A randomised controlled trial to test equivalence between retinyl palmitate and β carotene for vitamin A deficiency //

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Abstract

Objectives—To determine whether β carotene is therapeutically equivalent to retinyl palmitate in the formulation currently recommended by the World Health Organisation.

Design—Randomised blind equivalence trial.

Setting—Rural area in Senegal.

Subjects—Children aged 2-15 years suffering from vitamin A deficiency as defined by abnormal results on eye cytology were randomly allocated treatment with retinyl palmitate ($n=256$) and β carotene ($n=254$).

Main outcome measure—Reversion to normal results on eye cytology as defined by the reappearance of goblet cells and normalisation of the epithelial cells.

Results—Seven weeks after the supplement was given the percentages were 51.2% (124/242) children taking retinyl palmitate and 50.0% (123/246) of those taking β carotene, who had reverted to normal eye cytology, a difference of 1.2% (95% confidence interval 0.8% to 1.6%). According to an equivalence testing procedure, the two treatments were statistically equivalent; the null hypothesis of non-equivalence was rejected (one tailed p value = 0.03).

Conclusions— β Carotene supplementation seems to be a promising candidate for the alleviation of vitamin A deficiency. It could be given either as high dose capsule or through increased dietary intake. The challenge now is to improve dietary intake of vitamin A in programmes that are effective and sustainable at the community level.

Introduction

Up to 40 million children worldwide are estimated to suffer from vitamin A deficiency, which is associated with increased morbidity and mortality and leads to blindness.¹⁻³ These observations prompted the World Health Organisation to recommend appropriate prevention strategies^{4,5} based on oral administration of a large dose of vitamin A in the form of retinyl esters, particularly retinyl palmitate, to combat vitamin A deficiency and xerophthalmia. A dose of 200 000 IU retinyl palmitate to children over 1 year old is most widely used and has generally been regarded as a safe and potentially effective intervention according to biochemical,⁶ cytological,⁷ and clinical indicators.⁸ Prevention strategies differ only in the frequency of oral administration: every 3-6 months for periodic administration; immediately on diagnosis, the following day, and four weeks later for treating xerophthalmia.

In developing countries, animal products that provide retinyl esters are too expensive for most families to consume regularly in large quantities. Vegetables and fruit, which are cheap and good sources of vitamin A in the form of β carotene, are grown almost everywhere but are not consumed in adequate amounts.⁹ β Carotene is also considered to be virtually non-toxic (unlike retinyl esters, for which toxicity has been reported) and its metabolism differs from that of retinyl palmitate.^{10,11}

It has recently been suggested that vitamin A deficiency can be treated with a rich natural source of carotenoids.¹² In a preliminary study, Brazilian children were fed buriti palm fruit as a supplement in their normal diet. This fruit provides about 40% of a child's daily vitamin A requirement.¹³ After 20 days there was reversion of the clinical and subclinical signs of vitamin A deficiency in the study group.¹² Small daily doses of β carotene therefore show promise as a means of alleviating vitamin A deficiency. Since β carotene is the principal source of vitamin A in developing countries and is non-toxic, we compared retinyl palmitate and β carotene for treatment of vitamin A deficiency.

We conducted a randomised blind equivalence trial among 510 vitamin A deficient Senegalese children to test the equivalence¹⁴⁻¹⁷ between the effects of a large oral dose of either retinyl palmitate or β carotene in correcting vitamin A deficiency as defined by abnormal results on eye cytology.^{18,19} As no published reports describe the use of a single large dose of β carotene, the objective of our study was to determine whether β carotene was therapeutically equivalent to retinyl palmitate at the present formulation (200 000 IU) recommended by the World Health Organisation.⁵

Subjects and methods

SUBJECTS

The study was conducted between February and April 1991 among Senegalese children aged 2-15 years from 30 rural villages of the community of Malicounda in the Thies region. Previous surveys have been carried out in this community where vitamin A deficiency is present among preschool and also school age children.^{20,21} Children were divided into two age groups according to the World Health Organisation criteria for assessing vitamin A deficiency.⁵ All children from these randomly selected villages²⁰ were recorded by trained community workers and were enrolled in the survey, giving a total number of 1488 subjects. Civil status and ophthalmic and anthropometric data were recorded for each child.

To be eligible for the equivalence trial, children had to be resident in the Malicounda rural community, 2-15 years old, with or without eye infections, and suffering from xerophthalmia or cytological signs of vitamin A deficiency as defined by abnormal results of impression cytology.¹⁹ Of the 1488 who were enrolled, 510 children met our eligibility criteria and were included in the trial. The 510 children were randomly assigned to either retinyl palmitate or β carotene treatment groups by means of 510 serially numbered labels, which had been drawn from an envelope on the day before vitamin A supplementation and alternately allocated to retinyl palmitate and β carotene treatment groups.

Field work was conducted by a team consisting of a paediatrician, a nutritionist, an ophthalmologist, and two fieldworkers. The study was approved by the ethics committees of the Organisme de Recherche en Nutrition et en Alimentation Africaines and the Malicounda Développement Sahel and by the

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Senegalese Ministry of Health. Verbal informed consent was obtained from the parents at the time of enrolment as most were illiterate.

TREATMENT

The currently recommended method for vitamin A supplementation is oral administration of a large dose of preformed vitamin A capsules (200 000 IU retinyl palmitate).⁵ The nutritional relation between preformed vitamin A and carotenoids was calculated as β carotene is absorbed less well than retinyl esters (10% v 60% of intestinal absorption) from the intestine.²² The bioavailability of β carotene is not as good as that of retinyl esters (1 IU of preformed vitamin A = 3 IU of β carotene).¹³ Thus 200 000 IU preformed vitamin A are equivalent to 1 200 000 IU of provitamin A carotenoids. Consequently, the 510 eligible children received a single oral dose of either one capsule containing 200 000 IU of retinyl palmitate or six capsules simultaneously giving a total of 1 200 000 IU β carotene.

The ingestion of the six β carotene capsules required was not always easy with children 2-3 years old, even when the capsules were given with strawberry jam or orange juice. Therefore the ingestion of retinyl palmitate or β carotene capsules for this single treatment was supervised by the medical team, ensuring 100% compliance.

FOLLOW UP

The study was divided into two parts. The first examination, carried out in February 1991, included the examination of the 1488 children and the selection according to the previously defined inclusion criteria of the children eligible for the trial. Doses of either retinyl palmitate or β carotene were administered orally once the 510 subjects had been selected. The second examination was carried out in April 1991, seven weeks after supplementation, on only the eligible children. Seven weeks is the time required after administration of retinyl palmitate for cytological improvement of the eye, including the reappearance of goblet cells.⁷ The first and second examinations were performed during the dry season, just before the gathering and consumption of mangoes, which could have affected vitamin A status: mangoes are a rich source of carotenoids (100 g is estimated to contain 2100 IU vitamin A).²³

MEASURES AND ASSESSMENT CRITERIA

The sex and age of each of the 1488 children were recorded. Ocular examinations for vitamin A deficiency were based on clinical and cytological criteria. All eye examinations (n=488) for xerophthalmia⁵ were carried out by a well trained ophthalmologist in normal daylight with a portable slit lamp (Kowa SL 5).

None of the 1488 children showed signs of xerophthalmia. Other eye diseases, including inflammatory trachoma and conjunctivitis, were also diagnosed during the ophthalmic examination.²⁴ The impression cytology method was performed as previously described on both eyes of the 1488 children and on the 510 eligible children seven weeks after vitamin A supplementation.^{18,19} Each subject was scored according to the reading from the eye closest to normal. A normal cytological result was characterised by sheets of small epithelial cells and the presence of goblet cells. An abnormal cytological result was defined as either few or absent goblet cells and enlarged or keratinised epithelial cells. The impression cytology with transfer test is reproducible and is fairly sensitive in relation to serum retinol concentrations whose threshold was set at 0.35 μ mol/l in accordance with the prevailing World Health Organisation criteria.¹⁹ Since serum retinol concentrations are affected by nutritional status and

the collection of samples raises ethical difficulties and technical problems, we decided according to the required sample size for this clinical trial to use a simple, non-invasive, and cheap test such as impression cytology with transfer, which is rapidly gaining wide acceptance and validity.²⁵

Success of the treatment was indicated by normal results on eye cytology. All readings were taken by a single worker using a microscope without knowledge of the subject's treatment group to ensure a blind protocol.

The children's height was measured to the nearest centimetre with a calibrated board. Weight was determined to the nearest 0.1 kg with a bathroom scale (SECA, Hamburg, Germany) that was calibrated at the beginning of each session with a standard weight. All of the 1488 children were measured standing unshod and dressed (the children were lightly clothed owing to the climate). Standard deviation scores for height for age, weight for height, and weight for age were calculated according to the guidelines of the World Health Organisation: the cut off point for undernutrition is 2 SD below the median.²⁶

STATISTICAL METHODS

Many problems in which the true proportions of two populations are to be compared are drawn up in terms of a classic statistical procedure testing the null hypothesis of their equality. This formulation is inappropriate for experiments that aim to show that two treatments are equivalent rather than different. Consequently, a new testing procedure for such an equivalence trial has been proposed by Dunnett and Gent in which showing equivalence is the primary objective.¹⁴ Many authors have developed this methodology for trials.¹⁵⁻¹⁷

Hypothesis testing

Testing equivalence of two groups requires making an explicit statement of the hypothesis to be tested (the null hypothesis, H_0). Fundamentally, the idea is to "reverse" the role of H_0 and H_1 . That is, the hypothesis of equivalence is formulated as the alternative (H_1), so that the rejection of H_0 supports that notion.

Let P_{RP} and $P_{\beta car}$ be the true proportions of children who reverted to normality after a massive oral dose of retinyl palmitate or β carotene, respectively. The null hypothesis (H_0 of non-equivalence is accordingly $P_{RP} - P_{\beta car} \geq \delta$, and thus the alternative H_1 is $P_{RP} - P_{\beta car} < \delta$, where δ is the maximum difference between the effects of the treatments that could be considered to be clinically acceptable.

The problem was formulated as a one sided test since we were interested in whether or not the proportion of children treated with β carotene who reverted to normality ($P_{\beta car}$) was smaller than that with retinyl palmitate (P_{RP}). We decided to choose $\delta = 10\%$ such that if $P_{\beta car}$ fell in the interval $[P_{RP} - 10\%, 1]$ the β carotene treatment would be considered at least equivalent to retinyl palmitate.

Sample size requirement

The number of children required in this trial to show that β carotene was equivalent in efficacy to retinyl palmitate was calculated with the formula proposed by Makuch and Simon²⁷:

$$n = \frac{[z_\alpha + z_\beta]^2}{[\delta]^2} \times 2P_{RP}^*(a - P_{RP}^*)$$

—where z_α is the value of the standard normal distribution corresponding to a significant level of α (1.65 for a one sided test at the 0.05 level) and z_β is the value of the standard normal distribution corresponding to the desired level of power (0.84 for a power of 80%); P_{RP}^* is the assumed percentage of children who

revert to normality after a large oral dose of retinyl palmitate (45% according to a previous intervention survey conducted in Senegal by the same team²⁸); δ is the maximum allowable difference between the percentages of children who reverted to normality after a massive oral dose of retinyl palmitate or β carotene if they were equivalent ($\delta=10\%$); and n is the required number of children for each group ($n=310$). According to the formula, a sample of 620 vitamin A deficient children (two groups of 310) was necessary for this trial.

Statistical tests

We used the normal approximation of the distribution of the observed difference $p_{RP}-p_{\beta car}$ under the null hypothesis to construct the large sample test statistic.¹⁴

$$Z = \frac{[p_{RP}-p_{\beta car}-\delta]}{[\text{Variance}(p_{RP}-p_{\beta car})]^{1/2}}$$

We also investigated the percentage of children who reverted to normality in the two treatment groups for age, sex, nutritional status, and ocular infection, which are prognostic factors for the condition studied. Classic tests of interaction were used to assess whether the factors affected the observed difference in treatment.²⁹

Results

A total of 620 vitamin A deficient children were required for this trial. Due to practical conditions of work in the fields (the approach of the mango season), we stopped enrolment at 510 vitamin A deficient children from a total of 1488 children examined. This sample size of 510 resulted in a loss of power of 7%, according to Makuch and Simon's formula where $z_{\alpha}=1.65$ for $\alpha=5\%$ and $z_{\beta}=0.61$ for a power of 73%.²⁷

BASELINE CHARACTERISTICS

The 510 vitamin A deficient children were randomly allocated to either the retinyl palmitate group ($n=256$) or the β carotene group ($n=254$). The two groups were nearly identical in terms of the potential confounding variables of sex, age, nutritional status, and ocular infections at baseline examination (table I). Approximately a third of the vitamin A deficient children were classified by anthropometry as undernourished (<2 SD below the median). Stunting affected a somewhat larger proportion of the children than wasting, indicating that prolonged malnutrition was more common than acute malnutrition. Roughly half of the vitamin A deficient children also suffered from

TABLE I—Baseline characteristics of children with vitamin A deficiency as defined by abnormal eye cytology

Characteristics	No (%) given supplement	
	Retinyl palmitate (n=256)	β Carotene (n=254)
Sex:		
Boys	135 (52.7)	130 (51.2)
Girls	121 (47.3)	124 (48.8)
Age (years):		
2-6	165 (64.4)	166 (65.3)
7-15	91 (35.6)	88 (33.7)
Nutritional state*:		
Stunted	69 (27.0)	64 (25.2)
Wasted	9 (3.5)	9 (3.5)
Stunted-wasted	6 (2.3)	5 (2.0)
Normal	172 (67.2)	176 (69.3)
Eye infections:†		
Inflammatory trachoma	117	132
Conjunctivitis	34	28

*Stunted: height for age $<(\text{mean}-2 \text{ SD})$, weight for height $>(\text{mean}-2 \text{ SD})$.

Wasted: height for age $>(\text{mean}-2 \text{ SD})$, weight for height $<(\text{mean}-2 \text{ SD})$.

Stunted-wasted: height for age $<(\text{mean}-2 \text{ SD})$, weight for height $<(\text{mean}-2 \text{ SD})$.

Normal: height for age $>(\text{mean}-2 \text{ SD})$, weight for height $>(\text{mean}-2 \text{ SD})$.

†Some children had both infections.

TABLE II—Reversion to normal cytology after seven weeks

	Retinyl palmitate		β Carotene	
	No of children	No (%) who reverted to normality	No of children	No (%) who reverted to normality
Total	242	124 (51)	246	123 (50)
Sex*				
Boys	129	69 (54)	127	66 (52)
Girls	113	55 (49)	119	57 (48)
Age (years)*				
2-6	158	69 (44)	163	75 (46)
7-15	84	55 (66)	83	48 (58)
Nutritional state:*				
Undernourished†	78	39 (50)	77	36 (47)
Normal	164	85 (52)	169	87 (52)
Eye infections:*				
Present‡	127	51 (40)	142	57 (40)
Absent	115	73 (64)	104	66 (64)

*p Values for interaction test >0.20 .

†Height for age or weight for height, or both, <2 SD below reference mean.

‡Inflammatory trachoma or conjunctivitis.

ocular infections. Inflammatory trachoma (49% of the subjects) was more frequent than conjunctivitis (12%). These infections were similarly distributed in the two treatment groups (table I).

CYTOLOGICAL RESPONSE

After seven weeks, 488 children were available for re-examination (95% in the retinyl palmitate group and 97% in the β carotene group). Seventeen subjects did not present at the second examination and were thus lost to follow up. Impression cytology with transfer could not be performed on five further subjects at week 7 because of the child's non-acceptance.

The vitamin A status of both supplemented groups had improved. In the retinyl palmitate group 51.2% of children were normal after seven weeks and 50% were normal in the β carotene group, a difference of 1.2% (95% confidence interval 0.8% to 1.6%).

According to the equivalence testing procedure previously described, $z=-1.94$ and one tailed $p=0.03$. The null hypothesis of non-equivalence was thus rejected. Therefore β carotene was therapeutically equivalent to retinyl palmitate with respect to the cytological response to vitamin A supplementation. Furthermore, the groups did not differ in potential prognostic factors: sex, age, nutritional status, and ocular infections (table II). All p values for interaction tests were above 0.20.

SIDE EFFECTS

Large doses of either β carotene or retinyl palmitate were well tolerated. No complaints or complications such as headache, nausea, or vomiting, which often occur after large doses of retinyl palmitate, were reported during the survey.

Discussion

Vitamin A deficiency is well known to be a major problem among children of preschool age, and school age children also suffer from deficiency,²⁰ though policymakers rarely consider this group despite the consequences. This randomised blind equivalence trial showed that β carotene corrects vitamin A deficiency to the same extent as does retinyl palmitate, as shown by eye cytology. There was no evidence of a difference between the treatments according to factors (sex, age, nutritional status, and ocular infections) potentially affecting the outcome. Eye infections (inflammatory trachoma and conjunctivitis) described as confounding factors for the impression cytology with transfer test¹⁹ were common (55%). The reasons for these cytological changes, which are due to *Chlamydia trachomatis* or to an insufficient supply of vitamin A to the eye, are still unclear. However, we have previously shown that ocular inflammation led to moderate changes in the

validity data of impression cytology with transfer calculated according to a serum retinol cut off at 0.35 $\mu\text{mol/l}$, and that the test was even more sensitive when only the sample of children with eye infections was considered.¹⁹ After seven weeks, there was no evidence of difference between the treatments in children with or without eye infections, suggesting that β carotene has the same therapeutic effect as retinyl palmitate in children with trachoma or conjunctivitis.

Our findings are consistent with the preliminary survey conducted in Brazil in which 44 vitamin A deficient children were given buriti palm fruit supplements and showed replenishment of their hepatic reserves and partial or complete regression of clinical signs.¹² Several studies have also reported that Indonesian or African children with xerophthalmia consume foods rich in provitamin A less frequently than do clinically normal children.³⁰⁻³²

As both treatments had similar effects the 488 children could be considered as a whole. Seven weeks after vitamin A supplementation only 51% had normal findings on cytology, a figure similar to the 45% in a previous study.²⁸ These results contrast with studies conducted in areas of endemic vitamin A deficiency in India^{33,34} and in Indonesia,³⁵ where almost all children with abnormal cytology reverted to normal after retinyl palmitate treatment. The disparity with the African and Asian studies may be due to protein malnutrition. Vitamin A circulates on demand from the hepatic reserves to peripheral tissues by means of specific carrier proteins: retinol binding protein and transthyretin.³⁶ The two proteins are synthesised by the liver and are sensitive to acute variations in protein-energy status. If the diet is low in protein, vitamin A is not released from the liver and its transport is impaired owing to insufficient hepatic biosynthesis of the retinol binding protein and the transthyretin. Therefore, the satisfactory supply of vitamin A to peripheral targets (such as the eye) depends on the hepatic synthesis particularly of retinol binding protein and also transthyretin. This hepatic synthesis is inadequate in populations with mainly cereal diets poor in essential amino acids,³⁷ as in these villages of Senegal, where little fish and less meat are eaten. The transthyretin is widely implicated in the inflammatory reaction and its serum levels may therefore be considered as a nutritional index only in the absence of inflammation.³⁸ In our experience, however, the prognostic value of the transthyretin was not modified by the synthesis of defence factors involved in infectious diseases.³⁹ Mean serum retinol binding protein and transthyretin concentrations did not increase after vitamin A treatment in children in Senegal, who have only cereal diets.²⁸

Our study was conducted in a Senegalese area where vitamin A deficiency is common.²⁰ Food rich in vitamin A—meat, dairy products, and fish—is rarely consumed, and most dietary vitamin A is derived from vegetables rich in carotenoids. The main source is mango, which is available only during the harvest season (May to September) and is the most popular fruit for many Senegalese.⁴⁰ The cytological changes (reversion to normality) assessed in this trial can thus be attributed only to massive oral doses of retinyl palmitate or β carotene. Other natural vitamin A supplies or environmental factors were not confounding factors since the study was conducted before the mango season and vitamin A deficient children were randomly ascribed to either the retinyl palmitate or β carotene treatment groups.

We applied stringent criteria for success, which has not always been the case in other studies.¹² The randomised blind procedure and the complete compliance ensured by the medical team giving the vitamin A capsules ought to have reduced inaccuracy. Children were selected and followed up on the same cytological

criterion for vitamin A deficiency as impression cytology with transfer is an easy method to perform in the field in comparison to blood sampling and analyses and is also reproducible (intraobserver variation is low and results are independent of the site of sampling the conjunctival cells of the eye).⁴¹ Because of the time required for screening 1488 children in the field with impression cytology with transfer (this has not previously been done with such large numbers) and the approach of mango season, we stopped our enrolment when a sample size of 510 vitamin A deficient children was obtained. This sample size led to a loss of power of 7% compared with the optimal sample of 620 but did not change the outcome of this survey, although we decided to run a small risk of 5% of rejecting the null hypothesis of non-equivalence when it was actually true. Only 4% of subjects were lost to follow up; they were almost equally distributed between the two treatment groups and would not therefore be expected to influence the outcome.

VITAMIN A AND PUBLIC HEALTH

Since β carotene supplementation had a therapeutically similar effect to retinyl palmitate for vitamin A deficient children, the study has important public health implications in the struggle against vitamin A deficiency. β Carotene has advantages over retinyl palmitate in terms of toxicity, its availability in developing countries, and cost effectiveness.

Firstly, several studies have reported that large doses of retinyl palmitate can induce signs of raised intracranial tension such as bulging fontanelle, restlessness, fever, and vomiting in children and potential teratogenicity in pregnant women.¹¹ There has been no report of toxic symptoms resulting from excessive intake of carotene. The only known consequence is yellow pigmentation of the skin. Therefore β carotene could be given to pregnant and lactating women to increase their body stores and the vitamin A content of milk since β carotene is safer than retinyl palmitate and has no risk of teratogenicity. However, no side effects of retinyl palmitate were reported during our trial.

Secondly, β carotene is widely found in coloured fruits and vegetables, which are indeed the principal source of vitamin A. These products are widely available and less expensive than animal products in developing countries. Nutritional education and appropriate preservation technologies leading to increased consumption of carotenoids should be financially more advantageous than the consumption of retinyl esters, which are derived from animal products. Considering capsule supplementation, it is presently not possible to compare the cost of the two treatments because a capsule form of β carotene has never been used to treat vitamin A deficiency.¹² The price would depend on the quantity of capsules produced. In our study the cost per treatment was US\$0.78 for 1 200 000 IU of β carotene and US\$0.02 for 200 000 IU of retinyl palmitate.

The objective of our study was to determine whether single oral doses of β carotene were therapeutically equivalent of retinyl palmitate. β Carotene and retinyl palmitate capsules were given as a single treatment since the timing of vitamin A supplementation should take account of seasonal factors, such as the mango season. Therefore, further investigations are required to optimise the practical and financial constraints—altering the dose of β carotene, the type of preparation and packaging, and the route and frequency of administration.^{5,42}

CONCLUSION

Vitamin A deficiency can be treated with a “magic pill”—the administration of large oral doses of β carotene at the intervals defined by the World Health

Clinical implications

- Vitamin A deficiency, resulting in blindness, is a public health problem in developing countries
- Prevention strategy is generally based on oral administration of a large dose of retinyl palmitate associated with risk of toxicity
- A randomised blind equivalence trial conducted on 510 vitamin A deficient Senegalese children shows that β carotene is equivalent to retinyl palmitate
- β Carotene, virtually non-toxic, could be used as substitute for retinyl palmitate in large dose capsule
- The challenge is to improve dietary intake through vegetables which are widely available and the major source of carotenoids

Organisation for retinyl palmitate⁵ is thus a good method for vitamin A prophylaxis. Obviously, the best approach would be to encourage increased dietary intake of vitamin A through the consumption of carotenoid rich, coloured fruit and vegetables, which are the major sources of vitamin A in developing countries. β Carotene is non-toxic and as effective as retinyl palmitate. Additionally, there is growing evidence that β carotene enhances the immune response.⁴³ Consequently, β carotene could be used as a substitute for retinyl palmitate to address the problems of not only blindness but also mortality and morbidity, although there are as yet no surveys to assess impact on survival. Many foods rich in β carotene available in developing countries are not offered to children. The challenge now is to improve the dietary intake of vitamin A in programmes that are effective and sustainable at the community level to struggle against blindness and to improve child survival.

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