The role of vitamin A in enhancing humoral immunity produced by antirabies vaccine

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دور الفيتامين "أ" في تعزيز المناعة الخلطية التي ينتجها اللقاح المضاد للكَلَب

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الخلاصـة: قمنـا باختيار آثار الجرعات التكميلية من الفيتامين "أ" على عيار الأضداد لدى 40 من المتطوعين الأصحاء ممن تتراوح أعمارهم بين 10 أعـوام و35 عامـاً، والذين تلقّوا مقرراً علاجياً كاملاً مـن اللقـاح المضاد للكَلَب (5 حقـن على مـدى 30 يومـاً). وبعد تحديد حالة الفيتامين "أ" القاعدية في المصل عند 80 متطوعاً، عُقدت مقارنة بين 20 زوجاً لمعرفة مستوى الفيتامين "أ" في المصل، ومنسب كتلة الجسم، والسن، والجنس، والحالة الاجتماعية الاقتصادية، بحيث خصَّص شخص من كل زوج، بصورة عشوائية للمجموعة التجريبية أو المجموعة الشاهدة. وقد تلقّت المجموعة التجريبية الفيتامين "أ" واللقـاح المضاد للكَلَب. أما المجموعة الشاهدة فقد أُعطيت اللقاح فقط. وتبيَّن أن عيار اللقاح المضاد للكَلَب في مصل المجموعة التجريبية كان أعلى بصورة ملموسة منه في مصل المجموعة الشاهدة (2.1 مرة). ويُعَدُّ هذه النتيجة خطوة هامة نحو زيادة الاقتصاد في الجرعات المعطاة من اللقاحات المضادة للكَلَب.

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ABSTRACT We tested the effects of vitamin A supplementation on the antibody titre of 40 healthy volunteers (age range: 10–35 years), who had received a complete course of antirabies vaccine (5 injections over 30 days). After determining the baseline serum vitamin A status of 80 volunteers, 20 pairs were matched for serum vitamin A level, body mass index, age, sex and socioeconomic status. One person from each pair was randomly assigned to an experimental or control group. The experimental group received vitamin A and antirabies vaccine. Controls received only the vaccine. The experimental group had significantly greater (2.1 times) serum antirabies titre than controls. This finding is an important step towards improving the economy of dosage of antirabies vaccines.

Le rôle de la vitamine A dans le renforcement de l'immunité humorale produite par le vaccin antirabique

RESUME Nous avons testé les effets de la supplémentation en vitamine A sur le titre d'anticorps de 40 volontaires en bonne santé (âge compris entre 10 et 35 ans), à qui un cycle complet du vaccin antirabique avait été administré (5 injections sur 30 jours). Après avoir déterminé le statut de référence de 80 volontaires pour la vitamine A sérique, on a procédé à 20 appariements pour le taux de vitamine A sérique, l'indice de masse corporelle, l'âge, le sexe et le statut socio-économique. Une personne de chaque paire a été attribuée au hasard à un groupe expérimental ou un groupe de témoins. On a administré de la vitamine A et le vaccin antirabique au groupe expérimental. Les témoins n'ont reçu que le vaccin. Le groupe expérimental avait un titre sérique d'anticorps antirabiques plus important (2.1 fois) que les témoins. Ce résultat constitue une étape importante en vue d'améliorer l'économie dans la posologie du vaccin antirabique.

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Introduction

Rabies is an ancient disease of humans and animals [1]. The disease remains a continuing threat to humans in many parts of the world. Each year more than 30 000 deaths due to rabies virus are recorded, and almost 4 million people require post-exposure treatment [2]. Most of the cases are recorded in Africa, Asia and South America. The impact of rabies on human populations is aggravated by poor socioeconomic conditions, lack of education, insufficient health facilities and malnutrition in developing countries, including Pakistan. Data at Pakistan's National Institute of Health (NIH) show that the application of the recommended schedule of vaccination using both locally prepared and imported antirabies vaccine (human diploid cell, duck embryo) gives a 20%–30% variation in the required titre.

Vitamin A (retinol) is fat-soluble and is stored in body organs, principally in the liver [3,4]. Deficiency in vitamin A is defined by its tissue concentration; individuals with levels < 20 µg/dL are considered clinically deficient [4,5]. Deficiency in vitamin A results in increased risk of mortality from common childhood infections (such as diarrhoea, measles), maternal mortality, an increased risk of maternal transfer of human immunodeficiency virus (HIV), low immune responses, night blindness, Bitot spots and xerophthalmia [6–14]. The recognition that vitamin A is an immunoregulatory agent in animals and humans primarily stems from two types of observations [15]. First, vitamin A deficiency both in animals and humans has been associated with susceptibility to a variety of infections. The activity of polymorphonuclear cells, the predominant phagocytic cell type, is lower in vitamin A-deficient rats. Recent studies in mice have also shown T-helper subset cells to be adversely affected by vitamin A deficiency, and their function restored upon repletion of serum vitamin A. Secondly, the effect of vitamin A deficiency on increased morbidity and mortality from infectious diseases in humans has been noted. Repletion and supplementation with nontoxic doses of vitamin A as an adjuvant has been shown in many instances to stimulate immune response. Vitamin A deficiency is known to suppress both innate (nonspecific) and acquired (cellular and humoral) immunity [15–17].

Vitamin A supplementation has been shown to lead to clinically relevant immuno-enhancement [17–23]. The tetanus vaccine-induced antibody response in individuals given oral vitamin A supplement has been found to be equally effective in raising antitetanus titres in both clinically normal and xerophthalamic groups compared to individuals receiving a placebo [19]. We proposed, therefore, to study the possible correlation between baseline serum vitamin A level and immunoglobulin antibody titre in volunteers given a complete course of antirabies vaccine, and to establish whether administration of oral vitamin A simultaneously with antirabies vaccine would alter the immunoglobulin response in human subjects. We also wished to explore whether the results of the study could be used as a basis for determining a cost-effective antirabies vaccination dosage schedule.

Methods

A paired clinical trial was conducted at NIH Animal Bite Centre, Islamabad, Pakistan — a World Health Organization (WHO) collaborating centre for viral diagnostics and research. The study involved healthy volunteers from the Islamabad and Rawal-

pindi areas. Participants were non-smokers, not on any medication (including multivitamin therapy), and had not previously been exposed to antirabies vaccine. All procedures for data collection and analysis adopted in the study were in accordance with the ethical standards approved by NIH and WHO.

We recruited 80 volunteers for whom we measured baseline serum vitamin A (BSVA) and obtained information on their economic status, age, sex, weight and height. Using the BSVA, we formed an experimental and a control group, with each experimental participant of a specific BSVA level having a matching counterpart in the control group (to within 12 µg/dL). The pairs were then matched according to economic status based on monthly income: low (2000–3000 Pakistani rupees) (US$ 1 − 59.49 PKR), medium (4000 6000) and high (≥ 7000). Similarly, we matched the pairs on body mass index (BMI) (to within 4.2 kg/m^2), age (to within 7 years) and sex. This procedure yielded 20-matched pairs of 40 individuals, 20 of whom served as the experimental group (Group I) and the other 20 as controls (Group II). The remaining 40 unmatched individuals were excluded from the study.

Group I (the experimental group) received the usual 5-dose antirabies vaccine schedule — a 2.5 IU/mL dose on each of days 0, 3, 7, 14 and 30. They were also given 200 000 IU oral vitamin A— 100 000 IU on the first vaccination day, and 100 000 IU on the following day. Group II (the controls) received only the 5-dose antirabies vaccine (2.5 IU/mL/dose) according to the same schedule as Group I. All injections were given in the deltoid shoulder muscles [2]. The blood for supplemented serum vitamin A was collected from Group I subjects on the day of the last vaccine injection. The blood samples for analysis of serum antirabies titre (SART) were collected from all subjects 30 days after the last vaccine injection.

The blood samples for serum vitamin A analysis were collected from the anticubital vein. All laboratory steps were carried out in subdued light to avoid light-induced degradation of retinol. The vitamin A analysis was carried out using high performance liquid chromatography [24]. SART was measured by a manual enzyme-linked immunosorbent assay (ELISA) (Titre Tek Multiskan, Model: MCC/340, Eflab, Finland) equipped with a microplate reader (Flow). All sera samples were diluted 50% with serum that was previously tested free of antirabies titre. The dilution was made to bring the serum of Group I within the testing range of the Pasteur antirabies titre kit (ELISA).

All data from interviews, medical examination and serum measurements by high performance liquid chromatography and ELISA were analysed using an Excel 2000 software programme. The differences between the means were statistically evaluated using two-tailed t-tests.

Results

All selected volunteers fulfilled the prescribed conditions of the study protocol. The data given in Table I show that groups I and II participants matched closely in terms of age, sex, BMI, socioeconomic status and BSVA. The values for these parameters show statistically insignificant differences. All subjects selected for the study had a vitamin A levels above 20 µg/dL, which is a cut-off point between clinically deficient and normal vitamin A levels [19]. The serum vitamin A values prior to and after supplementation with vitamin A differed significantly ($P < 0.001$).

Table 1 **Characteristics of 40 volunteers studied to determine immune response effect of vitamin A on rabies vaccination, Islamabad, Pakistan**

Parameter (pairs)	Group I (experimental group, $n = 20$)	Group II (control group, $n = 20$)
Age (years)	26.000 ± 1.410	25.200 ± 1.510
Sex ratio M:F	15:5	15:5
Body mass index (kg/m²)	22.777 ± 0.495	23.179 ± 0.487
BSVA (µg/dL)	36.686 ± 2.690	36.684 ± 2.380
SSVA (µg/dL)	56.301 ± 1.730	–
SART	16.550 ± 1.100[a]	7.918 ± 0.578

[a]Statistically significant at $P < 0.001$.
Values are means ± standard error of the mean.
BSVA = baseline serum vitamin A.
SSVA = supplementary serum vitamin A.
SART = serum antirabies titre.

Group I also showed a significantly higher SART response compared to that of Group II. The mean IgG value was significantly (2.1 times) higher than that of Group II ($P < 0.001$). The mean SART value for male supplemented participants (16.5 IU/mL) was slightly higher than the value (15.62 IU/mL) for female participants, but the difference was statistically insignificant.

Discussion

Vitamin A deficiency is known to increase risk of mortality in individuals and has been found to be correlated with depressed immune response and other associated problems such as night blindness, Bitot spot, high maternal HIV transfer risk and xerophthalmia [5–14]. Conversely, vitamin A supplementation has been shown to reduce mortality and improve immune response [21,22].

In our study, an attempt was made to determine whether vitamin A could be used as adjuvant to antirabies vaccine in view of its known efficacy in enhancing immunocompetence [19]. The antirabies vaccine schedule presently in use is expensive and is spread over a month [2]. The advantage of using vitamin A as an adjuvant to antirabies vaccine is thus obvious.

As demonstrated, all participants involved in the study were closely matched in terms of sex, age, BMI and medical history. Furthermore their baseline serum vitamin A levels were within the clinically normal range. Vitamin A supplementation brought about a significant increase in the SART (IgG response) of volunteers. This booster effect of vitamin A on SART was significant, regardless of whether the BSVA status of the subjects was close to the borderline value of 20 µg/dL (the cut-off point for clinical vitamin A deficiency) or well above the value (e.g. 70 µg/dL). The economic status of the participants as measured in the study showed no relation to IgG response of the participants supplemented with vitamin A. The data presented here provide clear evidence that oral vitamin A supplementation enhances immunocompetence of people to antirabies

vaccine, suggesting a strong potential for the use of vitamin A as an adjuvant to antirabies vaccine. In spite of the supporting role of vitamin A in improving certain other disease conditions [15–17,20], very few studies have been carried out to assess the efficacy of vitamin A as a supplement in vaccination protocols [19,23]. The studies on tetanus vaccine have demonstrated that vitamin A supplementation brings about a significant increase in antibody titre in both clinically vitamin A-normal and deficient individuals [19]. There is only one study from Bangladesh that shows results that are contrary to our finding on immune response [23]. However, the methodology of the Bangladesh study differed from our study in that they gave intramuscular injections of vitamin A simultaneously with tetanus immunizations to vitamin A-deficient individuals. It is probable that vitamin A-deficient people react differently and may require more time to return to a normal range of serum vitamin A.

Further work is needed to study the response of clinically vitamin A-deficient individuals (BSVA < 20 µg/dL) to antirabies vaccine prior to and after vitamin A supplementation. In this context, it may be noted that the IgG response following tetanus immunization of xerophthalmic (vitamin A-deficient) individuals given vitamin A supplementation was as beneficial and as significant as that of clinically normal individuals [19]. In light of the present data, there is a strong possibility for establishing a more economical antirabies vaccine dosing protocol than the present 5-day schedule, by using vitamin A as an adjuvant to antirabies vaccine. Work is in progress at NIH in Pakistan on this, which it is hoped will open ways for improving sera production technology in general (antirabies antibodies, prepared immunoglobulin, antisnake venom) through the application of oral vitamin A.

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