or PCV7-TT and Heberpenta®-L concomitantly. However, the administration of VA-MENGOC-BC® induces a twofold increase, no statistically significant, of the protective activity against diphtheria toxin in seroneutralization assay. We suggest that VA-MENGOC-BC® can modulate the immune response against Heberpenta®-L antigens by generating dendritic cell maturation, recruiting B and T cells into the muscular tissues, and other immunological events. As outer membrane vesicles are good immune system activators and induce an inflammatory and cytokine environment with a reported Th1 pattern (Ochoa, 2013), these conditions should also favor the migration of clones of T and B cells specific against Heberpenta®-L antigens. If this assumption is true, the same or similar results must be observed in the immune responses generated by the other antigens contained in Heberpenta®-L. As we had presented previously and will discuss inmediately, VA-MENGOC-BC influenced in certain degree also the humoral responses against Hib and HB, but no statistical differences were found.

Heberpenta®-L alone induced an anti-HB IgG response statistically superior than negative control and similar to group administered with the three vaccines. However, there are

no statistical differences between GMC of this last group and negative control. Despite this result, as expressed previously, this statistical similitude is not biologically equal. Usually, a twofold increase is considered as positivity criterion, for example, in case of pneumococcal and Hib vaccines (Chang et al., 2013; Fernández-Santana et al., 2004). Other antigens, as conjugate *Salmonella* Typhi Vi polysaccharide, require a fourfold increment compared to the no conjugated polysaccharide (World Health Organization, 2013). In HB vaccines potency test, for instance, the threshold is calculated based on the response of the negative control group plus two times the standard deviation (World Health Organization, 2010). Analyzing our results, in case of group 1, a fourfold increasing in the geometrical mean was observed compared with negative control group, suggesting a remarked seroconversion. Such, it is possible to say that PCV7-TT and VA-MENGOC-BC® did not interfere with the immune response elicited by HB antigen in Heberpenta®-L.

In the case of response against Hib oligosaccharide, no statistical reduction was observed in the geometrical mean of the groups which received PCV7-TT compared with the immunized with Heberpenta®-L alone. However, the dispersion of the responses induced by the vaccines was high, probably affecting the statistical analysis. Even though a tendency toward a reduction in the MGC, the response induced by Heberpenta®-L co-administered with the other vaccines was highly enough comparing with the positive and the negative controls. A serious interference was one of our primary concerns, due to the carrier protein shared by Heberpenta®-L and PCV7-TT. Dagan and coworkers reported a carrier-specific impaired response against Hib polysaccharide when co-administered with pneumococcus-TT conjugate vaccine, but they also found a reduction in tetanus antitoxin (Dagan et al., 1998), which is not our case. Similar conclusions were reported by Fatton et al. (1999). However, other studies show an increased response against Hib-TT conjugate vaccine when administered with MenC-TT conjugates (Kitchin et al., 2006; Tejedor et al., 2006).

Even though it was not the principal aim of this study, we evaluated also the response against the seven pneumococcal polysaccharides contained in PCV7-TT vaccine. The results are presented as seroconversion percentages based on the positivity criterion of IgG concentrations equal or greater than 20 AU/ml. This value was selected based on the previous experience in potency tests for the evaluation of productive lots of PCV7-TT. This cutoff corresponds to around a fourfold increase in the concentration referred to the basal antibody concentration. We observed that the administration of the three vaccines in the same immunization schedule did not interfere with the immune response elicited by PCV7-TT. However, as this vaccine shares the TT with the pentavalent one, other studies must be conducted for exhaustive evaluation of the influence of co-administration at humoral and cellular levels. Probably, some differences shall be observed, especially in the T and B cell populations. Also, it is possible that isotype profiles can vary and the antibody avidity.

Certainly, it is not possible to extrapolate animal experiment results into the clinical studies (Dokmetjian et al., 2000); however, preclinical analysis is an important tool for decision taking and clinical design.

We consider that as the number of vaccines is growing, it is critical to evaluate the feasibility of dose and number of immunizations reductions. Truly, the immune system is very powerful, but the continued and repetitive challenges for vaccination with a due antigen can be, better than favorable, in a counteractive result.

Conclusions

This study helps us to understand the effect of the administration of the studied vaccines in the same immunization schedule. Based on the results presented herein, the administration of PCV7-TT in the same immunization schedule with Heberpenta®-L and VA-MENGOC-BC® does not represent a concern. Even so, this is a preclinical approach to the phenomenon, and these results suggest that not alarming interference must be expected in clinical studies. However, certain degree of emphasis must be taken in the case of the response against the Hib polysaccharide, also evaluating functional response. The dispersion of the data obtained in this experiment makes a consequent conclusion difficult. Other assay in rabbits with more animals per group and cellular studies are required. Sadly, the Hib component in Heberpenta®-L is not immunogenic in rodent, and it is not possible to assess the immune response in another suitable animal model.

Next steps in the preclinical research include the evaluation of co-administration of PCV7-TT with other vaccines included in the immunization programs. Also, we must evaluate the effect on the immune response against pertussis in mice. Other interesting question that we must assess is the humoral and cellular response elicited by PCV7-TT when it is co-administered with TT-containing vaccine (e.g., Heberpenta®-L and Vax-TET®).

In our criterion, the antigenic interferences are still a pending task in the Immunology, although many studies had been conducted until now.

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Declaration of interest

All authors are researchers and employees at Finlay Vaccine Institute, where PCV7-TT vaccine has been developed and produced.

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References

Borrow R, Balmer P, Roper M. 2006. The Immunological basis for immunization. Module 3: Tetanus. Update 2006. Geneva, Switzerland: WHO Press.

Chang J, Serrano Y, Garrido R, et al. 2013. Caracterización de conjugados inmunogénicos de polisacárido capsular Streptococcus pneumoniae serotipo 14. Vaccimonitor, 22(1),15–21.

Dagan R, Eskola J, Leclerc C, Leroy O. 1998. Reduced response to multiple sharing common protein epitopes that are administered simultaneously to infants. Infect Immun, 66(5),1093–2098.

Dagan R, Poolman J, Siegrist C. 2010. Glycoconjugate vaccines and immune interferences: A review. Vaccine, 28, 5513–5523.

Dokmetjian J, Della Valle C, Lavigne V, et al. 2000. A possible explanation for the discrepancy between ELISA and neutralising antibodies to tetanus toxin. Vaccine 18, 2698–703.

Dotres C, Puga R, Ricardo Y, et al. 2014. Safety and preliminary immunogenicity of Cuban pneumococcal conjugate vaccine candidate in healthy children: A randomized phase I clinical trial. Vaccine, 32, 5266–70.

Ehreth J. 2003. Value of vaccines. Vaccine, 21, 4111.

Fatton A, Cho Y, Chu C, et al. 1999. Epitopic overload at the site of injection may result in suppression of the immune response to combined capsular polysaccharide conjugate vaccines. Vaccine, 17, 126–133.

Fernández-Santana V, Cardoso F, Rodríguez A, et al. 2004. Antigenicity and immunogenicity of a synthetic oligosaccharide-protein conjugate vaccine against haemophilus influenzae type b. Infect Immun, 72(12),7115–7123.

Fletcher M, Fabre P, Debois H, Saliou P. 2004. Vaccines administered simultaneously: Directions for new combination vaccines based on an historical review of the literature. Int J Infect Dis, 8, 328–338.

González N, Paredes B, Pérez S, et al. 2015. Safety and immunogenicity of Cuban antipneumococcal conjugate vaccine PCV7-TT in healthy adults. MEDICC Rev, 17(4),32–37.

Kitchin N, Southern J, Morris R, et al. 2006. A randomized controlled study of the reactogenicity of an acellular pertussis-containing pentavalent infant vaccine compared to a quadrivalent whole cell pertussis-containing vaccine and oral poliomyelitis vaccine, when given concurrently with-meningococcal group C conjugate vaccine to healthy UK infants at 2, 3 and 4 months of age. Vaccine, 24(18),3964–3970.

López A, Montané E. 2010. Aspectos prácticos en la evaluación de ensayos clínicos con vacunas profilácticas frente a enfermedades infecciosas. Med Clin, 135(15),707–712.

Ochoa R. 2008. Inmunoepidemiología y estrategias de vacunación. Segunda Edición. La Habana, Cuba: Ediciones Finlay, pp. 1–79

Ochoa R. 2013. VA-MENGOC-BC®: 25 years of massive application. Vaccimonitor, 22(1),1–3.

Pobre K, Tashani M, Ridda I, et al. 2014. Carrier priming or suppression: Understanding carrier priming enhancement of anti-polysaccharide antibody response to conjugate vaccines. Vaccine, 32, 1423–1430.

Pöllabauer E, Petermann R, Ehrlich H. 2009. The influence of carrier protein on the immunogenicity of simultaneously administered conjugate vaccines in infants. Vaccine, 27, 1674–1679.

Sesardic D, Dawes C, McLellan K, et al. 1999. Non-pertussis components of combination vaccines: Problems with potency testing. Biologicals, 27, 177–181.

Tejedor J, Moro M, Ruiz-Contreras J, et al. 2006. Immunogenicity and reactogenicity of primary immunization with a hexavalent diphtheria-tetanus-acellular pertussis- hepatitis B- inactivated polio-Haemophilus influenzae type B vaccine coadministered with two doses of a meningococcal C-tetanus toxoid conjugate vaccine. Pediatr Infect Dis J, 25(8),713–720.

US National Institute of Health. 1947. Minimum Requirements: Diphtheria Toxoid. Fourth Revision ed. Bethesda, MD: NIH Press.

US National Institute of Health. 1952. Minimum requirements: tetanus toxoid. Fourth Revision ed. Bethesda, MD: NIH Press.

World Health Organization. 2010. Recommendations to Assure the Quality, Safety and Efficacy of Recombinant Hepatitis B Vaccines. Proposed replacement of: TRS 786, Annex 2 and TRS 889, Annex 4. Geneva, Switzerland: WHO Press.

World Health Organization. 2013. Guidelines on the quality, safety and efficacy of typhoid conjugate vaccines. Geneva, Switzerland: WHO Press.