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Adenoviral-Mediated Gene Delivery to Liver Isografts: Improved Model of Ex Vivo Gene Transfer

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PREVIOUS studies using adenoviral vectors in experimental liver transplant settings have demonstrated in vivo infectivity by perfusing grafts with viral vectors.[1,2] However, these techniques required high viral titers (1 to 5×10^{10} plaque-forming units [pfu]) to induce effective viral infection. In this study, we developed an improved gene delivery method that allows near maximal infectivity of adenoviral vector to liver grafts by trapping vector perfusate within the graft for the duration of the cold preservation period. Using this technique, we successfully delivered an adenovirus encoding the human inducible nitric oxide synthase (iNOS) gene to cold-preserved liver grafts. Nitric oxide (NO) has been shown to have protective properties,[3] due to its vasoprotective, antiinflammatory and antiapoptotic effects and its activity as a free radical scavenger. We therefore hypothesized that delivery of the iNOS gene to liver grafts will decrease ischemic damage in the early posttransplant period.

METHODS

Plaque-forming units (1×10^9) (multiplicity of infection [MOI] 0.4) of adenovirus encoding the *Lac*Z gene (AdLacZ, GenVec, Rockville, Md) were delivered to cold-preserved rat liver grafts by three different methods: (1) continuous perfusion via the portal vein (portal perfusion); (2) continuous perfusion via both the portal vein and hepatic artery (dual perfusion); and (3) trapping of viral perfusate in the liver vasculature by clamping outflow (clamp technique). Control animals were perfused without viral vector. *Lac*Z gene expression was detected by X-gal staining (expressed as percent positive cells), and by β-galactosidase enzyme assay (expressed as β-gal activity per total protein [U/g]). Using the delivery method providing the highest infectivity, duration of gene expression and associated liver injury were analyzed by killing animals at 1, 2, 7, 14, and 28 days posttransplant (n = 3 at each timepoint). Livers from control and experimental animals were stained with hematoxylin-eosin and evaluated for damage and inflammation.

Adenovirus encoding the human iNOS gene (AdiNOS, GenVec) was delivered to liver grafts using the clamp technique at a titer of 2×10^8 pfu. Animals were killed 48 hours posttransplant. The presence of iNOS mRNA in transplanted livers was analyzed by reverse transcriptase polymerase chain reaction (RT-PCR) and protein production was examined by immunohistochemistry using the polyclonal rabbit antihuman iNOS antibody NO53 (a gift from Merck & Co, Rahway, NJ).

RESULTS

Using 1×10^9 pfu AdLacZ (MOI 0.4), the transduction rate in 3-hour-preserved liver grafts 48 hours posttransplant was greatest with the clamp technique (21.5 ± 2.7% X-gal-positive cells and 81.1 ± 3.6 U/g β-gal activity, $P < 0.01$ vs portal perfusion), followed by dual perfusion (18.5 ± 1.8%, 66.6 ± 19.4 U/g, $P < .01$ vs portal perfusion) and portal perfusion (8.8 ± 2.5%, 19.7 ± 15.4 U/g). Further studies using the clamp technique demonstrated a near maximal gene transfer rate (29.9 ± 5.1%, 109.6 ± 6.9 U/g) at MOI 0.4 with prolonged cold preservation to 18 hours. *Lac*Z expression using the clamp technique was stable for 2 weeks and slowly declined to 7.8 ± 12.1% and 40.1 ± 49.4 U/g at day 28. Lack of an inflammatory response to the viral vector was confirmed by histopathologic examination. Liver enzymes peaked at 12 to 24 hours, but quickly normalized within 3 days posttransplant in both experimental and control animals. Gene transduction was induced selectively in hepatocytes with nearly no extrahepatic transgene expression in the lung and spleen. In AdiNOS-transduced livers, tissues stained with antihuman iNOS antibody demonstrated a transduction rate similar to that seen in AdLacZ-transduced livers. The presence of iNOS mRNA in AdiNOS-transduced livers was confirmed by RT-PCR analysis.

DISCUSSION

In this study, we demonstrated a highly efficient adenoviral gene delivery method to cold-preserved liver grafts that offers maximal infectivity with minimal technical manipulation. In the clamp technique, vector infusion via both the portal vein and hepatic artery allows delivery of viral vector to areas of microcirculation that are inaccessible by single perfusion, and vector-target cell contact is enhanced by trapping of vector in the liver microcirculation, likely accounting for the superior infectivity using this technique. The lack of an inflammatory response in this study may be related to the fact that transduction was limited almost

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exclusively to hepatocytes, with little expression in nonparenchymal cells and extrahepatic tissue. The viral proteins and the transgene may therefore be sequestered from the recipient immune system. The eventual loss of transgene expression is not clear, but may be due to silencing of the CMV promoter.[4] Finally, we have shown initial feasibility of adenoviral-mediated iNOS gene delivery. Studies are underway to investigate the effects of iNOS gene delivery in liver and other solid-organ transplants.

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