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Intravenous Statin Administration During Myocardial Infarction Compared With Oral Post-Infarct Administration

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ABSTRACT

BACKGROUND Beyond lipid-lowering, statins exert cardioprotective effects. High-dose statin treatment seems to reduce cardiovascular complications in high-risk patients. The ideal timing and administration regime remain unknown.

OBJECTIVES This study compared the cardioprotective effects of intravenous statin administration during myocardial infarction (MI) with oral administration immediately post-MI.

METHODS Hypercholesterolemic pigs underwent MI induction (90 min of ischemia) and were kept for 42 days. Animals were distributed in 3 arms (A): A1 received an intravenous bolus of atorvastatin during MI; A2 received an intravenous bolus of vehicle during MI; and A3 received oral atorvastatin within 2 h post-MI. A1 and A3 remained on daily oral atorvastatin for the following 42 days. Cardiac magnetic resonance analysis (days 3 and 42 post-MI) and molecular/histological studies were performed.

RESULTS At day 3, A1 showed a 10% reduction in infarct size compared with A3 and A2 and a 50% increase in myocardial salvage. At day 42, both A1 and A3 showed a significant decrease in scar size versus A2; however, A1 showed a further 24% reduction versus A3. Functional analyses revealed improved systolic performance in A1 compared with A2 and less wall motion abnormalities in the jeopardized myocardium versus both groups at day 42. A1 showed enhanced collagen content and AMP-activated protein kinase activation in the scar, increased vessel density in the penumbra, higher tumor necrosis factor α plasma levels and lower peripheral blood mononuclear cell activation versus both groups.

CONCLUSIONS Intravenous administration of atorvastatin during MI limits cardiac damage, improves cardiac function, and mitigates remodeling to a larger extent than when administered orally shortly after reperfusion. This therapeutic approach deserves to be investigated in ST-segment elevation MI patients. (J Am Coll Cardiol 2020;75:1386–402)

Several trials have suggested that high-dose statin treatment may reduce cardiovascular complications in patients undergoing invasive management. Through the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, statins reduce low-density lipoprotein (LDL) cholesterol levels and decrease cardiovascular morbidity and mortality (1). Moreover, intensive statin therapy

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has demonstrated clinical benefit in non-ST-segment elevation myocardial infarction (STEMI) patients, an effect also observed in patients subjected to elective coronary intervention procedures (2,3). In addition, statins seem to exert cardioprotective effects irrespective of their lipid-lowering properties. However, the ideal timing and administration regime are unknown. Post-interventional oral statin administration is a common practice in cardiology departments in accordance with current practice guidelines (4,5). Yet, the value of a loading dose of statin before intervention in reducing cardiovascular events in patients with acute coronary syndrome (ACS) remains to be demonstrated. As such, the SECURE-PCI (Statins Evaluation in Coronary Procedures and Revascularization Trial) (6) showed that routine administration of 2 early doses of atorvastatin (80 mg) is not superior to placebo in reducing major adverse cardiovascular events (MACE) at 30 days among patients presenting with ACS and scheduled to undergo an early invasive approach. Yet, a subgroup analysis of the STEMI patients revealed a significant reduction in MACE at

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30 days in those administered the statin regime, likely supporting statin-pleotropic effects. Indeed, beyond their lipid-lowering effects, statins have direct cardioprotective and vasculoprotective properties that may explain their beneficial effects in the acute phase of MI (7–10) and post-MI healing (11,12). Thus far, experimental studies have shown that statin treatment reduces infarct size when administered prior to or post-MI induction. In this context, our group demonstrated that administration of oral rosuvastatin early after reperfusion reduced infarct size (36% smaller infarcts) in a closed-chest pig model of MI (13). This effect was due to a reduction in apoptotic cell death and inflammatory cell infiltration, leading to improved cardiac function. The effects of rosuvastatin were not related to changes in low-density lipoprotein (LDL) cholesterol levels, but rather to the activation of cardioprotective kinases; thus, the effect was reversed by the addition of the isoprenoid geranylgeranyl pyrophosphate (13,14). Of note, we have recently evidenced that the intravenous administration of statin during early ischemia (mimicking first medical contact in the setting of STEMI) reduces the progression of ischemic injury (15). Additionally, we have shown that atorvastatin and β-OH-simvastatin (a modified active simvastatin) exert similar cardioprotective effects (16). However, whether intravenous statin treatment during ongoing myocardial ischemia optimizes cardiac protection compared with oral post-interventional statin therapy remains to be elucidated.

In this study, we have hypothesized that intravenous administration of atorvastatin during MI is superior to an oral dose of atorvastatin initiated shortly after revascularization. To adequately measure the impact of these interventions, we have used a preclinical pig model of MI with serial cardiac magnetic resonance (CMR) follow-up.

ABBREVIATIONS AND ACRONYMS

ACS = acute coronary syndrome

AMPK = AMP-activated protein kinase

CMR = cardiac magnetic resonance

IL = interleukin

LV = left ventricle/ventricular

MI = myocardial infarction

STEMI = ST-segment elevation myocardial infarction

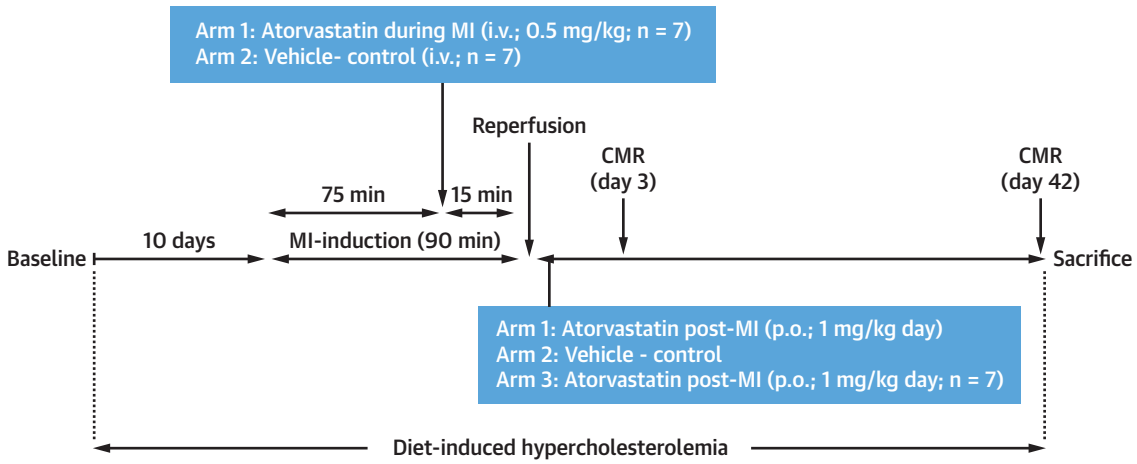
TNF = tumor necrosis factor

METHODS

The animal experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committees and authorized by the Animal Experimental Committee of the local government (#5601) in accordance to the Spanish law (RD 53/2013) and European Directive 2010/63/EU. In addition, the investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1985), has followed the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines and committed to the "3Rs" of laboratory animal research, and consequently, used the minimal number of animals to reach statistical conclusion (17). All animals were allowed to acclimate 7 days prior to any intervention.

EXPERIMENTAL DESIGN, DRUG ADMINISTRATION, AND MI INDUCTION. The experimental design is shown in **Figure 1**. Pigs (n = 21; 4-month-old sows; weight: 38 ± 3 kg) were fed a high cholesterol diet (Western-type hypercholesterolemic diet; 20% saturated fat, 2% cholesterol, 1% cholic acid) during 10 days. We have already reported that intake of this fat-rich diet for 10 days raises cholesterol to levels comparable to those found in hypercholesterolemic humans (18). At day 10, all pigs underwent MI induction, and then reperfusion was established and animals were kept for 42 days. Closed-chest MI was experimentally induced by percutaneous total balloon occlusion of the mid-portion of the left anterior descending artery, as previously described (19,20) (expanded in the Supplemental Appendix, Supplemental Methods). Although pigs have a sparse collateral circulation, we routinely monitored myocardial collateral circulation by fluoroscopy guidance in all pigs to ensure a comparable degree of ischemic damage after MI induction.

FIGURE 1 Experimental Design



CMR = cardiac magnetic resonance; i.v. = intravenous; MI = myocardial infarction; p.o. = per os.

Animals were randomly and blindly distributed in the following 3 study arms (7 animals/group). Arm 1: animals were administered an intravenous bolus of atorvastatin (a modified intravenous atorvastatin preparation, patent PCT/EP2018/058158; 0.5 mg/kg; n = 7) during ongoing ischemia (after 75 min of MI) (atorva-during-MI); arm 2: animals received an intravenous bolus of vehicle (dimethylformamide, diluted in saline) during MI (after 75 min of MI) (vehicle-control); and arm 3: animals were administered per os atorvastatin, initiated within the first 2 h post-MI (atorva-post-MI) (1 mg/kg). Animals in arms 1 and 3 remained on per os atorvastatin (1 mg/kg/day) therapy for the following 42 days, whereas animals in arm 2 received placebo pills. To ensure the blind administration of the therapies, an animal facility technician who was not involved in conducting the experimental protocol was responsible for daily pill administration.

The intravenous drug dosage was calculated according to a 40-mg oral dose of atorvastatin used in humans, and converted to pig dosing according to body surface area. We chose atorvastatin due to its lipophilicity and use in our previous ischemia studies (15), and because it is recommended in current clinical practice guidelines worldwide. In a subgroup of animals (n = 7 intravenous atorvastatin and n = 7 vehicle), we measured statin plasma levels reached by the intravenous administration of atorvastatin. Blood was collected, and K_2-ethylenediaminetetraacetic acid plasma samples were analyzed by high performance liquid chromatography tandem mass spectrometry. For this purpose, blood was serially drawn at 15, 45, and 75 min after the intravenous infusion of atorvastatin or vehicle.

At days 3 and 42 post-MI, animals were anesthetized and brought to the 3T-CMR facility for blind assessment of global and regional parameters. Animals were euthanized after the last CMR analysis (day 42), and blood and tissue samples were collected for molecular analysis.

3.0-T CMR ACQUISITION. CMR studies were conducted serially in all animals at days 3 (early remodeling phase) and 42 (late remodeling phase) post-MI. The studies were performed on a 3.0-T CMR system (Achieva, Philips, Best, the Netherlands) and CMR image acquisition was carried out by a CMR-specialized technician blinded to treatment. For CMR studies, animals were anesthetized with an intramuscular injection of a cocktail composed of ketamine, xylazine, and atropine and maintained by a continuous intravenous infusion of propofol to ensure mechanical ventilation. Once the animals were positioned in a head-first supine position with a flexible phased-array surface coil placed over the chest, electrocardiography gating was used to acquire still images of the heart. The following dedicated CMR sequences were acquired in all cases: “cine” (balanced steady-state free-precession) imaging sequence to assess wall motion (WM) and cardiac function, T2-weighted short-tau inversion recovery (T2w-STIR) sequence to assess myocardial edema, early gadolinium enhancement to analyze microvascular obstruction (no-reflow phenomenon), and late gadolinium enhancement (LGE) to assess the amount and extent of myocardial necrosis. All of the CMR studies followed

the same scheme. First, scout images (T1-TFE sequence) were obtained to localize the true axes of the heart and define a field of view involving the whole heart. Afterward, balanced steady-state free-precession cine imaging was performed in both horizontal and vertical long axes (4- and 2-chamber views) and in multiple contiguous short-axis images covering the whole left ventricle (LV). In the short-axis cine sequence, we acquired 24 cardiac phases of every slice to guarantee a correct evaluation of the WM and heart function. Once the cine sequences were acquired, a T2w-STIR sequence was obtained to assess myocardial edema. Thereafter, a gadolinium-based contrast agent was injected intravenously (Gd-GTPA, Magnevist, Berlex Laboratories Inc., Wayne, New Jersey) at a dose of 0.1 mmol/kg. The early gadolinium enhancement sequence was acquired 1 min after the administration of the contrast. The LGE sequences were obtained 10 min after the administration of contrast. Details of the technical parameters for CMR sequences have been previously published (19,20).

3T-CMR DATA ANALYSIS. Global functional and anatomical parameters. CMR data were independently analyzed using dedicated software (QMass MR version 7.6, Medis, Leiden, the Netherlands) by a CMR-trained radiologist (M.G.) blinded to the study medication. The protocol of analysis for global functional/anatomical parameters was performed as previously reported (19,21). Briefly, LV epicardial and endocardial borders were traced in each image of the cardiac phases representing the end-diastole and end-systole to obtain the left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV), and left ventricular ejection fraction (LVEF). LGE scar size was assessed by using manual planimetric segmentation on each slice. The same method was used for the assessment of myocardial edema. The area of myocardial edema was defined as the extent of the LV demonstrating high signal intensity on T2w-STIR images. The size of infarction (day 3) or scar (day 42) was quantified from the extent of myocardial enhancement in the LGE CMR sequence.

Cardiac remodeling parameters: WM and myocardial viability. LV regional analysis for each of the 16 American Heart Association myocardial segments was performed (22). The endocardial and epicardial borders of the LV were defined in all slices representing the end-diastolic and -systolic phases. Thereafter the end-systolic wall thickness (EST), the end-diastolic wall thickness and the WM (WM = EST − end-diastolic wall thickness) were automatically calculated as previously reported (23). In addition, we also determined the number of dysfunctional segments and myocardial viability as previously reported (24). Briefly, dysfunctional segments were considered those segments with a WM <2 mm whereas myocardial recovery was assessed as the percentage of segments with a WM at day 3 <2 mm (dysfunctional segments) that presented a WM at day 42 ≥2 mm (recovered segments). These measurements and analyses were performed only including the jeopardized segments (mid and apical antero/septal segments; target segments 7, 8, 13, and 14) and including those segments contralateral to the infracted region (basal, mid, and apical inferior/lateral segments; target segments 4, 5, 10, 11, 15, and 16).

SCAR SIZE ANALYSIS BY TRIPHENYLTETRAZOLIUM CHLORIDE STAINING. Although the investigation of scar size by triphenyltetrazolium chloride (TTC) staining late after reperfusion is not universally accepted, we performed the analysis with the intention of gathering some additional information. At day 42 and after CMR analysis, hearts were arrested with potassium chloride, rapidly excised, sliced, and stained with TTC to determine the size of the scar by planimetry using the National Institutes of Health software ImageJ.

MYOCARDIAL CHARACTERIZATION OF VESSEL DENSITY AND FIBROSIS: MOLECULAR AND HISTOLOGICAL APPROACHES. To investigate the effect of statin treatment on cardiac remodeling and scar tissue, we examined: 1) neovessel formation in the penumbra and remote myocardium via the analysis of angiogenic markers (CD105, von Willebrand factor [vWF], CD31) and lectin and vWF staining; and 2) myocardial fibrosis in the scar and remote myocardium by assessing mRNA levels for collagen 3A1, collagen 1A1; protein expression/activation of transforming growth factor (TGF)-β type II receptor (Abcam, Cambridge, United Kingdom), phosphorylated-Smad2/3 (Ser423/425, Santa Cruz, Dallas, Texas)], AMP-activated protein kinase (AMPK), and AMPK phosphorylated at Thr^{172} (P-AMPK); and, collagen deposition by Sirius red staining. We investigated the effects of statin treatment on AMPK activation given its previously reported cardioprotective effects (16,25).

For histological analyses, images were captured with a Nikon eclipse 80i microscope (Nikon, Tokyo, Japan) and digitalized by a Retiga-1300i (Teledyne QImaging, Surrey, British Columbia, Canada). Staining was calculated by a single blinded observer (S.B.-A.) from an average of 5-fields/sample and expressed as: (%) = (positive stained area/[total tissue area − vascular luminal areas]) × 100 using ImageJ.

TABLE 1 Serial Cardiac Magnetic Resonance Analyses

	Treatment	3 Days Post-MI	42 Days Post-MI
Anatomical parameters			
LV mass, g	Vehicle	70.1 ± 11.7	79.6 ± 13.8
	Atorva post-MI (p.o.)	60.3 ± 6.2	72.8 ± 7.4
	Atorva during MI (i.v.)	62.6 ± 8.9	71.4 ± 12.1
Edema, g LV	Vehicle	24.4 ± 2.8	
	Atorva post-MI (p.o.)	21.5 ± 2.3	
	Atorva during MI (i.v.)	20.7 ± 3.3	
Edema, % LV	Vehicle	35.5 ± 5.0	
	Atorva post-MI (p.o.)	35.8 ± 4.2	
	Atorva during MI (i.v.)	33.6 ± 7.0	
Infarct mass, g LV	Vehicle	20.2 ± 2.3	18.0 ± 2.1
	Atorva post-MI (p.o.)	17.4 ± 2.5	13.7 ± 2.3†
	Atorva during MI (i.v.)	12.2 ± 4.9*	10.3 ± 2.4*
Necrosis, % LV	Vehicle	29.3 ± 5.2	22.9 ± 2.5
	Atorva post-MI (p.o.)	29.0 ± 4.4	18.8 ± 2.5†
	Atorva during-MI (i.v.)	19.1 ± 6.6*	14.4 ± .4*
Myocardial salvage, g LV	Vehicle	4.3 ± 2.1	
	Atorva post-MI (p.o.)	4.1 ± 2.3	
	Atorva during MI (i.v.)	8.6 ± 2.4*	
Myocardial salvage index	Vehicle	17.2 ± 6.3	
	Atorva post-MI (p.o.)	19.0 ± 8.5	
	Atorva during MI (i.v.)	42.1 ± 18.3*	
No-reflow	Vehicle	1.9 ± 0.8	
	Atorva post-MI (p.o.)	2.2 ± 1.3	
	Atorva during MI (i.v.)	1.4 ± 0.7	
Functional parameters			
LVEF	Vehicle	49.1 ± 5.2	49.5 ± 5.8
	Atorva post-MI (p.o.)	52.4 ± 8.0	46.7 ± 9.9
	Atorva during MI (i.v.)	52.5 ± 6.6	54.0 ± 5.0
LVEDV	Vehicle	80.8 ± 11.1	110.8 ± 18.2‡
	Atorva post-MI (p.o.)	67.4 ± 10.5	97.0 ± 16.7‡
	Atorva during MI (i.v.)	69.5 ± 4.5	90.9 ± 15.1‡
LVESV	Vehicle	41.3 ± 8.1	55.2 ± 5.3‡
	Atorva post-MI (p.o.)	32.3 ± 8.0	52.1 ± 15.1‡
	Atorva during MI (i.v.)	33.2 ± 6.7	42.2 ± 10.2†

Values are mean ± SD. *$p < 0.05$ versus vehicle and atorvastatin post-MI. †$p < 0.05$ versus vehicle. ‡$p < 0.05$ versus day 3.

i.v. = intravenous. LV = left ventricle; LVEDV = left ventricular end-diastolic volume; LVEF = left ventricular ejection fraction; LVESV = left ventricular end-systolic volume.; MI = myocardial infarction; p.o. = per os.

INFLAMMATORY RESPONSE. Peripheral blood mononuclear cells isolation. Blood (20 ml) was collected into ethylenediaminetetraacetic acid tubes at baseline and 3 days post-MI to evaluate any potential effect of the treatment on MI-induced peripheral blood mononuclear cell (PBMC) activation (26). All blood samples were immediately subjected to Ficoll-paque Plus (Amersham Biosciences, Little Chalfont, United Kingdom) density gradient centrifugation to isolate PBMCs. Samples were counted and then directly frozen in liquid nitrogen and stored at −80°C until mRNA and protein analysis of monocyte-chemoattractant protein-1, cyclooxygenase-2, and Toll-like receptor-4 were performed.

Circulating cytokines and CRP measurement. We determined circulating levels of cytokines (interleukin [IL]-6 and tumor necrosis factor [TNF]-α) and CRP in plasma and serum, respectively, at baseline, acutely after MI induction (ischemia), and at 3 days post-MI by using commercially available enzyme-linked immunosorbent assay kits (porcine IL-6 and porcine TNF-α from R&D Systems, Minneapolis, Minnesota; porcine CRP from Abcam). According to the manufacturers, the minimum detection limits for IL-6, TNF-α, and CRP are 10 pg/ml, 3.7 pg/ml, and 2.15 ng/ml, respectively.

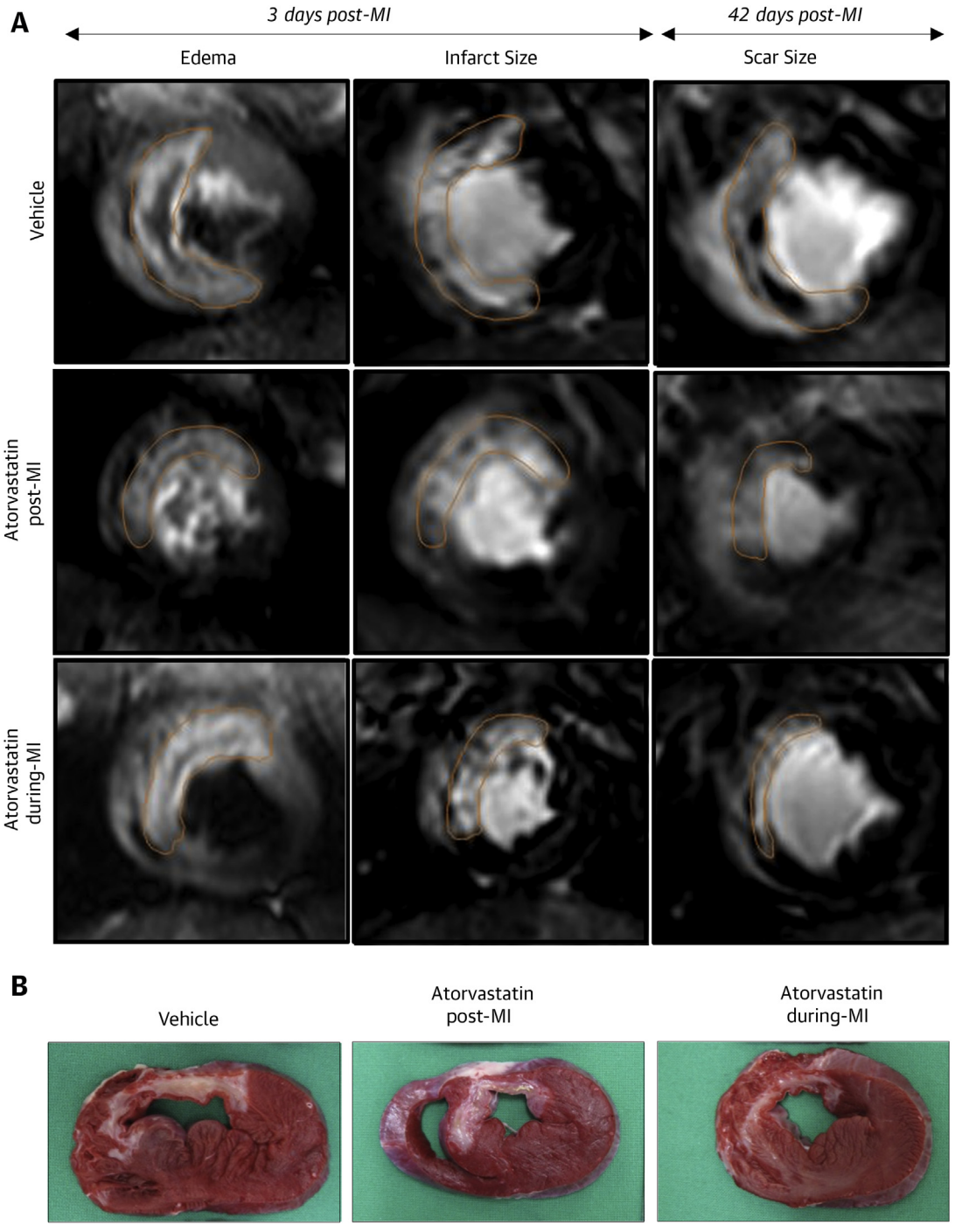
HEMATOLOGICAL AND BIOCHEMICAL FOLLOW-UP. Blood samples were collected at baseline, prior to MI, post-MI, and 3 and 42 days post-MI for lipid levels and liver and renal parameters assessment.

STATISTICAL ANALYSIS. After testing for normal distribution (Shapiro-Wilk test) repeated measures analysis of variance and paired Student's *t*-test was used to analyze variables within each group, whereas unpaired Student's *t*-test was used for all single time-point measurements to compare the 3 animal groups in the absence of multiple testing correction. A value of $p < 0.05$ was considered significant. Continuous variables are expressed as mean ± SD. The statistical analyses were performed with the statistical software package StatView (SAS Institute, Cary, North Carolina).

RESULTS

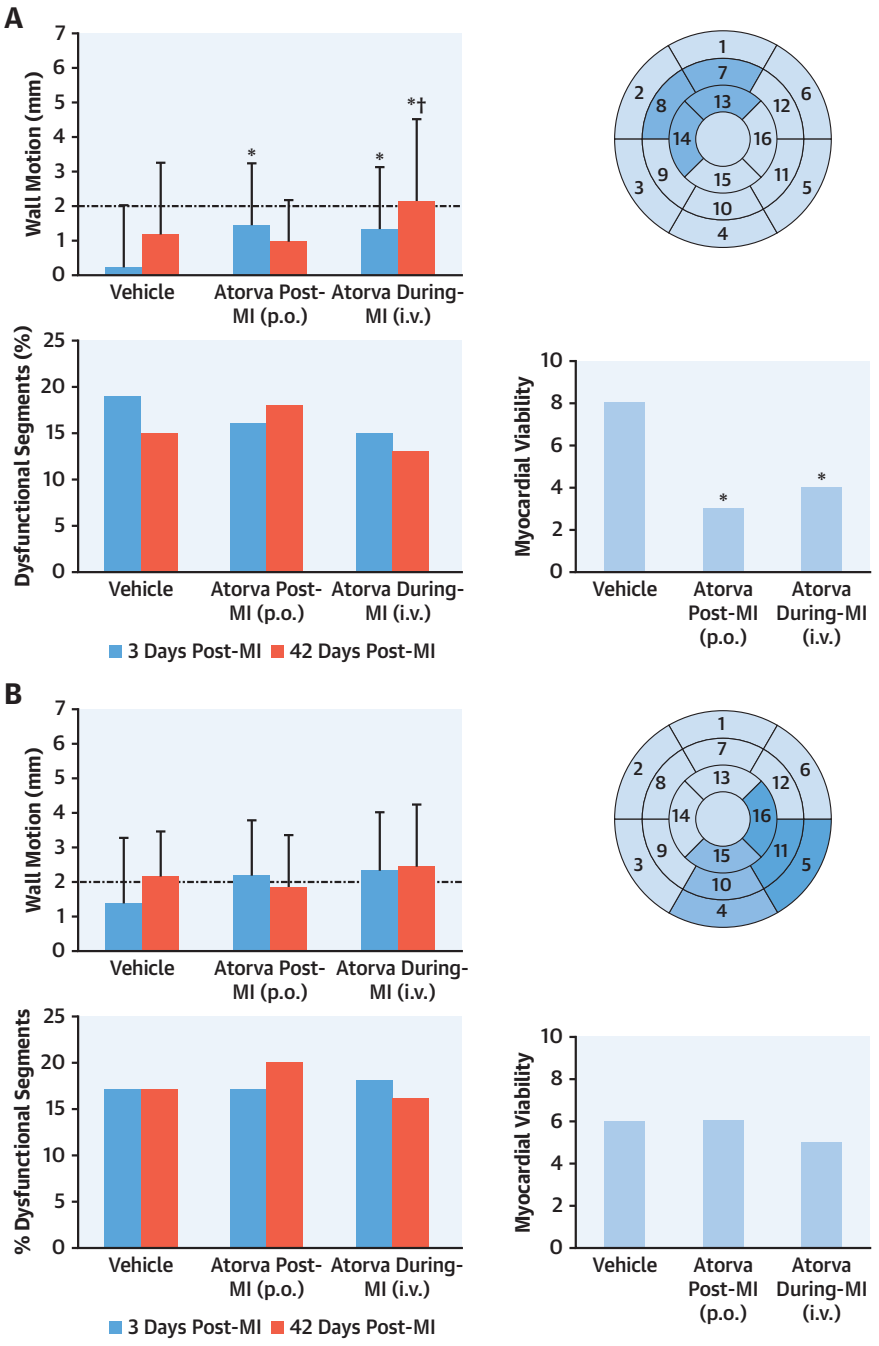
GLOBAL ANATOMICAL AND FUNCTIONAL PARAMETERS. Cardiac damage. The extent of area-at-risk (edema) and the no-reflow were similar between the 3 treatment arms at day 3 post-MI. Administration of intravenous atorvastatin during MI resulted in a significant and marked reduction on infarct size (either expressed as grams or % of LV) in comparison to both oral administration of atorvastatin post-MI and vehicle ($p < 0.05$ vs. both). Accordingly, administering atorvastatin during MI resulted in 50% increase in myocardial salvage ($p < 0.05$ vs. both). The 42-day CMR analysis revealed that orally treated atorvastatin animals after MI developed smaller scars as compared to vehicle-administered animals ($p < 0.05$) (Table 1, Figure 2A). Interestingly, atorva-during-MI animals consistently showed a more pronounced reduction in the size of the scar, with a further 24% reduction, compared with atorva-post-MI pigs ($p < 0.05$ vs. both groups). Histopathological

FIGURE 2 **Cardiac Damage Assessed by CMR at Days 3 and 42 Post-MI Induction**



(A) Cardiac magnetic resonance imaging (CMR) representative images of edema, infarct size (day 3), and scar size (day 42). **(B)** Gross pathological images. MI = myocardial infarction.

FIGURE 3 Regional Analysis of Functional Parameters According to the 16-Segment Model (American Heart Association)



Wall motion (WM) analysis, percentage of dysfunctional segments (WM <2 mm), and myocardial viability (percentage of dysfunctional segments at day 3 post-MI [WM <2 mm] that have been recovered [WM ≥2 mm] at day 42 post-MI) over time in the jeopardized **(A)** and remote **(B)** myocardium. Segments included in the analysis are highlighted in **blue**. The chi-square test was performed for the regional segmental analyses of the number of dysfunctional/recovered segments. WM = (end-systolic thickness) − (end-diastolic thickness). n = 7 animals/group. Segmental analysis: chi-square *$p < 0.05$ versus vehicle. †$p < 0.05$ versus all. atorva = atorvastatin; other abbreviations as in Figure 1.

analyses by TTC staining correlated ($r = 0.843$; $p < 0.005$) with CMR-assessed scar mass (**Figure 2B**). As observed in Supplemental Figure 1, no significant differences were detected in the collateral circulation among the 3 animal groups excluding the potential confounding effect of collaterals on the final size of infarction.

Cardiac function. No significant changes in LVEF were detected in the 3 animal groups between days 3 and 42 post-MI. Yet, there was a trend toward recovery in atorva-during-MI administered pigs ($p = 0.1$). In fact, although LV volumes (LVEDV and LVESV) significantly increased over time ($p < 0.05$ vs. day 3 post-MI), animals administered atorva-during-MI showed greater improvement in LVESV compared with control pigs ($p < 0.05$) (**Table 1**).

REGIONAL PARAMETERS ASSOCIATED WITH LV REMODELING. We initially evaluated WM abnormalities in the target segments (i.e., jeopardized myocardium; 28 segments/group) (**Figure 3A**). At day 3, segmental WM was severely impaired in vehicle animals (akinetic and dyskinetic; 0.2 mm), whereas they were mildly dysfunctional (hypokinetic) in all atorvastatin-treated pigs (1.4 mm). In line with these observations, the proportion of dysfunctional segments (WM <2.0 mm) was higher in the vehicle group (67% of the segments vs. ≈50% in atorvastatin-treated animals). At day 42 atorva-post-MI pigs persisted with WM at around 1.0 mm, while the atorva-during-MI pigs showed a marked improvement up to 2.1 mm ($p < 0.05$) (**Figure 3A**). We also assessed WM in the inferior-lateral myocardium (i.e., remote myocardium; $n = 42$ segments/group) (**Figure 3B**). No WM abnormalities were detected in the treatment arms, and only the vehicle-untreated group showed a nonsignificant impairment (WM = 1.4 mm).

IMPACT ON ANGIOGENESIS AND TGFβ/SMAD2/3/-COLLAGEN SIGNALING AND FIBROUS TISSUE DEPOSITION. As shown in **Figure 4A**, no changes were detected in the gene transcription of diverse markers of angiogenesis among the different animals and within the different cardiac regions, indicating a steady state in gene transcription. Lectin and vWF staining, however, revealed higher vessel density in the penumbra of all atorvastatin-treated pigs; yet, vWF staining (marker of mature endothelial cells) was found to be significantly enhanced in the penumbra of atorva-during-MI administered animals compared with that of atorva-post-MI pigs (**Figure 4B**). Gene levels of TGF-β receptor and the transcription factor Smad2 were significantly and similarly up-regulated in the scar region of all animals compared with the remote myocardium. No changes were detected as to Smad3 transcript levels throughout the different cardiac regions and among the different animal groups (**Figure 5A**). The mRNA expression of collagen 3A1 was enhanced in the scar region of atorvastatin-treated animals versus vehicle; yet, levels were significantly higher in atorva-during-MI treated pigs compared with atorva-post-MI animals ($p < 0.05$). Likewise, although collagen 1A1 mRNA was enhanced in the scar of all animals, atorva-during-MI pigs showed a 4-fold increase compared with the other 2 groups ($p < 0.05$). In line with this transcriptomic data, atorva-during-MI animals showed a higher activation of Smad2/3 protein in the scar region compared with all animals (**Figures 5B and 5C**). This increase was accordingly associated with a higher detection of collagen fibrils in the forming scar (**Figures 5D and 5E**).

IMPACT ON AMPK EXPRESSION AND ACTIVATION. As per AMPK mRNA levels, these were only enhanced in the scar region of atorva-during-MI pigs (**Figure 6A**). Furthermore, although AMPK activation was found to be enhanced in all atorvastatin-treated animals, the degree of activation was 2-fold higher in atorva-during-MI animals compared with atorva-post-MI pigs ($p < 0.05$) (**Figure 6B**).

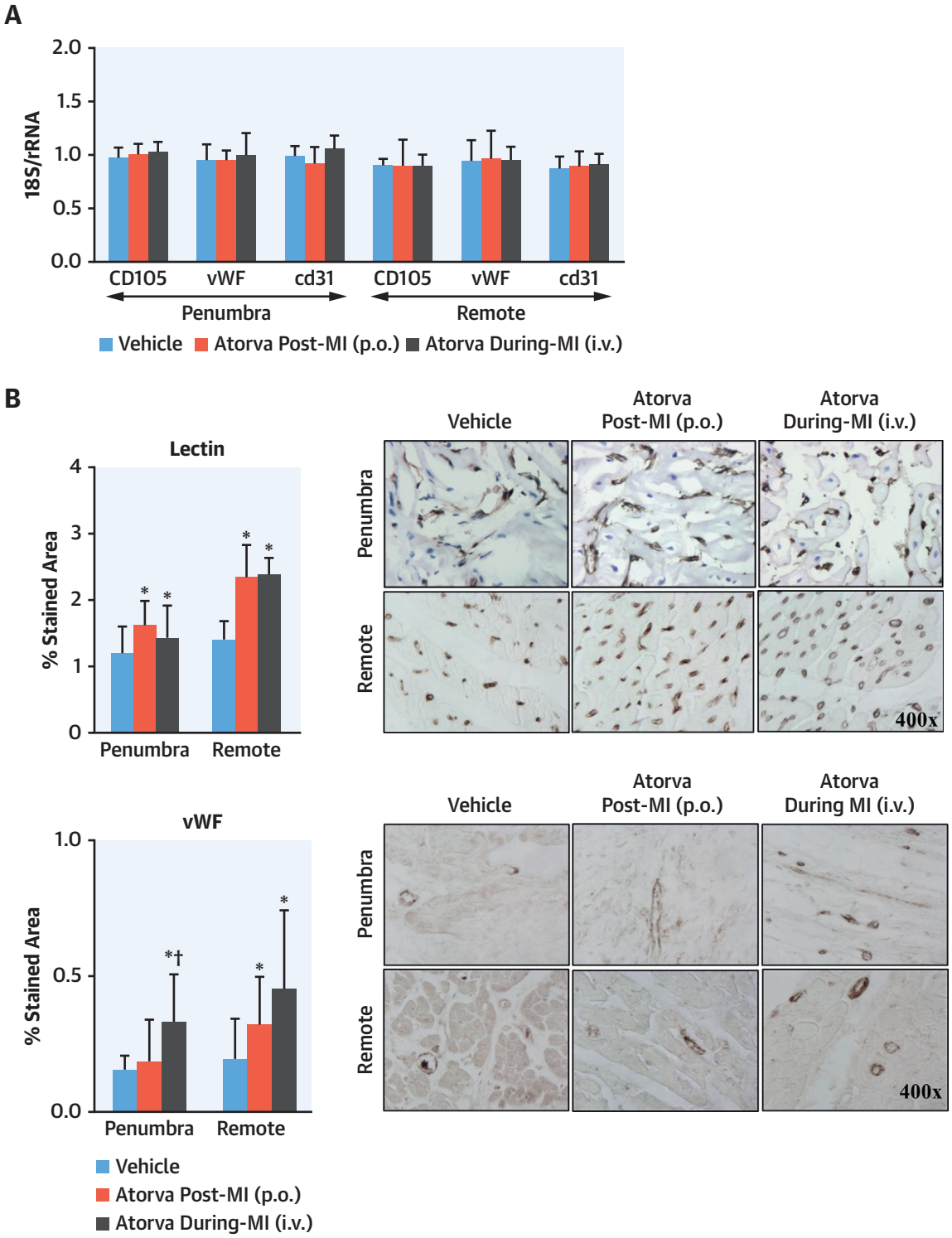
EFFECTS ON PBMCs ACTIVATION AND INFLAMMATORY CYTOKINES. MCP-1 and Toll-like receptor-4 mRNA levels were significantly down-regulated in PBMCs of atorva-during-MI pigs 3 days post-MI compared with the other 2 groups, reaching expression levels similar to those found at baseline (prior MI induction) (**Figure 7A**). The same pattern was observed in the protein expression of both inflammatory markers (**Figure 7B**). No changes were detected in cyclooxygenase-2 expression at day 3 (neither at the mRNA nor at the protein level) (**Figures 7A and 7B**).

As shown in **Figure 8**, IL-6 and TNF-α plasma levels were sharply increased post-MI in all 3 groups. Yet, TNF-α detection was almost 3-fold higher than that of vehicle and atorva-post-MI pigs. CRP was similarly enhanced at 3 days post-MI

ATORVASTATIN PLASMA LEVELS AND BIOCHEMICAL FOLLOW-UP. Plasma levels of atorvastatin were found to be markedly increased at 15 min post-infusion and then gradually declined for up to 75 min, always remaining above detectable levels (Supplemental Figure 2). Atorvastatin was undetectable in vehicle administered pigs.

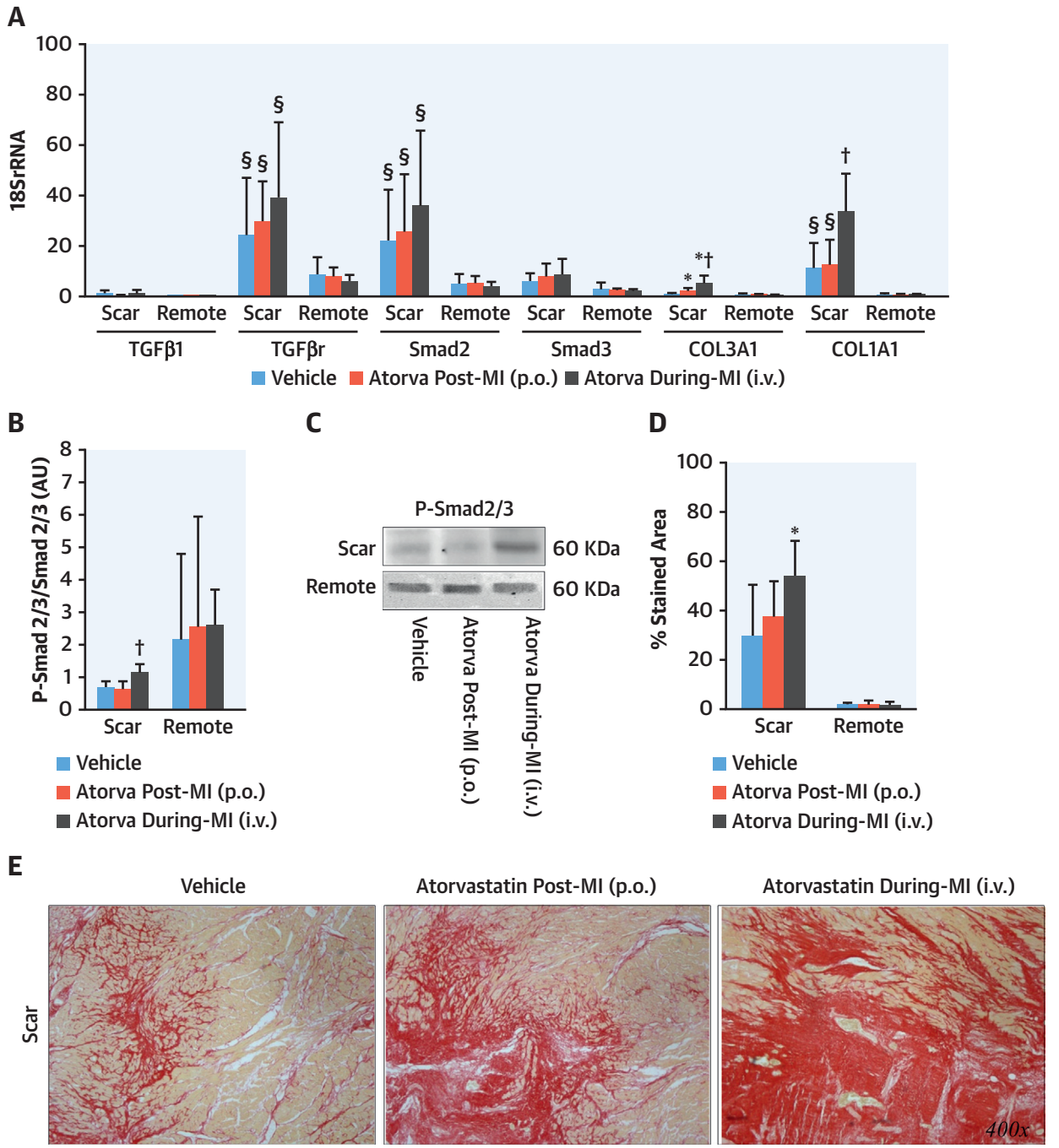
Blood cholesterol levels (total, LDL, and high-density lipoprotein cholesterol) (Supplemental Table 1) were raised by the hypercholesterolemic diet, and remained unchanged throughout the entire

FIGURE 4 Vessel Density in the Penumbra and Remote Cardiac Tissue



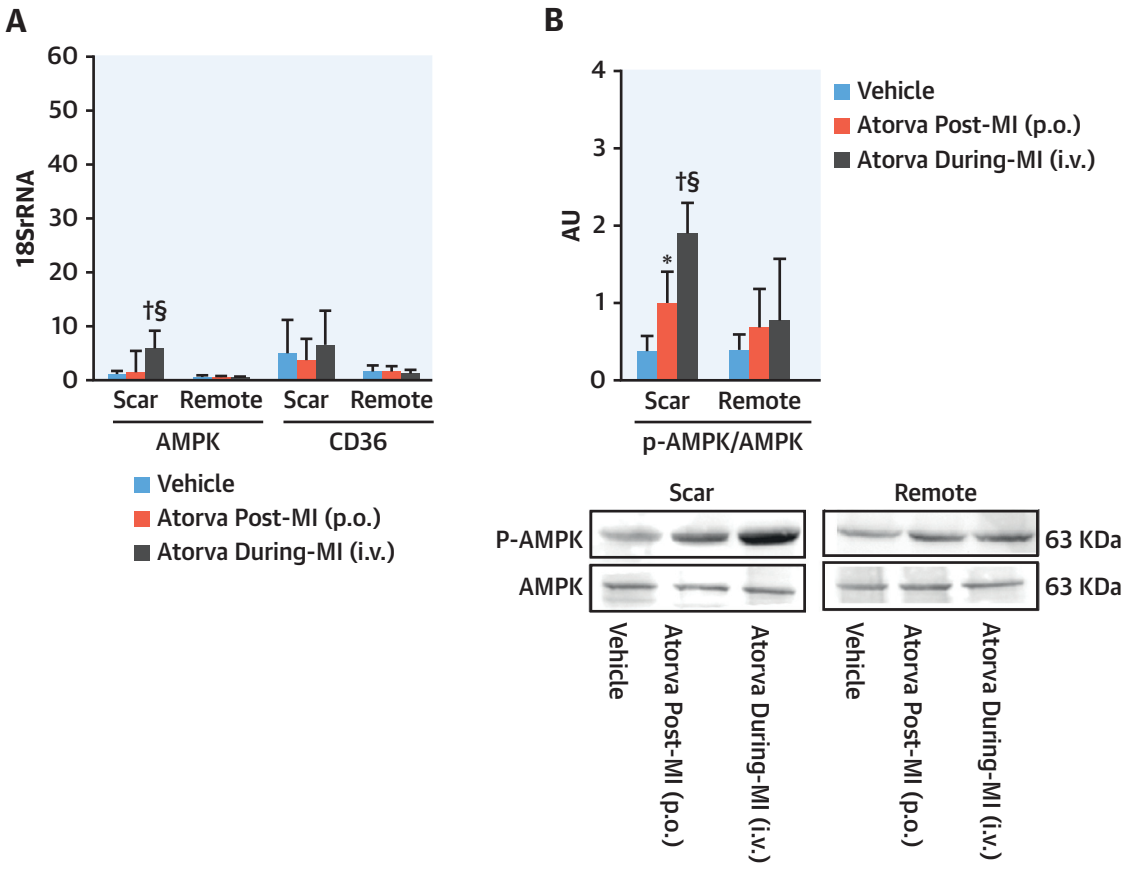
(A) Transcript levels of markers of new vessel formation (CD105, von Willebrand factor [vWF], and CD31). **(B)** Immunological staining against lectin and vWF. *p < 0.05 versus vehicle. †p < 0.05 versus vehicle and atorva-post-MI. Abbreviations as in Figure 1.

FIGURE 5 Myocardial Fibrosis in the Evolving Scar



(A) Transcript levels of fibrotic markers. **(B)** Activation of the pSmad2/3 signaling pathway. **(C)** Representative western blot image of Smad2/3 activation. **(D)** Sirius red staining. **(E)** Representative image of Sirius red. *p < 0.05 versus vehicle. †p < 0.05 versus all. §p < 0.05 versus remote myocardium. COL = collagen; TGF-β1 = tissue growth factor-β 1; TGF-βr = tissue growth factor β receptor; other abbreviations as in Figure 1.

FIGURE 6 Myocardial AMPK Expression and Activation



(A) Transcript levels of AMPK. **(B)** AMPK activation. *p < 0.05 versus vehicle. †p < 0.05 versus all. §p < 0.05 versus remote myocardium. AMPK = AMP-activated protein kinase; other abbreviations as in Figure 1.

study. No changes were observed in liver and kidney enzymes throughout the study (Supplemental Table 2).

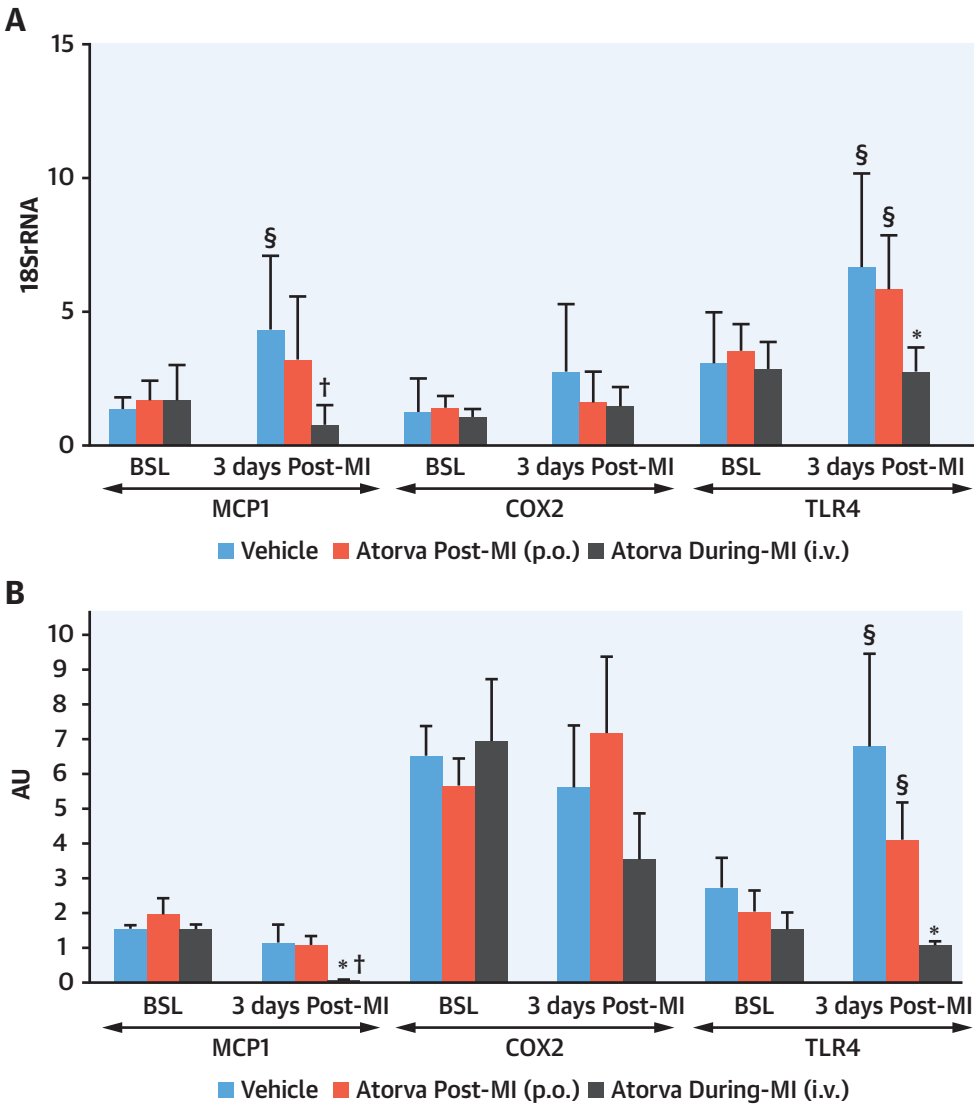
DISCUSSION

The improved management of MI patients has reduced morbidity and mortality in the acute phase; however, this success has resulted in an increased incidence of heart failure due to adverse post-MI LV remodeling (27). At present, treatment efforts are focused on limiting the damage in the setting of acute ischemic heart disease as well as on stimulating post-MI LV repair.

In this study, we have demonstrated that intravenous atorvastatin treatment during MI followed by oral administration thereafter limits myocardial damage, improves cardiac function, and reduces scar formation to a larger extent than when atorvastatin is administered orally in the first 2 h after coronary reperfusion. Such improved myocardial protection was associated with an up-regulation in collagen expression and enhanced AMPK activation in the evolving scar and an enhanced vessel density in the penumbra (**Central Illustration**).

Due to its elevated contrast-to-noise ratio, CMR allows for a precise differentiation between viable and necrotic tissue, facilitating the noninvasive quantification of myocardial necrosis and ultimately serving as the gold standard in cardioprotection research (28). In our study, animals receiving intravenous atorvastatin during ongoing MI exhibited significantly higher myocardial salvage index at 3 days post-MI than animals orally treated with atorvastatin early post-MI. In fact, the atorva-post-MI animals had similar myocardial salvage index than

FIGURE 7 Impact of Statin Treatment on Monocyte Expression of Inflammatory Markers

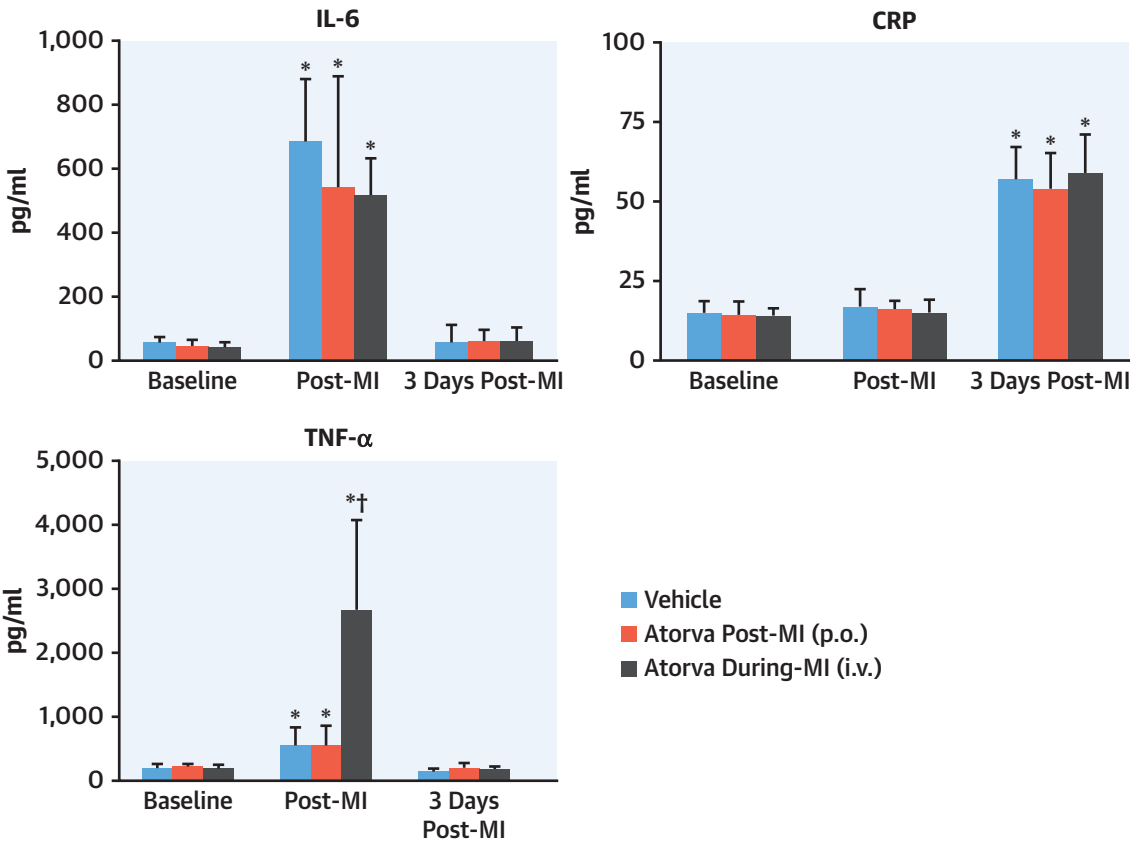


(A) Transcript levels. **(B)** Protein levels. *p < 0.05 versus vehicle. †p < 0.05 versus all. §p < 0.05 versus BSL. BSL = baseline; COX2 = cyclooxygenase-2; d = days; MPC = monocyte chemoattractant protein; TLR = toll-like receptor; other abbreviations as in Figure 1.

those treated with vehicle. In addition, at day 3 post-MI, atorva-during-MI animals had reduced infarct size and necrosis compared with all other animal treatment groups, and this effect persisted and was enlarged at day 42 post-MI. At day 42, orally only post-MI treated animals showed reduced scar size compared with vehicle, but in a significantly lower degree than atorvastatin-during-MI and, thereafter, orally treated animals. We have demonstrated that intravenous administration of atorvastatin during ischemia reduces the levels and activation of senescence-, apoptosis-, and cardioprotective/metabolic-related markers (16). These benefits in combination with the cardioprotective potential associated with enhanced TNF-α secretion may have contributed to activate the mechanisms that better preserve the damaged myocardium ultimately reducing scar size. Of note, our imaging data allows us to exclude the contribution of no-reflow phenomenon, myocardial collateral network, or edema formation on the differences observed in cardiac damage.

Regarding functional parameters, although no significance was detected, there was a clear trend toward improvement in LVEF in the intravenously treated

FIGURE 8 Levels of Circulating Inflammatory Markers

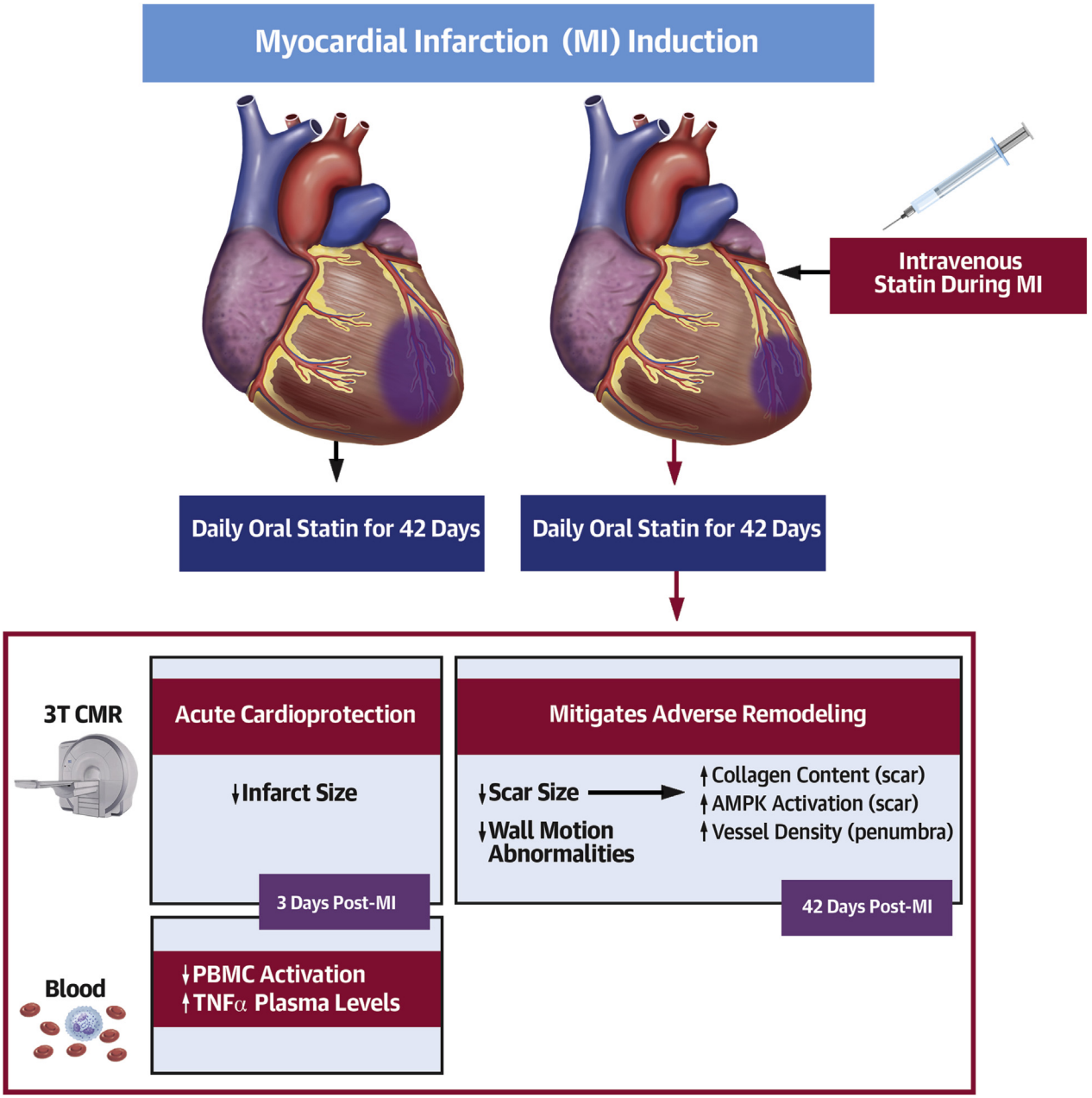


Levels of cytokines (interleukin [IL]-6 and tumor necrosis factor [TNF]-α) and CRP in plasma from all pigs. *p < 0.05 versus baseline values. †p < 0.05 versus vehicle and atorva-post-MI. d = days; other abbreviations as in Figure 1.

animals compared with the other 2 groups, largely derived from the significant recovery of systolic function (LVESV). Keeping in mind that LVESV is an important post-MI predictor of survival (29), these observations further underline the benefits derived from intravenous treatment during myocardial infarction. Beyond LVEF and volumes, CMR-detected regional function abnormalities have been related to post-MI death and/or major adverse cardiac events (30). Here, severe WM abnormalities were detected in the jeopardized myocardium of vehicle-administered animals compared with atorvastatin-treated pigs at 42 days post-MI. WM recovery in the jeopardized myocardium of intravenously treated animals was enhanced with respect to the other treatment arms at 42 days post-MI. However, the number of segments with improved WM in relation to 3 days post-MI was higher in vehicle-administered pigs compared with atorvastatin-treated animals, likely because they were remarkably dysfunctional at day 3; yet, WM within these segments remained below 2 mm, indicative of persistent dysfunctional contractility over time. Of note, regional WM analysis showed that despite contractile function impairment in the setting of ischemia, statin administration improved contractile dysfunction in the early phase post-MI and myocardial viability in later phases (42 days). Altogether, the overall functional/remodeling improvements in combination with lower size of infarction suggest that there would be a significant improvement in cardiac performance at longer follow-up periods in intravenous-during-MI atorvastatin-treated pigs.

Post-MI LV changes entail complex molecular interactions (31). Pharmacotherapeutic strategies aimed at mitigating adverse LV remodeling are currently under investigation. In this study, we have demonstrated that statin treatment regulates molecular markers of fibrosis. We previously showed, in the porcine model of hypercholesterolemia, that MI impairs the reparative fibrotic response mediated by

CENTRAL ILLUSTRATION **Cardiovascular Protection Afforded by Intravenous Administration of Atorvastatin During Myocardial Infarction Induction**



Mendieta, G. et al. J Am Coll Cardiol. 2020;75(12):1386–402.

Administration of intravenous atorvastatin during myocardial infarction (MI) induces acute protective effects both in the myocardium (lower infarct size) and systemically (diminished peripheral blood mononuclear cell [PBMC] activation and higher tumor necrosis factor [TNF]-α levels) and mitigates adverse left ventricular remodeling (smaller scar and less wall motion abnormalities) to a larger extent than when administered orally shortly after reperfusion. These benefits are associated with higher collagen content and activation of the AMP activated kinase (AMPK) in the evolving scar and enhanced vessel density in the penumbra. CMR = cardiac magnetic resonance.

TGF-β1-TGF-β receptor and Smad2/3 signaling pathways (18). The current study shows that intravenous administration of atorvastatin during MI enhances the TGF-β receptor signaling pathway, favoring a reparative fibrotic response in the developing scar. The TGF-β1-TGF-β receptor pathway regulates fibroblast differentiation into myofibroblasts, responsible together with downstream associated effectors Smad2/3 of regulating the transcription of extracellular matrix genes COL1A1-3A1, which in our study were significantly increased in the evolving scar of intravenously treated animals. However, intravenous atorvastatin-administered animals also show higher vWF staining in the penumbra zone, further indicating beneficial effects in the remodeling process. Altogether, our data indicates that the intravenous administration of statin during MI mitigates adverse cardiac remodeling, an unobserved benefit when administering oral statin early post-MI.

We have previously demonstrated that myocardial AMPK activation is associated with antiplatelet agent-related cardioprotective effects (23). In this study, we have observed enhanced AMPK activation in the developing scar of animals treated with statins versus vehicle. Yet, the intravenously statin-treated animals present a significantly higher degree of AMPK activation compared with the only orally treated statin group, which further supports the concept of intravenous treatment during MI as a strategy to enhance myocardial preservation.

Intravenous statin treatment during MI has also demonstrated to modulate the inflammatory response by acutely and sharply enhancing TNF-α. TNF-α is a cytokine released by multiple cells ranging from inflammatory cells to cardiomyocytes and fibroblasts. Transient increase in TNF-α has shown to protect against ischemia-reperfusion injury by interacting with myocardial TNF receptor type 2 and consequent downstream activation of the cardioprotective SAFE pathway (32–34). Whereas acute ischemia has shown to induce the secretion of preformed TNF-α from macrophages and mast cells, persistent ischemia (as in our study) has been demonstrated to induce cardiomyocyte release of TNF-α, which, in turn, induces SAFE pathway activation (35). Further studies are needed to evaluate whether such acute TNF-α induction leads to early myocardial STAT-3 activation. However, in the intravenously atorvastatin administered animals, we also detected a reduction in systemic PBMCs activation through the down-regulation of inflammatory mediators at 3 days post-MI. Indeed, excessive inflammatory cell activation worsens myocardial injury, and thus, the role of statins at a systemic level may also contribute to the beneficial effects observed in the cardiac remodeling response.

In this study, animals were fed a hypercholesterolemic diet prior to MI induction to model this common adverse cardiovascular comorbidity, often found in ACS patients. Animals were fed the hypercholesterolemic diet during the entire duration of the study (42 days) given that our objective was to interfere with the isoprenoid pathway (that is also regulated by 3-hidroxi-3-metilglutaril-coenzima A reductase), rather than to lower LDL cholesterol. Either reducing dietary cholesterol or a longer treatment period with atorvastatin would have reduced lipid levels; in fact, current guidelines advise lipid level control 3 months after treatment initiation and dietary modifications. Our intervention in the study, however, was free of adverse liver and/or renal effects.

STUDY LIMITATIONS. This study has several shortcomings worth discussing. First, the atorvastatin formulation used herein was readily prepared in the laboratory because a good manufacturing practice product was not required in this study; our efforts are currently centered on attaining a good manufacturing practice product. Second, our closed-chest total balloon occlusion MI model includes mechanical occlusion but not atherothrombosis as the triggering factor of ischemia; however, the porcine model is the best preclinical experimental model to investigate cardiac events due to the similarity in organ size, coronary anatomy, immunology, and physiology to humans. Third, induction of larger and more severe infarcts would have further evidenced the efficacy of intravenous atorvastatin-related cardioprotective effects; however, in this first statin long-term study, we adopted a conservative approach to reduce peri-intervention animal mortality. Fourth, we did not apply multiple testing corrections because of the low N (according to the ARRIVE guidelines in animal testing) and the many time points and groups analyzed. Finally, hypercholesterolemic patients are chronically treated with oral lipid-lowering doses of statins; however, in this study, our objective was to compare the effects of intravenous administration of statins during MI (not the "classic" lipid-lowering properties but rather assessing the effect of isoprenoid-blockage) versus oral post-MI therapy, in accordance with current clinical practice guidelines. Notwithstanding, the impact of being on statin treatment for lipid lowering prior to MI is presently being investigated.

CONCLUSIONS

In this study, we have demonstrated that intravenous administration of statin during MI followed by oral therapy thereafter is superior to the administration of atorvastatin orally shortly after reperfusion, in that it better protects the myocardium, significantly reduces scar size, and mitigates LV adverse remodeling. The potential therapeutic implications of this novel administration regime should be explored in the management of ACS patients.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS:

Intravenous administration of statin medication during MI is associated with less adverse cardiac remodeling than oral administration following myocardial reperfusion.

TRANSLATIONAL OUTLOOK:

Clinical studies should seek to establish the optimum timing and mode of statin administration in patients with acute MI.

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KEY WORDS cardioprotection, myocardial infarction, pigs, statin, timing

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