Method for Continuous Infusion into the Portal Vein of Mice

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Abstract | Recombinant retroviral vectors are attractive for in vivo gene transfer into the liver because they integrate into the host-cell genome, resulting in permanent gene expression. Gene-transfer efficiency can be improved by increasing the number of retroviral particles delivered to hepatocytes. For this purpose, we report a mouse model for continuous infusion into the portal circulation permitting large-volume vector administration, which will allow marked increase in gene-transfer efficiency. Continuous saline infusion was evaluated, using various parameters, and an infusion rate of 6 ml/24 h was found safe and well tolerated for at least 2 weeks. No significant changes in liver and kidney function and electrolyte balance were observed during the infusion. In addition to providing a valuable method for in vivo hepatic gene therapy, this model has a number of other potential applications, including mouse studies of hepatic tumor therapy, pharmacology, toxicology, and liver biology.

The liver is an important target organ for gene therapy because a number of genetic diseases result from an absence or deficiency of hepatocyte-derived gene products. For in vivo hepatic gene transfer, recombinant retroviral vectors are attractive because of their ability to integrate into the host-cell genome, which results in permanent gene expression (1–8).

Retrovirus-mediated gene transfer requires active cell division of the target cell (9), and vector delivery needs to be synchronized with cell replication (10). A number of methods have been used to induce hepatocyte proliferation in vivo, including 70% hepatectomy, hepatic expression of toxic gene products, and infusion of growth factors (1–3, 7, 8, 11–13).

Several approaches have been used to deliver the vector directly to the liver after induction of liver proliferation. Initial studies used retrovirus injected as a single bolus into the portal vein (5). Recently, it was documented that the number of retroviral particles delivered per hepatocyte (multiplicity of infection, MOI) correlates with the proportion of hepatocytes that are eventually transduced (i.e., gene-transfer efficiency) (14, 15). Thus, one way of improving gene-transfer efficiency would be the use of vectors of higher titer than can presently be obtained (10^6 to 10^8 colony-forming units [CFU]/ml). However, despite recent advances (14, 16), production of high titers remains technically difficult. Alternatively, high numbers of vector particles can be delivered to the liver by increasing the volume of retrovirus that is administered. To this end, a model of a sanguineous perfusion of the liver in situ was reported in mice (17, 18), but it is a technically complicated procedure, the volume that could be perfused was limited to 3 ml, and the perfusion could be performed only once. In contrast, repeated portal vein infusions in mice of up to 1 ml per day were made possible using a method previously developed in our laboratory that involves permanent placement of an indwelling cannula in the portal vein (12, 19).

We hypothesized that continuous vector administration into the portal circulation would further increase the volume that can be administered and thus optimize hepatic gene transfer. Although continuous intravenous infusion in mice has been reported, it did not involve the portal circulation (20–23). Portal vein infusion systems in rats have been described (24–26); however, the mouse model offers important advantages (27). In comparison with rats, mice are useful due to ease of handling and the availability of a multitude of genetic strains and genetically modified lines.

We describe a mouse model of continuous infusion of a large volume of fluids into the portal vein that allows introduction of a high number of viral particles into the liver. In addition, by continuous infusion, it is possible to synchronize vector delivery and DNA replication, when methods are used that induce a synchronous or prolonged hepatocyte proliferation. We recently documented that by using this method with new, safer strategies to induce liver proliferation developed in our laboratory, >30% of hepatocytes could be permanently transduced (1, 2). This gene-transfer efficiency is more than fourfold higher than that previously achieved in mice using retroviral vectors (12). The technical aspects of the continuous infusion system are described in detail, and the safety of large-volume infusion is documented.
Materials and Methods

Animals: Female 5- to 6-week-old C57BL/6 mice were purchased from Taconic Laboratory (Germantown, N.Y.) and treated according to the NIH guidelines and standards of the University of Washington for animal care and use. Blood was obtained from anesthetized mice by use of the retro-orbital technique, then mice were sacrificed by cervical dislocation. Total body and wet organ weights were determined, using an XL-3000 digital scale (Fisher, Santa Clara, Calif.).

Surgical technique: The proximal end of a 5-foot length of gas-sterilized silicone tube (0.02 i.d./0.037 o.d.; VWR, San Francisco, Calif.) was connected to a 5-ml syringe containing sterile saline. The distal end of the tube was then guided through a gas-sterilized mouse tether button assembly (Instech Labs, Plymouth Meeting, Pa.) that was used for protecting the cannula at the exit site. Mice were anesthetized by administration of 0.4 ml of 2,2,2-tribromoethanol (Avertin; Aldrich Chemical Co., Milwaukee, Wis. [20 mg/ml]). The hair over the abdomen and scapular region was shaved, and the skin was cleaned with 70% alcohol.

A midline abdominal incision was made, and a subcutaneous tunnel was created from the left side of the abdomen, using a forceps in craniodorsal direction, to the dorsal neck region. The neck skin was perforated in the midline. The distal end of the tube was then pulled through the previously created tunnel and was inserted into the abdominal cavity via an opening made in the abdominal muscle on the left side. Subsequently the intestines were displaced, and the portal vein was exposed. A gas-sterilized 8-mm-long PE-10 (0.011 i.d./0.024 o.d.) polyethylene tube (Clay Adams, Parsippany, N.J.) was inserted into the distal end of the tube, and the distal tip was sharpened by cutting it at a 45° angle. The tube was flushed, and occasional air bubbles were carefully removed. The tip was then inserted into the portal vein as previously described (19) and fixed in place with a drop of tissue adhesive (Histoacryl Blau; Braun, Melsungen, Germany), which provided hemostasis. Blood flow was tested by pulling the syringe plunger back slightly. The syringe was then mounted on a digital syringe pump with multiple syringe holder (Harvard Apparatus, South Natick, Mass.). To maintain cannula patency, slow infusion with sterile saline was started immediately at a rate of 0.1 ml/h.

The abdomen was closed with 4-0 silk suture. Mice were turned to the prone position for placement of a spring tether at the exit site of the silicone tube. The small exit site in the neck was slightly enlarged in the midline, and a pocket was created to allow subcutaneous attachment of a tether button. The button was anchored with three sutures on the neck muscle followed by skin closure. The tether was then connected to a low-torque swivel (Instech Labs) that was mounted on a laboratory ring stand above an open mouse cage, allowing free rotation of the animal without occlusion of the catheter lumen. The flexible spring tether allowed free movement around the cage.

After completion of the infusion, the mice were disconnected from the infusion system, and the tether was removed under general anesthesia. The cannula was ligated at the exit site, followed by skin closure. Cannula tips remained in situ for over 6 months without detectable complications.

Sham-operated mice underwent identical surgical manipulation as infused mice, except that the portal vein cannula was ligated and left in a previously created subcutaneous pocket.

Tissue analysis: For routine histologic examination, liver specimens were fixed in 10% (vol/vol) formalin, embedded in paraffin, and stained with hematoxylin and eosin.
Biochemical analysis: Serum electrolyte, total bilirubin, alkaline phosphatase, and creatinine values were measured by use of an RX Paramax Analyzer (Dade, Irvine, Calif.).

Alanine transaminase assay: Serum alanine transaminase (ALT) activity was determined, using a colorimetric diagnostic kit (Sigma Chemical Co., St. Louis, Mo.).

Results
Continuous infusion into the portal vein: Mice were cannulated and connected to the infusion system as described previously (Figure 1). The system allowed free rotation and movement around the cage. In experienced hands, the surgical procedure was associated with low mortality (<3%), which occurred within 24 h after surgery, and most likely was related to air emboli caused by small air bubbles that were present in the tubing. When the cannula was properly fixed at the portal vein insertion site and at the exit site in the neck, it remained in place at all times. Blood could be drawn throughout the infusion, in most instances by slightly pulling back the syringe plunger, indicating cannula patency. Collapse of the small-size portal vein likely accounted for the inability to draw blood in some animals.

Safety of large-volume continuous infusion: To determine optimal infusion rate, continuous saline infusion was carried out for 5 days at various infusion rates, ranging from 0.1 to 0.5 ml/h (n = 3 per group). All mice appeared healthy and active throughout the infusion period when the volume was not >0.3 ml/h. Infusion of larger volumes led to ascites and slower respirations, suggesting the presence of portal hypertension and hypervolemia with pulmonary edema. All subsequent infusions were, therefore, performed at the rate of 0.25 ml/h (6 ml/24 h). At this rate, an extended saline infusion of at least 14 days (duration of the experiment) was well tolerated. A 5-day infusion of a similar volume of recombinant retrovirus in culture medium (containing fetal bovine serum) was equally well tolerated.

Gross and histologic examination indicated that all livers were normal after 5 days of continuous infusion of saline or retrovirus at the rate of 6 ml/24 h (Figure 2). The kidneys, heart, lungs, intestines, and spleen were similarly examined, and abnormalities were not apparent (not shown). After 5 days of infusion, mean relative liver weight (proportion of total body weight) was slightly increased (5.6% versus 4.5% in normal untreated mice), whereas mean total body weight remained stable. This may have been caused by infiltrating cells or by osmotic swelling in the liver. However, histologic abnormalities or evidence of infiltration was not observed.

To determine whether the infusion had adverse effects...
on liver cell integrity and function, kidney function, and electrolytes, serum samples were obtained daily during saline infusion and thereafter, and ALT, a sensitive marker for liver injury, and total bilirubin, alkaline phosphatase, creatinine, sodium, potassium, and chloride were measured. The ALT activity was slightly high during the first 36 h of infusion, but was within the reference range during the remaining infusion period (36 to 120 h) (Figure 3A). Sham-operated mice also had slightly high ALT activity. Most likely, the observed low-level hepatic injury was not due to the infusion, but rather to the surgery or anesthesia.

Total bilirubin concentration was within the reference range (Figure 3B). During infusion, alkaline phosphatase values were slightly below normal (Figure 3C), but returned to pre-infusion values rapidly after the end of infusion. Creatinine concentration was within the reference range (Figure 3D). Serum electrolyte concentrations (Figures 3E–G) were not significantly different, compared with values for normal noninjured mice. Taken together, these results indicate that the procedure and a large-volume infusion are well tolerated without appreciable liver and kidney function deficits or electrolyte imbalance.

Discussion

We have described a novel technique for continuous portal vein infusion in mice. This method allows large-volume infusion without relevant side effects related to liver integrity and function, kidney function, and electrolyte balance. Although the mean daily oral intake of fluids of an adult mouse is approximately 3 ml (28), a 6-ml volume infused into the portal vein daily for at least 2 weeks was well tolerated without apparent morbidity.

The continuous infusion model is a valuable method for studies of in vivo retrovirus-mediated gene transfer into the liver. The number of viral particles delivered per hepatocyte could be increased at least 10-fold over previous vector delivery methods (12). In addition, our model allowed synchronization of vector delivery with DNA replication. As a result, we have recently reported that a marked increase in gene-transfer efficiency can be achieved (1, 2).

In addition to gene therapy, the mouse model described here may have a number of other potential applications, including pharmacologic and toxicologic studies, cancer therapy studies in mouse models of hepatic neoplasms (24–26), and metabolism and nutrition research (29). Furthermore, in studies of liver biology, the system described allows more efficient administration into the liver of a variety of growth factors and cytokines that have short intravascular half-lives (30, 31).

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References

Continuous Intraportal Infusion in Mice


