Вестник ВГУИТ/ Proceedings of VSUET ISSN 2226-910X E-ISSN 2310-1202
DOI: http://doi.org/10.20914/2310-1202-2024-1-84-88 Research article
УДК 640 Open Access Available online at vestnik-vsuet.ru

Metabolic processes in bone tissue under exposure to hyperthermia and administration of allogenic calcium-containing biomaterial "Lyoplast"

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Abstract. Stress increases the production of glucocorticoids, which enhance bone resorption processes. To treat bone tissue diseases, medicine uses drugs that regulate phosphorus-calcium metabolism. A promising biomaterial is a bone mineral component (BMC) of allogenic origin, containing hydroxyapatite and amorphous calcium phosphate, which enhances bone tissue regeneration. In this work the parameters of bone tissue metabolism were studied under daily stress exposure to high temperature and intramuscular administration of a suspension of bone mineral component "Lyoplast" to animals. There was an increase in cortisol and a decrease in alkaline phosphatase activity in the blood serum subjected to hyperthermia. Serum alkaline phosphatase activity in the hyperthermia group and the placebo group decreased by an average of 25%. The enzyme activity in animals that were injected with the bone component did not differ statistically from the control level. In animals exposed to hyperthermia due to the administration of a bone mineral component, the level of parathyroid hormone increased simultaneously with the calcitonin level. Serum parathyroid hormone levels were lower in the hyperthermia group than in the control one. A reciprocal relationship between two hormones, parathyroid hormone and calcitonin, has been established. Thus, the introduction of a suspension of the bone mineral component helps to reduce the intensity of osteoresorption. The use of biomaterial obtained by the original method helps to reduce the intensity of osteoresorption in the high-temperature model. With the introduction of a suspension of the bone mineral component, the osteodestructive effect of endogenous glucocorticoids is smoothed out and largely eliminated. Given the high potential for practical use of the bone mineral component, further research of its safety and effectiveness in other biological models is necessary with further implementation in clinical practice.

Keywords: high temperature, mineral component, bone tissue, cortisol, alkaline phosphatase, parathyroid hormone, calcitonin, osteoresorption.

Introduction

Excessive glucocorticoid (GK) content in the body is the main cause of secondary osteoporosis, when there is a rapid loss of bone mass, the risk of fractures increases. An increase in the level of GK can be not only exogenous, but also of endogenous nature, in particular, due to the development of stress [1]. Stress increases the production of steroid hormones glucocorticoids (GCs), which enhance the processes of bone resorption. At the cellular level, the effect of GCs disrupts the activity of osteoblasts, slowing down the process of differentiation of progenitor cells. GCs enhances osteoblast apoptosis, reduce the synthesis of collagen, prostaglandins and bone growth factors, which inhibits the process of bone formation [2–4].

For the treatment of bone tissue diseases in medicine drugs that regulate phosphorus-calcium metabolism in the body are used. One of the promising biomaterials is the allogenic BMC which contributes to an increase in bone tissue regeneration. The main BMC components are hydroxyapatite and amorphous calcium phosphate, which are widely used as a starting material for the synthesis of medical coatings. A feature of the biological (allogenic) BMC is good biocompatibility, self-absorption and stimulation of bone formation processes [5]. Some of its physical characteristics are described [6].

Therefore, the purpose of this research was to study the parameters of bone metabolism in animals exposed to elevated ambient temperatures and ectopic administration of BMC.

Methods

White adult outbred male laboratory rats (n = 24) were randomly assigned into 4 groups (6 animals per group): 1 – rats without influence (control group); 2 – rats induced high temperature (hT), 3 – rats induced hT and treated BMC (100 mg/kg) on 14th day (hT + BMC), 4 – rats induced hT and treated 0,9% saline on 14th day (placebo group). Animals had free access to food and water at all period (28 days) and were housed with a light/dark cycle of 12 h.

The simulation of high temperature conditions was carried out in a specialized thermal chamber as described earlier [7]. Briefly, there was a large number of small holes in the cell floor through which heated air was supplied from an external air source of heat. The air temperature was the same in all parts of the cell. The exposure time was 12 min,

Для цитирования
Писарева Е.В., Власов М.Ю., Волова Л.Т., Ищенко К.С., Сергеева С.С. Метаболические процессы в костной ткани при воздействии гипертермии и введении аллогенного кальцийсодержащего биоматериала «Лиопласт» // Вестник ВГУИТ. 2024. Т. 86. № 1. С. 84–88. doi:10.20914/2310-1202-2024-1-84-88

For citation
Pisareva E.V., Vlasov M.Yu, Volova L.T., Ishchenko K.S., Sergeeva S.S. Metabolic processes in bone tissue under exposure to hyperthermia and administration of allogenic calcium-containing biomaterial "Lyoplast". Vestnik VGUIT [Proceedings of VSUET]. 2024. vol. 86. no. 1. pp. 84–88. (in Russian). doi:10.20914/2310-1202-2024-1-84-88

the air temperature was 70 °C. BMC was obtained according to an original patented technique "Lyoplast" [8] and was injected intramuscularly into the rat's thigh muscle on day 14th as sterile suspension in saline. Animal welfare was ensured according to relevant international experimental animal rules and guidelines.

After 28 day the animals were stunned and killed by decapitation. Blood was taken from the trunk into tubes without anticoagulant and the sera separated after centrifugation at 4 °C. Sera were stored at -25 °C until required for assay.

Total and free calcium, inorganic phosphate (P_i), alkaline phosphatase (ALP) activity in sera were measured on Cobas Integra 400 (Roche, Switzerland). The concentrations of PTH, calcitonin and cortisol were determined on StatFax 3200 (Awareness Technology, USA) using a commercial enzyme-linked immunosorbent assay kits (Cloud-Clone Corp, USA). The Shapiro-Wilk test was used to assess for data normality. The data were analyzed by Student's t-test for independent means in SigmaPlot 12.0. The results are presented as mean and standard error of the mean (M ± SEM).

Results

The results of the study are presented in the table. After 28 days of hT influence on rats the level of cortisol in the sera of all groups increased 10-fold relative to the control group. The levels of ionized calcium are within normal limits, there are no significant changes in comparison with the control, however, a tendency for the level to decrease in the group with BMC by 5% was observed. Changes in total calcium levels are similar to ionized calcium. There is a statistically significant decrease in the level in the hT + BMC group.

Table 1.

Biochemical rat serum markers of bone metabolism, M±SEM

Index	Control	hT	hT + BMC	hT + saline
Cortisol, μ/dl	0,92 ± 0,04	9,07 ± 0,46 *	10,08 ± 0,51 *	9,55 ± 0,47 *
Total calcium, mmol/L	2,19 ± 0,11	2,22 ± 0,11	1,93 ± 0,10 *	2,24 ± 0,12
Free calcium, mmol/L	1,19 ± 0,05	1,19 ± 0,05	1,12 ± 0,04	1,20 ± 0,05
P_i, mmol/L	1,92 ± 0,10	1,58 ± 0,07 *	1,44 ± 0,07 *	1,60 ± 0,08 *
ALP, mU/L	191,80 ± 9,59	142,6 ± 7,13 *	191,40 ± 9,40	141,00 ± 7,10 *
Calcitonin, pg/ml	25,61 ± 1,28	85,3 ± 4,26 *	62,43 ± 3,12 *	84,10 ± 4,20 *
PTH, pg/ml	2,22 ± 0,11	1,89 ± 0,09 *	2,94 ± 0,14 *	1,89 ± 0,09 *

* – differences from control group are statistically significant with the level of significance $p < 0,05$

The content of P_i in the test groups is significantly lower than in the control group. In the placebo and hT groups, the levels were on average 17% lower than in control, at the same time the phosphorus content in the BMC injection group dropped by 25%. We also found an increase of calcitonin levels in all groups.

A significant decrease of serum ALP activity was revealed in the group with hT and the placebo group on average by 25%. At the same time, the level of activity in the group with BMC practically does not differ from the control level.

The serum PTH level was lower in the group hT then in control group. Instead of this the PTH level in group hT + BMC was higher than in control. Thus a reciprocal relationship between the two hormones (PTH and calcitonin) was determined (a decrease in hormone levels in groups with hT and placebo).

Discussion

Increased serum cortisol level is the body's response to stress caused by hyperthermia. Such a high level of glucocorticoids causes osteoresorption of bone tissue through an increase in the activity of osteoclasts. An increase in osteoclast activity occurs through the activation of the RANK pathway. By acting on it, GCs enhances the differentiation of hematopoietic stem cells into osteoclasts. At the same time, GCs affects osteoblasts through the Wnt signal (increase osteoblast apoptosis) and the RANKL pathway (reduce the differentiation of mesenchymal stromal cells into osteoblasts) [9]. Taken together, this triggers the pathogenic processes of glucocorticoid osteoporosis [10, 11].

The identical values of ionized calcium in the three groups are explained by the fact that calcium is a vital participant in most biochemical processes, without which this processes are disrupted. Calcium levels are regulated by many systems, and when it decreases, biochemical systems are activated to maintain normal calcium levels [12, 13]. A slight drop in the level of calcium is possibly associated with the increased activity of osteoblasts, one of the main functions of which is the involvement of phosphorus and calcium ions into the bone tissue [14].

Since it is the level of total calcium that more accurately demonstrates the biochemical processes associated with bone tissue, this decrease can be considered as an indicator of increased activity of the body's control systems. Presumably, the injection of BMC caused an increase in the level of calcium in the blood, in response to which the body increased the production of substances associated with a decrease in calcium, which, in turn, increased the activity of osteoblasts. The decline of P_i can be caused by many factors, the most probable of which is the active use of phosphorus ions for the synthesis of organic compounds involved in the response to heat stress followed bone resorption [15, 16].

ALP is one of the markers of bone tissue osteogenesis, and a decrease in its activity levels indicates a decrease in osteoblast activity. Based on this, it can be concluded that in the test groups without BMC injection, the activity of osteoblasts is significantly lower than in the control group, and the group with BMC showed activity equal to the control due to the positive effect of BMC. Also, the decrease of ALP activity can be indirectly explained by the decrease in the levels of phosphorus in the blood of animals, which disrupts the synthesis of some substances that require phosphorylation and dephosphorylation for transport [17]. The decreased activity of alkaline phosphatase in experimental groups of animals without BMC administration may be associated with a decrease of the intensity of osteosynthesis under the influence of a high level of glucocorticoids.

Thus the levels of calcium, phosphorus, and alkaline phosphatase activity indicate high osteoblast activity in the BMC group, while in other test groups the data indicate a decrease in osteogenesis activity and the prevalence of osteoresorption processes. A decrease in the content of total calcium by 5%, ionized calcium by 11% and phosphorus by 25% in the group with BMC injection demonstrates the main function of osteoblasts to incorporate these substances into bone tissue, which, against the background of osteoresorption processes occurring in all three groups, is a positive factor influence of BMC. The fact that the activity of alkaline phosphatase in this group practically did not change, while in other test groups the indicator decreased by 25%, emphasizes the activity of the bone remodeling process.

The release of calcitonin into the blood occurs when the level of calcium in the blood is elevated. This hormone affects osteoblasts, decreasing cell apoptosis and increasing the differentiation of preosteoblasts [18]. Since osteoblasts use calcium ions from the blood to incorporate them into bone tissue, the level of calcium in the blood decreases. The high levels of the hormone in the test groups are explained by osteoresorption of bone tissue, in which calcium is released into the blood. The processes of calcium level control are triggered and the production of this hormone increases, leading to an increase in remodeling activity.

However, in the hT + BMC group, the PTH level is significantly higher than in the other groups: 32.4% higher than in the control group and 55% higher than in other test groups. The level of PTH during the experiment in this group rose periodically, intermittently, and the hormone, in addition to the main function, showed a secondary function, promoting the activation of osteoblasts [19].

The main function of parathyroid hormone is the release of calcium from the bones when its level in the blood is low. This is accomplished by inhibition of osteoblast activity with a simultaneous increase in osteoclast activity, increased differentiation of progenitor cells into mature cells and a decrease in osteoclast apoptosis [20]. The secondary function of PTH is to reduce the production of inhibitors of the Wnt signal and increase osteoblast activity [21].

The content of calcitonin and PTH in blood serum in the groups with hyperthermia and placebo demonstrates the expected reciprocal dependence, however, in the group with BMC, the content of PTH did not fit this dependence, remaining high (34.3% compared with calcitonin and 55% compared with other groups) with a high level of calcitonin. This increase may be a manifestation of the secondary function of the hormone, when it activates osteoblasts with an intermittent effect and a high level, indirectly affecting the Wnt pathway by suppressing the synthesis of sclerostin and Dickopf-1 [22, 23].

In general, BMC has a positive effect on bone tissue during endogenous glucocorticoid osteoresorption caused by increased stress. By causing periodic increases in PTH levels, BMC presumably indirectly triggers bone remodeling through the osteoblast Wnt signaling pathway.

Conclusion

This research indicates that the use of the biomaterial obtained by the original method helps to reduce the intensity of osteoresorption in the high temperature model. With the introduction of BMC, the osteodestructive effect of endogenous (with daily short-term hyperthermia) intake of glucocorticoids is smoothed out and largely eliminated. Partial or complete normalization of the structure and organomineral composition of bone tissue is found as a result of normalization of the ratio of the intensity of bone remodeling processes.

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Contribution

proposed a scheme of the experiment and organized production trials

wrote the manuscript, correct it before filing in editing and is responsible for plagiarism

consultation during the study

review of the literature on an investigated problem, conducted an experiment, performed computations

proposed a scheme of the experiment and organized production trials

Conflict of interest

The authors declare no conflict of interest.

Received 18/01/2024 **Accepted in revised** 06/02/2024 **Accepted** 23/02/2024

Метаболические процессы в костной ткани при воздействии гипертермии и введении аллогенного кальцийсодержащего биоматериала «Лиопласт»

Аннотация. Стресс увеличивает выработку стероидных гормонов глюкокортикоидов, которые усиливают процессы резорбции кости. Для лечения заболеваний костной ткани в медицине используются препараты, регулирующие фосфорно-кальциевый обмен в организме. Перспективным биоматериалом является минеральный компонент кости аллогенного происхождения, содержащий гидроксиапатит и аморфный фосфат кальция, способствующий усилению регенерации костной ткани за счет хорошей биосовместимости и стимуляция процессов костеобразования. В настоящей работе изучены параметры метаболизма костной ткани при ежедневном стрессовом воздействии высокой температуры и внутримышечном введении суспензии минерального компонента кости «Лиопласт» животным. Отмечено повышение кортизола и снижение активности щелочной фосфатазы в сыворотке крови животных, подвергнутых гипертермии. Активность щелочной фосфатазы в сыворотке крови в группе с гипертермией и группе плацебо снижалась в среднем на 25%. Активность фермента в группе животных, которым вводили костный компонент, статистически не отличалась от контрольного уровня. У животных, подвергшихся гипертермии на фоне введения минерального компонента кости, уровень паратгормона повышался одновременно с уровнем кальцитонина. Уровень паратгормона в сыворотке крови был ниже в группе с гипертермией, чем в контрольной группе. При этом уровень в группе с гипертермическим воздействием и введением минерального компонента кости был выше, чем в контроле, тем самым была установлена реципрокная связь между двумя гормонами - паратгормоном и кальцитонином. Таким образом, введение суспензии минерального компонента кости способствует снижению интенсивности остеорезорбции. Использование биоматериала, полученного оригинальным методом, способствует снижению интенсивности остеорезорбции в высокотемпературной модели. При введении суспензии минерального компонента кости остеодеструктивный эффект эндогенного приема глюкокортикоидов сглаживается и в значительной степени устраняется. С учетом высокого потенциала практического применения минерального компонента кости необходимы исследования его безопасности и эффективности на других биологических моделях с дальнейшим внедрением в клиническую практику.

Ключевые слова: высокая температура, минеральный компонент, костная ткань, кортизол, щелочная фосфатаза, паратгормон, кальцитонин, остеорезорбция.

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