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Basic Investigation

Incomplete Retention After Direct Myocardial Injection

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Direct intramyocardial injection may permit local delivery of protein and gene therapy agents for myocardial and coronary artery disease. Little is known about the immediate fate of materials administered via percutaneous endomyocardial catheters or via surgical epicardial injection. In this study, we use a novel method to evaluate the acute retention of agents injected directly into the myocardium, compare epicardial with the percutaneous endocardial and postmortem delivery, and evaluate the influence of injectate volume on myocardial retention. Fifteen 40–50 kg pigs underwent overlapping myocardial injections using a percutaneous endomyocardial catheter, an epicardial needle via an open chest, and epicardial needle postmortem. Multiple distinct 15 μ neutron-activated microsphere species were used as tracers. Two or three myocardial walls were injected in each animal using 3.5 mm, 27–28 gauge needles at varying injectate volumes. Animals were sacrificed immediately. Myocardial walls were divided and multiple microsphere species were quantified. In an additional study, nine 70 kg pigs underwent percutaneous endomyocardial injections with replication-deficient adenovirus encoding for the production of lac-Z. The injectate volume was varied, while the viral particle number remained constant. The animals were sacrificed 5 days after the percutaneous injections; the heart, liver, and spleen were collected for β-galactosidase activity. Endomyocardial injection was associated with 43% ± 15% microsphere retention, compared with 15% ± 21% ($P < 0.01$) retention of open chest epicardial injection and 89% ± 60% ($P < 0.01$) for postmortem injection. Reducing the injectate volume from 100 to 10 μL improved microsphere retention ($P = 0.01$). There was a trend toward improved viral transfection associated with smaller injection volumes. Despite direct intramyocardial administration, a significant fraction of injectate is not retained locally. Catheter-based needle endomyocardial injection is associated with equivalent or superior injectate retention compared with open chest epicardial injection. Proportionately, more injectate may be retained at lower volumes. Loss may involve a combination of channel leakage, venous, and lymphatic return. *Cathet Cardiovasc Intervent 2002;55:392–397.*

Key words: catheter; endomyocardial; gene transfer; intramyocardial drug administration

INTRODUCTION

A variety of emerging molecular interventions may prove useful in the treatment of coronary artery and myocardial disease. Methods for delivery of therapeutic agents to the cardiac tissues include intravenous [1], intra-arterial [2], intracoronary [3], pericardial [4,5], and direct myocardial routes using an epicardial surgical [6] or catheter-based endocardial approach [7–9].

Direct myocardial injection is attractive because specific myocardial regions can be targeted and high local tissue concentrations, theoretically, can be achieved. In addition, local drug delivery might permit an overall dosage reduction, with attendant reduction in systemic toxicity [10,11]. However, little is known about the ability of myocardium to retain injected material after percutaneous endomyocardial catheter or direct epicardial injection.

In these large-animal experiments, we evaluate the acute retention of agents injected directly into the myocardium, both epicardially and via a new percutaneous endomyocardial catheter. In addition, we consider the

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effect of altered injectate volume on myocardial retention.

MATERIALS AND METHODS

Animals and Equipment

The University of Michigan Committee on the Use and Care of Animals approved all protocols. Healthy farm swine (40–70 kg) were sedated with xylazine (2.2 mg/kg) intramuscularly (i.m.), telazol (6.0 kg/mg) i.m., and atropine (0.04 mg/kg) i.v. and received continuous isoflurane (2%–2.5%) inhalation and mechanical ventilation.

Endomyocardial injections were performed with the Stiletto™ 28G Endomyocardial Injection System (Boston Scientific, Natick, MA). A transfemoral 9 Fr sheath was advanced into the left ventricle under fluoroscopic guidance and a coaxial 7 Fr steering guide directed the Stiletto needle injection catheter to the desired LV territory. The Stilletto was advanced to the endomyocardial surface using monoplane fluoroscopic guidance, and catheter tip stability was confirmed manually and fluoroscopically. A spring-loaded needle penetrates 3.5 mm into the endomyocardial wall. Injectate was introduced into the LV using a 1 mL or Hamilton 100 μL syringe (Hamilton, Reno, NV). Injectate was administered over a 15-sec interval. The needle was retracted and then the catheter was withdrawn and redirected to other sites.

Epicardial injections were performed systematically after all endocardial injections, at which point the sternum and pericardium were opened. Epicardial injections were performed using 27G butterfly needles, each angled 90°, yielding a 3.5 mm distal tip to give reproducible needle depth on a beating heart. For all injections, one operator controlled the needle and another performed injections.

Microsphere Retention Experiments

Neutron-activated microspheres (Biophysics Assay Laboratory, Wellesley Hills, MA) having a mean 15 μ diameter were used as tracers. A distinct heavy-metal tracer, gold, samarium, or iridium, was used for each method of injection. The microsphere concentration was 2.5×10^6/mL. The microsphere stock solution (3 mL) was mixed with 0.1 mL of ethylene blue dye (1%), bromcresol green dye (1%), or bromcresol purple dye (1%).

Three groups of five 40–50 kg pigs each underwent 10, 20, and 100 μL injections. Individual animals underwent three overlapping myocardial injections to the same myocardial segment using a fluoroscopically guided percutaneous endomyocardial catheter, open chest epicardial needle, and a postmortem epicardial control injection. Each needle remained in the myocardium for a total of 15 sec. After all injections were completed, the animals were sacrificed immediately with intravenous sodium pentobarbital (17 mg/kg), and the heart was collected. Injection sites were localized by gross examination of the endocardial surface. The myocardial walls were divided into three sections, anterior, inferior, and lateral, with localized dye tracer staining in the center of the sections. An overlapping injection of an equivalent volume of a distinct microsphere tracer was injected into the postmortem myocardial sections. In addition, myocardial sections remote to the microsphere injection sites (septum and right ventricle) were collected. The individual myocardial walls were sectioned and placed in tracer-free polypropylene vials. Control samples of the injectate were also placed directly into tracer-free vials and air-dried. All samples were shipped to a core laboratory (Biophysics Assay Laboratory) for neutron activation and analysis [12,13]. This permits quantification of multiple microsphere species in a single specimen.

Adenoviral Lac-Z Delivery Experiments

Nine 70 kg pigs underwent endomyocardial injection using the percutaneous myocardial injection catheter. A replication-defective adenovirus containing a lac-Z gene construct was used as a tracer. A constant number of viral particles (10^{10}) were administered during each injection, although injectate volume was varied. The injectate volume was 10 μL in three animals, 20 μL in three animals, and 100 μL in three animals. Under fluoroscopic guidance, the Stiletto Endomyocardial Injection System was used to deliver an array of 10 discrete injections, spaced 1 cm apart, to the anterior wall of each animal. Afterward, the catheter and sheath were removed, the femoral artery cut-down site was repaired, the animal was recovered and then sacrificed 5 days afterward. Tissue samples were collected and snap-frozen in liquid nitrogen. β-galactosidase expression was batch-assayed in homogenized samples of anterior LV wall, inferior LV wall, right ventricle, coronary sinus, liver, and spleen.

All samples were stored at −70°C until weighed and pulverized under liquid nitrogen using a SPEX 6800 freezer mill (SPEX CertiPrep, Metuchen, NJ). Frozen, pulverized tissue (50 mg) was solubilized in 500 mL of lysis buffer (250 mM Tris HCl, 0.5% Triton X-100, 50 mg/mL leupeptin, 100 mg/mL aprotinin, and 200 mg/mL Pefabloc, pH 7.4), centrifuged for 10 min at 14,000 rpm, and the supernatant was analyzed using the Stratagene High Sensitivity β-Galactosidase Staining Kit (catalog no. 200710; Stratagene, La Jolla, CA). The optical density was measured at 570–595 nm. The relative units of β-galactosidase per mg of tissue protein were multiplied by the myocardial tissue weight. Data were expressed as

total units of β-galactosidase (U) for myocardial tissue, or units β-galactosidase per mg tissue protein (U/mg) for coronary sinus, right ventricle, liver, and spleen.

Statistics

Continuous parameters were tested using analysis of variance and a two-tailed student's *t*-test using Bonferroni or Dunnet corrections for multiple comparisons as appropriate (SPSS for Windows version 8.0; SPSS, Chicago, IL).

RESULTS

Acute Injections

The endocardial and the epicardial injections were well tolerated in all animals. Injections induced isolated ventricular ectopic beats but no sustained dysrhythmia. Postmortem examination revealed no evidence of myocardial perforation or disruption of the mitral valve apparatus. Dye staining corresponding to endocardial and epicardial injections was identified in predicted myocardial segments.

Microsphere Retention

Less material was retained after endomyocardial injections or after epicardial injections compared with postmortem injections. When data from all injection volumes was pooled and compared with control specimens that were injected directly into a sample vial, microsphere retention was 43% ± 15% ($P < 0.01$ compared with postmortem controls) after endomyocardial, 15% ± 21% after epicardial ($P < 0.01$ compared with postmortem controls), and 89% ± 60% after postmortem injections. In addition, there was greater retention of injectate by the catheter-based endocardial compared with the epicardial injection approach ($P < 0.01$; Fig. 1).

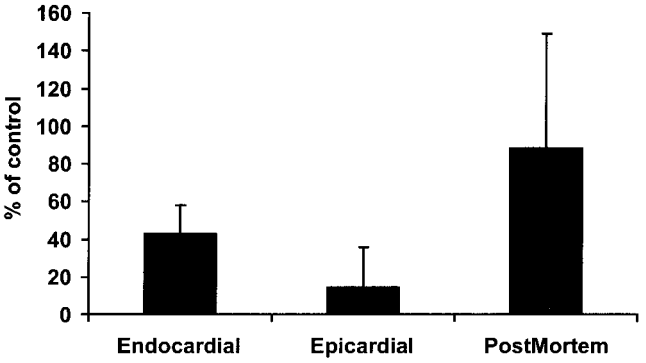

Fig. 1. The site of injection influences microsphere retention. Compared with postmortem control injections, there was significantly less material retained after endomyocardial injection ($p < 0.01$) or epicardial injection ($p < 0.01$). In addition, there was greater retention of microspheres associated with endomyocardial injection compared with epicardial injection ($p < 0.01$). Statistics are corrected for multiple comparisons as described in methods.

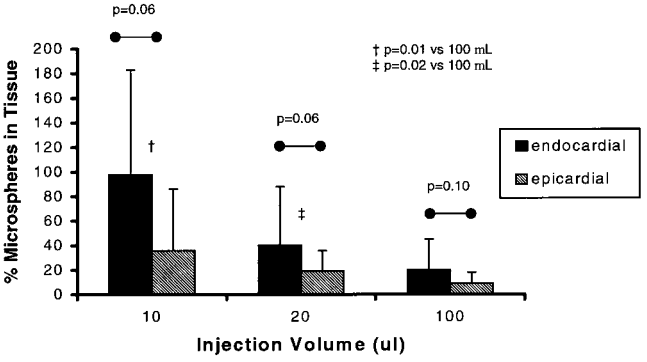

Fig. 2. The injection volume influences microsphere retention. Proportionally more microspheres were retained after smaller-volume injections than after larger volume injections. These differences were statistically significant for both endocardial and epicardial injection methods. In addition, there was superior retention of injectate after endocardial injections compared with epicardial injections. This approached statistical significance at each individual injection volume.

Injection Volume and Microsphere Retention

Proportionately more microspheres were retained after smaller-volume, 10 or 20 μl injections than after 100 μl injections ($P = 0.01$ and 0.02, respectively). Compared with control samples, the percentage of microspheres retained in the myocardium after 10, 20, and 100 μl endomyocardial injections was 98% ± 85, 40% ± 48%, and 20% ± 25%, respectively. The percentage of microspheres retained after 10, 20, and 100 μl epicardial injections was 36% ± 50%, 19% ± 17%, and 9% ± 9%, respectively. There was a trend toward superior retention after endocardial injections compared with epicardial injections. This difference approached statistical significance at each individual volume ($P = 0.06$ for 10 μl injections, $P = 0.07$ for 20 μl injections, and $P = 0.10$ for 100 μl injections). With all volumes pooled, there was a significant difference between retention of injectate after endocardial compared with epicardial injections, with a $P < 0.01$ after Dunnet correction for multiple comparisons (Fig. 2).

Viral Transfection After Endomyocardial Injection

An equivalent number of adenoviral particles encoding for the production of Lac-Z was injected. After correction for multiple comparisons, there was a trend toward improved viral transfection associated with the smaller injection volumes, 10 and 20 μl, compared with the 100 μl injection volume ($P = 0.08$ and 0.19, respectively; Fig. 3).

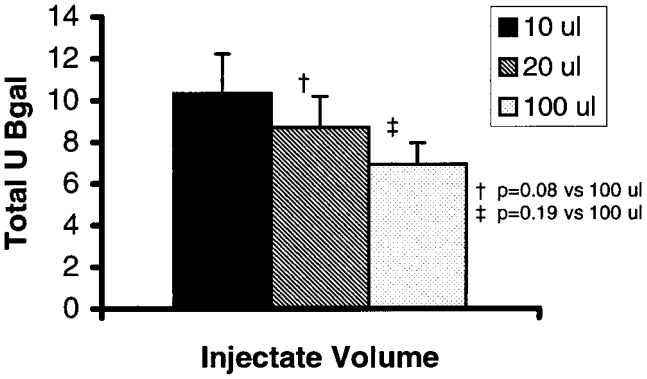

Fig. 3. Injection volume influences viral transfection. There was a trend towards improved viral transfection associated with the smaller-volume endomyocardial injections compared with the larger-volume endomyocardial injections.

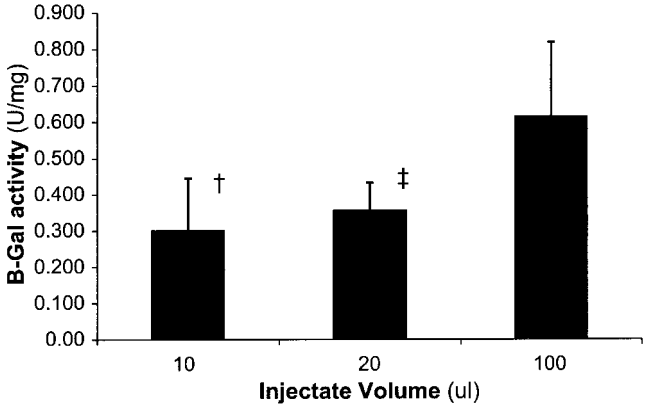

Fig. 4. Coronary sinus β-galactosidase activity. There was an insignificant trend towards greater viral transfection associated with the larger-volume injections compared with the smaller-volume injections. †: $p = 0.05$ compared with 100 μl volume injection; ‡: $p = 0.08$ compared with 100 μl volume injection.

Coronary Sinus Tissue

There was marginally more β-galactosidase activity in the coronary sinus tissue from the animals receiving 100 μl injections compared with those animals receiving lower volume injections, (Fig. 4). This may indicate that a greater proportion of the larger volume injections are lost to the coronary venous system.

Viral Transfection in Nontarget Organs

There was evidence of significant viral transfection in the liver and spleen (Table I).

Direct Leakage

Leakage of injected material from the injection site was observed during and immediately after all epicardial injections. The volume of this leaked material could not readily be quantified. During continuous intracardiac echocardiography of endomyocardial injections, direct leakage of microbubble-containing injectate was demonstrated (data not shown).

DISCUSSION

Injectate Leakage

The intramuscular route has long been used for systemic drug delivery [14,15]. Our data suggest that cardiac intramuscular delivery also leads to significant systemic exposure of injectate in that a substantial proportion of injected material immediately exits the myocardium. During direct epicardial injection, we observe obvious egress of material associated with cardiac systole after withdrawal of the injection needle. Moreover, using continuous intracardiac echocardiography, direct leakage of microbubble-containing injectate is easily demonstrated.

In addition to direct leakage, injectate may exit the myocardium via cardiac venous or lymphatic channels. The myocardium, like all muscle tissue, is highly vascular, with extensive vascular drainage [16]. Reporter gene detection in coronary sinus tissue, above regional background, suggests a component of venous return of administered viral vector [17,18]. Therefore, once escaping cardiac tissue, nontarget organs may be exposed to the injected material [9].

Retention Is Greater After Endomyocardial Than After Epicardial Injection

We found equivalent or superior retention of material injected into the myocardium via endocardial catheter compared with epicardial surgical needle. Transmural inhomogeneity of hemodynamic and physical forces may underlie this observation [19,20]. For example, subendocardial tissue is predisposed to ischemia and infarction earlier after coronary occlusion than is subepicardial tissue [21,22]. Conceivably similar forces act to extrude subepicardial more than subendocardial injectate. Similarly, it is possible that endocardial injection may afford deeper delivery of injectate.

Endomyocardial injection is clinically more attractive than direct surgical injection. Many patients who are candidates for protein- or gene-based direct myocardial therapy have undergone or will require cardiac surgery [23]. Transepicardial access generally entails higher patient morbidity and, for example, might endanger viable bypass grafts. Moreover, repeat direct myocardial drug administration is potentially more feasible via a transcatheter approach.

Methods That Might Improve Retention

We found that smaller-volume injection of more concentrated suspensions or solutions leads to improved

TABLE I. Non-Target Organ Total β-Galactosidase Activity

		Injection volume		
		10 μl	20 μl	100 μl
Tissue	*Liver*	**1.26** ± 0.21	**1.07** ± 0.20	**1.28** ± 0.28
β-Gal	*Spleen*	**0.31** ± 0.06	**0.24** ± 0.07	**0.65** ± 0.70
(U/mg)	*RV*	**0.34** ± 0.08	**0.39** ± 0.07	**0.40** ± 0.08
	CS	**0.30** ± 0.14	**0.36** ± 0.08	**0.62** ± 0.20

β-Gal, beta galctosidase activity; U, units; RV, right ventricle; CS, coronary sinus; μl, microliter.

myocardial retention, conceivably by reducing the physical or hemodynamic tissue displacement by injectate. Other methods that might improve retention include alterations in vehicle viscosity, osmolarity, or charge. Other investigators have even explored local alterations in tissue permeability, for example, by insonation [24] or electroporation [25,26].

Fluoroscopic Guidance of Endomyocardial Injections

We were able to access all myocardial segments using monoplane fluoroscopic guidance, and there was no evidence of sustained arrhythmia, myocardial perforation, or damage to the mitral valve apparatus. Similar safety and procedural success has been demonstrated using more elaborate geographic localization technologies, such as three-dimensional electromechanical mapping [7–9]. In comparison, a fluoroscopic-guided injection catheter is simpler, less expensive, and potentially faster.

Advantages and Goal of Direct Myocardial Delivery

Direct myocardial delivery of therapeutic agents for the treatment of coronary artery and myocardial disease is potentially advantageous. Compared with other forms of delivery, direct myocardial delivery is attractive because specific myocardial regions can be targeted, and higher local concentrations of agent can be achieved [9]. In one biodistribution study, less than 1% of radiolabeled basic fibroblast growth factor activity was recovered in the myocardium shortly after intracoronary or intravenous administration [27]. Conceivably, a smaller total dose of agent can be administered intramuscularly, thereby lowering systemic exposure and potential nontarget organ toxicity.

Study Limitations

The microsphere control counts have a high variability, possibly due to settling of the dense microspheres. While frequent agitation of the injectate was attempted, some settling of the microspheres within the injection system was possible.

These studies were performed in nonischemic myocardium. Direct myocardial injection tolerability and injectate retention in ischemic or nonviable myocardium may be different than the retention in normal cardiac tissue. In addition, the retention was evaluated in a single postinjection time point and retention over time was not evaluated. Finally, adenoviral transfection may be higher at higher viral particle concentrations; therefore, our finding of greater viral transfection at low injectate volumes conceivably may represent this inoculum effect in addition to more effective retention of a lower-volume injectate, as suggested in the microsphere studies.

Direct myocardial injection was well tolerated. A significant fraction of injectate is not retained locally. We found that injectate retention after catheter-based endomyocardial injection is equivalent or superior to open chest epicardial injection. Finally, injectate retention may be greater at lower injectate volumes. Catheter-based endomyocardial injection is a promising strategy for local drug delivery.

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