Myocardial Gene Transfer by Selective Pressure-Regulated Retroinfusion of Coronary Veins

Comparison With Surgical and Percutaneous Intramyocardial Gene Delivery

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OBJECTIVES We sought to study adenoviral gene delivery using percutaneous selective pressure-regulated retroinfusion and to compare it directly with surgical and percutaneous intramyocardial delivery (PIMD) for the first time.

BACKGROUND Intramyocardial delivery (IMD) has been recommended to be the preferred gene delivery strategy so far. However, surgical and percutaneous intramyocardial injection lead to incomplete retention of the injected viral vectors and to limited spatial myocardial distribution. Percutaneous selective pressure-regulated retroinfusion of the coronary veins was developed recently to provide an effective and more homogenous regional myocardial gene transfer.

METHODS In 15 pigs, adenoviral vectors (Ad2-CMV beta-galactosidase [β-gal] 5×10^9 pfu) were applied via surgical IMD (n = 5), PIMD (n = 5), and selective pressure-regulated retroinfusion (n = 5). Seven days after gene transfer, myocardial β-gal expression was measured by ELISA.

RESULTS Selective retroinfusion into the anterior cardiac vein substantially increased reporter gene expression (1,039 ± 79 pg β-gal/mg protein) in the targeted left anterior descending coronary artery territory when compared with surgical (448 ± 127, $p < 0.05$) and PIMD (842 ± 145, $p < 0.05$). Both IMD approaches showed an inhomogenous β-gal expression, particularly along the injection sites, while retroinfusion resulted in a more homogenous transmural gene expression.

CONCLUSIONS Percutaneous selective pressure-regulated retroinfusion compares favorably with surgical and percutaneous intramyocardial injection techniques by providing a more homogenous and even more efficient adenoviral gene delivery. (J Am Coll Cardiol 2004;44:1124–9) © 2004 by the American College of Cardiology Foundation

A variety of emerging molecular interventions are being designed to treat coronary artery disease and heart failure. Some of these strategies are cell-based (1–4), whereas others depend on proteins (5), naked complementary deoxyribonucleic acid integration (6), or viral vector gene transfer (7,8). Efficient substrate delivery to the targeted myocardial region is of crucial importance for each of these strategies. The majority of recent clinical studies aimed at inducing angiogenesis were based on intracoronary (8) or direct myocardial injection techniques (7,9). Both delivery strategies appeared to be safe in phase I and phase II clinical trials (7–9) but failed to demonstrate significant improvement of perfusion or clinical end points. The major drawback of intracoronary injection is limited retention of angiogenic substrates (10,11), whereas direct myocardial injection has to overcome limited spatial distribution (12). Although intramyocardial injection is considered to be the preferred delivery strategy so far, a more homogenous, but equally efficient, delivery might be required before regional myocardial blood supply or function can be substantially influenced by angiogenic or arteriogenic agents.

Percutaneous selective pressure-regulated retroinfusion of coronary veins (11,13,14) combines homogenous intravascular delivery with increased retention of angiogenic substrates. It has been shown previously to provide substantially higher and more homogenous gene expression after adenoviral reporter gene transfer when compared with intracoronary delivery (11). Moreover, favorable retention of angiogenic FGF-2 protein with functionally relevant induction of arterio- and angiogenesis was demonstrated recently in a pig model of chronic myocardial ischemia (15).

In this study, we addressed adenoviral gene transfer using percutaneous selective pressure-regulated retroinfusion and, for the first time, compared it directly with surgical and percutaneous intramyocardial delivery (PIMD) in a pig model.

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METHODS

The present investigation was carried out according to the "Guide for the Care and Use of Laboratory Animals" and

Abbreviations and Acronyms

ENDO = endomyocardial (probe or layer)
EPI = epicardial (probe or layer)
IMD = intramyocardial delivery
LAD = left anterior descending (coronary artery)
LV = left ventricle/ventricular
MID = midmyocardial (probe or layer)
PIMD = percutaneous intramyocardial delivery
β-gal = beta-galactosidase

was approved by the Bavarian Animal Care and Use Committee.

Fifteen German farm pigs (mean body weight, 26 ± 4 kg) were anesthetized and monitored as described previously (11,15). Catheter introducer sheaths were placed in the right carotid artery and right external jugular vein. Full anticoagulation was achieved by bolus injection of heparin 10,000 IU followed by continuous application of 5,000 IE/h. At the end of the experiment, all catheters and introducer sheaths were removed. Seven days later the animal was anesthetized again and intubated, and catheter introducer sheaths were placed as decribed above. The patency of the left anterior descending (LAD) artery was confirmed by coronary angiography. Thereafter, the heart was excised and the left ventricle (LV) was cut into slices of 1-cm thickness from the apex to the basis as described previously (15). Each slice was divided into eight transmural segments, and each segment was separated equally into an epicardial (EPI) probe, a midmyocardial (MID) probe, and a subendocardial (ENDO) probe (Fig. 1).

Catheterization procedures. In all animals, a 7-F guiding catheter was placed in the left coronary artery, and the LAD artery was wired. During the delivery of the adenoviral vectors, the LAD was occluded for 10 min by a percutaneous transluminal coronary angioplasty balloon distal to the first diagonal branch in all groups. In pigs treated by selective retroinfusion, the anterior interventricular vein was catheterized using a 6-F retroinfusion catheter (11,13,16,17).

Selective retroinfusion. The system of selective suction and pressure-regulated retroinfusion has been described in detail previously (11,13,16,17). For regional application of adenoviral vectors, a modified technique of continuous pressure-regulated retroinfusion was used with inactivation of the suction device (11). Briefly, the high-pressure reservoir (2.5 atm) was filled with saline solution (0.9%) that was kept at 37°C. The adenoviral vector solution (5×10^9 plaque forming unit [pfu] diluted in 20 ml saline) was delivered during 10 min of continuous retroinfusion.

Surgical intramyocardial delivery. In animals randomized for surgical intramyocardial delivery (IMD), a left thoracotomy was performed. After incision of the pericardium, the adenoviral vectors were injected with a 27-gauge needle. For each injection, the needle was advanced about 10 mm perpendicular to the surface of the heart into the myocardium. Sixteen injections of 100 μl adenoviral vector containing solution (5×10^9 pfu diluted in 1.6 ml saline) were applied distal to first diagonal branch into the LAD territory.

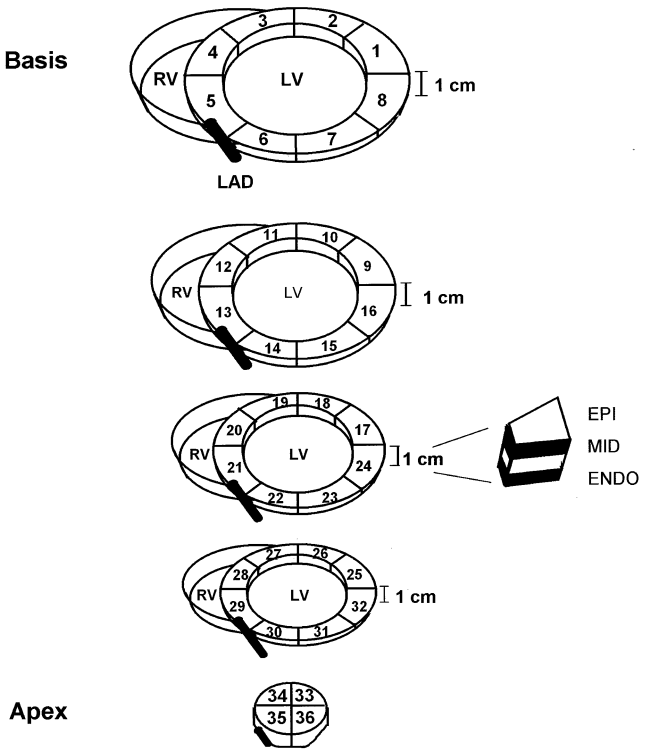

Figure 1. Harvesting of myocardial probes for measurement of regional myocardial beta-galactosidase concentration. After excision of the heart, the left ventricle (LV) was sliced parallel to the short axis from the apex to the basis. Each slice was divided into segments of equal size followed by dividing each segment into a epicardial (EPI), midmyocardial (MID), and subendocardial (ENDO) probe. RV = right ventricle.

PIMD. An endoventricular injection catheter (Steerjet, MicroHeart, Boston, Massachusetts) was placed across the aortic valve in the LV cavity; 16 injections of 100 μl adenoviral vector containing solution into the LV septum and anterior LV wall were performed under fluoroscopic control.

Adenoviral vectors and analysis of gene transfer. A replication-deficient, second generation adenoviral vector carrying the beta-galactosidase (β-gal)/lacZ reporter gene (Ad2-CMV-β-Gal, Genzyme, Boston, Massachusetts) was used. The β-gal concentration in each myocardial probe was quantified using an ELISA (18). For histologic assessment of β-gal, representative samples of the LAD region were acquired and processed as described previously (11).

Experimental groups and statistics. Three experimental groups (A to C) were randomly assigned. In group A (n = 5), the adenoviral vectors were injected using a surgical approach (i.e., an IMD). Percutaneous intramyocardial injection (i.e., a PIMD) was performed in group B (n = 5). In group C (n = 5), adenoviral vectors were delivered by retroinfusion.

All values are presented as mean ± SEM. Measurements

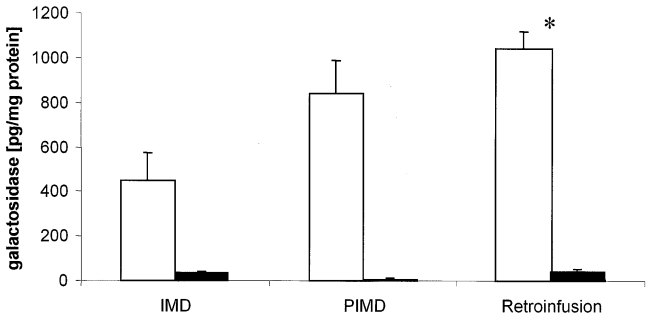

Figure 2. Mean transmyocardial beta-galactosidase expression in the left anterior descending coronary artery territory **(open bars)** and in the control, circumflex coronary artery area **(solid bars)** seven days after application of 5×10^9 pfu Ad2-CMV-beta-galactosidase. IMD = surgical intramyocardial delivery (group A); PIMD = percutaneous intramyocardial delivery (group B); retroinfusion = pressure-regulated retroinfusion of the anterior cardiac vein (group C). Mean ± SEM, n = 5/group. *$p < 0.05$ group C vs. group A and group B.

of β-gal expression were analyzed by nonparametric Kruskal-Wallis test. Whenever a statistically significant effect was obtained, we performed multiple comparison tests between the individual groups using the Mann-Whitney *U* test. A p value <0.05 was considered to be statistically significant.

RESULTS

The three groups were similar with regard to body weight and hemodynamics at baseline and day 7. Coronary angiograms performed at day 0 and day 7 showed no evidence for compromised coronary flow.

Efficiency of surgical IMD. At day 7 after surgical IMD of adenoviral vectors (group A), quantitative ELISA of β-gal expression revealed significant reporter gene expression in 20.2 ± 3.3% of all myocardial probes (n = 330) in the targeted LAD territory. The mean β-gal expression in the LAD territory was 448 ± 127 pg/mg protein (Fig. 2). There was a significantly higher β-gal expression in the EPI probes than in the MID probes and neglegible expression in the subendocardial probes (Figs. 3 and 4).

Efficiency of catheter-based PIMD. In group B with PIMD, significant β-gal expression was observed in 14.4 ± 0.6% of all myocardial probes (n = 321) in the targeted LAD territory. In contrast with group A, the highest levels of β-gal expression were found in the MID and subendocardial probes, whereas epicardial expression was very low (Figs. 3 and 4).

Efficiency of selective retrograde delivery into coronary veins. Retrograde delivery of adenoviral vectors resulted in significantly increased reporter gene expression in the target LAD territory (1,039 ± 79 pg β-gal/mg protein) compared with surgical (448 ± 127 pg/mg, $p < 0.05$) and percutaneous IMD (842 ± 145 pg/mg, $p < 0.05$) (Fig. 2). In addition, β-gal expression was present in significantly more probes of group C (46.2 ± 3.8% of n = 317 myocardial probes) than in group A (20.2 ± 3.3%, n = 330, $p < 0.05$) and group B (14.4 ± 0.6%, n = 321, $p < 0,05$). As expected from previous studies (11), there was a gradient from EPI to MID and ENDO layers (Figs. 3 and 4).

Histochemical analysis of β-gal expression. Histologic examination of the hearts transfected with the Ad_2-CMV-β-gal showed positive staining of cardiac myocytes (Fig. 5). After direct surgical intramyocardial injection, positive cardiomyocytes were detected, particularly along the injection sites in the subepicardial myocardium. Percutaneous direct intramyocardial injection revealed a similar gene expression pattern longitudinal along the injection channel, mainly in the midmyocardium and in subendocardial layers (Fig. 5A). In contrast, after retroinfusion, a more homogeneous distribution of positive staining cells was observed in all myocardial layers (Fig. 5B). There was no evidence for micro-infarctions in these probes determined by histologic assessment, arguing against false positive β-gal staining (19).

Transfection of non-targeted myocardium. Selective gene transfer to the target LAD territory after intramyocardial (IMD, group A and PIMD, group B) and retrograde delivery (retroinfusion, group C) was confirmed by very low amounts of β-gal expression in all probes of the control region (circumflex coronary artery territory) (Fig. 2).

DISCUSSION

Aiming at a catheter-based approach for efficient and homogeneous myocardial gene delivery, we developed a pressure-regulated system for selective retroinfusion of coronary veins (11), which allows a unique access to ischemic myocardium regardless of occluded or diffusely stenotic coronary arteries (11). In previous studies, we could demonstrate that selective pressure-regulated retroinfusion of coronary veins was more efficient than intracoronary arterial delivery with regard to adenoviral gene transfer (11) and to myocardial retention of angiogenic FGF-2 protein (15). In this study, adenoviral gene transfer by selective retroinfusion was compared directly with surgical and percutaneous intramyocardial injection, which have been recommended as the preferred gene delivery strategies so far (20). Interestingly, selective retroinfusion compared favorably with intramyocardial injection, not only by inducing a more homogeneous myocardial distribution but also by increasing the efficacy of adenoviral-mediated gene transfer. At the same time, selectivity of gene transfer by retroinfusion was similar to the intramyocardial injection techniques (Fig. 2).

After retrograde delivery into the coronary vein, the adenoviral vectors are exposed to the coronary venous system with a large endothelial surface. Apparently, widespread transmural transfection occurred after retrograde delivery, by contrast with distinct local transfection after intramyocardial injection (Fig. 3). This is in agreement with previous studies using selective pressure-regulated retroinfusion of adenoviral vectors (11) as well as of plasmids encoding for constitutive endothelial nitric oxide synthase (21). Recently, high-pressure retrograde delivery of plas-

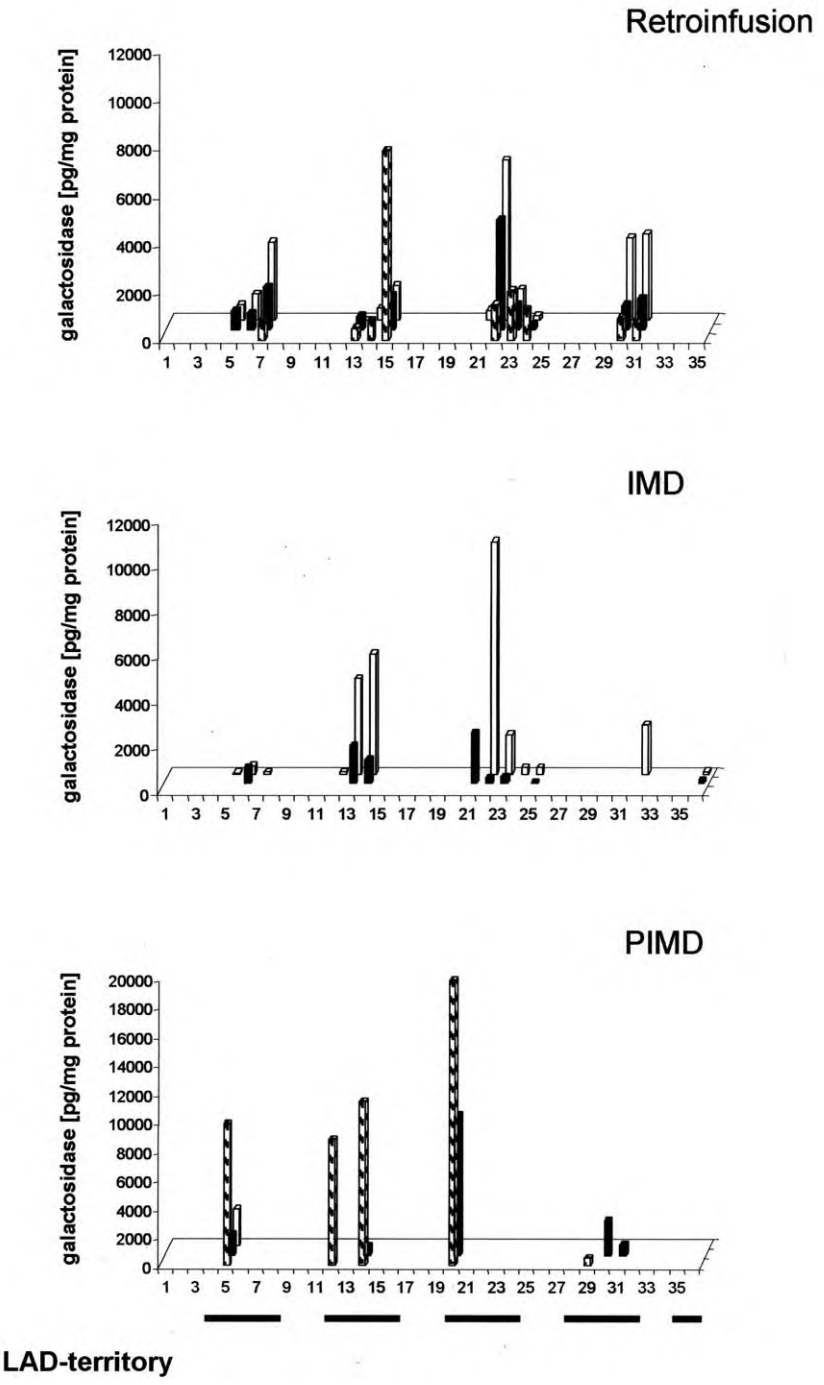

Figure 3. Representative maps of the regional myocardial beta-galactosidase protein expression after catheter-based selective pressure-regulated retroinfusion of the anterior cardiac vein (retroinfusion), surgical intramyocardial direct injection (IMD), and percutaneous intramyocardial injection (PIMD). Each map represents one animal. ENDO = subendocardial probe; EPI = epicardial probe; MID = midmyocardial probe. **Open bars** = EPI; **solid bars** = MID; **hatched bars** = ENDO.

mids encoding for human Del-1 has also been shown to provide widespread myocardial transfection (22).

In pigs treated by retrograde gene delivery, a gradient in gene expression from the EPI to the ENDO layers was observed in line with previous findings, probably due to the higher vascular resistance in the endomyocardium (11,23).

The difference in transfection between EPI and ENDO probes, however, was less pronounced for pigs treated by retroinfusion than for pigs treated by surgical IMD (Figs. 3 and 4) arguing again for a more homogeneous gene delivery by retroinfusion.

To the best of our knowledge, the transmural distribution of gene expression has not been studied after intramyocardial injection, particularly with regard to the difference between surgical epicardial injection and percutaneous endocardial injection. The finding of very low endocardial

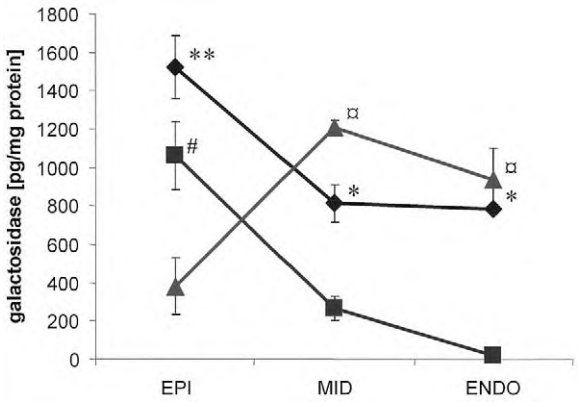

Figure 4. Mean beta-galactosidase expression after adenoviral transfection in the epicardial (EPI), midmyocardial (MID), and subendocardial (ENDO) layers of the targeted left anterior descending coronary territory. Mean ± SEM, n = 5/group. **$p < 0.05$ group C vs. group A and group B; *$p < 0.05$ group C vs. group A; #$p < 0.05$ group A vs. group B; $p < 0.05$ group B vs. group A. **Line with diamonds** = retroinfusion; **line with triangles** = percutaneous intramyocardial delivery; **line with squares** = surgical intramyocardial delivery.

levels after surgical epicardial injection (via IMD) is in agreement with other studies pointing out that there was immediate loss of material due to direct leakage (12). In addition, the injected material may exit the myocardium via cardiac veins or lymphatic channels (12). In light of the fact that part of the venous drainage is blocked during retrograde delivery, this might be another reason for increased retention (21) and adenoviral transfection by retroinfusion.

In contrast with surgical epicardial injection, percutaneous endocardial delivery was associated with very low epicardial levels of gene expression (Figs. 3 and 4). Although after endocardial delivery the overall gene expression was significantly higher than after epicardial delivery (Fig. 2), this was associated with a spotty inhomogeneous distribution. More sophisticated percutaneous endocardial injection systems (24) and a higher number of injections might improve transfection rates and homogeneity. However, histologic assessment of β-gal expression showing high levels of expression only at the site of injection (Fig. 5) argues against this strategy. If microparticle retention and adenoviral transfection were compared after percutaneous endomyocardial injection using different volumes of injection, very small injection volumes (10 μl) were associated with almost complete (98%) microparticle retention, by contrast with higher volumes (100 μl) with about 20% retention (12). However, there was only a non-significant trend toward improved transfection associated with the smaller injection volumes (12). Therefore, it is unlikely that the results of this study would have been changed substantially by using smaller injection volumes. More likely, smaller injection volumes would have increased further the inhomogeneity of gene expression after intramyocardial injection.

Selective regional myocardial infiltration by the percutaneous coronary venous route (25) is an alternative approach to direct intramyocardial injection and is taking advantage of the nondiseased coronary veins as an access to ischemic myocardium similar to selective retroinfusion. Whether adenoviral transfection, drug, or cell delivery (3) is the primary objective, epicardial injection using the coronary venous system faces the same limitations as pointed out for the techniques of intramyocardial injections used in this study.

In summary, percutaneous selective pressure-regulated retroinfusion compares favorably with surgical and percutaneous intramyocardial injection techniques by providing a

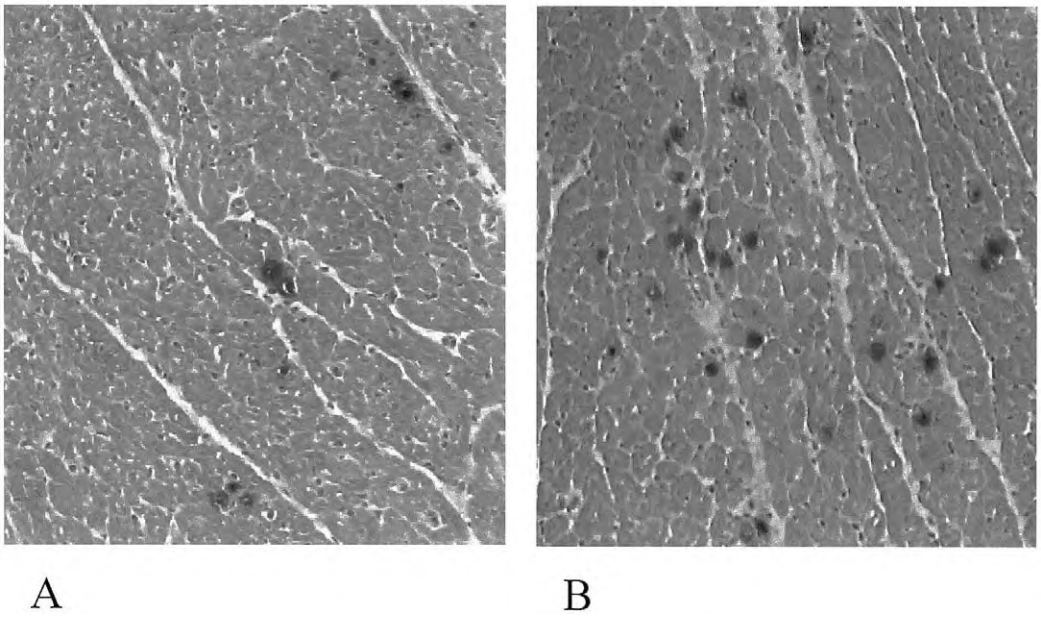

Figure 5. Histochemical detection of beta-galactosidase seven days after adenoviral transfection by percutaneous intramyocardial delivery **(A)** and selective pressure-regulated retroinfusion of the adenoviral vectors into the coronary vein **(B)** (original magnification × 80).

more homogenous and even more efficient adenoviral gene delivery.

With regard to the well-known safety of selective retroinfusion in patients with coronary artery disease (13,26), this is a promising percutaneous delivery technique for genes that might require a more homogenous transmural myocardial distribution. To the best of our knowledge, no data are available so far concerning whether biological activity is greater when a small area has high levels of gene expression or whether a larger area has lower or equal levels of gene expression. Therefore, ongoing preclinical studies will determine whether more homogenous and efficient myocardial gene transfer by selective pressure-regulated retroinfusion translates into higher biological activity of gene and cell delivery.

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