Ultrasound Responsive Carbon Monoxide Releasing Micelle

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Abstract:

Carbon monoxide releasing molecules (CORMs) have been intensively studied in the past decade due to the therapeutic effects of CO. The potential to control the release of CO to a specific target is one of the major advantages of CORM. In this work an ultrasound responsive micelle with pluronic as the shell and CORM-2 as the core were developed. The micelle released CO in the presence of cysteine. After the CO release stopped, applying ultrasound to the micelle suspension produced additional CO. About 1/3 of total CO can be controllably released by ultrasound. The mechanism of the ultrasound induced CO release is interpreted by the reaction between cysteine and CORM-2 inside the micelle.
Carbon monoxide (CO) is produced naturally during heme catabolism. As a gasotransmitter, CO plays important roles in many physiological functions in the mammalian body.\(^1\) Studies have shown that CO has beneficial effects including anti-inflammatory, anti-apoptotic, anticoagulative, anti-hypertensive, cell protective effects etc.\(^2\)-\(^10\) In the preclinical and animal studies, CO was delivered by inhalation or CO releasing molecules (CORMs). Inhaled, small-quantities of supplemental CO gas has been demonstrated in pre-clinical disease models to have therapeutic effects including reducing inflammatory and cardiovascular disorders etc.\(^11\) CORMs are a group of compounds capable of releasing controlled quantities of CO in cellular systems. The majority of CORMs studied are carbonyls of transition metals including both essential trace elements (manganese, iron, cobalt) and non-physiological metals (ruthenium, tungsten, rhenium).\(^1,4\)-\(^10\) Some nonmetallic CORMs have also been developed in recent years.\(^12\)-\(^18\) CORMs allows convenient administration of a certain dose of CO. In addition, the potential to control the release of CO to a specific target is the major advantage of CORMs over gaseous CO as a therapeutic agent. For this purpose, CORMs that response to different stimuli including light, magnetic field, enzyme, and reactive oxygen species (ROS) have been developed in recent years.\(^13\)-\(^24\)

Ultrasound imaging is one of the most widely used diagnostic methods. Ultrasound has also been used in tissue ablation, stone crushing, and transdermal drug delivery etc.\(^25\) In the past decade, ultrasound mediated drug delivery has been intensively studied.\(^26\)-\(^37\) Ultrasound either interrupts cell membrane, which increases cellular uptake of drugs, or interacts with the nano/micro drug carrier and releases the drug encapsulated. Given the deep penetration of ultrasound and well-developed ultrasound technology, ultrasound is an attractive approach for spatial and temporal control of drug delivery. No ultrasound mediated CO delivery has been reported before. Herein, we report a nanomaterial that releases CO upon ultrasonication. In addition, a new approach of ultrasound drug release based on prodrug reactivity is demonstrated.

Many CORMs react with common species in biological systems, e.g. water, cysteine, etc. to generate CO. They can be considered as prodrugs of CO. When they are encapsulated in nanocarriers, the CO releasing rate will be significantly reduced. If ultrasound can induce leakage on the shell of the nanocarrier, not only the encapsulated CORM can move out of the nanocarrier to react with the biospecies, but also the reactive biospecies can go inside to react with the CORM and release CO. (Figure 1) The latter mechanism is important since the compounds encapsulated in nanocarriers (in this case the CORM) are often hydrophobic and thus
difficult to escape from the nanocarrier. Since CO is a gas, it can be quickly released from inside of the nanocarrier. Therefore, the shell of the nanocarrier does not have to be significantly destructed to cause significant drug release, which means a relatively low intensity of ultrasound may be used. Several well-studied CORMs, e.g. CORM-A1 release CO by reacting with water, which is the most common species in biological systems. Theoretically, an ultrasound responsive nanocarrier containing this type of CORMs shall be able to release CO faster under ultrasound than without ultrasound. However, nanocarriers including micelles, nanoparticles etc. are often prepared by adding a solution of a hydrophobic drug and a surfactant into an aqueous solution. If a CORM releases CO by hydrolysis, it will not be stable during the preparation.

![Figure 1](image)

**Figure 1.** Illustration of the ultrasound mediated CO release based on a reaction between a CORM and a common biospecies.

CORM-2 \([\text{Ru}_2\text{Cl}_4(\text{CO})_6]\) is one the most extensively studied CORM. It has shown various therapeutic effects in many animal tests.\(^{38}\) It was thought that CORM-2 quickly releases CO via hydrolysis. However, recent studies by Poole and coworkers showed that CO release from CORM-2 was actually very slow in water and very quick in the presence of active sulfur compounds including cysteine and sodium dithionite.\(^{39}\) The latter is commonly used in the myoglobin test for measuring the concentration of CO. Since cysteine, glutathione, and proteins with free cysteine residues are abundant inside cells,\(^{40}\) CORM-2, which reacts with water very slowly but reacts with a common biospecies quickly and releases CO, can be used for preparing the ultrasound responsive CO releasing material. Pluronics are FDA approved drug delivery polymers, which form micelles in water. Previous studies have shown that pluronic micelles responded to ultrasound and released drugs encapsulated.\(^{35-37,41,42}\) Therefore, pluronics were used as the surfactant for the preparation of the CORM-2 micelle.
Pluronic micelle containing CORM-2 was prepared by adding an acetone solution of CORM-2 dropwise to an aqueous solution of pluronic F-127 quickly stirred. The ratio between CORM-2 and the pluronic was 1:5. The solution became cloudy indicating the formation of the micelle suspension. The suspension was stirred for 5 min and then lyophilized to yield a white powder. It should be noted that the CORM-2 used has a formula of Ru₂Cl₄(CO)₆·1/4 H₂O. We found that the water in the material greatly increased its solubility in acetone. When CORM-2 without water was used, it was difficult to dissolve. (Detailed procedure is given in the Experimental section.) The white powder obtained after lyophilization was a little hygroscopic and turned sticky after some days. Therefore, it was stored in a vial filled with nitrogen in refrigerator. In this case, no change of appearance was observed after weeks. Infrared spectroscopy showed strong CO stretching peaks at 2064 cm⁻¹ indicating that CORM-2 was not decomposed during the preparation. The size of the micelle was studied by dynamic light scattering (DLS) and Transmission electron microscopy (TEM). (Figure 2) DLS showed a mean size of 92 nm. TEM showed dark particles with diameters of ~20 nm, which is much smaller than that from DLS. Since the heavy metallic Ru(II) ion is much more sensitive to TEM than the pluronic shell, the dark particle observed by TEM is the CORM-2 core.

![IR spectrum and TEM image](image.png)

**Figure 2.** IR spectrum (left) and TEM image (right) of the CORM-2/pluronic micelle.

The CORM-2/pluronic micelle was resuspended in water and the CO release from the micelle was studied. CO release from CORMs is commonly measured by myoglobin test and/or head space method using a CO meter. As mentioned above, the sodium dithionite used in myoglobin test can react with CORM-2 and release CO. Therefore, we used the head space method with a setup commonly used in CORM research. Basically, a small amount of the CORM solution in an
open vial is sealed in a bigger container with a CO meter. The CO released from the CORM solution can be calculated using the following equation:

\[ N_{CO} = \frac{pV_g}{RT} + cV_l = p\left(\frac{V_g}{RT} + \frac{V_l}{k}\right) \]

[where \( p \) is partial pressure of CO (CO meter readings); \( V_g \) is volume of the gas phase; \( V_l \) is liquid phase; \( R \) is 0.08205 L-atm·mol⁻¹·K⁻¹; \( T \) is Temperature; \( c \) is CO concentration in the liquid phase; \( k \) is Henry’s law constant of CO in water (1052.63 L-atm·mol⁻¹ at 25 °C)].

As expected, no CO was detected when the aqueous suspension of CORM-2/pluronic micelle was tested. A solution of cysteine was then added to the CORM-2/pluronic micelle suspension until the final concentration of cysteine was 3.5 mM. The molar ratio of cysteine and CORM-2 was 10:1. A 3 mL suspension was put in the setup of CO measurement. After 15 min, the CO meter gave a steady reading of 4 ppm. Calculation showed that 5.9% of CORM-2 released CO after addition of the cysteine. In previous works, the cysteine concentration used for CO release from CORM-2 and its derivatives was 1-10 mM. The concentration of free cysteine in human blood plasma is about 1-2 mg/100 mL, which is 0.08-0.16 mM. Although not only free cysteine but also the cysteine in proteins and other reactive compounds such as glutathione may react with CORM-2, it is reasonable to use a relatively low concentration of cysteine. The concentration (3.5 mM) used in this test led to 4 ppm of CO in our measurement setup, further lowering the concentration will result in unreliable data since the resolution of the meter is 1 ppm.

To test whether ultrasound can assist the release of CO, the CORM-2/pluronic suspension containing the cysteine was ultrasonicated for 15 min. A commercial therapeutic ultrasound equipment was used to apply ultrasound to the micelle. Therapeutic ultrasound equipment has been used for in vitro evaluation of ultrasound responsive materials before. The micelle suspension was sealed in a container with the ultrasound probe ~3 mm below the surface. The frequency was 1 MHz; intensity was 2.5 w/cm²; and the duty cycle was 1:1. After the suspension was ultrasonicated, 3 mL of the solution was taken with a syringe from a small hole sealed with rubber, and then transferred to the head-space setup with a CO meter. A steady reading of 6 ppm was observed, which indicates an increase of 2 ppm comparing to the CO release (4 ppm) without ultrasonication. (Figure 3) Calculation showed that 8.9% of CORM-2 released CO. Ultrasonication for another 15 min did not yield a higher reading of CO. The experiment was
repeated seven times with calibrated gas meters operated by different people. Increase of CO release after ultrasonication was observed every time. The average reading of CO after addition of cysteine and before ultrasonication was 3.9±0.7 ppm, and that after ultrasonication was 6.3±0.8 ppm. We noticed that ultrasonication increased the temperature from room temperature (~25 °C) to ~31°C during the 15 min period. In fact, this is the reason that we used 1:1 duty cycle instead of continuous mode since the continuous mode raised the temperature to nearly 40 °C. To confirm that the CO release increase was not due to a thermal effect, the CORM-2/pluronic suspension with cysteine was kept at room temperature for 15 min, and then put in a heating bath at 31 °C for another 15 min. No increase of CO concentration was detected after heating. (Figure 3)

Figure 3. [CO] detected after addition of cysteine, ultrasonication, and heating.

The CO released from CORM-2/pluronic micelle by ultrasonication was about 50% of the CO released before ultrasonication, which means 1/3 of total CO can be delivered to the location under ultrasonication. The amount CORM-2 reacted (6% and 9%) in cysteine solution (3.5 mM) is comparable with the previously reported values for CORM-2 and its derivatives especially those with polymers. Previous work showed that vasodilatory activity level of 150-300 μM CORM-2 was comparable to a 10 μM CO solution on isolated rat afferent arterioles, which indicates that therapeutic effects can be generated from the CO released from a few percentage of CORM-2.

In summary, this work shows that CORM-2/pluronic micelle releases CO upon ultrasonication in the presence of cysteine. A common therapeutic ultrasound equipment is strong enough to
release significant amount of CO. This is the first ultrasound responsive CORM that has been reported. Using pluronic, which is a FDA approved drug delivery polymer, and CORM-2, which has been studied in many animal tests, as the components is a major advantage and could allow animal studies in near future. The low reactivity of CORM-2, especially when the concentration of the reactive sulfur species is low, is a concern. In addition, the side product of CORM-2 after CO release is likely to be hydrophobic and stay in the micelle. Part of the CORM-2 in the core may be covered by them and cannot react. In the future, ultrasound responsive CO releasing materials with high reactivity, high CO content, and low toxicity are required for clinical applications.

**Experimental Section:**

*General methods.* Unless otherwise noted, reagents and solvents were commercially available and used as received without any further purification. CORM-2 with a formula of \( \text{Ru}_2\text{Cl}_4(\text{CO})_8 \cdot \frac{1}{4} \text{H}_2\text{O} \) were purchased from Tocris. Pluronic F-127 was purchased from Sigma Aldrich. Ultrasonication was conducted using a Physio Sound UT ultrasound physical therapy machine. Dynamic light scattering (DLS) measurements were carried out on a SZ-100 Nanopartica Series Instrument manufactured by HORIBA. TEM image was taken with a Zeiss EM900 Transmission Electron Microscope. Partial pressure of CO was measured using a Honeywell BW Solo Gas Detector.

*Preparation of CORM-2/pluronic micelle.* Pluronic F-127 (50 mg) was dissolved in 5 mL deionized water at a concentration. To this solution, was added dropwise 0.5 mL acetone (anhydrous) solution containing 10 mg of CORM-2. The solution turned cloudy after addition. The mixture was left to stir for 5 min, then was lyophilized, which yielded a white powder. The white powder was somewhat hydroscopic. Therefore it was kept in a vial filled with some nitrogen in a refrigerator.

*Evaluation of the CO release from the CORM-2/pluronic micelle.* The CORM-2/pluronic micelle (60 mg) was dissolved in 55 mL of DI water and transferred to a 60 mL container with the ultrasound probe on the top of the container through a hole, and another hole sealed with rubber films. After the container was sealed, 2 mL of stock solutions of cysteine was added via the hole sealed with rubber using a syringe. At this time, the probe was about 3 mm below the surface of the whole solution. The final concentration of cysteine was 3.5 mM. After 15 min, 3 mL of the
mixture was taken by a syringe and transferred to the head-space setup, which is described in the main text, to measure the CO released from the micelle.

Ultrasound was then applied to the remaining mixture using a therapeutic ultrasound equipment. The frequency was 1 MHz; intensity was 2.5 w/cm\(^2\); and the duty cycle was 1:1. After 15 min of ultrasonication, 3 mL of the mixture was transferred to the head-space setup for CO measurement. Another 15 min of ultrasonication was applied to the remaining mixture and then CO content in 3 mL of the mixture was measured as above.

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Reference:
1. R. Motterlini, L. E. Otterbein, The therapeutic potential of carbon monoxide, *Nat. Rev. Drug. Discov.*, 2010, **9**, 728-743.
2. L. E. Otterbein, R. Foresti, R. Motterlini, Heme Oxygenase-1 and Carbon Monoxide in the Heart: The Balancing Act Between Danger Signaling and Pro-Survival, *Circ. Res.*, 2016, **118**, 1940-1959.
3. X. Ji, K. Damera, Y. Zheng, B. Yu, L. E. Otterbein, B. Wang Toward Carbon Monoxide-Based Therapeutics: Critical Drug Delivery and Developability Issues, *J. Pharm. Sci.*, 2016, **105**(2), 406-416.
4. D. Babu, R. Motterlini, R. A. Lefebvre, CO and CO-releasing molecules (CO-RMs) in acute gastrointestinal inflammation, *J. Pharmacol.*, 2015, **172**(6), 1557-1573.
5. S. Garcia-Gallego, G. J. L. Bernardes, *Angew. Chem. Int. Ed.*, 2014, **53**, 9712–9721.
6. R. Foresti, R. Motterlini, CO-releasing molecules: avoiding toxicity and exploiting the beneficial effects of CO for the treatment of cardiovascular disorders, *Future. Med. Chem.*, 2013, **5**(4), 367-369.
7. R. Motterlini, B. Haas, R. Foresti, Emerging concepts on the anti-inflammatory actions of carbon monoxide-releasing molecules (CO-RMs), *Med. Gas. Res.*, 2012, **2**, 28.
8. S. H. Heinemann, T. Hoshi, M. Westerhausen and A. Schiller, *Chem. Commun.*, 2014, **50**, 3644–3660.
9. C. C. Ramao, W. A. Blattler, J. D. Seixas, G. J. L. Bernardes, *Chem. Soc. Rev.*, 2012, **41**, 3571-3583.
10. B. E. Mann, *Organometallics*, 2012, **31**, 5728–5735.
11. C. P. Hopper, L. Meinel, C. Steiger, L. E. Otterbein, Where is the Clinical Breakthrough of Heme Oxygenase-1 / Carbon Monoxide Therapeutics?, *Curr. Pharm. Des.*, 2018, **24**(20), 2264-2282.

12. N. Abeyrathna, K. Washington, C. Bashur and Y. Liao, *Org Biomol Chem.*, 2017, **15**, 8692-8699.

13. (a) R. Motterlini, P. Sawle, J. Hammad, S. Bains, R. Alberto, R. Foresti and C. J. Green, *FASEB J.*, 2005, **19**, 284–286. (b) R. Alberto, K. Ortner, N. Wheatley, R. Schibli and A. P. Schubiger, *J. Am. Chem. Soc.*, 2001, **123**, 3135–3136.

14. T. I. Ayudhya, C. C. Raymond and N. N. Dingra, *Dalton Trans.*, 2017, **46**, 882–889.

15. D. Wang, E. Viennois, K. Ji, K. Damera, A. Draganov, Y. Zheng, C. Dai, D. Merlin and B. Wang, *Chem. Commun.*, 2014, **50**, 15890–15893.

16. P. Peng, C. Wang, Z. Shi, V. K. Johns, L. Ma, J. Oyer, A. Copik, R. Igarashi and Y. Liao, *Org. Biomol. Chem.*, 2013, **11**, 6671–6674.

17. (a) L. A. P. Antony, T. Slanina, P. Sobej, T. Solomek and P. Klan, *Org. Lett.*, 2013, **15**, 4552-5. (b) E. Palao, T. Slanina, L. Muchova, T. Solomek, L. Vitek and P. Klan, *J. Am. Chem. Soc.*, 2016, **138**, 126–133.

18. (a) M. Popova, T. Soboleva, S. Ayad, A. D. Benninghoff and L. M. Berreau, *J. Am. Chem. Soc.* 2018, **140**, 9721-9729. (b) S. N. Anderson, J. M. Richards, H. J. Esquer, A. D. Benninghoff, A. M. Arif and L. M. Berreau, *ChemistryOpen*, 2015, **4**, 590–594.

19. J. Ou, W. Zheng, Z. Xiao, Y. Yan, X. Jiang, Y. Dou, R. Jiang and X. Liu, *J. Mater. Chem. B*, 2017, **5**, 8161-8168.

20. A. C. Kautz, P. C. Kunz and C. Janiak, *Dalton Trans.*, 2016, **45**, 18045-18063.

21. O. S. Qureshi, A. Zeb, M. Akram, M. Kim, J. Kang, H. Kim, A. Majid, I. Han, S. Y. Chang, O. N. Bae and J. Kim, *Eur. J. Pharm. Biopharm.*, 2016. **108**, 178-195.

22. D. Nguyen, N. M. Adnan, S. Olivera and C. Boyer, *Macromol. Chem. Rapid Commun.*, 2016, **37**, 37, 739-744.

23. P. C. Kunz, H. Meyer, J. Barthel, S. Sollazzo, A. M. Schmidt and C. Janiak, *Chem. Commun.* 2013, **49**, 4896-4898.

24. G. Dordelmann, H. Pfeiffer, A. Birkner and U. Schatzschneider, *Inorg. Chem.*, 2011, **50**, 4363-4367.

25. A. Schroeder, J. Kost, Y. Barenholz, Ultrasound, liposomes, and drug delivery: principles for using ultrasound to control the release of drugs from liposomes, *Chem Phys Lipids*, 2009, **162**, 1–16.

26. H. Xia, Y. Zhao, R. Tong, Ultrasound-Mediated Polymeric Micelle Drug Delivery. *Adv Exp Med Biol.*, 2016, **880**, 365-84.

27. H.Y. Lin, J.L. Thomas, PEG-lipids and oligo(ethylene glycol) surfactants enhance the ultrasonic permeabilizability of liposomes, *Langmuir*, 2003, **19**, 1098–1105.

28. S. Mitragotri, Healing sound: the use of ultrasound in drug delivery and other therapeutic applications, *Nature Rev. Nat Rev Drug Discov*, 2005, **4**, 255–260.
29. H.J. Kim, H. Matsuda, H.S. Zhou, I. Honma, Ultrasound-triggered smart drug release from a poly(dimethylsiloxane)- mesoporous silica composite, *Adv Mater*, 2006, **18**, 3083–3088.

30. A.G. Skirtach, B.G. De Geest, A. Mamedov, A.A. Antipov, N.A. Kotov, G.B.J. Sukhorukov, Ultrasound stimulated release and catalysis using polyelectrolyte multilayer capsules, *J Mater Chem.*, 2007, **17**, 1050–2054.

31. K. Park, Focused ultrasound for targeted nanoparticle delivery to tumors, *J Control Release*, 2010, **146**, 263.

32. C. Oerlemans, W. Bult, M. Bos, G. Storm, J.F.W. Nijsen, W.E. Hennink, Polymeric micelles in anticancer therapy: targeting, imaging and triggered release, *Pharm Res.*, 2010, **27**, 2569–2589.

33. S. R. Sirsi, M. A. Borden, State-of-the-art materials for ultrasound-triggered drug delivery, *Adv Drug Deliv Rev.*, 2014, **72**, 3–14.

34. F. Kiessling, S. Fokong, J. Bzyl, W. Lederle, M. Palmowski, T. Lammer, Recent advances in molecular, multimodal and theranostic ultrasound imaging, *Adv Drug Deliv Rev.*, 2014, **72**, 15–27.

35. W. G. Pitt, G. A. Husseini, L. N. Kherbeck, Smart Materials for Drug Delivery, *RSC*, 2013, **6**, 169–171.

36. W. G. Pitt, G.A. Husseini, B. J. Staples, Ultrasonic drug delivery – a general review, *Expert Opin Drug Deliv*, 2004, **1**, 37–56.

37. G. A. Husseini, W. G. Pitt, Micelles and nanoparticles for ultrasonic drug and gene delivery, *Adv Drug Deliv Rev.*, 2008, **60**, 1137–1152.

38. For example: a) H. Yin, J. Fang, L. Liao, H. Nakamura, H. Maeda, Styrene-maleic acid copolymer-encapsulated CORM2, a water-soluble carbon monoxide (CO) donor with a constant CO-releasing property, exhibits therapeutic potential for inflammatory bowel disease, *J. Control Release*, 2014, **187**, 14–21. b) L. S. Nobre, H. Jeremias, C. C. Romão, L. M. Saraíva, Examining the antimicrobial activity and toxicity to animal cells of different types of CO-releasing molecules. *Dal Trans*, 2016, **45**, 1455–1466. c) R. Motterlini, B. E. Mann, R. Foresti, Therapeutic applications of carbon monoxide-releasing molecules. *Expert Opin Inv Drug*, 2005, **14**, 1305–1318. d) D. Donaghy, S. Yoo, T. Johnson, V. Nielsen, C. Olver, Carbon Monoxide-Releasing Molecule Enhances Coagulation and Decreases Fibrinolysis in Normal Canine Plasma, *Basic. Clin Pharmacol Toxicol*, 2018, **123**, 257–262.

39. S. McLean, B. E. Mann, R. K. Poole, Sulfite species enhance carbon monoxide release from CO-releasing molecules: implications for the deoxymyoglobin assay of activity, *Anal Biochem.*, 2012, **427**, 36–40.

40. G. Saito, J. A. Swanson, K. D. Lee, *Adv. Drug Delivery Rev.*, 2003, **55**, 199–215.

41. R. Tong, X. Lu, H. Xia, A facile mechanophore functionalization of an amphiphilic block copolymer towards remote ultrasound and redox dual stimulus responsiveness, *Chem Commun.*, 2014, **50**, 3575–3578.

42. P. Wu, Y. Jia, F. Qu, Y. Sun, P. Wang, K. Zhang, C. Xu, Q. Liu, X. Wang, Ultrasound-responsive polymeric micelles for sonoporation-assisted site-specific therapeutic action. *ACS Appl Mater Interfaces*, 2017, **9**, 25706–25716.
43. U. Hasegawa, A. J. Van Der Vlies, E. Simeoni, C. Wandrey and J. A. Hubbell, *J. Am. Chem. Soc.*, 2010, **132**, 18273-18280.

44. A. J. van der Vlies, R. Inubushi, H. Uyama, U. Hasegawa, Polymeric Framboidal Nanoparticles Loaded with a Carbon Monoxide Donor via Phenylboronic Acid-Catechol Complexation, *Bioconj Chem.*, 2016, **27**, 1500-8.

45. W. H. Stein, S. Moore, The free amino acids of human blood plasma, *J Biol Chem.*, 1954, **211**, 915-26.

46. S. Chang, T. Si, S. Zhang, M. A. Merrick, D. E. Cohn, R. X. Xu, Ultrasound mediated destruction of multifunctional microbubbles for image guided delivery of oxygen and drugs. Ultrason, *Sonochem.*, 2016, **28**, 31-38.

47. F.T. Botros, L.G. Navar, Interaction between endogenously produced carbon monoxide and nitric oxide in regulation of renal afferent arterioles, *Am J Physiol Heart Circ Physiol.*, 2006, **291**, H2772-2778.