Bioenergetics Measured by In Vivo ^{31}P MRSI in hind limb muscles correlates with functional performance before and after stem cell treatment in a mouse model of peripheral vascular disease

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Background and significance:

Peripheral vascular disease, characterized by atherosclerotic obstruction of blood supply resulting in skeletal muscle necrosis, accounts for a significant mortality and morbidity worldwide. Several therapeutic approaches, including delivery of angiogenic growth factors or vascular stem cells have been proposed for restoration of blood flow to the affected muscles and are currently being tested in clinical trials. One of the major challenges of such clinical trials is to adequately evaluate the efficacy and functional benefits of the applied therapy. Ideally, the efficacy scoring should be based on objective criteria and should be monitored continuously for a long follow-up period and therefore monitoring should be possible in a non-invasive way. Our goal was to investigate whether non-invasive magnetic resonance spectroscopy (MRS) combined with MRI could be used as a reliable and objective read-out for the functional benefits of stem cell therapy.

Methods:

Animal preparation: We used a mouse model of hind limb ischemia in which blood supply to the lower limb muscles was artificially interrupted by ligation of the left and right deep femoral artery. We first performed experiments on sham-operated animals as well as vehicle injected animals in order to establish the time-frame in which the muscle energy status changes. For subsequent evaluation of stem cell therapy, animals were randomized into three different treatment groups. The first group (N=4) received intramuscular (i.m.) injections of vehicle (PBS; *i.e.* phosphate buffered saline). The second group (N=2) received i.m. delivery of 1 x 10^6 red blood cell-depleted bone marrow (BMCs) cells and the third group (N=3) received the same amount of multipotent adult progenitor cells (MAPCs). The two cell populations of BMCs and MAPCs have been shown to contribute to blood vessels in animal models of angiogenesis [2-4]. Injections were performed immediately after ligation and only in the left limb at two different locations: the upper limb (adductor muscle) and the lower limb (gastrocnemius muscle). Functional improvement of limb muscle was assessed by a swim endurance test 9 days after surgery at which time MRS/MRI recordings were performed of the entire left hind limb in order to score the bioenergetic status and measure the pH of the limb muscles. Mice were sacrificed immediately after MRS/MRI study for histologic evaluation of cell engraftment.

MRS Experiments: *In vivo* ^{31}P MRS experiments were performed at the 9.4T horizontal animal magnet (Magnex Scientific, Abingdon, U.K.) interfaced with Unity INOVA console (Varian Inc., Palo Alto, CA). A multinuclear RF coil probe consisting of a solenoid ^{31}P coil and a saddle-shape ^{1}H coil was used. One-dimensional Fourier series window spectroscopic imaging method [1] was used to acquire multiple *in vivo* ^{31}P spectra from upper limb to lower limb. The total acquisition time for obtaining one MRSI data set was 6.25 minutes. The metabolite ratios of PCr/P_i and PCr/γ-ATP were determined and used to monitor the high phosphate energy status in the upper and lower limbs.

Results:

We first established the normal energetic status of hind limb muscles from sham-operated mice that did not undergo femoral artery ligation. The MRS/MRI study on ischemic limbs from PBS-injected mice 1 day after ligation revealed severely impaired muscle energetics and acidification compared to sham-operated mice. While muscle pH returned to normal by 9 days after ligation, PCr/P_i and PCr/γ-ATP ratios were still significantly lower than normal. The latter parameters further improved at 14 days after ligation. In accordance with the impaired muscle energetics, the swimming capacity of PBS-injected mice 9 days after ligation was significantly reduced to 25 ± 6 % of their normal swimming performance and spontaneously improved to 58 ± 15 % at 14 days after ligation. Furthermore, changes in the energy status were more prominent in the gastrocnemius muscle, in agreement with previous documentation that in this model, it is mainly the gastrocnemic muscle that is affected by ischemia [2-4]. Treatment with BMCs or MAPCs significantly improved PCr/P_i and PCr/γ-ATP ratios in the upper and – more prominently – in the lower limb muscle measured at 9 days after the ligation, an improvement that correlated with a significantly increased swimming performance (65 ± 9 % for BMCs with PCr/P_i = 17.5 and PCr/γ-ATP = 3.1 for lower limb muscle; 59 ± 13 % for MAPCs with PCr/P_i = 13.0 and PCr/γ-ATP = 3.2) compared to the PBS group (25 ± 6 % with PCr/P_i = 11.3 and PCr/γ-ATP = 2.5). Furthermore, the outcomes from MRS/MRI measurements were significantly better only in those animals that had adequate intra-muscular engraftment of stem cells, but not in one animal that showed an aberrant engraftment of cells outside the muscle.

Conclusions:

The bioenergetic changes measured noninvasively by *in vivo* ^{31}P MRS in ischemic hind limb muscles spatially correlated with the ischemic status of the muscle, temporally correlated with a functional performance test and successful stem cell engraftment. They adequately reflected functional improvement upon stem cell transplantation. Therefore, the non-invasive MRS/MRI technique could be useful in clinical trials to continuously monitor the effect of stem cell or other treatments in patients with peripheral vascular disease.

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