Published in final edited form as:

J Gene Med. 2012 ; 14(0): 609–620. doi:10.1002/jgm.2673.

AAV9 administered systemically after reperfusion preferentially targets cardiomyocytes in the infarct border zone with pharmacodynamics suitable for the attenuation of left ventricular remodeling

Prasad R. Konkalmatt¹, Feng Wang², Bryan A. Piras¹, Yaqin Xu¹, Daniel M. O'Connor³, Ronald J. Beyers¹, Frederick H. Epstein¹, Brian H. Annex², John A. Hossack¹, and Brent A. French^{1,2,4}

¹Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA

²Division of Cardiovascular Medicine, University of Virginia, Charlottesville, VA, USA

³Neuroscience Program, University of Virginia, Charlottesville, VA, USA

⁴Department of Radiology, University of Virginia, Charlottesville, VA, USA

Abstract

Objective—Adeno-associated virus serotype 9 (AAV9) vectors provide efficient and uniform gene expression to normal myocardium following systemic administration with kinetics that approach steady state within 2–3 weeks. However, due to the delayed onset of gene expression, AAV vectors have not previously been administered intravenously after reperfusion for post-infarct gene therapy applications. Here, we evaluate the therapeutic potential of post-MI gene delivery using intravenous AAV9.

Methods—AAV9 vectors expressing firefly luciferase, eGFP, or extracellular superoxide dismutase genes from the cTnT promoter (AcTnTLuc, AcTnTeGFP, AcTnTEcSOD) were employed. AcTnTLuc was administered intravenously at 10 minutes and at 1, 2 and 3 days post-ischemia/reperfusion (IR), and the kinetics of luciferase expression were assessed with bioluminescence imaging. AcTnTeGFP was used to evaluate the distribution of eGFP expression. High-resolution echocardiography was used to evaluate the effects of AcTnTEcSOD on left ventricular (LV) remodeling when injected 10 min post-IR.

Results—Compared to sham animals, luciferase expression at 2 days after vector administration was elevated by 4-, 24-, 210- and 213-fold in groups injected at 10 min, 1 day, 2 days and 3 days post-IR, respectively. Expression of cTnT-driven eGFP was strongest in cardiomyocytes bordering the infarct zone. In the efficacy study of EcSOD, post-infarct LV end-systolic and end-diastolic volumes at days 14 and 28 were significantly smaller in the EcSOD group compared to control.

Conclusion—Systemic administration of AAV9 vectors after IR both elevates and accelerates gene expression that preferentially targets cardiomyocytes in the border zone with pharmacodynamics suitable for the attenuation of LV remodeling.

Correspondence: Brent A. French, Ph.D., Department of Biomedical Engineering, 415 Lane Road, MR5 Building, Room 1219, University of Virginia, Charlottesville, VA 22903, bf4g@virginia.edu, Phone: (434) 924-5728, Fax: (434) 924-5923.

Conflict of Interest Statement

The authors have no disclosures to report relevant to this manuscript.

Keywords

AAV; Post myocardial infarction gene delivery; Cardiac gene therapy; ischemia and reperfusion injury; LV remodeling

Introduction

Myocardial ischemia/reperfusion (IR) injury often leads to progressive LV remodeling and eventual heart failure. LV remodeling resulting from myocardial infarction involves expansion of the infarct zone, extension of cell death in the border zone, overall dilation of the LV chamber and ultimately heart failure. LV remodeling (as assessed by changes in LV end-systolic and end-diastolic volumes) is immediately apparent within the first day after MI and continues for weeks in rodents and perhaps months in larger mammals [1]. Therefore, early intervention is necessary to protect the heart against LV remodeling following MI, particularly in small animal models where LV remodeling subsides within two weeks of reperfusion [2]. Effective gene therapy interventions to prevent LV remodeling may therefore benefit from gene delivery systems that preferentially transduce cardiomyocytes at risk and provide a rapid onset of gene expression. Adenoviral vectors provide robust and rapid onset of gene expression in the myocardium following direct injection into the LV. However, the utility of adenoviral vectors is limited due to the immunological recognition of low-level adenoviral gene expression by the host, leading to the clearance of transfected cells [3]. Furthermore, upon IV injection, adenoviral vectors accumulate primarily in the liver and have limited capacity to target the heart [4, 5].

Adeno-associated viral (AAV) vectors provide for sustained, long-term gene expression in a wide variety of tissues and cause minimal immunological complications compared to other viral vectors being tested for gene therapy [6]. In recent years, a variety of new AAV serotypes have been isolated [7, 8] that exhibit a wide range of tissue tropisms and provide for efficient transduction and long-term gene expression [9–11]. In particular, serotypes AAV6, AAV8 and AAV9 transduce cardiomyocytes preferentially following systemic administration and provide uniform gene delivery throughout the myocardium [10, 12–18]. The most widely studied serotype, AAV2, has a prolonged lag phase of 4–6 weeks before reaching maximum gene expression in the heart [19]. On the other hand, the more recently discovered AAV serotypes provide for an earlier onset of gene expression, approaching steady state levels within 2–3 weeks [12, 20]. However, the onset of gene expression provided by the newer serotypes of AAV still lags behind that achieved by adenoviral vectors. Thus, AAV2 vectors have typically been employed in preemptive gene therapy applications for MI and LV remodeling, with the AAV vector being administered several weeks before the induction of ischemia/reperfusion injury [21, 22]. Recently, AAV2 was directly injected into the myocardium shortly after IR to evaluate the ability of therapeutic gene delivery to preserve cardiac function in a porcine model [23, 24]. These studies showed that, despite the expected lag phase before gene expression, direct injection of AAV2 vectors could modulate the LV remodeling process in large animals, and could help preserve LV function. Although these studies are encouraging, delivering therapeutic genes by systemic administration would offer greater clinical relevance. However, due to the delayed onset of gene expression from conventional AAV vectors in normal myocardium, there are no reports, to date, on the use of AAV vectors to deliver gene therapy to the heart by systemic administration after ischemia and reperfusion.

In the present study, we provide evidence that AAV9 vector administration after ischemia and reperfusion provides preferential transduction to cardiomyocytes at risk in the infarct border zone, with the onset of gene expression occurring even earlier than that observed in

normal myocardium. Further, we show that post-IR delivery by IV injection of an AAV9 vector carrying EcSOD protects the heart against subsequent LV remodeling. These findings have potential clinical relevance because they establish a precedent for the intravenous administration of AAV-mediated, cardiac-targeted gene therapy post-reperfusion to protect the heart against subsequent LV remodeling and ultimately heart failure.

Materials and Methods

Plasmids

The AAV vectors containing the 418 bp chicken cardiac troponin-T (cTnT) promoter driving the expression of firefly luciferase (AcTnTLuc), eGFP (AcTnTeGFP) or EcSOD (AcTnTEcSOD) are diagrammed in Fig. 1, and their construction has previously been described [25, 26].

AAV vector production

AAV2-based vector genomes were cross-packaged into AAV9 capsids via the triple transfection of HEK 293 cells, then purified by ammonium sulfate fractionation and iodixanol gradient centrifugation [25, 27]. Titers of the AAV vectors (viral genomes/ml) were determined by quantitative real-time PCR [19, 25]. The following primers were used for amplifying luciferase: 5'-AGAACTGCCTGCGTGAGATT-3' (forward) and 5'-AAAACCGTGATGGAATGGAA-3' (reverse); eGFP: 5'-CACATGAAGCAGCACGACTT-3' (forward) and 5'-GAAGTTCACCTTGATGCCGT-3' (reverse); and EcSOD: 5'-CCTAGCAGACAGGCTTGACC-3' (forward) and 5'-CCATCCAGATCTCCAGCACT-3' (reverse). Known copy numbers (10^5 – 10^8) of the respective plasmids carrying the corresponding cDNAs were used to construct standard curves for quantification.

Myocardial IR and vector administration

Animal protocols used in the study were approved by the Institutional Animal Care and Use Committee and conformed to the "Guide for the Care and Use of Laboratory Animals" (NIH Publication 85–23, revised 1985). C57BL/6 mice (8–10 weeks old, weighing 20–25 g) were purchased from The Jackson Laboratories (Bar Harbor, ME) and maintained on a 12/12 hr light/dark cycle at 24°C and 60% humidity. The procedure employed to induce myocardial IR injury in mice has been described previously [28]. Briefly, mice were anesthetized with intraperitoneal (IP) injected sodium pentobarbital (100 mg/kg) and orally intubated. Artificial respiration was maintained at 80% inspired oxygen by using 100 strokes/min and a 2–3 ml tidal volume delivered through a loose connection from the rodent ventilator. The hearts were exposed through a left thoracotomy. Left anterior descending coronary artery (LAD) occlusion was accomplished by passing a suture beneath the LAD and tightening it over a piece of polyethylene-60 tubing. The LAD was occluded for 30 minutes in the preliminary studies of reporter gene expression and for 60 minutes in the LV remodeling study. Reperfusion was induced by removing the piece of tubing. For IV injection, mice were anesthetized with 1–1.2% isoflurane in oxygen while viral solution (50 μ l containing 1×10^{11} viral genome particles in all studies) was slowly injected via the jugular vein.

Bioluminescence imaging

Luciferase expression was serially assessed in live mice using an in vivo bioluminescence imaging system (IVIS 100 system, Caliper Life Sciences, Hopkinton, MA) as described previously [25, 29].

Quantitative luciferase activity assay

In the serial study, whole hearts were collected from mice after bioluminescence imaging and euthanasia at 7 weeks post-vector injection for luciferase activity assays. To compare the magnitude of gene expression between the previously ischemic and remote regions in mice injected 10 min post-reperfusion, the ischemic and remote zones of hearts explanted five days after vector injection were separated under a dissecting microscope for luciferase activity assays. Remote samples were obtained from the region furthest removed from the infarct (i.e., the basal septum). Luciferase activities (relative light units, RLU) in protein extracts from these tissues were determined using luciferase assay reagents from Promega Corp. (Madison, WI) and a FLUOstar Optima micro-plate reader (BMG Labtech, Durham, NC).

Determination of AAV vector genome copy number

Total genomic DNA was prepared from the mouse hearts by standard phenol-chloroform extraction. AAV vector genome copy numbers were determined by real-time quantitative PCR using the QuantiTect SYBR Green PCR kit (Qiagen Inc., Valencia, CA) and a Bio-Rad iCycler system (Bio-Rad Laboratories, Hercules, CA). The following primers were used for amplifying luciferase: 5'-AGAACTGCCTGCGTGAGATT-3' (forward) and 5'-AAAACCGTGATGGAATGGAA-3' (reverse). Known copy numbers (10^3 – 10^8) of the plasmid pAcTnTLuc were used to construct the standard curve. Results are expressed as the number of vector genomes per μ g of genomic DNA.

Histology and immunohistochemistry

Immunostaining for eGFP protein was performed on 6 μ m fixed-frozen sections. Five days following vector administration, animals were euthanized and hearts were collected and fixed in 3.7% para-formaldehyde for 1 h at 4°C. After washing in PBS (3 times, 5 min each), hearts were equilibrated with 30% sucrose in PBS overnight prior to freezing and sectioning. After incubation with hydrogen peroxide (0.5%) followed by avidin blocking, the sections were incubated overnight at 4°C with rabbit anti-GFP antibody (1:3000 dilution, Abcam Inc., Cambridge, MA). Biotinylated secondary antibody (5 μ g/ml, Vector Laboratories, Burlingame, CA) was then applied for 1 h at room temperature. After washing and incubation with avidin-biotin complex (Vector Laboratories), immunoreactivity was visualized by incubating the sections with the chromogen 3,3-diaminobenzidine tetrahydrochloride (DAB, Dako, Carpinteria CA) to produce a brown precipitate. Immunostained sections were counterstained with eosin before they were coverslipped for photography. Hearts were processed similarly to immunostain cardiomyocytes with a rabbit polyclonal antibody against myoglobin (Dako) using a Cy5-labeled goat anti-rabbit IgG (Life Technologies, Grand Island, NY) as secondary antibody. In the LV remodeling study, conventional hematoxylin and eosin (H&E) staining was performed on heart sections obtained 4 weeks post-MI.

Western immunoblotting

Flash-frozen tissue samples were homogenized in RIPA buffer, and equal amounts of protein (as determined by Bio-Rad Dc Protein Assay) were electrophoresed under reducing conditions on a polyacrylamide gel and then transferred onto PVDF membranes. After blocking, membranes were incubated overnight at 4°C with goat anti-GFP (BA-0702, Vector Laboratories Inc., Burlingame, CA) or rabbit anti-EcSOD (07-704, EMD Millipore Corp., Billerica, MA) followed by 1-h incubation at room temperature with rabbit anti-goat IgG conjugated with horseradish peroxidase (sc-2768, Santa Cruz Biotechnology Inc., Santa Cruz, CA) or goat anti-rabbit IgG conjugated with fluorescent dye (926-32211, LI-COR Biosciences, Lincoln, NE). Membranes were imaged via chemiluminescence or

fluorescence. To control for protein loading, GFP membranes were stripped and reprobed overnight at 4°C with rabbit anti-actin antibody (A2103, Sigma-Aldrich Inc, St. Louis, MO), followed by 1-h incubation at room temperature with goat anti-rabbit IgG conjugated with horseradish peroxidase (170–6615, Bio-Rad Laboratories). Similarly, EcSOD membranes were stripped and reprobed overnight at 4°C with rabbit anti-GAPDH antibody (600–401-A33, Rockland Immunochemicals Inc., Gilbertsville, PA), followed by 1-h incubation at room temperature with fluorescently-labeled goat anti-rabbit IgG. Signal intensities on Western blots were quantified by densitometry using ImageJ (NIH, Bethesda MD) and the primary signal in each lane was normalized to the loading control before being graphed relative to the mean of the negative control lanes.

Evaluation of cardiac function by echocardiography

A total of 17 mice were subjected to 60 min of coronary occlusion. Ten minutes following reperfusion, 8 mice were injected IV with AcTnTEcSOD while the remaining 9 mice served as controls. The procedure employed here to induce myocardial IR injury was the same as that described in “Myocardial IR and vector administration” except with reperfusion performed after 60 min of LAD occlusion. Mouse LV volumes and ejection fraction were obtained by echocardiography, as described previously, on the day before the surgery (baseline) and then on days 2, 7, 14, and 28 after surgery [30]. During echocardiography, mice were maintained under light anesthesia using an inhaled mixture of 1.5% isoflurane gas and atmospheric air. The mouse was placed in a supine position on a platform with an electrical heating pad and a tensor lamp was used to provide additional heat. Mouse core body temperature was monitored with a rectal temperature probe coupled to a digital thermometer and was maintained at $37.0 \pm 0.2^\circ\text{C}$. ECG signals were obtained by contacting the mouse limbs, coupled with electrically conductive gel, to ECG electrodes integrated into the heating pad. The chest area was depilated to improve the quality of the B-mode echocardiographic images. Care was taken not to apply excess pressure onto the chest during scanning in order to avoid heart deformation. B-mode cardiac image sequences were acquired using a Vevo 2100 high-resolution echocardiography scanner (VisualSonics Inc., Toronto, Ontario, Canada). For each mouse, a total of 6–7 serial parasternal LV short-axis views were acquired from the apex to the LV base at 1 mm intervals. The LV cross-sectional areas were obtained by tracing the end-diastolic and end-systolic endocardial borders at each slice position. The LV volumes were then calculated as the sums of the 1 mm-thick slice volumes contoured at end-systole (ESV) or end-diastole (EDV). For wall thickening analysis, the thicknesses of the anterior and inferior walls were determined from the B-mode images and wall thickening was calculated as in an M-mode analysis. Sphericity index was calculated from long-axis B-mode images at end-diastole by dividing the length of the LV from the apex to the mitral annulus by the short axis diameter of the LV at a point two-thirds the distance from the base to the apex.

Cardiac MR imaging

In preparation for late gadolinium enhanced (LGE) cardiac MR (CMR) imaging of myocardial infarction, a length of PE-20 tubing was surgically inserted into the IP cavity and connected to a syringe preloaded with a volume of gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) contrast agent necessary to deliver a 0.1 to 0.2 mmol/kg dose. All scans were performed on a 7 Tesla small bore scanner that was equipped with a circular polarized radio frequency body coil for mice and gradient system capable of 650 mT/m maximum strength and 6667 mT/m/ms maximum slew rate (Bruker, Ettlingen, Germany). All CMR was performed for three consecutive post-MI days. A multislice T2 preparation sequence for T2w edema imaging and a T1w inversion recovery sequence for LGE infarct imaging were performed as described in [31] and [32], respectively. Localizer imaging was performed to identify double-oblique short-axis views of the LV, followed by T2w edema

imaging to detect the edematous region within the entire LV. After T2w imaging, Gd-DTPA was injected for LGE infarct imaging. Ten minutes after injection, multislice inversion recovery imaging was performed to detect the location of the infarct region within the LV myocardium.

Statistical analyses

All data are expressed as mean $\pm$ SE. For the echocardiography study, two-way ANOVA was used to evaluate differences between and within the control group and the group treated with EcSOD vector at baseline and at serial time points after MI. *Post hoc* analyses (Bonferroni post-tests) were performed where appropriate. For other studies, statistical analyses were performed using Student's t-test.

Results

Pharmacodynamics of transgene expression and transduction in the heart following IV administration of AAV9 post-IR

In vivo bioluminescence imaging of mice that were injected IV at defined timepoints after reperfusion with the AAV9 vector expressing luciferase showed that light output was predominantly restricted to the left side of the chest cavity in all groups (see Fig. 2A for example images). A complete time course showing bioluminescence images for 2 mice from each group is included in Supplementary Data Figure S1. Groups that received vector after IR recorded higher light output compared to the sham-operated group at all time points (Fig. 2B, $n=4$ per group). When compared two days after vector administration, bioluminescence imaging showed that light output was numerically highest in the group that received vector on day 3 post-IR, followed by the groups that received vector at day 2, day 1 and 10 min post-IR (Fig. 2B). Compared to vector delivery in the sham-operated group, light output from the heart at 2 days after vector administration was elevated by 4-, 24-, 210- and 213-fold in groups injected at 10 min, 1 day, 2 days and 3 days post-IR, respectively (all comparisons $p<0.05$ vs. sham). The sham operated group approached steady-state levels of luciferase expression between 2–3 weeks. By contrast, luciferase expression in the groups that received vector 2 or 3 days post-IR exceeded steady-state expression levels in the sham-operated group after less than one week.

In vitro luciferase activity assays performed on protein extracts from hearts and livers collected at the end of the study showed that in all groups ($n=4$ per group), luciferase activity was significantly higher in the heart compared to liver (data not shown). Compared to the sham group, luciferase activity in the heart at 7 weeks post-vector injection was 4.1-, 5.6-, 4.5- and 2.1-fold higher in the groups that received vector at 10 min, 1 day, 2 days and 3 days post-IR, respectively (Fig. 2C). Importantly, vector genome copy numbers in the heart showed trends similar to the luciferase results, with levels that were 1.8-, 2.2-, 1.8- and 1.7-fold higher in the groups that received vector at 10 min, 1 day, 2 day and 3 day post-IR, respectively compared to the sham group (Fig. 2D). These data show that ischemia and reperfusion injury to the heart creates a more conducive environment for AAV transduction, as shown by the significantly elevated number of vector genomes present and the early and robust onset of reporter gene expression from the AAV9 vector relative to sham-operated (i.e., normal) heart.

Distribution of gene expression from AAV9 administered post-IR

The distribution of gene expression in the myocardium following vector administration 10 min post-IR (the most clinically relevant time point) was further assessed by IV injection of saline or AcTnTeGFP in sham-operated mice and in mice at 10 min post-IR ($n=3$ per group). Five days following IR and vector administration, eGFP expression was assessed by

Western blot analysis and immunohistochemistry. Western blot analysis showed that eGFP expression (as normalized to an actin loading control) in a representative mouse that received the AAV9 vector after IR (IR+AAV9) was 3.5-fold higher compared to a sham-operated mouse injected with the same vector (Sham+AAV9, Fig. 3A).

Immunohistochemistry on cryosections of the hearts confirmed no eGFP expression in the infarcted hearts of mice injected with saline (Figs. 3B and 3B1). In sham-operated mice that received AAV9 vector (Sham+AAV9), few cardiomyocytes stained positive for eGFP expression at this early time point (Figs. 3C and 3C1). In contrast, mice that received vector at 10 min post-IR (IR+AAV9) showed strong eGFP expression localized primarily within cardiomyocytes bordering the infarct zone (Figs. 3D and 3D2–3D4). A lower level of eGFP expression was also noted in the remote zone of the infarcted hearts (Fig. 3D1), but this expression was nevertheless higher than that observed in sham-operated mice that received AAV9 vector (Fig. 3C1). Co-localization of a cardiac-specific marker (myoglobin) and eGFP confirmed that eGFP expression was most abundant in cardiomyocytes located at the very edge of the infarct region (Fig. 4A–C). Note that the significant levels of gene expression detected in Figs. 3 and 4 only 5 days after vector injection represent a small fraction of the steady state gene expression levels anticipated at later time points (as demonstrated in Fig. 2).

To compare the magnitude of gene expression between the previously ischemic and remote regions of the heart, additional mice (n=4) were injected with AcTnTLuc at 10 min post-reperfusion. Five days following vector administration, hearts were explanted and luciferase activity assays were performed on tissue samples from the previously ischemic and remote regions of the hearts. Luciferase activity in the previously ischemic region was 4.3-fold higher ($p<0.05$) compared to the remote region of post-infarct hearts (Fig. 4D). Compared to normal myocardium in sham-operated mice, luciferase activities in the ischemic and remote regions were 15-fold ($p<0.01$) and 3.5-fold ($p<0.05$) higher, respectively (Fig. 4D).

These results show that the robust and accelerated onset of gene expression measured on day 5 following vector administration at 10 min post-IR was largely in cardiomyocytes bordering the infarct zone and also to a lesser extent in remote non-infarcted regions of the heart.

AAV9 administration after ischemia and reperfusion provides therapeutic levels of gene expression

We used an AAV9 vector carrying EcSOD under the control of the cTnT promoter (AcTnTEcSOD) to test the therapeutic benefit of AAV9 vector administration post-IR. Ten minutes post-IR, mice in the EcSOD group were injected IV with AcTnTEcSOD (n=8) while the control group (n=9) received no viral vector. Left ventricular end-diastolic volume (LVEDV) and end-systolic volume (LVESV) were measured using high-resolution echocardiography on the day before surgery (baseline) and on days 2, 7, 14 and 28 post-IR. Western blot analysis performed on hearts collected one day after the final echocardiography session indicated a 12.5-fold increase in EcSOD expression in EcSOD-treated mice over control mice after normalization for GAPDH expression (n=3 from each group, $p<0.05$, Fig. 5). Representative day 28 post-IR short axis echo images of control and EcSOD-treated mouse hearts at end-systole are shown in Fig. 6A&B. Representative H&E stained tissue sections are shown in Fig. 6C&D, illustrating the reduced chamber volumes found in EcSOD-treated hearts. Volumetric analyses between the two groups showed significant differences in relative LVEDV ($p<0.05$) and relative LVESV ($p<0.005$) as determined by two way ANOVA at days 14 and 28 post-IR (results expressed as fold changes relative to baseline). LVEDV (Fig. 6E) and LVESV (Fig. 6F) increased progressively in both control and EcSOD-treated groups with no significant differences at days 2 or 7 post-IR. In the control group, LVEDV and LVESV continued to increase

through day 14 (2.2 ± 0.2 and 5.0 ± 0.6 fold, respectively) and day 28 (2.3 ± 0.2 and 5.3 ± 0.4 fold, respectively). In contrast, the EcSOD group showed no significant increases in LVEDV or LVESV after day 7 post-MI. At day 14, the EcSOD group showed a 31% reduction in relative LVEDV ($p < 0.05$) and a 35% reduction in relative LVESV ($p < 0.01$) compared to the control group. The significant reductions in both relative LVEDV and LVESV in the EcSOD group persisted through 28 days post-IR, yielding final reductions of 31% in relative LVEDV ($p < 0.05$) and 35% in relative LVESV ($p < 0.001$) as compared to the control group (Fig. 6E&F). Due to the parallel changes in LVEDV and LVESV, no significant differences in LV ejection fraction (EF) were found at any time point. An analysis of sphericity index showed that the anatomic morphology of the heart was also significantly improved in the EcSOD-treated mice on day 28 post-IR relative to controls ($p < 0.05$, Fig. 6G).

These results were supported by a wall-thickening analysis performed on the anterior (infarcted) and inferior (remote) walls of B-mode images acquired at the mid-ventricular level (Table I). This M-mode style analysis performed at 28 days post-MI revealed that the inferior wall in the EcSOD-treated group was significantly thicker at both end-diastole and end-systole than in the control group ($p < 0.05$, both comparisons). It also detected trends towards improved wall thickening (contraction) in both the anterior and inferior walls, but these trends did not reach statistical significance. Overall, these results show that a single IV administration of AAV9 carrying AcTnTEcSOD at 10 minutes post-IR provides therapeutic levels of gene expression capable of attenuating global LV remodeling after myocardial infarction.

Demonstration that the infarct and surrounding border zone become edematous after MI

Following 60 min of coronary occlusion and reperfusion, CMR imaging was performed on days 1, 2 and 3 post-MI to delineate edematous and infarcted regions of myocardium ($n = 4$ mice per time point). From T2w and LGE images obtained at the same short-axis slice position, it was evident that the T2w hyperintense edematous region and LGE infarct region showed good spatial correspondence (Fig. 7). T2w hyperintense signals were strongest on day 2 post-MI. In all mice at all days, the infarct region was consistently confined within the edematous region, and the size of the infarct region was smaller than the edematous region. These results show that the border zone immediately surrounding the infarct region becomes significantly edematous shortly after MI.

Discussion

In the current study, we demonstrate that: 1) the onset of AAV9-mediated gene expression is accelerated when the vector is delivered after IR injury; 2) this enhanced expression is most pronounced in cardiomyocytes bordering the infarct region; 3) systemic administration ten minutes post-IR of an AAV9 vector expressing EcSOD significantly inhibits global LV remodeling subsequent to MI; and 4) the border zone becomes edematous shortly after MI, consistent with a localized increase in vascular permeability.

As shown in our previous work, AAV9-mediated gene expression can be effectively restricted to cardiomyocytes using the cardiac-specific cTnT promoter [25]. Using the AAV9 capsid in combination with the cTnT promoter, we showed that eGFP expression after systemic administration was virtually undetectable in both vascular smooth muscle and endothelial cells in the heart, even while it was expressed in $>95\%$ of cardiomyocytes [25]. Despite being the most efficient gene delivery platform currently available for cardiomyocytes, gene expression from AAV9 does not approach full strength in the normal heart until 2–3 weeks after vector administration (see sham in Fig. 2B). Conventional vectors packaged in the AAV2 capsid have shown an even more prolonged lag phase, taking

up to 8 weeks to reach a steady-state plateau of gene expression in the heart [19]. In order to accommodate this limitation, previous studies of AAV-mediated gene therapy for MI have typically employed a preemptive gene therapy approach in which AAV2 vectors carrying therapeutic genes were directly injected into the LV wall 4–6 weeks before the induction of myocardial ischemia [21, 22]. In a similarly designed study of preemptive delivery, our laboratory recently demonstrated that a single direct intramuscular injection into the LV wall of an AAV9 vector expressing EcSOD from the cTnT promoter four weeks before the induction of MI caused a 22-fold increase in EcSOD activity which significantly decreased infarct size [26].

The delay in reaching maximal gene expression in normal myocardium may also explain why only a few previous studies have attempted to protect the heart against LV remodeling by delivering AAV vectors after MI has already occurred. This delay is especially problematic in mice, where global LV remodeling starts within a day after reperfused MI and nears completion within 2 weeks [2]. Therefore, an early onset of therapeutic gene expression following vector administration is important in curtailing LV remodeling, particularly in mouse models of MI. Nevertheless, a few previous studies have explored the utility of administering AAV by direct injection into the LV wall after ischemia/reperfusion injury. Su et al. [33] directly injected an AAV1 vector carrying VEGF driven by a cardiac specific promoter into mouse myocardium after MI. Jacquier et al. [23] and Saeed et al. [24] directly injected AAV2 vectors carrying VEGF cDNA into swine myocardium after MI. Despite the prolonged lag phase to full gene expression documented in normal hearts, AAV2-mediated VEGF gene delivery after MI brought about significant improvements in cardiac function. However, none of these previous studies employed systemic administration, nor did they report the phenomenon of preferential transduction and early onset of gene expression in cardiomyocytes located in the infarct border zone. The results of the current study demonstrate that systemic administration of an AAV9 vector following ischemia/reperfusion injury provides for robust and early onset gene expression, particularly in the cardiomyocytes at risk bordering the infarct region. Since this is the first report documenting the phenomenon of preferential transduction of cardiomyocytes at risk following systemic administration of AAV vector after IR injury, it may warrant further investigation using other serotypes of AAV and in larger animal models of IR injury. The current study suggests that AAV9 vectors may have considerable potential to deliver therapeutic genes to the infarct border zone after MI, providing a means to genetically reprogram the subsequent LV remodeling process and the potential to avert heart failure in patients who survive a large MI.

Myocardial IR injury increases capillary permeability, both as a direct result of ischemia and as the indirect result of the local release of inflammatory mediators upon reperfusion [34, 35]. The increase in vascular permeability allows greater fluid passage into the extravascular space, disrupting the normal balance between capillary filtration and lymphatic reabsorption, resulting in the accumulation of fluid in the extravascular space (edema). The CMR experiments summarized in Fig. 7 confirm that edema develops quickly in the infarct border zone in this mouse model of reperfused myocardial infarction, providing evidence of increased capillary permeability in the border zone and suggesting a potential mechanism by which AAV9 vectors circulating in the bloodstream might gain enhanced access to viable cardiomyocytes within the edematous region. This mechanism of enhanced access to cardiomyocytes is supported by the fact that ischemia-induced elevations in gene expression (Fig. 2B&C) are accompanied by similar increases in the numbers of viral genomes/ μ g of genomic DNA (Fig. 2D).

The various serotypes of AAV accomplish transduction by first binding to different cell surface receptors. AAV2 uses heparin sulfate proteoglycan, FGFR1 and α v β 5 integrin [36–

38], AAV1 and 6 use $\alpha 2,3$ and $\alpha 2,6$ N-linked sialic acids [39], while AAV 8 and 9 use the 36/37 kDa laminin receptor [40]. Recently, Shen et al. showed that desialylated N-linked glycans with terminal galactosyl residues also serve as receptors for AAV9 [41]. Given that sialidase activity and free sialic acid are significantly increased in the plasma from patients with ischemia, it is plausible that endogenous sialidases are locally activated by ischemia and their activation may “unmask” receptors for AAV9 through the desialylation of N-linked glycans. Note that this potential mechanism for the ischemic enhancement of AAV9-mediated transduction may act in synergy with the mechanism of increased vascular permeability implicated here (Fig. 7), since the mechanism of receptor “unmasking” by endogenous sialidases can only be realized after AAV9 escapes the bloodstream, a process that is enhanced by increased vascular permeability.

Following receptor binding and viral entry into the cell, capsid uncoating and second strand DNA synthesis are the rate limiting steps for gene expression from the AAV genome. Previous studies have shown that the reagents that induce DNA damage and repair activity, such as hydroxyurea, UV irradiation and topoisomerase inhibitors, accelerate the onset of gene expression from AAV2 vectors [9, 19, 42, 43]. Recently, it was shown that another stress inducing factor, prolonged fasting, significantly improves AAV transduction in skeletal muscle, heart and liver following systemic administration of AAV2, 6 and 9 vectors [44]. The DNA damage that results from IR injury may well be a contributing mechanism for the observed increase in transduction efficiency, because DNA damage causes rapid relocalization of the heterotrimeric DNA repair complex consisting of *Mre11*, *Rad50* and *Nbs1* (MRN) to the site of DNA damage [45, 46]. Furthermore, degradation or re-localization of the MRN complex to sites of DNA damage appears to create a nuclear environment that is more conducive for AAV-mediated gene expression [43, 47]. Collectively, these studies suggest that rapid relocalization of the MRN DNA repair complex due to IR injury might be another mechanism contributing to the enhanced transduction of “at risk” cardiomyocytes in the border zone after MI.

The results of this study have implications for both basic science and clinical translation. From the perspective of basic cardiovascular science, the ability to selectively target gene expression to the infarct border zone after MI opens the possibility of examining the function of gene expression (or knockdown via siRNA) in a tissue-, region- and time-selective manner after MI. From the clinical perspective, the current study suggests the possibility of genetically reprogramming gene expression in the infarct border zone by simple IV administration after MI. In this manner, gene therapy protocols could be used in combination with conventional pharmacologic interventions or even cell-based therapies to improve long-term outcomes after MI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH R01s HL058582 and HL092305 to BAF, and by R01 EB001826 and S10 RR027333 to JAH.

References

1. Cohn JN, Ferrari R, Sharpe N. On behalf of an international forum on cardiac remodeling. Cardiac remodeling—concepts and clinical implications: a consensus paper from an international forum on cardiac remodeling. *J Am Coll Cardiol*. 2000; 35:569–582. [PubMed: 10716457]

2. Ross AJ, Yang Z, Berr SS, et al. Serial MRI evaluation of cardiac structure and function in mice after reperfused myocardial infarction. *Magnetic Resonance in Medicine*. 2002; 47:1158–1168. [PubMed: 12111962]
3. Yang Y, Nunes FA, Berencsi K, et al. Cellular immunity to viral antigens limits E1-deleted adenoviruses for gene therapy. *Proceedings of the National Academy of Sciences*. 1994; 91:4407–4411.
4. Dash R, Azab B, Shen X-N, et al. Developing an effective gene therapy for prostate cancer: New technologies with potential to translate from the laboratory into the clinic. *Discovery Medicine*. 2011; 11:46–56. [PubMed: 21276410]
5. Kang E, Yun C-O. Current advances in adenovirus nanocomplexes: more specificity and less immunogenicity. *BMB reports*. 2010; 43:781–788. [PubMed: 21189153]
6. Hernandez YJ, Wang J, Kearns WG, et al. Latent Adeno-Associated Virus Infection Elicits Humoral but Not Cell-Mediated Immune Responses in a Nonhuman Primate Model. *Journal of Virology*. 1999; 73:8549–8558. [PubMed: 10482608]
7. Gao G, Vandenberghe LH, Alvira MR, et al. Clades of Adeno-associated viruses are widely disseminated in human tissues. *Journal of Virology*. 2004; 78:6381–6388. [PubMed: 15163731]
8. Rutledge EA, Halbert CL, Russell DW. Infectious clones and vectors derived from adeno-associated virus (AAV) serotypes other than AAV type 2. *Journal of Virology*. 1998; 72:309–319. [PubMed: 9420229]
9. Du L, Kido M, Lee DV, et al. Differential myocardial gene delivery by recombinant serotype-specific adeno-associated viral vectors. *Molecular Therapy: the Journal of the American Society of Gene Therapy*. 2004; 10:604–608. [PubMed: 15336660]
10. Palomeque J, Chemaly ER, Colosi P, et al. Efficiency of eight different AAV serotypes in transducing rat myocardium in vivo. *Gene Therapy*. 2007; 14:989–997. [PubMed: 17251988]
11. Zincarelli C, Soltys S, Rengo G, et al. Analysis of AAV Serotypes 1–9 Mediated Gene Expression and Tropism in Mice After Systemic Injection. *Molecular Therapy*. 2008; 16:1073–1080. [PubMed: 18414476]
12. Inagaki K, Fuess S, Storm TA, et al. Robust systemic transduction with AAV9 vectors in mice: efficient global cardiac gene transfer superior to that of AAV8. *Molecular Therapy*. 2006; 14:45–53. [PubMed: 16713360]
13. Bostick B, Ghosh A, Yue Y, et al. Systemic AAV-9 transduction in mice is influenced by animal age but not by the route of administration. *Gene Therapy*. 2007; 14:1605–1609. [PubMed: 17898796]
14. Gregorevic P, Blankinship MJ, Allen JM, et al. Systemic delivery of genes to striated muscles using adeno-associated viral vectors. *Nature Medicine*. 2004; 10:828–834.
15. Townsend D, Blankinship MJ, Allen JM, et al. Systemic administration of micro-dystrophin restores cardiac geometry and prevents dobutamine-induced cardiac pump failure. *Molecular Therapy*. 2007; 15:1086–1092. [PubMed: 17440445]
16. Pacak CA, Mah CS, Thattaliyath BD, et al. Recombinant adeno-associated virus serotype 9 leads to preferential cardiac transduction in vivo. *Circulation Research*. 2006; 99:e3–9. [PubMed: 16873720]
17. Cataliotti A, Tonne JM, Bellavia D, et al. Long-Term Cardiac pro-B-Type Natriuretic Peptide Gene Delivery Prevents the Development of Hypertensive Heart Disease in Spontaneously Hypertensive Rats/Clinical Perspective. *Circulation*. 2011; 123:1297–1305. [PubMed: 21403100]
18. Kaya Z, Leib C, Werfel S, et al. Comparison of IL-10 and MCP-1–7ND gene transfer with AAV9 vectors for protection from murine autoimmune myocarditis. *Cardiovascular Research*. 2011; 91:116–123. [PubMed: 21354997]
19. Prasad K-MR, Xu Y, Yang Z, et al. Topoisomerase inhibition accelerates gene expression after adeno-associated virus-mediated gene transfer to the mammalian heart. *Molecular Therapy*. 2007; 15:764–771. [PubMed: 17299410]
20. Thomas CE, Storm TA, Huang Z, et al. Rapid uncoating of vector genomes is the key to efficient liver transduction with pseudotyped adeno-associated virus vectors. *Journal of Virology*. 2004; 78:3110–3122. [PubMed: 14990730]

21. Agrawal RS, Muangman S, Layne MD, et al. Pre-emptive gene therapy using recombinant adeno-associated virus delivery of extracellular superoxide dismutase protects heart against ischemic reperfusion injury, improves ventricular function and prolongs survival. *Gene Ther.* 2004; 11:962–969. [PubMed: 15029230]
22. Pachori AS, Melo LG, Zhang L, et al. Chronic recurrent myocardial ischemic injury is significantly attenuated by pre-emptive adeno-associated virus heme oxygenase-1 gene delivery. *J Am Coll Cardiol.* 2006; 47:635–643. [PubMed: 16458149]
23. Jacquier A, Higgins CB, Martin AJ, et al. Injection of Adeno-associated Viral Vector–Encoding Vascular Endothelial Growth Factor Gene in Infarcted Swine Myocardium: MR Measurements of Left Ventricular Function and Strain. *Radiology.* 2007; 245:196–205. [PubMed: 17885189]
24. Saeed M, Saloner D, Martin A, et al. Adeno-associated Viral Vector–Encoding Vascular Endothelial Growth Factor Gene: Effect on Cardiovascular MR Perfusion and Infarct Resorption Measurements in Swine. *Radiology.* 2007; 243:451–460. [PubMed: 17384240]
25. Prasad K-MR, Xu Y, Yang Z, et al. Robust Cardiomyocyte-Specific Gene Expression Following Systemic Injection of AAV: In Vivo Gene Delivery Follows a Poisson Distribution. *Gene Therapy.* 2011; 18:43–52. [PubMed: 20703310]
26. Prasad K-MR, Smith RS, Xu Y, et al. A single direct injection into the left ventricular wall of an adeno-associated virus 9 (AAV9) vector expressing extracellular superoxide dismutase from the cardiac troponin-T promoter protects mice against myocardial infarction. *J Gene Med.* 2011; 13:333–341. [PubMed: 21674736]
27. Ried MU, Girod A, Leike K, et al. Adeno-associated virus capsids displaying immunoglobulin-binding domains permit antibody-mediated vector retargeting to specific cell surface receptors. *Journal of Virology.* 2002; 76:4559–4566. [PubMed: 11932421]
28. Yang Z, Berr SS, Gilson WD, et al. Simultaneous evaluation of infarct size and cardiac function in intact mice by contrast-enhanced cardiac magnetic resonance imaging reveals contractile dysfunction in noninfarcted regions early after myocardial infarction. *Circulation.* 2004; 109:1161–1167. [PubMed: 14967719]
29. Wu JC, Inubushi M, Sundaresan G, et al. Optical imaging of cardiac reporter gene expression in living rats. *Circulation.* 2002; 105:1631–1634. [PubMed: 11940538]
30. Li Y, Garson CD, Xu Y, et al. High frequency ultrasound imaging detects cardiac dyssynchrony in noninfarcted regions of the murine left ventricle late after reperfused myocardial infarction. *Ultrasound Med Biol.* 2008; 34:1063–1075. [PubMed: 18313202]
31. Beyers RJ, Smith RS, Xu Y, et al. T2-weighted MRI of post-infarct myocardial edema in mice. *Magnetic Resonance in Medicine.* 2011; 67:201–209. [PubMed: 21630350]
32. Helm PA, Caravan P, French BA, et al. Postinfarction Myocardial Scarring in Mice: Molecular MR Imaging with Use of a Collagen-targeting Contrast Agent. *Radiology.* 2008; 247:788–796. [PubMed: 18403626]
33. Su H, Huang Y, Takagawa J, et al. AAV serotype-1 mediates early onset of gene expression in mouse hearts and results in better therapeutic effect. *Gene Therapy.* 2006; 13:1495–1502. [PubMed: 16775632]
34. Khandoga AG, Khandoga A, Anders H-J, et al. Postischemic vascular permeability requires both TLR-2 and TLR-4, but only TLR-2 mediates the transendothelial migration of leukocytes. *Shock.* 2009; 31:592–598. [PubMed: 19008784]
35. Weis SM. Vascular permeability in cardiovascular disease and cancer. *Current Opinion in Hematology.* 2008; 15:243–249. [PubMed: 18391792]
36. Summerford C, Samulski RJ. Membrane-associated heparan sulfate proteoglycan is a receptor for adeno-associated virus type 2 virions. *Journal of Virology.* 1998; 72:1438–1445. [PubMed: 9445046]
37. Summerford C, Bartlett JS, Samulski RJ. AlphaVbeta5 integrin: a co-receptor for adeno-associated virus type 2 infection. *Nature Medicine.* 1999; 5:78–82.
38. Qing K, Mah C, Hansen J, et al. Human fibroblast growth factor receptor 1 is a co-receptor for infection by adeno-associated virus 2. *Nature Medicine.* 1999; 5:71–77.

39. Wu Z, Miller E, Agbandje-McKenna M, et al. Alpha2,3 and alpha2,6 N-Linked Sialic Acids Facilitate Efficient Binding and Transduction by Adeno-Associated Virus Types 1 and 6. *Journal of Virology*. 2006; 80:9093–9103. [PubMed: 16940521]
40. Akache B, Grimm D, Pandey K, et al. The 37/67-kilodalton laminin receptor is a receptor for adeno-associated virus serotypes 8, 2, 3, and 9. *J Virol*. 2006; 80:9831–9836. [PubMed: 16973587]
41. Shen S, Bryant KD, Brown SM, et al. Terminal N-Linked Galactose Is the Primary Receptor for Adeno-associated Virus 9. *Journal of Biological Chemistry*. 2011; 286:13532–13540. [PubMed: 21330365]
42. Alexander IE, Russell DW, Spence AM, et al. Effects of gamma irradiation on the transduction of dividing and nondividing cells in brain and muscle of rats by adeno-associated virus vectors. *Hum Gene Ther*. 1996; 7:841–850. [PubMed: 8860836]
43. Russell DW, Alexander IE, Miller AD. DNA synthesis and topoisomerase inhibitors increase transduction by adeno-associated virus vectors. *Proc Natl Acad Sci U S A*. 1995; 92:5719–5723. [PubMed: 7777575]
44. Moulay G, Scherman D, Kichler A. Fasting Increases the In Vivo Gene Delivery of AAV Vectors. *Clin Transl Sci*. 2010; 3:333–336. [PubMed: 21207763]
45. Maser RS, Monsen KJ, Nelms BE, et al. hMre11 and hRad50 nuclear foci are induced during the normal cellular response to DNA double-strand breaks. *Mol Cell Biol*. 1997; 17:6087–6096. [PubMed: 9315668]
46. Mirzoeva OK, Petrini JH. DNA damage-dependent nuclear dynamics of the Mre11 complex. *Mol Cell Biol*. 2001; 21:281–288. [PubMed: 11113202]
47. Schwartz RA, Palacios JA, Cassell GD, et al. The Mre11/Rad50/Nbs1 Complex Limits Adeno-Associated Virus Transduction and Replication. *Journal of Virology*. 2007; 81:12936–12945. [PubMed: 17898048]

AcTnTLuc

AcTnTeGFP

AcTnTEcSOD

Fig. 1. Schematic representation of AAV vectors: AcTnTLuc, AcTnTeGFP and AcTnTEcSOD carry the firefly luciferase, eGFP and EcSOD cDNA, respectively, driven by the cardiac troponin-T (cTnT) promoter. AAV inverted terminal repeats (*ITR*) and SV40 polyadenylation sites (SV-pA) are indicated.

Fig. 2.

Experiments characterizing the effects of delaying the intravenous injection of AAV9 for various periods of time after reperfusion. Sham-operated mice (Sham) or mice that underwent 30 min IR (n=4 per group) were injected intravenously with AAV9 carrying AcTnTLuc, 1×10^{11} viral genomes/mouse at the indicated times (10 min, 1 day, 2 days or 3 days) after reperfusion. (A) Representative bioluminescence images acquired at 2 days following vector administration for two mice from each group. Additional bioluminescence images (of mice obtained at days 1, 2, 3, 6, 14, 21, 28 and 35 after vector administration) are shown in Supplementary Data (Fig. S1). (B) Graph showing the time course of luciferase expression in mice. For each group of mice, the mean values of bioluminescence as average radiance (photons/s.cm²-sr) were obtained from the regions of interest and plotted against time after AcTnTLuc vector injection. (C) Quantitative determination of luciferase activity in protein extracts from hearts collected 35 days after vector administration. Luciferase activities are expressed as relative light units per mg tissue (RLUs/mg tissue, *p<0.05 vs. sham). (D) AAV vector genome copy numbers in hearts following systemic administration of AcTnTeGFP (1×10^{11} viral genomes/mouse) in sham-operated mice (sham) or at the indicated times (10 min, 1 day, 2 days or 3 days) after reperfusion. Results are expressed as viral genomes/μg genomic DNA (*p<0.05 vs. sham).

Fig. 3.

Distribution of transgene expression within the heart as a result of AAV administered after IR injury: (A) Western blot showing the expression of eGFP in mouse hearts. Sham-operated mice (Sham) or mice that underwent 30 min of ischemia (IR) were injected with saline or AcTnTeGFP (AAV9) at 10 min following reperfusion. Five days later, protein extracts were prepared from the mouse hearts and eGFP expression was detected by immunoblot analysis. To control for sample-loading error, the blot was then stripped and re-probed with an antibody against actin. The fold-induction due to ischemia is graphed at right relative to the sham-operated control. Panels (B)-(D) show immunohistochemistry for eGFP performed in short-axis myocardial tissue sections from: (B) mice that underwent 30 min of coronary artery occlusion, but were injected IV with vehicle (IR+saline), (C) sham-operated mice that were injected with AcTnTeGFP (Sham+AAV9), and (D) mice that underwent 30 min of coronary artery occlusion and were injected with AcTnTeGFP 10 min after reperfusion (IR+AAV9). Five days following vector administration, eGFP expression in the mouse hearts was evaluated by immunohistochemistry on slides counterstained with eosin. Panels (B)-(D) show photomicrographs taken at 4 $\times$ magnification. Panels B1, C1, D1, D2, D3 and D4 show 40X magnifications of the areas indicated by rectangles in Panels B, C and D, respectively. Note that that combination of the red eosin counterstain and the brown DAB chromogen (labeling the antibody against eGFP) produce a reddish-brown color in eGFP-positive cells.

Fig. 4.

Post-MI AAV9 administration results in gene expression primarily localized to cardiomyocytes bordering the edge of the infarct. Mice were injected with AcTnTeGFP or AcTnTLuc at 10 min after IR injury. Five days later, (A) eGFP expression was detected by fluorescence microscopy (green). (B) Myoglobin expression in the same section was detected using an antibody against myoglobin (red). Note that the infarct region in the center of the field is evidenced by the loss of myosin (i.e., lack of red staining). (C) An overlay of panels A and B indicates in yellow to green the location of cardiomyocytes expressing various levels of eGFP (white arrows indicate two examples). (D) Hearts from mice injected with AcTnTLuc were collected 5 days following vector administration for quantitative

luciferase activity assays. The graph shows the results of quantitative luciferase activity assays on protein extracts from hearts of sham-operated mice (Sham) or mice that underwent 30 min of coronary occlusion. Remote (Rem) and ischemic (Isch) regions of hearts from mice that underwent 30 min of coronary occlusion prior to AAV9 injection were separated under a dissecting microscope for luciferase activity assay. Hearts were collected 5 days following vector administration for quantitative luciferase activity assays. Luciferase activities are expressed RLU/mg protein (* $p < 0.05$ vs. sham, ** $p < 0.05$ vs. sham or remote).

Fig. 5.

AAV9 carrying EcSOD administered after IR injury provides a 12.5-fold increase in EcSOD expression: Ten minutes after a 60 minute IR injury, mice were either injected IV with AcTnTEcSOD (EcSOD, n=8) or were used as controls (CTRL, n=9). Hearts were collected on day 29 (one day following the last echocardiography session described below in Fig. 6) and Western blot analysis performed on 3 hearts from each group revealed a 12.5-fold increase in GAPDH-normalized EcSOD expression in the EcSOD-treated group compared to the control group ($p < 0.05$).

Fig. 6.

AAV9 carrying EcSOD administered after IR injury attenuates LV remodeling: Ten minutes after a 60 minute IR injury, mice were either injected IV with AcTnTEcSOD (EcSOD, n=8) or were used as controls (CTRL, n=9). Representative short-axis echo images of mouse hearts at end-systole from the control (A) and EcSOD-treated (B) groups at Day 28 post-MI. For orientation, images are labeled on the anterior (Ant), inferior (Inf), septal (Sep), and lateral (Lat) walls. Note that end-systolic chamber volume is appreciably smaller, and that the ventricular walls are appreciably thicker in the EcSOD-treated heart (B) as compared to the control heart (A). One day after the final echocardiography session, hearts were explanted for histochemical staining. Representative paraffin-embedded sections from control and EcSOD-treated groups were stained with H&E (C and D, respectively). Scale bars = 1 mm. LV remodeling was measured by LV end-diastolic (E) and LV end-systolic (F) volumes. Measurements were obtained by high-resolution echocardiography performed at baseline (1 day before MI surgery) and at days 2, 7, 14 and 28 after MI. Results are reported as percent increase over baseline (*p<0.05 vs. CTRL on same day). Sphericity index (G) was also significantly improved in the EcSOD-treated group on Day 28 compared to the infarcted control group (CTRL, *p<0.05 vs. CTRL).

Fig. 7.

Cardiac MRI demonstrates that the infarct and surrounding border zone are edematous after IR. Myocardial infarct area and edematous regions were detected by T1w LGE and T2w CMR imaging (left and right columns), respectively. Representative short-axis images acquired at the mid-left ventricular level from the same mouse heart at 1, 2 and 3 days post-IR are shown. The infarct regions enhanced by T1w LGE lie within the areas at risk represented by the enhanced areas in the T2w images, demonstrating that edema exists within both the infarct and infarct border zone.

Table 1

Wall thickening analysis of Control vs. EcSOD-treated groups

	<u>End-Diastolic WT (mm)</u>		<u>End-Systolic WT (mm)</u>		<u>Wall Thickening (%)</u>	
	Control	EcSOD	Control	EcSOD	Control	EcSOD
Anterior	0.31±0.03	0.33±0.03	0.29±0.03	0.33±0.03	-7.1±2.9%	2.6±5.9%
Inferior	0.46±0.03	0.57±0.04 *	0.55±0.03	0.72±0.05 **	20.2±4.3%	27.8±5.5%

* p<0.05,

** p<0.01 compared to Control