Article

Redox imaging of skeletal muscle using *in vivo* DNP-MRI and its application to an animal model of local inflammation

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Abstract

Disorders of skeletal muscle are often associated with inflammation and alterations in redox status. A non-invasive technique that could localize and evaluate the severity of skeletal muscle inflammation based on its redox environment would be useful for disease identification and monitoring, and for the development of treatments; however, no such technique currently exists. We describe a method for redox imaging of skeletal muscle using dynamic nuclear polarization magnetic resonance imaging (DNP-MRI), and apply this method to an animal model of local inflammation. Female C57/BL6 mice received injections of 0.5% bupivacaine into their gastrocnemius muscles. Plasma biomarkers, myeloperoxidase activity, and histological sections were assessed at 4 and 24 h after bupivacaine injection to measure the inflammatory response. *In vivo* DNP-MRI was performed with the nitroxyl radicals carbamoyl-PROXYL (cell permeable) and carboxy-PROXYL (cell impermeable) as molecular imaging probes at 4 and 24 h after bupivacaine administration. The images obtained after carbamoyl-PROXYL administration were confirmed with the results of L-band EPR spectroscopy. The plasma biomarkers, myeloperoxidase activity, and histological findings indicated that bupivacaine injection caused acute muscle damage and inflammation. DNP-MRI images of mice treated with carbamoyl-PROXYL or

carboxy-PROXYL at 4 and 24 h after bupivacaine injection showed similar increases in image intensity and decay rate was significantly increased at 24 h. In addition, reduction rates in individual mice at 4 h and 24 h showed faster trends with bupivacaine injection than in their contralateral sides by image-based analysis. These findings indicate that *in vivo* DNP-MRI with nitroxyl radicals can non-invasively detect changes in the focal redox status of muscle resulting from locally-induced inflammation.

Keywords: dynamic nuclear polarization magnetic resonance imaging, oxidation reduction, inflammation, neutrophil activity, nitroxyl radical, skeletal muscle

Introduction

The skeletal muscle is a major producer and consumer of energy within the body. The many mitochondria present in skeletal muscle produce ATP via the electron transfer system, and are also an important source of free radicals [1]. Thus, oxidative stress may be closely related to the onset or progression of skeletal muscle disorders. Many acute or chronic muscle disorders, such polymyositis and muscular dystrophy, are incurable diseases with an inflammatory component involving infiltration of neutrophils, macrophages and lymphocytes into the muscle [2-4]. Acute neutrophil-mediated inflammation is a natural immune response; neutrophils infiltrate damaged tissues within minutes to hours after injury and produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide [5].

Bupivacaine (BPVC) is a clinically used local anesthetic agent; however, it is known to destroy skeletal muscle fibers and cause severe inflammation when injected [6, 7]. Within several hours of BPVC-induced muscle transient fiber destruction, inflammatory cells locally infiltrate into treated tissues; the muscle cell itself regenerates within several weeks [6]. Because of this effect, BPVC treatment has been used to create an animal model of muscle inflammation and regeneration [8]. While the alterations associated with inflammatory pathologies are commonly monitored by

testing blood biochemistry, imaging methods such as MRI and CT may be helpful for localizing damaged areas of muscle by allowing visualization of the associated edema and fibrosis [9]. However, these imaging methods are most suited to detecting the late-phase changes that occur after tissue damage. There is currently no non-invasive technique that can monitor the muscle inflammatory response *in vivo*; therefore, an imaging method for monitoring muscle inflammation based on its redox environment would be very helpful for the evaluation of muscle disorders and for the development of novel drugs.

In vivo dynamic nuclear polarization magnetic resonance imaging (DNP-MRI), also called proton-electron double resonance imaging or Overhauser MRI (OMRI), is a technique that allows imaging of the distribution of free radical species with the anatomical structures of living animals [10, 11]. DNP [12] enhances the MRI signal from nuclei such as ^1H by irradiating the tissue at the electron paramagnetic resonance (EPR) frequency of the free radical prior to applying the MRI pulse sequence, thus enhancing the image intensity where free radicals are present. The redox reaction is modulated by nitroxyl radicals, and its reduction rate depends on the redox environment, which is influenced by factors such as over-generation of ROS and decreased antioxidant defenses [13, 14]. *In vivo* DNP-MRI with nitroxyl radicals has been used to

obtain unique information about redox status [15-17], tissue oxygen partial pressure [18, 19], pH [20], and proteolytic activity [21] in living mice. Because the results of many *in vitro* studies suggest a relationship between oxidative stress and muscle disorders, a method for non-invasive monitoring of muscle redox status, such as DNP-MRI, could be a powerful tool for understanding the dynamics of redox alterations and for assessing the efficacy of drugs in the treatment of certain acute/chronic muscle disorders. Therefore, the objective of this study was to clarify an imaging method for the redox status of skeletal muscle using *in vivo* DNP-MRI with nitroxyl radicals as molecular imaging probes, and to apply this method to a BPVC-treated animal model.

Materials and methods

Chemicals

BPVC (0.5 % Marcaine) was obtained from Astrazeneca (Osaka, Japan). 3-carbamoyl-2,2,5,5-tetramethyl-1-pyrrolidine-1-oxyl (carbamoyl-PROXYL) and phorbol 12-myristate 13-acetate (PMA) were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). 3-carboxy-2,2,5,5-tetramethyl-pyrrolidine-1-oxyl (carboxyl-PROXYL) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). ¹⁵N-carbamoyl-PROXYL was obtained from Japan Redox Ltd (Fukuoka, Japan).

All other chemicals were commercially available, and of reagent grade quality.

Animals

Female C57/BL6 mice were purchased from Kyudo Co. (Saga, Japan) and housed in a climate-controlled room with a 12:12 h light:dark cycle.. The mice were allowed free access to water and food (MF diet, Oriental Yeast Co., Tokyo, Japan) and were acclimatized to their environment for 1 week prior to the experiments. At the time of the experiments, the mice were 7–9 weeks old and weighed 16–18 g. All animal care and experimental procedures were approved by the committee on the Ethics of Animal Experiments, Kyushu University, and were conducted in accordance with the Guidelines for Animal Experiments of Kyushu University.

Acute muscle inflammation model

Female C57/BL6 mice were anesthetized with 2% isoflurane. The hair was shaved from the skin over both gastrocnemius muscles and 50 μ L of 0.5% BPVC solution was injected intramuscularly into the right gastrocnemius. The contralateral gastrocnemius muscle was used as a control (untreated).

Plasma biochemistry

Female C57/BL6 mice were anesthetized with 2% isoflurane. Blood samples (0.5 mL) from control (N = 6) or BPVC-treated 4 (N = 6) and 24 h (N = 6) mice were collected from the postcaval vein for measurement of creatine phosphokinase (CPK), glutamic oxaloacetic transaminase (GOT), and lactate dehydrogenase (LDH) . Gastrocnemius muscles were also collected for histological assessment as shown in the section of histological evaluation. The blood samples were immediately centrifuged at $900 \times g$ for 15 min at 4 °C and the plasma was aliquoted and stored at -80 °C for later analysis. Plasma concentrations of CPK, GOT, and LDH were measured with an autoanalyzer (FUJI DRI-CHEM 4000, Fujifilm Corporation).

Histological evaluation

Both gastrocnemius muscles on BPVC treated side and contralateral muscle as a control were removed at 4 h (control N = 6, BPVC N = 6) and 24 h (control N = 6, BPVC N = 6) after BPVC injection, and portions of each muscle were either fixed in 10% formalin and paraffin embedded or sectioned frozen. The tissue sections were stained with hematoxylin and eosin (HE). Paraffin sections (2 μ m) were used for

histological evaluation (Fig. 3A) while the number of inflammatory cells (neutrophils and macrophages) were counted in frozen sections (6 μm). Total inflammatory cell numbers were obtained by counting the cells in eight fields of view (magnitude $\times 200$) in a coronal section (Fig. 3B).

Myeloperoxidase (MPO) activity

Neutrophil activity in the blood was estimated by measuring myeloperoxidase (MPO) activity, using a modification of the method previously described by Krawisz *et al.* [22]. Briefly, plasma samples (12 μL) obtained from control (N = 5) or BPVC-treated 4 (N = 6) and 24 h (N = 6) mice were diluted in 10 mM phosphate buffer (148 μL , pH 6.0) and incubated for 10 min at 37 $^{\circ}\text{C}$. After adding o-dianisidine (3.3 mg/mL) and H_2O_2 (0.385 M) in 10 mM phosphate buffer (pH 6.0) to each sample, the decay in absorbance at 450 nm was measured by spectrophotometer. MPO activity (U/mL) was calculated using a horseradish peroxidase standard curve.

Redox imaging of muscle tissue with *in vivo* DNP-MRI

In vivo redox imaging was performed with a custom *in vivo* DNP-MRI system, constructed using the external magnet of a commercial EPR spectrometer (JES-ES20,

JEOL Ltd.) [17]. The external magnetic field B_0 for EPR irradiation and MRI was fixed at 20 mT, and the radiofrequency of the EPR irradiation and MRI were 527.5 MHz and 793 kHz, respectively. A rectangular, one-turned curved surface coil (longitudinal direction 20 mm, lateral direction 32 mm) for EPR irradiation was made for muscle imaging in this study (Fig.1 A) [23]. The sensitivity area was confirmed using a 20-mL 4% agarose gel phantom containing 1 mM carbamoyl-PROXYL. In *in vivo* experiments, mice were anesthetized with 2% isoflurane 4 h (N = 8) or 24 h (N = 8) after BPVC treatment for intramuscular injection of carbamoyl-PROXYL or carboxyl-PROXYL and were then secured in sternal recumbency on a special holder with adhesive skin tape. The contralateral gastrocnemius muscle was used as a control. During the procedure, the body temperature of the mice was kept at 37 ± 1 °C with a heating pad. The holder was then placed in the resonator and *in vivo* DNP-MRI imaging of the lower abdomen and hind limbs was started immediately after intramuscular administration of either carbamoyl-PROXYL (2.5 mM, 50 μ L) or carboxyl-PROXYL (2.5 mM, 50 μ L). Pharmacokinetic DNP-MRI images were obtained at 2, 4, 7, 10, 13 min after injection. Normal MRI images were obtained without EPR irradiation. The *in vivo* redox map was obtained by the slope of the image intensity of each pixel in four pharmacokinetic images using a custom Excel macro program. The scanning conditions for the *in vivo*

DNP-MRI experiment were as follows: power of EPR irradiation, 9 W; flip angle, 90°; repetition time (T_R) $\times$ echo time (T_E) $\times$ EPR irradiation time (T_{EPR}), 1000 $\times$ 40 $\times$ 500 ms; number of averages, 1; slice thickness, 100 mm including whole thickness of the mouse; phase-encoding steps, 32; field of view (FOV), 40 $\times$ 40 mm; and matrix size, 64 $\times$ 64 after reconstruction.

***In vivo* redox measurement by L-band EPR spectroscopy**

To determine the muscle redox status in BPVC-treated mice, *in vivo* reduction of carbamoyl-PROXYL was monitored by L-band EPR spectroscopy [24]. BPVC-treated mice were anesthetized at 24 h after treatment with 1.5 % isoflurane and secured on the holder. First, 50 μ l of ^{15}N -carbamoyl-PROXYL (1 mM) in a phantom tube was used as an internal control marker, then 50 μ l of ^{14}N -carbamoyl-PROXYL (2.5 mM) was then injected into the same site as the BPVC injection. The reduction in the EPR signal was monitored every 30 s for 20 min ($N = 10$) by L-band EPR spectroscopy using the following parameters: microwave frequency, 1 GHz; microwave power, 10 mW; center of field, 37.8 mT; modulation width, 2 $\times$ 0.1 mT; sweep time, 10 sec; sweep width, 1 mT; receive gain, 3.2 $\times$ 100; and time constant, 0.03 sec. Animals without BPVC treatment were also measured as a control group ($N = 10$).

Assessment of leakage of nitroxyl radicals into the blood stream

A carbamoyl-PROXYL (50 μ L of 2.5 mM) solution was intramuscularly administered to control- (N = 5) or BPVC-treated (N = 5) mice. A blood sample was collected at 10 min after injection and the nitroxyl radical concentration in the blood was measured by X-band EPR.

Statistical analysis

The *in vivo* DNP-MRI data were analyzed using Image J software, which is available in the public domain [25, 26]. Data are presented as means $\pm$ standard deviations and differences in means were evaluated by t tests. Differences were considered statistically significant if $p < 0.05$.

Results and Discussion

To demonstrate that redox imaging is feasible in skeletal muscle, we first made the surface coil for highly sensitive local imaging. Previously, we described the surface coil with a flat coil for DNP-MRI measurements and it showed significant enhancement of image intensity on the surface of the coil [23]. To cover the legs of the mice for

muscle imaging, a curved surface coil was made as shown in Fig. 1A and S1. We used the custom curved surface coil in all experiments. The B_1 homogeneity of the custom surface coil for DNP-MRI signal distribution was estimated with the image of the 4% agarose phantom tube containing 1 mM carbamoyl-PROXYL as shown in figure 1A. The signal intensity was clearly enhanced by ESR irradiation (ESR ON) without heating and the high intensity area on the images of coronal and transverse fully covered the region for *in vivo* muscle DNP-MRI imaging (Fig 1B). We also confirmed that the location of the legs was within the area giving at least 20% of maximum sensitivity. In *in vivo* experiments, both legs were suitably positioned within the area of the surface coil, to give the best sensitivity and image (accumulation = 8) without ESR irradiation showed the anatomical structure of the lower limbs of the mice (Fig. 1C). Then, we tested *in vivo* DNP-MRI imaging in mice after intramuscular injection of nitroxyl radicals (50 μ L) into their hindlimbs. *In vivo* DNP-MRI images clearly showed the distribution of both nitroxyl radicals, carboxy-PROXYL and carbamoyl PROXYL in each leg, and the enhanced intensity that they generated decreased over time (Fig. 1D-E). In these experiments, we determined that the DNP-MRI imaging could simultaneously detect two redox reactions, for example, in control and treated sides, in the leg muscles of an individual mouse.

To confirm the acute toxicity of BPVC to muscle, we measured the plasma concentrations of conventional biochemical markers of muscle injury (CPK, GOT, and LDH) at 4 and 24 h after treatment with BPVC. The concentrations of CPK, GOT, and LDH all showed similar trends, with significant increases at 4 h and decreases at 24 h after BPVC injection (Fig. 2), which suggests that the muscle damage was induced immediately after injection of BPVC as has been shown previously in other studies [6, 7]. Histological assessment of the BPVC-treated muscle was performed to confirm the muscle damage and infiltration of inflammatory cells, as shown in figure 3. All control muscle specimens had histologically normal-appearing muscle fibers with a regular fiber arrangement and peripheral nuclei (Fig. 3A). In contrast, 4 h after BPVC treatment there was focal muscle necrosis in the center of the gastrocnemius, with hyaline degeneration of muscle fibers and infiltration of inflammatory cells. The muscle damage was primarily in the center of transverse sections of the muscle tissue (Fig. 3A dotted line) and extended approximately 8–10-mm horizontally (Fig. 3B). At 24 h after BPVC treatment, severe inflammatory cell infiltration was observed, in addition to the changes seen at 4 h. The number of inflammatory cells at the site of injury at 24 h after BPVC treatment was significantly greater than at 4 h after treatment (Fig. 3C). Neutrophil activity in the blood was estimated by measuring MPO activity. The activity of MPO in

plasma was significantly increased at 4 and 24 h after BPVC treatment compared with that of controls (Fig. 3D).

To non-invasively monitor redox status of the muscle, *in vivo* DNP-MRI was performed at 4 and 24 h after BPVC treatment in living mice. Two nitroxyl radicals, cell permeable (carbamoyl-PROXYL) and cell impermeable (carboxyl-PROXYL), were used in the *in vivo* DNP-MRI experiments to confirm whether the redox reaction mainly occurs in the intracellular or extracellular compartment. Nitroxyl radical solution was carefully injected to the same region of the mouse leg that had received BPVC pretreatment. After injection of carbamoyl-PROXYL, image intensities of both control and BPVC-treated legs (at 4 or 24 h) were clearly enhanced, and this intensity decreased over time. Although the image intensities of both legs were similarly decreased at 4 h after BPVC treatment, the image intensity of the BPVC-treated leg at 24 h showed faster decay than that of the untreated leg (Fig. 4A). The redox maps clearly illustrated differences in decay rates with and distributions between control and BPVC treated legs. The average decay rate observed in the BPVC-treated legs of 8 mice was significantly increased at 24 h but not at 4 h. We next calculated the differences in decay rates of BPVC-treated and contralateral (non-treated) legs in individual mice (Fig. 4A, right panels). In these panels, a symbol above the line marked “even” indicates that the decay

rate of the BPVC-treated leg was faster than that of the control leg. Interestingly, this analysis in individual mice showed a trend toward a faster decay rate with BPVC treatment even at 4 h and more clearly, consistent with the averaged data, at 24 h. In the mice injected with carboxy-PROXYL, the difference in the rate of reduction of image intensity between the control and BPVC-treated regions was also evident (Fig. 4B). The average decay rate in the BPVC-treated legs was significantly greater than in the untreated legs at 24 h, and the plots in individual mice also showed trends similar to those observed with carbamoyl-PROXYL (Fig. 4B, right panels). Though intramuscularly injected probe appeared to distribute in the area of interest in the muscle tissue, the degree of overlap between probe distribution and the area of induced inflammation area may cause variability in decay rates measured *in vivo*. These data suggest that changes in redox status that occur with muscle injury can be detected by *in vivo* DNP-MRI, and that this change in redox status may occur in the interstitial space within the muscle tissue rather than within the muscle cells.

We confirmed the signal decay of carbamoyl-PROXYL in BPVC-treated mice with *in vivo* L-band EPR spectroscopy (Fig. 5). Figure 5A shows the typical *in vivo* EPR spectra of ^{14}N -carbamoyl-PROXYL (center peak) with ^{15}N -carbamoyl-PROXYL for normalization of each measurement in control or BPVC-treated mice. After

administration of carbamoyl-PROXYL, the signal intensity rapidly increased and then decreased over time. The rate of reduction in signal intensity in BPVC-treated mice 24 h after carbamoyl-PROXYL administration was also faster than that of the control group (Fig. 5B), which corresponded well with the results of our *in vivo* DNP-MRI experiment.

Tissue vascular permeability is enhanced by inflammation due to constriction of the vascular endothelial cells. The blood nitroxyl radical concentration at 10 min after the administration of nitroxyl radicals (carbamoyl-PROXYL and carboxy-PROXYL) was measured by X-band EPR at 24 h after BPVC treatment to confirm the effect of vascular permeability on the decay of nitroxyl radicals *in vivo* (Fig. 6). There was no difference in blood nitroxyl radical concentration between the control and BPVC-treated mice, though the results of *in vivo* DNP-MRI showed a clear difference in redox status. These results suggest that the rapid decay of the nitroxyl radicals at 24 h after BPVC treatment is mainly due to the redox reaction in the muscle tissue.

In general, MPO is released by activated neutrophils and macrophages both inside the phagosomes and extracellularly, catalyzing the production of H_2O_2 and $HOCl$. In contrast, nitroxyl radicals have been shown to reduce the damage caused by MPO-derived oxidants *in vitro* and to inhibit its chlorinating activity [27, 28]. The data

from our *in vitro* study demonstrated that activated neutrophils could reduce nitroxyl radicals to their non-radical forms (Fig. S2). Blood MPO concentrations were significantly increased at 4 h (Fig. 3D) after BPVC treatment. Consistent with this, in the *in vivo* image-based analysis, nitroxyl radical reduction showed a trend of being faster from 4 h and was significantly faster at 24 h in BPVC-treated as compared with untreated leg muscles. Thus, we suggest that the inflammatory cells infiltrating the muscle after BPVC treatment contributed to these *in vivo* redox differences, and that *in vivo* DNP-MRI imaging can detect this redox status within the affected area of the muscle tissue. In addition, our results demonstrate that the cell-permeable nitroxyl radical, carbamoyl-PROXYL, had a rapid decay rate compared with that of the cell-impermeable carboxyl-PROXYL (Fig. 4). This indicates that carbamoyl-PROXYL decay rates might not only reflect redox conditions in the interstitial space, but also provide evidence of intracellular redox status. Duguez *et al.* reported that muscle regeneration within several days after BPVC treatment is accompanied by marked stimulation of mitochondrial biogenesis that occurs with the onset of muscle cell differentiation [29], though BPVC may also act as a short-term uncoupler of mitochondrial oxidative phosphorylation [30-32]. Mitochondrial biogenesis is an important determinant of tissue redox status due to electron transfer for ATP and free

radical production [1]. It is thought that increased mitochondrial biogenesis in the 24-hr BPVC-treated model may also contribute to *in vivo* redox changes in BPVC-treated muscle.

In the present study, we demonstrated that *in vivo* DNP-MRI with nitroxyl radicals with a custom surface coil could provide useful images in mouse muscle tissue. This supports the value of detecting redox alterations of muscle tissue induced by local inflammation secondary to BPVC injection. Redox imaging by DNP-MRI enables simultaneous analysis of the redox environment in both control and BPVC-treated sides in individual mice. This serves to reduce the variability caused by deviations between individual animals or separate imaging measurements. The redox maps and analysis of decay rates in individual mice clearly illustrated the localization of redox alterations as well as differences between control and inflammation-induced legs. Such redox imaging data were obtained using a lower number of experiments than those required for *in vivo* spectroscopic experiments. Because many muscle disorders are associated with systemic inflammation, the ability of *in vivo* DNP-MRI to non-invasively detect the focal redox status of muscle, as we have demonstrated in this study, suggests that it could be a valuable tool for the diagnosis of muscle diseases and assessment of novel treatment strategies in preclinical studies.

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Figure legends

Fig. 1 Redox imaging of the hind limbs of mice after intramuscular injection of nitroxyl radicals. (A) Diagram of the custom surface coil for muscle imaging and schematic of the phantom study. (B) Sensitivity map of the 4% agarose phantom containing 1 mM carbamoyl-PROXYL by DNP-MRI imaging with the surface coil. (C) The *in vivo* experimental set-up. Both legs were placed on the surface coil for ESR irradiation. (*in vivo* DNP images of the mouse; accumulation = 8). Fifty μ L of two nitroxyl radicals, carboxy-PROXYL and carbamoyl-PROXYL were intramuscularly injected into the left and right hind limbs, respectively. (D) *In vivo* DNP-MRI images

were obtained at the indicated times. (E) Plot of image intensities for both legs. (N = 4).

Fig. 2 Response of biochemical markers to BPVC treatment. (A) creatine phosphokinase (CPK), (B) glutamic oxaloacetic transaminase (GOT), and (C) lactate dehydrogenase (LDH) plasma concentrations in mice 4 and 24 h after BPVC treatment (N = 5–6 per group). * $p < 0.01$. ** $p < 0.05$.

Fig. 3 Pathological evidence of muscle injury after BPVC treatment. (A) Hematoxylin and eosin (HE) staining of paraffin sections of the gastrocnemius at 4 and 24 h after treatment with 0.5 % BPVC solution. The dotted lines in the overview slides (top) delineate the damaged area. (B) Coronal section of muscle tissue (C) Number of inflammatory cells (neutrophils and macrophages). Inflammatory cells in the HE-stained sections were counted in eight fields of view in the same sample. (D) Plasma MPO activity at 4 and 24 h after BPVC treatment. (N= 5–6 per group). * $p < 0.01$. ** $p < 0.05$.

Fig. 4. Redox imaging of muscle in BPVC-treated mice using *in vivo* DNP-MRI. (A) *In vivo* DNP-MRI images and redox maps at 4 and 24 h after BPVC treatment in mice.

Images were taken after injection of 2.5 mM carbamoyl-PROXYL (50 μ L) into both legs. Redox maps were calculated using four pharmacokinetic *in vivo* DNP-MRI images. Decay rates were calculated for the DNP-MRI images of untreated and BPVC-treated legs. Plots showing differences between decay rates in BPVC treated vs untreated (contralateral) legs in individual mice. (B) *in vivo* DNP-MRI images and redox map at 4 and 24 h after BPVC treatment in mice. Images were taken after injection of 2.5 mM carboxy-PROXYL (50 μ L) into both legs. (N = 8 per group). * $p < 0.01$. (Other parameters are plotted as for carbamoyl-PROXYL.)

Fig. 5 *In vivo* redox spectroscopic analysis of mice by L-band ESR. (A) Typical *in vivo* ESR spectra of mice in control and 24 h BPVC-treated mice. ESR signals were collected every 30 s and normalized using the area of ^{15}N -carbamoyl-PROXYL as an internal standard. (B) Decay rate of carbamoyl-PROXYL. N = 10 per group. * $p < 0.05$.

Fig. 6 Assessment of leakage of nitroxyl radicals into blood. Blood levels of nitroxyl radicals after intramuscular administration of (A) carbamoyl-PROXYL and (B) carboxyl-PROXYL to 24 h BPVC-treated legs. Blood radical concentrations were monitored by X-band-EPR. N = 5 per group.

Figure1

Figure2

Figure3

Figure4

A Carbamoyl-PROXYL

B Carboxy-PROXYL

Figure5

Figure6

Noninvasive monitoring of muscle redox status

Surface coil

in vivo DNP-MRI images

2 min

7 min

13 min

Local inflammation by bupivacaine

Redox Map

Normal Inflammation