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COMPARISON OF THE DYNAMICS OF CHANGES IN BLOOD BIOCHEMICAL PARAMETERS DEPENDING ON THE USED STIMULATOR OF REPARATIVE OSTEOGENESIS AT DIFFERENT PERIODS OF BONE WOUND HEALING

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Abstract

Injuries and violence are a global health burden with a high economic cost to health system. To compare efficacy of several stimulators of reparative osteogenesis, complex experimental study of 120 white Wistar male rats weighing 180-200 g and aged 10 - 12 months has been performed. Group I, control, (n =30) included animals which were not exposed to any manipulations. Group II (n =30) consisted of rats with fibula experimental fracture. The observation of its regeneration took place without any intervention. Group III (n = 30) comprised animals in which, from the second day after fibula fracture, phonophoresis with an ointment containing native bee venom, was administered. Group IV (n = 30) was presented by rats with fibula fracture in which once every three days an injection of plasma enriched with platelets was performed at the fracture site. The phasing of bone fusion during fracture was accompanied by fluctuations in the activity of calcium metabolism and positively correlated with the content of PO_4^{3-}, as the main conductor of Ca^{2+}. Changes of alkaline phosphatase activity were noticed. It was proved that bee venom administration by phonophoresis, as well as the administration of platelet-rich plasma into the fracture site, accelerates the process of bone tissue regeneration. The use of both regeneration stimulants has been shown to be effective.

Keywords: *fibula fracture, platelet-rich plasma (PRP), native bee venom, calcium metabolism, phosphorus metabolism, alkaline phosphatase metabolism*

Introduction

According to the past decades WHO's statistics [1], a moderate decrease in the number of injuries and violence took place, which, as a consequence, entails a downfall in disability in the European population. Injuries resulting from traffic accidents, falling from a height, at work or as a result of someone's illegal actions are among the main causes of death, disability and invalidization, especially among men of working age [2, 3]. Traumata cause significant damage to health, posing a threat to economic and social development of a region as a whole [3–6]. According to the data of the journal "Injuries in the European Union" there are 23 hospitalizations for every death and 163 admissions to emergency departments [7]. The mortality rate in the European Region in 2015 was 530.000 [1], and the number of hospital admissions and emergency department visits was 12 and 86 million, respectively.

Based on the gross data, let us consider the economic aspect of temporary or permanent disability of the able-bodied population of this country.

Industrial injuries cause significant economic losses, damaging the workers health, reducing the technical and economic effectiveness of enterprises, which negatively affects the socio-economic potential and interstate rating of the country [8]. According to the official state statistical reports, more than 20 thousand workers are injured annually in the workplace, many of them become disabled [9]. Every year, the Social Insurance Fund for Accidents in the Event of Disability pays more than UAH 2 billion, and this amount is only increasing [10].

In the general structure of primary disability, trauma ranks third. The intensive rate of primary disability due to injury is 6 - 7 per 10.000 workers and employees. Treatment of patients with multiple and concomitant trauma presents significant difficulties. More than 20% of victims become disabled [11, 12]. According to various authors, in our time, a violation of reparative osteogenesis is observed in 10 - 20% of cases of bone injuries [13, 14, 15]. According to a study conducted in 2008-2009 by the "Prof. M. I. Sitenko Institute of Spine and Joint Pathology Academy of Medical Sciences of Ukraine" and by "Institute of Traumatology and Orthopedics of Academy of Medical Sciences of Ukraine" delayed consolidation was observed in 32.7% of the injured [16] persons. The data given prove that the problem also has socio-economic relevance, associated with long-term treatment and rehabilitation of these patients [17]. At the same time, the means used to activate reparative osteogenesis have unequal efficacy [18].

Methods

A complex experimental study of 120 white Wistar male rats weighing 180-200 g and aged 10 - 12 months has been performed. The experimental animals were kept in conditions following the standard rules for the ordering, equipment, and maintenance of experimental biological clinics (vivariums), the rules for the care of experimental animals and work with them established by the Directive of the European Parliament and of the Council (2010/63 / EU) [20] and the order of the Ministry Health of Ukraine (06.04.2012, No. 249) "On approval of the Procedure for conducting experiments and experiments on animals by scientific institutions [19].

In accordance with the tasks of the work, the animals were divided into 4 groups.

Group I (n =30) included rats, kept under standard conditions of the vivarium, and were not exposed to any manipulations. The data obtained in the study of this group animals served as a control. Group II (n =30) consisted of rats with fibula experimental fracture. The observation of its regeneration took place without any intervention. Group III (n = 30) comprised animals in which, from the second day after fibula fracture, phonophoresis with an ointment containing native bee venom, (1.0 g contained 0.3 mg of standardized bee venom) was performed. Ultrasonic radiation power flux density was 2 W / sec², the duration of the procedure was 2 minutes, every other day. Group IV (n = 30) was presented by rats with fibula fracture in which once every three days an injection of plasma enriched with platelets in an amount of 0.1 ml was performed at the fracture site till taking out of the experiment.

Fibula fracture in rats was simulated by surgery. After introducing the rat into drug sleep, operative access to the fibula was made and osteotomy was performed. Then the wound was sutured tightly.

The animals were taken out of the experiment on the 7th, 14th, 21st days by decapitation under light ether anesthesia. After decapitation, 4.0–5.0 ml of blood was taken for subsequent biochemical studies. Biochemical methods were used to determine the content of calcium ions in blood plasma (fluorometric method), phosphorus (photometric method with molybdenum in an acidic medium), and alkaline phosphatase activity (nitrophenyl method).

Results

In group II (30 rats) on the 7th day of observation, the biochemical parameters of blood in bone tissue repair, is accompanied by a change in the parameters of calcium and phosphorus metabolism. Content of Ca^{2+} in the blood serum practically does not differ from the control data (Table 1). At the same time, phosphorus content is significantly lower than in the control. It can be assumed that the depletion of phosphorus metabolism is not restored and the deposition of Ca^{2+}, including at the site of fracture recovery, although it remains activated, still does not provide rapid tissue ossification, although there is a large amount of calcium in the blood plasma. Since the activity of alkaline phosphatase is significantly lower than that of the control group, it can be assumed that the tension in the flow of calcium into the repair zone remains.

Blood sampling on the 14th day of the experiment showed that there is no difference in the content of Ca^{2+} and (PO_4^{3-}) in blood plasma compared with the data of the previous period of observation; the same applies to the activity of alkaline phosphatase, so, it can be assumed that there is some stabilization of these types of metabolism. In the control group, on the 21st day, the data of biochemical studies (Table 1) indicate that the experimental animals retain a sharply increased activity of alkaline phosphatase, although it is significantly lower than after 14 days of the experiment. Ca^{2+} content also remains near the control data and those of the previous observation periods. So the intensity of calcium metabolism does not change in comparison with the 14th day of the experiment, there is no intensification of its deposition in the fracture area, as evidenced by the maintenance of the same level of (PO_4^{3-}). Thus, the results of our studies have shown that the reparative processes in the fracture area, in the absence of external influences, occurs according to the chondrogenic type of osteogenesis. The state of calcium metabolism is disturbed due to decreased calcium absorption because of shifts in phosphorus metabolism.

In group III (30 rats) on the 7th day when applying phonopharesis with a combined preparation of bee venom certain changes of blood biochemical indexes were revealed.

The indicators of calcium and phosphorus metabolism, and the activity of alkaline phosphatase, given in Table 2, indicate that the content of Ca^{2+} in the blood serum doubles in comparison with the control data. At the same time, phosphorus content slightly increases in relation to the group with a fracture that was not affected, but at the same time it is one third lower than the data in the control group. The activity of alkaline phosphatase is lower than in the control group and it is practically equal to the II group indicators. When observing this group of animals on the 14th day of the experiment, there is a sharp decrease of Ca^{2+} and (PO_4^{3-}) in plasma, concentration of calcium is two times less than in the control, it is also less in the group with fracture, which was not affected, decrease in the content of phosphorus is insignificant. Alkaline phosphatase remains practically at the same level as in the previous period. So, the mechanisms ensuring the absorption of calcium by tissues is normalized, in addition, it becomes more balanced. Obviously, this is due to the restoration, albeit inconclusive, of the integrity of the dense substance of the damaged bone. On the 21st day there is a sharp rise in the concentration of Ca^{2+} to a level more than twice as high as in the control and the group with a fracture, which was not exposed to external influences. However, a slight decrease of phosphorus and alkaline phosphatase continues. This indicates that the flow of calcium into blood remains very intense, while its assimilation comes to normal. Summing up the data of this observation period, it should be noted that there is a significant increase of Ca^{2+} concentration, up to a level more than twice as high as the control and group with a fracture without any inner influences.

In the IVth group (30 rats) on the 7th day, there were changes in biochemical parameters

characterizing calcium metabolism. Ca^{2+} concentration in plasma was slightly reduced in comparison with the control group and the group with a fracture without exposure, and two times lower than in the group treated with phonophoresis with a bee venom preparation. A somewhat different picture took place with the concentration of phosphorus. Its concentration was reduced in comparison with healthy animals, as well as with animals in groups with a fracture, which was not exposed to external influences and a fracture, which was affected by phonophoresis with a combined preparation of bee venom. As for the activity of alkaline phosphatase, it was insignificantly reduced in relation to the control group indicators, but remains one third higher than this indicator in animals of the IInd group, and the fracture which healed when using phonophoresis with bee venom.

Biochemical parameters on the 14th day of the experiment changed as it is shown in Table 3. Concentration of Ca^{2+} in rats' plasma is practically equal to it in the control group. Compared with the seven-day period of the treatment method under study application, Ca^{2+} concentration is higher but not significantly and still does not reach the level of the control group. Compared to the fracture group, which was not exposed to external influences, the level of Ca^{2+} in this group is insignificantly lower, in the group where phonophoresis with bee venom was used Ca^{2+} concentration was nearly twice as high.

PO_4^{3-} concentration in comparison with the control group is one and a half times higher and two times higher in comparison with the group where platelet-rich plasma injections were used. The content of phosphorus in plasma of animals with untreated fracture and treated with phonophoresis with a bee venom preparation within 14 days after surgery is approximately equal and in relation to the control group is twice less, and in relation to the group of fractures treated with platelet-rich plasma, they are reduced by three times.

The activity of alkaline phosphatase changed as follows. On the 14th day, the activity of alkaline phosphatase was 1/5 higher than in the control group, and almost two times higher than in the 7-day period of the same group. The activity of alkaline phosphatase in the group with the use of platelet-rich plasma to the of the experiment was almost twice as high. Ca^{2+} decrease and PO_4^{3-} and alkaline phosphatase increase allows us to suggests that the absorption and use of Ca^{2+} in these animals is very active, which contributes to bone regeneration. 21 days biochemical data show that Ca^{2+} concentration is approximately 20% lower than in the control group and approximately the same as in 14 days of the experiment. It is also reduced in comparison with the 7-day period of the experiment in the same group.

If we compare these biochemical data with those on the 21st day of the experiment in the group of animals with untreated fracture, and that where the fracture's treatment was made with phonophoresis and bee venom preparation, it turns out that Ca^{2+} concentration was the lowest. In comparison with the group where no specific treatment of the fracture was carried out, the concentration was reduced by almost a third, and more than two times lower than the group with the use of phonophoresis

Phosphorus ions on the 21st day after a significant increase in the 14th day, practically returned to the value of the 7th day, but still remained one third lower than in the control group. But among the same indicators of the other two groups on the 21st day of the experiment, it remains the highest, by about a quarter. But the activity of alkaline phosphorus

Phosphorus on the 21st day after a significant increase in the 14th day, practically returned to the value of 7 days, but still remained one third lower than the control group. But among the same indicators of the other two groups on the 21st day of the experiment, it remains the highest, by about a quarter.

The activity of alkaline phosphatase at this stage of the experiment is the same as in the control group, but in relation to the 14-day period, with injection treatment, it is reduced by a quarter. When compared with the two remaining groups, on the 21st day, the level of alkaline phosphatase activity is one third higher. It can be assumed that the intensification of calcium metabolism is normalized in connection with bone restoration.

Conclusions

The state of calcium metabolism, in particular its release from the depot and assimilation in bone

tissue, is an important factor in the process of regulation of bone repair.

The phasing of the process of bone fusion during fracture is accompanied by fluctuations in the activity of calcium metabolism in the form of intensification in the phase of inflammation and remodeling (by 10-15%), and a decrease in its activity in the phase of formation of tissue-specific elements.

Fluctuations in the content of calcium in blood are positively correlated with the content of PO_4^{3-}, as the main conductor of Ca^{2+}.

Changes of alkaline phosphatase activity, which provides mobilization of PO_4^{3-} from compounds, is a factor of calcium metabolism intensity regulation, since its activity in the phases of the regeneration process, in which proliferation and differentiation of bone tissue cells occurs, is reduced by 30–35%.

It was proved that bee venom administration by phonophoresis, as well as the administration of platelet-rich plasma into the fracture site, accelerates the process of bone tissue regeneration by ≤ 30%. The use of both regeneration stimulants has been shown to be effective.

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Table 1. Indicators of calcium and phosphorus metabolism and alkaline phosphatase in rats with a fracture of fibula, M ± m

Indicator / group		Ca^{2+}, mmol / l	PO_4^{3-}, mmol / l	APh, U/L
Control		2.66±0.02	3.0±0.74	369.19±12.41
Fracture of tubular bone	7 days	2.45±0.02	1.72±0.35*	221.18±28.32*
	14 days	2.70±0.04	1.60±0.21*	263.06±30.06*
	21 days	2.82±1.33	1.28±0.11*	206.19±28.25*

*p ≤ 0.05 in relation to the norm

Table 2. Indicators of calcium, phosphorus and alkaline phosphatase metabolism in rats with a fracture of fibula when exposed to its fusion by phonophoresis with native bee venom (M ± m)

Indicator / Group		Ca^{2+}, mmol/l	PO_4^{3-}, mmol/l	APh, U/L
Control		2.66±0.41	3.0±0.74	369.19±12.41
Fracture+Phonophoresis with native bee venom	7 days	5.50±0.067*	1.82±0.22	244.84±14.79*
	14 days	2.39±0.052*	1.54±0.046	242.84±4.56*
	21 days	5.60±0.035*	1.41±0.33	213.68±4.32*

Table 3. The state of indicators of calcium metabolism under various external influences, M ± m

Indicators / group		Ca^{2+}, mmol / l	PO_4^{3-}, mmol / l	APh, U/L
Control		2.66 ± 0.41	3.0 ± 0.74	369.19 ± 12.41
Fracture of tubular bone, n=30	7 days	2.45 ± 0.02	1.72 ± 0.35 *	221.18 ± 28.32*
	14 days	2.70 ± 0.04	1.60 ± 0.21*	263.06± 30.006*
	21 days	2.82 ± 1.33	1.28 ± 0.11 *	206.19 ± 28.25*
Fracture + phonophoresis with native bee venom	7 days	2.82 ± 1.33	1.28 ± 0.11 *	206.19± 28.25*
	14 days	2.39 ± 0.052 *	1.54 ± 0.046 *	242.84 ± 4.56*
	21 days	5.60 ± 0.035 *	1.41 ± 0.33	213.68 ± 4.32
Fracture + Platelet Rich Plasma Injections	7 days	2.93 ± 0.12	1.80 ± 0.23	98.0 ± 23.6 *
	14 days	2.42 ± 0.32 4	4.45 ± 0.47 *	456.0 ± 46.9
	21 days	2.03 ± 0.18	1.92 ± 0.16	326.0 ± 36.3

p ≤ 0.05 between control and different options for influencing the course of the fracture