**EFFECT OF NITRIC OXIDE DONOR SNAC ON THE MICROCIRCULATION OF DENERVATED SKELETAL MUSCLE DURING REPERFUSION**

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**Introduction:** The protective effect of exogenous nitric oxide (NO) on the innervated skeletal muscle during early reperfusion after 5-hour ischemia has been demonstrated in our previous study. Because an intriguing interrelation exists among denervation, ischemia-induced reperfusion injury, and NO production, this study was designed to clarify the effect of exogenous NO donor on the microcirculation during early reperfusion in a denervated skeletal muscle model which is more relevant to most clinical practice.

**Methods (approved by IACUC):** Thirty two denervated rat cremaster muscles subjected to 3-hour ischemia and 90-minute reperfusion were divided into two groups; one received systemic infusion of NO donor, s-nitroso-n-acetylcysteine (SNAC 100 nmol/min), and the other received phosphate-buffered saline (PBS). During reperfusion, the arteriolar diameters were measured with intravital microscopy and the blood flow of the main vessel pedicle of the cremaster muscle was measured with laser Doppler flowmetry. Analysis of variance (ANOVA) was used for statistical analysis.

**Results:** The average diameter in 10-20 µm arterioles throughout 90 min of reperfusion was between 107% and 123% of baseline in the SNAC group and between 55% and 84% in the PBS group. These values in 21-40 µm and 41-70 µm arterioles were between 100% and 110% in the SNAC group and between 70% and 90% in the PBS group from 20- to 90-min of reperfusion. Compared to the PBS group, the SNAC group had a significantly greater vessel diameter in both 10-20 µm (p<0.001) and 21-40 µm arterioles (p<0.01 to p<0.001) from 10- to 90-min of reperfusion, and in 41-70 µm arterioles (p<0.02 to <0.001) from 20- to 90-min of reperfusion. The overall blood flow of the reperfused cremaster muscle in the SNAC group increased from 37% of baseline at 10 min to 108% at 40 min of reperfusion, and remained above baseline throughout the experiment, with a highest level of 155% at 60 min of reperfusion. In contrast, the average blood flow in the PBS group was only between 27% and 68% of baseline during 90 min of reperfusion. Compared to the PBS group, the average blood flow in the SNAC group was significantly (p<0.03 to p<0.001) greater from 40- to 90-min of reperfusion.

**Discussion:** In the present study, we implemented an animal model which denervates both the somatic nerve to skeletal muscle and the autonomic nerve to intramuscular blood vessels. Denervation accentuates the reperfusion injury of skeletal muscle via three proposed mechanisms: 1) vasoconstriction; 2) loss of response to vasoactive metabolite produced during ischemia; and 3) venous stasis with interstitial edema. Nitric oxide, as a vasodilator, antiadhesion, and antithrombotic agent, has received much attention in relieving the deleterious effect of reperfusion injury in skeletal muscle. The results of our experiment suggest that 1) Exogenous NO donor (SNAC) improves microcirculation of reperfused denervated skeletal muscle; and 2) This protection against reperfusion injury of skeletal muscle is independent of the nerve.

This experiment used direct regional measurement of arteriolar diameter by intravital microscopy and indirect global measurement of blood flow by laser Doppler flowmetry as a complement to each other, hoping to reflect the more actual extent of the changes of blood flow and the intervention effect of exogenous NO donor. There are merits and drawbacks in both methods. Vessel diameter measurement allows direct observation of dynamic changes in the microcirculation throughout the whole ischemia/reperfusion course, but its regional observation field limits its ability to document the gross damage inflicted. Though vessel diameter is the main determinant of geometric factor affecting blood vessel resistance according to Poiseuille’s Law, there are many other rheological factors affecting vessel resistance, which intravital microscopic observation can not detect, such as plasma viscosity, blood cell size and shape, deformability, aggregability, and hematocrit. The advantage of blood flow measurement using a laser Doppler flowmetry probe on the vascular pedicle is its ability to detect the impairment of blood flow delivered to the whole muscle during reperfusion. However, the use of laser Doppler for the indirect detection of blood cell flow is subjected to many variables that may affect the measuring result, such as occasional motion on the experimental animal under various stages of anesthesia, and maintenance of constant distance between the laser probe and vascular pedicle to eliminate spatial variation throughout the whole procedure. The results using these two measuring methods seem to correlate, which suggests that random selection of sites measured with intravitral microscopy does reflect the overall extent of ischemia/reperfusion injury as measured with laser Doppler flowmetry.

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