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Regulation of reparative processes of proximal femur fractures by correction of arterial hypertension (experimental study)

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Objective. To study the regulation of reparative processes of proximal femur fractures at intramedullary osteosynthesis against concomitant arterial hypertension correction (by IFN-γ and biochemical parameters). Methods. The experiment involved 36 rats from two groups: healthy normotensive rats and rats with genetically determined arterial hypertension (SHR). Animals of both groups were divided into subgroups. The rats of subgroups 1.2, 2.2, and 2.4 underwent closed mini-invasive intramedullary osteosynthesis after a simulated proximal femur fracture. Rats of subgroups 2.3 and 2.4 were corrected for arterial hypertension with enalapril. The levels of IFN-γ, total protein, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase were determined in the serum of the animals. In the animal groups bone density indices were measured and the relative area of newly formed bone tissue in the bone callus was determined. Results. The level of IFN-γ was elevated after surgery in animals of all groups. The level of this cytokine was higher in SHR rats compared to the same values in intact animals. Administration of enalapril decreased the concentration of IFN-γ. A multidirectional change in the levels of biochemical indices in the blood of animals was demonstrated. It was found that bone mineral density was significantly reduced in the SHR animals group compared to intact animals. According to histomorphometric analysis, the largest relative area of newly formed bone tissue in the bone callus was in intact rats. The relative area of bone trabeculae in the animal group receiving enalapril therapy was greater than in the untreated group. Conclusions. Correction of concomitant arterial hypertension leads to optimization of repair processes of the proximal femur fractures in an experiment.

Мета. Вивчення регуляції репаративних процесів за переломів проксимального відділу стегнової кістки з інтрамедулярним остеосинтезом на тлі корекції супутньої артеріальної гіпертензії (АГ). Методи. В експерименті було задіяно 36 щурів із двох груп: здорові нормотензивні особини і з генетично детермінованою артеріальною гіпертензією (SHR). Тварини обох груп розділили на підгрупи. Щурам підгруп 1.2, 2.2, 2.4 виконано закритий мініінвазивний інтрамедулярний остеосинтез після моделювання перелому проксимального відділу стегнової кістки. Тваринам підгруп 2.3 і 2.4 проводили корекцію артеріальної гіпертензії еналаприлом. У сироватці крові визначали рівні ІФН-γ, загального білка, аспартатамінотрансферази, аланінамінотрансферази та лужної фосфатази. Вимірювали щільність кісткової тканини та відносну площу новоутвореної кісткової тканини в кістковому мозолі. Результати. Рівень ІФН-γ був підвищений після хірургічного втручання у тварин усіх груп, щурів лінії SHR вище порівняно з аналогічними показниками у інтактних тварин. Застосування еналаприлу знижувало концентрацію ІФН-γ. Продемонстровано різноспрямовану зміну рівнів біохімічних показників у крові. Встановлено, що мінеральна щільність кісткової тканини вірогідно знижена в групі тварин SHR порівняно з інтактними. За даними гістоморфометричного аналізу найбільша відносна площа новоутвореної кісткової тканини в кістковому мозолі була в інтактних щурів. Водночас відносна площа кісткових трабекул у групі, яка отримувала терапію еналаприлом, більша, ніж у групі без лікування. Висновки. Корекція супутньої артеріальної гіпертензії приводить до оптимізації процесів репарації переломів проксимального відділу стегнової кістки в експерименті. Ключові слова. Репаративні процеси, переломи проксимального відділу стегнової кістки, супутні захворювання, артеріальна гіпертензія, інтерферон, біохімічні показники.

Key words. Repair processes, proximal femur fractures, concomitant diseases, arterial hypertension, interferon, biochemical parameters

Introduction

Hip fractures remain an important problem for patients and place a heavy burden on the healthcare system. Projections show that yearly hip fractures will increase yearly from 1.66 million in 1990 to 6.26 million by 2050 [1]. In the United States, 10 % of all bone fractures fail to heal properly without intervention, resulting in nonunion [2]. Intramedullary osteosynthesis is considered the most effective treatment for proximal femoral fractures. Patients with fractures and comorbidities have an increased risk of mortality [3]. Fracture treatment should be combined with chronic disease management [4]. One of the most common diseases in the world is arterial hypertension (AH): more than 1.3 billion people have high blood pressure. Disease development is associated with inflammatory dysregulation and immune activation [5]. In turn, chronic inflammation may be associated with loss of bone mass [6]. Pharmacological correction of blood pressure is important to optimize fracture healing in patients with concomitant AH [7]. Angiotensin-converting enzyme (ACE) inhibitors are widely used to treat AH. Thus, it is interesting to study the regulation of fracture repair disorders in patients by correcting concomitant hypertension. Since the healing process involves complex interactions between different cells and signaling molecules, studies at the molecular level are relevant. Also important is the information on the main indicators of metabolic processes in fracture healing, used to correct treatment tactics.

Objective: to study the regulation of reparative processes of proximal femur fractures at intramedullary osteosynthesis against concomitant AH correction (by interferon-γ (IFN-γ) and biochemical parameters).

Material and methods

Thirty-six rats weighing about 250 grams aged 9–10 months were involved in the experiment. All manipulations were carried out taking into account the Law of Ukraine On the Protection of Animals from Cruelty (No. 3447-IV dated 02/21/2006) and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), and approved the Bioethics Committee of Kharkiv Medical Academy of Postgraduate Education (protocol No. 2 dated September 6, 2022). The surgeries were performed under general anesthesia with zoletil (tiletamine hydrochloride and zolazepam hydrochloride, France) at a rate of 10 mg/kg body weight.

The animals were categorized into two groups. The first group consisted of 12 healthy normotensive rats. Animals in this group were randomized into two subgroups:

– intact (subgroup 1.1 – Int);

– rats that underwent closed mini-invasive intramedullary osteosynthesis after a simulated proximal femur fracture (subgroup 1.2 — Int + Frac). The fracture was performed without a soft tissue incision using crampons, which mechanically impacted the proximal femur perpendicular to the axis of the bone until a fracture appeared. After osteosynthesis, the stability of fixation was checked for the absence of rotational mobility of the bone fragments, and the absence of mobility restrictions in the knee and hip joints.

The second group of animals was represented by 24 spontaneously hypertensive rats (SHR) that have genetically high blood pressure. The rats in this group were randomized into 4 subgroups:

– SHR rats without exposures (subgroup 2.1 — SHR);

– SHR rats that underwent closed mini-invasive intramedullary osteosynthesis after a simulated proximal femur fracture (subgroup 2.2 — SHR + Frac);

– SHR rats treated for AH with enalapril (subgroup 2.3 — SHR + Enal). Enalapril solution (ACE inhibitor drug class) at a rate of 5 mg/kg body weight was administered into the stomach with a probe daily, the day after surgery and until the day of euthanasia;

– SHR rats that underwent closed mini-invasive intramedullary osteosynthesis after simulated proximal femur fracture followed by enalapril administration (subgroup 2.4 — SHR + Frac + Enal).

The rats were removed from the experiment on day 14. Blood for research was taken from the heart by open puncture. The level of IFN-γ was determined in serum using the Vector-Best kit (Ukraine) by enzyme immunoassay method. Levels of total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) in blood serum were determined using diagnostic kits for clinical biochemistry SpineLab (Ukraine).

Bone sections containing bone callus were isolated and fixed in a 10 % neutral formalin solution. Specimens were decalcified, dehydrated, and embedded in paraffin, and sections were stained with hematoxylin-eosin and Van Gieson according to conventional histological techniques. Preparations were analyzed in the field of view of the PrimoStar microscope (Germany) and photographed with a digital camera. The relative area of newly formed bone tissue in 10 fields of view of bone callus histopreparations

of each animal was measured using ImageJ software (USA) according to the methodological recommendations [8]. The mean value of bone trabeculae area was calculated for each animal and then in the three groups of operated animals. The obtained data were visualized as graphs using GraphPad Prism 9 (USA). Femurs were isolated in the Int and SHR animal groups. Bone remodeling disorders were determined by directly measuring bone density calculated as the ratio of bone mass to bone volume [9].

The statistical analysis was performed by the one-way ANOVA using the Statistica software 12.0 (USA). The significance of the differences between groups was evaluated using the non-parametric Kruskal–Wallis test for independent samples. The results were presented as mean ± SE, where SE was the standard error of the arithmetic mean. Differences were considered statistically significant at $p < 0.05$. Histograms were plotted by GraphPad Prism 9 (USA).

Results

According to histomorphometric analysis, the largest relative area of newly formed bone tissue in the bone callus was in intact rats. It exceeded the same index in groups: in SHR by 1.98 times, in SHR+Enal — by 1.25 times ($p < 0.05$). At the same time, the relative area of bone trabeculae in the group of animals receiving therapy was 1.58 times higher than in the group without AH treatment (Fig. 1).

When bone mineral density was examined, it was significantly decreased in the group of animals of subgroup 2.1 with genetically determined AH (SHR) compared to intact animals of subgroup 1.1 ((1.453 ± 0.037) g/cm^3 and (1.577 ± 0.035) g/cm^3, respectively, $p < 0.05$), confirming the impaired bone remodeling in SHR group.

Fig. 2 presents the concentrations of IFN-γ, total protein, AST, ALT, and ALP in animal blood serum.

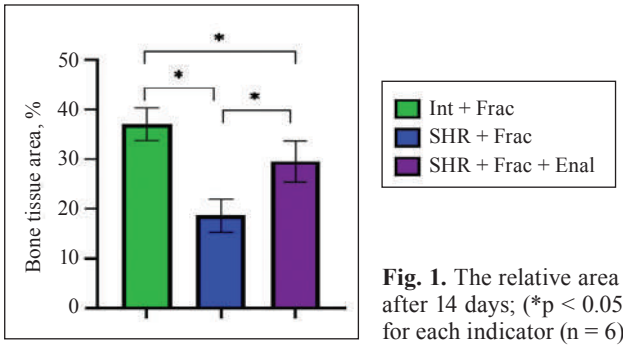

Fig. 1. The relative area of bone trabeculae (%) in the bone callus in animals of three groups after 14 days; (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6)

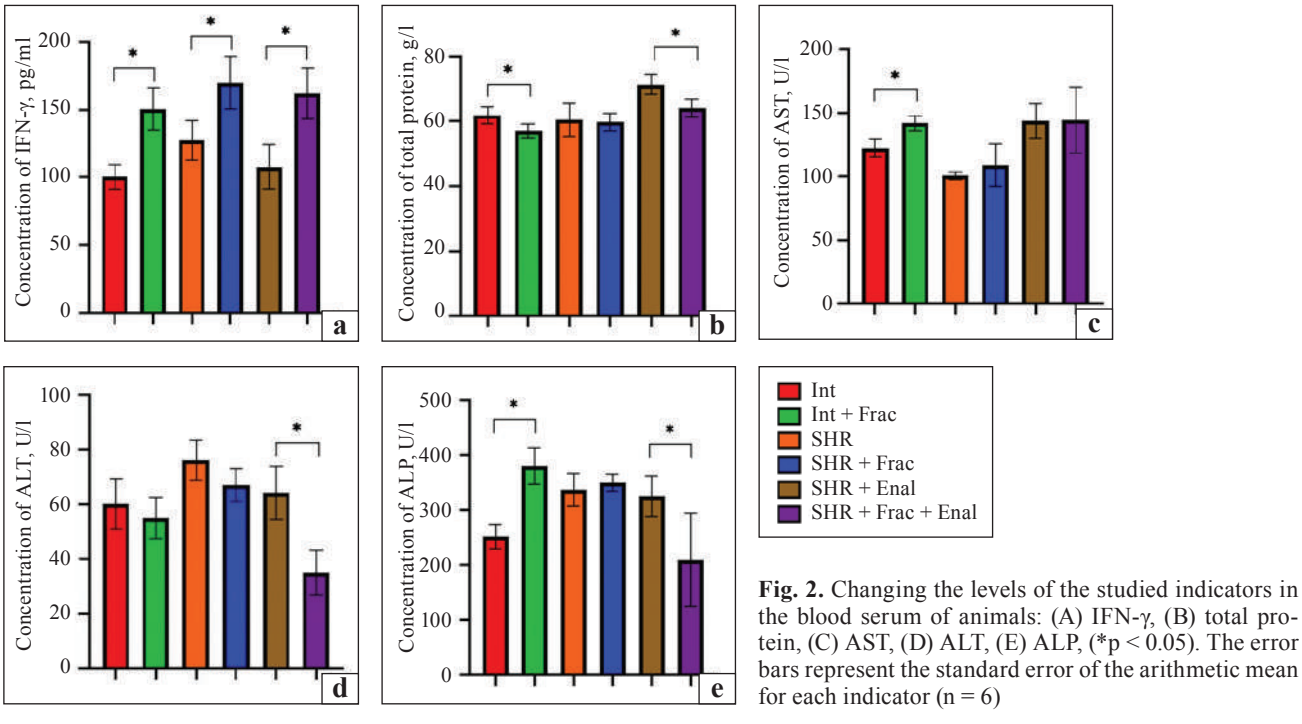

Fig. 2. Changing the levels of the studied indicators in the blood serum of animals: (A) IFN-γ, (B) total protein, (C) AST, (D) ALT, (E) ALP, (*$p < 0.05$). The error bars represent the standard error of the arithmetic mean for each indicator (n = 6)

Discussion

Immune cell signaling is important not only in the initial stage of inflammation but also in the later stages of soft and hard callus formation [10]. Thus, IFN-γ is one of the pro-inflammatory cytokines associated with increased fracture risk [11]. On the other hand, IFN-γ may be involved in inhibiting osteoclast formation [12]. In our work, IFN-γ levels were elevated after surgical intervention in animals of all groups. In addition to the production of this cytokine in inflammatory diseases, IFN-γ can also participate in anti-inflammatory mechanisms by activating apoptosis [13]. Our results contradict the findings of a paper in which blood levels of IFN-γ decreased after trauma and remained low during fracture healing [11].

In our study, IFN-γ levels were elevated in SHR rats, which seems to be related to the ability of this cytokine together with IL-17 to promote hypertension by inducing oxidative stress-induced damage, and endothelial dysfunction [14]. The use of enalapril decreased the concentration of IFN-γ. Previously, systolic blood pressure (SBP) decreased in SHR animal groups that received enalapril. There were no differences in SBP values 14 days after surgery compared to preoperative values in healthy rats and SHR rats [15].

Blood biochemical parameters were analyzed to monitor the fracture healing process for correction of treatment tactics. This paper shows that total protein levels were reduced after surgery in the SHR animal group with AH correction (SHR + Frac + Enal group). This may be due to the processes of synthesis and breakdown as a result of trauma and tissue repair [16]. Enzyme levels were analyzed because of their role as markers of tissue destruction. Thus, the increase in AST concentrations observed in our study after fractures in healthy animals (Int + Frac group) seems to reflect tissue damage (plasma membrane destruction or apoptosis), plasma membrane bleb formation, increased tissue expression and macroenzymes (AST complexes with plasma proteins) [17].

ALT levels in the serum of SHR rats were elevated compared with the concentration of this enzyme in healthy animals. This is possible because elevated levels of the liver enzymes ALT and AST are associated with many factors, including an increased risk of AH [18].

Our study also observed increasing ALP levels after fractures in healthy rats. This may be because IFN-γ at the middle and late stages of osteogenic differentiation can significantly increase the ALP and osteocalcin expression, effectively promoting osteogenic differentiation [19]. Our data are consistent with the literature findings of increased serum ALP levels two weeks after hip fracture surgery [20]. Determination of ALP activity in the blood is used to diagnose bone and liver diseases. An association between elevated ALP and an increased risk of cardiovascular disease or all-cause mortality has been shown [21]. The decrease of ALT and ALP concentrations in the serum of SHR rats treated with enalapril can apparently be explained by the treatment of AH.

The data we obtained on the relative area of newly formed bone tissue in the bone callus (Fig. 1) indicated a positive effect of enalapril on the healing of proximal femur fractures in animals with AH (SHR + Frac + Enal group). The histologic findings support this conclusion [22].

Conclusions

It is shown that rats with genetically determined AH have reduced reparative function in the proximal femur fracture. An increased level of IFN-γ is observed. Sufficient bone density is not achieved in the remodeling process.

The use of the drug enalapril in the modeling of proximal femur fractures with intramedullary osteosynthesis is due to an increase in the concentration of IFN-γ and changes in blood biochemical parameters, which contributes to an increase in the relative area of newly formed bone tissue in the bone callus.

Correction of concomitant AH leads to optimization of repair processes of the proximal femur fractures in an experiment.

Conflict of interest. The authors declare no conflict of interest.

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РЕГУЛЯЦІЯ РЕПАРАТИВНИХ ПРОЦЕСІВ У РАЗІ ПЕРЕЛОМІВ ПРОКСИМАЛЬНОГО ВІДДІЛУ СТЕГНОВОЇ КІСТКИ ШЛЯХОМ КОРЕКЦІЇ АРТЕРІАЛЬНОЇ ГІПЕРТЕНЗІЇ (ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ)

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