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Letters

RESEARCH LETTER

Murine Ischemia Reperfusion Without Endotracheal Intubation or Ventilation

A Tutorial

Mouse models of myocardial infarction (MI) are essential preclinical platforms, and left coronary artery (LCA) ligation is the standard method for inducing MI in the mouse heart (1). Although the classical approach requires endotracheal intubation prior to thoracotomy to maintain oxygenation, in 2010, Gao et al (2,3) introduced an innovative approach to induce MI in the mouse that circumvented the need for intubation by "externalizing" the heart and quickly ligating the LCA. Eliminating the need for intubation reduced airway injury, barotrauma, and infection risk. Despite these benefits, some technical limitations and obstacles to adoption remained. Their report focused primarily on permanent LCA ligation as opposed to ischemia-reperfusion (I/R), which is a more clinically relevant model because most patients presenting with ST-segment elevation MI now receive reperfusion therapy. Furthermore, details for operating on female mice and tying the quick release knot required for I/R were also lacking. Here, we present technical improvements for performing I/R without intubation, include female-specific details, and include a series of high-resolution videos of this procedure (**Figure 1A**, Videos 1, 2, and 3) to aid other researchers in adopting this technique.

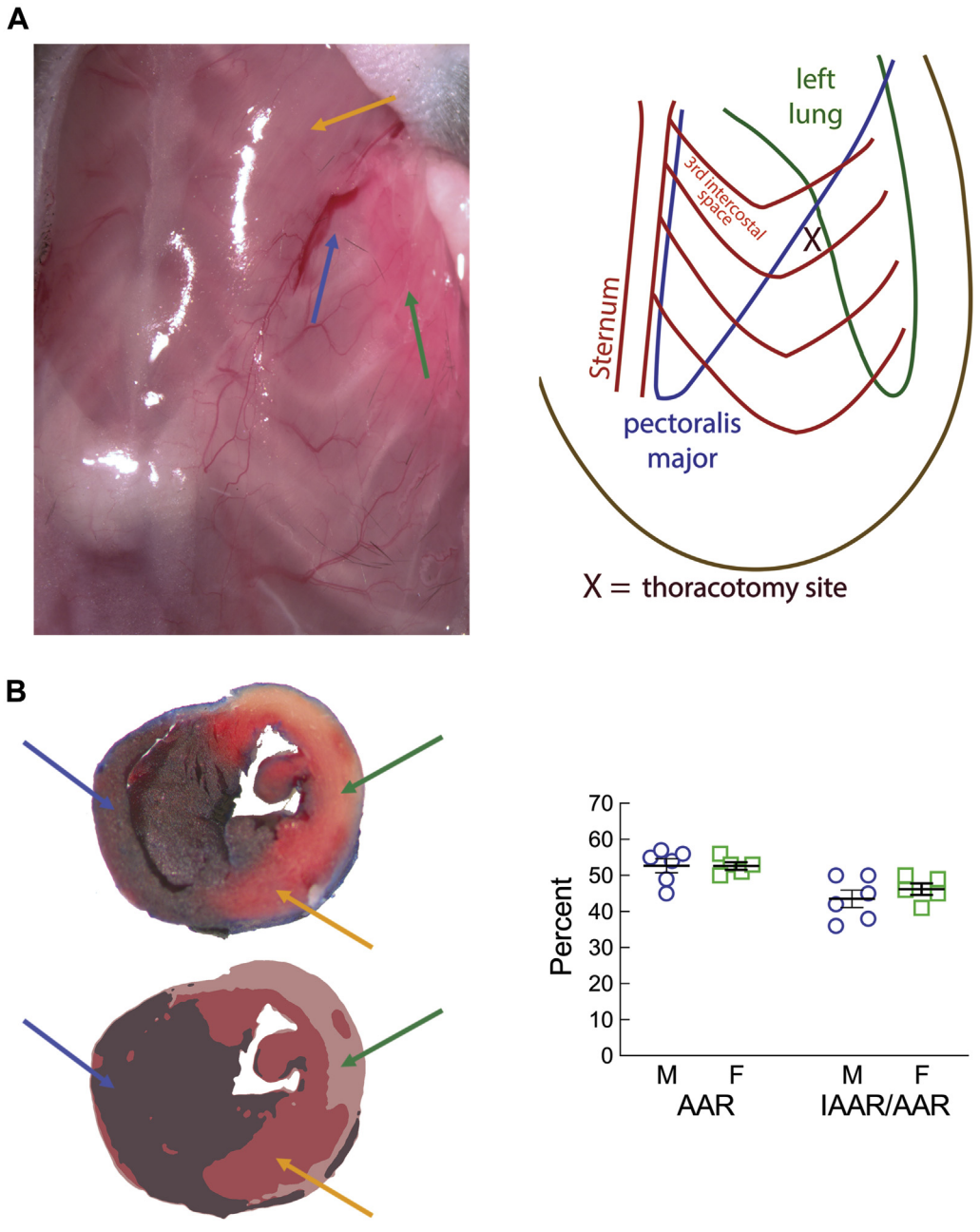
A full version of our method is included as the Supplemental Appendix. This study was carried out in adherence to the Public Health Service Guide for the Care and Use of Laboratory Animals, and all procedures were approved by the University of Minnesota Institutional Animal Care and Use Committee. Innovations of our technique include that: 1) we place the mouse head inside a latex-wrapped nose cone to maintain continuous positive airway pressure and oxygenation throughout the procedure (4); 2) we tunnel the LCA-ligating 8-0 suture under the skin superficial to the manubrium prior to thoracotomy; 3) we perform the thoracotomy at the third intercostal space, as opposed to the fourth intercostal space (**Figure 1A**), to externalize more of the left ventricle to expose the proximal LCA; 4) we return the heart inside the thoracic cavity before tying the ischemia-inducing quick release knot, limiting global hypoxia time to <20 seconds; 5) once the chest is closed and anesthesia is stopped, we continue supplemental oxygen until the mouse is breathing rapidly and responds to toe pinch; and finally, 6) we induce reperfusion by placing traction on the tunneled end of the 8-0 suture until the other end begins to move—indicating successful release of the knot and reperfusion.

With our technique, we produced an area at risk of approximately 50% and an infarct size of approximately 45% (**Figure 1B**), consistent with other studies using intubation (1). For this study, our overall survival was 86% (7 of 9 male mice, 5 out of 5 female mice). The 2 deaths occurred intraoperatively during LCA ligation—in general, this is when nearly all I/R fatalities occur. Of note, our procedure can also be adapted to the closed-chest model of chronic I/R by replacing the quick release knot with the snare-forming PE-10 tubing (5).

There are additional considerations for non-intubating I/R in female mice. Because of their lower body weights, female mice require less anesthesia, and timely isoflurane dose adjustment is critical to prevent excessive bradypnea and bradycardia. Female mice are more susceptible to tension pneumothorax after chest closure, and repeat pneumothorax evacuation may be necessary during the immediate postoperative period. Progressive bradypnea despite cessation of anesthesia is the primary symptom of worsening tension pneumothorax. Finally, because female mice have smaller muscles and thinner fascia than male mice, they are more susceptible to bleeding and therefore require more careful surgical handling to prevent catastrophic intraoperative bleeding.

Despite our improvements to the nonintubating method of murine I/R, some limitations remain. The LCA anatomy is variable and direct identification is not always possible; therefore, LCA ligation is primarily achieved by anatomic landmarks. Also,

FIGURE 1 **Murine Ischemia-Reperfusion Without Intubation**



(A) Exposed mouse thorax demonstrating thoracotomy site **(blue arrow)** between the pectoralis major **(orange arrow)** and left lung **(green arrow)**; schematic of the thoracic landmarks (Video 1, induction of ischemia; Video 2, induction of reperfusion; and Video 3, the quick release knot.). **(B)** Representative tetrazolium-stained heart cross-section, with color segmentation in ImageJ 1.53c and quantitation of the area at risk (AAR) and infarct AAR (IAAR) and AAR (IAAR/AAR) generated in male and female C57BL/6J mice. **Blue arrows** indicate the area not at risk, **orange arrows** indicate the nonischemic area at risk, and **green arrows** indicate the infarct.

because the heart is no longer visible after the quick release knot has been tensioned, apical paling cannot be used to verify ligation success. Verification of procedural success can only be performed postoperatively by electrocardiography, echocardiography, or tetrazolium staining.

In summary, we described improvements to the nonintubating method of murine I/R and compiled a

tutorial to assist new investigators who are learning this technique.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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APPENDIX For an expanded Methods section and supplemental videos, please see the online version of this paper.