Efficient and controlled gene expression in mouse pancreatic islets by arterial delivery of tetracycline-inducible adenoviral vectors

Rui Takahashi, Hisamitsu Ishihara, Kazuma Takahashi, Akira Tamura, Suguru Yamaguchi, Takahiro Yamada, Hideki Katagiri1 and Yoshitomo Oka

Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, 2-1 Seiryo-machi, Aoba-ku, Sendai, Miyagi 980-8575, Japan
1Advanced Therapeutics for Metabolic Diseases, Center for Translational and Advanced Animal Research, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan

(Requests for offprints should be addressed to H Ishihara; Email: hisamitsu-ishihara@mail.tains.tohoku.ac.jp)

Abstract

Gene transfer with adenovirus vectors has been used extensively for pancreatic islet research. However, infection efficiency varies among reports. We reevaluated the infection efficiency, defined here as the percentage of islet cells expressing transgenes, in mouse islets. When the isolated islets were infected with adenoviruses, the infection efficiency was found to be 30–40% and the transduced cells were distributed in the islet periphery. Collagenase treatment of isolated islets before infection increased the infection efficiency to 70%, but with suppression of glucose-stimulated insulin secretion. To explore more efficient strategies, we employed arterial delivery of virus particles to islets in situ. Delivery of adenovirus (∼10⁸ particles per pancreas) through the celiac and superior mesenteric arteries is highly efficient, resulting in more than 90% transduction without impairing glucose-stimulated insulin secretion. Arterial delivery of an adenovirus harboring glycerol kinase cDNA allowed us to observe glycerol-stimulated insulin secretion from mouse islets, which was not observed when we employed the conventional method. Furthermore, the arterial delivery method combined with a tetracycline-inducible adenovirus system induced efficient and controlled transgene expression. Our data provide new insights into gene transduction methods using recombinant adenoviruses in mouse islets, and are therefore anticipated to contribute to future basic and clinical islet research applications.

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Introduction

Gene therapy holds promise for the treatment of many human diseases, including diabetes mellitus (Yechoor & Chan 2005). Ex vivo gene transfer to pancreatic β-cells offers a potential means of preventing β-cell death via the expression of immunoregulatory, cytoprotective, or anti-apoptotic genes (Fernandes et al. 2004, Tran et al. 2004). In addition, the induction of transcriptional regulators and growth factors by gene therapy could stimulate β-cell differentiation and regeneration (Brun et al. 2004, Cozar-Castellano et al. 2004). Among several gene transfer vehicles, adenovirus vectors are attractive because they have the capacity for large DNA inserts, can be produced at high titters and have abilities to transduce non-dividing cells. In addition to potential therapeutic uses, adenovirus vectors are valuable experimental tools for genetic manipulation of islet cells. However, using adenovirus vectors, infection efficiency, defined here as the percentage of cells expressing transgenes, are reportedly variable, ranging from 10 to 80% in human (Giannoukakis et al. 1999, Leibowitz et al. 1999, Barbu et al. 2005, Rao et al. 2005), rat (Becker et al. 1994, Weber et al. 1997, Ishihara et al. 2003, Zhou et al. 2003, Diraison et al. 2004), and mouse (Csete et al. 1995, Bertera et al. 2003, Garcia-Ocana et al. 2003, Leclerc et al. 2004, Diao et al. 2005) islets.

There exist large numbers of genetically modified mice and basic aspects of mouse pancreatic islets have been intensively studied. Therefore, mouse islets are a suitable model for applying adenovirus technology, with the goal of clinical application of ex vivo gene transfer for this endocrine organ. For certain purposes, including transfection of cytoprotective genes for islet transplantation, it is essential that most of the cells express the transgenes. High efficiency is also desirable for studying islet biology and essential for suppressing gene expression in islets with adenovirus-mediated shRNA expression (Bain et al. 2004, Diao et al. 2005). In this study, we first reevaluated the transfection efficiency of recombinant adenoviruses in mouse islets and then explored the usefulness of different strategies, collagenase treatment before infection, and arterial delivery of virus particles in situ. We observed markedly improved infection efficiency with these methods. In particular, arterial delivery combined with a tetracycline-inducible
Adenovirus system induced reliable and controlled expression of foreign genes in mouse islets.

Material and methods

Mouse

C57BL/6 mice, 12–16 weeks of age, were used. All animal experiments were approved by the Tohoku University Institutional Animal Care and Use Committee (#15–110).

Recombinant adenoviruses

AdRIP-HArGlyK expressing hemagglutinin (HA) epitope-tagged rat glycerol kinase under control of the rat insulin 1 promoter was described previously (Takahashi et al. 2006). The cytomegalovirus (CMV) promoter containing the Tet operator sequence (CTO) and the enhanced green fluorescent protein (eGFP) cDNA were excised from pcDNA5/TO (Invitrogen) and pEGFP-1 (BD Biosciences Clontech) respectively, and then ligated. The Tet-repressor cDNA was excised from pcDNA6/TR (Invitrogen) and ligated to the CAG promoter unit (Niwa et al. 1991). The CMV-eGFP expression unit was excised from pEGFP-1. These expression units were cloned into a cosmid vector pAdex1cw (Miyake et al. 1996). These cosmid vectors containing the expression units and adenovirus DNA-terminal protein complex were then co-transfected into HEK293 cells, which were then seeded onto 96-well plates. After 10 days, cytopathic effects were seen in several wells. Recombinant adenoviruses were extracted from 96-well plates and amplified first in HEK293 cells in 24-well plates and then in cells in 3 × 150 mm dishes. Usually, we pick up adenoviruses from 96-well plates in which no more than 20 wells show cytopathic effects. In addition, careful examination of adenovirus DNA structure by enzyme digestion excludes contamination with adenoviruses lacking some portion of their genome. Therefore, we consider the purity of the virus preparation to be similar to that obtained from purified plaques. Amplified adenoviruses were purified with CsCl gradient ultracentrifugation. The resulting viruses were designated AdCAG-TR for the Tet repressor expressing virus, AdCMV-eGFP for the eGFP-expressing virus under the CMV promoter, and AdCTO-eGFP for the eGFP-expressing virus under the CMV promoter with the Tet operator. Virus infectious titers (plaque-forming unit (pfu)) were determined by a previously described method (Miyake et al. 1996). Amounts of virus particles were calculated from OD260 with a formula in which 1 OD260 corresponds to 1·1 × 10^{12} particles/ml (Maizel et al. 1968). Viral particle to pfu ratios of these virus stocks were 20–80. Infectious titer (pfu) is more closely related to viral infectivity and thus used to present the virus amount in this study.

Isolation of mouse islets and infection with recombinant adenoviruses

Islets were isolated by retrograde infusion of 1·3 ml cold Hanks’-balanced salt solution containing 1·0 mg/ml collagenase (Sigma-Aldrich) from the common bile duct and harvested by hand under microscopy. One hundred islets, immediately after isolation or after overnight culture, were placed in 1 ml RPMI media containing 10% fetal bovine serum, 11 mM glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin, to which recombinant adenoviruses were added at an m.o.i. of 300 (assuming 2500 cells per islet; 7·5 × 10^7 pfu of viruses/100 islets per milliliter), and cultured at 37 °C for 2 h. Islets were then washed twice with the media and cultured for 48 h in RPMI media. In some of the experiments, islets cultured overnight were treated with 1 ml Hanks’-balanced salt solution containing 1·0 mg/ml collagenase for 10 min at 37 °C, washed twice with PBS and then infected with recombinant adenoviruses as mentioned earlier.

Arterial delivery of adenovirus vectors

For arterial delivery of virus particles, polyethylene tubes 0·3 mm in diameter were inserted into the celiac artery (CA) and the superior mesenteric artery (SMA) (Fig. 1). Viruses (0·5 to 5 × 10^8 pfu) were diluted in 1·3 ml saline. After clamping the portal vein and the hepatic artery as well as the splenic artery, an approximately 0·9 ml viral solution was injected into the pancreas via the CA and 0·4 ml through the SMA. The pancreas was left at room temperature for 10 min and then 1·3 ml cold Hanks’-balanced salt solution containing 1·0 mg/ml collagenase was infused through the common bile duct. Islets were harvested by hand and cultured for 48 h.

GFP expression and immunocytochemistry of islet sections

Islets were fixed with 4% paraformaldehyde for 15 min at 4 °C, immersed in 5% sucrose/PBS for 30 min and 30% sucrose/PBS for 1 h. Samples were then embedded in TISSU MOUNT (Chiba Medical, Saitama, Japan) and quick-frozen in liquid nitrogen. Islet sections (6 μm) were made using a cryostat and examined for eGFP fluorescence with a Leica fluorescent microscope (DFC350FX). Sections were also stained with anti-insulin and anti-glucagon antibodies (Sigma-Aldrich).

Flow cytometric analysis of GFP expression

The infected islet cells were quantitatively analyzed by flow cytometry (FACS Calibur; BD Bioscience Clontech) on at least 1 × 10^6 cells per sample. Forty-eight hours after infection, islet cells were dispersed by
treatment with 0.25% trypsin per 1 mM EDTA for 5 min at 37 °C. The percentage of eGFP-positive cells (in the M2 range) was determined after compensating for autofluorescence (in the M1 range) using uninfected cells as a negative control.

**Measurement of insulin secretion**

Islets (ten islets per tube) infected with recombinant adenoviruses were incubated over a period of 60 min in 1 ml Krebs–Ringer bicarbonate Hepes buffer (KRBH, 140 mM NaCl, 3.6 mM KCl, 0.5 mM NaH₂PO₄, 0.5 mM MgSO₄, 1.5 mM CaCl₂, 2 mM NaHCO₃, 10 mM Hepes (pH 7.4), 0.25% BSA) containing 2.5 mM glucose, 2.5 mM glucose plus 10 mM glycerol, 15 mM glucose or 20 mM glucose. Experiments were conducted with three to five tubes for each condition. Insulin was detected using RIA kits (LINCO, St Charles, MO, USA).

**Statistical analysis**

Data are presented as mean ± S.E.M. when otherwise stated. Differences between groups were assessed by Student’s *t*-test for paired or unpaired data.

**Results**

**Adenovirus-mediated gene transfer using the conventional method**

We reevaluated the efficiency of adenovirus infection in isolated mouse islets. An optimal titer of adenoviruses was first determined employing AdCMVeGFP expressing the enhanced Green fluorescent protein (eGFP). Analysis using a fluorescence-activated cell sorter (FACS) showed the percentage of islet cells expressing eGFP to be increased, as virus titer rose from multiplicity of infection (m.o.i.) of 100–900 (Fig. 2A). In addition, the expression levels, as evaluated by geometric mean fluorescence, were increased from 46.46 ± 0.54 (m.o.i. = 100), 71.98 ± 5.79 (m.o.i. = 300) to 111.26 ± 6.56 (m.o.i. = 900; arbitrary unit n = 3). However, the infection of islets with AdCMV-eGFP at an m.o.i. of 900 resulted in reduced glucose (20 mM)-stimulated insulin secretion when compared with that in non-infected islets (data not shown), as was noted previously (Rao et al. 2005). In contrast, a more than fourfold increase in glucose-stimulated insulin secretion was observed in both non-infected islets and those infected with AdCMV-eGFP at an m.o.i. of 100 or 300 (data not shown). Therefore, for the subsequent studies, we used AdCMV-eGFP at an m.o.i. of 300.

Many other parameters affect infection efficiency, such as islet size and duration of exposure to the adenovirus. As described previously (Leclerc et al. 2004), we noticed that cells at the centers of islets with a diameter >150 μm were difficult to infect. Therefore, islets with a diameter between approximately 80 and 120 μm were employed for the present study. Very little necrosis of cells at the islet core (central necrosis) was seen in islets with this size range (data not shown). As to the duration of exposure to the adenovirus, we observed that 2-h exposure to the virus at 37 °C resulted in greater numbers of infected cells than 1-h exposure, while no further increases were seen with overnight exposure (data not shown).

Another factor that could affect the infection efficiency is the timing of adenovirus infection. Therefore, we examined whether overnight culture affects the infection efficiency in mouse islets. Islets were infected
Figure 2 Adenovirus-mediated eGFP expression in mouse islets. (A) Mouse islets were infected with AdCMV-eGFP immediately after isolation at an m.o.i. of 100, 300, or 900. Percentages of eGFP-positive cells were analyzed by FACS 48 h after infection. Data are means ± s.d. of three independent experiments. (B–D) Immunostaining of sections of mouse islets infected with AdCMV-eGFP (300 m.o.i.) immediately after isolation. Sections of islets were directly observed for eGFP at low magnification (×100) (B) or stained with anti-insulin (C) or anti-glucagon (D) and observed at high magnification (×400). (E–G) Immunostaining of sections of mouse islets infected with AdCMV-eGFP (300 m.o.i.) after an overnight culture. Sections of islets were directly observed for eGFP at low magnification (×100) (E) or stained with anti-insulin (F) or anti-glucagon (G) and observed at high magnification (×400). Bars, 100 μm in B and E, and 20 μm in C, D, F and G.
with AdCMV-eGFP either immediately after isolation or after an overnight culture. Some eGFP-positive cells were observed in the inner parts of islets when islets were infected immediately after isolation (Fig. 2B–D), while eGFP-positive cells were located almost exclusively in the surface layer of islets when infected after an overnight culture (Fig. 2E–G). Quantitative analysis using a FACS demonstrated that eGFP-positive cell numbers tended to be greater in islets infected immediately after isolation (39.8 ± 2.4%, n = 3) than in those infected after an overnight culture (35.6 ± 1.2%, n = 3), but the difference did not reach statistical significance. Immunostaining with an insulin or glucagon antibody revealed that small percentages of β-cells were infected either immediately after isolation (Fig. 2C) or after an overnight culture (Fig. 2F), while very high percentages of α-cells, nearly 100%, were infected. The latter observation was especially apparent in islets infected immediately after isolation (Fig. 2D and G).

Islets were fragmented or damaged with a few cells detaching from the islet surface immediately after isolation (data not shown). In contrast, islets appeared to have recovered, forming compact spheres, after an overnight culture. We speculated that the somewhat higher infection efficiency in islets immediately after isolation might be related to islet surface damage, which allows virus entry into the inner parts of islets.

Increased efficiency of adenovirus infection after collagenase treatment

To improve the infection efficiency, islets cultured overnight were treated with 1.0 mg/ml collagenase for 10 min at 37 °C before adenovirus infection. Infected islets are then cultured for 48 h. We observed eGFP-positive cells even at the islet center (Fig. 3A–C) and recognized a marked increase in the percentage of eGFP-positive cells; average infection efficiency was recognized a marked increase in the percentage of positive cells even at the islet center (Fig. 3A–C) and islets are then cultured for 48 h. We observed eGFP-positive cell numbers tended to have recovered, forming compact spheres, after an overnight culture. We speculated that the somewhat higher infection efficiency in islets immediately after isolation might be related to islet surface damage, which allows virus entry into the inner parts of islets.

Arterial delivery of recombinant adenovirus increased gene transduction efficiency

Next, to devise a better infection method, we examined gene transduction efficiency with adenovirus delivery to mouse islets in situ through arteries perfusing the pancreas. Arterial delivery of recombinant adenoviruses has been used to infect islets of obese Zucker diabetic fatty rats (Wang et al. 1998). However, neither the details of the method used nor its infection efficiency have been described. Furthermore, arterial delivery has not been applied for mouse islet infection. Therefore, we tested the feasibility of delivering recombinant adenoviruses through both the celiac artery (CA) and the superior mesenteric artery (SMA). The SMA perfuses mainly the ventral portion of the pancreas, while the CA supplies the pancreatic body and tail. With the portal vein and the hepatic artery as well as the splenic artery clamped, 3.4 × 10⁸ plaque forming units (pfu) of AdCMV-eGFP virus were delivered through these arteries: 70% through the CA and 30% through the SMA (Fig. 1). The pancreas was then left at room temperature for 10 min. The islets were next isolated by retrograde collagenase infusion through the common bile duct. More than 90% of islets showed viral infection with this method emitting green fluorescence (Fig. 4A). Importantly, eGFP-positive cells were distributed throughout almost all infected islets, and the intensity of green fluorescence was homogenous (Fig. 4A–C). Furthermore, both β-cells (Fig. 4B) and α-cells (Fig. 4C) were effectively infected. Quantitative assessment of efficiency, determined using FACS, revealed 90.37 ± 2.0% (n = 3) of islet cells to be infected with the adenovirus. The expression levels of eGFP in individual cells were very high as shown by the rightward shift of the peak fluorescence in the FACS analysis (Fig. 4D compared with Fig. 3D). In addition, glucose-stimulated insulin secretion was unaffected by infection with adenovirus delivered through the CA and the SMA, being essentially the same as that in non-infected control islets (Fig. 4E).

We then studied the impact of rat glycerol kinase expression (rGlyK), by the arterial delivery method, on non-treated islets (Fig. 3F, open columns versus closed columns). The decrease in insulin secretion was not attributable to reduced insulin contents, since insulin contents did not differ between these islets (226.3 ± 46.5 ng/islet, n = 3 experiments, for control and infected islets respectively). Considering that the fold-increase in glucose-stimulated insulin secretion and insulin content in infected islets were similar to those in non-infected islets, glucose-sensing, and the associated intracellular signaling were thought to be unaffected, while insulin exocytosis was impaired by adenovirus infection after collagenase treatment.
β-cell-secretory functions. We previously reported that glycerol stimulates insulin secretion from INS-1E cells and rat β-cells overexpressing rGlyK (Takahashi et al. 2006). However, when mouse islets were infected immediately after isolation with AdRIP-HArGlyK expressing rGlyK (Fig. 5A), we detected no glycerol-stimulated insulin secretion (Fig. 5B). We considered this to be due to low efficiency of adenovirus infection into mouse β-cells with the conventional method (Fig. 5A). Therefore, mouse islets were infected with AdRIP-HArGlyK via delivery through the CA and the SMA. When islets were infected with arterially delivered viruses, almost all β-cells expressed HA-GlyK (Fig. 5C). As expected, glycerol (10 mM) evoked insulin secretion from mouse islets infected with AdRIP-HArGlyK delivered through these arteries (Fig. 5D). These data, taken together, indicate that adenovirus administration through the CA and the SMA allows very high infection efficiency without adverse effects on islet-secretory activities.

**Controlled gene expression with tetracycline-inducible adenoviruses**

In comparing the effects of different adenovirus transductions, it is essential to use fixed amounts of adenoviruses, which do not differ between control and experimental groups. However, this is not an easy task. To create comparable conditions when using the arterial delivery method, a tetracycline-inducible system was applied. This system employs an adenovirus expressing the Tet repressor (AdCAG-TR) and another virus with a
promoter containing the Tet operator. AdCTO-eGFP (1.5×10^8 pfu) expressing eGFP under control of the CMV promoter containing the Tet operator was delivered together with AdCAG-TR (0.5×10^8 pfu) into the pancreas through the CA and the SMA. Islets were then isolated, divided into two groups and incubated in media with or without doxycycline (2 mg/ml). Islets treated with doxycycline exhibited strong eGFP expression, while only weak expression in a limited number of cells was observed in islets infected with these viruses but not treated with doxycycline (Fig. 6). These data indicate that the arterial delivery method combined with the tetracycline-inducible system assure efficient and controlled gene expression in islets.

**Discussion**

We studied the effects of different infection methods on adenovirus infection efficiency in mouse islets, including virus delivery through the CA and the SMA. Several factors influence the efficiency of adenovirus infection and cause variable levels of cytotoxicity. Results should always be interpreted with caution, and limited infection efficiency and possible cytotoxicity must be taken into account.

The efficiency of adenovirus infection in pancreatic islets varies among reports. Since not all factors in previous studies were provided, reasons for the different efficiencies are unclear. In this study, we showed that collagenase treatment of islets before exposure to viruses improves the efficiency. High efficiencies in several studies (Becker et al. 1994, Giannoukakis et al. 1999, Ishihara et al. 2003, Zhou et al. 2003, Diao et al. 2005) could be due to surface damage by collagenase treatment during isolation. Necrosis of cells at the islet core (central necrosis) is sometimes observed and could affect infection efficiency (Ilieva et al. 1999, Giuliani et al. 2005). When efficiency is estimated by counting dispersed islet cells expressing transgenes, central necrosis could...
result in an erroneous increase in efficiency. This is because there is a possibility that uninfected necrotic cells located in the islet core were lost during dispersion. Central necrosis occurs more often in relatively larger islets (Ilieva et al. 1999). In this respect, rat or human islets appear to be more susceptible to central necrosis than mouse islets. In mice, as demonstrated in this study using islets 80–120 µm in diameter, central necrosis is not a major problem.

Several methods have been employed to enhance adenoviral transgene expression. Adenoviruses enter cells via the coxsackievirus and adenovirus receptor (CAR). Use of a modified adenovirus that has an Arg-Gly-Asp (RGD) motif in the adenovirus fiber knob markedly improves infection efficiency in nonhuman primate isolated islets (Bilbao et al. 2002). We infected mouse islets with an eGFP-expressing adenovirus containing the RGD motif but observed no improvement in efficiency (R Takahashi, H Ishihara and H Mizuguchi, unpublished observation). Very high infection efficiency in monolayer culture of mouse islet cells suggests sufficient expression levels of CAR in mouse islet cells (Narushima et al. 2004). In addition, several agents were reported to enhance transgene expression in other cell types (Jornot et al. 2002, Huang et al. 2005, Triplett et al. 2005), and these agents may also be useful for pancreatic islet cells.

Figure 5 Impact of efficient gene expression, by arterial delivery of recombinant adenovirus, on insulin secretion. (A) Islets infected with AdRIP-HArGlyK immediately after isolation were cultured for 48 h, dispersed and stained with anti-HA or anti-insulin. Experiments were repeated thrice with essentially similar results. (B) Glucose (Glc; 20 mM) or glycerol (Gly; 10 mM)-stimulated insulin secretion from islets infected with AdRIP-HArGlyK immediately after isolation (closed columns) and non-infected control islets (open columns). The value of insulin secretion from control islets at 2.5 mM glucose (6.30 ± 0.77 ng/h per ten islets, n = 4) was taken as 100%. Data are means ± S.E.M. from four independent experiments. (C) Islets infected with AdRIP-HArGlyK, delivered through the CA and the SMA, were cultured for 48 h, dispersed and stained with anti-HA or anti-insulin. Experiments were repeated twice with essentially similar results. (D) Glucose (20 mM) or glycerol (10 mM)-stimulated insulin secretion from islets infected with arterially delivered AdRIP-HArGlyK (closed columns) and non-infected control islets (open columns). The value of insulin secretion from control islets at 2.5 mM glucose (3.51 ± 0.63 ng/h per ten islets, n = 3) was taken as 100%. Data are means ± S.E.M. from three independent experiments. *P < 0.05.

Figure 6 Doxycycline-inducible eGFP expression via adenoviruses delivered through the CA and the SMA. AdCAG-TR (0.5 × 10⁸ pfu) and AdCTO-eGFP (1.5 × 10⁸ pfu) were injected into the pancreas through the CA and the SMA. Expression of eGFP was induced by treatment with doxycycline (DOX, 2 µg/ml) for 48 h. The pictures shown are representative of three independent experiments. Bars, 20 µm.
Arterial delivery of adenovirus was demonstrated to be highly efficient, although the procedure is not as easy as infection of isolated islets in vitro. Since nearly 100% infection is not possible with conventional infection methods, this method could be useful when nearly 100% infection is desirable, such as gene silencing by expression of short hairpin RNA using adenoviral vectors. It is also likely that this method can be more easily applied to larger animals, including humans. In the present study, more than 90% infection was achieved by arterial delivery of approximately $3 \times 10^8$ (plaque forming units (pfu)) viruses. This is much less than the amount used in Zucker diabetic fatty rats ($1 \times 10^{12}$ pfu; Wang et al. 1998), even after taking into account the differences in animal sizes. The mouse pancreas contains more than 1000 islets, including small islets. On the assumption that average cell number in each islet is 1000 (not 2500 because of the presence of small islets), the administration of $3 \times 10^8$ viruses into a pancreas corresponds to an m.o.i. of 300. In fact, the m.o.i. in our experiments must have been <300, since the virus solution was distributed not only to islets but also to acinar cells. This might explain the lack of adverse effects on insulin secretion in association with virus infection by this method. Although the arterial delivery method is promising and the conditions we used in this study are acceptable in terms of the transfection efficiency, functional impact, and lack of adverse effects, further studies are needed to optimize conditions. In this regard, we found that the delivery of viruses at more than $3 \times 10^8$ pfu resulted in blunted glucose-stimulated insulin secretion and that more than 3 ml adenovirus solution cannot be infused when portal vein, hepatic artery, and splenic artery are clamped.

We also demonstrated combining arterial delivery of the virus with a tetracycline-inducible expression system to enhance the feasibility of the arterial delivery method. This method allows us to use exactly the same amount of adenovirus in the control and experimental groups, avoiding false results due to possible differences in titers of control and experimental viruses. As discussed in an earlier report (Irminger et al. 1996), there is inherent inaccuracy in determining adenovirus titers. The inducible adenovirus vector system is also advantageous since phenotype changes can be analyzed according to the degree of gain or loss of gene expression.

In conclusion, the present data provide new insight into gene transduction using recombinant adenovirus in mouse islets. Our data demonstrate that adenovirus administration through the CA and the SMA yielded very high transduction efficiency in mouse islets. This method could easily be applied to human pancreas removed from donors prior to isolating islets. Several genes have been demonstrated to improve islet transplantation performance (Bertera et al. 2003, García-Ocana et al. 2003, Fernandes et al. 2004). Our data could thus contribute to future studies in β-cell research, and may well be applicable to islet transplantation.

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Arterial delivery of adenoviral vectors


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