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RESEARCH LETTER

Magnetic Resonance Imaging of the Regenerating Neonatal Mouse Heart

Mala Gunadasa-Rohling, BSc
Megan Masters, BSc, MSc, PhD
Mahon L. Maguire, MA, MSci, PhD
Sean C. Smart, BSc, MSc, PhD
Jürgen E. Schneider, PhD
Paul R. Riley, BSc, PhD

After myocardial infarction (MI), the human heart is unable to regenerate lost tissue, leading to scarring, pathological remodeling, and progression to heart failure. The study of animal models that can intrinsically regenerate the heart, therefore, offers therapeutic insight into targeting tissue restoration. In 2011, the first evidence of mammalian heart regeneration was reported by Porrello et al.[1] After surgical resection of ≈15% of the left ventricle apex of a postnatal day 1 (P1) neonatal mouse, the heart fully regenerated by 21 days after injury, whereas if the procedure was repeated 1 week later on a P7 mouse heart, fibrosis and scarring ensued, recapitulating the adult wound-healing response. The mechanism of regeneration observed involved proliferation of resident cardiomyocytes, which is analogous to that described in the adult zebrafish heart.[2] Since the original study, others have described neonatal myocardial regeneration after alternative insults, such as MI.[3] However, controversy also surrounds the extent of heart regeneration during the first weeks of life, whereby it was reported that regeneration did not occur in the P1 heart and was replaced by long-term fibrosis (180 days) with extensive cardiac remodeling.[4]

The major issue to date with all of these studies is that they are based on sacrificing individual animals at specific time points after injury, followed by histological assessment of the heart. This provides no insight into the extent of the initial injury, whether the heart was indeed injured from the outset or the regenerative process over time. Moreover, an important question remains as to how the regenerating mammalian heart copes in terms of functional output and remodeling during scar resolution and tissue restoration, as is directly relevant to human patients with ischemic heart disease subjected to regenerative therapies. To address both of these key issues, we developed noninvasive MRI, which enabled tracking of individual newborn mice after MI. Although MRI is well established for quantifying and monitoring cardiac function in adult mice, its longitudinal application in neonates requires significant refinement and has not been reported at the early P1 time point or after injury.[5]

We measured left-ventricular function at baseline (before surgery, day 0) and days 4, 7, 14, and 21 after MI for P1 and P7 age groups versus matched sham-operated controls. We assessed left-ventricular end-diastolic volume, end-systolic volume, ejection fraction, stroke volume, heart rate, and left-ventricular mass/body mass index (Figure; stroke volume and heart rate not shown). Representative images at days 4 and 21 after MI revealed extensive growth of the heart and apparent maintenance of wall thickness in P1 injured hearts versus sham controls, compared to left-ventricular dilation and wall thinning after injury at P7 (Figure, A and B). End-diastolic volume for the P7 MI group was significantly higher than in sham controls at most time points after injury (Figure, C), which is consistent with chamber dilation and remodeling. For the P1 group, there was no difference immediately after injury, but a rise in end-diastolic volume in the injury group was observed,

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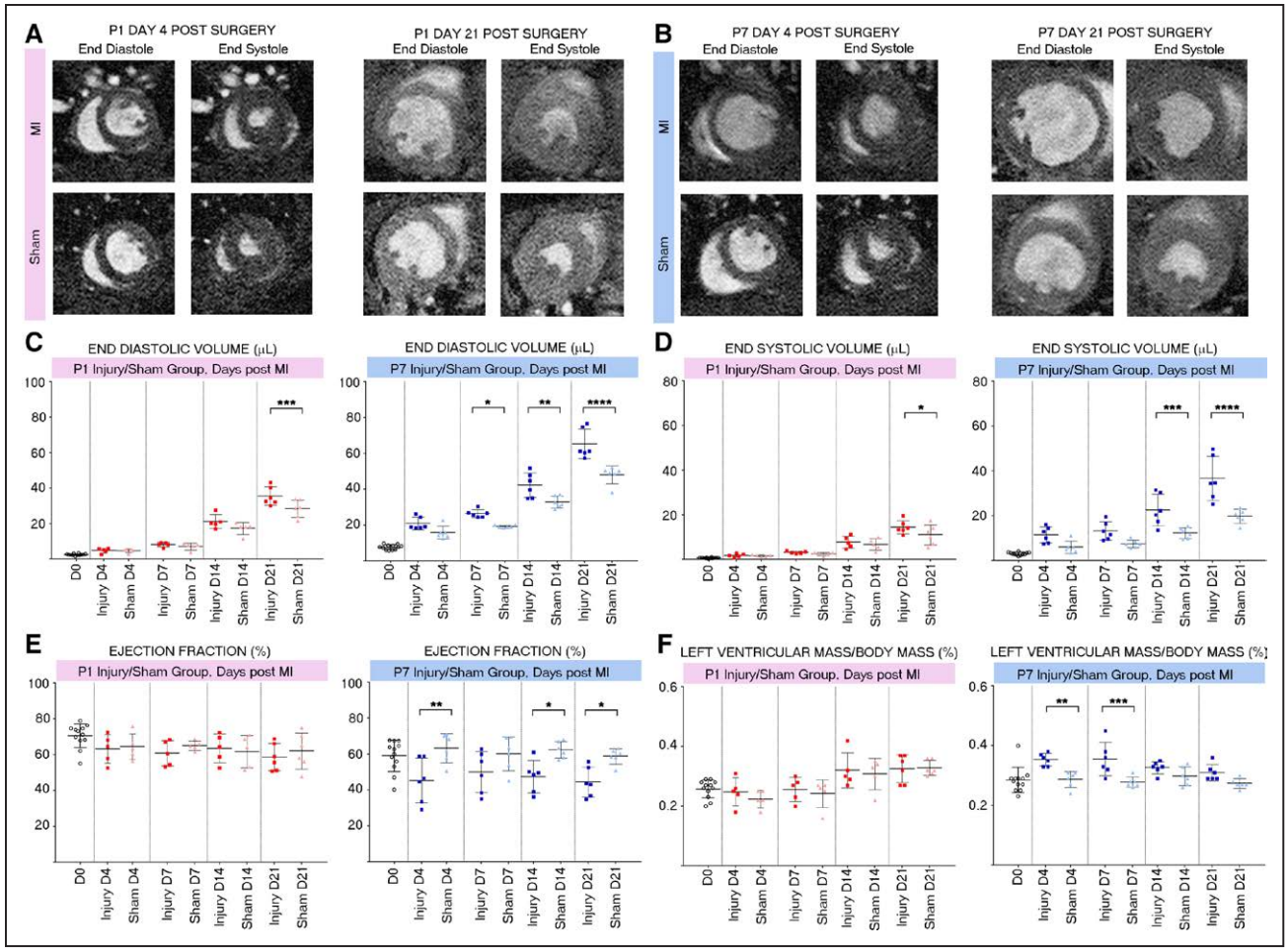

Figure. Functional MRI parameters at baseline and days 4, 7, 14, and 21 after MI and sham-operated P1 versus P7 mice.
A and **B**, Midventricular short-axis cine-MRI frames for P1 (**A**) and P7 (**B**) hearts in diastole and systole at days 4 and 21 after MI. **C** through **F**, MRI values for end diastolic volume (**C**), end systolic volume (**D**), ejection fraction (**E**), and left ventricular mass to body mass ratio (**F**) are indicated. Each time point shows a comparison between the injury and sham groups within each P1 and P7 cohort across the duration of study. One-way ANOVA with Sidak's multiple comparisons test. Significant differences between injury and sham cohorts are indicated as **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001; N=5 or 6 for all groups. MI indicates myocardial infarction; MRI, magnetic resonance imaging; and P, postnatal day.

which became significant by day 21 (Figure, C), inferring that regeneration may be incomplete in contrast to previous reports.[3] End-systolic volume values for the P7 injured group were also significantly higher across later time points (Figure, D). The P1 injured group revealed small increases in end-systolic volume, which were significant only by day 21 (Figure, D). The ejection fraction was significantly lower for the P7 injury group, compared with controls, for most of the time points analyzed (Figure, E): there was a decrease in ejection fraction immediately after MI, an apparent recovery at day 7, followed by further significant decreases in ejection fraction through days 14 and 21, suggesting impaired recovery of normal heart function over time. In contrast, the P1 group revealed no significant differences in ejection fraction between injured and sham hearts at all time points (Figure, E. Moreover, ejection fraction was maintained at ≈60% as previously reported for intact neonatal mice.[5] Maintenance of function coincides with the regenerative response at P1 and is likely linked to the compensatory growth of the heart from this stage and throughout the analyses. Stroke volume increased linearly for both P1 and P7 hearts as the mice aged, with a significant increase in the P1 MI group at day 14 (not shown). There were no significant differences in heart rate across all groups (not shown). The left-ventricular mass/body mass index was significantly increased in the P7 group at days 4 and 7 and remained elevated until end point after injury (Figure, F), which is consistent with an adult cardiomyocyte hypertrophy response, whereas no significant differences were observed at P1 (Figure, F).

Collectively, these data reveal compensatory changes in cardiac function with the restoration of tissue and resolution of injury for P1 neonates and sustained injury responses for the P7 cohort. This study resolves the controversy surrounding neonatal mouse heart regeneration and establishes a functional platform for live capture of the regenerative process and for future testing of genetic or therapeutic interventions.

All animal experiments were carried out according to the UK Home Office project license PPL30/2987 compliant with the UK Animals (Scientific Procedures) Act 1986 and approved by the local Biological Services Ethical Review Process.

ARTICLE INFORMATION

The data, analytical methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure. These are available on request from paul.riley@dpag.ox.ac.uk and J.E.Schneider@leeds.ac.uk.

Correspondence

Paul R. Riley, BSc, PhD, Department of Physiology, Anatomy and Genetics Sherrington Bldg, University of Oxford, South Parks Rd, Oxford OX1 3PT, United Kingdom; or Jürgen E. Schneider, PhD, Division of Biomedical Imaging, Leeds Institute of Cardiovascular and Metabolic Medicine, LIGHT Laboratories, Clarendon Way, University of Leeds, Leeds LS2 9JT, United Kingdom. Email paul.riley@dpag.ox.ac.uk or J.E.Schneider@leeds.ac.uk

Affiliations

Department of Physiology, Anatomy and Genetics, British Heart Foundation, Oxbridge Centre of Regenerative Medicine, University of Oxford, UK (M.G.-R., M.M., J.E.S., P.R.R.). British Heart Foundation, Experimental Magnetic Resonance Unit, Wellcome Trust Centre for Human Genetics, Roosevelt Dr, Oxford, UK (M.L.M., J.E.S.). RDM Cardiovascular Medicine, John Radcliffe Hospital, Headley Way, Headington, Oxford UK (M.L.M., J.E.S.). Department of Oncology, Cancer Research UK, Medical Research Council, Oxford Institute for Radiation Oncology, University of Oxford, UK (S.C.S.). Division of Biomedical Imaging, Leeds Institute of Cardiovascular and Metabolic Medicine, LIGHT Laboratories, Clarendon Way, University of Leeds, UK (J.E.S.).

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Disclosures

PRR is cofounder and equity holder in OxStem Cardio, an Oxford University spin-out that seeks to exploit therapeutic strategies stimulating endogenous repair in cardiovascular regenerative medicine.

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