The Volume of Mesenchymal Stem Cells in the Peripheral Blood in Horses is Associated with Physical Stress

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Introduction

Peripheral blood is one of many possible sources of mesenchymal stem cells (MSCs). It is the easiest source from which to obtain them. However, the number of MSCs in peripheral blood is naturaly very low. Our objective was to determine whether physical exercise increases the amount of precursor MSCs in the peripheral blood of horses.

Materials and methods

In our study we used five warmblooded sports horses. Peripheral blood was collected from the *vena jugularis*. Peripheral blood was collected from five horses in three different phases. The first phase was at rest (the horses were not exercised work for three days, peripheral blood was collected in the morning in the stables). The second phase was two hours after exercise and the third phase was 20 hours after exercise. The exercise was one hour trotting and galloping with a rider. The collection of peripheral blood was 10 ml in a syringe with heparin. The peripheral blood was transported to the laboratory within one hour. In the laboratory the peripheral blood was separated. The mononuclear cells (MNCs) were separated from the peripheral blood immediately after collection with the help of Histopaque. Afterwards the mononuclear cells were cultured in A-MEM 89%, FBS 10%, PenStrep 1% (37°C, 5% CO_2). The number, viability, and CD markers (CD44, CD90, CD105) of the MSCs were determined using Bürker´s chamber and flow cytometry (FACS). A change of cultivation medium was performed after 3-4 days. During the three weeks of cultivation the morphology of the mesenchymal stem cells (MSCs) was controlled. After three weeks of cultivation the MSCs were harvested using 0.05-0.25% trypsin-EDTA and the total number, expression of CD markers CD44, CD90, CD105 (performed on the FACS) and viability analysed.

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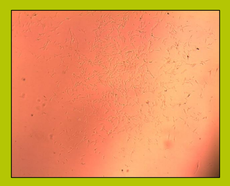
Day 5



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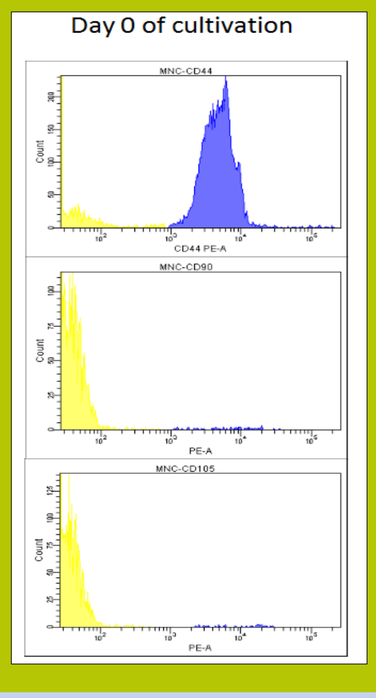

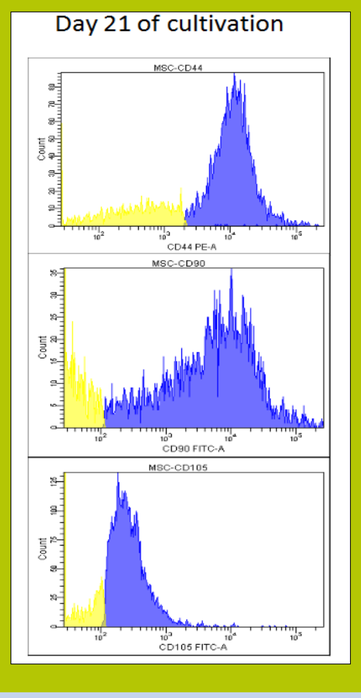

Number, CD markers and viability of MSCs in different phases

	Name of horse	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
The first phase	Z	0.8 x 10^6	97.9	49.2	0.0	98.9
	S	0	88.6	4.5	3.3	95.8
	K	0	86.8	4.4	2.2	94.1
	M	0	90.6	7.0	2.5	95.5
	J	0	92.2	4.7	5.1	96.3
The second phase	Name of horse	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
	Z	0	95.4	8.0	4.8	90.4
	S	0	96.9	9.7	4.1	90.9
	K	1.2 x 10^6	98.0	36.4	1.5	92.6
	M	0.7 x 10^6	82.3	88.1	0.0	77.7
	J	0	86.0	7.8	5.6	51.5
The third phase	Name of horse	Number of MSCs	CD44/%	CD90/%	CD105/%	Viability/%
	Z	0	61.1	10.7	4.0	39.1
	S	1.4 x 10^6	95.6	66.7	1.5	78.7
	K	1.6 x 10^6	93.0	69.6	0.1	85.5
	M	1.9 x 10^6	94.5	36.0	2.9	72.1
	J	0.8 x 10^6	96.8	67.5	0.0	84.0

Results

<u>The first phase (at rest):</u>

The MSCs were isolated *in vitro* in only one horse. This was about 10 days after the diagnosis of a hoof abscess. Fibroblasts were isolated from two horses. In the remaining two horses MSCs did not adhere at all.

<u>The second phase (2h after exercise):</u>

MSCs were isolated *in vitro* in the two horses in the previous group who were negative for MSC cultivation. Fibroblasts were isolated from one horse. MSCs did not adhere to the horse that was positive in the first phase.

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<u>The third phase (20h after exercise):</u>

MSCs were isolated *in vitro* in the four horses. MSCs did not adhere to the horse that was positive in the first phase.

Conclusions

From our work, it is obvious that physical stress has an effect on the migration of MSCs from the place of origin (niche) into the peripheral blood. It is a result of physiological tissue regeneration. For the horse (Z) that had a hoof abscess, we isolated enough MSCs in the peripheral blood even without physical exercise. After healing the hoof abscess no MSCs in phase 2 or 3 were isolated.

In our study is evident that physical stress and inflammation are factors for the migration MSCs to peripheral blood. Our preliminary data suggest, that it may be possible to collect MSCs from the peripheral blood of a horse under a physiological condition.