Collagen type I and III metabolism in assessment of mandible fractures healing

Borys J[1], Grabowska SZ[1], Antonowicz B[1], Dryl D[1], Citko A[2], Rogowski F[2]

[1] Department of Maxillofacial Surgery, Medical University of Białystok, Poland
[2] Department of Nuclear Medicine, Medical University of Białystok, Poland

Abstract

Purpose: The aim of this work was estimation of the PIIINP, PICP and ICTP concentrations in blood serum during non-complicated mandible fracture healing; settlement of dependences between kinetics of changes of examined markers with reference to particular bone fracture phases and applied treatment methods; the determination of usefulness of collagen metabolism markers type III and I for the monitoring of mandibular fracture healing.

Material and methods: The material was blood serum of men aged 20-30 years, which were treated on mandible fractures in Maxillofacial Clinic Medical University of Białystok. Depending on the treating method, examined patients were divided into two groups. Patients treated with non-operative method were I group (n=31), II group was made of patients treated with surgery (n=33). The concentrations of markers measured on the 2nd, 14th, 42nd, 90th day after trauma and in II group these substances were measured additionally on the 2nd and 14th day after surgery. Control group consisted of 20 healthy men the same age. Concentrations of markers were measured with the radioimmunological method (RIA).

Results: Regular process of mandible fracture healing in men in various periods occurs with PICP, PIIINP and ICTP concentration changes in blood serum.

Conclusions: Changes of maker concentration show that, mandible fracture healing treated non-operatively is a more dynamic process than stable osteosynthesis method applied. Lack of positive correlation of the PIIINP and PICP concentration in blood serum of patients in two examinated groups can indicate on the different machanisms of mandible farcture healing connected with different methods of the treatment.

Key words: fracture healing, biochemical markers, PIIINP – N-terminal propeptide of type III procollagen, PICP – C-terminal propeptide of type I procollagen, ICTP – C-terminal telopeptide of type I collagen.

Introduction

The process of bone fracture healing can be assessed by using many present diagnostic methods [1-4]. However, they are of limited usefulness in clinical practice due to their invasive character or late manifestation of changes occurring during bone regeneration. Thus, there is a necessity for non-invasive and repetitive methods enabling assessment of fracture healing at early stages [5-8]. Taking into account the importance of collagen synthesis in the process of bone healing such requirements can be fulfilled by biochemical markers of collagen metabolism, i.e. collagen synthesis and degradation products released into the blood.

Experimental studies have shown that during fracture healing with immobilization of bone fragments, collagen type I and III are synthesized in variable quantitative and temporary relations [2,3,9]. In the estimation of type III collagen available for examination, the N-terminal propeptide of type III procollagen (PIIINP) is a marker of collagen III type metabolism, i.e. both its synthesis and degradation [10].

The C-terminal propeptide of type I procollagen (PICP) is a marker of type I collagen biosynthesis and also a marker of bone tissue formation. The propeptide is released during collagen synthesis from precursor molecule [5,8,11]. The C-terminal telopeptide of type I collagen ICTP which contains net binding,

ADDRESS FOR CORRESPONDENCE:
Jan Borys M.D.
Department of Maxillofacial Surgery
Medical University of Białystok
ul. M. Skłodowskiej-Curie 24a
15-276 Białystok, Poland

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is a marker of bone resorption. The telopeptide is formed during degradation of mature fibres of type I collagen [5,8,11,12].

The markers are of non-invasive character, are repetitive and have the possibility of duplication of various biochemical determinations. The possibility of assessment of resorption and bone tissue formation, i.e. basic processes taking place in fracture healing, is their advantage.

The evaluation of fracture healing using markers of collagen type III and I have been a subject of single experimental [1] and clinical [13-18] studies. Clinical studies covered a small number of patients, diversified as far as the kind of fracture, methods of treatment, age and sex of patients were concerned.

Including above-mentioned data, a study on men with mandible fractures in various stages of regular healing was conducted. The aims of the study were:

– estimation of the PIIINP, PICP, and ICTP concentrations in blood serum during non-complicated mandible fracture healing

– settlement of dependences between kinetics of changes of examined markers with reference to particular bone fracture phases and applied treatment methods

– determination of usefulness of collagen metabolism markers type III and I for monitoring of mandible fracture healing.

Material and methods

The examined group comprised of 64 men, aged 20-30, treated in the Maxillofacial Clinic, Medical University of Białystok due to mandible fractures. The following criteria qualified patients for the study: mandible fracture diagnosed on the basis of clinical and radiological examinations; lack of skin wounds and other bodily injuries including fractures and the central nervous system damage; case history, physical examination and basic laboratory blood and urine tests did not show past or coexisting systemic and metabolic diseases; the patients were not treated for bone fractures; did not take hormones, anticoagulants, anticonvulsants, diuretics, calcium and magnesium preparations, vit. D prior the trauma, they were not alcohol- and narcotic-addicted; were not under the influence of alcohol on the day of accident; had clinically satisfactory state and paradontal tissues; gave a written consent to 4 or 6 times of blood collection for test in appropriate time intervals.

Depending on the method of treatment of mandible fracture, the patients were divided into 2 groups: I – the patients treated with non-operative method (n=31) and II – the patients treated operatively (n=33). Single mandible fractures were observed in 18 patients (58.1%) and multiple fractures – in 13 patients (41.9%) of the first group. The most frequent localization of mandible angle (36.1%), mandible body in the region of molar teeth (17%), and condyloid process (14.9%). Multiple fractures were presented in 22 patients (60.6%) and simple fractures in 11 patients (39.4%) of the second group. Bone injuries concerned mainly mandible angle (25%), condyloid process (21.1%), and the region of molars (17.3%).

Non-operative treatment was based on application of splints and elastic intermaxillary traction, which was changed into permanent fixation after bone fragments reduction. The period of intermaxillary immobilization was 6 weeks.

Mandible fractures were treated operatively in the II group. The procedure was conducted under general anesthesia with intratracheal intubation and the section was performed intraorally or extraorally. Bone fragments were repositioned and fixed using miniplates, which were fixed with screws. One-sided osteosynthesis was performed in 11 patients, both-sided – in 22. Additional intermaxillary immobilization stretched on the splints, to fix occlusion prior to the trauma, was applied in the patients of this group in the first 24 hours after the surgery. Screws and plates, used for osteosynthesis, were removed after 3 months.

The patients with mandible body fracture, due to contact of fracture fissure with oral environment and those treated surgically, received antibiotic for 5-7 days. Clinical and radiological evaluation revealed bone fracture healing without complications. During both hospitalization and ambulatory treatment, blood was taken in assigned periods to determine PIIINP, PICP, and ICTP; in groups I and II – on the 2nd, 14th, 42nd, and 90th days after the trauma and in the II group – moreover on the 2nd and 14th days after operation. The days of markers determination in the course of mandible fracture healing were settled on the basis of the duration of 4 healing phases (in the mechanism of spontaneous concrescence). They resembled the following phases of the process: the 2nd day – phase I (inflammatory), the 14th day – phase 2 (granulation), the 42nd day – phase 3 (callus, i.e. clinical concrescence), and the 90th day – phase 4 (callus reconstruction) [19-21]. The examinations on the 2nd and 14th days after operation were to establish the influence of the operation and soft tissue healing on these markers concentration. It should be noticed that the material taken on the 90th day after the trauma was collected before miniplates removal.

The blood was collected from the elbow vein, on empty stomach between 7 and 8 a.m. to the testing tubes to obtain clots. After clotting, the blood was centrifuged and serum were frozen at the temperature of -80^0C until metabolism markers determination of collagen types III and I was conducted. Similarly, the blood of healthy men (volunteers) aged 20-30, the control group, was collected between 7 and 8 a.m. on empty stomach.

Blood serum PIIINP, PICP and ICTP concentrations were determined with radioimmunological method, using ready RIA kits, Orion Diagnostica (Finland). The results were presented as arithmetic means for particular groups of men and defined periods of fracture healing with regard to standard deviation – SD. Statistical differences between the examined groups and the control group on consecutive days of healing, were conducted using Student-t test for unpaired trials. Unpaired t-test was used to compare the results of particular days of healing. Differences at the level of confidence $p<0.05$ were considered significant. In order to establish mutual relationships between PIIINP, PICP, and ICTP concentrations in the course of fracture healing, a correlation coefficient was calculated, by comparing mean concentration of consecutive days of fracture healing. The correlation was considered significant (positive or negative) coefficient r values exceeding 0.9.

Figure 1. **PIIINP concentrations in blood serum in I group of patients in examined days of healing and control group (K)**

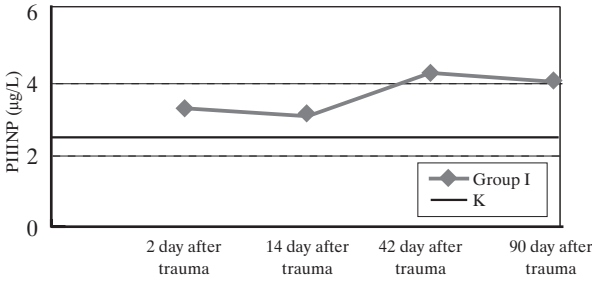

Figure 2. **PIIINP concentrations in blood serum in II group of patients in examined days of healing and control group (K)**

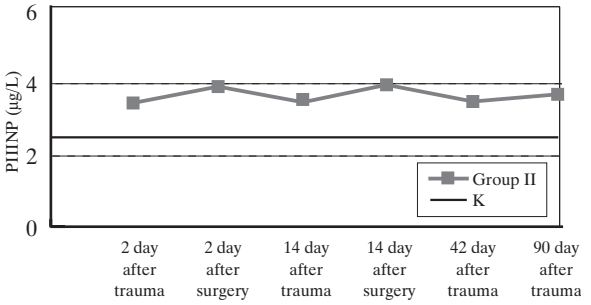

Table 1. **PIIINP concentrations in the blood serum in particular days of healing in I group of patients and control group**

The days of examination after trauma	2	14	42	90	Control group
PIIINP concentration (μg/L) ±SD	3.32 (±0.94)	3.10 (±0.93)	4.30 (±1.24)	4.09 (±1.21)	2.49 (±0.67)
Level of significance (p)	p<0.002	p<0.02	p<0.0001	p<0.0001	-

Table 2. **Statistical differences between PIIINP concentrations in particular days of healing after trauma in I group of patients (NS – statistically non-significant)**

The days of examination after trauma	2:14	2:42	2:90	14:42	14:90	42:90
Level of significance (p)	NS	p<0.001	p<0.01	p<0.0001	p<0.001	NS

Table 3. **PIIINP concentrations in the blood serum in particular days of healing in II group of patients and control group**

The days of examination	2nd day after trauma	2nd day after surgery	14th day after trauma	14th day after surgery	42nd day after trauma	90th day after trauma	Control group
PIIINP concentration (μg/L) ±SD	3.38 (±0.94)	3.83 (±1.09)	3.40 (±0.99)	3.88 (±1.16)	3.42 (±1.02)	3.62 (±1.02)	2.49 (±0.67)
Level of significance (p)	p<0.001	p<0.0001	p<0.001	p<0.0001	p<0.001	p<0.0001	-

The Senate Committee for Ethics and Supervision of Studies on People, Medical University of Białystok gave its consent for the study.

Results

Having done statistical calculation with the division of patients into subgroups with regard to simple and multiple mandible fractures, we did not observe any significant differences in connection with treatment method. Thus, the results were presented in two groups, group I – patients treated with non-operative method and group II – operative method.

PIIINP

Group I. The blood serum of patients treated with non-operative method in the course of mandible fracture healing showed the increase in PIIINP concentrations on all examination days as compared to the control group (K). On the 2nd and 14th days after the trauma, PIIINP concentrations were approximate. The highest concentration of propeptide was observed on the 42nd day after the trauma. As compared to this period, PIIINP concentrations in the last examination (the 90th day after the injury) were only slightly lower (*Fig. 1*).

Propeptide concentrations increase, observed in all periods of mandible fracture healing in patients treated with non-operative method were statistically significant as compared to PIIINP concentrations in group K (*Tab. 1*).

Statistically significant differences were obtained comparing PIIINP concentrations between days 2:42, 2:90, 14:42, 14:90 (*Tab. 2*).

Group II. The patients treated operatively showed, similarly to group I, PIIINP concentrations increase in all examination days of fracture healing as compared to group K. Propeptide concentrations on the 2nd, 14th, 42nd, and 90th days after the trauma were very close. The highest concentrations of PIIINP were observed on both days after operation (the 2nd and 14th). The concentrations were on similar levels (*Fig. 2*).

Propeptide concentrations increase, observed on particular examination days of mandible fracture healing, after the injury and after the operation, was statistically significant, as compared to PIIINP concentrations in group K (*Tab. 3*). There were no statistically significant differences comparing PIIINP concentrations between examination days after the trauma.

We did not find positive correlation comparing mean concentrations of PIIINP obtained on particular examination days after the injury in patients of both groups.

Figure 3. **PICP concentrations in blood serum in I group of patients in examined days of healing and control group (K)**

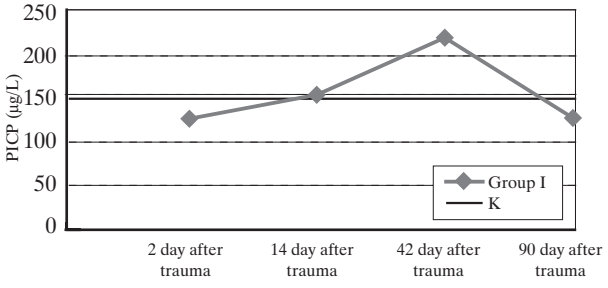

Figure 4. **PICP concentrations in blood serum in II group of patients in examined days of healing and control group (K)**

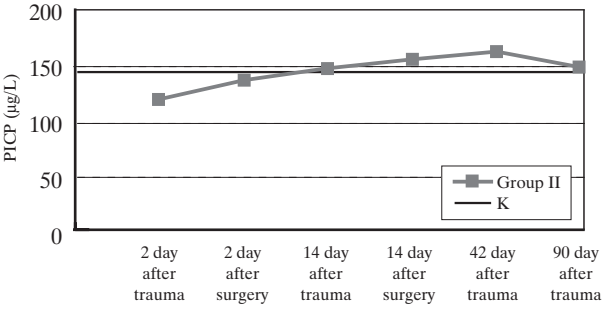

Table 4. **PICP concentrations in the blood serum in particular days of healing in I group of patients and control group (NS – statistically non-significant)**

The days of examination after trauma	2	14	42	90	Control group
PICP concentration (μg/L) ±SD	124.9 (±34.0)	151.2 (±36.4)	215.5 (±49.6)	126.9 (±34.2)	146.5 (±38.8)
Level of significance (p)	p<0.04	NS	p<0.0001	NS	-

Table 5. **Statistical differences between PICP concentrations in particular days of healing after trauma in I group of patients (NS – statistically non-significant)**

The days of examination after trauma	2:14	2:42	2:90	14:42	14:90	42:90
Level of significance (p)	p<0.005	p<0.0001	NS	p<0.0001	NS	p<0.0001

Table 6. **PICP concentrations in the blood serum in particular days of healing in II group of patients and control group (NS – statistically non-significant)**

The days of examination	2nd day after trauma	2nd day after surgery	14th day after trauma	14th day after surgery	42nd day after trauma	90th day after trauma	Control group
PICP concentration (μg/L) ±SD	121.2 (±32.2)	138.7 (±37.7)	149.3 (±34.9)	157.9 (±39.4)	165.9 (±36.0)	150.8 (±34.5)	146.5 (±38.8)
Level of significance (p)	p<0.01	NS	NS	NS	NS	NS	-

PICP

Group I. Blood serum of the patients showed PICP concentrations decrease on the 2nd day after the trauma, as compared to group K. Next two periods – on the 14th and 42nd day after the injury showed gradual increase in propeptide concentrations. The highest propeptide concentrations were noted on the 42nd day of fracture healing. In the last period, PICP concentrations again showed the decrease (*Fig. 3*).

The statistical analysis presented the decrease in PICP concentrations on the 2nd day and propeptide concentration increase on the 42nd day after the trauma, which were significant in relation to their concentrations in group K. However, differences of PICP concentrations observed between the controls and examined groups on other examination days were not statistically significant (*Tab. 4*).

Comparing PICP concentrations between test days we observed statistically significant increase in propeptide concentrations between 2:14, 2:42, and 14:42 days and statistically significant decrease between 42:90 days after the injury (*Tab. 5*).

Group II. Blood serum of the patients treated with stable osteosynthesis method showed lower PICP concentrations on the 2nd day after the trauma and on the 2nd after operation as compared to group K. The lowest values of propeptide concentrations were noted on the 2nd day after the injury. Since that time up to the 42nd day after injury, we observed constant increase in PICP concentrations. On the 90th day of examination, PICP decreased again and was at the level of PICP concentrations in group K. The highest PICP values were observed, as in non-operative patients, on the 42nd day of fracture healing (*Fig. 4*).

In comparison with PICP concentrations in group K, the decrease in propeptide concentrations on the second after trauma was statistically significant. Differences of PICP concentrations on other fracture healing days in patients operatively treated were not significant (*Tab. 6*).

Statistically significant elevation of propeptide concentrations between 2:14, 2:42, and 2:90 days was observed in group II, as compared with PICP concentrations on particular days after trauma (*Tab. 7*).

Figure 5. **ICTP concentrations in blood serum in I group of patients in examined days of healing and control group (K)**

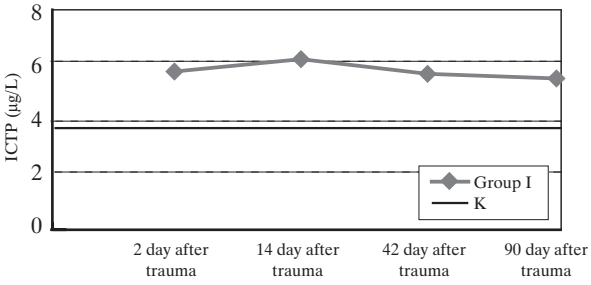

Figure 6. **ICTP concentrations in blood serum in II group of patients in examined days of healing and control group (K)**

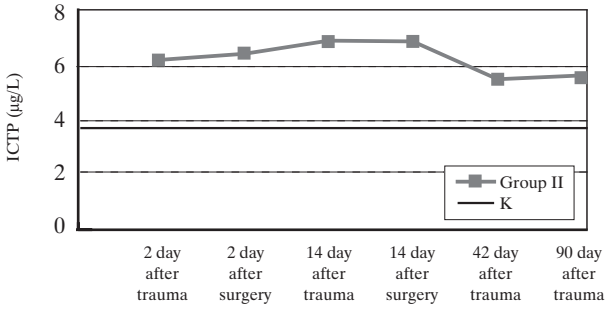

Table 7. **Statistical differences between PICP concentrations in particular days of healing after trauma in II group of patients (NS – statistically non-significant)**

The days of examination after trauma	2:14	2:42	2:90	14:42	14:90	42:90
Level of significance (p)	p<0.002	p<0.0001	p<0.001	NS	NS	NS

Table 8. **ICTP concentrations in the blood serum in particular days of healing in I group of patients and control group**

The days of examination after trauma	2	14	42	90	Control group
ICTP concentration (µg/L) ±SD	5.71 (±1.8)	6.12 (±1.56)	5.62 (±1.65)	5.42 (±1.54)	3.63 (±0.86)
Level of significance (p)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	-

Table 9. **ICTP concentrations in the blood serum in particular days of healing in II group of patients and control group**

The days of examination	2nd day after trauma	2nd day after surgery	14th day after trauma	14th day after surgery	42nd day after trauma	90th day after trauma	Control group
ICTP concentration (µg/L) ±SD	6.12 (±1.8)	6.39 (±1.85)	6.82 (±1.74)	6.83 (±1.88)	5.39 (±1.52)	5.58 (±1.59)	3.63 (±0.86)
Level of significance (p)	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	p<0.0001	-

Table 10. **Statistical differences between ICTP concentrations in particular days of healing after trauma in II group of patients (NS – statistically non-significant)**

The days of examination after trauma	2:14	2:42	2:90	14:42	14:90	42:90
Level of significance (p)	NS	NS	NS	p<0.001	p<0.005	NS

Positive correlation was not presented while comparing mean PICP concentrations on consecutive examination days in patients treated non-operatively and operatively.

ICTP

Group I. On particular test days of mandible fracture healing, elevated ICTP concentrations were observed in blood serum of patients treated with non-operative method, as compared to group K. The highest ICTP concentrations were noted on the 14th day after the injury. Next days presented ICTP concentrations close to telopeptide concentrations of the first period of the study (*Fig. 5*).

ICTP concentration increase, shown on each test day after the injury was statistically significant in relation to its concentrations in group K (*Tab. 8*). However, comparing ICTP concentrations between days of examination, we did not observe any statistical significance.

Group II. Elevated ICTP concentrations, compared to group K, were noted in blood serum of the patients with surgically treated mandible fracture, as it was in group I. Telopeptide concentrations increased gradually from the first day up to the 14th day after operation, reaching the highest concentration at this period. Next two periods of examination showed lowering of telopeptide concentrations and was at approximate level, however it was higher than telopeptide concentration in group K (*Fig. 6*). In relation to ICTP concentrations in group K, telopeptide concentration increase observed in mandible fracture healing was statistically significant (*Tab. 9*).

We compared ICTP concentrations between days after the trauma and revealed statistically significant lowering of ICTP between 14:42 and 14:90 days (*Tab. 10*). Positive correlation r=0.96 was observed, comparing mean ICTP of particular days of examination after the injury in patients of groups I and II.

However, we did not find any significant correlation between mean concentrations of examined markers of collagen type I and III metabolism in the course of mandible fracture healing in patients of both groups.

Discussion

An important component of fracture healing process is the synthesis of collagen types I and III in variable quantitative and temporary relations. Measuring products of collagen synthesis and degradation released into the blood, we can conclude about collagen metabolism type I and III in people.

In our studies, we evaluated concentrations of PIIINP, PICP, and ICTP in blood serum of men in the course of regular healing of mandible fractures, treated with non-operative (group I) and operative (group II) methods.

Significant elevation of PIIINP concentrations was observed in blood serum of the patients of both groups in all periods of fracture healing. In group I, the PIIINP concentrations was similar on the 2nd and 14th days after the trauma, on the 42nd day its concentrations significantly increased and was only slightly lower on the last day of examination.

The patients of group II revealed PIIINP concentrations increase on similar levels. Higher elevation of PIIINP concentrations in this group was observed only on the 2nd and 14th days after the operation.

The results point to the fact, that the trauma and/or the course of mandible fracture healing in men affect collagen type III metabolism increase and its activity is connected with the method of treatment.

The evaluation of bone fracture healing process based on PIIINP concentration measurement in blood serum has been the subject of only few studies [14-17]. Jerring et al. [14] and Kurdy et al. [16] obtained similar results to ours concerning group I, as far as the values and duration of maintenance of PIIINP concentration increase in blood serum in long bone healing with non-operative method were concerned.

Two basic mechanisms of reconstruction of bone tissue continuity can be distinguished in fracture healing: so called spontaneous concrescence and primary concrescence [22,23]. Fracture healing through spontaneous concrescence occurs in conditions of limited mobility of fragments, which can be obtained while applying non-operative methods to fracture treatment. Spontaneous concrescence is produced due to participation of osteogenic cells of internal layer of periosteum, osteogenic cells of endosteum, and bone marrow [22,23]. The exact adhesion and "ideal" immobilization of fragments, which can be obtained by applying stable osteosynthesis with compression are the conditions of reconstructing the continuity of fractured bone through primary concrescence. Giving up compression, exact adhesion of the plate to the bone and its elasticity in fracture healing was the basis of introducing indirect concrescence. This way of healing, the reconstruction of bone continuity occurs through primary accretion (under the plate) and that similar to spontaneous concrescence (on the side opposite to the plate) [23].

Our studies show no correlation between mean PIIINP concentrations obtained on particular days of examination after injury in patients treated with non-operative and operative methods. That can suggest that fracture healing treated with these methods occur through different mechanisms.

In opposition to present studies, Jerring et al. [15] observed significant increase in PIIINP concentrations in regular fracture healing of tibial bone treated non-operatively and operatively on the 1st and 2nd weeks after injury. Further examinations presented propeptide concentrations remaining elevated up to the end of the study i.e. on the 26th week after the trauma. The authors did not state any differences in dynamics of PIIINP concentration changes depending on fracture healing treatment method. These discrepancies might be due to the fact, that results of PIIINP concentrations referred to the 1st day after the injury and not to the control group, the type of bone fracture, small number of examined patients, different age and sex [15].

The results cited authors seem to confirm our conclusion that the course of regular mandible fracture healing may affect collagen type III metabolism increase.

A mature bone tissue contains a small amount of collagen type III, mainly in blood vessels and Havers canals [3]. In the course of long bone fracture healing in animals, type III collagen appears earlier and is more abundant than collagen type I [3,9]. The authors' results may thus account for dynamics of PIIINP concentration changes observed in our studies.

The role of type III collagen in fracture healing has not been determined yet. It is assumed that type III collagen may play a role of "organic framework" which enables migration, bone cell adhesion and blood vessels in-growing [3,9]. Beside fracture regeneration process, type III collagen is synthesized as the first in skin wound healing [24-27] and tendon healing [3]. It is assumed that type III collagen is a significant component of most body regenerative processes [3,10,24-28].

PIIINP concentrations determined in blood serum reflect both synthesis and degradation of type III collagen. It is impossible to determine proportions of PIIINP that comes from synthesis or degradation of type III collagen on the basis of propeptide measurement in blood serum and using available RIA kits. To solve the problem we should know molecular structure and metabolism of PIIINP propeptide and work out specific methods enabling separate evaluation of collagen type III synthesis and degradation.

Significant PIIINP concentration increase in both groups of patients observed on the 2nd day after the trauma can be the result of general reaction to the injury from both the skeletal system and soft tissues. Waydhas et al. results seem to confirm the assumption. They observed significant elevation of PIIINP in blood serum of patients with severe bodily injuries in the period from 3 to 14 days after trauma. Moreover, they pointed to the fact that PIIINP concentration was decidedly higher in patients with coexisting bone fracture [28].

Comparing our studies to fracture healing phases through spontaneous concrescence mechanism we can only suppose that PIIINP concentration increase on the 2nd day after the injury can occur due to the propeptide release from damaged blood vessels and soft tissues at the side and surroundings of the fracture. Considering the order of appearance of type III and I collagen in animal models, we can assume that PIIINP concentrations

observed on the 14th day of our studies is a result of collagen type III synthesis. As collagen type I is the only component of mineralization process in bone organic matrix [3,29], observed increase in PIIINP concentrations on the 42nd day, i.e. in the period of clinical mandible concrescence, can be the result of degradation of earlier synthesized type III collagen. Wen et al. studies stated the necessity of the replacement of collagen type III by type I in this period of bone fracture healing. Their studies showed, that the lack of exchange in quantitative relations of both types of collagen is the cause of bone concrescence disturbances. The disturbances are reflected by insufficient mineralization in microstructural evaluation [29].

Multimaki et al. studies presented the highest mRNA level for type I collagen in the period of callus remodelling and lamellar bone formation. The level of mRNA for collagen type III was minimum in this period of healing [2]. Having the data in mind, we can only assume, that high PIIINP concentration observed on the 90th day after mandible fracture is the result of type III collagen degradation. It also points out, that the process of fracture healing is in the course of duration. As far as mandible is concerned, the period of callus reconstruction is 1 year [30].

Assuming the possibility of mandible fracture healing in patients of group II through the primary and/or indirect concrescence, the explanation of PIIINP increase on the 14th, 42nd, and 90th days after the trauma requires further experimental studies to determine the kind and possible quantitative relations of collagen types I and III.

Analyzing the results of PICP concentrations in serum of patients of groups I and II in the course of mandible fracture healing, its significant lowering was observed on the 2nd day after injury. Since that time, up to the 42nd day after the trauma, constant increase in serum PICP concentrations of patients of both groups was observed. The highest PICP concentrations were also presented in both groups of patients on the 42nd day. It should be noticed, that absolute values of PICP concentrations were evidently higher in group I than in group II. Statistically significant PICP concentration increase was shown, as compared to the controls, only in group I. The 90th day did not give any particular differences of PICP concentrations in both groups as far as propeptide concentrations in the control group were concerned.

The results point to the fact that regular course of mandible fracture healing in men can affect changes of PICP concentrations and the dynamics of these changes, like in the case of PIIINP concentrations, is connected to the method of treatment.

Kurdy et al. [16] observed the dynamics of PICP concentration changes similar to ours in group I. Their study concerned patients with regular healing of tibial fracture treated non-operatively. However, Jerring et al. did not notice PICP concentration lowering in the first days of regular healing of radial bone fracture [14] and tibia [15] in patients treated non-operatively. They observed significant increase in PICP concentrations on the 2nd and 5th weeks of radial bone healing [14] and on the 2nd and 6th weeks of tibial bone healing [15]. PICP concentrations obtained in the course of long bone fracture healing referred to the 1st day of examination (i.e. the day of admission) Jerring et al. [14,15], and not to propeptide concentrations in the control group. Analogically, the relation of group I results and PICP concentrations on the 2nd day after the trauma (the first examination) and differences of duration of mandible and particular long bone fracture healing show our results comparable to those of Jerring et al. [14,15].

Jerring et al. observed significant PICP concentration increase in single cases of delayed concrescence of long bones on the 1st and 2nd weeks after the injury [15]. Other studies of delayed concrescence of long bones [17] showed significant PICP concentration increase on the 5th week. However, they observed statistically significant decrease in propeptide concentrations on the 10th and 20th weeks [15,17]. The cited authors conclude of usefulness of PICP concentration determination as a non-invasive method to evaluate regular and disturbed processes of fracture healing in people.

There was no positive correlation in present studies between mean concentrations of PICP and PIIINP if patients treated non-operatively and operatively. It can suggest that fracture healing treated with non-operative method occurs through spontaneous concrescence and with stable osteosynthesis through primary and/or indirect accretion [18,23]. The mechanism of healing through primary concrescence, bone continuity reconstruction is based on "exaggerated reconstruction" [23]. Conditions, which have to be fulfilled in this method of healing exclude or limit the participation of hematoma and soft tissues in the closest surrounding in bone regeneration. They also eliminate beneficial activity of mechanical stress on bone tissue [19,20,23]. It can be reflected in our studies in differences of PIIINP and PICP concentration changes observed in both groups. Group I revealed dynamics of these changes more evidently than group II. Suggestions of our studies are in accordance with Lotz et al. observations [18] concerning PICP concentrations in serum of patients with femoral bone fracture treated with stable osteosynthesis method with compression as well as other surgical methods that enable healing through spontaneous concrescence.

Jerring et al. [15], unlike Lotz et al. [18] and our data, did not observe differences in dynamics of PICP concentrations in regular tibial fracture healing, treated operatively and non-operatively, depending on treatment method. The differences can occur due to relation of PICP concentrations to the 1st day after the trauma and various operative methods used in the treatment.

Our studies revealed significant decrease in PICP concentrations on the 2nd day after injury in patients of both groups. Taking into account that PICP concentrations in blood serum of young men are maintained mainly due to continuous metabolism of type I collagen in the skeletal system [8], PICP concentration lowering in our studies was not expected in this period. Thus, a question arises whether PICP concentration decrease in blood serum can be the result of general reaction to the trauma and/or to fracture. Protein biosynthesis decrease is one of constant component of general reaction to injury. The period changes of existence in blood serum connected with the reaction of the acute phase in people is described in the range of 1 to 4 days after the injury [30]. PICP concentration lowering observed in our studies fits the time intervals. Kurdy et al. [16]

tried to explain the decrease in PICP concentrations in blood serum of patients with "temporary suppression" of osteoblast activity in response to the trauma, which is the bone fracture.

According to observed PIIINP and PICP concentrations in blood serum of men in the course of mandible fracture healing, PICP concentration increase appears later than PIIINP. Antonowicz showed similar dynamics of PICP concentrations in rat serum in experimental mandible fracture healing [1]. It is in accordance with other authors' results that evaluated collagen types in models of bone fractures in animals using polyclonal antibodies [3,9], immunofluorescent and histological examinations [3] and the expression of collagen genes [2]. It indicates the similarity of type I and III collagen appearance in regeneration of people and animals bone fractures. The coexistence of various types of collagen can suggest that they complement each other as for functioning in the course of fracture healing [9,19-21]. However, their role during healing process has not been described in details yet.

It should be noticed that PIIINP and PICP are not tissue specific markers. The propeptide release takes place during extracellular changes of collagen of the skin, ligaments, and fascia [5,8,10]. Thus, we should consider other than bone tissue sources of PIIINP and PICP. It is stressed that in generally healthy people, bone metabolism is faster than that of other kinds of connective tissue. Thus, it is assumed that bone tissue metabolisms characterized by PICP concentrations in these cases [8] and that the participation of PICP and PIIINP, other than of bone origin, can be increased only in diseases with intensive fibrosis [8,31,32].

Significant increase in PICP and PIIINP concentrations in blood serum was observed only after extensive surgeries, mainly of the abdominal cavity [24,26]. In the course of soft tissue wound healing, e.g. after hernia operation, there were no significant changes of the propeptide concentrations [26]. In our studies in both groups, mandible fractures were open fractures and had contact with the oral cavity environment through mucous membrane injuries or only through periodontal fissure. In compliance with the rules, sections of soft tissues in the region of the facial skeleton were very economical. Moreover, we used two additional determinations of markers concentrations on the 2nd and 14th day after surgery to evaluate the influence of soft tissues on PICP and PIIINP blood serum concentrations of patients treated operatively. The values obtained on these days and studies mentioned above show that the influence of soft tissue healing on PICP and PIIINP concentrations in blood serum in our studies were not significant.

The significant increase in ICTP concentrations was observed in both groups in all periods of mandible fracture healing as compared to the controls. We compared the dynamics of ICTP concentrations in both groups and revealed that telopeptide concentration increase from the 2nd to 14th days after the trauma in group II was evidently higher than in group I. In consecutive two periods after the injury (the 42nd and 90th days), serum ICTP concentrations were similar in both groups.

The results can suggest that the trauma and/or the course of healing in men influence serum ICTP concentration increase regardless the method of treatment.

A similar dynamics of ICTP concentration elevation, observed in our studies, was also presented by Jerring et al. [15], Kurdy et al. [16], and Lotz et al. [18] in the course of long bones healing. Their results point to the fact that fracture and regular course of healing of a relatively small bone, the mandible, causes similar changes of ICTP and other markers of collagen metabolism concentrations as in the case of long bones.

In delayed concrescence of long bones, ICTP concentrations did not differ significantly from telopeptide concentrations observed in regular healing process of these bones [15,17].

In contrast to PIIINP and PICP, concentrations of ICTP were on similar levels in both examined groups. It was confirmed by the positive correlation.

The influence of trauma and/or fracture healing on the skeletal system or a fractured bone has been the subject of few studies on animals and people. Einhorn et al., on the basis of histomorphometric examination on animals, stated general reaction of the skeletal system, caused by long bone fracture, to the trauma. The skeletal system reaction, i.e. the elevation of bone metabolism index, was almost immediate and lasted up to the 3rd week [33]. Osteopenia was diagnosed in a fractured extremity histologically and radiologically in animals [19,20,34]. People also revealed osteopenia, diagnosed histomorphometrically [35] and densitometrically in a fractured extremity. Bone mineral density decrease maintained for several months after the fracture [36]. The results can confirm ICTP concentration increase observed in our studies.

ICTP and PICP concentrations analysis showed that two first weeks of mandible fracture healing are characterized by degradation increase and type I collagen synthesis decrease. The concentrations of PICP and ICTP, observed in group I on the 42nd day after injury, can suggest the superiority of type I collagen synthesis over its degradation. On the 90th day in group I and on the 42nd and 90th days in group II, both processes are in balance.

Resorption and osteogenic processes are closely linked in regular conditions [8]. However, in metabolic bone diseases, there is no such linking. The superiority of resorption over formation leads to eventual loss of bone mass [6,8].

The results of cited authors [15,16,18] and our studies show lack of linking of synthesis and degradation of collagen type I in early periods of regular bone healing. Kurdy et al. [16] observation can be partially explained by so called regional acceleratory phenomenon (RAP) [19,20].

Based on the data it can be assumed that early loss of type I collagen in patients with bone fracture is not a specific feature connected with the process of mandible fracture healing but general reaction to the trauma. Other authors have the same opinion [15,17], however, the subject requires further investigation.

Moreover, we should take into account that age, sex, condiments, certain drugs, coexisting diseases (mainly metabolic) [8,37] as well as paradontal diseases [38] have a great impact on the concentration of markers of collagen type I and III metabolism determined in blood serum. The concentrations of resorption and bone formation markers in men are highest between 20 and 30 years of age and correspond to the peak of bone mass [37]. The above-mentioned data were the basis for conducting the study on men in one age interval and

determining other strict qualifying criteria described in Material and methods.

Conclusions

1. Regular process of mandible fracture healing in men in various periods occurs with PICP, PIIINP, and ICTP concentration changes in blood serum. It indicates that the trauma and/or normal bone healing finds its reflection in changes of examined collagen metabolism markers type I and III in men's blood serum.

2. As the evaluation of marker concentration changes show that, mandible fracture healing treated non-operatively is a more dynamic process than stable osteosynthesis method applied.

3. Lack of positive correlation PIIINP and PICP concentration in blood serum of patients with mandible fracture treated non-operatively and operatively can suggest, that fracture healing treated with conservative-orthopedic methods occurs through spontaneous concrescence while treated with stable osteosynthesis through primary and/or indirect concrescence. However, the subject requires further investigation.

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