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Research article

External use of radon and sulfide mineral waters in the treatment of experimental arthrosis

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Abstract: The article presents the results of studies of the effect of radon and sulfide mineral waters (MW) on Wistar rats with experimental arthrosis. materials and methods. Rats were randomized into 4 groups. Group 1 consisted of intact rats (control group). In the remaining three groups, a model of knee arthrosis was reproduced using dexamethasone injections. Group 2 consisted of rats with untreated pathology. Group 3 consisted of rats using radon MW procedures, and group 4 consisted of rats using sulfide MW. Results. Morphological studies have determined that using radon and sulfide MW has a curative effect on the structural and functional organization of the joint and cartilage - the manifestations of inflammation in the knee joints are significantly reduced, dystrophic manifestations disappear, and reparative processes in cartilage improve. There are signs of an improvement in the state of metabolic processes in the body of rats: the balance in the lipid peroxidation and antioxidant systems is restored, and the indicators of the processes of energy supply of transmembrane transport (according to the activity of magnesium-dependent Na+/K+-ATP-ase and Ca2+-ATP-ase) and protein exchange.Conclusion. Based on the data obtained, it was concluded that both applied MWs have a unidirectional, but somewhat different in strength, curative effect on the course of experimental arthrosis, which is due to different mechanisms of the biological action of radon and hydrogen sulfide.

Keywords: experimental arthrosis; morphological and metabolic indicators; radon; hydrogen sulfide; mineral water.

1. Introduction

Destructive-degenerative diseases of the musculoskeletal system are an essential medical problem [1, 2]. Arthritis and osteoarthrosis are considered the most common pathology of the joints, while the risk of developing this pathology increases significantly with age [3]. According to forecasts, back in 2015, it was believed that osteoarthritis would become the eighth cause of disability in men and the fourth in women soon. [4]. Today, this negative trend in the spread of arthrosis has further increased [5]. Thus, at the present time in Ukraine, diseases of the musculoskeletal system are the most widespread phenomenon among the able-bodied and socially active part of the population [6] In addition, the development of post-traumatic arthrosis in the younger part of the country's population is becoming widespread [7]. Therefore, the problem of effective treatment of such patients becomes relevant in medical, social, and economic aspects [8, 9].

Drug treatment of arthrosis primarily involves the use of corticosteroids and painkillers, which relieves debilitating pain and improves reparative processes in cartilage, but these drugs have some disadvantages (insufficient effectiveness and side effects) [10 - 12.]. The latest means and drugs of a new generation, which are introduced into the treatment, are more effective and cause fewer side effects inherent to corticosteroids, but still do not remove them completely [13, 14].

It should be noted that the etiology of arthrosis has not been fully elucidated. It is generally accepted that arthrosis and osteoarthritis are mainly the results of "wear and tear" of the articular cartilage. But according to modern concepts, arthrosis is accompanied by the loss of cartilage, not only due to mechanical stress. In the pathogenesis of arthrosis, the main links are considered to be: a violation of the vascular system of cartilage, which causes oxygen-substrate insufficiency and leads to the activation of proteolytic enzymes [15] and the development of inflammatory processes. [16]. The increased production of pro-inflammatory mediators in the cartilage and synovial tissue contributes to the destruction of the cartilage, which, in turn, increases the inflammation of the joint, forming a vicious circle that makes the pathology chronic. [17, 18]. All components of the joint are affected, leading to pathological changes in tissues and their metabolic functions. Therefore, to correct the pathological process's main links and systemic disorders, it is necessary to increase the number of prescribed medicines, which may increase in side effects. [19].

In modern medical practice, balneotherapy is widely used, devoid of the above side effects. [20 - 23]. Each MW used in balneotherapy to correct arthrosis is unique in its physical and chemical composition (features of the ionic, macrocomponent, microelement composition, the presence of gases, and biologically active substances). Despite belonging to the same balneological group or type of mineral waters (carbonic, boric, silicic, sulfide, arsenic, radon, ferrous, iodine-bromine, etc.), MW may differ in the strength of biological, physiological, and therapeutic effects [24 - 28]. In this aspect, the authors' latest work on the status of trace elements in the development of arthritis attracts attention [29].

But it is possible to assess the presence and strength of the corrective action of each of the MW only by examining its effect directly on the patient's body. Taking into account that it is impossible to carry out such research on humans from ethical standards, today in academic medicine, the top place is occupied by research using laboratory animals, namely rats. [30 - 33]. Therefore, a comparative evaluation of the effectiveness of sulfide and radon MW was carried out on rats with a model of knee joint arthrosis, which made it possible to justify using these MW in the complex rehabilitation treatment of arthrosis based on sanatorium-and-spa institutions.

The goal is to determine the influence of radon and sulfide mineral waters on the links of the pathogenesis of experimental arthrosis in rats, to justify further therapeutic use.

Materials and methods.

The experiment was carried out on 42 mature white Wistar female rats of outbred breeding with body weight from 180.0 g to 200.0 g. Experimental studies were conducted in accordance with the rules established by the Directive of the European Parliament and the Council (2010/63/EU), by the order of the Ministry of Education and Science, Youth and Sports of Ukraine No. 249 of March 1, 2012 "On Approval of the Procedure for conducting scientific experiments, experiments on animals by scientific institutions" [34]. During the experiment, the animals were in the experimental biological clinic (vivarium) of the State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine", Odesa in the conditions of free access to food and water. The animals were kept in standard laboratory conditions: photoperiod - light /darkness 12:12; air temperature - 22 ± 2 ° C; humidity - 55 ± 10%. The experimental studies were the reproduction of experimental arthrosis, the conduct of a treatment course using MW, and physiological studies carried out during an expeditionary trip with experimental animals directly to the corresponding sanatoriums (where MW wells were located). On the basis of the sanatoriums, a room for a temporary laboratory was equipped (taking into account the necessary conditions for conducting experimental studies). Upon completion of the experiment, the experimental animals, specialists, and the physiological laboratory were transported by special transport to Odessa within 8-10 hours. The conduct of experimental work was reviewed and approved by the Bioethics Commission (Protocol No. 11 dated 06.15.2018 and Protocol No. 2 dated 01.02.2019 of State Institution "Ukrainian Research Institute of Medical Rehabilitation and Resort Therapy of the Ministry of Health of Ukraine". All rats were randomized into four groups. 1st group (12 rats) consisted of intact animals, the data of which served as a control. In rats of groups 2nd, 3rd, and 4th, an experimental model of arthrosis was reproduced.

The arthrosis model was reproduced by injecting 0.1 ml of dexamethasone into the knee joint of rats daily for three days. The model was verified on the basis of daily measurements of the volume of the joint, its temperature, and morphological changes. The development of an active pathological process was registered on the seventh day from the beginning of the experiment. During the further experiment, the rats of the second group (10 pieces) did not receive treatment. Rats of the 3rd and 4th groups received baths with appropriate mineral water on the injured limb. The duration of one procedure was 20 minutes, every other day, 5 procedures per course. Rats of the 3rd group (10 pieces) received baths from the MW well No. 604-E of the "Radon" sanatorium in the city of Khmilnyk (Vinnytsia region, Ukraine). It is characterized as very weakly radon, low-mineralized calcium-sodium, weakly alkaline, cold. The concentration of radon (Rn) ranged from 484 Vq/l to 674 Vq/l, with the balneological norm for radon MW exceeding 185 Vq/l. Total mineralization was 1.81 g/l. The content of hydrocarbons was 1.0614 g/l, sulfate ions — 0.1527 g/l, chloride ions — 0.1136 g/l. The content of sodium and potassium was 0.2524 g/l, calcium – 0.1980 g/l, and magnesium – 0.0353 g/l.

Rats of the 4th group (10 pieces) received baths from the MW well No. 13D of the "Medobory" sanatorium (Konopkivka village, Ternopil region, Ukraine) characterized as medium sulfide (sulfide-hydrosulfide), weakly mineralized sulfate-hydrocarbonate calcium, weakly acid-neutral, cold. The content of hydrogen sulfide (H2S) was 76.55 mg/l, with the balneological norm for hydrogen sulfide (sulfide) mineral waters of 10.00 mg/l. Total mineralization was 0.86 g/l. The content of hydrocarbonate ions was 0.4626 g/l, sulfate ions — 0.1743 g/l, chloride ions — 0.0035 g/l. The content of sodium and potassium was 0.0094 g/l, calcium ions —0.1893 g/l, and magnesium ions 0.0174 g/l.

At the end of the experiment, changes in the pathological joint and metabolism were evaluated. The volume of the joint was determined macroscopically, and structural changes in the knee joint were determined microscopically. For microscopic studies during an autopsy, a piece of the affected joint was removed. After fixation, it was decalcified with a 5% solution of nitric acid and passed through alcohols of increasing concentration, and poured into celloidin. Histological sections were made, 7-9 μm thick, stained with hematoxylin-eosin.

The content of seromucoids, urea, creatinine, concentration of total protein and protein fractions was determined in blood serum. The state of the antioxidant system (AOS) was also studied - by catalase activity, the state of lipid peroxidation (LPO) - by the content of malondialdehyde (MDA). The state of the transmembrane energy-dependent transport system was assessed by the activity of Ca2+-ATPase and Na+/K+-ATPase.

At the end of the study, the animals were removed from the experiment by decapitation under ether anesthesia. The methodical methods and techniques used in the research were approved by order of the Ministry of Health of Ukraine No. 692 of 09/28/2009 [35, 36].

All data were processed using the statistical package Statistica 10.0 (Statsoft/Dell, Tulsa, OK, USA). The descriptive statistics of the data in tables include mean ± standard error of the mean (SEM). Significance was assessed by using the one-way ANOVA followed by t-test. Values were considered statistically significant when P value is less than 0.05.

Results

After the completion of the experiment, on the 16th day of the experiment, the rats of the 2nd group had an increase in the diameter of the knee joint by 24% compared with the 1st control group. Palpation examination of the joint revealed a rise in its temperature and pain. The rats moved around the cage without leaning on the diseased limb. At the microscopic examination, the fibrous joint capsule is edematous; fibroblasts have juicy nuclei and lymphoid infiltration. The articular space looks like an enlarged gap. Perchondrium with areas of swelling. The substance of the perichondrium is homogeneous. Cells of the surface layer with flat, elongated nuclei, unevenly spaced. Cartilage papillae are smoothed. Hyaline cartilage of articular surfaces of unequal thickness. The main substance of the cartilage is edematous in places. Chondrocytes are located throughout the mass of cartilage, with poorly stained cytoplasm and enlarged light-stained nuclei. Nests of chondrocytes have an edematous indistinct capsule. There are chondrocytes with nuclear pycnosis. In the interbeam spaces of the subcartilaginous zone of the bone, there are very dense accumulations of lymphoid elements and erythrocytes. So, we have seen structural signs of the formation and development of dystrophic and inflammatory processes in the joints.

Table 1. Diameter of the knee joint of rats after completing the course, (M ± m)

Indicators	1st group n=12	2nd group n=10	3rd group n=10	4th group n=10
The diameter of the knee joint, cm	0,79 ± 0,05	0,98 ± 0,09*	0,80 ± 0,11	0,81 ± 0,09

Note: * - significant changes in indicators compared with the 1st group (p<0.05).

The diameter of the knee joint in the treated rats did not differ from the animals of the control group (Table 1). A palpable increase in the temperature of the joint or its tenderness has not been established. Rats used the affected limb without restriction.

Microscopic studies in rats of 3rd group with an arthrosis model, which received applications with radon MW, established that the joint capsule is dense, fibroblasts with elongated dark nuclei. Lymphoid infiltration is not determined. The width of the joint space is defined as a moderate gap. The perichondrium is of the same thickness throughout; its main substance is homogeneous and dense. In the deep layers, the density of chondrocyte cells is quite high, outwardly they do not differ from the norm. Single cells enter the underlying bone, strengthening the cartilage connection with it. Hyaline cartilage throughout the articular surface of the same thickness; its surface is smooth. Chondrocytes are pale-colored and their nuclei are of moderate size and color. Nests of chondroblasts with a thin, pale colored capsule, chondroblasts themselves are pale-colored with pale enlarged nuclei. The organization of the cartilage stem layer was restored. In the bone, there are interbeam spaces with a moderate amount of lymphoid elements and lipid inclusions of various sizes. That is, dystrophic changes in the tissues of the joint are significantly reduced (approaching the norm), and manifestations of inflammation in the joint are eliminated.

Histological studies of the structure of the joint of rats of the 4th group with a model of arthrosis, which received a course of procedures with sulfide MB, established the following: the tissues of the joint capsule are dense and of a normal appearance. The joint gap is thin. The surface of the joint is smooth. The thickness of the cartilage throughout is thin; there is not much interstitial substance, it is uniform. Chondrocytes in deep layers are collected in nests. The nuclei of chondrocytes are of medium size and color density. There is a moderate amount of chondroblasts in the superficial layers, they are evenly distributed; the nuclei are small, dark. Consequently, the process of cartilage renewal improves to a certain extent and the manifestations of inflammation are eliminated.

When analyzing biochemical parameters in 2nd group rats with a model of arthrosis of the knee joints, inhibition of the activity of the antioxidant system was established, as evidenced by a significant decrease in catalase activity by 15% and a significant increase in the activity of prooxidant processes: the MDA content increased by 50% , which is a sign of LPO/AOS imbalance (Table 2). Significantly - by 42% and 19% increases the content of urea and creatinine, which indicates the inhibition of the state of the body's detoxification processes and signs of increased catabolism of nitrogen-containing compounds. A significant decrease in the content of total protein was determined - by 11%, and albumin and β-globulin - by 58% and 32%, respectively, at the same time, the content of $\alpha 1$, $\alpha 2$, γ - globulins - by 54%, 47%, and 26%. Consequently, protein metabolism is shifted towards the formation of inflammatory proteins. The level of seromucoids also increases (by 53%), which indicates the activation of inflammatory reactions in the body of rats. Negative changes in the state of energy supply of transmembrane transport have been established, namely, inhibition of the activity of Na+/K+-ATPase enzymes by 53% and Ca2+-ATPase by 23%.

Table 2. Biochemical indicators of rats after completing the course, (M ± m)

Indicators	1st group n=12	2nd group n=10	3rd group n=10	4th group n=10
MDA, nmol/(min·mg)	5,94 ± 0,21	8,94 ± 0,78*	6,19± 0,43	6,17 ± 0,27
Catalase, %	76,71 ± 1,52	65,11 ± 1,68*	73,19 ± 4,03	73,80 ± 2,29
Creatinin, mkmol/l	47,80 ± 0,63	57,11 ± 1,70*	51,04 ± 1,78	50,68 ± 1,21
Urea, mmol/l	2,80 ± 0,27	3,99 ± 0,26*	6,37 ± 0,36*	3,07 ± 0,14
Ca^{2+}- ATPuse, mg P/g tissue	9,11 ± 0,29	7,12 ± 0,15*	7,88 ± 0,31*	9,19 ± 0,44
Na^{+}/K^{+}- ATPuse, mg P/g tissue	6,40 ± 0,62	2,98 ± 0,19*	3,44 ± 0,10*	4,21 ± 0,47*
Total protein, g / l	68,70 ± 2,74	61,50 ± 0,93*	60,26 ± 0,84*	66,44 ± 4,02
Albumin, g/l	25,80 ± 1,9	11,00 ± 0,32*	24,23 ± 1,13	33,91 ± 2,95*
α-1 Globulin, g/l	8,28 ± 1,06	12,80 ± 0,80*	3,14 ± 0,45*	6,16 ± 1,00
α-2 Globulin,g/l	10,70 ± 0,92	15,75 ± 0,75*	12,15 ± 0,56	10,26 ± 0,58
β- Globulin, g/l	11,80 ± 0,79	8,00 ± 0,71*	14,78 ± 0,63*	6,52 ± 0,98*
γ- Globulin, g/l	11,10 ± 0,73	14,00 ± 0,41*	5,70 ± 0,35*	9,60 ± 1,01
Seromucoids, U/L	0,200 ± 0,009	0,307 ± 0,007*	0,184 ± 0,006	0,220 ± 0,008

Note: * - significant changes in indicators of 2nd, 3rd and 4th groups compared with the 1st group ($p<0.05$).

Upon completion of the course of procedures with radon MW, in rats of the 3rd group, the restoration of the content of MDA and catalase activity in the blood to the level of indicators of the 1st control group was established, which indicates the restoration of balance in the LPO/AOS system. There is also a recovery of the level of seromucoids, as evidenced by the absence of significant changes ($p>0.05$) compared with the 1st control group. The creatinine content recovered, but the urea content remained at the level of rats with untreated pathology and significantly differed from the corresponding control indicator ($p<0.05$). Positive changes were determined in terms of indicators characterizing the state of energy supply of transmembrane transport, which is manifested by the restoration of the activity of Ca2+-ATPase ($p<0.05$) and Na+/K+-ATPase. To a lesser extent, this concerns the state of protein metabolism: total protein content remains low (at the level of the indicator of 2nd group). The content of protein fractions - $\alpha 1$-globulins and γ-globulins also remain low. At the same time, there is a normalization of the content of albumin and protein fractions - $\alpha 2$-globulin and β-globulin ($p> 0.05$), which together indicates a limitation of the inflammatory process.

When analyzing the results of a course of procedures with sulfide MW on the biochemical parameters of rats of the 4th group, more pronounced positive changes were established than in rats of the 3rd group (Table 2). The balance in the LPO/AOS system was restored (catalase activity and MDA content did not differ from the control group). The content of creatinine, urea, and the level of seromucoids also recovered. At the same time, normalization of the content of total protein, albumin, and most protein fractions is observed. The state of energy supply of transmembrane transport is almost completely normalized, as evidenced by the restoration of Ca^{2+}-ATPase activity ($p<0.05$) and significant signs of normalization of Na^+/K^+-ATPase activity.

Discussion.

Our previous studies have established positive changes in the structure of cartilage, metabolism and urinary system in rats with a model of arthrosis with a course of external use of natural remedies (NR): brine with a high content of magnesium and bromine, sodium chloride brine, and glauconite clay. [37, 38]. The nature of these changes was unidirectional, but each of the applied NR had its own features of corrective influence.

The results of the studies presented in this paper also indicate that the use of MW with a high content of radon or hydrogen sulfide positively affected the course of the pathological process in experimental arthrosis of the knee joint. But there was a significant difference between them. If in the structural characteristics of the articular cartilage this difference consisted in a greater restoration of the cartilage structure with the use of radon MW, then a more significant positive effect on the state of metabolism was observed with the use of sulfide MW, as evidenced by changes in the reactions of energy supply of transmembrane transport and the intensity of protein metabolism.

The difference in the action of the MW used in the study is connected, in our opinion, with the peculiarities of the mechanisms of the biological activity of biologically active components, namely, hydrogen sulfide and radon. [39, 22]. Hydrogen sulfide is characterized by a vasodilating effect and a direct effect on the activity of redox enzymes. [40]. Hydrogen sulfide in concentrations of 25 - 150 mg/l has a great regenerative capacity, which increases the antioxidant activity of tissues, primarily the liver, reduces redox potential, optimizes bioenergetic processes, has cytoprotective and anti-inflammatory effects [41, 42]. Radon MW is characterized by the formation of compounds in the body caused by ionizing alpha radiation. These compounds most likely play the role of regulatory molecules that change the activity of the body's functional systems [22, 43].

In this aspect, the concept of hormesis and its role in hydrothermal treatment methods should be mentioned [44]. The biological mechanisms of the restorative action during the external use of MW in the rehabilitation of certain pathologies have not yet been fully studied. But it is known that when conducting balneotherapy, neuroendocrine and immunological reactions are involved, causing anti-inflammatory, analgesic, antioxidant, chondroprotective, and anabolic effects in the aggregate of neuroendocrine-immune regulation in various pathological conditions. Hormesis can play a leading role in the biological mechanisms of the corrective action of sulfide and radon MW.

Conclusions

1. Procedures with the use of radon MW lead to a significant reduction in dystrophic damage to the joint and eliminate the manifestations of inflammation in it. This is accompanied by positive changes in the studied parameters of metabolism: the balance in the LPO/AOS system is restored (restoration of catalase activity and the content of malondialdehyde), the content of MDA is normalized, the content of seromucoids and creatinine is restored (but the content of urea remains increased), Ca^{2+}-ATPase approaches the norm and Na^+ / K^+-ATP-ases, signs of restoration of the blood protein spectrum are determined.

2. The use of procedures with sulfide MW leads to the elimination of inflammation and stimulation of the process of cartilage restoration, accompanied by the normalization of metabolic processes: the balance of the LPO / AOS system, the state of the body's detoxification processes and the activity of enzymes for energy supply of transmembrane transport and the intensity of protein metabolism are restored.

The data obtained by the authors allow us to conclude that radon MW and sulfide MW have curative properties that are effective when applied externally against the background of the development of experimental arthrosis. Based on the conclusions, a conclusion was made on the recommendation to conduct further clinical trials of radon MW based on the Radon sanatorium and sulfide MW based on the Medobory sanatorium in order to be able to use them in balneological practice..

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