Simultaneous Vitamin A Administration at Routine Immunization Contact Enhances Antibody Response to Diphtheria Vaccine in Infants Younger than Six Months

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ABSTRACT A randomized, double-blind, placebo-controlled trial was conducted to evaluate the effect of simultaneous vitamin A supplementation and diphtheria, pertussis and tetanus (DPT) vaccination on the antibody levels. Infants aged 6–17 wk (n = 56) were randomly given 15 mg oral vitamin A or placebo at the time of their DPT immunization. Three such doses were given at monthly intervals. Immunoglobulin (Ig) G antibodies to diphtheria, pertussis and tetanus were assayed on enrollment and 1 mo after the third dose. Baseline antibody concentrations to diphtheria, pertussis and tetanus did not differ between the vitamin A–supplemented and placebo-treated groups. The postdose antibody to diphtheria level was significantly greater in the vitamin A than in the placebo-treated group. The geometric mean ± sem antibody levels (mg/L) were 22.9 ± 1.2 and 11.0 ± 1.3 in the vitamin A and placebo groups, respectively (P = 0.029). The postsupplementation concentrations of antibodies to pertussis and tetanus did not differ between the two groups. These results suggest that antibody response to diphtheria vaccination was potentiated by simultaneous vitamin A administration and DPT immunization. J. Nutr. 129: 2192–2195, 1999.

KEY WORDS: infants • vitamin A • diphtheria • pertussis • tetanus • antibody • immunization

Improving the vitamin A status of deficient children and treating cases of measles with a large dose of vitamin A, even in populations in which xerophthalmia is rare, result in substantial reductions in childhood morbidity and mortality (Arthur et al. 1992, Barclay et al. 1987, Barreto et al. 1994, Ghana VAST Study Team 1993, Glaziou and Mackerras 1993, Hussey and Klein 1990). The exact mechanism by which vitamin A reduces morbidity and mortality is unknown. However, vitamin A improves the immune responses to a variety of antigens (Dennert 1984, Nauss 1986, Olson 1994, Semba 1994). Because of the potential beneficial effect of vitamin A on child survival, routine administration of vitamin A every 6 mo to children >6 mo of age has been incorporated into child survival programs. Recently, vitamin A administration in infants <6 mo of age using the routine Expanded Program on Immunization (EPI)† contact has also been suggested (WHO 1982). However, the effect of simultaneous administration of large-dose vitamin A administration and diphtheria, pertussis and tetanus (DPT) vaccination in infants <6 mo of age on the seroconversion to these vaccines is unknown. In this study, we examined the effect of simultaneous vitamin A administration and DPT vaccination on the antibody response to these vaccines.

SUBJECTS AND METHODS

Subjects. This study was conducted in the EPI clinic of the International Center for Diarrhoeal Disease Research, Dhaka, Bangladesh (ICDDR,B: Center for Health and Population Research). ICDDR,B treats ~120,000 patients with diarrhea each year; most of these are children. All children <2 y of age are routinely immunized at discharge (Islam et al. 1992) on the basis of the missed opportunity concept (WHO 1989). Infants aged 6–17 wk who were scheduled to receive their first dose of DPT and oral polio (OPV) vaccines were enrolled after written informed consent was received from their parents. Infants with diarrhea, respiratory tract infections or other infections were not included. The study was approved by the Ethical Committee of the ICDDR,B.

Study design. This study was part of a double-blind, randomized placebo-controlled trial. Infants (n = 200) were randomly assigned to receive either 15 mg (50,000 IU) of oral vitamin A or placebo. The infants were fed retinyl palmitate or placebo and then immunized with DPT and OPV vaccines. The second and third doses of supplementation were given along with the vaccine after 4 and 8 wk, respectively. The manufacturer of the DPT vaccine was Pasteur Marieux (Paris, France). We used two lots of vaccine produced on March 8, 1993 and April 5, 1993, respectively. A vaccine cold chain monitor
RESULTS

Fifty-six infants were studied, 33 in the vitamin A group and 23 in the placebo group (Table 1). The mean age of these infants was 2.5 mo and 93% were breast-fed. The serum retinol concentrations at entry did not differ between the two groups; 89% had serum retinol < 0.70 μmol/L and 38% had serum retinol < 0.53 μmol/L (Table 2). Among the infants with low serum retinol, 86% had normal C-reactive protein, and the rest had a slightly elevated serum C-reactive protein. The serum concentrations had increased in both groups after 3 mo when they were > 3.5 μmol/L (P < 0.001; paired t test). The postsupplementation mean serum retinol concentration tended to be greater in the vitamin A group (P = 0.22). Baseline antibodies to diphtheria, pertussis and tetanus did not differ between the vitamin A and the placebo groups (Table 3). The antibody concentrations at 6 mo were higher in the vitamin A group than in the placebo group (P = 0.029). The postdose diphtheria antibody concentration was 3.3 times the initial value in the vitamin A group and 2.2 times that in the placebo group. The post-vitamin A supplementation IgG antibodies to tetanus and pertussis were not different between the two groups (Fig. 1). After adjusting for age, sex, nutritional status, baseline retinol and C-reactive protein, the greater response to diphtheria antibody in vitamin A–supplemented infants remained significant (P = 0.019).

Age of the child was also associated with a greater antibody response (P = 0.05). Association of other variables with postvaccination antibody levels was not significant.

DISCUSSION

Vitamin A and its metabolites enhance the antibody response to T-cell–dependent antigens, increase lymphocyte proliferation and cytokine production, inhibit apoptosis and maintain mucosal integrity (Blomhoff et al. 1992, Ross 1992, Semba 1994). Physiologic concentrations of retinol augment IgM synthesis by cord-blood mononuclear cells and IgG synthesis by mononuclear cells in the peripheral blood of adults (Israel et al. 1991, Wang et al. 1993). The increased production of immunoglobulin is mediated by increased T-helper cell numbers and enhanced production of cytokines, which in turn induce B cells to differentiate into greater numbers of immunoglobulin-secreting cells (Wang and Ballow 1993, Wang et al. 1993). However, not all responses to T-cell–dependent antigens are increased by vitamin A, nor is the production of all cytokines increased, e.g., γ-interferon is decreased. This study showed enhanced antibody response to diphtheria after simultaneous vitamin A administration and DPT immunization. Vitamin A deficiency is associated with a reduction of both humoral (Carman et al. 1989, Pasatiempo et al. 1989, Smith et al. 1987, Smith and Hayes 1987) and cell-mediated immunity (Chandra and Au 1981, Mark et al. 1983, Nauss et al. 1979 and 1985), which may explain in large part the increased mortality in vitamin A–deficient children (Sommer et al. 1983 and 1984). The beneficial effect of vitamin A supplementation on mortality reduction is well established. In our earlier report from the same cohort, we demonstrated that infants who received vitamin A experienced respiratory tract infections. Venous blood (1 mL) for serum retinol and antibody titers assay was obtained on enrollment and again 1 mo after the third dose. This study reported here was part of the larger trial described above (n = 200) in which the effects of large doses of vitamin A on acute toxicity and morbidity were evaluated. Blood was preserved from those infants whose mothers consented to blood drawing. Because all of the available paired (pre- vs. post-treatment) samples were analyzed, and the overall trial was randomized and double blind, selection bias was unlikely. We had paired samples from 56 infants, a number adequate to detect a difference in postsupplementation antibody concentrations between the supplemented and placebo groups at the 5% probability level with 80% power. This sample estimation was calculated on the basis of a previous study in which vitamin A supplementation was shown to increase the tetanus immunoglobulin (IgG) antibody (Semba et al. 1992). The baseline characteristics of the 56 infants did not differ from those of the other infants in the original cohort studied (n = 200). The effects of vitamin A supplementation on acute toxicity (Mahalanabis et al. 1997), morbidity (Rahman et al. 1996) and seroconversion to oral polio vaccine (Rahman et al. 1998) in the same cohort of 200 children were reported earlier.

Laboratory methods. Serum IgG antibodies to diphtheria, tetanus and pertussis were assayed by ELISA as previously described (Englund et al. 1997, Siber et al. 1991). Briefly, ELISA plates were coated with a 1 mg/L solution of diphtheria toxoid, tetanus toxoid or pertussis toxin (Massachusetts Public Health Biologic Laboratories, Boston, MA) in PBS. Assays utilized a standard of pooled adult sera calibrated by the method of Zollinger and Boslego (1981). Results are reported as mg/L. During analysis, the passively acquired maternal antibody titer (baseline value) was subtracted (half-life was taken as 1 mo) to calculate the antibody titer measured at 3 mo (Robertson 1993).

Serum retinol was analyzed using HPLC (Waters, Millipore, Bedford, MA) (Cattagni and Bieri 1983).

Statistical analysis. Analyses were done using the statistical package for Social Science (SPSS/PC+, Chicago, IL). Categorical analysis was done using the χ-square test. Comparison of continuous variables was done with Student’s t test for normally distributed data and the Mann-Whitney test for skewed data. Because antibody levels were highly skewed, a t test was done after log transformation, and the antibody levels are presented as geometric means. A paired t test was done to compare the postsupplementation serum retinol value with the baseline value.
infections for significantly fewer days compared with the placebo group, although the number of episodes was similar (Rahman et al. 1996). This suggests that the severity of infections was less in vitamin A–supplemented infants, which may be attributed to enhanced immune function.

In this study there was no effect of simultaneous vitamin A administration on antibody responses to pertussis and tetanus vaccines. In an earlier study in Bangladesh, Brown et al. (1980) found no effect of vitamin A on the antibody response to tetanus toxoid. In contrast, Semba et al. (1992) reported that vitamin A supplementation increased the response to tetanus vaccine in children aged 3–6 y. One explanation for the different findings between these two studies is that Brown et al. gave intramuscular injections of vitamin A simultaneously with intramuscular tetanus immunizations to vitamin A–deficient children. Vitamin A acts in a hormone-responsive manner by upregulating the immune system (Blomhoff 1992, Semba 1994). The immune system of a vitamin A–deficient child might require more time to respond after vitamin A supplementation. Therefore, it is possible that there was not adequate time to observe an immune-enhancing effect in that study. The other difference was that the children in the study infants was only 2.5 mo at the time of enrollment, making them much younger than the children of previous studies. In very young infants, antibody response to tetanus may be affected by passive immunity, especially if their mothers were immunized with two doses of tetanus toxoid during pregnancy to prevent neonatal tetanus.

One of the important reasons why Brown et al. (1980) did not find a significant effect of vitamin A supplementation on immune response to tetanus toxoid could be that their method of assay was the mouse protection assay. In this assay, the patient’s serum or plasma is mixed with a known quantity of tetanus toxoid and then injected into a mouse to see if the mouse dies. This is a relatively crude assay, and the mouse protection assay did not detect even a primary antibody response to tetanus toxoid in that study. An ELISA detects (with great accuracy) a primary IgG response to tetanus toxoid.

Why tetanus and pertussis antibody responses were not enhanced, despite an immune-enhancing effect on diphtheria, is not clear. The diphtheria and tetanus vaccines are toxoid, whereas the pertussis vaccine is a whole-cell derivative. This difference in vaccine preparation could be one factor. The immune response to vaccines can differ on the basis of the type of antigen because this can affect the type of antigen-presenting cells (e.g., dentritic or macrophase) that will be involved. These antigen-presenting cells can produce different cytokines.

### TABLE 2

<table>
<thead>
<tr>
<th>Serum retinol μmol/L</th>
<th>Before</th>
<th>After</th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.35, n (%)</td>
<td>18 (54.5)</td>
<td>2 (6.1)</td>
<td>11 (47.8)</td>
<td>4 (17.4)</td>
</tr>
<tr>
<td>0.35–0.52, n (%)</td>
<td>6 (18.2)</td>
<td>4 (12.1)</td>
<td>4 (17.4)</td>
<td>2 (8.7)</td>
</tr>
<tr>
<td>0.53–0.69, n (%)</td>
<td>4 (12.1)</td>
<td>8 (24.2)</td>
<td>7 (30.4)</td>
<td>8 (34.8)</td>
</tr>
<tr>
<td>≥0.70, n (%)</td>
<td>5 (15.2)</td>
<td>19 (57.6)</td>
<td>1 (4.4)</td>
<td>9 (39.1)</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>0.41 ± 0.26</td>
<td>0.75 ± 0.24</td>
<td>0.39 ± 0.22</td>
<td>0.67 ± 0.28</td>
</tr>
</tbody>
</table>

1 Chi-square for trend = 21.9; P < 0.001 (compared with before value).
2 Chi-square for trend = 9.2; P = 0.002 (compared with before value).
3 Significantly different from before values, (P < 0.001), paired t test.

| TABLE 3

Immunoglobulin (Ig)G antibodies against diphtheria, pertussis, and tetanus vaccine in vitamin A–supplemented and placebo-treated infants1

<table>
<thead>
<tr>
<th></th>
<th>Vitamin A</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 33</td>
<td>n = 23</td>
</tr>
<tr>
<td>Diphtheria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1.34 ± 1.1</td>
<td>1.77 ± 1.2</td>
</tr>
<tr>
<td>Postdose</td>
<td>22.9 ± 1.2</td>
<td>11.0 ± 1.3</td>
</tr>
<tr>
<td>Pertussis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.23 ± 1.2</td>
<td>0.31 ± 1.2</td>
</tr>
<tr>
<td>Postdose</td>
<td>4.1 ± 1.3</td>
<td>5.7 ± 1.3</td>
</tr>
<tr>
<td>Tetanus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>7.5 ± 1.3</td>
<td>6.1 ± 1.3</td>
</tr>
<tr>
<td>Postdose</td>
<td>112 ± 1.2</td>
<td>119 ± 1.2</td>
</tr>
</tbody>
</table>

1 Values are geometric means ± SEM.
2 Different from vitamin A–treated group; P = 0.029.
patterns [e.g., macrophages will produce interleukin (IL)-12; IL-12 production is impaired by vitamin A deficiency]. Thus, the difference in responses among the vaccines may be related to an underlying specific effect of vitamin A on, for example, IL-12 production. If IL-12 is not needed for the response to pertussis and tetanus, this may explain the lack of response to vitamin A. Another explanation could be a difference in vaccine potency. One of the limitations of our study is that we did not measure the vaccine potency. However, the rise of IgG antibody in both the vitamin A-supplemented and placebo groups suggests that the vaccine potency was good. Also, the enhanced immune response to diphtheria indicates that the vaccine potency was well maintained. In an earlier report on the same cohort of children, we found that the combined cell-mediated immune responses to diphtheria, tetanus and tuberculosis were greater in vitamin A-replete infants who received vitamin A supplementation than in infants who received placebo (Rahman et al. 1997). Small sample size is also a possible reason for not observing a significant difference in vaccine responses. However, in this study, there was not even a trend for differences between the two groups.

Our data demonstrate that vitamin A supplementation at routine immunization enhances the antibody response to diphtheria. Although the antibody responses to tetanus and pertussis were not affected in terms of increased antibody levels, a previous report based on cutaneous responses suggests a beneficial effect of giving vitamin A with these antigens (Rahman et al. 1997). Because vitamin A deficiency occurs early in life in developing countries, vitamin A administration at EPI contact might be beneficial in improving both humoral and cell-mediated immunity. Administration of vitamin A as part of routine EPI visits has the potential to enhance immune responses while reducing the program cost of administering vitamin A at a separate time.

LITERATURE CITED


