Comparison between adenosine and isoflurane for assessing the coronary flow reserve in mouse models of left ventricular pressure and volume overload

Jieyun You,* Jian Wu,* Junbo Ge, and Yunzeng Zou

Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital and Institutes of Biomedical Sciences, Fudan University, Shanghai, China

Submitted 14 August 2012; accepted in final form 17 September 2012

You J, Wu J, Ge J, Zou Y. Comparison between adenosine and isoflurane for assessing the coronary flow reserve in mouse models of left ventricular pressure and volume overload. Am J Physiol Heart Circ Physiol 303: H1199–H1207, 2012. First published September 21, 2012; doi:10.1152/ajpheart.00612.2012.—Adenosine and high-concentration isoflurane are commonly used to induce hyperemia for assessment of coronary flow reserve (CFR) in mice, but high-concentration isoflurane may exacerbate cardiac dysfunction, leading to impaired CFR. However, there is no study to find comparing the effects of adenosine and isoflurane on CFR and corresponding cardiac function. High-resolution echocardiography and invasive hemodynamic assessment were performed in 20 mice 2 wk after transverse aortic constriction (TAC), aortic regurgitation (AR), and corresponding sham operation. CFR was calculated as the ratio of hyperemic to basal peak diastolic velocity (CFRpdv) or diastolic velocity-time integral (CFRdvti). In the sham-operated mice, no differences were observed between the effects of adenosine and isoflurane on CFR, left ventricular systolic function (left ventricular ejection fraction and fractional shortening), left ventricular end-systolic pressure, maximal contraction and relaxation velocity (+dp/dt and −dp/dt), alteration of left ventricular pressure (∆LVP), or ± dp/dt (Δdp/dt). But adenosine-derived results were significantly higher than isoflurane-derived ones in both the TAC and the AR groups. Moreover, CFRpdv or CFRdvti was positively correlated with both LVEF and LVFS. Compared with adenosine-derived CFR, isoflurane-derived CFR may be underestimated in the TAC and the AR mice, which is probably associated with suppressed cardiac function.

pressure overload; volume overload; adenosine; coronary flow reserve; isoflurane

CORONARY MICROcirculation dysfunction, a cause of impaired blood supply to the myocardium, is critically involved in the evolution of heart diseases (22). Coronary flow reserve (CFR) is an important functional parameter to validate coronary microcirculation, which is associated with myocardial viability, cardiac function, and remodeling (2). It is routinely measured by transthoracic Doppler echocardiography (17) and calculated as the ratio of hyperemic to basal coronary flow or velocity (17, 28). In clinical practice, CFR has been widely used for detecting coronary stenosis and confirming successful revascularization (7). Impaired CFR, an independent prognostic predictor for long-term cardiovascular events, has been reported in patients with coronary artery disease, hypertension, cardiomyopathy, diabetes mellitus, obesity, hyperlipidemia, and syndrome X (2, 18, 19, 21). In mice, hyperemic coronary flow can be elicited by intravenous adenosine infusion (27) or by high-concentration isoflurane inhalation (11). Compared with adenosine, a conventional vasodilator (24, 27), 2.5% isoflurane is increasingly applied to dilate coronary arterioles and provoke hyperemic coronary flow for its convenience by obviating tail vein cannulation (1, 11, 12, 15).

Previous literature reported that isoflurane- and adenosine-derived CFR were comparable in normal or type 2 diabetic mice (15). Nevertheless, CFR is closely related with cardiac function (10, 20). Given that isoflurane can depress cardiac function through myofilament desensitization (5), the findings in mice without significant cardiac overloading could not be simply extrapolated to mice with loading changes (e.g., pressure or volume overload) or cardiac remodeling (concentric or eccentric hypertrophy). Moreover, although cardiac structure and function were comparable under 1 and 2% isoflurane anesthesia in normal mice (32), it is still unknown whether high-concentration isoflurane exacerbates cardiac dysfunction in mice with cardiovascular disease, resulting in miscalculation of CFR.

In this study, by using high-frequency ultrasound, we aimed to 1) validate the feasibility of CFR measurement in mouse models with pressure [transverse aortic constriction (TAC)] and volume overload [aortic regurgitation (AR)] derived by adenosine and high-concentration isoflurane and 2) compare the effects of adenosine and isoflurane on CFR and evaluate the correlations between CFR and cardiac function.

MATERIALS AND METHODS

Mice and Surgery

A total of 23 wild-type C57BL/6J mice (male, 8–11 wk old, 25.06 ± 0.40 g, purchased from Shanghai Branch of National Rodent Laboratory Animal Resources, Shanghai, China) were enrolled in this study. Mice were housed at 24 ± 2°C under a 12:12-h light-dark cycle with ad libitum access to water and standard laboratory mouse chow. Animal experiments were performed in accordance with Guide for the Care and Use of Laboratory Animals (revised 1996; National Institutes of Health, Bethesda, MD), and the study protocol was approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

TAC was produced in eight mice, as we described previously (16, 31). Briefly, the mice were anesthetized by intraperitoneal injection of a mixture of ketamine (150 mg/kg) and xylazine (10 mg/kg), endotracheally intubated, and ventilated (type 7025; Harvard Apparatus, March-Hugstetten, Germany). Parasternal thoracotomy was performed in the second intercostal space. The transverse aorta was first isolated, and a blunted 27-gauge needle was tied to the aorta between the origins of innominate artery and left common carotid artery and then removed promptly to yield a constriction. Finally, the chest wall was closed with a 4-0 silk suture, and meloxicam (0.13 mg each) was administered subcutaneously for analgesia. The corresponding sham-operated mice (sham; n = 4) underwent the same procedure except for ligation of the aorta.

AR was performed under the guidance of ultrasound imaging in seven mice, as described by Zhou et al. (33). Briefly, the mice were anesthetized the same way as those subjected to TAC operation. A plastic catheter was introduced via the right common carotid artery
and advanced to the aortic orifice. A metal wire was then guided through the catheter to disrupt the aortic valves until Doppler recording showed significant diastolic retrograde flow in the aorta arch. Finally, the catheter, together with the metal wire, was withdrawn, and the right common carotid artery was ligated. Meloxicam (0.13 mg each) was administered subcutaneously for analgesia. The corresponding sham-operated mice (shamM; n = 4) underwent the same procedure except for destruction of the aortic valves.

Of all the mice, one TAC and one AR mouse died during surgery, and one TAC mouse died within 2 wk after surgery. The data of the dead were excluded from statistics.

**Echocardiography**

At 2 wk after surgery, transthoracic echocardiography was performed using a high-frequency ultrasound system Vevo770 (Visual-Sonics, Toronto, ON, Canada) with a 30-MHz central frequency scanhead, which provided a spatial resolution of ~115 (lateral) by ~55 μm (axial).

**Aortic arch flow.** The B-mode aortic arch was visualized in a right parasternal long-axis view (9, 31), with Doppler sample volume positioned in the aortic arch between innominate artery and left common carotid artery (31, 33). The aortic arch Doppler flow spectrum was recorded as the peak systolic velocity of aortic arch flow (PSVa), systolic velocity-time integral of aortic arch flow (SVTia), peak diastolic velocity of aortic arch flow (PDVa), and diastolic velocity-time integral of aortic arch flow (DVTai).

**Left ventricular structure and function.** The M-mode recording was made at the middle level of left ventricle in a left parasternal long-axis view. Left ventricular structure and systolic function were measured as described previously (29, 31), including left ventricular end-diastolic (LVEDD) and end-systolic dimensions (LVEDS), left ventricular anterior wall end-diastolic (LVAWTd) and end-systolic thickness (LVAWTts), left ventricular posterior wall end-diastolic (LVPWTd) and end-systolic thickness (LVPWts), and left ventricular ejection fraction (LVEF) and fractional shortening (LVFS).

**Left coronary artery flow.** The B-mode left coronary artery (LCA) was imaged in a left parasternal long-axis view, with Doppler sample volume placed in the proximal LCA, as we described previously (30). CFR was calculated as the ratio of hyperemic to basal peak diastolic velocity (CFRpdv) or hyperemic to basal diastolic velocity-time integral of LCA flow (CFRdvt) (23, 28, 30).

**Invasive Hemodynamic Study**

Left ventricular hemodynamics was evaluated 2 wk after surgery, as we described previously (16, 31). Briefly, a micromanometer (Millar 1.4F, SPR 835; Millar Instruments, Houston, TX) was inserted through the right common carotid artery into the aorta and carefully advanced into the left ventricle in the TAC mice and their corresponding sham group, whereas the micromanometer was inserted via the left common carotid artery in the AR mice and their corresponding sham group because the right common carotid artery was already occluded during operation. The transducer was connected to a Power Laboratory system (AD Instruments, Castle Hill, Australia) to record heart rate, left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), and maximal contraction and relaxation velocity (+dp/dt and −dp/dt). ΔLVP was calculated as the difference between LVESP and LVEDP and Δdp/dt as the difference between +dp/dt and −dp/dt.

**Measurement of Basal and Hyperemic Parameters**

In the same mouse the basal cardiac structure, cardiac function, Doppler peak diastolic velocity (PDVc) and diastolic velocity-time integral of LCA (DVTCc), and left ventricular pressure were measured during 1% isoflurane inhalation, whereas the hyperemic ones were measured ≥3 min after persistent 2.5% isoflurane inhalation or adenosine infusion (140 μg·kg⁻¹·min⁻¹, under 1% isoflurane) via tail vein (11, 28, 30). The elution period of 2.5% isoflurane or adenosine was ≥5 min. All procedures were performed on a thermostatic animal platform, with the heat rate controlled ~500 beats/min (29, 30).

**Statistics**

Echocardiographic assessment had good intra- and interobserver agreement, as reported in our previous studies (29, 30). Parameters were presented as means ± SE unless otherwise stated. Correlation analyses were performed by the software SigmaPlot 12.0 (Jandel Scientific, San Rafael, CA) and other analyses by SPSS 15.0 (SPSS, Chicago, IL). Multiple comparisons were conducted using one-way or two-way ANOVA with least significant difference test. Pearson's correlation analysis was performed to evaluate associations between continuous variables. P < 0.05 was considered statistically significant.

**RESULTS**

**Establishment of the TAC and the AR Models**

Doppler flow of the aortic arch, echocardiographic inner dimension and wall thickness of left ventricle, and left ventricular pressure at 2 wk after operation confirmed successful TAC and AR establishment (31, 33). PSVa, SVTia, and LVESP were all strikingly increased in the TAC mice compared with the sham mice (Figs. 1 and 2 and Table 1). Thickened ventricular wall and impaired ±dp/dt were presented in both the TAC and the AR mice, and dilated left ventricular inner dimension was observed in the AR mice (Fig. 1 and Table 1).

**Greater Decreased CFR Using 2.5% Isoflurane Inhalation Compared With Intravenous Adenosine Infusion in Both the TAC and the AR Mice**

In both the shamM and the shamM mice, 2.5% isoflurane inhalation and adenosine infusion remarkably increased PDVc and DVTCc over basal condition, and no significant difference was found in isoflurane- or adenosine-derived CFR (Fig. 3).

The TAC mice showed increased basal PDVc and DVTCc compared with the shamM mice (PDVc: 450 ± 15 vs. 253 ± 33 mm/s, DVTCc: 1.96 ± 0.12 vs. 1.15 ± 0.10 cm, both P < 0.05). After 2.5% isoflurane inhalation, hyperemic PDVc and DVTCc increased significantly over the basal condition (PDVc:...
EFFECTS OF ADENOSINE AND ISOFLURANE ON CFR IN MICE

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00612.2012 • www.ajpheart.org
Fig. 2. Left ventricular hemodynamics during 1% ISO inhalation (A), 2.5% ISO inhalation (B), and AD infusion (C). Left ventricular end-systolic pressure (LVESP; D), left ventricular end-diastolic pressure (LVEDP; E), the difference between LVESP and LVEDP (ΔLVP; F), maximal contraction velocity (+dp/dt; G), maximal relaxation velocity (−dp/dt; H), and the difference between +dp/dt and −dp/dt (Δdp/dt; I) during 1% ISO inhalation, 2.5% ISO inhalation, and AD infusion. *P < 0.05 vs. the corresponding values during 1% ISO inhalation in the same group; †P < 0.05 vs. the corresponding values during 2.5% ISO inhalation in the same group.
626 ± 19 vs. 450 ± 15 mm/s, DVTIc: 3.08 ± 0.07 vs. 1.96 ± 0.12 cm, both \( P < 0.05 \), and a greater increase was observed after adenosine infusion compared with 2.5% isoflurane inhalation (PDVc: 749 ± 27 vs. 626 ± 19 mm/s, DVTIc: 3.71 ± 0.09 vs. 3.08 ± 0.07 cm, both \( P < 0.05 \)). Hyperemic PDVc and DVTIc induced by adenosine infusion in the TAC mice were comparable with those in the sham mice (PDVc: 749 ± 27 vs. 741 ± 54 mm/s, DVTIc: 3.71 ± 0.09 vs. 4.10 ± 0.13 cm, both \( P > 0.05 \)). As a result, CFRpdv and CFRdvti induced by 2.5% isoflurane inhalation were lower than those induced by adenosine infusion (CFRpdv: 1.40 ± 0.02 vs. 1.68 ± 0.10, CFRdvti: 1.59 ± 0.06 vs. 1.95 ± 0.17, both \( P < 0.05 \); Fig. 3).

The AR mice also demonstrated increased basal PDVc and DVTIc compared with the sham mice (PDVc: 553 ± 7 vs. 324 ± 33 mm/s, DVTIc: 2.29 ± 0.15 vs. 1.50 ± 0.05 cm, both \( P < 0.05 \)). After 2.5% isoflurane induction, hyperemic PDVc and DVTIc tended to increase over basal condition, albeit without statistical significance (PDVc: 583 ± 60 vs. 553 ± 7 mm/s, DVTIc: 2.67 ± 0.29 vs. 2.29 ± 0.15 cm, both \( P > 0.05 \)). In contrast, hyperemic PDVc and DVTIc were increased significantly after adenosine infusion (PDVc: 827 ± 95 vs. 553 ± 7 mm/s, DVTIc: 3.72 ± 0.52 vs. 2.29 ± 0.15 cm, both \( P < 0.05 \)), reaching the level in the sham mice (PDVc: 827 ± 95 vs. 838 ± 20 mm/s, DVTIc: 3.72 ± 0.52 vs. 4.61 ± 0.30 cm, both \( P > 0.05 \)). As a result, CFRpdv and CFRdvti induced by 2.5% isoflurane were lower than those induced by adenosine infusion (CFRpdv: 1.05 ± 0.11 vs. 1.49 ± 0.17, CFRdvti: 1.21 ± 0.18 vs. 1.66 ± 0.28, both \( P < 0.05 \); Fig. 3).

**Greater Impairment of Cardiac Function During 2.5% Isoflurane Inhalation Compared With Adenosine Infusion in Both the TAC and the AR Mice**

Either in the sham, or the sham, mice, no significant difference was found in left ventricular systolic function (LVEF and LVFS), left ventricular pressure (LVESP, LVEDP, and \( \Delta LP \)), and contraction and relaxation velocity (+dp/dt, −dp/dt, and \( \Delta dp/dt \)) during 1% isoflurane inhalation, 2.5% isoflurane, or adenosine infusion (all \( P > 0.05 \)) (Figs. 1 and 2).

The TAC mice showed comparable left ventricular systolic function (LVEF and LVFS), left ventricular pressure (LVESP, LVEDP, and \( \Delta LP \)), and contraction and relaxation velocity (+dp/dt, −dp/dt, and \( \Delta dp/dt \)) during 1% isoflurane inhalation and adenosine infusion. However, compared with 1% isoflurane inhalation or adenosine infusion, 2.5% isoflurane inhalation induced a decrease in left ventricular systolic function (LVEF: 49.21 ± 5.56 vs. 60.11 ± 1.68 or 67.03 ± 0.85%, LVFS: 24.72 ± 3.25 vs. 31.46 ± 1.15 or 36.04 ± 0.66%, all \( P < 0.05 \)), left ventricular pressure (LVESP: 130.68 ± 3.02 vs. 155.16 ± 3.59 or 156.30 ± 4.81 mmHg, \( \Delta LP \): 126.38 ± 3.04 vs. 151.02 ± 3.52 or 152.12 ± 4.90 mmHg, all \( P < 0.05 \)), and contraction and relaxation velocity (+dp/dt: 5.120 ± 412 vs. 7.408 ± 621 or 7.302 ± 694 mmHg/s, −dp/dt: 4.787 ± 284 vs. 5.925 ± 307 or 6.103 ± 283 mmHg/s, \( \Delta dp/dt \): 9.907 ± 630 vs. 13.333 ± 737 or 13.405 ± 822 mmHg/s, all \( P < 0.05 \)) (Figs. 1 and 2).

In the AR mice, similar results were obtained in left ventricular systolic function (LVEF and LVFS), left ventricular pressure (LVESP, LVEDP, and \( \Delta LP \)), and contraction and relaxation velocity (+dp/dt, −dp/dt, and \( \Delta dp/dt \)) during 1% isoflurane inhalation and adenosine infusion. However, compared with 1% isoflurane inhalation or adenosine infusion, 2.5% isoflurane inhalation induced attenuated left ventricular systolic function (LVEF: 36.83 ± 3.67 vs. 58.73 ± 2.54 or 64.55 ± 2.32%, LVFS: 17.99 ± 1.95 vs. 31.31 ± 1.78 or 35.34 ± 1.66%, all \( P < 0.05 \)), left ventricular pressure (LVESP: 91.84 ± 1.75 vs. 113.11 ± 5.25 or 113.93 ± 5.28 mmHg, \( \Delta LP \): 54.75 ± 3.69 vs. 76.96 ± 6.43 or 77.60 ± 4.90 mmHg, all \( P < 0.05 \)), and contraction and relaxation velocity (+dp/dt: 2.916 ± 351 vs.
Fig. 3. Coronary flow velocity waveform during 1% ISO inhalation (A), 2.5% ISO inhalation (B), and AD infusion (C). Peak diastolic velocity of left coronary artery flow (PDVc; D) and diastolic velocity-time integral of left coronary artery flow (DVTIc; E) during 1% ISO inhalation, 2.5% ISO inhalation, and AD infusion. Hyperemic to basal peak diastolic velocity of left coronary artery flow (CFRpdv; F) and hyperemic to basal diastolic velocity-time integral of left coronary artery flow (CFRdvti; G) derived from 2.5% ISO inhalation and AD infusion. D and E: *P < 0.05 vs. the corresponding values during 1% ISO inhalation in the same group; †P < 0.05 vs. the corresponding values during 2.5% ISO inhalation. F and G: *P < 0.05 vs. the corresponding values during 2.5% ISO inhalation.
Association Between Cardiac Function and CFR

We further assessed the association between CFR and cardiac function by Pearson’s correlation analysis. CFRpdv or CFRdvti was positively associated with LVEF and LVFS during both adenosine infusion and 2.5% isoflurane inhalation (all \( P < 0.05 \)) (Figs. 1 and 2).

**DISCUSSION**

Two mouse models of cardiovascular disease were employed in this study. TAC produced pressure overload by afterload increase, and AR induced volume overload by preload increment. Both models induced cardiac remodeling 2 wk after operation, indicating aggravated cardiac work and oxygen consumption of the overloaded heart. Interestingly, both models demonstrated impaired CFR mainly through increased basal coronary flow.

Using high-frequency ultrasound, we showed that CFR measurement was feasible in mice with pressure and volume overload. Adenosine infusion and high-concentration isoflurane inhalation were sufficient to induce hyperemic coronary flow in mice (12, 15). Similar to previous studies (12, 32), we found that 2.5% isoflurane- and adenosine-derived CFR or cardiac function were comparable in the sham-operated mice. However, isoflurane as a volatile anesthetic is known to depress cardiac function through myofilament desensitization (5). Therefore, high attention should be paid to the possible depressing effect of high-concentration isoflurane on cardiac function and CFR in cardiac remodeling mice. Indeed, we found that 2.5% isoflurane-derived CFR was significantly lower than adenosine-derived CFR in the TAC and the AR mice. At the same time, cardiac function was significantly suppressed during 2.5% isoflurane inhalation compared with during 1% isoflurane inhalation or adenosine infusion in those mice, especially in the AR mice. We also found that CFR was closely related to cardiac function, which was in accord with previous studies in humans (20) and mice (10). All of the evidence suggests that high-concentration isoflurane-induced cardiac dysfunction may lead to underestimation of CFR.

CFR was calculated as the ratio of hyperemic to basal PDV or DVTI. Since adenosine- and isoflurane-derived CFR were calculated using the same basal coronary flow in the same mice, the greater impairment of CFR by 2.5% isoflurane was accounted for restricted hyperemic coronary flow. In the present study, a lower hyperemic coronary flow in the TAC and the AR mice, in concomitance with greater impairment of cardiac function, was observed during 2.5% isoflurane inhalation compared with during adenosine infusion. Studies have shown that pressure gradient between aorta and distal capillaries provides the driving force for coronary perfusion and that decompression of coronary microcirculation during ventricular diastole produces a dominant suction pressure, accelerating diastolic antegrade flow (4, 25). Thus, direct evidence from coronary perfusion pressure may further explain the different effects of adenosine and high-concentration isoflurane on CFR. However, direct measurement of the coronary perfusion is impossible because of the small size of murine LCA. Instead, we recorded alterations of left ventricular pressure and dp/dt throughout the cardiac cycle. Compared with 1% isoflurane inhalation or adenosine infusion, 2.5% isoflurane inhalation caused lower ΔLVP and Δdp/dt due to cardiac dysfunction, leading to decreased suction pressure from distal microcirculation, impaired hyperemic coronary flow, and eventually, underestimated CFR.

Our findings have important pragmatic implications. Since vasodilators that suppress the cardiac function might lead to bias in the evaluation of coronary microcirculation, they should be used cautiously in CFR estimation, especially in patients with cardiac remodeling and dysfunction. Meanwhile, a number of general anesthetics (including isoflurane) have been found to have negative inotropic effects on myocardial contractility, especially in patients with left ventricular impairment (6). The exacerbation of heart failure during anesthesia might be attributed partially to the suppressive effect of such agents on cardiac function and CFR. Because of higher perioperative morbidity and mortality (14), patients with cardiovascular diseases should be monitored extensively and cared for during perioperative anesthesia.
Considering significant impairment of coronary endothelial function and preservation of nonendothelial function at 2 wk after persistent overload (26), one would speculate that adenosine and high-concentration isoflurane might activate different receptors in coronary arterioles to induce disparate CFR in mice of cardiac remodeling. However, both adenosine and isoflurane exert their vasodilation via endothelial and nonendothelial (smooth muscle) pathways (3, 8, 13). Therefore, it is unlikely that the two agents caused different hyperemic flow velocity and CFR by activating different signal pathways of vasodilation.

**Study Limitations**

We cannot rule out the possibility that hypometabolism, as a result of moderate anesthesia from 2.5% isoflurane, may decrease oxygen demand and coronary flow. However, all procedures were performed on a thermostatic animal platform. Moreover, the sham-operated animals also experienced corresponding anesthesia but didn’t show decreased CFR. Thus, the effect of hypometabolism might be negligible.

**Conclusion**

Measurement of CFR was feasible in the TAC and the AR mouse models, using adenosine and isoflurane as vasodilators. Compared with adenosine, high-concentration isoflurane may underestimate CFR, which is probably associated with suppressed cardiac function.

**ACKNOWLEDGMENTS**

We are grateful to Dr. Yu-Qing Zhou at the Hospital for Sick Children, Toronto, ON, Canada, for help in developing animal models and training in high-frequency ultrasound imaging. We thank Qingshun Sun from the College of Foreign Languages and Literature, Fudan University, for helpful and thorough proofreading. We also thank Jinguo Jia at the Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, and Guoping Zhang at the Institutes of Biomedical Sciences, Fudan University, for their kindness with technical expertise.

**GRANTS**

This work was supported by National Science Fund for Distinguished Young Scholars Grant 30525018 (to Y. Zou), National Natural Science Fund of China Grant 30930043 (to Y. Zou), National Natural Science Fund of China Grant 81200086 (to J. Wu), and Natural Science Fund of Zhongshan Hospital Grant 2012ZZQN29 (to J. Wu).

**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

J.Y., J.W., J.G., and Y.Z. did the conception and design of the research; J.Y. and J.W. performed the experiments; J.Y. analyzed the data; J.Y. and J.W. interpreted the results of the experiments; J.Y. and J.W. prepared the figures; J.Y. and J.W. drafted the manuscript; J.Y., J.W., J.G., and Y.Z. approved the final version of the manuscript; Y.Z. edited and revised the manuscript.

**REFERENCES**


