pH DA MEDULA ÓSSEA

pH OF BONE MARROW

pH КОСТНОГО МОЗГА

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RESUMO

A medula óssea é um "nicho" para as células-tronco; determinar o valor do pH da medula óssea é de grande importância. É muito difícil, quase impossível, realizar a determinação intravital do pH. Na prática cirúrgica, há situações em que os médicos não têm como salvar o membro inferior do paciente devido à ameaça à vida. Nesses casos, é possível extrair a medula óssea da cavidade da medula óssea do fêmur amputado e examiná-la. O objetivo desta pesquisa foi estudar as peculiaridades do equilíbrio ácido-base da medula óssea de ossos longos e realizar uma análise comparativa dos dados obtidos e dos indicadores de ossos chatos. O estudo incluiu 40 amostras de teste de medula óssea. Amputações dos membros inferiores foram realizadas devido à gangrena dos pés. A medula óssea foi extraída do fêmur. A medula dos ossos chatos foi obtida por punção esternal. No teste de pH da punção esternal, os dados variaram dentro do intervalo do pH do sangue, isto é, entre 7,35-7,45 e 7,8. Os dados de pH e composição gasosa da punção esternal foram idênticos aos indicadores sanguíneos. Os dados da medula óssea obtidos do osso longo eram completamente diferentes. O equilíbrio ácido-base variou estritamente de 6,7 a 6,9.

Palavras-chave: *medula óssea, punção esternal, medula óssea de ossos longos, células-tronco da medula óssea.*

ABSTRACT

The bone marrow is a "niche" for stem cells; determining the bone marrow pH value is of great importance. It is very difficult, almost impossible, to carry out the intravital determination of pH. In surgical practice, there are situations when doctors have no way to save the patient's lower limb because of the threat to life. In such cases, it is possible to extract the bone marrow from the bone marrow cavity of the amputated femur and examine it. The purpose of this research was to study the acid-base balance peculiarities of the marrow of long bones and carry out a comparative analysis of the obtained data and the indicators of flat bones. The study included 40 test samples of bone marrow. Lower limb amputations were performed because of foot gangrene. Bone marrow was extracted from the femur. The marrow of flat bones was obtained by a sternal puncture. In the pH test of the sternal puncture, the data varied within the range of the blood pH, i.e., between 7.35-7.45 and 7.8. The pH and gas composition data of the sternal puncture were identical to the blood indicators. The data of bone marrow obtained from the long bone was completely different. The acid-base balance strictly ranged from 6.7 to 6.9.

Keywords: *bone marrow, sternal puncture, bone marrow of long bones, stem cells of the bone marrow.*

АННОТАЦИЯ

Костный мозг является «нишей» стволовых клеток и определить значение pH костного мозга имеет большое значение. Прижизненное определение pH представляет большие сложности, практически невозможно. В хирургической практике бывают ситуации, когда сохранить нижнюю конечность пациента невозможно из-за угрозы его жизни. В таких случаях можно извлечь костный мозг из костномозговой полости ампутированной бедренной кости и исследовать. Цель изучить особенности кислотно-щелочного состояния костного мозга трубчатой кости провести сравнительную характеристику с показателями плоских костей. Исследовались 40 образцов костного мозга. В результате гангрены стопы пациентам была произведена ампутация нижней конечности. Из бедренной кости извлекался

костный мозг. Костный мозг плоских костей получали путем стеральной пункции. При исследовании pH стеральной пункции данные колебались в пределах pH крови т.е. 7,35-7,45 до 7,8. Данные pH и газового состава стеральной пункции были идентичны показателям крови. Данные костного мозга, полученного из трубчатой кости были совершенно иные. Кислотно-щелочное равновесие было жестким и колебалось в пределах 6,7 до 6,9.

Keywords: костный мозг, стеральная пункция, костный мозг трубчатых костей, стволовые клетки костного мозга.

1. INTRODUCTION

Acid-base balance or blood pH has an important function in the body (Nikolaeva *et al.*, 2010). A deviation from the norm in one direction or another indicate possible serious consequences for human health (Paltsev, 2004). The acid-base index of the bone marrow was not determined either in practical medicine or for scientific purposes. This indicator can be crucial both for the prognosis of the treatment of patients and for the assessment of the bone marrow functional status (Nikolaeva, 2015; Nikolaeva *et al.*, 2015). Control and change of the pH can create different activities in the bone marrow, namely, stem cells, and, probably, influence the differentiation of stem cells (Ummenhofer *et al.*, 1994; Miller *et al.*, 2010). The blood pH of 7.4 indicates that the balance is weakly alkaline, and the human health condition is normal. If the level is below normal, this condition indicates an increase in acidity is called "acidic blood" or acidosis. Acidosis is more common than alkalosis. Acidosis can be caused by alcoholism or a complication of diabetes mellitus, i.e., the body tissues and organs receive oxygen in insufficient quantities (Orlowski *et al.*, 1989; Paladino *et al.*, 2008). Between the blood, tissue fluid, and lymph, there is a constant metabolic conversion and transport of water carrying dissolved metabolic products, hormones, gases, and biologically active substances. Consequently, the internal environment of the body is a single system of humoral transport that includes general circulation and movement in a sequential chain: blood - tissue fluid - tissue (cell) - tissue fluid - lymph - blood. It is reasonable to include the bone marrow in this sequential chain. Organisms and cells are usually very resistant even to significant changes in the environment pH. Intracellular processes, on the contrary, are highly sensitive to pH and take place in an environment, whose pH is strictly regulated (although some pH fluctuations can also be observed inside the cell, for example, at the membrane surface). Most intracellular processes take place at neutral pH values when their rate is maximal. In biological tissues and organs,

constant pH value is maintained by effective circulatory buffers, which, by their chemical nature, prevent pH changes that occur during metabolic processes (Shirakabe *et al.*, 2012; Rudkin *et al.*, 2012; Strandberg *et al.*, 2012).

The purposes of the study are 1. To determine the bone marrow pH of long and flat bones, to conduct a comparative analysis of the bone marrow acid-base status depending on the place of extraction. 2. To test the possibility of using a blood acid-base and gas composition analyzer (Radiometer Medical Aps, Akandevej 21 DK-2700 Bronshøj, Denmark) for determining the acid-base indicator of the bone marrow.

2. MATERIALS AND METHODS

Patients' characteristics. The work included 40 samples; all patients were informed and gave their consent to the tests. The age of patients ranged from 50 to 75 years, and the average age was 63 years. Twenty-five patients were men (62.5%), 15 were women (37.5%). Most patients (96%) had type 2 diabetes mellitus and diabetic foot syndrome (DFS) of mixed form. The patients underwent amputation of the lower limb as a result of foot gangrene. The bone marrow was extracted from the femur into a sterile test-tube and then transported to a laboratory.

Bone marrow was obtained by sternal puncture, which was performed in hematological patients and, for comparison reasons, in patients for whom a hematological disease was excluded. The marrow of long bones was obtained after amputation of the lower limb, generally at the level of the femur (upper or lower third of the thigh). Amputation of the lower limb was carried out by life-saving indication; the overwhelming number of patients had type 2 diabetes mellitus. The bone marrow was taken according to the sternal puncture standard: the anterior wall of the sternum was pierced with a Kassirsky's needle at the level of the 3d-4th rib along the midclavicular line or in the presternum area. The needle passed through the compact substance of the sternum frontal surface and entered the bone marrow space, with a sense of a dip. Then the

mandrin was removed from the sternal needle and replaced with a 20 ml syringe to aspirate the bone contents. Creation of vacuum allowed receiving 1-2 ml of blood. The syringe contents were sent to the laboratory. The use of the hospital analyzer was a great risk because of the possibility to take it out of operation (Nicoll and Rochester, 2014; Vos *et al.*, 2006; Sediame *et al.*, 1999). The removal of conglomerates of cells and adipose tissue was performed by means of a 10-minute settling of the sample; at that, the fat of the bone marrow rose to the upper layer. Without affecting the upper layer, the lower phase containing the bone marrow nuclear cells was collected in a new tube. Additionally, to avoid the analyzer damage, the bone marrow was twice filtered through 4-layer gauze. The acid-base indicator was determined with a blood acid-base and gas composition analyzer (Radiometer Medical Aps, Akandevvej 21 DK-2700 Bronshøj, Denmark). Despite the fact that this article discusses acid-base balance, data on the bone marrow gas composition are also of interest (Papadea *et al.*, 2002). The first results were repeated every 30 minutes during 5 hours; the result of the pH did not change. The difficulty with the collection of samples was that amputation is not a regular surgery; it is not frequent. There was approximately 1 sample every 5-6 months; thus, the collection of bone marrow samples lasted for 6-7 years. Sternal puncture data was collected during the examination of patients for the presence of hematological disease when the bone marrow was taken from the flat bone. If the hematological diagnosis was not confirmed, the resulting sample was taken into account in the study. The need to take into account the blood pH appeared with the first samples of bone marrow from sternal puncture. The study of the sternal puncture results showed that the biochemical parameters, including pH, coincide with those of blood.

3. RESULTS

The pH data of the sternal puncture varied in the range of the blood pH, i.e., from 7.35-7.45 to 7.8. The pH and gas composition indicators of the sternal puncture were identical to those of blood (Nolan *et al.*, 2010; Bland and Altman, 1986; Franssen *et al.*, 2013). The bone marrow data obtained from long bones were completely different. The acid-base balance strictly ranged from 6.7 to 6.9. There were cases when the bone marrow pH went beyond these limits; this occurred when blood entered the sample. However, the samples for the study were taken

without blood. In the marrow of the long bones, there were rare pink foci of hemopoiesis; these parts of tissue were not removed. The blood pH of 7.4 indicates that the balance is weakly alkaline, and the human condition is normal. If the level is below the norm, this condition is called acidosis ("acidic blood") and indicates an increase in acidity. Comparing the pH of the sternal puncture (i.e., the marrow of flat bones) and the marrow pH of long bones, we see a sharp difference between the two bone marrow samples. While the bone marrow of flat bones (sternal puncture) has a weakly alkaline reaction similar to that of blood (Hurren, 2000; Grisham and Hastings, 1991; Bland and Altman, 1999), the bone marrow of long bones has an acid reaction of 6.7 - 6.9. Different acid-base environment indicates differences in functional activity (Kelly, 2010; Kissoon *et al.*, 1993; 1994). In the first place, these bone marrow samples are different in cell composition. The study included tests for the presence of stem cells. In the sternal puncture samples, only hematopoietic stem cells were detected; in the bone marrow of long bones, there were both hematopoietic and mesenchymal cells. There is an interesting fact: the hematopoietic cells of long bones exist in a weakly acidic environment, and in flat bones, the conditions are weakly alkaline, like blood pH (Schuster, 1984; Mullner *et al.*, 1997; Shinozaki *et al.*, 2011). It can be assumed that hematopoietic cells originate in the bone marrow of long bones, and their further differentiation or maturation occur in flat bones, where they become functionally active. It is clear that it depends, among other things, on the pH of the environment in which the stem cells are located. The acid-base indicator was determined with a blood acid-base and gas composition analyzer (Radiometer Medical Aps, Akandevvej 21 DK-2700 Bronshøj, Denmark). To eliminate data distortion, the bone marrow pH evaluation was carried out immediately after removal from the femur, after 10-15 minutes, and then repeated every 30 minutes. The pH indicator of long bones did not change for 5 hours; it was weakly acidic (6.7-6.9). These pH values are necessary for stable vital activity of the bone marrow. It is advisable to consider these data for successful bone marrow transplantation. If the bone marrow cells get into unusual conditions, including pH, they may start to differentiate prematurely and bring no therapeutic effect. In the long term, it is necessary to investigate the behavior of bone marrow cells by creating environmental conditions with various (acidic and alkaline) pH values. By changing the bone marrow pH, it is

possible to determine the functional state of the cells depending on the acid-base balance of the bone marrow. When taking bone marrow from long bones, the bone marrow consistency was different, from liquid to dense like jelly. However, this condition did not affect the pH rate. Sometimes, blood clots entered the bone marrow during the surgery. If it was impossible to remove them, such samples were omitted in the study results because their pH readings differed from 'clean' ones. The study of the bone marrow pH requires an accumulation of indicators to identify standards and norms. Active blood reaction is an extremely important homeostatic constant of the body; it ensures redox processes, the activity of enzymes, the direction and intensity of all types of metabolism. Hence, these statements are true with respect to the pH of the bone marrow. The constancy of the acid-base status is maintained by both physicochemical (buffering systems) and physiological compensation mechanisms (lungs, kidneys, liver, other organs). This study takes into account the pH of the bone marrow in certain diseases. It can be assumed that these pH values are the norm for the bone marrow. The majority of the patients (96%) had the diagnosis of diabetes mellitus, but the bone marrow pH in other patients (they did not have the diagnosis of diabetes) was also in the range of 6.7-6.9. Thus, it can be assumed that the pH of the bone marrow does not depend on the diagnosis. The results of the study showed that the bone marrow pH does not depend on age (the age of the patients varied from 50 to 75 years). The body kept the pH in constant numbers regardless of age and disease.

4. DISCUSSIONS

Many research centers carry out studies on bone marrow and stem cells. Researchers have accumulated broad experience in the use of cellular technology for the treatment of many diseases, but there is still much to discover. Its use is significant and necessary, but further study of the bone marrow is even more important. This work is devoted to the pH of the bone marrow. How important is this topic? Constant pH of the environment creates optimal conditions for the metabolism of all body reactions. Bone marrow also requires certain pH values, namely in the range from 6.7 to 6.9. Like any cells from a tissue or organism, the bone marrow cells, after they are removed from the femur and placed in a culture medium, must have the same conditions for vital activity as they had in vivo. Creating such conditions is essential for the preservation of

cells, their long existence, proliferation, and differentiation. The environment of the cells should have necessary nutritional and hormonal factors, including a certain pH; only then the growth and survival of the cells are possible. The pH indicators required for cell multiplication have a specific range and vary depending on the type of cells. For example, the growth of diploid fibroblasts requires a pH of 7.30. It can be assumed that the weakly acidic environment of the bone marrow is necessary to keep cells in an inactive state. It is of interest to study the consistency of the bone marrow. Why is it so different? The bone marrow is sometimes tremeloid, almost watery, but sometimes it is as dense as a thick jelly. That may depend on the functional state of the bone marrow. Still, regardless of the consistency, the bone marrow pH does not go beyond the established range of 6.7-6.9. The bone marrow of the femur includes hematopoietic and mesenchymal stem cells; flat bones include mainly hematopoietic cells. There is a hypothesis that primary cells are mesenchymal cells, which differentiate into hematopoietic cells upon a change in pH. Between these two types of cells, there are intermediate, so-called pre-hematopoietic cells that complete the differentiation into hematopoietic cells in flat bones or in the blood. Why is the "niche" of bone marrow in tubular bones? Everything is reasoned in the human body, and the millennial evolution has provided places with maximal protection from the external environment. If flat bones can be perforated, for example, with a needle during sternal puncture, long bones, especially their cortical bone part, can be separated with great difficulty with a Gigli wire saw. The bone marrow of long bones is enclosed in a solid, isolated container that is difficult to open. What can our body hide in it? Only something very important for its existence. These are stem cells that, under conditions that threaten the life of the organism, create reparative mechanisms to restore damaged tissues and organs. Research works devoted to influencing on the differentiation of cells and tissues is especially relevant and important in the current situation of active biotechnology development, when the creation of individual organs become real. Modern technologies allow obtaining stem cells from many organs and tissues. Each cell culture has its own proliferative and differentiating potential. These two indicators determine the value of cells. Undoubtedly, the bone marrow cells of the femur have the highest proliferative and differentiation potential because they are the most vital cells for the body. Bone

marrow transplantations are performed across the whole world, which saves the lives of seriously ill patients. Despite the fact that bone marrow banks are becoming common, it is still difficult to find compatible bone marrow. To solve this problem, bone marrow banks should be significantly increased. It is possible through using the bone marrow from amputated limbs (tubular bones). Every year, there are many operations - amputations of limbs, which are disposed of after surgery. But the actual use of the bone marrow taken from long bones can help in solving many problems. First of all, it is necessary to continue the studies of the bone marrow of the femur; lifetime receipt of the bone marrow is a rare opportunity. The study omitted the sample obtained from a patient who had an oncological diagnosis and who already had metastases. This sample showed complete replacement of the mesenchymal stem cells from the tubular bone marrow, and the number of hematopoietic cells exceeded the norm by ten times. One sample of bone marrow from an oncological patient is surely not enough for proper statistics, but it was the only one for the 6 years of sampling. It is necessary to accumulate data and draw further conclusions. The study also did not take into account the result of the femur bone marrow of a young 18-year-old patient. This patient got into an accident and got severe limb injuries. Doctors could not save the limb and amputated it at the hip level. The tests showed that at the age of 18, the bone marrow pH was stable and equal to 6.84.

5. CONCLUSIONS

The ionic composition of the blood, including its pH, has long been studied. Blood pH values depend on the vessel type. For veins, it can be between 7.32 and 7.42; for arteries, it varies from 7.36 to 7.43. In medical practice, the states when blood pH is below 6.8 or more than 7.8 are considered dangerous to human life. Control or change of the pH can stimulate a shift in the cellular composition of the bone marrow in the desired direction, influence the differentiation of cells and their functional state. The obtained data are subject to further analysis and collection of information on the acid-base balance of the bone marrow. This study revealed the following facts:

1. The bone marrow of long and flat bones has constant pH values. However, these values are different: the pH of flat bones' marrow is similar to the blood pH, and the pH of the long bones' marrow is acidic and equal to 6.7-6.9.

2. Hematopoietic stem cells are found in both acidic and weakly alkaline environments.

3. Mesenchymal stem cells, which are the possible source of hematopoietic stem cells, are mainly found in long bones, where the environment pH is 6.7-6.9.

4. A blood acid-base and gas composition analyzer (Radiometer Medical Aps, Akandevej 21 DK-2700 Bronshøj, Denmark) can be used for determining the acid-base indicator of bone marrow. To avoid damage to the analyzer, the bone marrow was filtered through 2-4 layers of gauze to remove fatty conglomerates. All bone marrow samples were tested for cultures, and there was no microflora growth. This was done to keep the analyzer in working condition.

In some tissues and organs, a small change in the environment pH causes biological activity. This paper discusses the specifics of the acid-base balance of bone marrow in long bones. The stem cells of the bone marrow determine the differentiation of cells in different directions. Therefore, it is necessary to know the extent to which a change in pH affects the physical properties and physiological activity of the bone marrow. The study of the acid-base state of the bone marrow continues. Taking into account the development of cellular technologies, the possibility to control the stem cells differentiation and direct it to the desired result using the environment pH is timely and relevant.

6. CONFLICT OF INTERESTS

The author does not declare any conflict of interests.

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