Antibody responses to a combination vaccine against *Haemophilus influenzae* type b, diphtheria, pertussis, and tetanus*

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Forty-one children were studied in order to provide information on antibody responses to H. influenzae *type b polyribophosphate (PRP), given in combination with diphtheria–pertussis–tetanus vaccine (DPT). When PRP was administered alone, 9 of 15 children demonstrated fourfold or greater increases in titres of anti-PRP antibody. In contrast, in the group receiving a combination vaccine consisting of DPT and PRP only 1 of 13 children showed a similar rise in anti-PRP antibody. It was concluded that, in the population studied, the combination vaccine was less effective than PRP alone. The reasons for this difference and its potential significance are discussed.*

The possibility of using polyribophosphate (PRP), the capsular polysaccharide of *H. influenzae* type b, to immunize against the serious diseases caused by this bacterium has been the subject of recent reports (1-3). Smith et al. (3) have demonstrated that administration of 5 μg of PRP resulted in higher antibody titres in most middle-income Caucasian children in Massachusetts, USA. Meningitis, the most serious disease caused by *H. influenzae* type b, is a significant problem in developing as well as in industrialized nations. The present study was therefore initiated to measure the antibody responses of children living in a region characterized by widespread poverty and a high prevalence of malnutrition.

A combination vaccine consisting of PRP and diphtheria–pertussis–tetanus antigen (DPT) would be an efficient and convenient method of administration; it was therefore decided to determine whether simultaneous administration of DPT and PRP would affect the production of anti-PRP antibodies.

Responses to polysaccharide antigen among children suffering from malnutrition is of additional interest, since such observations might help to define their immunological responsiveness and thus determine the suitability of this vaccine for the prevention of systemic disease caused by *H. influenzae* in this population.

METHODS

The study was conducted in Lima, Peru during January–March, 1973. The population of children studied was aged between 18 months and 4 years in March, 1973. All had previously been immunized with diphtheria, pertussis, and tetanus vaccine. They were living in a poor community in which malnutrition was common. A more detailed description of the socioeconomic status and medical care of these children has been given by Graham (4).

Forty-one children were selected for the study; all had height quotients of less than 75, meaning that their height "age" was less than 75% of their true age. This implied chronic malnutrition in most of them, with the possible exception of a few who might have had a genetically determined very short stature. Parental consent was obtained after the parents had been interviewed and informed of the

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nature of the study. The children were randomly assigned to one of 3 vaccination groups as follows:

1. 5 μg PRP, Eli Lilly lot CT-2525-2A.

2. A mixture of 5 μg PRP and 5 units DPT, Parke-Davis Bio. 0294 lot 996112a (the vaccines were drawn up into the same syringe, mixed, and injected within a minute).

3. 5 units DPT.

The vaccine was injected intramuscularly into the deltoid. Serum was obtained before vaccination and 2 weeks afterwards and was stored at −70°C. After the study had been completed the serum was transported by air to the USA where antibody assays were performed.

Antibody to PRP was assayed by the radioimmunoassay method (5) in the laboratories of the Children's Hospital Medical Center, Boston, and those to diphtheria, pertussis, and tetanus were assayed at the Eli Lilly Laboratories, Indianapolis. Antibody to diphtheria toxin was determined by a modification of the microtissue culture assay of Quevillon and Chagnon (6). Antibody to tetanus toxoid was determined by agglutination of sensitized normal human O erythrocytes with purified tetanus toxoid according to the chromium chloride method of Baker (7). Antibody to *Bordetella pertussis* was determined by agglutination of methylene blue-treated organisms (8) in microtitration trays (Cooke Engineering Co., Alexandria, VA, USA). The haematocrit and total serum proteins were determined at the time of the second visit. Total serum proteins were estimated in duplicate by refractometry (9). Measurements of height, weight, and subcutaneous fat (intrascapular and triceps) were performed at the time of the first visit.

RESULTS AND CONCLUSIONS

Comparison of the children in the 3 vaccination groups revealed no significant differences in age, sex, physical characteristics, haematocrit, or total serum protein concentration. Fourfold or greater responses to either DPT, PRP, or a mixture of DPT and PRP are shown in Table 1. PRP stimulated antibody production in significantly more children when it was given alone than when it was given mixed with DPT ($\chi^2 = 8.30$; $P < 0.001$). The geometric mean titre of anti-PRP antibody in the group that received PRP alone was 392 ng/ml 2 weeks following immunization, whereas the mean titres in the groups receiving the combination vaccine and DPT alone were 32 ng/ml and 20 ng/ml, respectively (Table 2).

Table 1. Number of children demonstrating fourfold or greater rises in antibody titre

Vaccine	No. of children	Diphtheria	Pertussis	Tetanus	PRP
DPT	13	6 (46.2)[a]	5 (38.5)	7 (53.9)	0 (0.0)
DPT + PRP	13	8 (61.5)	1 (7.7)	2 (15.4)	1 (7.7)
PRP	15	2 (13.3)	1 (6.7)	1 (6.7)	9 (60.0)

[a] Figures in parentheses are percentages.

Table 2. Geometric mean concentrations of anti-PRP antibody before and 2 weeks after vaccination

Vaccine	No. of children	Antibody titre (ng/ml)	
		before vaccination	2 weeks after vaccination
PRP	15	31	392
DPT + PRP	13	19	32
DPT	13	13	20

Fewer of the children that received the combination vaccine showed fourfold or greater increases in antibody to pertussis and tetanus than those that received DPT alone; these differences, however, were not statistically significant.

Interpretation of the results obtained in this study must be cautious owing to the small sample size. Nevertheless, the criteria used for antibody responsiveness (a fourfold or greater rise in post-immunization titre) demonstrated significantly greater rises in both the number of children responding and the titres of anti-PRP antibody when PRP was administered alone, compared with results obtained with the combination vaccine. PRP was immunogenic in 60% of the children studied, but the geometric mean titre (392 ng/ml) was significantly lower than that (1 100 ng/ml) of children of similar age immunized in Massachusetts (3). Previous studies (10) have indicated the protective concentration of anti-PRP antibodies to be 100–200 ng/ml. The observed differences in geometric mean post-immunization titres between the Peruvian and the Massachusetts children may be attributed to differences in the population vaccinated, or to differences in the lots of PRP vaccine used in the two studies. When PRP was combined with DPT it was not immunogenic; only

one of 13 children showed a fourfold antibody response. The poor responses of the children that received the combination vaccine could have important consequences, since this method of administration has been advocated both for its convenience and for the possibility of enhancing the anti-PRP response by means of the pertussis component of DPT.

The precise mechanism of the interference will require additional studies to determine if the effect is:

1. A direct effect of DPT resulting in local inactivation of PRP.

2. The result of suppression of antibody production *in vivo*.

3. The result of using a particular lot or brand of DPT or PRP.

4. Characteristic of all polysaccarides, or only of PRP.

When different concentrations of PRP and DPT were mixed at 24°C and 37°C for as long as 18 h they produced identical lines when assayed by Ouchterlony gel diffusion, as did PRP alone. Therefore, the antigenicity of the PRP was not lost as a result of mixing with DPT. However, the possibility that the immunogenic properties of PRP might have been modified, despite preservation of antigenicity, cannot be ruled out. Moreover, since only a single dose of PRP was given, further studies will be necessary to determine the effect of multiple doses.

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RÉSUMÉ

PRODUCTION D'ANTICORPS EN RÉPONSE À UN VACCIN ASSOCIÉ CONTRE *HAEMOPHILUS INFLUENZAE* TYPE b, LA DIPHTÉRIE, LA COQUELUCHE ET LE TÉTANOS

Nous avons étudié chez des enfants péruviens la formation d'anticorps sous l'effet du polyribophosphate (PRP). Cette substance est le polysaccharide capsulaire qui caractérise *Haemophilus influenzae* type b. D'après des travaux antérieurs il semblait que les anticorps contre le PRP pourraient empêcher la survenue de maladies générales à *H. influenzae*, notamment la complication la plus sérieuse, la méningite. Cette étude a eu pour but particulier d'observer les effets d'une association du PRP avec les antigènes diphtériques, coquelucheux et tétaniques (DPT) en ce qui concerne l'augmentation du taux des anticorps contre ces quatre immunogènes.

Réalisées à Lima (Pérou), ces études ont porté sur 41 enfants de 18 mois à 4 ans, qui ont été vaccinés selon l'une des trois modalités suivantes:

1. 5 μg de PRP, Eli Lilly, lot CT-2525-2A.

2. un mélange de 5 μg de PRP et de 5 unités de DPT, Parke-Davis Bio. 0294 lot 996112a (aspirés et mélangés dans la même seringue, les vaccins étaient injectés en l'espace d'une minute).

3. 5 unités de DPT.

Les injections ont été pratiquées dans le muscle deltoïde.

Les trois groupes d'enfants vaccinés ne différaient pas notablement en ce qui concerne l'âge, le sexe, les caractères physiques, l'hématocrite ou les concentrations des protéines sériques totales. L'analyse des réponses en anticorps a montré des différences entre les sujets ayant reçu du PRP seul et ceux à qui l'on avait administré un mélange de DPT et de PRP. En effet, on a observé chez les premiers à la fois un nombre plus grand de réponses positives (définies par le quadruplement des taux d'anticorps) et une plus grande concentration moyenne des anticorps anti-PRP post-vaccinaux. Ainsi, après administration de PRP seul, 9/15 (60%) des sujets accusaient une augmentation de ces titres, alors que dans le groupe ayant reçu le vaccin associant DPT et PRP, une élévation similaire du taux des anticorps anti-PRP se voyait seulement chez 1/13 (8%) des sujets. En outre, lorsque le PRP était administré isolément, la moyenne géométrique de la concentration postvaccinale d'anticorps anti-PRP était égale à 392 nanogrammes par ml, contre 32 nanogrammes par ml et 20 nanogrammes par ml respectivement chez les enfants ayant reçu le mélange DPT et PRP ou le DPT seul.

Aucune différence sensible n'a été observée après la vaccination dans les titres des anticorps antidiphtériques, anticoquelucheux et antitétaniques des trois groupes de vaccination.

On en conclut que, dans la population étudiée, l'activité du vaccin associé est moindre que celle du PRP seul. Des travaux supplémentaires sont nécessaires pour préciser le mécanisme de l'interférence qu'on observe lorsqu'on associe le DPT au PRP.

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