Short communication

Retinyl palmitate supplementation by inhalation of an aerosol improves vitamin A status of preschool children in Gondar (Ethiopia)

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We report successful vitamin A supplementation by inhalation of retinyl palmitate in a placebo-controlled pilot study in twenty-five preschool children (2–5 years of age) in the rural district of Gondar, Ethiopia. Preschool children (*n* 161) were randomly selected from 220 households. Out of this cohort, twenty-five children were randomly assigned to each of two treatment groups: one receiving retinyl palmitate by inhalation of two puffs of an aerosol containing 1 mg (3000 IU) per delivery to give a total of 2 mg (6000 IU); and the other receiving an aerosol without retinyl palmitate. Both treatments were administered every 2 weeks for 3 months. Serum retinol and retinol-binding protein concentrations in the vitamin A-treated group were 0·68 (SD 0·31) µmol/l and 59·4 (SD 24·2) mg/l before and 1·43 (SD 0·46) µmol/l ($P < 0.01$) and 97·3 (SD 31·2) mg/l ($P < 0.05$) 3 months after supplementation with retinyl palmitate, suggesting that this novel method of delivery of retinyl palmitate by inhalation is effective in improving vitamin A status.

Retinyl palmitate: Vitamin A: Xerophthalmia

The effectiveness of various therapeutic protocols of vitamin A administration has been examined in numerous studies using tablets or capsules. In India and Indonesia, provision of extra vitamin A resulted in considerable reduction of mortality (about 40 %) in preschool children (Bhandari *et al.* 1994; Humphrey *et al.* 1996). While the effect of high oral doses (67 mg (200 000 IU) every 3 months) has been questioned, it is evident that a low dose given once weekly apparently has little effect on morbidity due to gastrointestinal and respiratory infections (Ramakrishnan *et al.* 1995*a,b*). Indeed, recurrent diarrhoeal episodes resulting in impaired absorption of vitamin A, or the existence of protein–energy malnutrition (PEM) with subsequent reduced retinol-binding protein (RBP) synthesis may explain in part the poor effect seen with oral vitamin A administration at low doses (Ramakrishnan *et al.* 1995*b*).

The present investigation aimed to evaluate the effect of inhalation of the vitamin as an alternative route to oral administration. This procedure bypasses the possible problems of poor gastrointestinal absorption and/or impaired release of retinol from liver stores that may occur in PEM due to insufficient RBP synthesis (Rahmathullah *et al.* 1991).

In addition, this method can supply vitamin A directly to a very sensitive target tissue, the respiratory mucosa, that undergoes severe morphological and functional alterations as a consequence of vitamin A deficiency (Stofft *et al.* 1992).

The aim of the present study was, therefore, to test this novel mode of administration using retinyl palmitate in an aerosol.

Materials and methods

The study, a placebo-controlled randomized supplementation trial, was carried out between October and January in the rural district (Azozo) of Gondar, Ethiopia. This area was chosen because of its known prevalence of endemic vitamin A deficiency (DeSole *et al.* 1987).

From 220 households 161 children (2–5 years of age) were selected at random for the study; at the first visit to the local clinic, nutritional assessment and stool examination (parasites or ova) were carried out. Children with parasites (*n* 141) were treated routinely with mebendazole (one capsule) at this first visit to the clinic 4 d before the start of the trial. The effect of the treatment on parasitic infection was

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not further controlled. Heparinized blood was obtained for assessment of vitamin A, RBP and transthyretin (TTR) concentrations 4 d before the start of the study and 4 d after the last inhalation.

Twenty-five children (four without mebendazole treatment), selected at random, received aerosol treatment with retinyl palmitate, 2 mg (6000 IU) every 2 weeks, over 3 months, while twenty-five control children (three without mebendazole treatment) received aerosol without retinyl palmitate, as a placebo. The aerosol was administered through the mouth with a 300 ml spacer during the breath-in mode, which allows a controlled application. The mean particle size is below 5 μm ensuring good lung deposition of the vitamin (Roche *et al.* 1997).

The inhalation trials and blood sampling were performed by trained field workers and no adverse effects or reactions were observed during inhalation. The children complied well with the treatment. Plasma retinol was measured by a reverse-phase HPLC method (Biesalski *et al.* 1983). RBP concentrations were determined by using radio-immuno diffusion employing LC partigen (Behringwerke AG, Marburg, Germany). TTR levels were assessed with a kit (Antibody set II; Inestar Corp., Stilwater, MN, USA).

The trial was approved by the Gondar College of Medical Science Ethics Committee and informed consent was obtained from the parents of the children participating in the study.

The aerosol

The composition of the aerosol (Hermes Arzneimittel GmbH, München, Germany) was as follows: retinyl palmitate (stabilized with butylated hydroxyanisole and butylated hydroxytoluene), 1,1,2-trifluoroethane (198·3 g/kg) dichloro-difluoromethane and 1,2-dichloro-1,1,2,2-tetrafluoroethane (800 g/kg). The metering valve discharged a mean dose of 1 mg (3000 IU) retinyl palmitate (0·97 (SD 0·038) mg) as measured by repeated administration to a filter and detection subsequent to extraction. DL-α-Tocopherol (0·75 mg) was added to the aerosol as an antioxidant. The placebo was identical in composition to the agent, but did not contain the active substance (retinyl ester).

Statistical analysis

Data were analysed with Prism 2.0 software (GraphPad, Inc., San Diego, CA, USA). The Wilcoxon non-parametric test for testing dependent variables and the Mann Whitney test for the independent variables were used for statistical analysis.

Results

The mean baseline serum retinol concentration derived from the 161 children was 0·74 (SD 0·46) μmol/l. Fourteen children out of 161 (8·7 %) exhibited extremely low serum retinol concentrations ($< 0·35$ μmol/l) suggesting severe vitamin A deficiency while seventy-eight children (48 %) had low serum concentrations ($< 0·70$ μmol/l) signifying inadequate vitamin A status according to WHO (World Health Organization, 1984). Mean serum retinol concentrations were not different in the test and control groups before
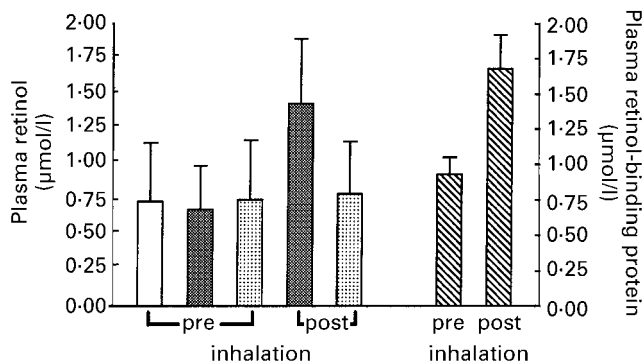
Fig. 1. Plasma retinol (□, ▨, ▨) and retinol-binding protein (▨) concentrations of preschool children before and after 3 months treatment with an aerosol containing retinyl palmitate (▨) or a placebo (▨). (□), Plasma retinol level in the whole group of preschool children (*n* 161) before selection of the study and placebo groups (*n* 25). Values are means, with standard deviations indicated by vertical bars.

inhalation (0·68 (SD 0·31) μmol/l *v.* 0·75 (SD 0·42) μmol/l) showing a distribution of vitamin A deficiency similar to that for the total group. However, serum retinol concentration increased considerably (1·43 (SD 0·46) μmol/l, $P < 0·001$ compared with the initial value) following supplementation (six inhalations, totalling 12 mg (36 000 IU) retinyl palmitate) in the test group compared with the control group (0·79 (SD 0·37) μmol/l) (Fig. 1). RBP concentration was low before the treatment (pre: 0·93 (SD 0·12) μmol/l) and increased after inhalation (post: 1·68 (SD 0·24) μmol/l). The concentration in controls (pre: 0·89 (SD 0·14) μmol/l, post: 0·9 (SD 0·11) μmol/l) remained low. Supplementary vitamin A did not affect TTR concentrations in the treatment group (pre: 157·2 (SD 43·7) mg/l *v.* post: 170·9 (SD 35·1) mg/l) and TTR levels of the control group (pre: 165·7 (SD 36·1), post: 168·1 (SD 28) mg/l) did not differ from those of the vitamin A-treated group. There was no obvious effect of mebendazole treatment for parasites on vitamin A status in the placebo group.

Discussion

In the present study we were able to show successful treatment of vitamin A deficiency with retinyl palmitate administration by the inhalation of an aerosol. The levels of retinol and RBP, which were initially deficient, increased to within normal ranges in the vitamin A-treated group but not in the placebo group. The increases in the concentrations of retinol and RBP in the bloodstream following vitamin A supplementation show that the children were indeed vitamin A deficient and that the vitamin was taken up by the liver following the application by inhalation. In addition, the increase in RBP concentration clearly demonstrates that the children did not suffer from impaired hepatic protein synthesis due to PEM. This is also supported by the unaltered TTR levels in both groups. Indeed, it is well known that TTR metabolism is regulated independently and that changes in the concentration of TTR are usually associated with alterations in nutritional status. Consequently, the considerable decreases in retinol and RBP concentrations, in the face of unchanged TTR levels, indicate that these alterations were

entirely due to vitamin A deficiency or its supplementation. The fact that the release of RBP from the liver increased following aerosol inhalation strongly indicates that the observed vitamin A deficiency is primarily due to low intake or malabsorption of vitamin A and not due to PEM (Blaner, 1989).

We postulate that retinol might have reached the liver following inhalation in two different ways: (1) an unknown quantity of the inhaled retinyl palmitate in the aerosol might have been swallowed and subsequently absorbed via the intestine. From there, it would be transported in the bloodstream, incorporated into chylomicrons and delivered to the liver; (2) another portion might have been absorbed via the respiratory mucosa of the respiratory tract and consequently have appeared as retinyl palmitate in the bloodstream without incorporation into chylomicrons.

The first possibility cannot be excluded although the use of a spacer as an inhalation device and the adequate particle size (2–5 μm) ensure good lung deposition of drugs even in subjects with poor coordination for inhalation (Roche et al. 1997). The second possible route for retinyl ester delivery to the bloodstream, and at least to the liver, is the absorption via the respiratory tract. Indeed, retinyl esters can be absorbed as such from the respiratory tract and occur in the bloodstream following inhalation, as we demonstrated recently in a rat experiment (Biesalski, 1996). Retinyl esters circulating in lipid droplets in the blood following intravenous application, and not bound to chylomicrons, can be taken up into the liver and further tissues (Gerlach et al. 1989; Hultin et al. 1996). This allows a direct supply of vitamin A to tissues, circumventing the homeostatic control of retinol release from the liver and problems of RBP synthesis in the case of PEM. The hypothesis that retinyl esters can replace retinol–RBP as the main complex that supplies the vitamin A-dependent cells is strengthened by a recent finding of two female siblings with a mutation of the RBP gene (Biesalski et al. 1999). These siblings (aged 14 and 16 years) had retinol levels below 0·2 μmol/l and undetectable RBP levels, but normal or slightly elevated fasting retinyl ester levels (0·14–0·22 μmol/l) and normal intestinal absorption of vitamin A. In spite of this low retinol concentration, both girls were normally developed and, with the exception of night blindness and reduced visual acuity (Seeliger et al. 1999), had no other signs of vitamin A deficiency that would normally manifest at such low levels of serum retinol over a long time period.

Besides the uptake of retinyl esters into tissues, the application of vitamin A by inhalation may offer an additional benefit: the respiratory mucosa and other cells of the respiratory tract which critically depend on vitamin A can be supplied via this route. Under normal dietary conditions, retinyl esters are found in different cells of the respiratory tract. They are either formed by esterification of intracellular retinol, bound to cellular RBP (Napoli, 1996), or they are taken up as such from the bloodstream (Biesalski, 1996). From these intracellular pools of retinyl esters, retinol may be acquired following hydrolysis of the retinyl esters by means of the cholate-independent retinyl ester hydrolase which predominates in the lung (Napoli et al. 1989). Retinol bound to cellular RBP may then be further oxidized to retinoic acid which enters the nucleus bound to cellular retinoic acid binding protein to interact with retinoic acid receptors and their target genes.

The importance of the intracellular retinyl esters was recently shown in rat lung fibroblasts (McGowan et al. 1997). The authors inhibited the hydrolysis of retinyl esters and consequently the formation of retinoic acid in lung fibroblasts. As a result of this inhibition, the expression and the steady-state level of the tropoelastin mRNA were reduced. These findings suggest that intracellular retinyl esters are important sources for retinol and retinoic acid respectively. During moderate vitamin A deficiency, the retinyl ester stores of the respiratory epithelium become rapidly depleted, while plasma retinol levels are only slightly decreased (Biesalski et al. 1990; Biesalski & Weiser, 1990). This depletion of retinyl ester stores in the respiratory mucosa results in a loss of cilia and an increase in mucus-secreting cells with impairment of lung function (Biesalski & Stofft, 1992; Stofft et al. 1992). It is also known that an impairment of the mucociliary clearance increases the susceptibility to infectious respiratory diseases frequently associated with marginal vitamin A deficiency (Sommer et al. 1984), and conversely, that infections of the respiratory- and gastrointestinal tracts increase the risk of vitamin A deficiency (Sommer et al. 1986). Interestingly, vitamin A status following supplementation with 15 mg retinyl palmitate monthly for 2·5 months was not improved in the presence of respiratory tract infections (Rahman et al. 1996).

It is essential to examine whether the uptake of retinyl esters in the respiratory mucosa might be associated with formation of excess retinol or retinoic acid in the cells with the formation exceeding the binding capacity of cellular RBP or cellular retinoic acid binding protein. In experimental studies with application of retinol margarinate by inhalation, the uptake of retinyl esters showed great variation in different sites of the lung tissue (Biesalski, 1996), yet the cellular concentration of retinol remained essentially unchanged. This indicates that the formation of retinol is strictly controlled despite high or low uptake of retinyl esters. In this connection it is also important to emphasize that long-term topical administration of high-concentration vitamin A in liquids containing 5 mg (15 000 IU)/ml is an established therapy for atrophic rhinitis, rhinitis sicca and further metaplastic changes of the nasal epithelium (Simm, 1980). The applications lead to the normalization of the epithelium and reappearance of a normal function without any reported side-effects.

Consequently administration of retinyl esters by inhalation and their subsequent uptake into cells of the respiratory tract may serve to establish an intracellular pool of retinol after controlled hydrolysis of the retinyl esters. A further advantage of the route is that absorption of the retinyl ester occurs via the respiratory mucosa. Thus, inhalation of retinyl esters might be suitable for vitamin A therapy in the presence of malnutrition, respiratory-tract diseases and diarrhoea when normal gastric absorption might be problematic.

We found that administration of vitamin A in the form of an aerosol is an effective, safe and routinely manageable method to elevate vitamin A and RBP levels. Consequently, this procedure may serve as an alternative method for vitamin A therapy during chronic or acute episodes of malnutrition,

malabsorption or in the case of insufficient compliance with other therapies, and it might be useful in treating respiratory diseases associated with vitamin A deficiency.

Acknowledgements

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