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Supporting Information

Mesoporous Silica Nanoparticles for Glutathione-Triggered Long-Range and Stable Releasing Hydrogen Sulfide

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EXPERIMENTAL SECTION

Chemicals and Reagents:

Diallyl trisulfide (DATS) was purchased from Shanghai Harvest Pharmaceutical Co., Ltd. (Shanghai, China). Tetraethylorthosilicate (TEOS) (AR) ethanol and concentrated ammonia solution (28 wt %), and cetyltrimethylammonium bromide (CTAB) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Cyanine 3 (Cy3) and Cyanine 7 (Cy7) were gained from Beijing OKEANOS TECH Co., Ltd. (Shanghai, China). GSH, monobromobimane (MBB), triton-X100, phalloidin and other chemical reagents were obtained from Sigma-Aldrich. Other reagents were of analytical grade and used as purchased. All the solutions were prepared by Milli-Q water and deaerated with high-purity nitrogen before experiments.

***Apparatus and Measurements*:**

The field-emission scanning electron microscopy (FESEM) images were characterized by a NanoSEM 450 (FEI Nova, USA) and a FE-SEM S-4800 (Hitachi, Japan) field-emission scanning electron microscope. The high-resolution transmission electron microscopy (HRTEM) images were recorded by a JEM-2100F (JEOL, Japan) and a Tecnai T20 (FEI, USA) transmission electron microscope. The absorption spectrum was recorded by a UV-vis spectrometer (U-3900H, Hitachi, Japan). N_2 adsorption-desorption were measured with a Micromeritics Tristar 3020 analyzer (Micromeritics, USA). Before the measurements, the sample was degassed in vacuum at 180 °C for at least 6 h. The Brunauer-Emmett-Teller (BET) method was utilized to calculate the surface areas. The pore size distributions were retrieved by using the Barrett-Joyner-Halanda (BJH) method. All experiments were performed at room temperature and the pH value was measured with a pH meter.

Preparation of mesoporous silica nanoparticles (MSN):

The synthesis of MSN was carried out by sol-gel method.[1] Briefly, 2.1 mL of NaOH (2 M) and 0.6 g of CTAB (99%) were mixed in 300 mL of deionized water. The mixture was heated at 80 °C and stirred at 200 rpm for 1 h, followed by addition of 3 mL of TEOS (98%). After another 2 h, the precipitate was collected by centrifugation (14000 rpm, 10 min) and washed with water and ethanol for twice. Finally, the pore-generating template, CTAB, was removed by stirred for 2 h at 80 °C in ethanol solution (100 mL) and ammonium hydroxide (1 mL), then washed with water and ethanol for several times. Finally, the obtained MSN was dried under vacuum to give the white powder.

Synthesis of DATS-loaded MSN (DATS-MSN):

Drug loading was carried out by the previously modified method.[2] In the procedure, 10 mg of MSNs was dispersed in 2 mL of distilled water. Then, 8 mg of DATS was dispersed in MSN dispersion and the mixture with a ratio of drug/carrier 0.8/1 was stirred at room temperature for 12 h. The composite nanoparticles loaded with DATS were collected by centrifugation (14000 rpm, 10 min). Drug-loaded nanoparticles were washed with distilled water to remove DATS on the surface of MSNs.

The amount of DATS unloaded in supernatant was analyzed by UV-vis at the wavelength of 210 nm. Unloaded DATS was subtracted from initial total DATS to calculate the mass of DATS loaded in MSNs. The drug loading content (mass of drug loaded in nanoparticles /mass of drug loaded nanoparticles*100 %) and entrapment efficiency (mass of drug loaded in nanoparticles / initial total mass of drug *100 %) were calculated.

To study the release ability, DATS-loaded MSNs were dispersed in 1 mL of PBS (0.01 M, pH 7.4). The dispersion was transferred into a dialysis bag (cut off molecular weight 14,000 g/mol) kept in a beaker with 100 mL of PBS. The system was treated with ample stir at 37 ^{o}C. At fixed time intervals, 2 mL of PBS in beaker was taken out and analyzed by UV-vis at the wavelength of 210 nm to calculate the amount of DATS, and 2 mL of PBS was added to keep the volume constant. The releasing content was calculated by the formula: Releasing content (%) = (mass of drug in the release medium / mass of drug loaded in nanoparticles)*100 %.[3]

Characterization:

The structure of DATS-MSN was analyzed by TEM and HRTEM images. The diameters of samples were acquired by averaging size of 50 nanoparticles in TEM images. Dynamic light scattering (DLS) Autosizer 4700 (Malvern, MA, UK) was used to measure the size distribution. Polydispersity index (PDI) was the ratio of volume average diameter (dv) to number average diameter (dn) in DLS measurements. The samples for DLS were tested at 25 °C with concentration of 5 μg/mL. .

In vitro H_2S release of DATS-MSN:

In vitro, H_2S production from DATS-MSN was mainly assessed by high-performance liquid chromatography (HPLC) analysis according to the method reported by Shen et al.[4] Briefly, DATS-MSN (100 μg/mL) was added to glass chambers containing PBS (100 mM, 4 mL), and GSH (2 mM or 200 μM or 20 μM) was separately added. Then every 30 μL of the sample was withdrawn, mixed with 70 μL of Tris-HCl (100 mM, pH 9.5, 0.1 mM of DTPA) and 50 μL of MBB solution (10 mM, in CH_3CN) to form the stable sulfide-dibimane derivative. The reaction was stopped after 30 min by adding 50 μL of 200 mM ice-cold 5-sulfosalicylic acid. 100 μL supernatant were transferred to an HPLC vial equipped, analyzed by an Agilent Technologies HPLC (1260 infinity, CA, USA)

with fluorescence detection (λ_{ex}: 390 nm and λ_{em}: 475 nm) and an Eclipse XDB-C18 column (150 * 4.6 mm, 5 μm). Concentration of H_2S was determined using a standard curve of Na_2S (0-100 μM). Effect of time (0 to 12 h), pH (8.0, 7.4 and 6.0) and temperature (37, 20 and 4 ^oC) on the release of H_2S from DATS-MSN was also studied.

HPLC analysis could not be used to evaluate the very early stage of the release process due to technical limitations. So the early release course was assessed by H_2S-selective microelectrode assay: DATS-MSN (10 μg/mL), DATS (10 μg/mL) and NaHS (10 μM) was separately added to a glass chamber (World Precision Instruments, WPI, USA) containing PBS (100 mM, pH 7.4, 4 mL) at 37 °C. Then 2 mM of GSH was added and H_2S formation was detected using ISO-H_2S-2 sensor attached to an Apollo 1100 Free Radical Analyser (WPI, FL, USA). H_2S release was real-time displayed by picoamps (PA) current curve.

Cytotoxicity assays of DATS-MSN:

All animal experiments were approved by Institutional Review Board and Institutional Animal Care and Use Committee Protocols of Fudan University.

Cytotoxicity assay of DATS-MSN was assessed using primary neonatal cardiomyocytes obtained from the cardiac tissue of newborn Sprague-Dawley rats (1-3 d) according to the previously described method.[5] Isolated cardiomyocytes were seeded at a density of $1*10^5$ cells/mL in Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12, Gibco Invitrogen, CA, USA) supplemented with 10 % of fetal bovine serum, 100 U/mL of penicillin, 100 mg/mL of streptomycin, and 100 mM of 5-bromodeoxyuridine. Cells were cultured in a humidified incubator at 37 °C with 95% air and 5% CO_2 for 72 h.

The DATS-MSN was diluted with culture medium to gain a concentration range from 0 to 100 μg/mL, and was added to 96-well flat-bottomed tissue-culture plate with cardiomyocytes seeded. After incubation for 24 h, medium was removed, and cells were washed with PBS, then the medium was replaced with cell counting kit-8 (CCK-8) solution (Dojindo Laboratories, Kumamoto, Japan). The absorbance of individual wells was measured at 450 nm by a microplate reader (Molecular Devices, FlexStation 3, CA, USA). The results were expressed as the mean percentage of cell viability relative to control.

In vitro cellular uptake:

Fluorophore Cy3 (-NHS) was covalent conjugated to the MSN framework (-NH_2) as follows: 5 mg of Cy3 (-NHS) and 100 mg of MSN-NHS samples (connected by APS) were diluted and mixed in 50 mL of PBS (pH 7.4). After stirring for 24 h at room temperature, the Cy3-MSN complex was washed with PBS thrice, followed by centrifuging for 30 min at 12000 rpm and dialyzed in PBS for 48 h.

Neonatal rat cardiomyocytes were treated with the Cy3-MSN (50 μg/mL) for 4 h. After washed with PBS, the cells were fixed by 4% paraformaldehyde for 15 min, penetrated by 0.1% triton-X 100 for 20 min, stained by phalloidin (conjugated with AF488) for 20 min and DAPI for 15 min. Then the Cy3-MSN inside cells were visualized using a fluoresce microscope (Olympus IX-71, Japan).

Protection effect of DATS-MSN from hypoxia/reoxygenation induced damage in rat cardiomyocytes:

Neonatal cardiomyocytes were seeded into 96-well flat-bottomed tissue-culture plate at 5×10^3 cells/well, and incubated for 24 h in DMEM/F-12 with glucose and serum at 5% CO_2 and 37 °C. Then DATS-MSN (100 μg/mL) + GSH (2 mM), DATS (100 μg/mL) + GSH (2 mM), NaHS (100 μM) and saline of same volume (Control) were separately added to culture medium. After incubation for 4 h, medium was removed and replaced with DMEM/F-12 without glucose and serum. Then the cells were exposed to hypoxia (94% N_2, 5% CO_2, 1% O_2) for 4 h in a CO_2 incubator (Forma SERIES II WATER JACKET, Thermo Scientific, MA, USA), followed by reoxygenation (5% CO_2) for 1 h. After which, the cell viability was evaluated by CCK-8 assay and compared among 4 groups.

After the hypoxia/reoxygenation procedure, lactate dehydrogenase (LDH) activities of each group were also measured and compared to evaluate cytotoxicity using an assay kit (JianCheng, Nanjing, China) according to the manufacturer's instructions. The absorbance was determined by a micro plate reader at 440 nm.

In vivo H_2S release of DATS-MSN in plasma:

For *in vivo* experiments, male Sprague-Dawley rats (weight, 200 to 220 g, n = 6) were anesthetized with medetomidine hydrochloride (250 μg/kg, IP.) and ketamine hydrochloride (50 mg/kg, IP.). Carotid arteries were cannulated for blood withdrawal. DATS-MSN (10 mg/kg), DATS (10 mg/kg) and NaHS (100 μmol/kg) were separately administrated by tail vein injection. Blood (0.2 mL) was withdrawn at time intervals (0 to 9 h) after administration. The blood collected was anticoagulated with heparin sodium (50 U/mL) and centrifuged (3000 rpm, 15 min) to obtain plasma. H_2S concentration of DATS-MSN in plasma was measured by HPLC method described above, except for extra centrifuging of samples at 12000 rpm at 4 °C for 10 min after 5-sulfosalicylic acid added.

In vivo imaging of fluorophore labelled MSN:

Fluorophore Cy7 (-NHS) was covalent conjugated to the MSN framework (-NH2) as follows: 5 mg of Cy7 (-NHS) and 100 mg of MSN-NHS samples (connected by APS) were diluted and mixed in 50 mL of PBS (pH 7.4). After stirring for 24 h at room temperature, the Cy7-MSN complex was washed with PBS thrice, followed by centrifuging for 30 min at 12000 rpm and dialyzed in PBS for 48 h.

In vivo imaging was performed using the IVIS® Imaging System Spectrum CT and analyzed by the IVIS Living Imaging 4.3.2 software (PerkinElmer, MA, USA). To evaluate the biodistribution of MSN, male nude mice (20 g, 6-8 weeks old, n=6) and male Sprague-Dawley rats (weight, 200 to 220 g, n=6) were used. CY7-MSN (5 mg/kg) was administrated to both mice and rats by tail vein injection. At the time points of 2 h, 24 h or 7 d after injection, mice were placed in the chamber of the spectrum CT. A Cy7 filter set was used to acquire the fluorescence light and optical fluorescence images were obtained (λ_{ex}: 747 nm and λ_{em}: 774 nm). After surface tomography, 3D reconstruction of Fluorescence Light Imaging Tomography (FLIT) was performed by the imaging software. Meanwhile, at 2 h, 24 h and 7 d, Sprague-Dawley rats were sequentially sacrificed, then the heart, liver, spleen, lung, kidney and brain were immediately harvested for *ex vivo* fluorescence imaging. The fluorescence signal intensity of the organs were normalized and quantified as photons per centimeter squared per second per steradian (p/cm^2/s/sr).

H_2S concentration in different organs:

Sprague-Dawley rats (n=6) administrated by DATS-MSN (10 mg/kg) or saline were sacrificed at 2 h, 24 h and 7 d. Heart, liver, spleen, lung, kidney and brain were quickly incised and stored at -80 °C. Then small pieces of tissues (about 50 mg) were homogenated in 500 μL of PBS (pH of 7.4). H_2S concentration of the homogenate was determined by HPLC analysis. The protein content of tissue was measured by the bicinchoninic acid (BCA) method using a BCA Protein Assay Kit (Pierce, IL, USA). The H_2S content of samples were quantified by protein content and expressed in proportion relative to control.

Effect of DATS-MSN on heart rates, blood pressure and cardiac function:

Sprague-Dawley rats (n = 6) were anesthetized and cannulated as described above. Carotid arteries catheters were connected to Model SMUP-E4 Bioelectric Signals Processing System (MFLab301) to display rat real time heart rates and blood pressure, which were recorded every 5 min. DATS-MSN (10 mg/kg) or NaHS (100 μmol/kg) were administrated by tail vein injection. Then change of blood pressure and heart rates was evaluated.

Rat cardiac function was evaluated by echocardiography at 1 h after drug administration. Same volume of saline was injected into control group. Transthoracic echocardiography was performed using the Philips IE 33 system and a 12-4 MHz linear transducer (S12-4, Philips, AMS, NED). Left ventricular internal dimension in systole (LVIDs), left ventricular internal dimension in diastole (LVIDd), left ventricular posterior wall in diastole (LVPWd), left ventricular interventricular septum in diastole (LVSd), left ventricular end-diastolic volume (LVEDV) and left ventricular end-systolic volume (LVESV) were obtained from the M-mode tracings. Ejection

fraction (EF) and fractional shortening (FS) were also derived to evaluate cardiac function. All echocardiographic measurements were performed by skilled observer blindly.

Hematological, serological and histological examinations:

At 2 h, 24 h and 7 d after injection of DATS-MSN (10 mg/kg) and saline into the rats (n = 6), blood was collected for hematological analysis and evaluation of hepatic and renal function. For hematological analysis, blood was preserved in sodium ethylenediaminetetra-acetic acid tubes. The red blood cells (RBC), white blood cells (WBC), platelets (PLT), the hemoglobin level (HGB), hematocrit (HCT) and white blood cell distribution (neutrophils, lymphocytes) were all determined by Automatic Hematological Analyzer (XE-2100, SYSMEX, Japan). The levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine (CRE) and urea nitrogen in the serum (BUN) were determined using Automatic Analyzer (7600-120, HITACHI, Japan) according to the instruction provided by the commercial assay kits.

The rats (n=6) were sacrificed at the same time point, and major organs including heart, liver, spleen, lung, kidney and brain were collected, fixed in 4.0% paraformaldehyde, paraffin-embedded, sectioned and stained with hematoxylin and eosin (H&E) using standard methods.

Protection effect of DATS-MSN from ischemic/reperfusion (I/R) injury in isolated rat hearts:

Male Sprague-Dawley rats (weight, 200 to 220 g, n=6 for each group) were anesthetized and heparinized (0.2 mL) by IP. injection. Hearts were rapidly excised and mounted on the Langendorff mode. Retrograde perfusion was established at a pressure of 75 mm Hg with an oxygenated (95% O_2, 5% CO_2), 37 °C Krebs-Henseleit (K-H) buffer containing NaCl (118 mM), KCl (4.7 mM), $CaCl_2$•$2H_2O$ (2.5 mM), MgSO4•$7H_2O$ (1.7 mM), $NaHCO_3$ (25 mM), KH_2PO_4 (1.2 mM), glucose (5.5 mM). Then a water-filled latex balloon connected to a pressure transducer was inserted into the left ventricle through the incised left atrium and mitral valve, and the volume was adjusted to maintain left ventricular end-diastolic pressure to 8-12 mmHg. The pressure transducer was connected to the computer and the following parameters were derived by Model SMUP-PC Bioelectric Signals Processing System (Cardio Version 2.00): heart rate (HR), left ventricle developed pressure (LVDP, systolic minus diastolic pressure) and maximum rate of left ventricular pressure development (+dp/dt max, -dp/dt max). Perfused K-H buffer was collected to evaluate coronary flow (CF, mL/min) and to measure LDH and creatine kinase (CK) by assay kit.

After stabilizing for 30 min, the items mentioned above were recorded as baseline. Then the following 4 buffers were perfused for 1 h: (1) Control: normal K-H buffer, (2) NaHS group: K-H buffer + NaHS (100 μM), (3) DATS group: K-H buffer + DATS (100 μg/mL) + GSH (2 mM) and (4) DATS-MSN group: K-H buffer + DATS-

MSN (100 μg/mL) + GSH (2 mM). All solutions were formulated just before the experiment. After the perfusion, hearts of all groups were exposed for 30 min global ischemia followed by 1 h reperfusion with normal K-H buffer. At the end of the reperfusion, the recovery of hemodynamic data was evaluated in percentage of the baseline value. Then the isolated rat hearts were fixed in 4.0% paraformaldehyde, paraffin-embedded, sectioned and performed a TUNEL assay. The cell nuclei were stained with 3,3-diaminobenzidine (DAB) color development kits (Roche, Basil, CH) according to the manufacturer's instructions. Cell nuclei stained brown were defined as TUNEL-positive nuclei and were monitored using a fluorescence microscope (Olympus IX-71, Japan). The proportion of TUNEL positive nuclei per 500 nuclei was quantified at *400 magnification.

Statistical analysis:

All statistics were performed using SPSS Statistics Base 17.0 for Windows. Continuous data were expressed as mean ± standard errors (SEM). One-way analysis of variance (ANOVA) was used to examine statistical comparisons between groups. The significant difference between two groups was analyzed by Student's t test. A value of $P < 0.05$ was considered to be significant. All authors had full access to, and take full responsibility for the integrity of the data.

References:

1 I.I. Slowing, B.G. Trewyn and V.S. Lin, *J. Am. Chem. Soc.*, 2007, **129**, 8845-8849.

2 H. Tang, J. Guo, Y. Sun, B. Chang, Q. Ren and W. Yang, *Int. J. Pharm.*, 2011, **421**, 388-396.

3 H. Tang, S. Shen, J. Guo, B. Chang, X. Jiang and W. Yang, *J. Mater. Chem.*, 2012, **22**, 16095-16103.

4 X. Shen, C.B. Pattillo, S. Pardue, S.C. Bir, R. Wang and C.G. Kevil, *Free Radical Biol. Med.*, 2011, **50**, 1021-1031.

5 J. Sadoshima, L. Jahn, T. Takahashi, T.J. Kulik and S. Izumo, *J. Biol. Chem.*, 1992, **267**, 10551-10560.

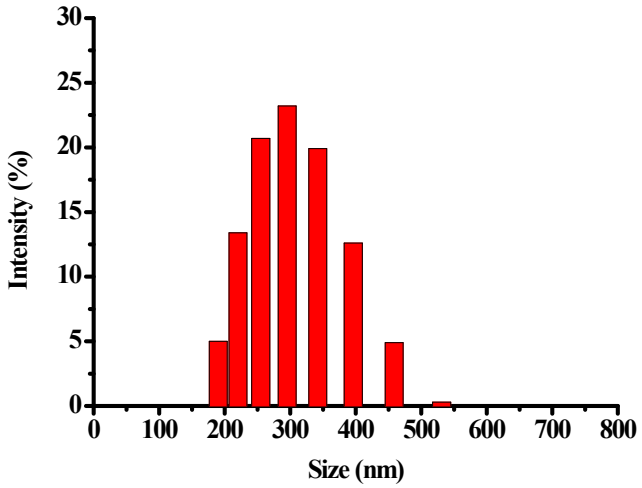

Fig.S1. Dynamic light scattering (DLS) result of DATS-MSN.

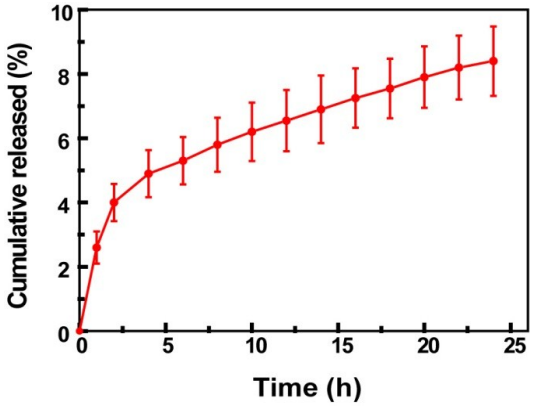

Fig.S2. Release of DATS from DATS-MSN at different times in PBS solution (pH 7.4, 37 °C), DATS release was measured by UV-vis (mean ± SEM, n=3).

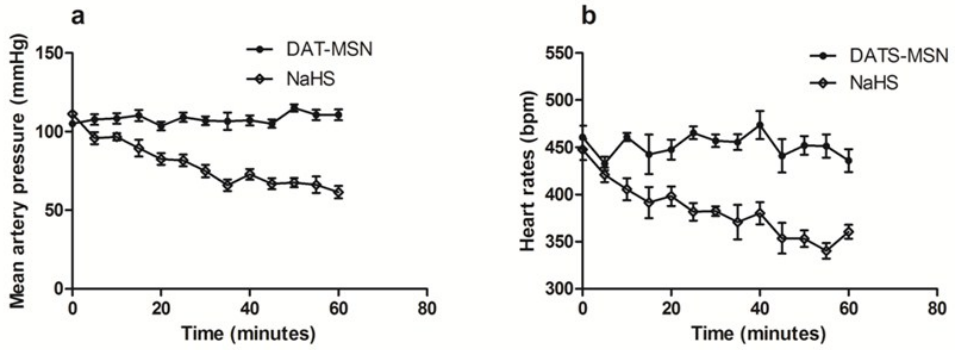

Fig.S3. *In vivo* safety evaluation of DATS-MSN in cardiovascular system. DATS-MSN (10 mg/kg) and NaHS (100 μmol/kg) on rat (a) blood pressure and (b) heart rates in 1 h after tail vein injection (mean ± SEM, n = 6).

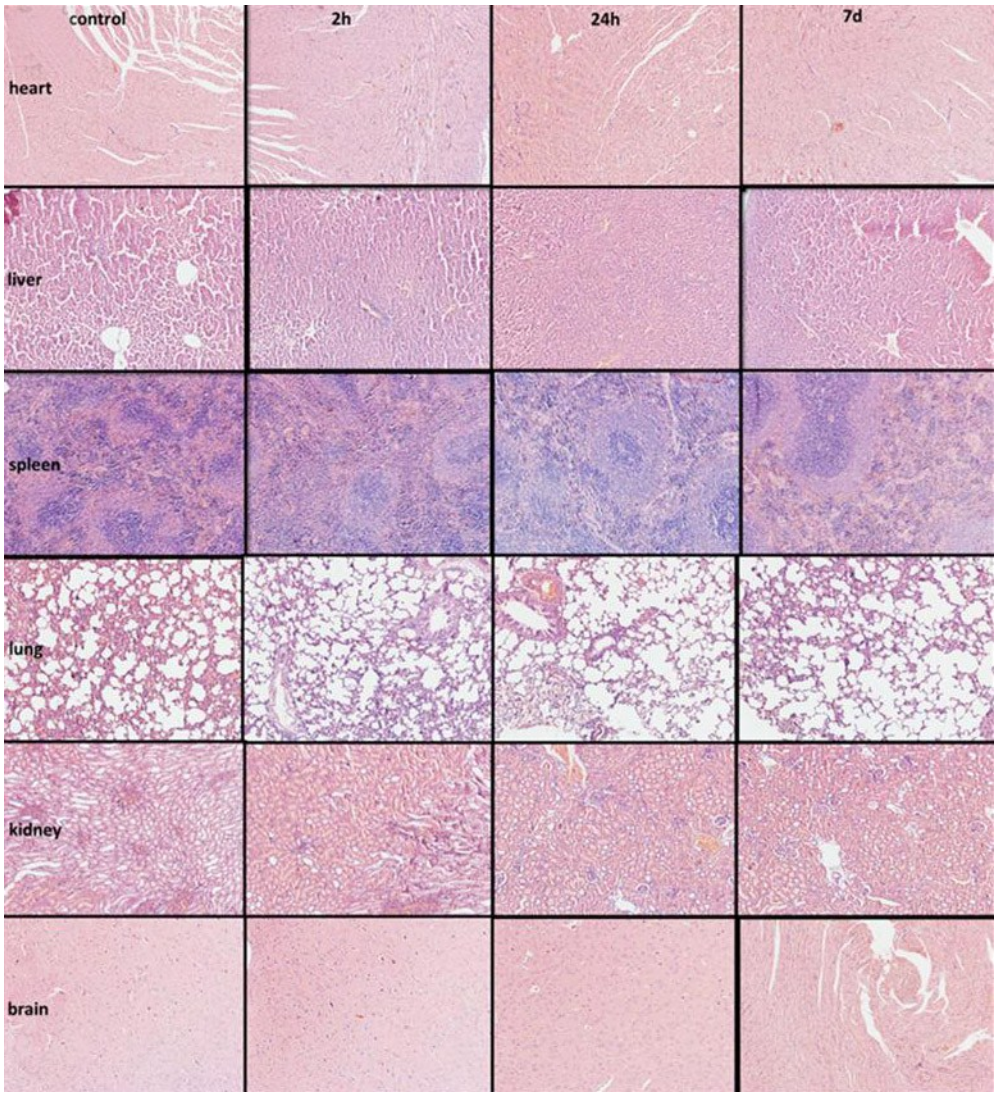

Fig.S4. Histological examination of tissues (heart, liver, spleen, lung, kidney and brain) from rats at 2 h, 24 h and 7 d post injection of DATS-MSN (10 mg/kg).

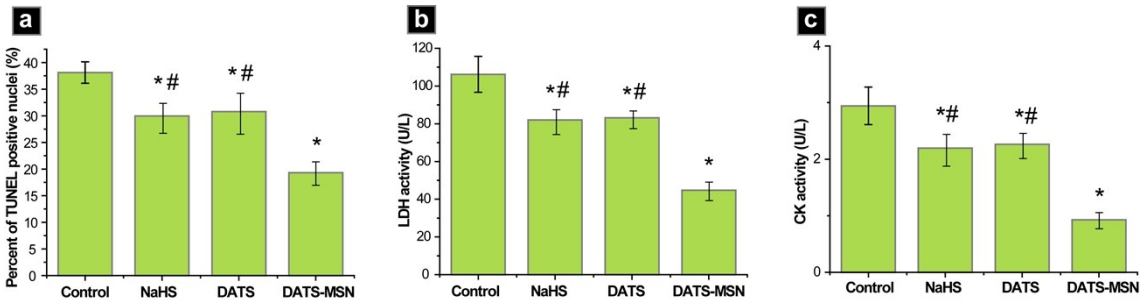

Fig.S5. Myocardial protection effect of the pretreatment of DATS-MSN (100 μg/mL) from ischemic/reperfusion injury compared with NaHS (100 μM), DATS (100 μg/mL) and control: (a) Percent of TUNEL positive nuclei, (b) Lactate dehydrogenase (LDH) activity and (c) Creatine kinase (CK) activity (mean ± SEM, n = 6). *: $P < 0.05$ compared with Control, #: $P < 0.05$ compared with DATS-MSN group.

TABLES

Table 1: Cardiac structure and function treated by DATS-MSN and NaHS by echocardiography

	Control	NaHS (100 μmol/kg)	DATS-MSN (10 mg/kg)
EF %	97.13 ± 1.93	96.59 ± 1.77	97.69 ± 1.49
FS %	78.23 ± 1.86	78.25 ± 1.30	79.08 ± 1.26
LVEDV (mL)	148.24 ± 8.22	143.55 ± 7.79	161.54 ± 13.12
LVESV (mL)	4.13 ± 0.26	4.78 ± 0.38	3.77 ± 0.47
LVIDd (mm)	407.38 ± 7.95	393.47 ± 7.36	409.52 ± 8.52
LVIDs (mm)	104.61 ± 9.56	103.08 ± 4.43	91.17 ± 5.87
LVPWd (mm)	169.83 ± 4.85	165.59 ± 5.72	165.59 ± 11.26
LVSd (mm)	160.57 ± 4.47	171.24 ± 15.30	167.16 ± 11.23

Data were presented as mean ± SEM (n = 6). There was no significant difference of any data between groups. EF: ejection fraction, FS: fractional shortening, LVEDV: left ventricular end-diastolic volume, LVESV: left ventricular end-systolic volume, LVIDd: left ventricular internal dimension in diastole, LVIDs: Left ventricular internal dimension in systole, LVPWd: left ventricular posterior wall in diastole and LVSd: left ventricular interventricular septum in diastole.

Table 2: Hematological and serological results of rats treated with DATS-MSN

	Control	2h	24h	7d
WBC [K μL^{-1}]	6.59 ± 1.61	6.40 ± 1.41	6.37 ± 2.51	5.60 ± 1.61
NEU %	7.44 ± 3.80	7.65 ± 3.37	6.13 ± 2.34	5.02 ± 3.06
LY %	90.38 ± 3.71	88.95 ± 5.74	91.60 ± 3.70	89.44 ± 4.28
RBC [M μL^{-1}]	6.87 ± 0.09	6.95 ± 0.35	7.02 ± 0.27	7.19 ± 0.35
HGB [g dL^{-1}]	145.69 ± 11.12	140.30 ± 9.33	145.60 ± 3.57	144.32 ± 5.09
HCT %	42.98 ± 3.72	41.36 ± 1.96	39.29 ± 2.67	42.38 ± 2.39
PLT [K μL^{-1}]	936.62 ± 187.93	908.32 ± 114.86	915.55 ± 84.81	938.79 ± 136.63
ALT [U L^{-1}]	37.71 ± 8.33	36.42 ± 12.20	36.98 ± 11.90	30.29 ± 7.77
AST [U L^{-1}]	88.30 ± 9.17	93.43 ± 11.56	94.78 ± 19.94	84.18 ± 6.91
BUN [mg dL^{-1}]	6.03 ± 0.35	6.11 ± 0.77	5.81 ± 0.70	6.39 ± 1.10
CRE [mg dL^{-1}]	18.01 ± 2.01	20.00 ± 3.13	18.13 ± 1.73	19.38 ± 2.89

Data were presented as mean ± SEM (n = 6) .There was no significant difference of any data among groups. WBC: white blood cells, NEU: neutrophils, LY: lymphocytes, RBC: red blood cells, HGB: hemoglobin level, HCT: hematocrit, PLT: platelets, ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: urea nitrogen and CRE: serum creatinine.

Table 3: Recovery of hemodynamic parameters after ischemic/reperfusion injury

	Control	DATS-MSN (100μg/mL) + GSH (2mM)	NaHS (100μM)	DATS (100μg/mL) + GSH (2mM)
HR (%)	77.6 ± 3.8	81.3 ± 10.5	73.1 ± 6.3	76.3 ± 7.8
LVDP (%)	44.3 ± 2.9	81.7 ± 8.1*	45.8 ± 2.7#	41.7 ± 3.3*#
+dp/dt max (%)	45.9 ± 2.7	81.5 ± 7.0*	59.8 ± 3.6*#	54.5 ± 5.2*#
- dp/dt max (%)	71.6 ± 3.2	79.2 ± 3.6	70.9 ± 4.1	72.2 ± 3.3
CF (%)	44.6 ± 3.1	89.4 ± 7.9*	64.7 ± 2.9*#	59.4 ± 3.8*#

Data were presented as mean ± SEM (n = 6). HR: heart rate, LVDP: left ventricle developed pressure, dp/dt max: maximum rate of left ventricular pressure development and CF: coronary flow. *: $P < 0.05$ compared with Control, #: $P < 0.05$ compared with DATS-MSN group.