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Current and Novel Experimental Methods in Ischemia/ Reperfusion Research: From Cutting-Edge Models to Innovative Therapies

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Editorial

Tissue ischemia is encountered frequently in clinical medicine and remains a key focus in current research. As early as 1880, catastrophic coronary arterial occlusion was used to demonstrate the effects of ischemia, but it was not until recently that the distinct and separate injury associated with reperfusion was acknowledged[1]. Ischemia/reperfusion injury (IRI) research is growing at a rapid pace. In this collection, we highlight important new additions to the field, from cutting-edge models to novel therapies in varied pre-clinical models. This compilation brings together useful tools for any basic science researcher. For instance, the cerebral or cardiac ischemia models proposed here can be imaged using the 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) staining methods described by Liepinsh[2]. Similarly, Paro et al.[3] and Compton et al.[4] utilize laser Doppler to confirm ischemia and reperfusion states in separate tissues.

Coronary occlusion is the leading cause of sudden cardiac death[5]. Many *in vivo* models attempt to recapitulate this injury in non-physiologic ways (e.g. by cryoablation or cautery).[6] Through micromanipulation, Lv et al.[6] demonstrate that the left anterior descending artery (LAD) can be reliably ligated and reperfused in a low-cost reproducible mouse model of coronary IRI[6]. The left ventricle is exposed *via* thoracotomy, and a dissecting microscope allows the identification of the LAD and the passage of a suture around the artery. A slipknot is used for the reversible occlusion. Troponin, echocardiography, and TTC staining can be used to quantify the IRI. This model has broad applications, from therapeutics to mechanistic studies in this genetically modifiable species.

Stroke is the second-most common cause of mortality, and middle cerebral artery (MCA) occlusion is the most common form of stroke[7,8]. Currently, there is a lack of effective treatments for recovery following the acute stages of stroke. Previous approaches to increase vascular growth and return the necessary blood supply have had disappointing results. Paro et al.[3] adapt a current neurosurgical procedure for the novel treatment of ischemic stroke[3]. In this technical approach, intravascular occlusion and reperfusion are achieved using a suture monofilament passed from

the carotid artery to the MCA. Changes in the blood flow are confirmed with laser Doppler. The temporalis muscle is then rotated through a craniotomy to lie on the exposed MCA territory brain surface. This procedure modulates angiogenesis growth factors and leads to increased density of new vessel growth within ischemic lesions.

Moving from *in vivo* to *ex vivo*, Barajas et al.[9] expand a time-tested model, the Langendorff heart preparation, to use with newborn developing mouse hearts[9]. This allows for the study of genetically modifiable subjects during key developmental time points. Prior developmental studies were limited to larger animals. The newborn heart displays differences in metabolism and physiologic and pharmacologic IRI responses. This method will not only support the development of pediatric IRI models but may also help address long-standing developmental questions. The newborn mouse heart is too small to measure the cardiac work with a balloon and requires the utilization of a force transducer attached to the ventricular apex, which represents the classic approach described by Langendorff in 1895[10].

The high-quality assessment of viable, at-risk, and necrotic tissue areas after IRI is paramount for assessing interventions. Such quantification is applicable in the three articles described above. Many tools for this process exist; however, an elegantly simple and inexpensive step-wise method is detailed by Liepinsh et al.[2] for the heart and brain. This is a useful methodology in any *ex vivo* and *in vivo* researcher's toolbox. The proper handling of the tissues includes ensuring standardized staining pressure and time, as well as standardized freezing and storage times. The macro photography settings should be adjusted manually; incorrect exposure and white balance, as well as blur and reflections, can be carefully prevented with their detailed setup.

Cell–cell signals in response to IRI occur across cell types and between cells both in physical contact and at longer distances from each other[11]. Li et al.[12] highlight this phenomenon by utilizing an endothelial-cardiomyocyte cross talk-enabled but physically isolated cell co-culture[12]. This method allows for the high-fidelity *in vitro* analysis of a single cell line. In their study, the demonstration of endothelial cell attenuation of cardiomyocyte IRI was dependent on cell–cell distancing. This demonstrates the clear advantages of non-mixed co-cultures and highlights to future researchers the importance of controlling for physical environments. While this model lends itself immediately to the investigation of cardiac endothelial–cardiomyocyte interactions, expansion to any other tissue–organ interaction is possible.

Moving on from cardiac and cerebral tissues, Compton et al.[4] explore the effect of light therapy on limb IRI[4]. There is no definitive optimal tourniquet inflation pressure or time, and muscle and neuronal injury from surgical tourniquets are well documented and may lead to patient morbidity[13]. The authors mimic clinical settings using tourniquets for unilateral limb ischemia confirmed with laser Doppler in mice. In their study, 5 min of near-infrared light treatment per hour of ischemic time altered macrophage proliferation, inflammatory markers, and levels of tissue necrosis. This clinically applicable model and non-invasive therapy extends the current boundaries of "blood-less" surgical field practices.

Following the heart and brain, the kidneys are among the most studied IRI organs. The kidney is extremely sensitive to malperfusion, and Godoy et al.[14] highlight that the

same procedure, even if altered slightly, can lead to varied pathologic states[14]. Utilizing a retroperitoneal approach to expose and clamp the renal pedicle for as little as 30 min leads to significant damage. Through the alteration of the reperfusion time, various states of recovery and disease can be studied, including tubular obstruction, tubular dilation, neutrophil infiltration, and even focal fibrosis. This model preserves the contralateral kidney for comparison, which may aid in injury marker characterization specifically in relation to renal–renal cross talk.

In summary, this compilation of IRI methods provides a widely applicable toolbox for researchers utilizing many different tissues and preparations from *in vitro* to *ex vivo* and *in vivo* models. From the novel reimagining of surgical techniques to expanding time-tested methods, the established experts in IRI research provide novel, cutting-edge investigative tools. However, a commonality among these studies seems to be the improved translatability to clinical models through the development of relevant techniques. From mimicking surgical settings with tourniquets to MCA and LAD occlusion, the improvement of preclinical models will undoubtedly aid in the future clinical application of our work.

Disclosures

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