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Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance

Mark F. Berry, Adam J. Engler, Y. Joseph Woo, Timothy J. Pirolli, Vasant Jayasankar, Kevin J. Morine, Timothy J. Gardner, Dennis E. Discher, and H. Lee Sweeney. Mesenchymal stem cell injection after myocardial infarction improves myocardial compliance. *Am J Physiol Heart Circ Physiol* 290: H2196–H2203, 2006. First published February 10, 2006; doi:10.1152/ajpheart.01017.2005.—Cellular therapy for myocardial injury has improved ventricular function in both animal and clinical studies, though the mechanism of benefit is unclear. This study was undertaken to examine the effects of cellular injection after infarction on myocardial elasticity. Coronary artery ligation of Lewis rats was followed by direct injection of human mesenchymal stem cells (MSCs) into the acutely ischemic myocardium. Two weeks postinfarct, myocardial elasticity was mapped by atomic force microscopy. MSC-injected hearts near the infarct region were twofold stiffer than myocardium from noninfarcted animals but softer than myocardium from vehicle-treated infarcted animals. After 8 wk, the following variables were evaluated: MSC engraftment and differentiation, increased myocardial thickness, and preservation of systolic and diastolic cardiac function. In summary, MSC injection after myocardial infarction did not regenerate contracting cardiomyocytes but reduced the stiffness of the subsequent scar and attenuated postinfarction remodeling, preserving some cardiac function. Improving heart muscle compliance could be a functional benefit of cellular cardiomypoplasty.

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**MATERIALS AND METHODS**

Animal care. Animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). The study was reviewed and approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Surgical preparation. Twenty-seven male Lewis inbred rats (250–300 g; Charles River, Wilmington, MA) were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg), intubated, and mechanically ventilated with 0.5% isoflurane. The proximal left anterior descending coronary artery (LAD) was encircled with a suture via a left thoracotomy. In two animals, the suture was removed without being tied. The suture was ligated in the other 25 animals, of which 11 received five MSC injections [2 × 10⁶ total cells (Osiris Therapeutics, Baltimore, MD) diluted in 200 μl of Dulbecco’s modified Eagle’s medium (DMEM)] directly into central infarct and

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border zone areas of the myocardium, 12 received DMEM injections, and two received no injections. Injection of cells and DMEM was performed in a randomized and blinded fashion by a single investigator. All animals were closed and recovered.

Atomic force microscopy characterization. Eight of the animals that underwent the surgery described above (the two that did not undergo ligation, two that had cells injected, two that had DMEM injected, and the two that underwent ligation but no injections) were euthanized 2 wk later, after which the hearts were removed to obtain atomic force microscopy (AFM) measurements of elasticity in the infarct and border region. Infarcted and control tissue from these eight animals was sectioned parallel to the longitudinal axis of the left ventricle (LV) from ~5 mm above the LAD ligation point to a point ~15 mm downstream of the ligation (see Fig. 3A) to yield tissue samples 15-mm long, 1-mm wide, and 1-mm thick. The basal surface was mounted and immobilized on coverglass with adhesive tape, exposing the apical surface for probing. Tissue samples were submerged in DMEM, placed in an Asylum 1-D AFM (Asylum Research, Santa Barbara, CA), and indented with a blunted pyramid-tipped cantilever (Veeco, Santa Barbara, CA) having a nominal spring constant of 60 pN/nm, checked by a thermal calibration. Nine-hundred seventy-four total force-indentation measurements (see Fig. 3B, inset) were made on a spatially controlled stage (±0.1 mm) at 15–20 positions per animal along the length of the infarct (Fig. 3A,

![Control vs MSC](image)

Fig. 1. Mesenchymal stem cells (MSCs) engrafted into the infarct region 8 wk postinfarction. A, B, D, and E: hematoxylin and eosin staining qualitatively demonstrates presence of more cells in infarct region at 8 wk for MSC-injected rats vs. control animals (A and D, ×100 magnification; B and E, ×400). Infarct regions shown are approximately same distance from border zone for both MSC and control animals. C and F: merged images of double staining of sections for human lamin (red) and nonspecific nuclear stain 4′-6-diamidino-2-phenylindole (DAPI, blue) demonstrate the presence of human cells in infarct region of MSC-injected rats but not control animals (×400).
x-axis). At each of these lateral positions, measurements were varied along the width of the sample (Fig. 3A, y-axis) to obtain data over the entire sample surface. Each force-indentation plot (Fig. 3B, inset, black curve) was fit to a Hertz cone model (red curve) to determine an elastic modulus, E (6, 27). Elastic moduli were binned at 1- to 2-mm intervals and averaged for each animal (n = 2 animals for each sample group). Samples were indented at rates <2 μm/s, which is generally sufficient to explore elastic rather than viscoelastic properties of cells and ECM (16). Normal heart muscle from the noninfarcted animals was used to determine a baseline elastic modulus, whereas the “border zone slope modulus” was determined from data one deviation below the normal and stiff, infarcted regions and above the noninfarcted region.

**Hemodynamic measurements.** The remaining animals that underwent ligation and either cell (n = 8) or DMEM (n = 8) injection were recovered for 8 wk, when they were again anesthetized, intubated, and mechanically ventilated. A median sternotomy was performed, and a 2-Fr pressure-volume conductance catheter (Millar Instruments, Houston, TX) was inserted into the LV through the apex to obtain hemodynamic data, which were analyzed with ARIA 1 Pressure-Volume Analysis software (Millar Instruments) (4). The following parameters were chosen for analysis: maximum LV pressure, ejection fraction, maximum change in pressure over time (dP/dtmax), the dP/dtmax-end-diastolic volume (EDV) relationship, and maximal elastance as indexes of systolic function; minimum dP/dt, and the time constant of relaxation (τ) as indexes of diastolic function; and preload recruitable stroke work as an index of contractility. At the completion of the hemodynamic measurement, hearts were arrested in diastole by injection of 0.1 ml of KCl (1 meq/ml), and the LV cavity was filled with optimal cutting temperature (OCT)-embedding compound retrograde through the transected aortic root. The distended heart was placed in a container of OCT, frozen in liquid nitrogen, and stored at −80°C. Heart sections (10-μm thick) were prepared with a cryostat through the infarct region from the point of ligation to the apex of the heart at 2.5-mm intervals.

**Histological and viability measurements.** Sections were fixed with formaldehyde, permeabilized using 0.5% Triton X-100 in phosphate-buffered saline (PBS), and blocked with 5% bovine serum albumin in PBS. Sections were incubated with antibodies to either human lamin A/C or human cardiac muscle troponin T (Santa Cruz Biotechnology, Santa Cruz, CA). Sections were then rinsed and incubated with secondary antibodies (Jackson ImmunoResearch; West Grove, PA) and mounted with Vectashield mounting medium with 4’-6-diamidino-2-phenylindole (Vector, Burlingame, CA).

Ventricular geometry was reconstructed from photomicrographs of hematoxylin and eosin-stained heart sections. Measurements were performed on two sections obtained from the midpoint between the LAD ligation and the apex of the heart for each animal and averaged for each geometric parameter. Wall thickness measurements were performed on two separate border zone areas, and ventricular diameter measurements were performed in both the anterior-posterior and septal-lateral axes for each section. For all analyses, the border zone was defined as the viable myocardial tissue immediately adjacent to the infarct scar. Infarct thickness was measured at the midpoint of the infarct region, which was the point in the infarct region that was equidistant from each infarct border. All thickness measurements were made in a radial direction, perpendicular to the ventricular wall. Infarct size in each heart was calculated by averaging the percentages of both inner and outer infarct scar circumferences relative to the LV free wall on two separate sections for each heart. Additionally, heart sections were stained with Masson Accustain Trichrome stain (Sigma, St. Louis, MO) to distinguish areas of connective tissue. The extent of fibrosis in the infarct region of each heart was measured as previously described (40). The percentage of blue staining, indicative of fibrosis, was measured (10 fields randomly selected from the infarct area on each section) from the infarct area on two sections from each heart and averaged. The value was expressed as the ratio of Trichrome-stained fibrosis area to total infarct area. Finally, TdT-mediated dUTP nick-end labeling (TUNEL) assay was performed on heart sections with a TdT-FragEL DNA Fragmentation Detection Kit (Oncogene Research Products, Boston, MA) to quantify apoptosis. Counterstaining with methyl green was performed to visualize normal nuclei. Measurements of the apoptotic nuclei percentage were obtained from the border zone area of two sections from each heart and averaged (4 fields randomly selected from the border zone of each heart).

Immunohistochemistry to assess angiogenesis in the border zone was performed by incubating sections with a mouse monoclonal antibody to the endothelial cell marker von Willebrand factor (Cedar Lane Laboratories, Hornby, ON, Canada), followed by a secondary antibody linked to alkaline phosphatase. Detection was performed with the 5-bromo-4-chloro-3-indolyl phosphate/Nitro Blue Tetrazolium Liquid Substrate System (Sigma). The sections were then counterstained with eosin, and quantitative assessment of the number of endothelial cells per high-powered field was determined in five representative border zone fields in a blinded fashion.

To compare matrix metalloproteinase (MMP) activity in cell-injected animals and control animals, myocardial biopsy specimens from the infarct border zone of three animals in each group were pulverized, homogenized in 10 vol of protein extraction buffer [50 mmol/L Tris (pH 7.5), 1 mmol/L CaCl2, and 0.5% Triton X-100], sheared with a 25-gauge needle, and centrifuged for 1 min at 6,000 rpm to remove cellular debris. The supernatants were assayed for total protein content (Bio-Rad Protein Assay), and 100 μg of each sample

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**Image Notes:**

- **Fig. 2. A:** human cardiomyocyte troponin T (green) and DAPI (blue) show that engrafted cells are expressing troponin T at 8 wk postinfarct. **B:** hearts lacking MSC injections do not have troponin T expression. All images are at ×400. Note that control hearts do contain some nonspecific background green staining in region of fibrotic infarct; however, fluorescent signal intensity observed in control hearts was much less relative to MSC-injected hearts, and green staining in control hearts did not appear to be associated with an adjacent DAPI-stained nucleus.
were assayed for MMP activity using the Type 1 Collagenase Activity Assay Kit (Chemicon) according to the manufacturer’s instructions. Comparison of absorbance to \( p \)-aminophenylmercuric acetate-activated MMP-1 control samples (provided by the manufacturer) processed in parallel was performed to quantify the absorbance data, with levels of activity reported as nanograms of control MMP activity.

**Statistical analysis.** A single investigator blinded to the treatment group performed all hemodynamic and histological analyses and measurements. All values are expressed as means \( \pm \) SE. The unpaired Student’s \( t \)-test with the use of a two-tailed distribution was used to calculate the statistical significance between the means of the two groups. A \( P \) value of \(<0.05\) was considered to be significant.

**RESULTS**

**Animal mortality.** Three of the animals in the study died in the immediate postoperative period; one had received cell injections and two had received DMEM injections. Animals that died had their chests immediately reopened, and none had an obvious cause of death or appeared qualitatively to have had significantly different infarct sizes from all other animals. Deaths were presumed to be from a malignant arrhythmia or acute respiratory insufficiency related to the thoracotomy and heart manipulation.

**Cell engraftment and protein expression.** Qualitative histological analysis of the infarct region showed increased cellularity in MSC-injected animals (Fig. 1, D and E) versus control animals (Fig. 1, A and B) 8 wk postinfarction. Immunofluorescent staining for human lamin, a nuclear structural protein, verified the presence of the injected human MSCs (Fig. 1F), although the engraftment pattern was not uniformly distributed. Some engrafted cells expressed the cardiomyocyte-specific protein troponin T (Fig. 2, A and B) but lacked morphological structures similar to cardiomyocytes and structural integration with native cardiomyocytes (Fig. 1, D and E).

**Local ventricular stiffness determined by AFM.** The baseline elastic modulus for normal heart muscle from noninfarcted

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**Fig. 3.** A: dashed white circle indicates ischemic area after left anterior descending coronary artery ligation. Black rectangle indicates section of tissue removed and mounted for atomic force microscopy (AFM) probing of elasticity (E). The \( x \)- and \( y \)-axes and asterisk indicate sample orientation on AFM stage and ligation point, respectively. Scale bar is 2 mm. B: AFM indentations were made as a function of length along tissue sections for normal, infarcted, DMEM-injected, and MSC-injected rats (\( n = 2\) animals for each group). Elastic modulus for infarcted animals increased dramatically at point of ligation and plateaued at 55 \( \pm \) 15 kPa in control infarcted animals and at 40 \( \pm \) 10 kPa in MSC-injected animals. Gradients in elastic modulus resulting from border zone softening, represented by blue and black solid lines, were 8.5 kPa/mm for control infarcted animals, whereas the MSC-injected animals had a shallower gradient of 4.3 kPa/mm (dotted lines, extrapolated data). Gray shaded zone represents the normal stiffness required for differentiation of skeletal muscle reported by Engler et al. (7).

**Inset:** sample force-indentation curve. MI, myocardial infarction.

**Fig. 4.** Tissue sections 8 wk postinfarct from control animals (A) and MSC-injected animals (B) (hematoxylin and eosin staining) are shown. Solid arrows indicate where infarct thickness was measured, and dashed arrows indicate where border zone thickness was measured. Average left ventricular (LV) chamber diameter, border zone wall thickness, and infarct wall thickness (C) are significantly improved in MSC-injected animals (\( n = 8\)) compared with controls (\( n = 8\)). *\( P = 0.009\), †\( P = 0.0008\), and ‡\( P = 0.049\) compared with control.
animals was 18/11006 kPa. Infarcted animals receiving no treatment or simply receiving DMEM injections formed significant fibrosis, with a similar threefold increase in the elastic modulus in both control groups (55/11006 15 kPa; P/11005 0.49) (Fig. 3). Tissue elasticity proximal to the occluded vessel was normal, but the tissue modulus was sharply increased from the LAD ligation point to a point 6 mm distally. Fibrosis was less dramatic further away from the ligation point (6–9 mm) in the infarct border zone, and passive tissue stiffness softened at a rate of 8.5 kPa/mm until approaching normal myocardium. At the point of ligation, MSC-injected animals also exhibited a sharp increase but had a significantly softer tissue modulus (40 ± 10 kPa). Although the elevated stiffness of the infarct occurred over a similar area compared with control animals, softening of the border zone was more gradual (4.3 kPa/mm) and eases the modulus mismatch between stiff, infarcted regions and normal tissue. Overall, MSC injections resulted in a 30% reduction in modulus compared with controls (P < 0.001), and the myocardium was only twofold stiffer compared with normal. It is important to note the heterogeneity observed in both MSC-injected and control cases (illustrated by large error bars in the binned data), which is indicative of a very localized fibrosis-induced stiffening, effectively creating a “composite” tissue with less modulus mismatch.

**Ventricular remodeling.** Infarct size was not significantly different between the MSC-injected animals and the control animals [46 ± 2 (MSC) vs. 46 ± 4% (control) of LV free wall]. MSC-injected animals had greater preservation of normal LV geometry compared with control animals, including improved LV chamber diameter and wall thickness of both the border zone and infarct myocardium (Fig. 4). The geometrical improvements seen in the MSC-injected rats were associated with a 20% reduction in fibrosis compared with control hearts in the infarct region (Fig. 5). TUNEL staining demonstrated that MSC-injected hearts also had decreased apoptosis in the myocardial border zone (Fig. 6). Endothelial cell density was equivalent in the border zone myocardium of the MSC-injected and control animals [67.2 ± 4.1 (MSC) vs. 65.2 ± 4.5 (control) von Willebrand factor-positive cells per high-power field; P = 0.74, n = 7 in each group], as was MMP activity [19.6 ± 8.4 (MSC) vs. 14.2 ± 4.2 ng (control) of MMP-1 activity; P = 0.60, n = 3 in each group]. In vivo conductance catheter measurements of LV end-diastolic volume trended toward less ventricular dilation (Table 1). Therefore, cells remained embedded in the ventricular wall, softening it mechanically rather than differentiating or undergoing apoptosis.

**Hemodynamics and cardiac function.** MSC-injected rats had significant hemodynamic preservation compared with control...
animals (Table 1 and Fig. 7). Pressure-volume loops obtained during inferior vena cava (IVC) occlusion (Fig. 7A) show increased LV end-systolic pressure and decreased EDV for MSC-injected rats compared with controls. MSC-injected rats also showed improved LV systolic function in maximum pressure, dP/dt max, and dP/dt max-EDV relationship, as determined by pressure-volume loops during IVC occlusion (Table 1 and Fig. 7B). MSC-injected rats had a larger slope and decreased intercept for the dP/dt max-EDV relationship. The relationship between stroke work and EDV, also determined from pressure-volume loops during IVC occlusion, demonstrated that MSC-injected rats had improved preload recruitable stroke work and cardiac contractility (Table 1 and Fig. 7C). In addition, MSC animals had higher maximal elastance (Table 1), which is another index of global cardiac contractility determined by the end-systolic pressure-volume relationship. Finally, MSC animals had better diastolic function, indicated by minimum dP/dt and a trend toward improvement in the relaxation time constant τ (Table 1).

**DISCUSSION**

Ventricular remodeling after myocardial infarction generally involves cardiomyocyte necrosis and apoptosis, expansion of the infarct border zone, ventricular dilation, wall thinning, progressive fibrosis, and ultimately, further loss of function and the clinical syndrome of heart failure (2, 3, 11, 13, 19, 21, 30).

### Table 1. Rats injected with MSCs have improved hemodynamics and contractility versus control rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>MSCs</th>
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<tr>
<td>n</td>
<td>8</td>
<td>8</td>
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<tr>
<td>EDV, μl</td>
<td>283±16</td>
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<td>Heart rate, beats/min</td>
<td>265±14</td>
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<td>Ejection fraction, %</td>
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<td>52±3</td>
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<td>1,610±90</td>
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<td>Minimum left ventricular dP/dt, mmHg/s</td>
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<td>-1,230±110</td>
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<tr>
<td>τ, ms</td>
<td>30±5</td>
<td>25±3</td>
<td>0.4</td>
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<tr>
<td>Maximal elastance, mmHg/μl</td>
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<td>0.03</td>
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<td>Slope maximum dP/dt-EDV, mmHg/s-1/μL^-1</td>
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<td>Maximum dP/dt-EDV volume intercept, μl</td>
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<td>PRSW, mmHg</td>
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<td>PRSW volume intercept, μl</td>
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Values are means ± SE; n, number of animals. MSCs, mesenchymal stem cells; EDV, end-diastolic volume; dP/dt, change in left ventricular pressure over time; τ, time constant of relaxation; PRSW, preload recruitable stroke work; NS, not significant.

Fig. 7. A: representative pressure-volume loops for control (left) and MSC-injected (right) animals. Loops for control animals are shifted downward and rightward compared with MSC-injected animals. B: maximum change in pressure over time (dP/dt max)-end-diastolic volume (EDV) relationships show that MSC-injected animals have a leftward and upward shift compared with control animals. Each line on graph represents data points for dP/dt max and EDV measured from individual pressure-volume loops obtained during reduction of preload volume by inferior vena cava (IVC) occlusion. C: representative stroke work (SW)-EDV relationships show that MSC-injected animals are shifted leftward and upward compared with control animals. Each line on graph represents data points for SW and EDV measured from individual pressure-volume loops obtained during reduction of preload volume by IVC occlusion.
Cellular cardiomyoplasty appears promising as a heart failure treatment, particularly with MSCs that could differentiate into cardiomyocytes (14, 23, 28, 34). In this study, MSCs engrafted and expressed muscle proteins but did not appear to form contractile elements that integrated with native tissue. Cell injection also did not stimulate significant angiogenesis because endothelial cell density was equivalent in the border zone myocardium of both the MSC and control groups, although it should be noted that interpreting endothelial cell density is limited in that vascularization could be different even with identical endothelial cell density if cells are organized differently in the two groups. Cell injection also did not change MMP activity in the border zone, though it is important to acknowledge that MMP activity could be impacted more in the infarct region where more dramatic effects on myocardial stiffness occur. Still, cellular engraftment attenuated postinfarction remodeling and preserved cardiac function, as seen in previous studies. Cellular engraftment appeared to provide functional benefit by effectively softening the tissue by increasing cellularity in the infarct scar and making it a more heterogeneous, “composite” tissue rather than by regenerating functional cardiomyocytes. Injecting cells significantly improved the subsequent elastic moduli of the infarct, as well as more gradually softening the border zone. Having a more compliant infarct scar early after infarction was subsequently associated with less remodeling, with reduced thinning of the infarct and border zone myocardium, border zone apoptosis, and dilation of the LV cavity. Whereas fibrosis of infarcted myocardium is needed to prevent myocardial rupture, the resultant dense scar that the remaining noninfarcted portions of the ventricle have to contract against may drive some of the subsequent detrimental ventricular remodeling. The results of this study appear to be the first observations that directly link changes in postinfarction remodeling to having a more compliant infarct scar.

The importance of matrix mechanics on cellular and tissue function is not restricted to cardiomyocytes and the heart. Similar phenomena have been reported with smooth muscle cells, epithelial cells, fibroblasts, endothelial cells, and neurons (5, 8, 9, 15, 22, 25, 37). A narrow optimum in matrix stiffness, recently shown to be required for myofibrillogenesis in myotubes, has also been extended to MSC differentiation, which is recently shown to be required for myofibrillogenesis in myotubes (7, 29). Further matrix softening with other antifibrotic therapies may be required before a proper microenvironment for MSC differentiation will exist such that preinfarct ventricle function can be restored. In addition, cardiomyocyte contact has been shown to induce MSCs to express a cardiomyocyte phenotype (24). More recently, however, it has been shown that bone marrow cells fuse with cardiac muscle cells and do not truly “transdifferentiate” into cardiomyocytes (1). The cells in our study that expressed cardiac muscle proteins were located in the infarct region and isolated from viable myocardium, making it unlikely that they were expressing a cardiomyocyte phenotype due to cell fusion. However, even with the expression of cardiac proteins, the engrafted cells did not receive the appropriate cues to fully differentiate into cardiomyocytes. Additional signaling in the form of genetic modification or from extracellular mediators may have to be provided to the engrafted cells to completely push them toward a cardiomyocyte phenotype. Also, whether cell fusion or simply cell contact is needed, transplantation of enough cells to completely fill the infarct region to ensure a bridge of cells from border zone to border zone may be needed to result in the engrafted cells eventually becoming cardiomyocytes that effectively participate in the contractile process.

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