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Original Paper

Protective Effects of Modeled Superoxide Dismutase Coordination Compound (MSODa) Against Ischemia/Reperfusion Injury in Rat Skeletal Muscle

Xin-Tao Wang Ye Tian Wen-Xiao Xu Li-Huang Cui Shou-Yang Xiang Song-Cen Lü

Department of Orthopedic Surgery, the 2nd-Affiliated Hospital of Harbin medical University, Harbin, China

Key Words
Superoxide dismutase • Free radicals • Adherent molecules • Ischemia/reperfusion • Skeletal muscle

Abstract
Background/Aim: Ischemia/reperfusion (I/R) injury of skeletal muscles is common pathophysiology during surgeries and the superoxide dismutase (SOD) plays a critical role in this process. SOD-modeled coordination compound (MSODa) may simulate the protective effects as SOD. ***Methods***: Therefore, this study was designed to explore the protective effects and underlying mechanism of MSODa on malondialdehyde (MDA) and integrin-β2 (CD11b/CD18) in plasma, myeloperoxidase (MPO) and intercellular cell adhesion molecule-1 (ICAM-1) in tissue, and morphological changes before and after I/R injury. The rat model of I/R in hind limb was established and randomly divided into sham, ischemia, I/R, I/R-treated with saline, SOD, and MSODa, respectively. ***Results***: These results showed that averaged values for MDA, MPO, CD11b/CD18, and ICAM-1 were significantly increased ($P < 0.01$ vs ischemia alone) in a time-dependent fashion along with marked tissue remodeling, such as abnormal arrangement of muscular fibers, interstitial edema, vasodilation with no-reflow, inflammatory cells adherent and infiltration, structural changes in mitochondrial, and decrease in glycogens as well. However, all parameter changes induced by I/R injury were reversed, at least partially, by MSODa and SOD treatments and intriguingly, the beneficial/protective effects of MSODa was superior to SOD with an early onset. ***Conclusion***: This novel finding demonstrates that MSODa improves I/R injury of skeletal muscles due at least partially to inhibition of adherent molecule expression and reduction of oxygen free radical formation during I/R pathophysiological processes and this protective action of MSODa was superior to SOD, highlighting the bright future for MSODa in clinical management of tissue I/R injury.

Dr. Lü

Department of Orthopedics, the 2nd Affiliated Hospital of Harbin Medical University, #249 Xue-Fu Road, Harbin 150081, (China)
Tel. +86 451-86296062, Fax +86 451-86296058, E-Mail lvsongcen@yahoo.com

Introduction

The skeletal muscle ischemia-reperfusion (I/R) injury is a pathophysiological process during surgeries and post-surgical management in clinical practice, commonly seen in limb reattachment [1], limb vascular damage [2], osteofascial compartment syndrome [3-5], and free flap transplantation [6], which will directly affect the succeed of the operation especially in the division of orthopedics and the quality of life. It has well been accepted that skeletal muscle I/R injury is caused by free radicals produced from oxidative stress [7] and leukocytes play a key role in the pathogenesis of skeletal muscle I/R injury [8-11], which is preconditioned by the expression of adhesion molecular on leukocytes and endothelia [12-14]. Large scale of evidences have also demonstrated that the superoxide dismutase (SOD) is an important to control reactive oxygen species during I/R injury [15, 16], but the relevance to human disease conditions mentioned above and the potential mechanism underlying I/R injury is not clarified. Moreover, looking for a therapeutic agent to replacement of SOD would be worthy of attempting due to the sources and cost for utilizing the SOD. Therefore, this study was designed to explore the protective effects of both SOD and modeled SOD coordination compound (MSODa) [17], an artificially synthesed compound $Cu_2(C_3N_2H_4)_6(C_3H_2H_2)Cl_3$ based upon the natural Cu-Zn (Cu-ZnSOD) showing similar property and the structure with molecular weight at only 708.5, and extracellular activity compared with natural SOD, on plasma MDA, tissue MPO, CD11b/CD18 positive neutrophils, and ICAM-1 positive vasculatures, and morphological changes before and after skeletal muscle I/R injury in rats. The current observation demonstrates that the protective effects of MSODa against I/R injury-induced by oxidative stress is superior to SOD.

Materials and Methods

Experimental animals

For duplicate I/R models of skeletal muscle of rats, adult male Wistar rats with body weight between 200-220 g were purchased from Experimental Animal Center of the 2nd Affiliated Hospital of Harbin Medical University (Certification №: SCXK-20-120-002). All rats were maintained in the animal facility with 12 hour light cycle. Experimental protocols used in this experiment have been preapproved by the Institutional Animal Care and Use Committee of School of Medical Science, Harbin Medical University. All animal experiments were conducted by following the Principles of laboratory animal care (NIH publication no. 85–23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm), as well as specific national laws where applicable.

Chemicals

Monoclonal antibodies for ICAM-1 (MCA733), CD11b-RPE (MCA711PE), and CD18-FITC (MCA775F) were purchased from AbD Serotec® Bio Comp (Oxford, UK). Biotin goat anti mouse IgG antibody, poly-D lysine, SABC kits, and DAB color rendering kits were ordered from Boster Bio (Wuhan, China). SOD (specific activity: 8000 units/mg), MSODa (MW: 708.5, molecular formula: $Cu_2(C_3N_2H_4)_6(C_3N_2H_3\text{-})Cl_3$; sterile injection: 100 μg/ml), MDA, and MPO kits were all purchased from the regular commercial sources.

Equipments

FACSort Cell Sorter Flow Cytometer (B.D., New Jersey, USA), emission electromicroscope (JEM-1220, Japanese Electronics Co., Lid., Kyoto, Japan), grating spectrophotometer (model 722, Analytical Instrument Comp., Shanghai, China), high speed centrifuge (model H-1600, Analytical Instruments, Comp., Shanghai, China), light microscope (Olympus, Tokyo, Japan), thermostatic waterbath (Medical equipments Comp., Beijing, China), and electrically heated drying oven (Hongda Medical Instruments Comp., Nantong, China) were used for designed measurements and tests.

Ischemia/reperfusion model

The skeletal muscle I/R model rats were prepared according to the procedures described in details previously [12]. Briefly, the rats were anesthetized by intraperitoneal 1% pentobarbital sodium. After

completely relaxation, the skin on the left hind leg was sheared and sterilized with medical iodine and 70% alcohol, cut open along the knee, and blunts deserted subcutaneous tissue and muscles until completely expositing the joint. Following these procedures, both the sciatic and femoral nerves were cut, the pretibial and posttibial blood vessels were carefully separated toward proximal ends until the crotch of femoral artery and vein, and then the branches of femoral blood vessels were ligated and cut, the meanwhile, the periosteum on the distal femur was peeled with caution. At this point, the ischemia model was established by closing both femoral artery and vein together using proper size of non-invasive vascular clamp for 4 h. Whereas, for the I/R injury model was established by reopen the clamp at 1, 2, 4, 8, and 12 h after ischemia. The sham operation was also performed upon the similar procedures without using vascular clamp. For the saline and test groups, 500 ml saline, SOD (16000 unit/kg), or MSODa (400 μg/kg) were given intravenously at 5 min prior to the reperfusion, respectively.

CD11b/CD18 expression neutrophils

10 μl CD11b-RPE, 10 μl CD18-FITC, and μl PBS were added, respectively, into the test tubes containing 100 μl heparinized whole blood to label monoclonal antibody MRC OX-42 and WT, well mixed with shaker in the darker room; and incubated at room temperature for 25 min, and then adding red blood cell cracking liquid (10 times diluted) and standing for 10 min followed by centrifugation at 1500/rpm at room temperature for 5 min. After removing the supernatant, the sample was washed with PBS twice and shaked well with additional 800 μl PBS before the cytometry detection. By using Cellquest software, the rate of expression of CD11b/CD18 was analyzed (the percentage of CD11b/CD18 fluorescent positive neutrophils in total number of neutrophils in the tested sample).

ICAM-1 expression in vasculature of skeletal muscle

The tissue block was continuously sliced at 3 μM and placed on the poly-D lysine coated coverslips, and then incubated at 37°C for before use; For dewaxing, the tissue slice was treated with xylene for 4 times with 10 min each, washed another 4 times with anhydrous alcohol to remove the xylene, and then washed once with 95% alcohol followed by ddH_2O; The tissue slice was merged into mixed solution of 0.3% methanol and hydrogen peroxide for 30 min and washed by PBS for 3 times with 5 min each; The slice was then place into the container with sodium citrate and heated up to 92°C~98°C for 10~15 min, after cooling at room temperature for 20 min, the tissue slice was sealed using 5%~10% goat serum at room temperature for 20 min; After completely removing the goat serum, the slice was treated with 50 μl PBS diluted primary antibody MCA-733 at 4°C overnight; following 3 times wash with PBS at 5 min each, biotinylated goat again mouse secondary antibody was added and incubated at room temperature for 30 min; Following 3 times wash with PBS, the tissue slice was treated with DAB for appropriate time upon the observation under the light microscope, and stained with hematoxylin for the microscopy analysis. Continuous 20 with no overlapping field of visions was investigated (40 **x**) randomly and ICMA-1 positive vasculatures (%) in total observed blood vessels were calculated.

MDA determination

0.5 ml whole blood was taken from femoral vein, heparinized and centrifuged at 3000 rpm for 10 min to get the plasma. The amount of Methane Dicarboxylic Aldehyde (MDA) in plasma was determined by glucosinolates pentobarbital sodium and detail procedures of the measurements followed the instruction of the commercial kit using spectrophotometer and the OD value was directly read and presented as nmol/L.

MPO measurement

For this measurement, 5% tissue homogenate was prepared with the medium at the ratio of 9:1 and myeloperoxidase (MPO) in tissue was measured by reading the OD value at 460 nm using spectrophotometer and expressed as MPO unit/gram wet tissue.

Microscopy

The tissue was fixed with 10~15% formaldehyde for 24 h, and underwent with dehydration with gradient alcohol, transparent processing with xylene, embedded with paraffin and then the tissue block was sectioned to 4 μm tissues slices, which was dewaxed and stained with hematoxylin, and then the slices were underwent transparent treatment, sealing for light microscope investigation.

Electromicroscopy

The tissue was cut into 1 cubic millimeter block, fixed with 2% glutaraldehyde for 1 h, washed with 0.1% PBS buffer for 3 times; the above tissue block was fixed with 0.1% goose acid for 2h, and then dehydrated with gradient acetone, merged into epoxy resin over night; The fixed tissue block was sectioned into 50~100 nM slices and treated with 1% venturi blue for electronic microscope observation.

Statistical analysis

Averaged data were presented as the mean ± SD. The statistical analysis was performed with SAS software package and the student *t*-test, chi-square test, and ANOVA were applied where appreciate. The P values less than 0.05 were considered as the significant difference.

Results

Effects of MSODa on plasma MDA before and after ischemia/reperfusion injury of skeletal muscle

The MDA occurs naturally and is commonly used as a biomarker to measure the level of oxidative stress in an organism [18, 19], especially in I/R injury in both myocardial [20] and skeletal muscles [21]. In this regard, the MDA was tested and the results showed (Table 1) that, compared with sham, the amount of MDA was dramatically increased (4.94 ± 0.53 nM, $P < 0.01$) in the plasma of ischemic rats, which was further enhanced, at least double in amount, time-dependently following reperfusion in either I/R and saline-treated models and peaked at 8 h after I/R. Even though the increased values of MDA were also confirmed in SOD- and MSODa-treated groups, the values were significantly less than those seen in I/R

Table 1. Effects of SOD and SOD-modeled coordination compound (MSODa) on increased plasma malondialdehyde (MDA) and skeletal muscular myeloperoxidase (MPO) in each group before and after ischemia, ischemia/reperfusion (I/R) injury, and . Averaged data were expressed as mean ± SD, $n = 6$ rats for each group. *$P < 0.05$ and **$P < 0.01$ vs sham, †$P < 0.05$ and ††$P < 0.01$ vs ischemia, ‡$P < 0.05$ and ‡‡$P < 0.01$ vs I/R, §$P < 0.05$ and §§$P < 0.01$ vs SOD. Note: Sham: sham operation group; I: ischemia group; I/R: ischemia/reperfusion group; Saline: I/R rats treated with normal saline group; SOD: I/R rats treated with SOD; MSODa: I/R rats treated with MSODa

	MDA (nmol/L) after reperfusion					
Group		1h	2h	4h	8h	12h
Sham	2.42 ± 0.19					
I	4.94 ± 0.53**					
I/R		8.31 ± 1.46**††	9.43 ± 0.75**††	10.2 ± 1.04**††	12.6 ± 0.71**††	11.3 ± 0.60**††
Saline		8.43 ± 1.21**††	9.08 ± 1.28**††	9.76 ± 0.99**††	12.1 ± 1.30**††	10.9 ± 0.84**††
SOD		4.73 ± 0.67**‡‡	6.17 ± 0.57**‡‡	6.36 ± 0.72**‡‡	10.3 ± 1.05**††‡‡	10.0 ± 1.00**††‡‡
MSODa		5.73 ± 0.51**‡‡	7.00 ± 0.65**††‡‡	8.13 ± 0.67**††‡‡	7.36 ± 0.91**††‡‡§§	7.18 ± 0.62**††‡‡§§
	MPO (Units/g) after reperfusion					
Sham	0.41 ± 0.03					
I	0.68 ± 0.05*					
I/R		1.05 ± 0.08**††	1.24 ± 0.08**††	1.74 ± 0.11**††	2.41 ± 0.11**††	2.42 ± 0.11**††
Saline		1.05 ± 0.08**††	1.16 ± 0.09**††	1.70 ± 0.25**††	2.41 ± 0.14**††	2.33 ± 0.15**††
SOD		0.65 ± 0.08**††	0.68 ± 0.09**††	0.82 ± 0.11**†‡	1.14 ± 0.07**††‡‡	1.14 ± 0.08**††‡‡
MSODa		0.69 ± 0.08**††‡‡	0.71 ± 0.11**††‡‡	0.85 ± 0.09**†‡‡	1.05 ± 0.07**††‡‡	1.05 ± 0.07**††‡‡

Table 2. Effects of SO) and SOD-modeled coordination compound (MSODa) on increased CD11b/CD18 and intercellular adhesion molecule-1 (ICAM-1) expression in each group before and after ischemia, ischemia/reperfusion (I/R) injury, . Averaged data were expressed as mean ± SD, n = 6 rats for each group. *P < 0.05 and **P < 0.01 vs sham, $^{\dagger}P$ < 0.05 and $^{\dagger\dagger}P$ < 0.01 vs ischemia, $^{\ddagger}P$ < 0.05 and $^{\ddagger\ddagger}P$ < 0.01 vs I/R, $^{\S}P$ < 0.05 and $^{\S\S}P$ < 0.01 vs SOD. Note: Sham: sham operation group; I: ischemia group; I/R: ischemia/reperfusion group; Saline: I/R rats treated with normal saline group; SOD: I/R rats treated with SOD; MSODa: I/R rats treated with MSODa

		CD11b/CD18 (%) after reperfusion				
Group		1h	2h	4h	8h	12h
Sham	22.8 ± 4.94					
I	24.3 ± 4.40					
I/R		39.0 ± 4.82**††	46.7 ± 5.42**††	56.6 ± 10.7**††	69.1 ± 11.8**††	48.8 ± 8.49**††
Saline		39.2 ± 3.69**††	46.2 ± 5.47**††	55.6 ± 10.2**††	68.1 ± 12.8**††	48.6 ± 12.4**††
SOD		26.4 ± 5.54‡‡	38.3 ± 3.91**†‡‡	51.9 ± 6.34**††‡	60.0 ± 11.2**††‡	43.9 ± 12.5**††‡
MSODa		26.5 ± 5.85‡‡	34.2 ± 7.22*‡‡	39.1 ± 6.15**††‡‡§	54.3 ± 4.84**††‡‡§	25.9 ± 4.42‡‡§§

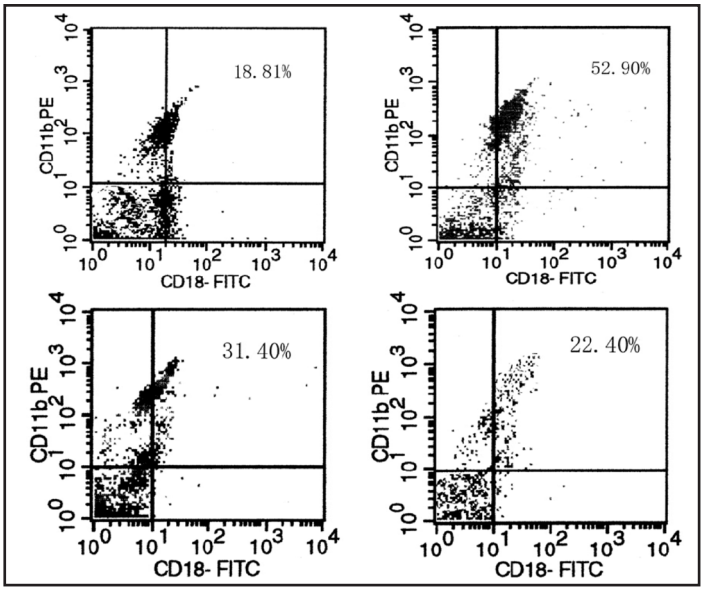

Fig. 1. Cytometry evaluation of the positive rate of CD11b/CD18 after ischemia (A), at 12 h after I/R injury (B), I/R model rats treated with SOD (C) or MSODa (D).

and saline-treated model rats. Intriguingly, the MSODa-treated rats displayed even better plasma measurement of MDA (P < 0.01 vs SOD) at 8-12 h after I/R.

Effects of MSODa tissue MPO before and after ischemia/reperfusion injury of skeletal muscle

The MPO is a peroxidase enzyme and most abundantly expressed in neutrophil granulocytes. Since 2003, the MPO has long been served as a sensitive predictor for I/R injury of both cardiac and skeletal muscles [21, 22]. To evaluate the injured level of skeletal muscle after I/R, the MPO was measured and the averaged data showed that increased MPO was observed in ischemic tissue (0.68 ± 0.05, P < 0.05 vs sham) and greatly increased from 150% up to 350% at 2 h and 8 h followed by the reperfusion with a similar trends during entire reperfusion compared with the alternation of MDA in the same preparation (Table 1). In SOD- and MSODa-treated rats, the tissue levels of MPO were reduced markedly and identically (P < 0.01 vs I/R or saline).

Effects of MSODa on CD11b/CD18 expression on neutrophils before and after ischemia/reperfusion injury of skeletal muscle

The CD11b/CD11 belongs to the integrin superfamily and plays a key role in mediating neutrophil-endothelia interaction through ICAM-1 [23, 24] during I/R injury in skeletal

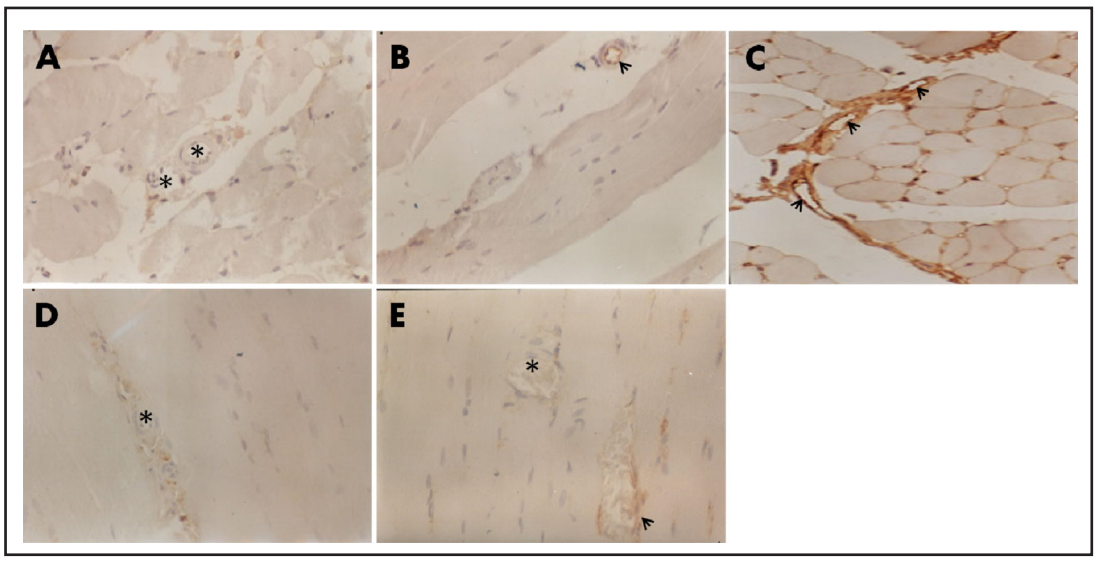

Fig. 2. Immunohistochemical analysis of an angiogenesis in skeletal muscle after I/R injury in rats using SRBC method. (A) control (sham) rats without ICAM-1-positive angiogenesis; (B) ischemic rats with a few ICAM-1-positive angiogenesis (indicated by arrow head); (C) 12 h I/R injury inducing a significant increase in ICAM-1-positve vasculatures (indicated by arrow head); (D – E) protective effect of MSODa on I/R injury showing weak (indicated by asterisk) or negative ICAM-1 immunoactivity. All images are presented at 400×.

muscle. Therefore, monitoring the dynamic changes of CD11b/CD18 during I/R would provide directly evidence to evaluate the severity of I/R injury by using cytometry. Our data showed that the CD11b/CD18 was significantly upregulated during ischemia ($P > 0.01$ vs sham, Table 2), but the reperfusion significantly increased the positive rate of CD11b/CD18 (~160 up to 200%) in a time-dependent fashion. Surprisingly, the pretreatment of I/R rats with SOD or MSODa, especially with the later, dramatically reduced increased CD11b/CD18, even back to the normal level in MSODa-treated group at the end of reperfusion process (Table 1 and Fig. 1).

Effects of MSODa on vasculature ICAM-1 expression before and after ischemia/reperfusion injury of skeletal muscle

The ICAM-1 is an adhesion molecule located on the surface of endothelial cell to mediate the adhesion process with CD11b/CD18 (integrin-β2) on the neutrophil, and sequent cell migration and infiltration [25]. Recent observation has shown that Inhibition of ICAM-1-dependent binding using monoclonal antibodies has been shown to be efficacious in ameliorating I/R injury by preventing the influx of neutrophils into the ischemic tissue [26]. To test if SOD and MSODa reduce ICAM-1 expression, the immunofluorescent staining was performed (Fig. 2) and the results showed that positive immuostaining vasculature was observed in sham-control rats (Fig. 2A, as indicated by *), while, weak and heavy staining of positive immuostaining vasculatures were confirmed in ischemia and I/R model rats (Fig. 2B and C, as indicated by arrow heads), and interestingly, ICAM-1 positive vasculature was almost disappeared when pretreatment with SOD and MSODa (Fig. 2D and E, as indicated by * or arrow head). Averaged data showed that tissue ICAM-1 expression was increased to 23.1 ± 2.38 after ischemia as compared with sham, and this data were then further and time-dependent increased by ~270% at 12 h after the reperfusion followed by ischemia (Table 2).

Morphological changes in skeletal muscle induced by ischemia/reperfusion injury before and after MSODa treatment

As compared with the sham control rats, the skeletal muscle underwent a significant morphological alternation during I/R. At 2 h after I/R (Fig. 3B and E), the space between

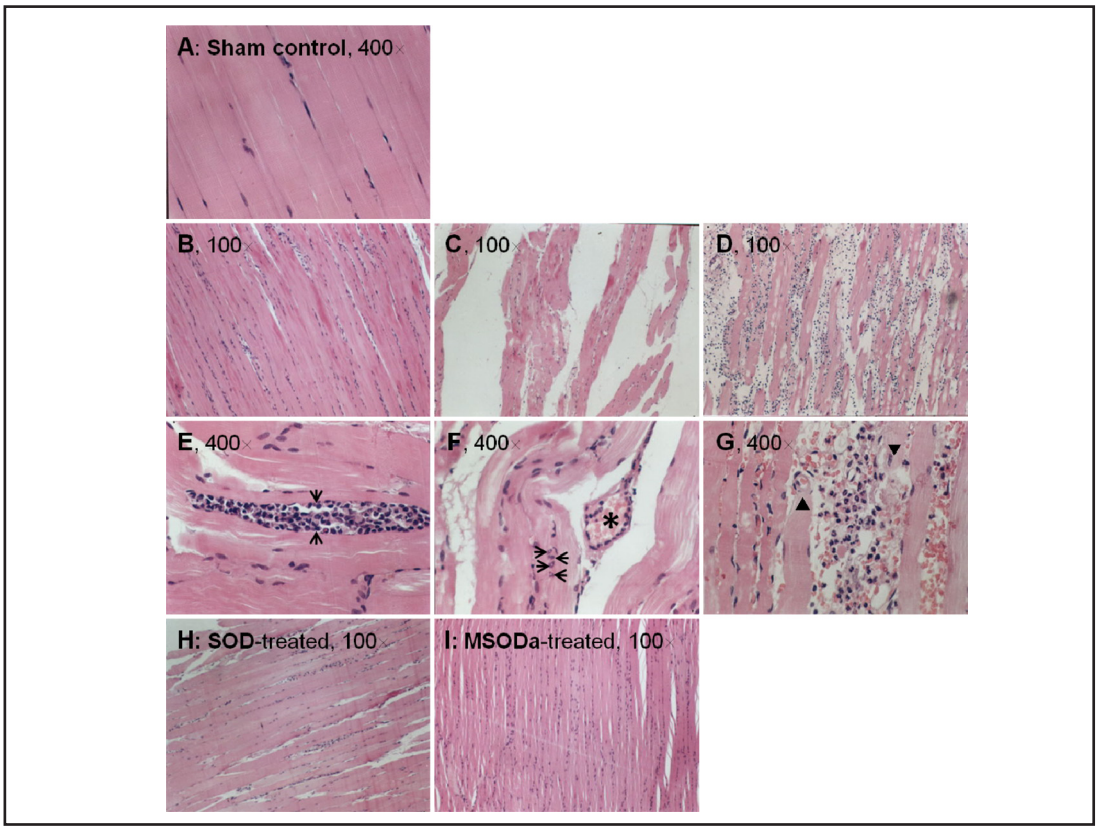

Fig. 3. Morphological changes of skeletal muscle after I/R injury under the light microscope. (A) sham control rats showing normal arrangement of skeletal muscles with clear bands and no white blood cell infiltration; (B and E) morphological changes at 2 h after muscular I/R injury under low (100×) and high magnification (400×); neutrophil accumulation can be observed in the vasculature under high power (between the arrow heads); (C and F) morphological changes at 4 h after muscular I/R injury showing abnormal arrangement of muscular fibers with enlarged intracellular space and significant interstitial edema under both amplifications, significant adhesion of neutrophils on the surface of endothelium (indicated by the asterisk) and tissue infiltration (indicated by arrow heads) identified under high power; (D and G) morphological alternations at 8 h after muscular I/R injury showing significant migration of white blood cells into interstitial space and tissue infiltration under both amplifications; irregular shape and enlargement of skeletal muscle with unclear bands and broken ends (indicated by ▼ or ▲), marked focal myolysis with large amount of neutrophil accumulation; (H) protective effect of SOD on I/R-induced morphological injury observed in skeletal muscles of rats showing near normal arrangement of skeletal muscles with slight enlarged intramuscular space and interstitial edema and not significant white blood cell accumulation within vasculature and tissue infiltration; (I) protective effect of MSODa on I/R-induced morphological injury observed in skeletal muscles of rats showing near normal arrangement of skeletal muscle with clear bands, slight interstitial edema without cell infiltration.

muscular fibers were enlarged, obvious interstitial edema could be observed, and large amount of white blood cells were accumulated in venules with no reflow. Upon 4 h after I/R (Fig. 3C and F), based on the morphological changes occurred at 2 h after I/R, the muscular fiber gap was further aggravated, venules was dilated with significant white blood cells adhesion on the entire surface of the endothelium with clear tissue migration of neutrophil. Along with the reperfusion up to 8 h (Fig. 3D and G), the myolysis and broken muscular fibers (as indicated by ▲ or ▼) were observed with blur bands and more severe muscular edema. Intriguingly, all morphological changes induced by I/R mentioned above were dramatically improved in SOD-treated or MSODa-treated rats (Fig. 3H or 3I).

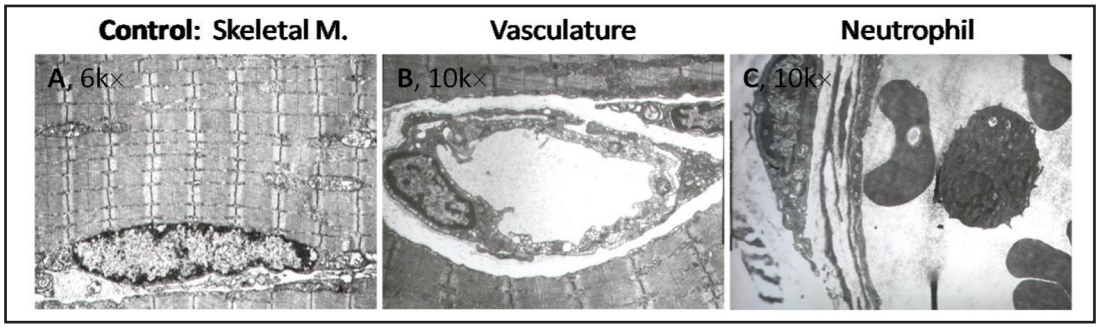

Fig. 4. Normal ultrastructure of skeletal muscles, vasculature, and white blood cells in control rats. (A) the skeletal muscles show normal arrangement with clear bands with a spindle shape of nucleus and large amount of mitochondria around nucleus and glycogens between intermuscular muscle fibers. (B) The endothelial integrity and the diameter of vessel are in good shape. (C) Neutrophils show near round shape with slight wrinkles on the surface.

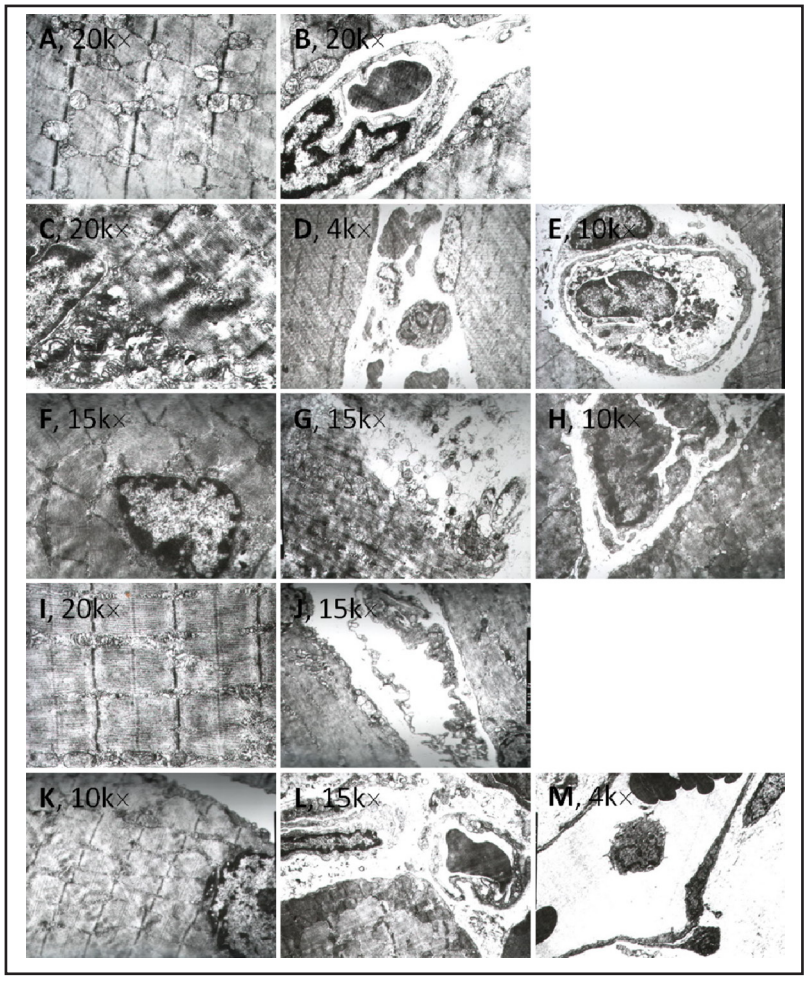

Fig. 5. Changes in ultrastructure in of skeletal muscles after ischemia/reperfusion injury, and protective effects of SOD and MSODa in rats. (A and B) typical changes after ischemia; (C – E) typical changes at 4h after I/R injury; (F – H) typical changes at 8h after I/R injury; (I – J) Protective effects of SOD on I/R injury; (K – M) Protective effects of MSODa on I/R injury.

Effects of MSODa on ultrastructure changes in skeletal muscle before and after ischemia/reperfusion injury

In order to verify the morphological changes induced by I/R and protective effects of SOD or MSODa, the ultrastructural examination was also conducted using emission electromicroscope (EM) and the results indicated that, in sham control rats, the sarcomere arrangement was in alignment with clear bands, the spindle shape nucleus surrounded with mitochondria, and abundant glycogens between muscular fibers (Fig. 4A); The endothelial

integrity of vasculature was observed with proper diameter (Fig. 4B); the ball-shaped neutrophil could also be seen in the center of the lumen with slight wrinkles on the surface (Fig. 4C).

Undergoing the ischemia (Fig. 5A and B), the arrangement of muscular fibers were remain in order with slight fuzzy bands, weak mitochondrial swelling, and reduced glycogens, as well as the vessel wall thickening with contracted status and the heterochromatin marginalization and less pinocytosis vesicles. As expected, all morphological alternations mentioned above were aggravated progressively along with the reperfusion, manifested as increased gap of muscular fibers with significant fuzzy bands, focal muscular dissolution, vacuolar mitochondria, few glycogens surroundings, neutrophil tissue infiltration, and no reflow phenomenon with a likelihood irregular and stiffness of neutrophil membrane, and intermittent endothelium (Fig. 5C-E) at 4 h after I/R; Furthermore, by increasing the reperfusion time up to 12 h after ischemia (Fig. 5F-H), the more severer morphological changes were occurred, showing that the shape of the nucleus for skeletal muscle was changed with obvious migration into muscular tissue, the structure of sarcomere became dimness, and the muscular fibers were not in alignment with dramatic fuzzy bands, large scale of dissolution, and mitochondria vacuolar degeneration; additionally, the nucleus of endothelia projected into the lumen, the vascular integrity was further and significantly damaged.

Surprisingly, the mentioned morphological changes were significantly improved by pretreatment of I/R rats with either SOD (Fig. 5 I and J) or MSODa (Fig. 5K-M).

Discussion

Major findings and potential clinical impacts

Using an established rat model of experimentally induced I/R injury of skeletal muscle in the left hind leg , we have shown that the SOD and MSODa, an artificially synthesed compound $Cu_2(C_3N_2H_4)_6(C_3H_2H_2)Cl_3$ based upon the natural Cu-Zn (Cu-ZnSOD), exhibit a significant protective effects against I/R-induced oxidative stress through the reduction in expression of integrin (CD11b/CD18) and adhesion molecule (ICAM-1) on neutrophil and endothelium leading to less adhesion, migration, and tissue infiltration, and resulting in an attenuation of neutrophil-mediated no reflow and tissue dissolution. Importantly, the dynamic changes in pathophysiological expression of both integrin and adhesion molecule are well paralleled with the time course of MDA and MPO changing, and morphological alternations as well during I/R injury. The MDA and MPO are the metabolic products of lipid peroxidation, and the correlation analysis for MDA vs CD11b/CD18 and ICAM-1 demonstrated that the regression coefficients (r^2) are 0.34 and 0.24, respectively, for SOD, and 0.48 and 0.39, respectively, in the case of MSODa. These observations strongly suggest that SOD and MSODa play a critical role in protective against I/R injury in rat skeletal muscle preparation and interestingly, the advantage and prospective of MSODa may become the first priority in clinical application due mainly to its SOD-like activity, no cost-quality tradeoff profile, and physicochemical characteristics, such as molecular size, easiness crossing the membrane, and lesser immunogenicity as compared with native SOD [27].

I/R mediated oxidative stress in skeletal muscle

The oxidative stress and free radicals are key players in I/R-mediated microvasculature [28] and muscular cell injuries [29, 30]. Whereas, the neutrophils contribute significantly to I/R-mediated tissue and cellular damage [31, 32] via toxic free radicals release during adhesion, migration and tissue infiltration. During this pathophysiological processes of I/R, the adhesion molecules including CD11b/CD18 on neutrophil [33, 34] and ICAM-1 [34, 35] on endothelium are the most important mediators. Our current study showed that both plasma CD11b/CD18 and tissue ICAM-1 increased significantly and progressively during I/R, consistent and paralleled well with the time course of MDA and MPO measurements,

and morphological observations as well regarding the alternations in skeletal muscle, vasculature, and neutrophil, suggesting the great contribution of neutrophil-orientated free radicals to I/R injury and the close involvement of both CD11b/CD18 and ICAM-1 in this pathophysiological process.

Advantage and prospective of MSODa

The SOD are the native enzymes that alternately catalyze the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Mice lacking SOD develop a wide range of pathology, including cancer, an acceleration of age-related muscle mass loss, and earlier incidence of cataracts and reduced lifespan, even die several days after birth [36-39]. Thus, the SOD is an integral part of antioxidant defense in nearly all living cells exposed to oxygen. In our observation, the SOD significantly reduces the plasma MDA and tissue MPO, at the meantime, attenuates the expression of adhesion molecules, including CD11b/CD18 on neutrophils and ICAM-1 on endothelium, and importantly, reverses in parallel the morphological alternations. Surprisingly, the MSODa, a synthesized modeled coordination compound upon the active center of Cu-ZnSOD displays even better protective effects in all aspects against I/R injury in skeletal muscle preparation. Moreover, other property of MSODa, such as dramatically smaller size of the molecule (M.W.: 708.5) compared with SOD (32000~40000), which makes MSODa easily being transported through the membrane. Additionally, the immunogenicity is an other key issue to limit native SOD to be widely used in clinically because it is commonly obtained from bovine liver, however, the immunogenicity for MSODa would be significant lesser than that of native SOD, which would greatly benefit to all patients with indications. Finally, the cost for utilizing the MSODa is tremendously reduced compared with getting the native SOD.

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Disclosure Statement

No conflict of interests.

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