Physical exercise enhances angiogenesis during bone defect healing in mice

INTRODUCTION:

Already in the 18th century John Hunter reported the important role of blood vessels during bone formation (1). In the meantime, numerous studies have impressively demonstrated the importance of angiogenesis for fracture healing and bone regeneration (2). However, there is only limited information on whether physical exercise stimulates angiogenesis during bone repair, although angiogenesis has been shown to be affected by exercise during regeneration processes in a variety of other tissues (3). The aim of the present study was to investigate the impact of physical exercise on angiogenesis during the healing of a cranial bone defect in mice.

METHODS:

We used two groups of mice to analyze angiogenesis during bone healing. Mice of the first group were housed in cages supplied with running wheels (exercise group; n=7), while those of the second group were kept in standard cages without running wheels (controls; n=7). The running distance of each animal was calculated by a digital rev counter. Using a cranial bone window model, we evaluated angiogenesis during bone healing by intravital fluorescence microscopy (IVM) at days 0, 3, 6, 9, 12, 15, 18, and 21 (Figure 1). Additionally, we performed histomorphometric analyses at days 3, 6, 9, and 15 to characterize the type and time course of bone repair.

All data are given as means ± standard error of the mean (SEM). U-Test (Mann-Whitney) was used to compare IVM results of the two study groups. In addition, we performed correlation analyses (Spearman) between results of the IVM and the daily running distance in the exercise group.

The study was approved by the local governmental animal care committee and was conducted in accordance with the national legislation on protection of animals and the NIH Guidelines for the Care and Use of Laboratory Animals.

RESULTS:

Mice of the exercise group run a mean distance of 3.6km/d. IVM showed an accelerated decrease of bone defect area in the exercise group when compared to the control group (Figure 2). This was associated with a significantly higher blood vessel diameter in animals undergoing exercise at days 9 and 12 (Figure 2) and a significant correlation between running distance and blood vessel density at day 9 (r=0.74).

Histomorphometry showed a typical pattern of intramembranous bone repair. In both groups, osseous bridging of the defect was observed at day 9 (Figure 1). This was associated with the formation of a neo-periosteum, which covered the new woven bone and contained a dense network of newly formed blood vessels. At day 15 the major fraction of fibrous tissue was replaced by bone undergoing extensive remodeling.

DISCUSSION:

Data of the IVM showed an acceleration of bone defect healing under exercise associated with a significantly higher blood vessel diameter. In addition, we found a positive correlation between the daily running distance and vascular density at day 9. These results indicate that exercise stimulates angiogenesis and bone healing during intramembranous bone repair.

Previous studies in long bones have shown that exercise affects bone repair through an alteration of the mechanobiology within the fracture gap (4). Results of the present study demonstrate that physical exercise is affecting bone repair also independently of mechanical factors. This finding is in accordance with results of studies investigating the effect of exercise on angiogenesis during regeneration of other tissues (3).

It has to be considered that we evaluated by IVM the surface of the calvarian bone defect. Histological analyses showed the formation of a neo-periosteum, which contained those blood vessels, that were visible by IVM. It is well accepted that periosteal blood supply is an important prerequisite determining the outcome of bone repair. Therefore, we feel that the evaluation of the microcirculation in the periosteum and not in deeper parts of the bone is rather an interesting aspect than a limitation of the presented cranial window model.

REFERENCES:

3. Bloor CM. Angiogenesis 2005

Figures:

Figure 1: (A) IVM of the bone defect (dotted line) at day 9 (representative control animal). At this time point, a dense network of newly formed blood vessels including numerous blood vessel sprouts is detectable, which covers not only the defect, but the calvarium of the complete bone chamber including the surrounding bone surface. (B) Histology shows a typical pattern of intramembranous bone repair. At day 9 we found extensive periosteal bone formation bridging the defect (arrow), while only some remaining fibrous tissue (ft) was detectable in the center of the defect. The newly formed bone (wb) was covered by a connective tissue membrane demonstrating the characteristics of periosteum (p).

Figure 2: Analyses of defect area, blood vessel density, and blood vessel diameter by IVM at days 0, 3, 6, 9, 12, 15, 18, and 21 in animals undergoing exercise (triangles) and controls (rectangles). All data are given as means ± standard error of the mean (SEM). *p<0.05 versus controls (U-Test).