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Effects of Systemic Isotretinoin on Serum Markers of Collagen Synthesis and Degradation

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In the present investigation, collagen synthesis and degradation were studied by measuring the carboxyterminal propeptide of type I procollagen (PICP), the aminoterminal propeptide of type III procollagen (PIIINP) and a type I collagen-specific degradation peptide (ICTP) in the sera of 43 male patients, treated for acne with isotretinoin or with tetracycline. The values were compared with those observed in 24 acne patients without treatment and in healthy controls. The treatment with isotretinoin did not seem to affect these parameters in a cross-sectional setting, whereas tetracycline treatment was associated with slightly decreased levels of ICTP. Since there were marked variations in the PICP, PIIINP and ICTP levels between individual subjects, a follow-up study, including male and female patients, others than in the first part of the study, was conducted. Two other biochemical markers of bone metabolism, osteocalcin, reflecting osteoblastic activity, and tartrate-resistant acid phosphatase (TRAP), reflecting osteoclastic activity, were also analyzed. In females, all these parameters were lower than in males. In addition, the changes in females were more pronounced; in particular, PIIINP and TRAP were significantly increased in females during retinoid treatment ($p < 0.05$ and $p < 0.01$, respectively). Importantly, no increase was found in the synthesis of type I collagen during retinoid treatment, suggesting that the commonly used retinoid dosages do not stimulate the synthesis of type I collagen *in vivo*.

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Retinoids are extensively used in dermatology. Acitretin and etretinate are widely used for the treatment of psoriasis, and isotretinoin is in routine use for the treatment of severe acne (1). The side-effects of retinoid treatment are, however, evident. Changes in the bones and skeleton indicate that epithelial tissues are not the only targets of retinoid action. In addition, retinoids have been shown to modulate connective tissue in various conditions, such as in lichen sclerosus et atrophicus, scleroderma, keloids and solar damage (2). In most of these conditions, retinoids have been shown to enhance the formation of connective tissue. However, several cell culture experiments have shown that, in certain cell lines and cell culture conditions, retinoids can also be inhibitory to connective tissue, especially to collagen synthesis and degradation (3).

In recent years, the development of new assays for monitoring collagen synthesis and degradation has made it possible to follow changes in collagen metabolism in humans *in vivo* (4, 5). We have now measured the propeptides of type I and type III procollagen and a specific degradation product of type I collagen (ICTP) in dermatological patients receiving isotretinoin. These changes were compared with those in two biochemical markers of bone metabolism, tartrate-resistant acid phosphatase (TRAP), which reflects osteoclastic activity, and osteocalcin, reflecting osteoblastic activity.

PATIENTS AND METHODS

Patients and treatments

In the first part of the study, 86 dermatological patients and healthy controls were examined. All the patients were males and of about the same age, in order to minimize the effects of sex and age variations. The patients and controls were divided into four groups: 32 isotretinoin-treated (range 18–23 years) (Roaccutane®, Roche; mean dose 0.48 mg/kg/d) and 11 tetracycline-treated (range 19–47 years) (mean dose 8.1 mg/kg/d) patients with acne were compared with 24 acne patients (range 17–30 years) receiving no systemic treatment and with 19 controls (range 19–24 years). The controls were healthy or had some minor dermatological or venereological disease not considered to affect collagen or bone metabolism. The serum samples were obtained during an outpatient visit to the Department of Dermatology at Central Military Hospital in Helsinki.

In the second part of the study, 35 dermatological patients suffering from moderate or severe acne were examined. Their clinical characteristics and the doses of isotretinoin are shown in Table I. The serum samples were taken before and during the treatments. In some patients, three to five consecutive samples were obtained. The consecutive *samples were taken at the same time of the day, in order to eliminate possible diurnal variations of the parameters. Individual values obtained for the measured parameters without treatment have not varied markedly from day to day in previous studies (6). The samples were kept at −20°C until analyzed.*

Methods

The concentrations of the carboxyterminal propeptide of type I procollagen (PICP) (5) and of the aminoterminal propeptide of type III procollagen (PIIINP) (4) were determined by equilibrium radioimmunoassays with reagents provided by Orion Diagnostica (Oulunsalo, Finland). In both assays, the intra- and interassay coefficients of variation are about or less than 5% at the concentrations observed. The stabilities of these antigens during storage at −20°C have been documented previously (4, 5). The reference intervals for serum PICP, obtained from healthy Finnish blood donors, are 40 to 200 μg/l for men, and 50 to 170 μg/l for women (5). There is an inverse correlation between age and the concentration of PICP in blood in adult men, but not in women. The reference interval for PIIINP is 1.7 to 4.2 μg/l, with no difference between men and women and with no changes upon aging in adults.

The specific degradation product of bone type I collagen, the pyridinoline cross-linked carboxyterminal telopeptide (ICTP), was measured by an equilibrium radioimmunoassay. The ICTP concentration in normal adult human serum varies between 1.7 and 5.0 μg/l. The characterization of this assay will be published separately (J. Risteli et al., in preparation). Liver enzymes (ALAT and ASAT) were measured frequently in patients receiving retinoid treatment.

The concentration of osteocalcin (bone gammacarboxyglutamic acid-containing protein, BGP; reference interval 4.0 to 11.0 μg/l) was measured by radioimmunoassay (OSTK-PR radioimmunoassay kit, CIS Bio International, Gif-sur-Yvette, France).

Table I. *Clinical details of the follow-up patients treated with isotretinoin*

	n	Age mean (range)	Weight mean (range)	Isotretinoin mg/kg/d mean (range)	Cumulative[a] isotretinoin dose mean (range)
Males	24	20.1 (15–29)	71.2 (60–90)	0.56 (0.44–0.67)	1642 (480–2800)
Females	11	23.1 (15–37)	62.6 (51–80)	0.65 (0.50–0.78)	1652 (1080–2900)

[a] The cumulative dose of isotretinoin corresponds to that which the patients had received until PICP, PIIINP, ICTP, osteocalcin and TRAP were determined during the treatment (see Fig. 3).

The serum activity of the tartrate-resistant acid phosphatase (reference interval 1.6–8.8 U/l) was assessed by the method of Lau and coworkers (7). One-way analysis of variance, multipe range analysis, linear regression, the Mann-Whitney U-test and Wilcoxon's signed rank test were used for statistical evaluation of the results.

RESULTS

Cross-sectional study

In the first part of the study, PICP, ICTP and PIIINP were measured in the sera of 86 dermatological patients and healthy subjects. The individual PICP, ICTP and PIIINP levels are shown in Fig. 1A–C. The patients were divided into four groups: isotretinoin-treated and tetracycline-treated acne patients were compared with acne patients receiving no systemic treatment and with controls. We made this division in order to study the effects of isotretinoin on collagen synthesis and degradation, and to compare the changes during tetracycline treatment, which has also been shown to affect collagen metabolism (8, 9).

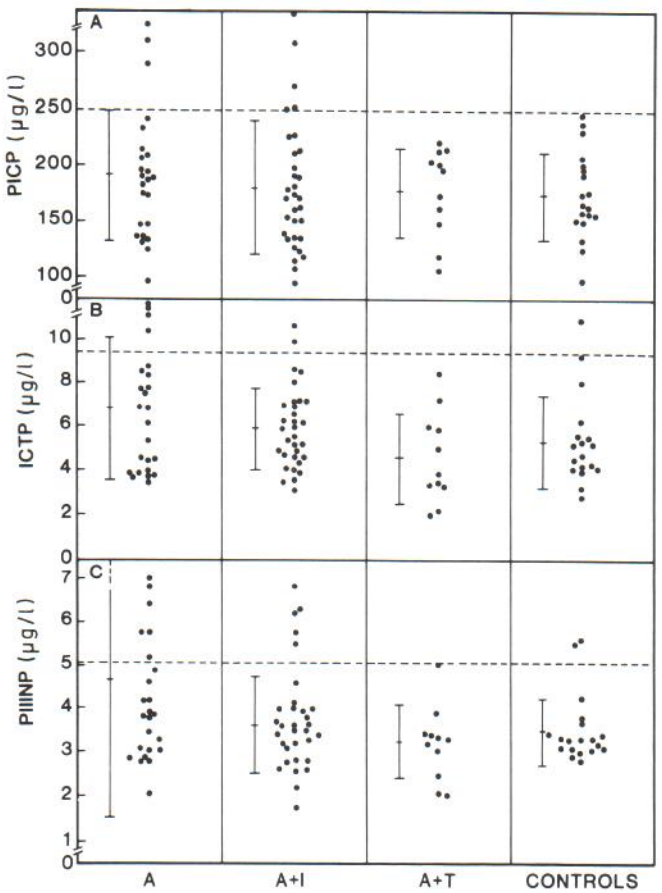

Fig. 1. Concentrations of the carboxyterminal propeptide of type I procollagen (*PICP*) (A), the type I collagen-specific degradation product (*ICTP*) (B) and the aminoterminal propeptide of type III procollagen (*PIIINP*) (C) in the sera of acne patients without systemic treatment (*A*), acne patients treated with isotretinoin (*A + I*), acne patients treated with tetracycline (*A + T*) and controls. The broken horizontal line shows the mean + 2 SD of the controls. In each group mean ± SD is also shown.

The concentrations of PICP were about the same in all groups, and no statistical difference was found between the groups (Fig. 1A). In untreated acne patients, three values were above the mean + 2 SD of the controls, and in acne patients treated with isotretinoin, five values were above the mean + 2 SD.

The ICTP values were highest in the acne group (without treatment) (mean = 6.9 µg/l) and lowest in acne patients treated with tetracycline, in whom the mean value was 4.6 µg/l (the difference between these groups was almost statistically significant ($p = 0.0625$) (Fig. 1B). The mean level of PIIINP was again highest in the acne group (4.71 µg/l) and lowest in acne patients treated with tetracycline (3.2 µg/l) (Fig. 1C). It should be noted that, both in the acne group and in acne patients treated with isotretinoin, there were numerous values above the mean + 2 SD of the controls. The liver enzymes, ALAT and ASAT, were measured in acne patients who had been treated with isotretinoin, and these values were within the normal range, including those of the patients who showed increased levels of PIIINP.

In the isotretinoin-treated acne group, the PICP, PIIINP and ICTP levels did not correlate with the dose of isotretinoin (mg/kg/day).

Follow-up study

In the second part of the study, PICP, PIIINP, ICTP, TRAP and osteocalcin concentrations were measured before and during the isotretinoin treatment. Variations in all the measured parameters occurred during the treatments. PICP concentrations seemed to decrease in most patients, while the average PIIINP, ICTP, TRAP and osteocalcin concentrations seemed to increase during the treatment with isotretinoin when individual patients were followed (Fig. 2A–D).

The osteocalcin and TRAP concentrations seemed to vary in parallel with each other, but no obvious correlation between

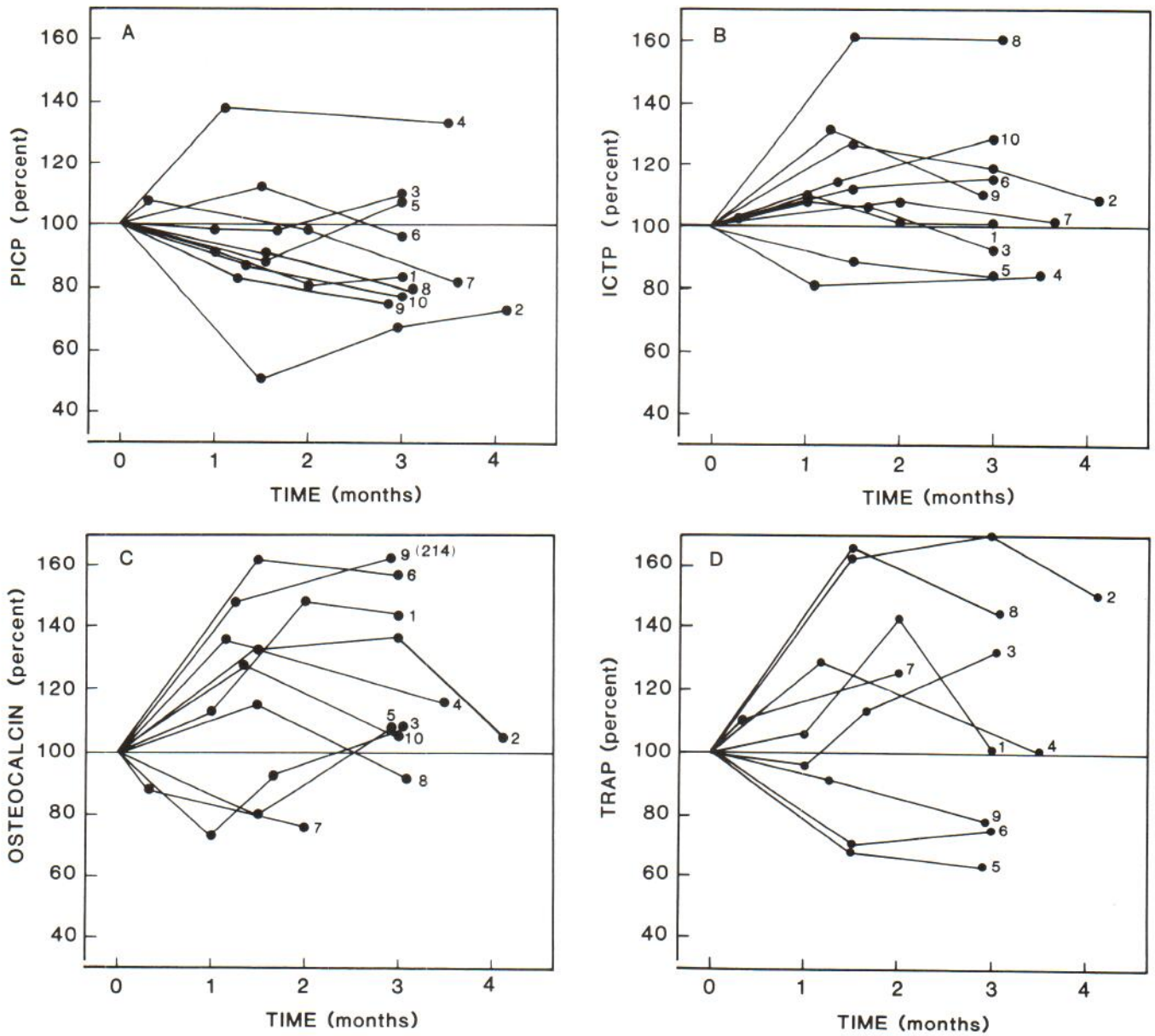

Fig. 2. Changes in *PICP* (A), *ICTP* (B), *osteocalcin* (C) and *TRAP* (D) in 10 patients during treatment with isotretinoin. The values are expressed as percentage of the pre-treatment values. Patients 3 and 10 were females and rest were males. The dose of isotretinoin varied from 0.47 mg/kg/d to 0.78 mg/kg/d.

the changes in PICP and ICTP was noted. Female patients had generally lower pre-treatment concentrations of all the measured parameters (Fig. 3A–E). Both before and after the treatment, the PICP levels were significantly lower in females than in males ($p < 0.01$ and $p < 0.05$ respectively). In females, the PIIINP levels after treatment were significantly higher than before the treatment ($p < 0.05$). ALAT values were measured in all the patients in this part of the study. In one female patient, there was an increase in ALAT values during isotretinoin treatment from 30 to 98 U/l. The PIIINP level did not increase markedly in this patient during isotretinoin (from 3.60 to 3.91 μg/l). The ICTP levels in females were significantly lower, both before and after the treatment, compared to the male levels ($p < 0.05$). No differences in osteocalcin concentrations were observed when the levels before and after the treatment (first determination) in females and males were compared. There was a significant increase in TRAP concentrations in females when the pre-treatment levels and the levels after the treatment were compared ($p < 0.01$). Such increases were not observed in males.

Correlations between different parameters

As demonstrated in Table II, there were significant correlations between the various parameters, in both the isotretinoin and the non-isotretinoin group. The correlation coefficients were remarkably similar in both groups.

DISCUSSION

In the first part of the study, the concentrations of markers of

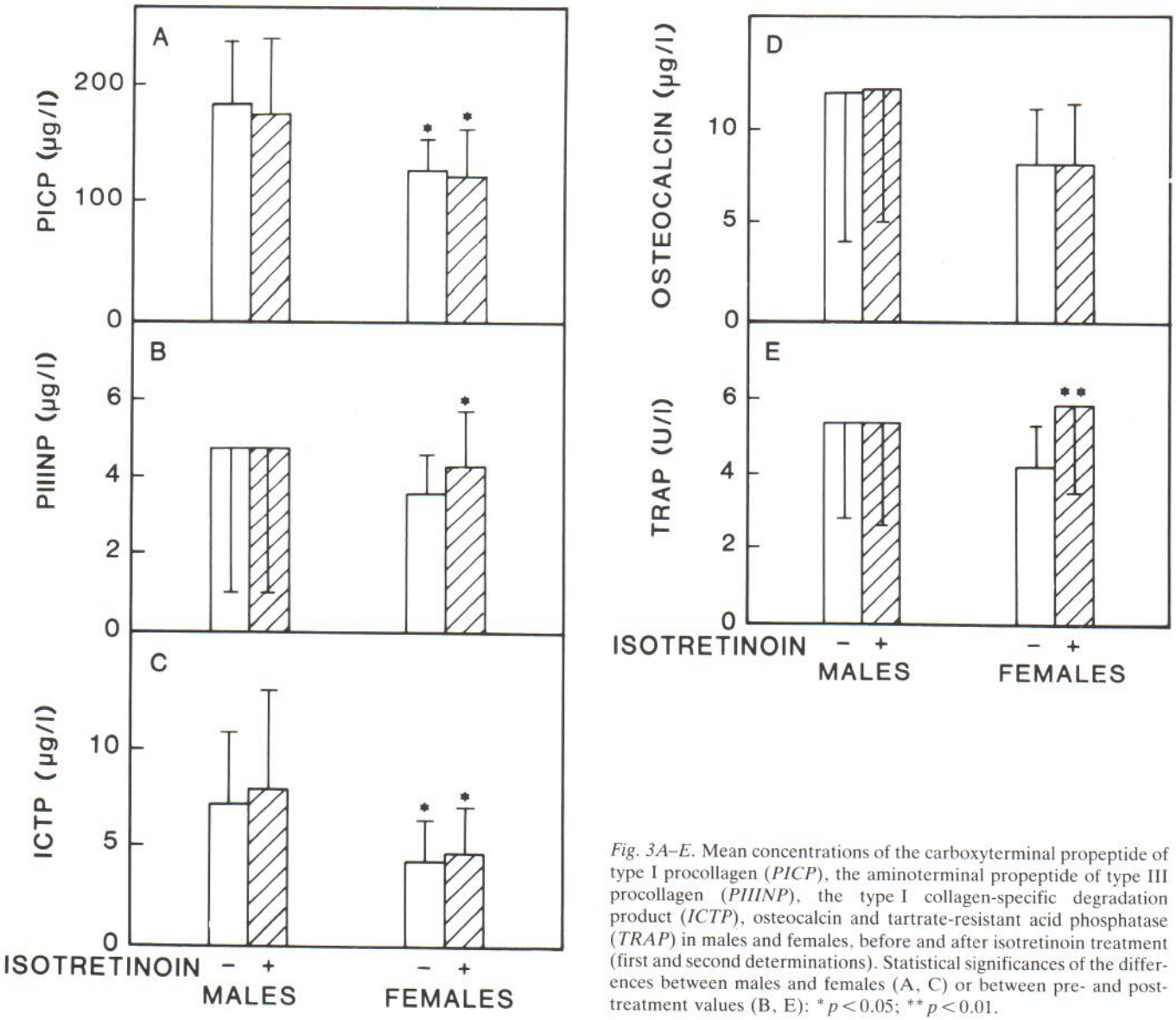

Fig. 3A–E. Mean concentrations of the carboxyterminal propeptide of type I procollagen (*PICP*), the aminoterminal propeptide of type III procollagen (*PIIINP*), the type I collagen-specific degradation product (*ICTP*), osteocalcin and tartrate-resistant acid phosphatase (*TRAP*) in males and females, before and after isotretinoin treatment (first and second determinations). Statistical significances of the differences between males and females (A, C) or between pre- and post-treatment values (B, E): $^{*}p < 0.05$; $^{**}p < 0.01$.

collagen synthesis and degradation were measured in randomly sampled acne patients and in controls who had a minor dermato-venereological problem or were healthy. PIIINP and ICTP concentrations were higher in acne patients without systemic treatment than in the other patient groups and systemic isotretinoin did not seem to affect these parameters specifically.

Interestingly, ICTP values were lowest in the tetracycline-treated patients, which suggests that, *in vivo,* tetracycline may decrease the degradation of type I collagen, as has also been observed in *in vitro* studies (8).

In order to study the effects of isotretinoin on collagen and other bone markers, a follow-up study was conducted.

Female patients had lower levels of PICP, ICTP, PIIINP, osteocalcin and TRAP than males before isotretinoin treatment. This may be due to slower turnover rates of collagen and other proteins in bones and could also reflect the smaller bone mass, compared with males. During isotretinoin treatment, the changes in males were small. In contrast, there was a significant increase in PIIINP and TRAP in females. Since severe liver damage could partially explain increased levels of PIIINP, liver-function tests were performed and, in one patient, an increase in liver enzymes was found.

The reason for the increased TRAP levels in females during retinoid treatment is not known. The TRAP mostly reflects osteoclastic acitivty, and it is possible that retinoids either directly stimulate the osteoclasts, or stimulate differentiation. It should also be noted that females who used retinoids also had hormonal treatments (oral contraceptives), which may partially explain the changes in TRAP.

Surprisingly, our results did not show any constant increase in type I collagen synthesis as determined by the propeptide assay. This cannot be due to invalidity of the assay, since we have recently shown that systemic glucocorticoids markedly decreased the serum levels of PICP (6). Furthermore, other studies, as well as our preliminary studies, have shown that PICP is increased in bone diseases, and that most of the serum PICP is derived from bones (10–12). In fact, in most patients,

Table II. *Correlation between the concentrations of the carboxy-terminal propeptide of type I procollagen (PICP), the cross-linked telopeptide of type I collagen (ICTP), the aminoterminal propeptide of type III procollagen (PIIINP), and osteocalcin and the activity of tartrate-resistant acid phosphatase (TRAP) in serum*

The samples were taken before (non-isotretinoin) and during isotretinoin treatment in the same patients. The number of samples in the isotretinoin group was larger than in the non-isotretinoin group, since in some patients three to five consecutive samples were obtained during isotretinoin treatment.

Correlation between	Isotretinoin		Non-isotretinoin	
	r	*p*	r	*p*
ICTP and PICP	0.65	<0.001	0.67	<0.001
ICTP and PIIINP	0.89	<0.001	0.89	<0.001
ICTP and osteocalcin	0.81	<0.001	0.90	<0.001
ICTP and TRAP	0.84	<0.001	0.80	<0.001
PICP and PIIINP	0.71	<0.001	0.68	<0.01
PICP and osteocalcin	0.69	<0.001	0.64	<0.01
PICP and TRAP	0.53	<0.01	0.42	ns
PIIINP and osteocalcin	0.83	<0.001	0.93	<0.001
PIIINP and TRAP	0.90	<0.001	0.84	<0.001
Osteocalcin and TRAP	0.76	<0.001	0.85	<0.001

ICTP levels, which reflect the degradation of type I collagen, were increased during retinoid treatment. However, it should be noted that there were marked individual variations in the levels of PICP and ICTP during retinoid treatment and, by following individual patients, it was possible to find some patients (nos. 3, 4 and 5) who demonstrated both increased synthesis of type I collagen and its decreased degradation. Hence it is possible that, in certain patients, isotretinoin may lead to the accumulation of collagen and thus to hyperostosis. However, x-ray examinations should be carried out to ascertain that hyperostosis is excluded.

Retinoids have been shown, in cell culture conditions, to stimulate osteocalcin synthesis by osteoblastic cells in concentrations which are clinically relevant (13). Here, when individual patients were followed, the majority presented an increased in osteocalcin levels. However, when the mean levels of the first determinations were compared, no stimulation was observed during retinoid treatment. Again, a certain subgroup of patients with increased levels of osteocalcin and decreased levels of TRAP could be found (patients 6 and 9), which could lead to bone changes if the subjects had a longer therapy.

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