Vaccines: World Health Organization versus Federal Drug Administration recommended formula

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اللقاحات: مقارنة بين التركيبات التي توصي بها منظمة الصحة العالمية وتلك التي توصي بها الإدارة الفدرالية للأدوية

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خلاصة: إن اللقاحات التي يتم إنتاجها وفقاً لتركيبات منظمة الصحة العالمية تختلف في تركيزها عن تلك التي تستعمل في الولايات المتحدة الأمريكية وفقاً لتركيبات الإدارة الفدرالية للأدوية. ولقد كان هدفنا مقارنة قدرة كل من التركيبتين على توليد المناعة. فتم تقسيم عدد من الرضع البالغين من العمر ستة أسابيع في ثلاث مجموعات بطريقة عشوائية، ليتلقوا ثلاث جرعات من اللقاحات في سن ستة أسابيع ثم ثلاثة أشهر ثم خمسة اشهر. وضمّت هذه اللقاحات المستدمية النزلية "ب" (HbOC) واللقاح الثلاثي (ضد الخانوق والكزاز والشاهوق) واللقاح الفموي ضد شلل الأطفال، حسب تركيبات كل من الجانبين. وبعد مضي شهر من إعطاء الجرعة الثالثة من اللقاحات تم قياس مستويات الأضداد بالنسبة لفسفات البولي ريبوزيل ريبيتول (PRP) والكزاز والخانوق وفيروس شلل الأطفال. وبالرغم من أن مستضدات الخانوق والكزاز في تركيبات الإدارة الفدرالية للأدوية تبلغ نصف تركيزاتها في تركيبات منظمة الصحة العالمية، إلا أن أضداد الكزاز وأضداد الخانوق كانت أعلى بدرجة يعتد بها إحصائياً. ولم توجد أية اختلافات بين مجموعات الأطفال فيما يتعلق باللقاح الفموي ضد شلل الأطفال.

ABSTRACT Vaccines produced in accordance with WHO formulas, differ in concentration from those used in United States according to FDA formulas. We aimed to compare the immunogenicity of both formulas. Infants who were 6 weeks old were randomly put into 3 groups to receive 3 doses of vaccines at 6 weeks, 3 months and 5 months of age. The vaccines consisted of *Haemophilus influenzae* type b vaccine, diphtheria–tetanus–pertussis and oral polio vaccine. Antibody levels for polyribosylribitol phosphate (PRP), tetanus, diphtheria and poliovirus were measured 1 month after the third dose of vaccines. Although diphtheria and tetanus antigens in the FDA formula are half the concentration of the WHO formula, anti-tetanus and anti-diphtheria antibodies were significantly higher. No difference was found between groups regarding oral poliovirus vaccine.

Vaccins: formules recommandées par l'Organisation mondiale de la Santé versus par la « Federal Drug Administration (FDA)»

RESUME Les vaccins produits selon les formules de l'OMS ont une concentration différente de celles distribuées aux Etats-Unis d'Amérique selon les formules de la FDA. Notre objectif était de comparer l'immunogénicité des deux formules. Des nouveau-nés âgés de 6 semaines ont été répartis au hasard dans trois groupes pour recevoir 3 doses de vaccins à l'âge de 6 semaines, 3 mois et 5 mois. Il s'agissait des vaccins contre *Haemophilus influenza* de type b, contre la diphtérie/le tétanos/la coqueluche et contre la poliomyélite (oral). Les niveaux d'anticorps ont été mesurés un mois après la troisième dose de vaccins à la recherche de phosphate de polyribosylribitol (PRP), du tétanos, de la diphtérie et du poliovirus. Bien que la concentration des antigènes diphtériques et tétaniques dans la formule de la FDA soit la moitié de celle de la formule de l'OMS, les anticorps antitétaniques et antidiphtériques étaient considérablement plus élevés. Aucune différence n'a été trouvée entre les groupes en ce qui concerne le vaccin antipoliomyélitique oral.

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Introduction

Vaccines distributed in the United States of America (USA) which are licensed by the Federal Drug Administration (FDA) may contain a different formula and concentration of antigens than the same vaccines distributed in other parts of the world. Vaccines used in all countries except the USA are manufactured according World Health Organization (WHO) specifications, and may contain a higher or lower concentration of antigens than the FDA-recommended concentrations. For example, WHO-recommended diphtheria–tetanus–pertussis (WHO DTP) vaccines contain twice the amount of diphtheria and tetanus antigens as compared to FDA DTP (Table 1). WHO-recommended oral poliovirus vaccines (WHO OPV) contain a lower concentration of the three poliovirus types compared to the FDA-recommended formula (Table 2). What are the effects of these differences on immunogenicity? Can we use the high titre OPV from the FDA to solve the problem of vaccine failure in Saudi Arabia or other developing countries [*1*]?

Table 1 **Comparison of diphtheria–tetanus–pertuesie vaccine formulae recommended by the Federal Drug Administration (FDA) and the World Health Organization (WHO)**

Antigen	DiTePer anatoxal® (Berna) WHO-recommended formula	Tri-immunol® (Lederle) FDA-recommended formula
Diphtheria	25 Lf	12.5 Lf
Tetanus	10 Lf	5 Lf
Pertussis	4 IU	4 IU

Material and methods

Our study was part of a protocol of vaccinating Saudi children with *Haemophilus influenzae* type b (Hib) vaccine with either FDA DTP and OPV used in USA, or with WHO DTP and OPV used in countries like Saudi Arabia. The study was carried out in 1992 and 1993 in three health centres in

Table 2 **Comparison of oral poliovirus vaccines recommended by the Federal Drug Administration (FDA) and the World Health Organization (WHO)**

Name	Orimune® (Lederle)	Polio Sabin® (Smith, Kline & French)
Formula	FDA recommended	WHO recommended
Ratio between poliovirus types 1, 2, 3	30:4:20	10:1:3
Dose ratio ($\log_{10}$ $TCID_{50}$)	6.5:5.6:6.3	6.0:5.0:5.5[a]
Dose in $TCID_{50}$		
Type 1	3 000 000	1 000 000
Type 2	400 000	100 000
Type 3	2 000 000	300 000
Stabilizer	Sorbitol	Magnesium chloride

[a] *WHO formula for type 3 is now 5.8* $\log_{10}$
TCID = tissue culture infectious dose

Hafr El-Batin, Riyadh and Jizan. A total of 210 children who attended the well-baby clinics were divided randomly into three groups using an envelope method. Each group had a sample size of 50 children after some were removed from the study by their parents. The first group received Hib vaccine HbOC (HibTITER®, Lederle) with the WHO-recommended formula of DTP and OPV. The second group received HibTITER® and the FDA-recommended formula of DTP (Tri-immunol®, Lederle) and OPV (Orimune®). The third group received only the WHO-recommended formula of DTP and OPV without the Hib vaccine. Children were vaccinated at 6 weeks, 3 months and 5 months of age.

FDA-recommended formula DTP (Tri-immunol®) and OPV (Orimune®) were supplied by Lederle, New York (now Wyeth-Lederle Vaccines and Pediatrics). Tri-immunol® was supplied as a multidose vial containing 15 doses. Each tube of Orimune® contained a single dose in a volume of 0.5 mL. The WHO-recommended formula OPV used was (Polio Sabin®, Smith, Kline & French) from Smith Kline, Belgium. Each tube contained 10 doses, and 1 dose equalled 2 drops. The differences between the FDA- and WHO-recommended formulas for DTP and OPV vaccines are summarized in Tables 1 and 2. All vaccines were from a single lot and were stored according to standard procedures.

A minimum blood sample of 3 mL was collected at 6 months of age, 1 month after the third dose of the vaccines. Sera were identified by barcode. An antibody assay was performed on each sample in Lederle Laboratories, New York. Only the serology results of DTP and poliovirus are presented in this paper. Results of Hib have been published in a previous report [*2*].

Antibodies against tetanus and diphtheria were measured using an enzyme-linked immunosorbent assay (ELISA) and the results were expressed in international units (IU/mL). A level of 0.01 IU/mL of antitoxin was regarded as the protective level for tetanus and diphtheria [*3,4*]. A triplicate microneutralization assay method was used to measure poliovirus antibodies and the results were expressed in the reciprocal of titre, 0.125 being considered positive.

SPSS was used for statistical analysis. Logarithmic transformation of the antibody level as IU or as the reciprocal of the titres was used for *ANOVA* and Student *t*-test analysis, and means were expressed as geometric mean titre (GMT). A chi-squared test was used to compare the groups regarding the proportion of children with a protective titre.

Results

Table 3 shows the amounts of post-vaccination anti-tetanus and anti-diphtheria antibodies. After the third dose of the FDA DTP vaccine, the antibodies in the second group were significantly higher than the WHO DTP vaccine: $P = 0.047$ in the case of tetanus and $P = 0.0001$ for diphtheria. No significant difference was found between the proportion of children with protective antibody levels in different groups. Regarding poliovirus antibodies, there was consistency in the pattern of the response to the three virus types across the three groups. GMT was highest for type 2, followed by type 1 and then type 3 (Table 4). There was a higher GMT in the second group, but this did not reach a statistically significant level except between antibody level for type 3 virus in group 2 and group 1 ($P = 0.041$). Another exception was a higher GMT for type 1 virus in the first group. No significant differences were found between the three groups when the titres were expressed in IU (Table 5). After the third dose, the proportion of

Table 3 **Antibodies against tetanus and diphtheria in the DPT formula recommended by the Federal Drug Administration (FDA) and the World Health Organization (WHO)**

Vaccine	Group 1 *Haemophilus influenzae* type b and WHO-recommended formula	Group 2 *Haemophilus influenzae* type b and FDA-recommended formula	Group 3 Control	*ANOVA P*-value
Tetanus				0.047
Number	47	50	53	
Geometric mean titre	6.3	9.9	6.4	
95% confidence intervals	4.9–8.1	7.5–13.1	4.6–8.9	
% > 0.01	100	100	100	
% > 0.1	100	100	100	
Diphtheria				0.0001
Number	47	50	53	
Geometric mean titre	0.8	1.3	0.4	
95% confidence intervals	0.6–1.2	1.0–1.7	0.3–0.6	
% > 0.01	100	100	98.1	
% > 0.1	91.5	100	86.8	

Tetanus t-test results P-value: WHO vs control = 0.919; FDA vs control = 0.045; WHO vs FDA = 0.016
Diphtheria t-test results P-value: WHO vs control = 0.009; FDA vs control = 0.0001; WHO vs FDA = 0.043

Table 4 **Comparison of geometric mean titre (GMT) in the three groups using the reciprocal of the titre against the three polioviruses**

Poliovirus type	Group 1 (n = 48) Hib + WHO[a] GMT (95% CI)	Group 2 (n = 47) Hib + FDA[b] GMT (95% CI)	Group 3 (n = 47) WHO GMT (95% CI)
Type 1	256.98 (154.0–417.9)	207.34 (134.0–320.0)	137.90 (81.0–233.0)
Type 2	729.00 (476.0–1117.0)	1164.30 (770.0–1759.0)	914.74 (615.0–1360.0)
Type 3	58.84[c] (36.4–95.0)	117.08 (73.7–185.9)	85.40 (55.7–131.0)

[a]*Hib + WHO* = Haemophilus influenzae *type b vaccine + World Health Organization-recommended formula for poliovirus vaccine*
[b]*Hib + FDA* = Haemophilus influenzae *type b vaccine + Federal Drug Administration-recommended formula for poliovirus vaccine*
[c]*P = 0.041*
CI = confidence intervals

Table 5 **Geometric mean titre (GMT) comparison of the three groups using antibody level against the three polioviruses expressed in international units**

Poliovirus type	Group 1 (n = 46) Hib + WHO[a] GMT (95% CI)	Group 2 (n = 47) Hib + FDA[b] GMT (95% CI)	Group 3 (n = 47) WHO GMT (95% CI)
Type 1	9.20 (5.5–15.39)	7.04 (4.5–10.97)	5.40 (3.10–9.08)
Type 2	23.45 (15.29–35.90)	32.34 (21.26–49.19)	42.48 (28.38–63.59)
Type 3	1.10 (0.688–1.763)	1.69 (1.079–2.656)	1.26 (0.810–1.967)

[a]*Hib + WHO* = Haemophilus influenzae *type b vaccine + World Health Organization-recommended formula for poliovirus vaccine*
[b]*Hib + FDA* = Haemophilus influenzae *type b vaccine + Federal Drug Administration-recommended formula for poliovirus vaccine*
CI = confidence intervals

Table 6 **Proportion of children with detectable poliovirus antibodies in the three study groups**

Virus type	Group 1 Hib + WHO[a]		Group 2 Hib + FDA[b]		Group 3 WHO	
	No.	%	No.	%	No.	%
Type 1	45/48	93.8	45/47	95.7	41/47	87.2
Type 2	47/48	97.9	47/47	100.0	47/47	100.0
Type 3	39/48	81.3	43/47	91.5	41/47	87.2

[a]*Hib + WHO* = Haemophilus influenzae *type b vaccine + World Health Organization-recommended formula for poliovirus vaccine*
[b]*Hib + FDA* = Haemophilus influenzae *type b vaccine + Federal Drug Administration-recommended formula for poliovirus vaccine*

children with a detectable poliovirus antibody level was higher in the second study group for the three types of poliovirus, but the differences were not statistically significant (Table 6).

With regard to GMT, when groups 1 and 3 (WHO OPV) were pooled together, there was no statistically significant difference found compared to the FDA group and the proportion with positive antibody levels. When children vaccinated in these groups with WHO OPV were compared with results of our 1991 survey [*1*], a significant difference was found for the three poliovirus types regarding seropositivity (Table 7).

Discussion

In our study, we found that the formula or immunogenicity of DTP or OPV did not affect the GMT of anti-PRP [*2*]. Priming within certain intervals is more likely than co-administration of the carrier protein vaccine to enhance the response to PRP [*5*]. The effect of priming with a different DTP formula can be studied to evaluate the enhancement of the PRP response, if any.

Table 7 **Comparison between proportion of children with positive poliovirus antibody in the present study and in a 1991 survey**

Poliovirus type	Present study (n = 95)	1991 survey[a] (n = 209)	P-value
Type 1	90.5%	78.9%	0.01
Type 2	98.9%	88.0%	0.001
Type 3	84.2%	65.1%	0.0006

Source: [1]

Although the FDA-recommended formula of DTP contained 50% less tetanus and diphtheria compared to the WHO-recommended formula, the immunogenicity was higher after the third dose. Perhaps the response after the third dose was enhanced because children were primed with a lower dose in the FDA group. It may also be a reflection of the quality of the antigen, which can evoke strong priming and a good immunological memory. In Pichichero's study on acellular pertussis, the control group was vaccinated with whole cell pertussis in the form of whole cell diphtheria–tetanus–pertussis (WDTP) of FDA-recommended formula [*6*]. GMT of tetanus was significantly higher in children vaccinated with WDTP of FDA-recommended formula (6.2 U/mL), compared to children vaccinated with acellular diphtheria–tetanus–pertussis containing the WHO-recommended formula (1.2 U/mL) ($P < 0.001$). The diphtheria antibody was higher but did not reach a significant level. The increase in diphtheria antibody in our study may be attributed in part to the diphtheria carrier protein in the Hib vaccine in groups 1 and 2. Antigen quality or dose may play an important role as the diphtheria antibody was significantly higher in children vaccinated with HbOC and FDA DTP as compared to children vaccinated with HbOC and WHO DTP (P = 0.043).

A study conducted in Australia showed that the increase in diphtheria or tetanus concentration was not met with an increase in the reported systemic or local clinical signs associated with the first three doses [*7*]. The advantage of a vaccine with a lower concentration is that it provides the optimum dose that results in the required immune response.

There was a significant improvement in the proportion of children with protective levels for the three poliovirus types who were vaccinated with three doses of the WHO OPV (group 1 and 3) compared to that in our previous serosurvey [*1*]. Compared with 1991, children with a positive antibody titre for type 3 increased from 65.1% to 81.3% in group 1 and from 78.9% to 87.2% in group 3 (Tables 6 and 7). This may be due to the increased interval between the doses from 1 month to 2 months and the timing of the study, which was conducted in winter [*8–10*]. We should not underestimate the effect of the improving quality of life and services in Saudi Arabia, and the fact that the present work was a controlled study conducted under standard operating procedures.

Environmental factors are the most important determinant of the seroresponse to OPV. In the United Kingdom (UK), using WHO OPV vaccine at 2, 3 and 4 months of age, seroresponse is 99%, 97% and 100% for poliovirus types 1, 2 and 3 respectively [*11*]. The early completion of immunization and the short interval of time between doses have been linked to an impaired response to OPV in developing countries, but this negative effect has not been seen in the UK [*8*]. Standard of living is the most important factor in explaining why people who are in living in the same country can show different responses to OPV [*12*].

Since it is difficult to change environmental factors in the short term, in developing countries efforts should be directed towards understanding factors that affect the seroresponse.

Although there is an apparent higher titre in the FDA OPV compared to the WHO-recommended formula, recent comparative testing of the vaccines has suggested that the potency of the two vaccines is comparable [*9*]. There are still potential advantages to the FDA-recommended formula, such as the higher ratio of type 3 in relation to type 2, and the single dose container of 0.5 mL that guarantees an optimal amount for administration. However, the stabilizer (magnesium chloride) in the WHO-recommended formula is more suitable for tropical countries.

A study conducted in Brazil and Gambia showed that a formula with a two-fold increase in type 1 poliovirus and a ratio of 20:1:6 of the three poliovirus types was associated with a significant increase in seroconversion for type 1. In the same study, a two-fold increase in the dose of type 3 was not associated with a significant increase in seroconversion although more than 1400 children were included in the study [*10*]. In our study, the type 3 poliovirus in the FDA-recommended formula was six times the amount in the WHO-recommended formula and the ratio of type 3 to type 2 was higher. This may explain why the GMT for type 3 was significantly higher in the group vaccinated with FDA OPV (117.08), as compared to the first group vaccinated with WHO OPV (58.84) ($P = 0.04$) (Table 4). The study was conducted when the WHO-recommended formula contained 300 000 $TCID_{50}$ of type 3 poliovirus. The new WHO-recommended formula contains 600 000 $TCID_{50}$ and the ratio of type 3 to type 2 is 6:1. We do not think that this will make a significant difference judging from the results of the WHO study in Brazil and Gambia [*10*].

Even if the FDA OPV appears to be superior to the WHO-recommended formula, the feasibility of its use in developing countries must be taken into consideration. Factors in this decision include the cost of the vaccine and other vaccination alternatives. Other alternatives include the use of enhanced injectable poliovirus vaccine, increasing the number of oral doses, the development of an optimum formula to be distributed by WHO, the use of a monotype vaccine, or a combination of two of the above [*10,13,14*]. The use of OPV is essential in any immunization strategy in developing countries for its known advantages [*15*]. It is even more important in countries like Saudi Arabia where imported poliomyelitis will be always a potential hazard due the large number of expatriates and pilgrims coming into the country.

Conclusions

Seroresponse to OPV is a function of the vaccine, the host and environmental factors. These factors should be identified and monitored, especially in developing countries. An extended programme of immunization should be complemented with supplemental immunization strategies such as immunization days. Our study shows that adherence to standard operating procedures and applying the WHO strategy for poliomyelitis eradication can improve the seroresponse to WHO OPV.

Our study showed that the immunogenic response did not correlate with the concentration of the antigen in the case of DTP. Vaccines with a lower concentration of antigens like FDA DTP may produce a higher immune response. Highly concentrated vaccines such as FDA OPV may not be the solution for vaccine failure in developing

countries. Immune response is not only a function of antigen concentration, but it is a multifactoral event involving human and environmental factors. Since DTP is the core vaccine for most of the bacterial combinations, the minimum requirements and specifications of both formulas should be re-evaluated, including comparing their respective immunogenicity in humans.

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