



## **Dynamics of morphogenesis under the influence of melatonin and mexidol in the context of induced carcinogenesis**

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*Introduction.* The article deals with the effect of mexidol and melatonin in the separate and combined use, on the process of carcinogenesis of soft tissue induced by benzo(a)pyrene at mice.

*Materials and Methods.* 120 mice were divided into four groups: three experimental groups of 30 mice, 30 mice are the control group on carcinogen. Animals from the all four groups were applied with benzo(a)pyrene at a dose of 0.2 ml per mouse, 2 times a week.

*Results.* It is found that the histological examination has showed a pattern similar to malignant fibrous histiocytoma. In the application of melatonin, the number of animals with induced tumors was 57 %, under the influence of mexidol frequency of formation of tumor nodes was 50 %. With combined use of mexidol and melatonin, the number of mice which had progressing tumor nodes was 36.6 %.

*Discussion and Conclusions.* While induced tumor growth of soft tissue, we can identify the various stages of the morphogenesis of malignancy.

**Keywords:** induced carcinogenesis, mesenchymal tumors, morphogenesis, melatonin, mexidol

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## **Динамика морфогенеза под влиянием мелатонина и мексидола в условиях индуцированного канцерогенеза**

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*Введение.* Статья исследует влияние мексидола и мелатонина при отдельном и комбинированном применении на процесс канцерогенеза мягких тканей мышей, индуцированного бензо(а)пиреном.

*Материалы и методы.* 120 мышей были разделены на 4 группы: 3 экспериментальные (по 30 особей) и контрольная группа, индуцированная канцерогеном, (30 мышей). Животным из всех четырех групп вводили бензо(а)пирен в дозировке 0,2 мл, 2 раза в неделю.

*Результаты исследования.* Установлено, что при гистологическом исследовании наблюдалась картина, сходная со злокачественной фиброзной гистиоцитомой. При применении мелатонина количество особей с индуцированными опухолями составляло 57 %, под влиянием мексидола частота образования опухолевых узлов составляла 50 %. При комбинированном использовании мексидола и мелатонина доля особей с прогрессирующими опухолевыми узлами достигала 36,6 %.

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*Обсуждение и заключения.* При индуцированном опухолевом росте мягких тканей мы можем идентифицировать различные стадии морфогенеза злокачественных образований.

**Ключевые слова:** индуцированный канцерогенез, мезенхимальная опухоль, морфогенез, мелатонин, мексидол

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## Introduction

The relevance of research. It is well known that malignant neoplasms occupy a leading place in the structure of morbidity and mortality of the population. According to the WHO for 2012, the incidence of malignant neoplasms in the Russian Federation was 480.2 per 100,000 population, and the death rate was 288.3 per 100,000 population [1]. At present, malignant neoplasias in Russia occupy the second place among all causes of death of the population – 14.9 % [2].

As is known, carcinogenesis is a multi-stage process during which a normal stem cell is transformed into an atypical one. Typically, the stages of tumor growth are presented in the form of pre-tumor hyperplasia, dysplasia, cancer “in place”, invasive growth and metastasis. The process of tumor transformation is accompanied by metabolic disturbances, changes in many body systems, including nervous, immune and endocrine systems [3]. Presence of premalignant lesions, processes of hyperplasia and cell proliferation in the initial stage of neoplasia are one of the fundamental properties of various stages of tumor growth. Further invasive growth of neoplasms seems to be a complex morphogenetic program in which proliferative processes are integrated into such as migration, natural selection, induction of cell division [4]. Any stage of tumor growth is potentially reversible, which encourages the search for oncostatic drugs that can affect the different stages of carcinogenesis.

Currently, one of the widely accepted theories of tumor growth is the free radical theory, which explains the morphogen-

esis of many pathological processes, such as cardiovascular pathology, immunodeficiency states, brain function disorders, cataracts, neoplasms and some others [5].

According to this theory, during the process of morphogenesis of tumor growth, on the one hand, the processes of lipid peroxidation are accelerated and activated, and on the other hand, there is a decrease in the reserves of the body's own antioxidant system [3].

The peroxide-oxygen concept of tumor growth postulates the need to create inside the cells hyperoxic conditions for their further malignancy. The relatively high level of free radicals that arise at the same time, in fact, creates conditions conducive to tumor transformation in the cell.

Much attention is paid to the search for new effective oncostatic drugs, taking into account the staged nature of the neoplasia processes, especially under conditions of experimental tumor growth.

One of the representatives of synthetic antioxidants is mexidol – a 3-hydroxypyridine derivative. The drug has a wide range of biological effects: it increases activity towards oxygen-dependent pathological processes, inhibits the formation of lipid peroxidation products, and also the formation of superoxide and hydroxyl radicals. At the heart of their antioxidant effect is the ability to suppress the rates of oxidative stress, the synthesis of free radicals at the initiation stage, thereby reducing the possibility of developing neoplasia [6]. In particular, in the literature available to us, information on the oncostatic effect of antioxidants in conditions of induced tumor growth is not fully presented and contradictory.

It is known that melatonin also has antioxidant properties [7]. Melatonin is the main hormone, which is synthesized mainly at night, in the tissue of the pineal gland.

The main functions of melatonin in the body are antioxidant, immunomodulating and biorhythmic [8].

The results of many years and many studies is the fact that the most significant physiological functions of melatonin are: suppression of cell proliferation and antitumor effect in experimental neoplasia, stimulation of metabolic processes; Inhibitory effect on the metabolism of pigment substances, sedative effect on the central nervous system, controlling the effect with seasonal and circadian rhythms. In addition, one of the most important functions of melatonin is the active absorption of most endogenous free radicals [9].

For a more complete understanding of the effect of melatonin and the mexidol antioxidant on the processes of tumor growth, experimental studies on a significant number of experimental models using substances of diverse organ specificity and a different mechanism of action are needed to reveal the mechanisms and patterns of their possible suppressive effect on tumor growth processes [10].

### Materials and Methods

120 mice were divided into four groups: three experimental groups of 30 mice, 30 mice are the control group on carcinogen. All experimental animals 4 groups were introduced by benzopyrene one time. Benzopyrene is the carcinogen of the chemical origin. Benzopyrene was introduced at a dose of 2 mg per mouse in a volume of 0.1 ml, subcutaneously in the area of the lumbar region, previously carcinogen was dissolved in sterile olive oil. The next day after the introduction of benzopyrene, experimental animals of the 1<sup>st</sup> experimental group received melatonin in a dose of 2 mg/L.

For the experiment, there was daily prepared solution of melatonin ex tem-

pore. In order to prepare a solution, the drug was dissolved in 2–3 drops of 96 % ethanol and then was diluted with tap water and brought to the desired concentration. Experimental animals of the 2nd experimental group during the whole day received mexidol in a dose of 0.1 mg/kg together with drinking water. The mice of the 3<sup>rd</sup> group were simultaneously given melatonin at a dose of 2 mg/l and mexidol in a dose of 0.1 mg/kg, during the day along with drinking water. The control group was submitted to the mice of the 4<sup>th</sup> group who received drinking water without the addition of the studied drugs.

All experimental animals, and also mice that have died and withdrawn from the experiment at the end of the period, were subjected to autopsy and macroscopic examination. In autopsy were investigated by details - the skin, induced neoplasms of the skin and tissue of internal organs. It was counted the total number of tumor nodes on the skin and in the soft tissues, and also identified their options and dimensions. Tissue of all induced tumors were excised and then fixed in 10 % formalin solution. All the pieces were exposed to standard histopathological treatment and were drenched in paraffin. Subsequently, histological sections with thickness of 5–7 mkm were stained with hematoxylin and eosin, fuchsin van gieson and studied microscopically.

Staining by Van-Gieson is used to identify collagen of connective tissue fibers.

### Results

After 6 months the mice that were exposed by benzopyrene, in 73 % of cases (22 out of 30 animals), there were soft tissue tumors. Thus, in the course of the experiment it was possible to trace the stages of morphogenesis of tumor growth. Since 126 days, since the introduction of benzopyrene in experimental animals described the group, on the skin of the back, at the injection of the carcinogen, were detected tumor nodules rounded-oval, with clear boundaries, grayish-white color, with a rough sur-



face, dense texture, displace on palpation. With increasing tumor sites in size, they became tightly fixed to the underlying

tissues. On tumor-like formations, in all cases, hair was missing and the skin was bruised (fig.1)



Fig. 1 Malignant tumor of soft tissues in the experimental mice of the control group

Р и с. 1. Злокачественные опухоли мягких тканей у мышей контрольной группы

After a latent period of tumor development, during 121–140 days, in the control group most of tumors were tumor nodules

with a large diameter. We also often marked phenomena of collapse, hemorrhage and destruction of the tumor tissue (fig. 2).



Fig. 2. Malignant tumor of soft tissues in the experimental mice of the control group

Р и с. 2. Злокачественные опухоли мягких тканей у мышей контрольной группы

In the experimental group with the use of mexidol macroscopically, the tissue induced tu-

mors only in a few cases we observed the process of ulceration of the tumor tissue (fig. 3).



Fig. 3. Malignant soft tissue tumors from the mice that received mexidol

Р и с. 3. Злокачественные опухоли мягких тканей у мышей, получавших мексидол

In the group with the use of melatonin in one case revealed the development of

secondary changes in the form of the ulcer (fig. 4).



Fig. 4. Malignant soft tissue tumors from the mice that received melatonin

Р и с. 4. Злокачественные опухоли мягких тканей у мышей, получавших мелатонин





When the joint introduction of mexidol and melatonin in experimental animals, in all

cases, on the surface of tumor sites were: hair, skin integrity was not compromised (fig. 5).

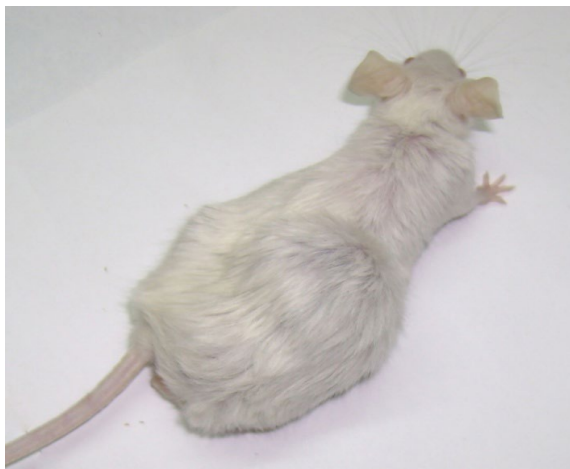


Fig. 5. Malignant soft tissue tumors from the mice that received melatonin and mexidol

Р и с. 5. Злокачественные опухоли мягких тканей у мышей, получавших мелатонин и мексидол

At the macroscopic study of induced tumor nodes, the dead mice of the control group, the tumors were firmly adherent with epidermis and surrounding tissue. Almost all tumors had foci of hemorrhage and necrosis. In mice of the experimental group also had tumors that had an expansive growth form, quite clear, the capsule, and in relation to the surrounding tissues squeezed them, pushed away, but not ingrown. Skin tumor in these nodes quite easily separated.

Microscopically, the introduction of benzpyrene subcutaneously in mice was diagnosed with a tumor of the soft tissues fibrohistiocytoma origin, the type of malignant fibrous histiocytoma.

Histologically, the tumor is built of fibroblasts, myofibroblasts, oval or rounded histiocytes. In places the tumor cells develop into short bundles, forming a typical moiré structure (fig. 6).

In tumor nodes in the control group, were microscopically identified two forms of malignant fibrous histiocytoma: superficial and deep. At a superficial der-

mal localization of fibrous histiocytoma is more pronounced cellular polymorphism, with the presence of stroma, inflammatory infiltrates, and secondary changes such as hemorrhage and necrosis (fig. 7).

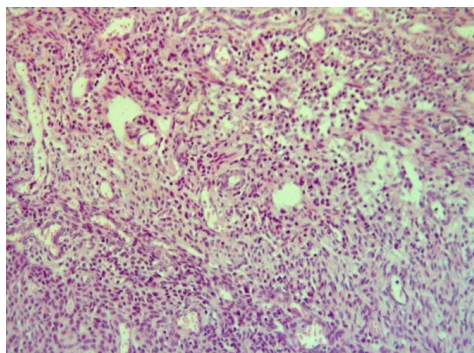


Fig. 6. Malignant tumor of soft tissue fibrohistiocytoma origin in the mice of the control group. The formation of moiré structures. Hematoxylin and eosin, x 200

Р и с. 6. Злокачественная опухоль мягких тканей фиброгистиоцитомного происхождения у мышей контрольной группы. Образование муаровых структур. Гематоксилин и эозин, x 200

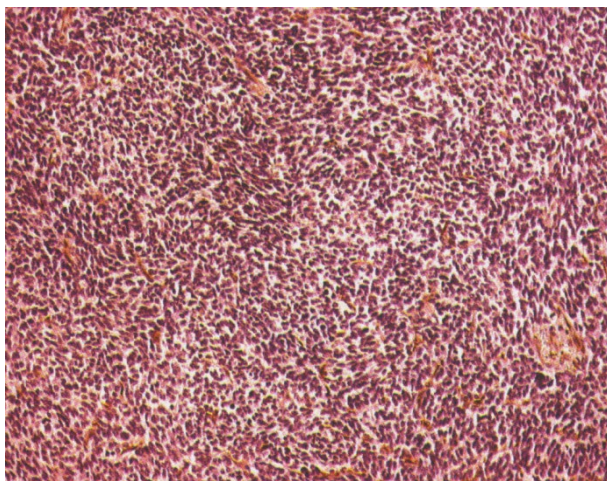


Fig. 7. Development of secondary processes in soft-tissue tumors in animals of the control group. Massive areas of hemorrhages in the stroma of the tumor tissue. A hematoxylin and eosin, x 200

Р и с. 7. Развитие вторичных процессов в опухолях мягких тканей у животных контрольной группы. Массивные участки кровоизлияний в строме опухолевой ткани. Гематоксилин и эозин, x 200

With the deep form of a malignant fibrous histiocytoma prevailed myofibroblastic component. In the tumor were also identified hemangiopericytoma

structure, multi-core osteoclastogenic, foam cells, cell Tutone (fig. 8) also were foci of myxomatosis and hyalinosis stroma (fig. 9).

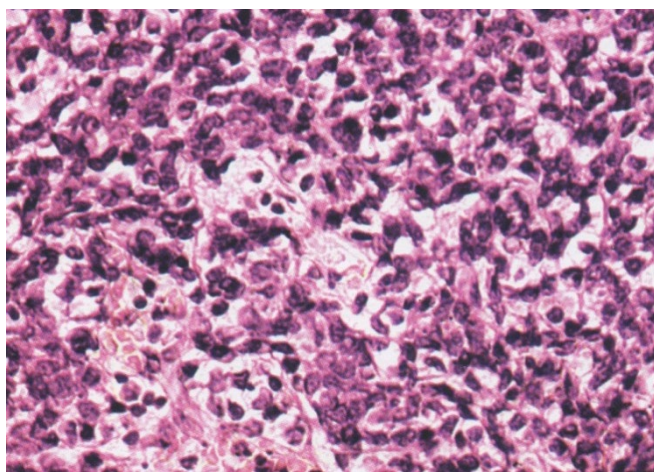


Fig. 8. The deep form of a malignant fibrous histiocytoma. Dominated by a pronounced cellular irregularities in the form of polymorphism and anaplasia of the tumor cells. A hematoxylin and eosin, x 200

Р и с. 8. Глубокая форма злокачественной фиброзной гистиоцитомы. Доминирована ярко выраженными клеточными нарушениями в виде полиморфизма и анаплазии опухолевых клеток. Гематоксилин и эозин, x 200



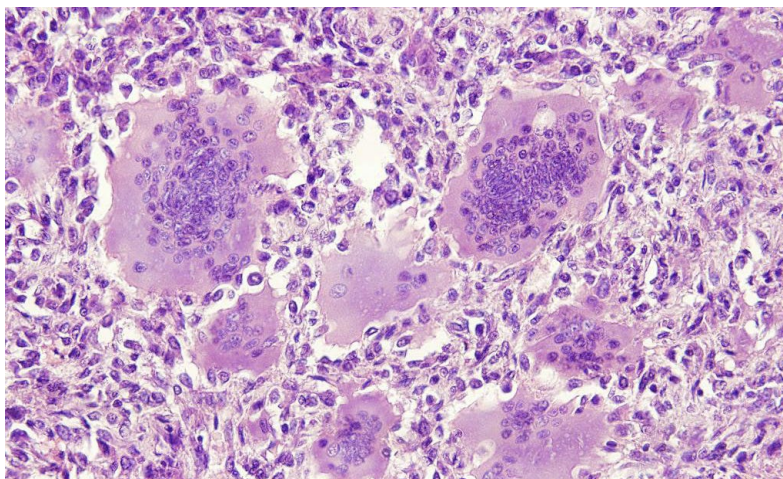


Fig. 9. The deep form of a malignant tumor of the soft tissues fibrohistiocytoma origin. Foci of hyalinosis stroma. A hematoxylin and eosin, x 200

Р и с. 9. Глубокая форма злокачественной опухоли мягких тканей фиброгистиоцитомного происхождения. Очаги гиалиноза стромы. Гематоксиллин и эозин, x 200

Were often revealed angiomatous form of malignant histiocytoma, which microscopically is characterized by three main matching component. First, it is cystic cavities with hemorrhagic content from the solid to the centers of red blood cells organizes hematoma (fig. 10); second, solid clusters of oval and elongated cells resembling

gistiitarnaya (fig. 11), and, finally, the presence of foci of reactive inflammatory infiltrates containing lymphocytes, plasma cells, sometimes foam cells and deposits of hemosiderin. On the periphery nodes among the inflammatory infiltrate were often discovered reactive lymphoid follicles with centers of reproduction.

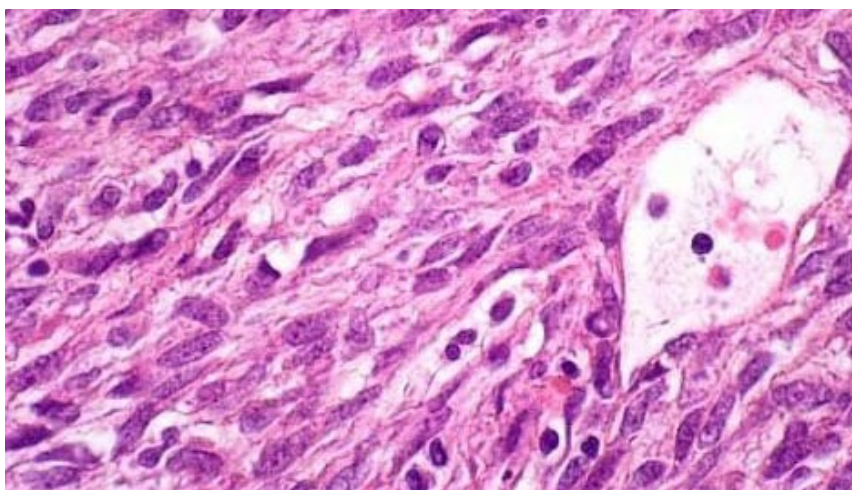


Fig. 10. Malignant fibrous histiocytoma. Angiomatous form. The formation of cystic cavities. A hematoxylin and eosin, x 400

Р и с. 10 Злокачественная фиброзная гистиоцитома. Ангиоматозная форма. Формирование кистозных полостей. Гематоксиллин и эозин, x 400



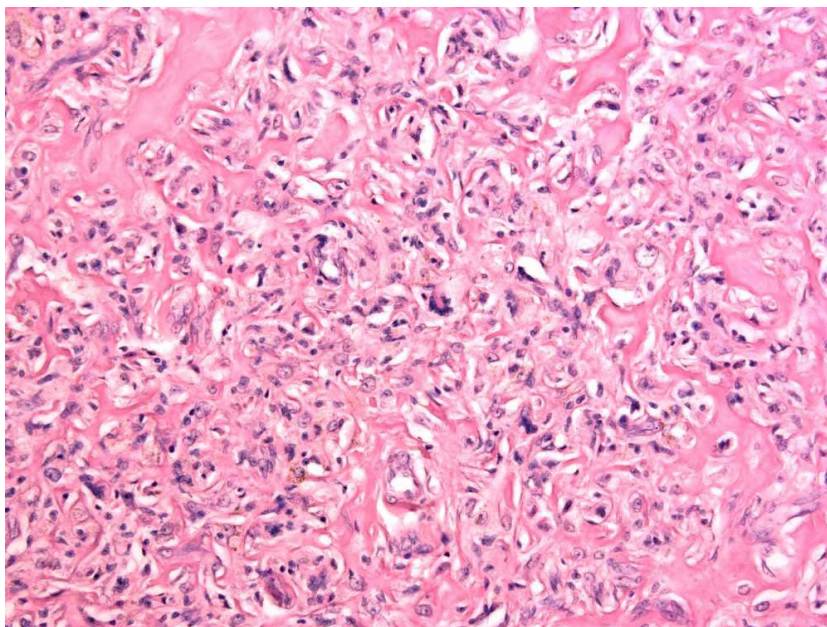


Fig. 11. Malignant fibrous histiocytoma. Angiomatous form. Proliferation of oval or elongated cells that looks like histiocytes. A hematoxylin and eosin, x 200

Р и с. 11. Злокачественная фиброзная гистиоцитома. Ангиоматозная форма. Проплиферация овальных или продолговатых элементов, выглядящих как гистиоцит. Гематоксилин и эозин, x 200

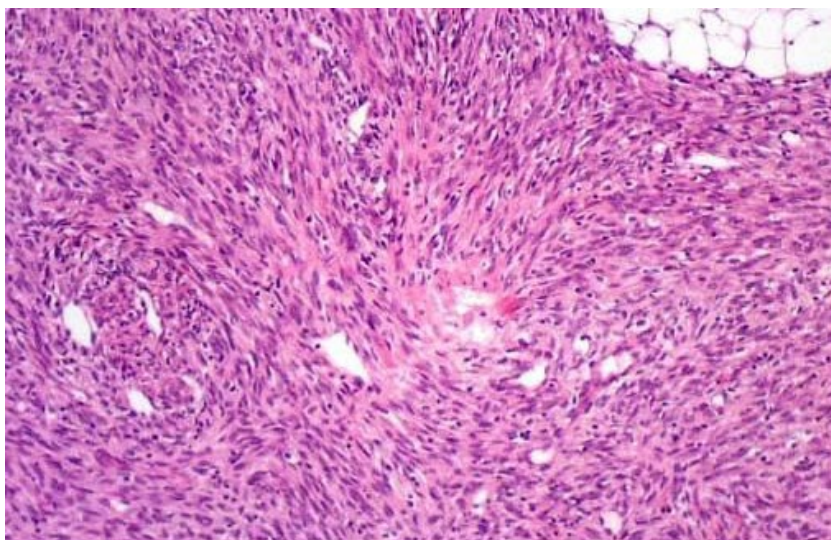


Fig. 12. Malignant fibrous histiocytoma of combined application of melatonin and mexidol. Fibroblastoid cellular elements. A hematoxylin and eosin, x 200

Р и с. 12. Злокачественная фиброзная гистиоцитома в результате комбинированного применения мелатонина и мексидола. Фибробластоидные клеточные элементы. Гематоксилин и эозин, x 200



Microscopic structure of tumor tissue, on the background of combined application of melatonin and mexidol was characteristic polymorphic cellular composition, in particular, the formation of fusiform cells, the presence of fibroblastoid and histopathologic elements (fig. 12). In some cases, we identified cells con-

taining lipids, large multinucleated macrophages type cells Tutone and type of osteoclasts. Was also found isolated pockets lymphomacrophagal interstitial infiltration and foci of the connective-tissue substitution (fig. 13). Areas of myxomatosis and mucosis of stroma was not detected.

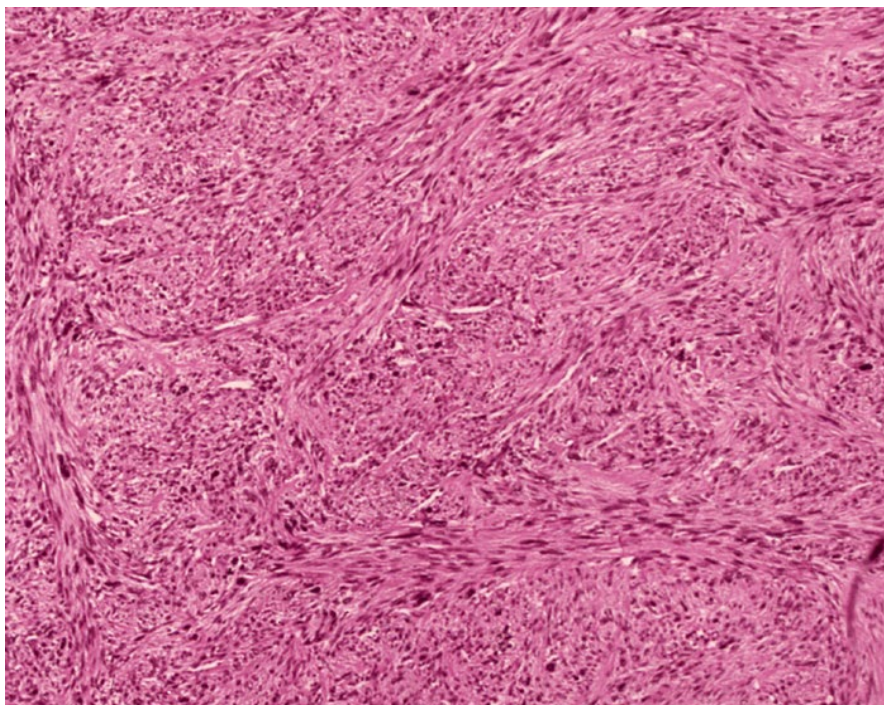


Fig. 13. Malignant fibrous histiocytoma of combined application of melatonin and mexidol. Extensive foci of fibrosis. A hematoxylin and eosin, x 200

Р и с. 13. Злокачественная фиброзная гистиоцитома комбинированного применения мелатонина и мексидола. Обширные очаги фиброза. Гематоксилин и эозин, x 200

Cells dominated by a pronounced polymorphism, a change in the relationship between the nucleus and the cytoplasm, the chaotic distribution of chromatin. A distinctive feature is the presence of mitoses in the cells of the tumors. In the stromal part of the tumor tissue were identified as lymphocytes, foam cells, hemosiderin, reactive macrophages.

Depending on the prevalence in tumor tissue cellular and fibrous structures were diagnosed with different pathological variants of malignant histiocytoma. Was often characteristic (fibrous) forms of soft-tissue malignant tumor fibrohistiocytoma origin submitted by cellular elements in the form histopathologic cells (fig. 14). Specific, that spindle-shaped tumor cells formed a characteristic “moire” of education.



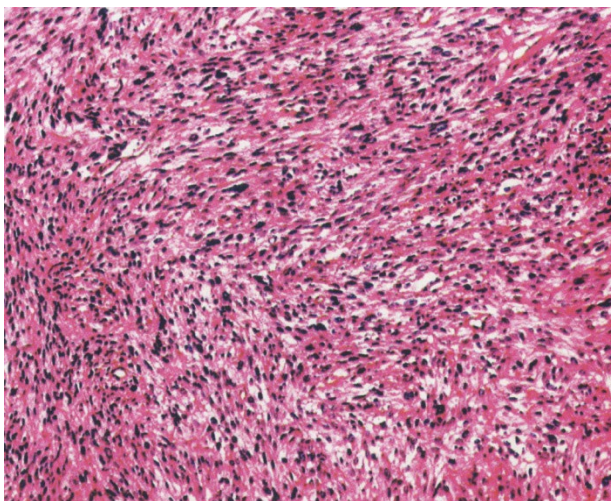


Fig. 14. Malignant fibrous histiocytoma of combined application of melatonin and mexidol. Fibroblastoid and histiocyte cell shape. A hematoxylin and eosin, x 200

Р и с. 14. Злокачественная фиброзная гистиоцитома в результате комбинированного применения мелатонина и мексидола. Фибробластоидная форма клеток и форма в виде листьев папоротника (гистиоцитерис). Гематоксилин и эозин, x 200

In some cases, tumor tissue has revealed a large number of giant multinu-

cleated osteoclasts, with the presence of nuclei of irregular configuration (fig. 15).

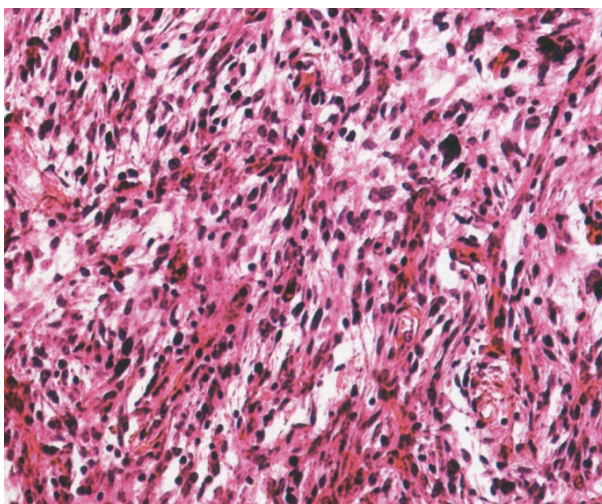


Fig. 15. Malignant tumor fibrohistiocytoma of the animal of combined application of melatonin and mexidol. Large multinucleated cell type of osteoclasts with diverse forms nuclei. A hematoxylin and eosin, x 200

Р и с. 15. Злокачественная фиброгистоцитомная опухоль животного в результате комбинированного применения мелатонина и мексидола. Большой многоядерный тип клеток с различными формами ядер остеокластов. Гематоксилин и эозин, x 200



Thus, on the background of mexidol and melatonin in tumor sites that were induced by benzopyrene in experimental animals, formed the characteristic (fibrous) forms of malignant histiocytoma presented fusiform cell shapes with cell irregularities, and polymorphism. However, in the stroma of the tumor tissue was not identified pockets of mucus and myxomatosis, and areas of necrosis and hemorrhages were found in isolated cases. These morphologic signs are a manifestation of secondary changes in the tumor tissue. Reduction or absence of such secondary changes shows, in our opinion, about the possible slowdown of tumor progression.

## Discussion and Conclusions

1. When induced tumor growth of soft tissue identify the various stages of the morphogenesis of malignancy: expansive growing tumor nodules, tumors with invasive growth and neoplasia with fuzzy boundaries, extensive lesions are ulcerated with the presence of secondary changes, necrosis and hemorrhages.

2. The combined use of melatonin and mexidol has the greatest onkotic effect that is manifested by positive dynamics of pathological changes: the absence of foci of mucus and myxomatosis in the stroma of the tumor tissue, and the presence of isolated areas of necrosis and hemorrhage.

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*Contribution of the co-authors:*

N. A. Plotnikova: scientific guidance, analysis and revision of the final text; O. N. Deryabina: writing the draft, analysis of literature data; S. B. Radynova: reviewing the relevant literature, word processing.

*All authors have read and approved the final version of the manuscript.*

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