The treatment of tibia fracture by mesenchymal stem cells (Clinical case)

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Abstract. There are many complications related to the treatment of small breed dogs’ fractures. Patient complaints: The owners contacted us after a four-year-old female toy terrier fell from a height of 2 meters, which resulted in a double tibia fracture. The first osteosynthesis procedure was effective only while healing of one fracture. The two subsequent procedures of osteosynthesis were ineffective during bone fusion in the area of the second fracture. The diagnosis was as the idiopathic disturbance of tibia fracture healing. Interventions: two months after the last osteosynthesis procedure and the absence of all signs of bone fusion, in order to avoid limb amputation, it was decided to inject allogeneic mesenchymal stem cells into the fracture area. Results: The slight signs of bone fusion were observed after three weeks of allogeneic mesenchymal stem cells injections into the area of the fracture. The X-ray examination after one and half months of cell therapy demonstrated the complete fusion of fracture. Conclusion: Considering the efficacy of healing of compound fractures or healing in breeds with impaired osteogenesis, the treatment with the application of mesenchymal stem cells is more optimal.

1. Introduction

The reparative bone regeneration or reparative osteogenesis is a physiological process of bone rehabilitation after damage. Disturbances of reparative osteogenesis are an important problem in decorative dogs. Frequent internal fractures are often accompanied by a disorder of tissue vascularization and retardation or complete lack of the process of reparative osteogenesis in the fracture zone. Various factors as a genetically determined disturbance of osteogenesis, wrong choice of bone stabilization method and its technically wrong implementation, a large distance between fracture fragments, systemic diseases, infections and etc., can be the causes [1]. At the moment the main tool of fractures’ treatment is surgery which often does not lead to the expected results. In this regard, veterinary specialists and a group of scientists are constantly in search of effective methods of treatment of this group of pathology. All these factors induce a constant interest in the application of regenerative therapy in vivo [2] based on the mesenchymal stem cells. Due to the whole complex of unique properties as direct differentiation into bone tissue cells (osteoblasts), the ability to excrete biologically active substances affecting the suppression of the inflammatory process at the area of the lesion, the implication of regional haematological and mesenchymal stem cells to it, which also has an impact on the formation of osteoblasts and a new chain of blood vessels. As a result, these factors lead to the regeneration of...
bone tissue [3, 4, 5, 6, 7]. Nevertheless, the unqualified use of cell therapy approaches leads to a large amount of conflicting data. Therefore, we have a great interest in the clinical application of MSCs in the case of disturbances of reparative regeneration of bone tissue.

1.1. Description of the clinical case
The owners of four years old toy terrier (doe) have approached us. The initial appointment to the veterinary occurred as a result of animal fall from a two-meter height on 28.01.20. The diagnosis was made as a compound double fracture of the right femur right pelvic limb. On 02.01.2020 surgery was performed for reposition of right tibia and fixation of the fracture with titanium wire according to the generally accepted technique. On 03.04.2020 during the daily examination was detected hygroma in the area of surgical intervention. Wherein, the overall health condition of the animal was deemed stable and satisfactory. The support function of the right hind limb was not restored. A medical puncture with fluid evacuation (lymphoextravasate) was performed. On 03/27/2020, the X-ray examination revealed insufficient consolidation of the double fracture. Thereby, the veterinary specialist decided to preserve the metal structure for a longer period. During planned examinations and examinations by 03/05/2020, a concomitant diagnosis as idiopathic disturbance of tibia fracture fusion was made. A repeated surgery was conducted for replacing the titanium wire with a titanium plate for improving bone stabilization. This was confirmed by repeated surgery on 05/06/2020 for replacing the spoke with a titanium plate, according to standard technical approaches. The surgical intervention has revealed osteodystrophy of bone tissue at the area of the fracture. In the area of a simple fracture, the presence of callus formation was recorded. On 06/03/2020 the owners again asked for veterinary help. It is known from the anamnesis that the animal has already begun to use the hind right limb, but suddenly began limping. The X-ray examination has noticed the presence of a lesion on the titanium plate and as a result an additional trauma of fracture. From the anamnesis, it was revealed that the reason for metal structure breaking is not known. The further replacement of the titanium plate was scheduled and carried out on 06/06/2020. At that time the broken plate and screws were removed and replaced with a angled titanium plate. During surgery, the pseudarthrosis was excised, the edges of the bone wound were renewed, and the missing part of the bone was replaced with cancellous substance from the ilium. As a result of numerous surgical interventions, the length of the sore paw was reduced about one centimetre. There was a complete absence of signs of fracture healing during re-examinations for two months. Also, significant muscle atrophy in the right hind paw was observed. Consequently, veterinary specialists and employees of our laboratory made decisions on the use of regenerative cell therapy based on allogeneic MSCs. The procedure was scheduled for 08/19/2020. Allogeneic cell therapy was chosen due to the patient's idiopathic disturbances of bone restoration.

2. Materials and methods
MSCs were derived from adipose tissue (AT), which was taken in a veterinary clinic from a clinically healthy female mongrel dog during ovariohysterectomy with the prior informed consent of the owners. The animal was selected according to the presence of all routine vaccinations and treatments against ecto- and endoparasites, for deriving AT. The donor's age at the time of AT collection was 14 months. AT was obtained from the uterine ligaments by a veterinary specialist in compliance with all the rules of asepsis and antiseptics. The volume of the donor AT sample was 5 grams. For isolation MSCs and study their properties and characteristics, we used our earlier developed technique [4]. The cultivation of MSCs was carried out without fetal bovine serum (FBS) for six hours before their injection. Then the cells were removed in a standard way [4] and transported in physiological solution in a volume of 300 μl for 2.5 hours before the cell therapy procedure.
Figure 1. (a) – Radiograph before insertion of MSCs into the fracture site; (b) – X-ray image three weeks after MSC injection; (c) – radiograph 1.5 months after the introduction of MSCs; (d) – preparation for the introduction of MSCs at the fracture site.

The insertion of MSCs into the fracture zone, in the amount of $2 \times 10^6$ cells, was carried out under the conditions of a veterinary clinic, in compliance with all aseptic and antiseptic conditions, preliminarily injecting a 21G needle (figure 1 (b)) into the fracture site, under the control of an X-ray apparatus. In this case, the dog was under a muscle relaxant. The cell preparation was injected slowly at 300 μl / 30 sec. In order to objectively evaluate the result of cell therapy, the pharmacological drugs were not prescribed for a patient while the whole period of observation. For 48 hours, the animal has remained in the veterinary clinic under observation. Evaluation of the efficacy of cell therapy based on MSCs was carried out by periodic X-ray examinations, biochemical, and general clinical blood examinations. The X-ray examination was performed before MSC injection into the fracture site, (figure 1(a)), three weeks,
and one and a half months after MSC injection. In this case, the assessment was carried out using direct dorsal and left lateral radiographs. The observation period for the patient, at the time of manuscript writing was 3 months. The patient will be still monitored in the future.

3. Results
It is known from the anamnesis that a day after the injection of MSCs, the pain symptoms disappeared and the dog began to move on the sore paw. Clinical examination during the whole period of observation showed the restoration of the functional abilities of the hind right paw. The volume and physiological capacity of muscle tissue have been restored by 50%. The data of biochemical and general clinical blood tests, during the observation period, did not reveal any rejection in the physiological state of the animal. The X-ray results three weeks after the injection of MSCs into the fracture area showed the onset of callus formation figure 1(c). The X-ray conducted 1.5 months after MSC injection showed complete fusion of the bone tissue at the fracture area (figure 1(d)).

4. Discussion
We have made decisions to use allogeneic MSCs after studying the anamnesis of a patient with a concomitant diagnosis of idiopathic disturbances of bone restoration of the right hind paw. In decorative dogs, genetically determined disorders of osteogenesis processes are often observed and their own MSCs are usually involved in this process. Therefore, such dogs often have an urgent need for allogeneic MSCs. Thus, the introduction of allogeneic MSCs into the fracture area, in an amount of $2 \times 10^6$ cells, during the day led to the pain relief in the dog, which has been confirmed by numerous scientific data indicating the properties of MSCs. The anti-inflammatory properties of MSCs allowed our patient to stand up on a sore paw. The results of X-ray examinations already three weeks after the introduction of MSCs showed the callus formation, which indicates the regenerative abilities of MSCs in bone tissue. The efficacy of the regenerative properties of bone tissue MSCs is confirmed by X-ray examination carried out after 1.5 months. The results demonstrated a complete reduction of the bone tissue. During the whole period of patient observation, there were not observed rejection from physiological norms that was confirmed by general clinical and biochemical blood tests. Thus, the application of allogeneic MSCs in the treatment of pathologies of bone tissue fusion had a positive result in our patient, which also confirms the immune safety of allogeneic cell therapy based on MSCs.

5. Conclusion
Disturbance of bone tissue regeneration is a significant problem not only in veterinary medicine but also in medicine. The application of cell medicine is currently considered one of the most effective approaches in the treatment of this pathology. of the effectiveness of the healing of compound refraction or healing of fractures in breeds with disturbed osteogenesis, we consider the application of MSCs optimal from the point of view of the efficacy of the healing of compound refraction or healing of fractures in breeds with disturbed osteogenesis. Further work in the field of cellular regenerative therapy in the future will optimize methodological and technical approaches.

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