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Short communication

Therapeutic effect of adipose-derived mesenchymal stem cell injection in horses suffering from bone spavin

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Abstract

In this article we demonstrate the efficiency of autologous transplantations of adipose-derived mesenchymal stem cells for equine bone spavin treatment. Horses qualified to the study were divided into three groups: (i) research – treated with intra-articular injections of autologous stem cells, (ii) comparison treated with steroid drugs and (iii) control – untreated. All animals underwent comprehensive clinical examination before and after treatment. Our research confirms the long-term beneficial influence resulting from stem cell therapy in horse bone spavin treatment, in contrast to routine steroid usage.

Key words: equine bone spavin, adipose stem cells, lameness

Introduction

Equine bone spavin is one of the best described locomotive system disorders in horses. Bone spavin affects mainly adult horses (8-10 years old), however juvenile spavin, affecting young horses (less than 3 years old), is also distinguished. The conservative treatment is based on intraarticular injection of corticosteroids, which leads to a remarkable decrease in inflammation symptoms and significant improvement in clinical status (Bellamy et al. 2009). However, treatment of bone spavin with corticosteroids is only extemporary, decreasing pain and inflammation. Moreover, application of steroids in sport horses is severely limited due to anti-doping tests (Ho et al. 2006). Therefore, we attempted to treat horse bone spavin with autologous, adipose-derived mesenchymal stem cells (Ad-MSC). Such cells reside in subcutaneous adipose tissue, which is the most available and commonly used in veterinary medicine (Marycz et al. 2012). We conclude that adipose stem cell injections could also be an efficient procedure for treatment of animal locomotive system disorders such as bone spavin.

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Materials and Methods

We used 16 horses (from 8 to 14 years old) with early symptoms of horse bone spavin, detected by clinical signs and diagnostic anesthesia. The research group consisted of 10 horses, the comparison group 3, and the control group 3. In the research group, intraarticular injections of autologous stem cells were given. The comparison group animals received 1 ml of betamethasone, applied intraarticularly, while horses from the control group were only limited in movement. Tissue collection was carried out at the base of the research horses' tails and was followed by immediate isolation of cells using the method described previously (Grzesiak et al. 2011). In order to obtain a sufficient number of cells for the injections, AdMSCs were cultivated *in vitro*. The cell yield was suspended in sterile normal saline for infusion, at a concentration of 5x106 cells/mL. Stem cell solutions (1 mL) were injected directly into the damaged joint. Clinical examinations were made at day 0 and after 30, 60, 90 and 180 days after injection. Assessments of synovial fluid were done at day 0, 90 and 180 after stem cell application. Scintigraphic tests were made in 6 horses (2 individuals from each group), at day 0, 90 and 180, with 99mTechnetium-labeled phosphates for detection of the inflammatory process.

Results and Discussion

At day 0, all horses were severely lame. In the comparison group significant improvement and no signs of lameness were noticed after 30 and 60 days. In the control group the degree of lameness did not change. In the research group, no changes were noticed after 30 days, but after 60 days the degree of lameness decreased greatly. On day 90, none of the horses from the research and comparison groups showed symptoms of lameness, while in the control group they symptoms were easily observed. Clinical examination after 180 days showed a slight decrease in lameness in the control group, an increase in lameness in the comparison group and no signs of lameness in the research group. Synovial fluid analysis on day 0 from all individuals showed signs of inflammatory process. After 90 days, both in the comparison and in the research group, the results were significantly improved; the synovial fluid showed no signs of inflammation. The number of neutrophils was lower in the control group after 90 days, which may indicate conversion from acute inflammation to chronic. After 180 days, the values obtained for the research group were within the normal range; however, the leukocyte number was still decreasing. Compared to the results obtained after 90 days, for the comparison group, several trends were noticed. First of all we observed a significant increase in inflammatory cell number and a high level of total protein. Moreover, the synovial fluid of the comparison horses had low viscosity and changed color and turbidity, suggesting inflammation progression. After 180 days no changes were detected in the control group.

Although after 90 days of the study, no differences between groups were noticed, results obtained from physical evaluation and synovial fluid evaluation indicate good outcomes and suggest the positive, long-term effect of stem cell therapy. In the scintigraphic examination on day 0, a high concentration of radionuclide in the tarsal joint was detected in all horses, indicating ongoing inflammation. After 90 days of treatment, both in the research and in the comparison group, no signs of joint inflammation were noticed, whereas in the control group inflammation process was prominent. After 180 days, inflammatory response was still observed in the control group. Moreover, scintigraphic imaging showed that in the comparison group the inflammatory process emerged again, while in the research group no signs of inflammation were observed.

Modern treatment with intra-articular injections of autologous, adipose-derived mesenchymal stem cells meets contemporary requirements for horse bone spavin therapy. This method is safe, provides a long-term anti-inflammatory effect, influences the improvement of joint mechanics and does not cause any dangerous adverse effects. However, the application procedure, including precise qualification of cell quality and quantity, or even an optimal form of implantation, still needs further refinements and thus should be elaborated in further research.

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