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Assessment of the risk of osteosarcoma recurrence in canine long bone replants

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Abstract
This paper presents data on the risk of recurrence of osteosarcoma in the tissue exposed to radiation and freezed bone replants, used for replacing of bone defects in organ-preserving surgical operations on canine long bones. The best option of reconstructive surgery during replacement of bone graft in patients with bone tumors is obtaining of graft directly during the operation from the resected bone after pretreatment. One way of anticancer treatment of replanted bone is its single-phase exposition to radiation *in vitro* at the dose of 200 Gray (Gy) after preliminary freezing in liquid nitrogen vapor. Despite the ongoing treatment, there is a certain risk of recurrence of osteosarcoma in tissues of the replant.

Keywords: Dog; morphology; osteosarcoma; replant; osteogenesis; incorporation; relapse.

Introduction

Tumors of the musculoskeletal system are among the most topical problems of clinical oncology [1-4]. The incidence of osteosarcoma in dogs is 80-90% of all bone tumors [5,6]. In recent years effective chemotherapy regimens were developed, which certainly allow increased survival in patients. However surgical treatment is the main way to influence the primary tumor, which aims not only to remove the affected bone but also to preserve the limb [5,7-12]. In an attempt to treat bone tumors, various intact operations and various methods of bone defects replacement were offered: autografts, allografts, and endoprosthesis [2,13]. Each of these methods has its own advantages and disadvantages. One option for reconstructive surgery for tumors of the long bones is the replacement of the bone defect by a replant obtained from the resected bone after pretreatment directly during the operation [5]. In this regard, the seek for previously unstudied conditions of bone repair using single-phase bone replants exposition to radiation *in vitro* at the dose of 200 Gray (Gy) with preliminary freezing and definition of the risk of recurrence of the tumor seems to be relevant.

The objective of this study is to determine the risk of osteosarcoma recurrence in the tissue of frozen and exposed radiation replant.

Methods

The work was performed at the Department of Clinical Veterinary Medicine, Peoples' Friendship University of Russia, in the veterinary clinic "Biocontrol", and diagnostic laboratory "Neovet". 35 bones replant of dogs undergoing comprehensive treatment for osteosarcoma were studied. The average age of animals was 7 years.

Animals underwent segmental resection of the affected bone and skeletization, then the extracted bone fragment was frozen in the Dewar vessel in liquid nitrogen vapor at 195°C for 10 minutes followed by its exposition to radiation using "Agate-R" device at the dose of 200 Gy. Then all soft tissues were removed from the replant followed by the curettage of all contents of the medullary canal and fastened to the parent bone by plates or Ilizarov apparatus. After death or euthanasia of animals, the remote replant was sawed through lengthwise of the bone, getting a plate with a thickness of about 0.5 cm from the center of the bone. Portions of bone were cut out and labeled. The preparation was placed in 10% formalin solution, fixed, decalcified, treated by the conventional technique, and embedded in paraffin. Then the preparation was sectioned with microtome, deparaffinized, and stained with hematoxylin, eosin, and Van Gieson's picrofuchsin [14]. Stained sections were placed

under the cover glass using Canada balsam and subjected to microscopy.

The period of observation was conventionally divided into stages in order to determine the risk of recurrence, depending on the length of replant incorporation: up to 30 days, from 30 to 120 days, from 120 to 240 days, and over 240 days with the number of investigated replants 7, 15, 8, and 5 respectively.

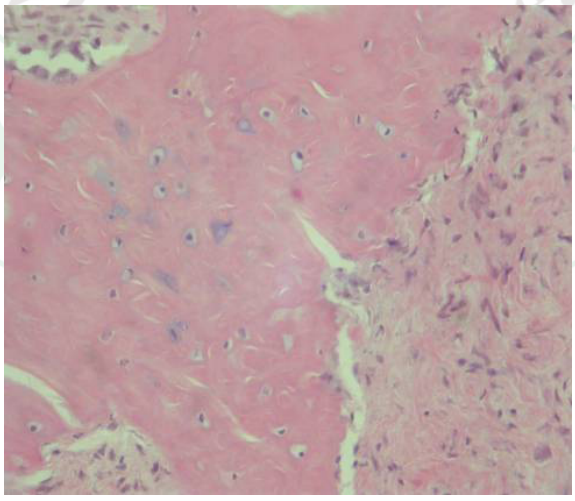
In our studies we have shown that in the period of up to 30 days the main changes detected at this stage of restructuring were accompanied by necrobiotic changes of preexisting bone and tumor. We also revealed lacunar resorption of bone trabeculae with the growth of fibrous tissue in interplate spaces. It's difficult to visualize structure of osteosarcoma and preexisting bone due to necrobiotic changes. On the contrary it's easy to determine tissue not subjected to necrobiosis. Bone tissue surrounding replant shows signs of reactive osteogenesis. Signs of lacunar resorption were revealed. A relapse of osteosarcoma wasn't revealed in any case.

The period from 30 to 120 days characterized by the fact that the formation of callus is the main process that occurs at this stage. Lacunar resorption is completed, bone lacunes are filled with fibrous tissue. Osteoclasts are in the phase of attenuation of vital activity, as evidenced by their isolation and rounded contours. Fibrous tissue is formed. Necrobiosis processes are completed. The recurrence of osteosarcoma is observed in 20% of patients. Small fragments of tumor cells formed by atypical osteoblast-like polymorphic cells are revealed on the histograms of replants. Signs of indirect atypical osteogenesis are revealed in cells (Figure 1).

The studies have shown that fibrosis is the main process occurring in replants in the period from 120 to 240 days. Massive fields of fibrous tissue are visualized in many preparations, while periosteum is often fibrosed. Reactive changes in bone are a common complication observed in 25% of cases. Relapsed osteosarcoma at this term developed in 50% of cases. Tumor field with signs of atypical osteogenesis was revealed on histological examination. Pathological bone osteoid is presented by uneven bone beams of different maturity and calcification. Cellular component of the tumor cells is constituted by atypical polymorphic type osteoblasts and giant multinucleated osteoclast-like cells (Figure 2).

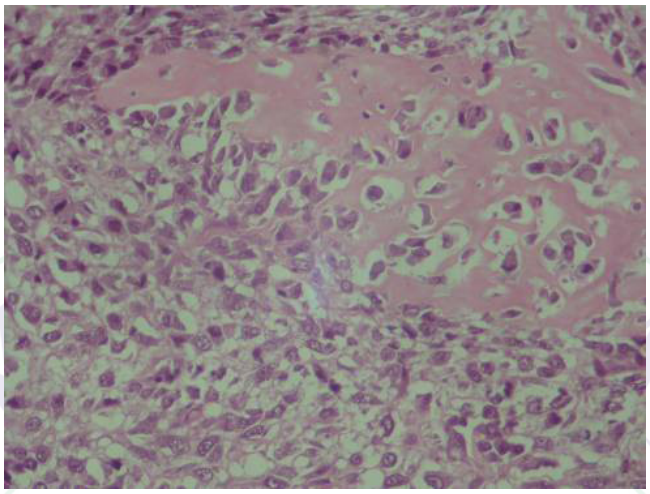
In the period of more than 240 days, any changes revealed in replant are presented by various complications, including leukocytic infiltration of replant tissue, reactive osteogenesis, and metallosis. Relapse tumor was observed in 80% of cases. On the histograms there were identified separately located atypical cells with marked signs of indirect atypical osteogenesis. Pathological osteoid is represented as fields of oxyphilous substance (Figure 3).

Figure 1: Microphotography. Recurrence of osteosarcoma in replant tissue in the period from 30 to 120 days.



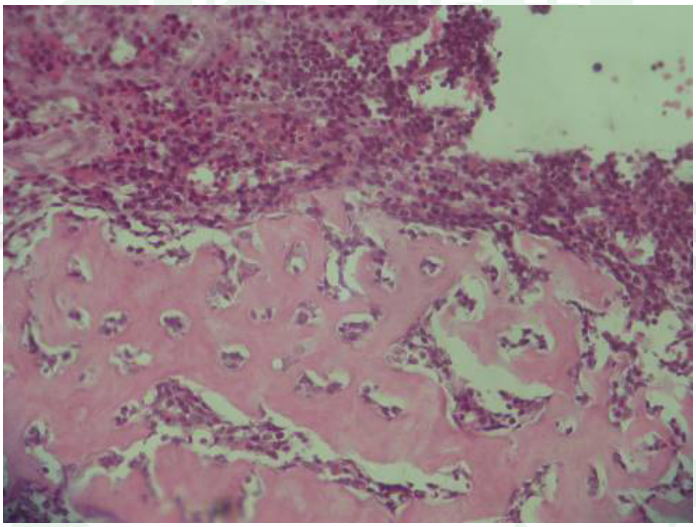
Small fragments of tumor cells formed by atypical osteoblast-like polymorphic cells with signs of indirect atypical osteogenesis. Staining by hematoxylin and eosin. Magnification: eyepiece lens 10X and objective lens 20X.

Figure 2: Microphotography. Recurrence of osteosarcoma in replant tissue in the period from 120 to 240 days.



Tumor fields with atypical symptoms of osteogenesis. Pathological bone osteoid is presented by bone beams of different maturity and calcification. Tumor cells are presented by atypical osteoblast-like polymorphic cells and giant multinucleated osteoclast-like cells. Staining by hematoxylin and eosin. Magnification: eyepiece lens 10X and objective lens 40X.

Figure 3: Microphotography. Recurrence of osteosarcoma in replant tissue in the period of more than 240 days.



Discrete atypical cells with marked signs of indirect atypical osteogenesis. Pathological osteoid is represented as fields of oxyphilous substance. Staining by hematoxylin and eosin. Magnification: eyepiece lens 10X and objective lens 20X.

Results

On assessing the risk of osteosarcoma recurrence at different stages of replants restructuring, it was found that the recurrence develops in the period from 30 to 120 days in 20% of cases (n = 3 from 15), in the period from 120 to 240 days in 50% of cases (n = 4 of 8), and in the period of more than 240 days in 80% of cases (n = 4 of 5). The first term of detection of recurrence is between 30 and 120 days. The longer replant rebuilds, the higher the risk of recurrence of osteosarcoma in its tissues (Table 1).

Thus, the first osteosarcoma recurrence in replanted tissues can be detected in the period of 30 days of observation, while in the period of more than 240 days, 80% of replants contain recurrent tumor tissue. Relapse in the

Table 1: Dependence of the risk of osteosarcoma recurrence on the duration of replant incorporation.

Duration of replant incorporation (days)	Number of tested animals	Number of animals with osteosarcoma recurrence in replant	% osteosarcoma recurrence in replant
Up to 30	7	0	0
From 30 to 120	15	3	20
From 120 to 240	8	4	50
More than 240	5	4	80

later stages of replant incorporation depends on the effectiveness of the specific therapy, and may indicate a lack of response to the treatment. Relapse of the tumor in the early period may be caused by inadequate preoperative therapy or violation of anticancer treatment of replant.

Conclusion

Osteosarcoma recurrence in the period of replant incorporation of more than 240 days was 80%, in the period from 120 to 240 days was 50%, and in the period from 30 to 120 days was 20%.

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