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ACNE TREATMENT WITH ORAL ZINC AND VITAMIN A: EFFECTS ON THE SERUM LEVELS OF ZINC AND RETINOL BINDING PROTEIN (RBP)

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Abstract. The serum levels of zinc, vitamin A and retinol binding protein (RBP) were studied in 75 acne patients before and during oral treatment with zinc, vitamin A or placebo. In the zinc-treated patients an increase in the mean serum zinc level was seen after 2 weeks, when also the first clinical improvement occurred. After 4 weeks the zinc level had increased by about 30% and no further significant increase was observed during 3 months of treatment. In 33 healthy subjects there was an increase of 14% after 4 weeks of zinc therapy. Vitamin A and placebo induced no significant changes in the serum zinc status. Prior to therapy the serum levels of vitamin A and RBP were lower in the acne patients than in the controls. Zinc+vitamin A treatment raised the serum RBP value to normal after 4 weeks. In patients given vitamin A alone, a probable increase in RBP was achieved. Zinc and placebo treatment did not change the serum level of RBP.

In recent years a beneficial effect of oral zinc therapy on acne vulgaris has been reported (4, 10, 12). The basis for this effect of zinc is unknown, but it is probably of an anti-inflammatory nature (3). Zinc is also of importance for epithelialization and for a large number of enzyme activities affecting the metabolism of other compounds (for ref. see 19). For example, zinc appears necessary for several stages in the metabolism of vitamin A[1] (5, 21). This interaction has drawn our attention, as the importance of vitamin A in relation to acne vulgaris has been repeatedly discussed over the years (2). We have recently reported low serum levels of the retinol-binding protein (RBP) in acne patients (11-, a finding in agreement with reports on low vitamin A levels (13). In most of our cases, however, treatment with oral vitamin A palmitate was not very effective against the acne lesions (10).

In the present study, we have observed the serum zinc, vitamin A, and RBP levels in acne patients during treatment with oral zinc and/or vitamin A. As controls, placebo-treated acne patients and zinc/vitamin A treated healthy adolescents were included. The clinical results of these same treatments have been reported earlier (10).

SUBJECTS AND METHODS

Subjects

A total of 75 acne patients, 53 males and 22 females, between 15 and 25 years of age, took part in the study that was undertaken mainly between February and May. They all had acne of grades II–IV according to Pillsbury et al. (18). No tetracyclines or other antibiotics had been used during the preceding 3 months. Aside from their acne, the patients were apparently healthy and none of them had diabetes or signs of liver or kidney disease. The material is essentially the same as that described in the study on the clinical effects of zinc and vitamin A treatment (10) but with the addition of 16 male patients treated with either vitamin A alone or zinc + vitamin A (see below). One boy and 2 girls under 15 years of age and 2 girls who were taking oral contraceptives were excluded. The exclusions were made because pubertal development, as well as contraceptives, can influence at least the RBP values (9). As a control group 19 male and 14 female healthy students (aged 22–27 years) were included in this study.

Treatment

The patients were randomly placed in one of the following groups:

Zinc

Zinc sulphate ($ZnSO_4$+7 H_2O) effervescent tablets (Solvezinc®), 0.2 g corresponding to 45 mg Zn^{++}, were given three times daily.

Placebo

Placebo effervescent tablets without $ZnSO_4$. The tablets looked and tasted the same as the zinc-containing formula. They were dissolved in a glass of water and taken after meals 3 times daily.

Vitamin A

An emulsified solution of retinol palmitate (Arovit, Roche) was used with a concentration of 150 000 IU vitamin A per

[1] Vitamin A is used generically to include all compounds that are biologically and structurally related to retinol (=vitamin A alcohol).

Table 1. *Serum zinc values in acne patients during treatment (μmoles/l (±S.E.M.))*

Treatment	No. of patients[a]	Weeks of treatment 0	2	4	6	8	12
Zinc	21	14.1±0.4	17.0±0.4[b]	18.2±0.6[b]	19.0±0.9	19.6±0.7	18.1±0.7
Zinc + vitamin A	18	13.9±0.4	16.5±0.3[b]	18.4±0.6[b]	18.2±0.6	19.1±0.7	18.5±0.8
Vitamin A	15	15.1±0.4	14.4±0.2	14.4±0.2	–	–	–
Placebo	15	14.5±0.4	15.1±0.5	14.8±0.4	–	–	–

[a] A total number of 69 patients. Values were not available at all times in the 6 missing cases.
[b] Indicates that a value is significantly ($p<0.05$–0.001) greater than the values nearest to the left (see text for details).

ml. The patients took 25 drops twice daily if their body weight was 60 kg or less or 30 drops twice daily if their body weight was over 60 kg. The daily dose of vitamin A was thus approximately 300000 IU (90 mg retinol).

Zinc plus vitamin A

Zinc sulphate and vitamin A were given as described above, but together. The healthy subjects were given this treatment for 4 weeks.

The treatment of the patients was continued for 12 weeks. If a patient had become worse or showed insignificant improvement after 4 weeks on either zinc or vitamin A, the combined treatment was given. In this case, week number five was registered as the first week of combined treatment.

Examination procedures

The patients were seen in the morning and were fasting for at least 14 hours when the venous blood samples were drawn. The counting of the lesions and estimation of the severy of the acne have been described previously (10). Serum was collected by centrifugation after storage in the dark for a few hours. Subsequent storage was at −20°C in the dark prior to processing.

Analyses

Zinc. Serum samples were collected for zinc determination and special precautions were observed to avoid contamination. The analyses were done using a Varian 1100 atomic absorption spectrophotometer as outlined previously (9).

RBP. The serum concentration of RBP was determined by radial immunodiffusion as described in detail elsewhere (9, 25). For each sample the mean value of three determinations was used. Since our previous reports appeared concerning serum RBP levels in acne patients and in healthy adolescents (9, 11), the standardization of the method has been modified. The present standardization yields RBP values that are numerically increased by 17%.

Vitamin A. A Turner spectrofluorometer (Model 430) was used for the quantitation of vitamin A, essentially as described by Thompson et al. by employing a correction formula (24). Serum (100 μl) was extracted with hexan and the fluorescence at 475 nm was measured with excitaion at both 335 nm and 367 nm. These wavelengths yielded the best resolution according to the principle of the method. Factors for the conversion of fluorescence into equivalents of concentraton of retinol were derived from analyses on chromatographed solutions of retinol and phytofluene. Subsequently, a solution of quinine bisulphate was used as standard. The analyses were performed in duplicates.

Statistics

The mean and standard error of the mean (S.E.M.) are used throughout. Correlation coefficients, regression

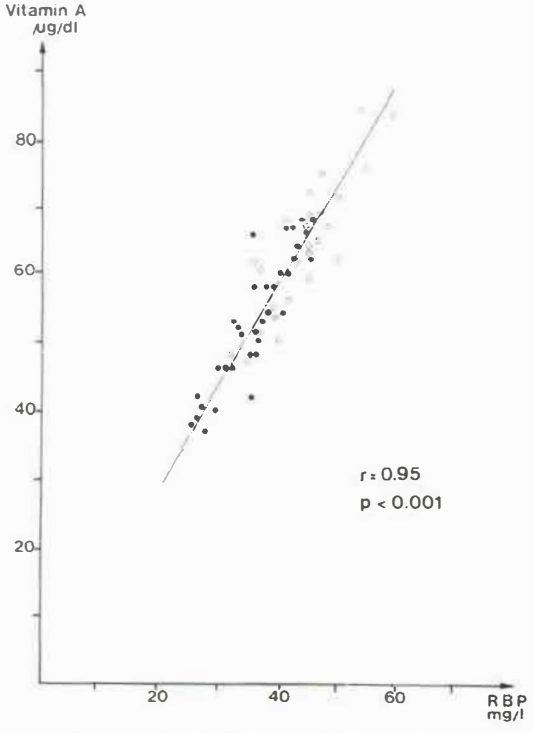

Fig. 1. The correlation between fasting serum concentrations of vitamin A and retinol binding protein (RBP) in untreated acne patients and healthy controls. ○, Healthy subjects; ●, acne patients. The equation of the regression line was $Y=1.46x+1$.

Table II. *Serum RBP values in acne patients during treatment (mg/l) (±S.E.M.)*

Treatment	No. of patients[a]	Weeks of treatment 0	2	4	8
Zinc	14	39.6±2.4	39.8±2.5	39.7±2.6	40.6±2.1
Zinc + vitamin A	19	39.3±1.5	42.8±1.5*	46.3±1.8**	43.2±1.4*
Vitamin A	10	37.4±1.3	39.7±1.6	41.3±1.4*	–
Placebo	8	39.5±1.4	39.9±1.9	37.6±1.6	–

[a] A total number of 51 patients. Only those patients are included for whom values were available at all times.
* Indicates $p<0.05$ and ** $p<0.01$ for statistical difference vis. initial value.

analysis, and Student's *t*-test for significance were calculated on a digital computer according to accepted principles (23).

RESULTS

Serum zinc

In the untreated acne patients, the zinc concentration (mean ± S.E.M.) in serum was 14.2±0.2 μmol/l, which was similar to that of the healthy controls (13.9±0.3 μmol/l). The values appeared to be normally distributed.

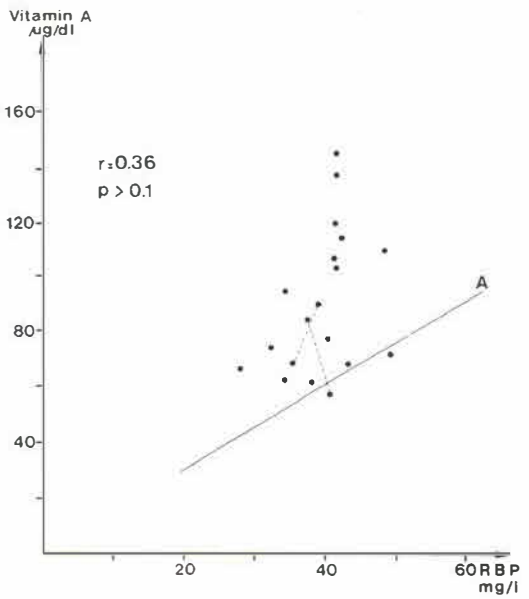

Fig. 2. The correlation between fasting serum concentrations of vitamin A and retinol binding protein (RBP) in patients treated with vitamin A palmitate. The letter A indicates the regression line from Fig. 1, for untreated subjects. Blood samples were obtained at 2 and/or 4 weeks of treatment. Serial values from the same individual have been connected by broken lines.

As can be seen in Table I the serum zinc level had increased significantly in the acne patients after 2 weeks of treatment with either zinc, or zinc + vitamin A ($p<0.001$). It continued to increase the following 2 weeks ($p<0.05$–0.01). After 4 weeks of treatment there was no further significant increase in the serum zinc level. Neither vitamin A alone nor placebo treatment induced any significant change in the serum zinc concentration. The mean serum zinc level in the healthy controls, only studied after 4 weeks of zinc + vitamin A treatment, had increased significantly after this treatment to 15.8±0.4 μmol/l ($p<0.005$), but the increase was smaller than in the similarly treated acne patients ($p<0.01$).

Serum vitamin A and RBP

The RBP level in serum before treatment was significantly depressed in acne patients, as compared with healthy subjects (38.6±0.7 vs. 43.0±1.1, $p<0.001$). The vitamin A level before treatment was closely correlated to the RBP value in both groups (Fig. 1).

In a pilot study on acne patients treated with vitamin A, the serum level of vitamin A was found to vary considerably and was poorly correlated to the RBP level (Fig. 2). This was in contrast to the findings in placebo- and zinc-treated patients, who showed the same correlation as in non-treated subjects. Due to the inconstancy of the vitamin A level during vitamin A treatment it was not studied routinely.

The effects of various treatments on the serum RBP level of acne patients are summarized in Table II. During zinc and placebo therapy, the mean RBP level remained unchanged and below the normal. The combined zinc and vitamin A therapy induced a significant elevation of the mean RBP level already at 2 weeks. The individual values at 0 and 4 weeks

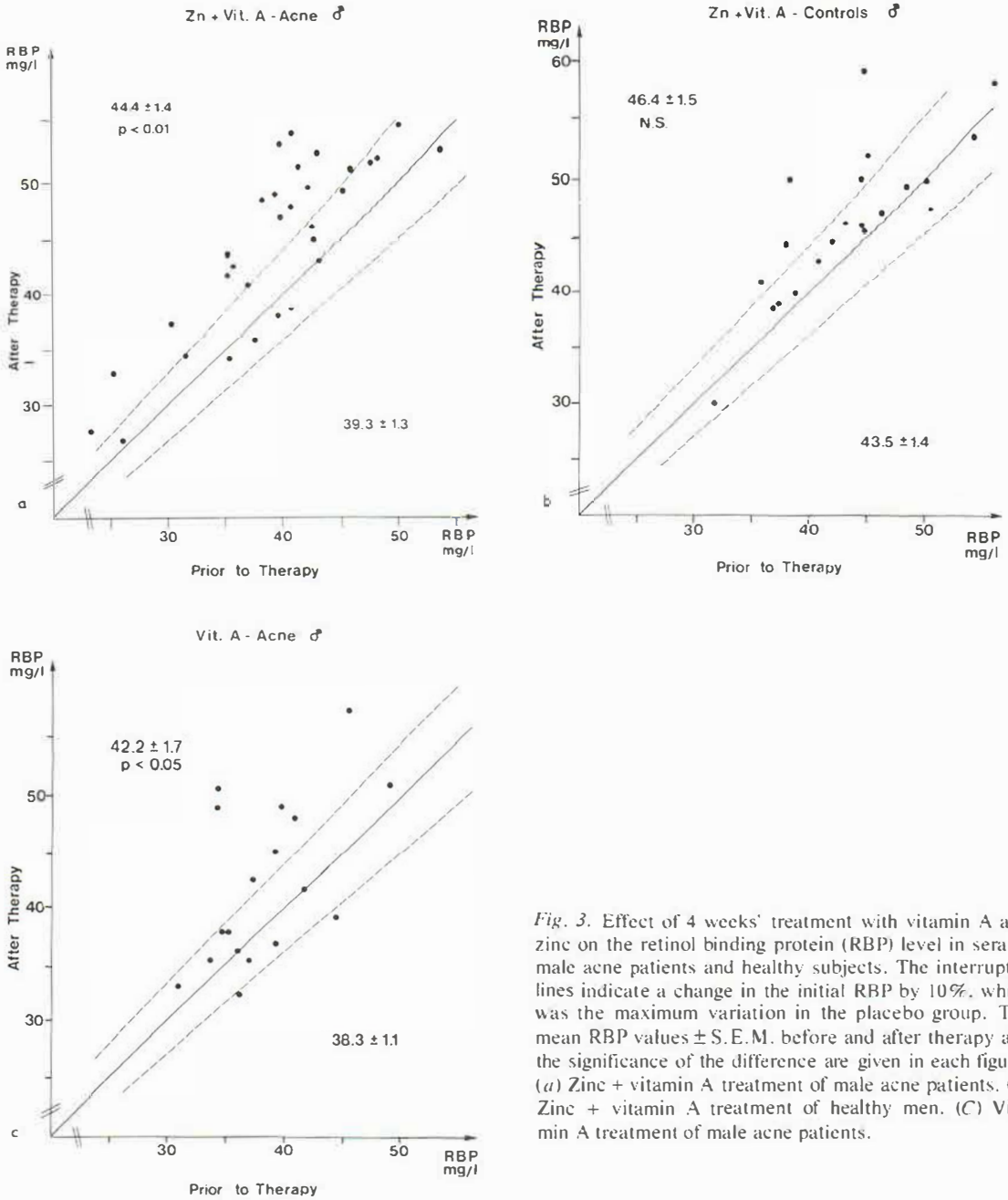

Fig. 3. Effect of 4 weeks' treatment with vitamin A and zinc on the retinol binding protein (RBP) level in sera of male acne patients and healthy subjects. The interrupted lines indicate a change in the initial RBP by 10%, which was the maximum variation in the placebo group. The mean RBP values ± S.E.M. before and after therapy and the significance of the difference are given in each figure. (*a*) Zinc + vitamin A treatment of male acne patients. (*b*) Zinc + vitamin A treatment of healthy men. (*C*) Vitamin A treatment of male acne patients.

in a total of 31 male acne patients treated with zinc + vitamin A are shown in Fig. 3*a*. For comparison, values from 21 similarly treated healthy male controls are shown in Fig. 3*b*. Women were excluded, as variations in the serum RBP level induced by the menstrual cycle could simulate effects of treatment (26). An individual increase in the serum RBP level exceeding 10% can be seen in 56% of the acne patients and in 28 per cent of the healthy controls. A significant increase in the mean RBP value was observed only in the acne group. Vitamin A therapy alone induced a probably significant rise in the serum RBP level within 4 weeks of treatment but it remained low in some of

the patients, with an initial low RBP level (Table II and Fig. 3*c*). There was no correlation between the increase in serum RBP and the clinical improvement in the acne patients.

DISCUSSION

The clinical results of this study have been reported elsewhere, showing a significant effect of oral zinc therapy on acne lesions as compared with placebo (10). It was also shown that the effect of a combined zinc and vitamin A therapy was not superior to that of zinc alone. Furthermore, oral vitamin A treatment was not significantly more effective than placebo, although occasional patients improved quite distinctly.

We have previously demonstrated a slight decrease in the serum zinc level in male patients with severe acne (11). In the present mixed acne material, we found the mean serum zinc value to be normal. This level increased 20% after 2 weeks and 36% after 8 weeks in the zinc-treated acne patients. A similar increase has been found by others (28). No change in serum zinc status was found after placebo or vitamin A treatment, nor was it changed after treatment with tetracyclines (12). These results differ from those of Weissman et al. (28) who reported significantly increased serum zinc levels also in placebo treated acne patients. Healthy adolescents showed a significant increase in the serum zinc level when given zinc supplementation. The increase was less pronounced than in acne patients. Part of this discrepancy can be due to differences in the motivation to take the zinc tablets rather than to a different response to zinc supplementation per se.

A clear clinical improvement of zinc-treated patients was often observed at 4 weeks and, at this time, most patients had attained their maximum zinc level. However, a healing effect of zinc was also seen in some patients yet without any clear increase in the serum zinc level. Whether this reflects an inaccuracy of serum zinc as a measure of zinc status in the individual case (1) or reflects the existence of pharmacological effects of zinc unrelated to the relief of a deficiency, cannot yet be decided.

In spite of the rather large dose of zinc sulphate given, we observed no signs of toxicity in the acne patients. In agreement with this, the highest individual zinc value was found to be below the supposed toxic level of serum zinc (17). There was no significant tendency towards a progressive increase in serum zinc levels over 3 months of therapy. Thus the tendency to hyperzincemia reported to arise during zinc supplementation in patients with acrodermatitis enteropathica was not observed in acne patients (15). Further studies are needed, however, to establish the optimal zinc levels in serum and tissues.

The serum RBP values are significantly lower in acne patients than in healthy controls (11). The present report confirms that a low serum RBP level reflects a low vitamin A concentration in blood samples of fasting acne patients. Zinc treatment did not affect the low RBP levels of acne patients in this study, in spite of the good clinical results. In another acne study, the RBP mean values were also unchanged after 3 months of treatment with either zinc or tetracycline (unpublished observation). These findings suggest that the low serum RBP levels in acne patients are not secondary to the skin inflammation. Vitamin A therapy induced a probably significant increase in the serum RBP level. The zinc + vitamin A-treated patients showed a numerically greater increase in serum RBP but we could not ascertain any significant difference between the effects on RBP of the two treatments. The vitamin A + zinc treated healthy controls showed no significant increase in serum RBP. This finding is in agreement with the opinion that the RBP level of an already well nourished healthy person will not be influenced even if he/she is given a high dosage of vitamin A (27).

RBP is the physiological mediator of vitamin A to the tissues and its level in plasma is carefully regulated (7). In the postabsorptive state vitamin A is also associated with lipoproteins, which convey the vitamin to the liver. Excessive increases in this fraction of vitamin A result in symptoms of hypervitaminosis A (22). The amount of vitamin A given in our study exceeds the daily requirement for the vitamin by 50 times (20). Thus, it was not surprising to find moderately increased levels of lipoprotein associated vitamin A in several of the treated patients. In none, however, were signs of vitamin A intoxication observed. In fact, the risk for hypervitaminosis A appears to be insignificant even during prolonged vitamin A therapy, provided that the daily dose is kept below 300000 units (6, 8).

The release of RBP from the liver is impeded by vitamin A deficiency and possibly also by zinc deficiency in experimental animals (14). Our results do not provide any conclusive evidence that zinc defi-

ciency is a major cause to the low RBP and vitamin A levels in acne patients. We favour the view that the depressed level of RBP in many of the patients is mostly an effect of a moderate vitamin A deficiency. However, correction of this deficiency does not appear to be necessary to produce healing of acne. In those cases where vitamin A treatment appears to be effective, it probably acts via a pharmacological effect on the skin mediated by lipoprotein-associated vitamin A in addition to the normalization of serum RBP. This possibility is now under further investigation.

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