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Research Article

Experimental evaluation of osteogenic activity of certain hormonal drugs

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Abstract

Introduction: The necessity of extra medicamental stimulation of bone tissue regeneration in addition to basic surgical method of treating fractures is usually determined by osteoporosis or high severity of injury. One of the lesser-studied options for osteogenic hormone therapy of fractures is the use of human chorionic gonadotropin (hCG). **The aim of the study**: Conducting an experimental evaluation of the osteogenic activity of hCG in its systemic and local application based on a model of perforated fracture of femoral bone in laboratory white rats.

Materials and Methods: The experimental perforated femoral fracture in 64 male Wistar laboratory rats was modeled by drilling a circular bone defect with a 1.4 mm diameter. The osteogenic effect of the studied hormonal drugs (hCG and thyrocalcitonin) compared with a reference agent from the bisphosphonate group – zoledronic acid, after a course of injectable treatment was assessed according to the rate of bone defect recovery using digital radiography. In addition, the same method was used to experimentally evaluate the local osteogenic activity of hCG and zoledronate introduced into the bone marrow canal of the damaged bone via a patented original intramedullary metal construction for osteosynthesis.

Results and Discussion: Upon administering the studied hormonal drugs by injection, significant osteogenic activity was observed, with a more pronounced effect for hCG, although still inferior to that of the reference pharmacotherapy. The intra-bone local application of hCG showed results practically equal to the osteogenic effect of zoledronate.

Conclusion: As a result of the experiment, a high therapeutic effect of hCG on bone tissue regeneration was discovered, which, with local application (intramedullary implants soaked in hCG solution with a concentration of 5000 IU/mL), reached the level of zoledronate's effect (intramedullary implants soaked in solution with a concentration of 0.8 mg/mL), but without the toxic side effects typically associated with the reference pharmacotherapy.

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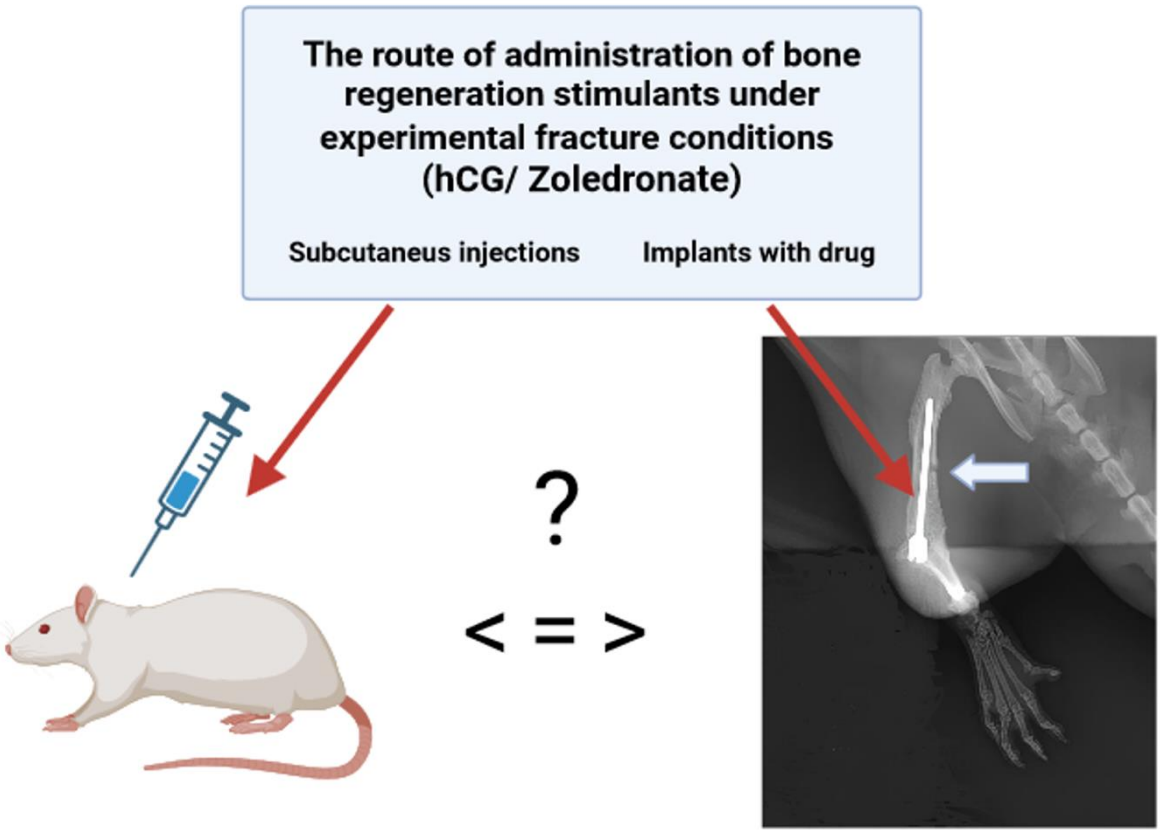
Graphical abstract



Keywords

bone fracture, osteoporosis, bone regeneration stimulators, human chorionic gonadotropin, thyrocalcitonin, zoledronate, rats, experiment

Introduction

Bone fractures are a serious public health issue worldwide. According to the World Health Organization (WHO), over 170 million fractures are reported around the world each year. In the Russian Federation, morbidity structure, the total incidence of fractures of various localizations remains at 3,000 cases per year per 100,000 population, accounting for nearly 23% of all injuries (Okladnikov and Nikitina 2023).

Moreover, a fracture almost always leads to a temporary loss of work capacity for a significant period and often requires hospitalization and inpatient treatment. Bone fractures can result in chronic pain, disability, and a reduced quality of life, as well as an increased risk of premature death (Akimova et al. 2009).

The standard practice for treating fractures involves the use of various immobilization methods: plaster casts, splints, and osteosynthesis devices. Although many pharmacological methods for accelerating bone tissue regeneration are known, they are relatively infrequently used in practice. This may be due to the insufficient knowledge in this area, lack of awareness among practicing physicians, and their concerns about the risk of adverse drug reactions.

The need for pharmacological stimulation of bone tissue regeneration, in addition to the main surgical treatment methods, typically arises in cases of pathological fractures caused by osteoporosis or in severe polytraumas resulting from road accidents, falls from height, or combat wounds (Belaya et al. 2021).

Nowadays, medications from various pharmacological groups can be used to treat osteoporosis and accelerate fracture healing. Among them, bisphosphonates (alendronate, risedronate, zoledronate) are considered the most effective, as they inhibit bone tissue resorption by suppressing osteoclast activity. However, their long-term use is associated with the risk of developing osteonecrosis of the jaw, atypical femoral fractures, and other side effects, which limits their use (Black and Rosen 2016; Belaya et al. 2021).

Another highly effective agent is denosumab – a monoclonal antibody to RANKL reducing osteoclast activity. Despite its effectiveness, discontinuation of denosumab is associated with a rapid decrease in bone mineral density and an increased risk of multiple vertebral compression fractures (Cummings et al. 2018).

Teriparatide, a parathyroid hormone analogue, stimulates the formation of new bone tissue, but its use is limited by its high cost and restrictions on the duration of therapy due to the potential risk of osteosarcoma (Vahle et al. 2004; Belaya et al. 2021).

Given the drawbacks of existing drugs, there is a growing need to search for new therapeutic approaches aimed not only at inhibiting resorption but also at stimulating bone tissue formation with minimal side effects. One of the promising areas of research in this field is the study of the little-investigated ability of human chorionic gonadotropin (hCG) to stimulate bone tissue regeneration.

Human chorionic gonadotropin (hCG) is a hormonal drug produced by the placenta during pregnancy, widely used in reproductive medicine to support the luteal phase and stimulate ovulation (Cole 2012). Recent studies suggest that hCG may influence bone metabolism. It is believed to stimulate osteoblast proliferation, collagen synthesis, and suppress apoptosis of bone cells (Mansell et al. 2007). However, data on the effect of hCG on bone tissue regeneration in osteoporosis and fractures are limited and requires further investigation.

Studying the potential of hCG in the treatment of bone diseases opens new possibilities for osteoporosis therapy and the acceleration of fracture consolidation. Its use may be particularly relevant for patients with contraindications to existing medications.

Materials and Methods

Animals

The experiments were conducted on 64 male Wistar rats aged 10–11 months, weighing 310–360 g. The animals were kept under the supervision of a licensed veterinarian in controlled conditions with a 12-hour light and 12-hour dark cycle, with free access to water and food, in accordance with the principles outlined in the Guide for the Care and Use of Laboratory Animals.

The study was approved by the Ethics Committee of Yaroslavl State Medical University (Minutes №19 of 26 October 2017).

Methodology for creating a bone defect

Under general anesthesia (Zoletil, 30 mg/kg intraperitoneally), a perforated defect with a diameter of 1.4 mm was drilled in the mid-third of the femur through both cortical layers of all animals using a Kirschner wire and a screwdriver.

Medication therapy

In the first series of experiments, the effects of pharmaceutical drugs on the regeneration of bone tissue and their safety during injection administration were studied. For this purpose, 40 rats were divided into 4 experimental groups of 10 animals each. The first group received daily subcutaneous injections of calcitonin (Miacalcic, Novartis Pharma, Switzerland) at a dosage of 50 IU/kg. The second group received subcutaneous injections of human chorionic gonadotropin (hCG) (Moscow Endocrine Plant, Russia) at a dosage of 500 IU/kg. The third group received a single subcutaneous injection of a bisphosphonate drug – zoledronic acid (Resoclastin, Pharmstandard-UfaVITA, Russia) at a dosage of 150 mcg/kg. The fourth group served as the control one and received daily subcutaneous injections of physiological saline (sodium chloride solution).

To prevent purulent complications, all rats were additionally given intraperitoneal injections of 4% gentamicin solution (Dalchimpharm, Russia) at a dosage of 4 mg/kg once daily for 7 days after surgery.

In the second series of experiments, the effectiveness and safety of hCG and zoledronic acid were evaluated when applied locally. Calcitonin preparations, which had demonstrated the lowest osteogenic activity in the first series of experiments, were no longer available on the Russian market by the start of these experiments and were therefore excluded.

Targeted delivery of medications to the fracture area was achieved using a previously patented intramedullary metal implant described in the utility model patent RU 187285 (Smirnov

et al.2019). This design is intended for use in laboratory practice with rats to study the localized effects of various medications on bone tissue regeneration. The metal implant is an intramedullary implant made from a medical-grade titanium alloy. The implant features threading along the entire length of the pin and a porous hydroxyapatite coating, which allows the structure to carry a greater amount of the drug on its surface. Drugs were applied to the porous coating of the implant by soaking it in solutions of the respective drugs for 24 hours, followed by drying for 24 hours at +4 to +8°C under sterile conditions. During the soaking stage, an hCG solution with a concentration of 5000 IU/mL was used for drug application, and a zoledronic acid solution with a concentration of 0.8 mg/mL was used for its application. For the control group, physiological saline (sodium chloride solution) was used during the soaking stage. To prevent purulent complications, all implants were additionally soaked in a gentamicin antibiotic solution with a concentration of 40 mg/mL and dried again. In the first group, implants with dehydrated hCG in the porous coating were introduced into the medullary canal of the damaged bone. In the second group, implants with dehydrated zoledronic acid were used. The third group served as the control one.

An X-ray image showing the intramedullary implant placed in the femoral bone canal of a rat is shown in Figure 1.

Monitoring of regeneration and treatment safety

The rate of bone tissue recovery was monitored using digital radiography (Toshiba Radrex X-ray machine, Japan) by assessing the reduction in the bone defect area (amount of bone tissue growth) and the frequency of complete defect closure by the end of the experiment (Fig. 2). Radiography was conducted on days 1, 14, and 28 after the experimental fracture was created. Digital images were processed, and necessary measurements were conducted using the Radiant DICOM Viewer software (Poland). The safety of the treatment was evaluated based on the survival rate and the average weight gain of the animals in the group by the end of the experiment.

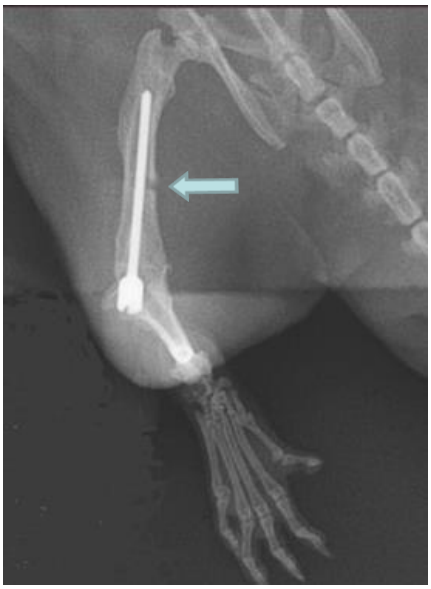


Figure 1. X-ray image of a rat's femur with a perforated fracture (indicated by an arrow) and an installed metal implant.

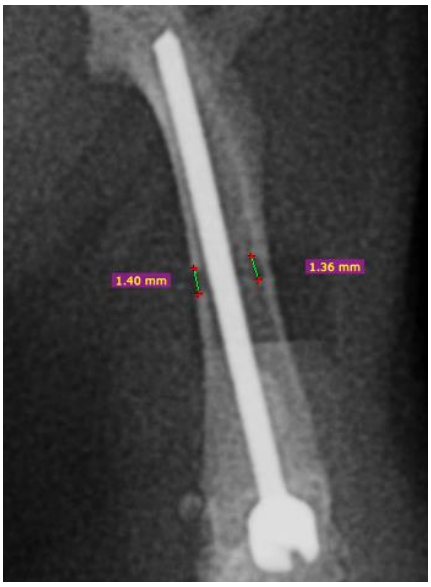


Figure 2. X-ray image with an example of fracture bone measurements.

Statistical analysis

Statistical data analysis was performed using SPSS Statistics 27 programme (IBM, USA). Given the small sample size and the absence of a normal distribution for most of the obtained values, the Kruskal-Wallis H-test was used to assess intergroup differences, followed by pairwise comparisons using the Mann-Whitney U-test. Differences were considered statistically significant at $p<0.05$. The results are presented as mean values ± standard error of the mean (SEM).

Results

The results of evaluating the effectiveness of the drugs when administered subcutaneously are shown in Figures 3 and 4. By the 14th day of the experiment, the increase in bone tissue was significantly higher in all experimental groups compared to the control group: by 143.5% in the calcitonin group, by 187.5% in the hCG group, and by 154.9% in the group receiving zoledronic acid ($p<0.001$). Moreover, the effect of hCG significantly exceeded the effects of calcitonin and zoledronic acid by 18.1% and 12.7%, respectively ($p<0.05$). The increase in bone tissue in the zoledronate and calcitonin groups did not differ significantly from each other.

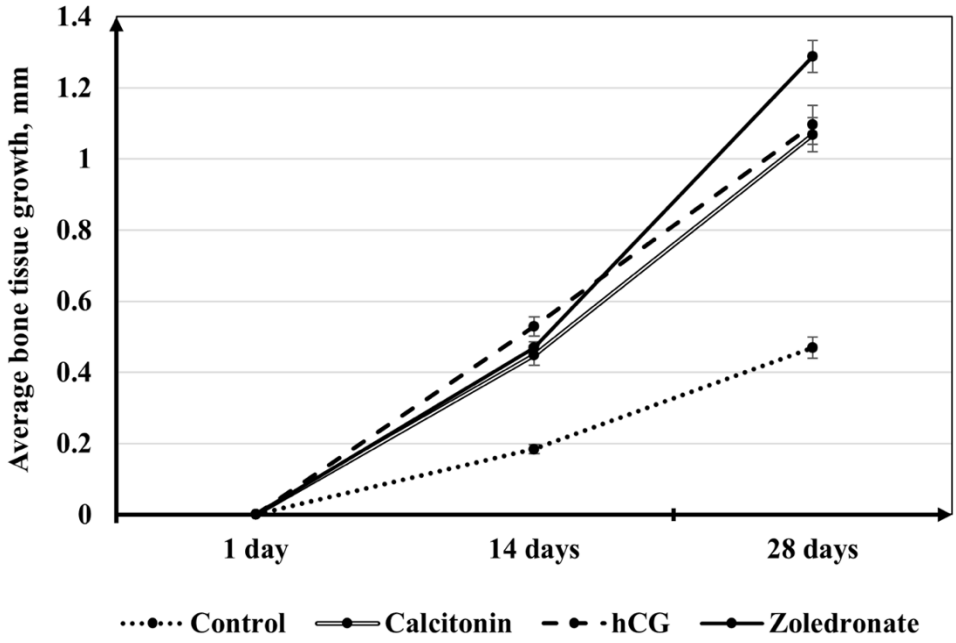


Figure 3. The dynamics of the impact of subcutaneous administration of calcitonin, hCG and zoledronate on bone tissue growth; n=10 for each group.

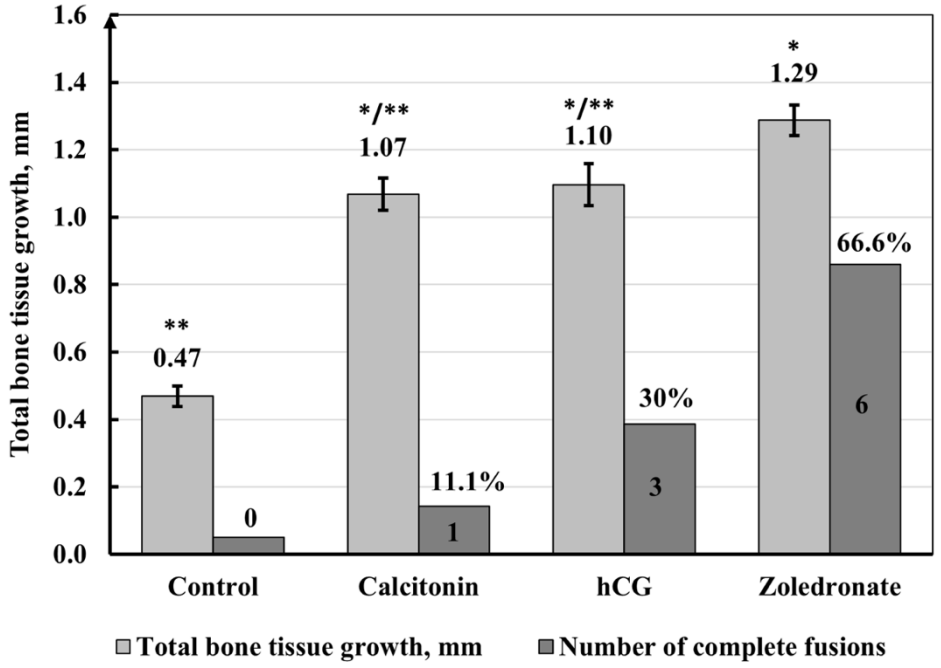


Figure 4. The impact of subcutaneous administration of calcitonin, hCG and zoledronate on the value of bone tissue growth and the number of complete defect fusions at the end of the experiment; n=10 for each group. ***Note:*** * – significant difference from the control group; ** – significant difference from the reference drug (zoledronate).

From days 14 to 28 of the experiment, the rate of bone tissue growth in the calcitonin and hCG groups remained almost unchanged compared to the first two weeks; in the zoledronic acid

group, however, the regeneration rate increased significantly by the end of the experiment, and on day 28, bone tissue growth in this group was the highest. Thus, the bone tissue growth in the zoledronate group exceeded that of the control group by 174.6%, of the hCG group – by 133.7%, and of the calcitonin group – by 127.7% ($p<0.001$); the effect of zoledronate surpassed that of hCG by 17.5% and calcitonin – by 20.5% ($p<0.05$). No statistically significant differences were observed between the hCG and calcitonin groups.

With administration of the studied drugs via injection, no cases of complete bone defect fusion were recorded in the control group by the end of the experiment. Stimulation of bone regeneration under the influence of calcitonin resulted in complete defect fusion in 11.1% of individuals, in 30% with the use of hCG and in 66.6% with zoledronic acid.

The safety study of the treatment methods used for the experimental fracture (Fig. 5) showed that hCG therapy is quite safe, as 100% survival was observed in the groups of rats receiving these drugs, similar to the control group. Also, hCG therapy had a positive effect on the animals' weight gain rate compared to the control group (+34%; $p<0.001$). In the calcitonin and zoledronate groups, 9 out of 10 laboratory animals were alive at the end of the experiment, while the weight gain rate in the surviving animals was lower than in the control group by 18.3% ($p<0.05$) and 29.7% ($p=0.001$) respectively, which may indicate a toxic systemic effect of the drugs.

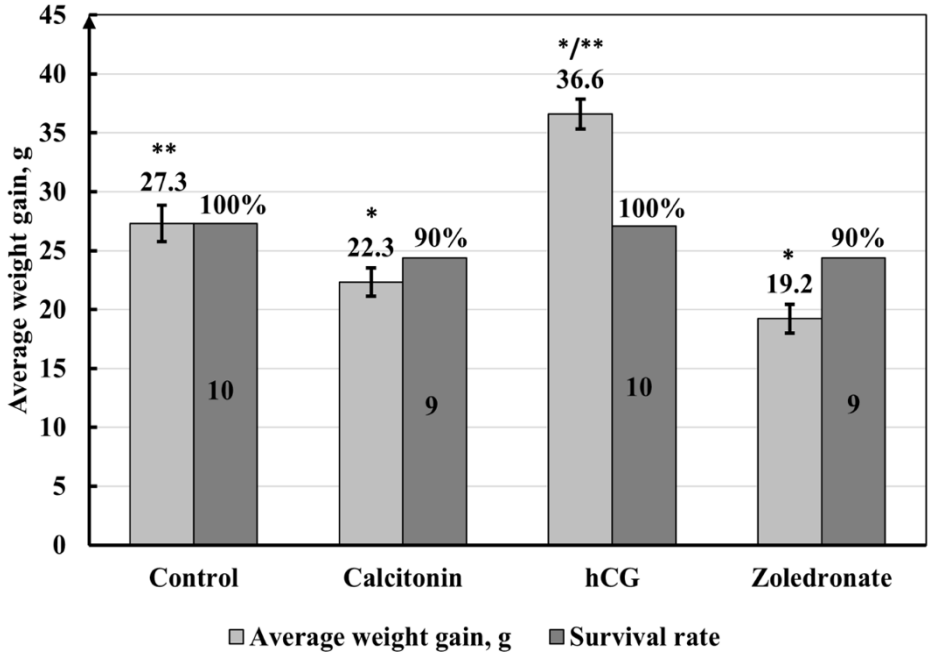


Figure 5. The impact of administration of calcitonin, hCG and zoledronate via injection on the survival rate and body weight dynamics of the animals; n=10 for each group. ***Note:*** * – significant difference from the control group; ** – significant difference from the reference drug (zoledronate).

The results of the experiment on the local application of hCG and zoledronic acid using titanium metal implants are shown in Figures 6, 7, and 8.

According to the obtained results, after 2 weeks of treatment, hCG and zoledronic acid significantly accelerated bone tissue growth compared to the control group by 118.1% and 161.1%, respectively ($p<0.001$). Zoledronate turned out to be more effective than hCG by 19.7% with $p<0.05$. Interestingly, compared to the results of administering drug via injection, the mere use of intramedullary metal implants had a certain therapeutic effect, resulting in a significant increase in bone tissue growth at this stage of the experiment by 22.8% ($p<0.05$). Local application of zoledronic acid was also significantly more effective than injectable application by 25.7% with $p<0.001$. In the groups treated with hCG, no statistically significant difference was observed between local and injectable applications.

After 4 weeks, in the group with metal implants coated with hCG, the bone regenerate was larger than that in the control group by 103.8%, and in the zoledronic acid group – by 140.1%. The effect of zoledronate surpassed the effect of hCG by 17.8% ($p<0.001$). In the group with metal implants without drug coating, the increase in bone tissue was significantly different from that in the group receiving only saline injections by 22.8% ($p<0.05$). In the groups with metal implants coated with hCG and zoledronic acid, the rate of regeneration was also somewhat higher than in the groups with administering drug via injection, but this difference did not reach statistically significant values.

However, with local application of hCG and zoledronic acid, the frequency of complete bone defect fusion by the end of the experiment was significantly higher: 50% and 100%, compared to 30% and 66.6%, respectively, with systemic application of the same drugs. In the control groups, no cases of complete defect fusion were observed.

The results of the safety assessment of treatment using intramedullary metal implants are shown in Figure 8.

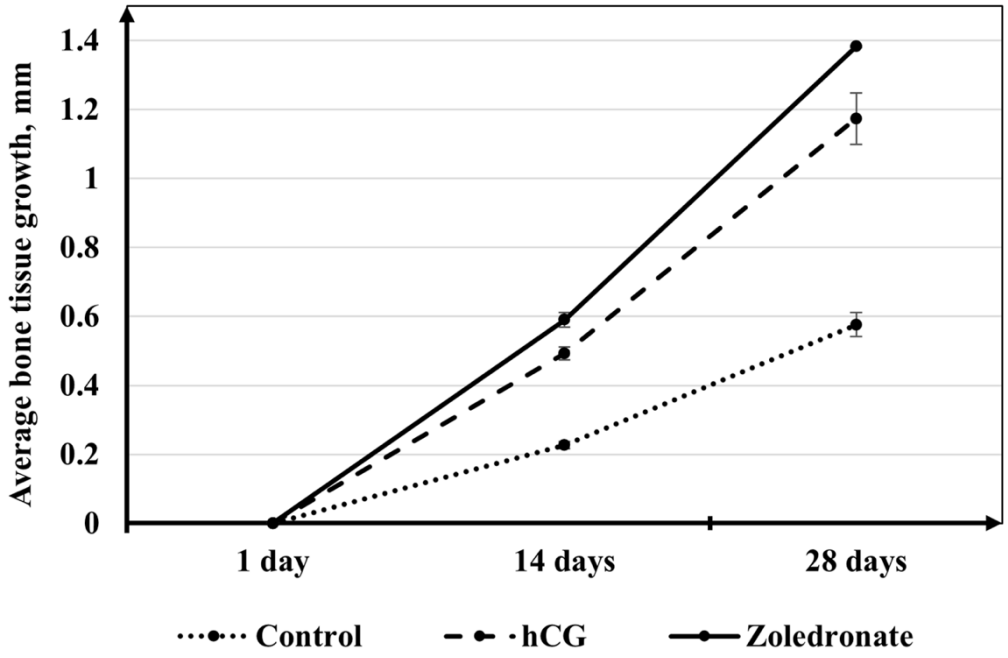


Figure 6. The impact of using intramedullary metal implants with the application of hCG and zoledronic acid on bone tissue growth; n=8 for each group.

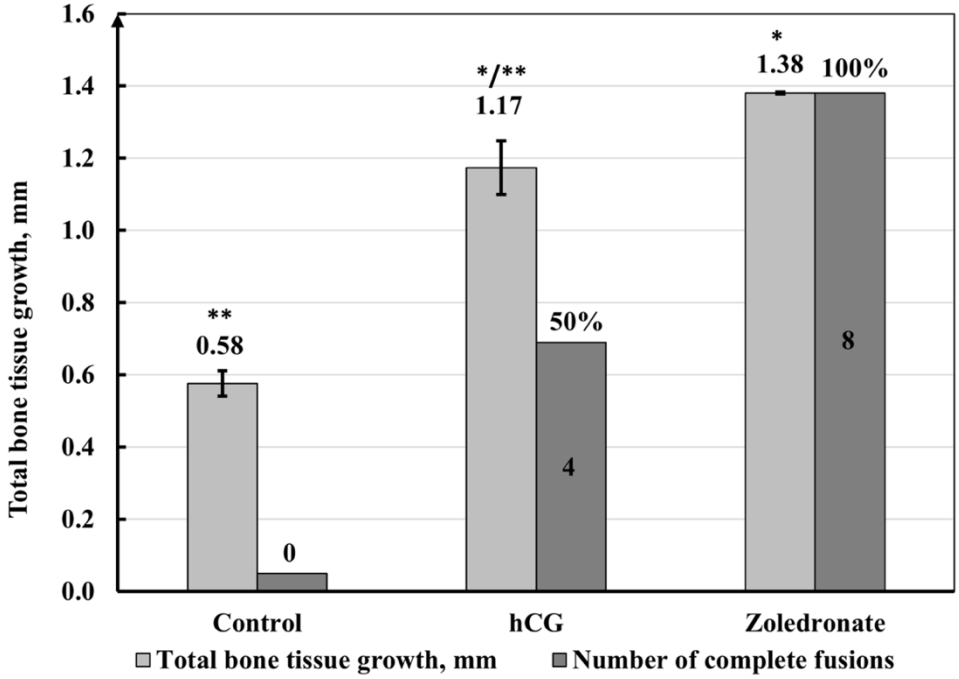


Figure 7. The impact of using intramedullary metal implants with the application of hCG and zoledronate on the increase value in bone tissue and the frequency of complete bone defect fusion by the end of the experiment; n=8 for each group. ***Note*:** * – significant difference from the control group; ** — significant difference from the reference drug (zoledronate).

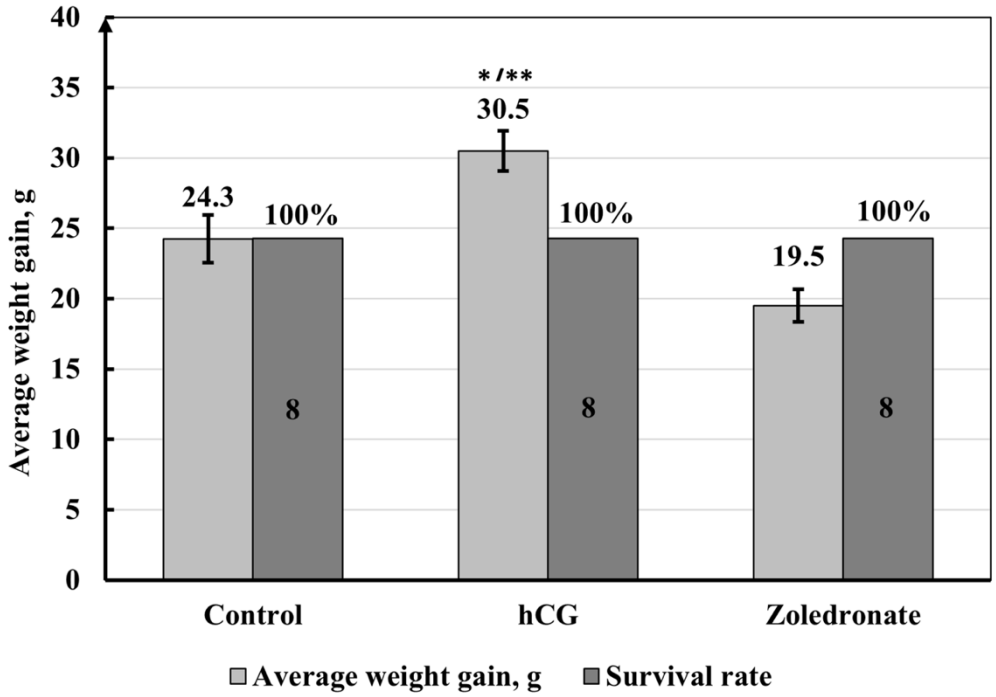


Figure 8. The effect of applying hCG and zoledronic acid to intramedullary metal implants on the survival and body weight dynamics of animals; n=8 for each group. ***Note*:** * – significant difference from the control group; ** – significant difference from the reference drug (zoledronate).

When the studied drugs were applied locally, the survival rate of the animals was 100% in all experimental groups, whereas in the group of rats that received zoledronate injections in the previous series of experiments, one animal died. Also, with local application of zoledronic acid, body weight gain was somewhat slower compared to the control group, but this difference was not statistically significant. These findings may indicate greater safety of local bisphosphonate application compared to their systemic use. The body weight gain of animals in the group with intramedullary implants carrying hCG was significantly higher than in the control group by 25.5% ($p<0.05$), but lower than in the group with injectable hCG by 16.6% ($p<0.05$), which could be explained by a smaller systemic anabolic effect due to local application and a smaller overall course dose.

These findings may indicate greater safety of local bisphosphonate application compared to their systemic use. The body weight gain of animals in the group with intramedullary implants carrying hCG was significantly higher than that in the control group by 25.5% ($p<0.05$), but was 16.6% lower compared to the group with injectable hCG ($p<0.05$). This may be explained by a reduced systemic anabolic effect due to local application and a smaller overall cumulative dose.

Conclusion

Overall, the results of the experiments confirmed that hCG (500 IU/kg, subcutaneously) demonstrates a pronounced pharmacological activity associated with its ability to accelerate the regeneration of bone tissue damaged by fractures. This effect was significantly stronger than that of Miacalcic (50 UI/kg, subcutaneously) and comparable in strength to that of a representative of the zoledronic acid group (150 mcg/kg, subcutaneously). However, unlike bisphosphonates, hCG did not cause toxic side effects, which are most prominent with systemic use of these drugs.

The results of the study on the intramedullary application of hCG (concentration of solution 5000 UI/mL) and zoledronate (concentration of solution 0.8 mg/mL), applied to the surface of an intramedullary metal implant for osteosynthesis, demonstrated the advantages of this method of administering osteogenesis stimulators compared to their systemic injection-based application. These advantages included better tolerability and a reduction in the frequency of side effects. Furthermore, the findings from this series of experiments led to the conclusion that the osteogenic activity of hCG has a direct type of restorative effect on damaged bone tissue and is not solely a result of the substance's stimulating effect on the secretion of sex hormones.

The most logical explanation for the mechanism of the noted osteogenic activity of hCG is its recently discovered ability to boost the regeneration of various damaged tissues in the body through the direct stimulation of pluripotent stem cells, as discovered in studies by several authors conducted both in vivo and in vitro (Tucker 2006; Mansell et al. 2007).

Additional information

Conflict of interest

The authors declare the absence of a conflict of interests.

Funding

The authors have no funding to report.

Data availability

All of the data that support the findings of this study are available in the main text.

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