

Site-specific kidney-targeted plasmid DNA transfer using nonviral techniques

Review Article

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Abbreviations: hemagglutinating virus of Japan, (HVJ); anionic artificial viral envelope, (AVE); chloramphenicol acetyltransferase, (CAT); Epstein-Barr virus, (EBV); EBV nuclear antigen-1, (EBNA-1); transforming growth factor, (TGF)- β 1; platelet-derived growth factor, (PDGF)-B; N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride, (DOTMA); cytomegalovirus, (CMV); dioleoylphosphatidyl ethanolamine, (DOPE); 2,3-dioleoyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate, (DOSPA), 3-[N-(N,N-dimethylaminoethane)carbonyl] cholesterol, (DAC-Chol); 3-dimethyl-hydroxyethylammonium bromide, (DMRIE); Polyethyleneimine, (PEI); heat shock protein, (HSP); peritubular capillaries, (PTC); erythropoietin, (Epo); carbonic anhydrase, (CA); liposome-polycation-DNA complexes, (LPD)

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Summary

Kidney-targeted gene transfer has the potential to be one of the most important tools for broadening our understanding of renal disease processes and for revolutionizing the treatment of renal diseases. We reviewed the literature on kidney-targeted plasmid DNA transfer using nonviral techniques: naked plasmid DNA, cationic lipid/DNA complex, hemagglutinating virus of Japan (HVJ)-liposome complex, anionic artificial viral envelope-type HVJ-liposome complex, cationic polymer/DNA complex, electroporation, ultrasound-microbubble, and hydrodynamics-based transfection. Gene-transfer methods using nonviral techniques are administered via renal arterial, renal venous, pelvic, ureteric, or direct routes into the glomerulus, tubules, or interstitial fibroblasts. Gene transfer can be achieved with varying degrees of transfection efficiency and duration of gene expression. Thus, we can select the most effective gene transfer technique to deliver the appropriate therapeutic gene to the particular sites involved in various renal diseases

I. Introduction

We can access the kidney by four different local routes: the renal artery, anterogradely; the renal vein, retrogradely; the urinary tract (ureter or pelvis), retrogradely; and by direct injection.

Viruses (adenovirus, adeno-associated virus, retrovirus and others) are used as a vector to transfer exogenous genes into kidneys. Although these vectors can achieve high gene transfer efficiency, it is still possible that the protein encoded by viral genes induce immunological responses. On the other hand, nonviral techniques have several advantages over transfer using viral vectors. A large quantity of highly purified plasmid

DNA is easily and inexpensively obtained. Because there are fewer size constraints than with current viral vectors, plasmid vectors can carry larger genes. Plasmid vectors are less likelihood of inducing an immunological response than with viral vectors.

The local route to the kidney and the kind of nonviral techniques used principally determine the transgene expression sites (Tomita et al, 1992; Isaka et al, 1993; Akami et al, 1994; Arai et al, 1995; Boletta et al, 1997; Lai et al, 1997, 1998; Tsujie et al, 2000; Foglieni et al, 2000; Tsujie et al, 2001a, 2001b; Maruyama et al, 2002a; Lan et al, 2003; Kita et al, 2003). Tubules have been targeted by the injection of naked plasmid DNA into the intra-renal parenchyma (Lai et al, 1997), cationic lipid/DNA complex

via the pelvic route (Lai et al, 1997, 1998) or renal artery (Lai et al, 1997), anionic artificial viral envelope (AVE)-type hemagglutinating virus of Japan (HVJ)-liposome complex via the renal artery (Kita et al, 2003), or cationic polymer/DNA complex via the renal artery (Boletta et al, 1997; Foglieni et al, 2000), or by ultrasound-Optison-mediated naked plasmid DNA transfer via the renal artery (Lan et al, 2003).

The glomerulus has been targeted by delivering an HVJ-liposome complex via the renal artery (Tomita et al, 1992; Isaka et al, 1993; Akami et al, 1994; Arai et al, 1995), an AVE-type HVJ-liposome complex via the renal artery (Tsujiie et al, 2001a), by electroporation-mediated naked plasmid DNA transfer via the renal artery (Tsujiie et al, 2001b), and by ultrasound-Optison-mediated naked plasmid DNA transfer via the renal artery (Lan et al, 2003).

Interstitial fibroblasts have been targeted by injecting an AVE-type HVJ-liposome complex retrogradely via the ureter (Tsujiie et al, 2000), by the retrograde injection of naked plasmid DNA via the renal vein using hydrodynamics-based transfection (Maruyama et al, 2002a), or by ultrasound-Optison-mediated naked plasmid DNA transfer via the renal artery (Lan et al, 2003).

Most nonviral techniques of kidney-targeted gene transfer have resulted in transient gene expression for relatively short periods of time: the HVJ-liposome (Tomita et al, 1992; Isaka et al, 1993; Arai et al, 1995), electroporation-mediated naked plasmid DNA transfer (Tsujiie et al, 2001b), and ultrasound-Optison-mediated naked plasmid DNA transfer (Lan et al, 2003) methods have resulted in expression for 1 week or less. The cationic polymer/DNA complex (Boletta et al, 1997) and AVE-type HVJ-liposome delivered retrogradely via the ureter (Tsujiie et al, 2000) have resulted in 2 weeks of expression. Genes delivered by cationic lipid/DNA complex were expressed for 5 weeks (Lai et al, 1997, 1998), and AVE-type HVJ-liposome complex delivered via the renal artery for 8 weeks (Tsujiie et al, 2001a). Recently, the technique of retrograde injection via the renal vein has achieved long-term gene expression, lasting more than 24 weeks (Maruyama et al, 2002a).

Thus, the recent history of kidney-targeted plasmid DNA transfer shows the rapid development of nonviral techniques for use in gene therapy. In this article, we review kidney-targeted plasmid DNA transfer using nonviral techniques via several routes into different sites, their transfection efficiency, preclinical studies using these methods, safety concerns, and future clinical applications.

II. Kidney-targeted gene transfer via the renal artery

Table 1 provides an overview of studies that have used kidney-targeted plasmid DNA transfer via the renal artery.

A. Liposomes

1 HVJ-liposome

Tomita et al, (1992) first reported that the HVJ-

liposome is efficient for glomerulus-targeted gene transfer. Its expression is transient. Four days after the injection of HVJ-liposome, SV40 large T antigen is detected immunohistochemically in 15% of the glomerular cells in the kidney. Isaka et al, (1993) demonstrated a 35% transfection efficiency of the glomerulus with plasmids carrying the gene for chloramphenicol acetyltransferase (CAT), transforming growth factor (TGF)- β , and platelet-derived growth factor (PDGF)-B genes, driven by the chicken β -actin promoter. The blood urea nitrogen and plasma creatinine concentrations are not changed significantly by this procedure (Tomita et al, 1992; Isaka et al, 1993). Proteinuria is not observed in the rats treated with the HVJ-liposome method (Tomita et al, 1992; Isaka et al, 1993). Isaka et al (1993) demonstrated that the introduction of either the TGF- β 1 or PDGF-B gene into the kidney induces glomerulosclerosis. Akami et al, (1994) reported the HVJ-liposome mediated gene transfer of a human CD59 expression plasmid, pSR CD59, into the canine kidney. Human CD59 was detected immunohistochemically in the canine glomerular cells for 7 days with a maximum on day 2. Yamada et al (1995) identified a silencer in the mouse renin gene promoter region by transferring renin gene promoter-CAT fusion constructs into the kidney using the HVJ-liposome technique. Arai et al, (1995) demonstrated human renin expression in approximately 30% of the glomeruli on day 3 after the introduction of the human renin and angiotensinogen genes. In addition, on day 7 after the transfection of the renin and angiotensinogen genes, the extracellular matrix had expanded in the glomeruli and α -smooth muscle was expressed in the mesangial cells. These findings suggest that the locally activated renin angiotensin system induces glomerular sclerosis and a phenotypic change in mesangial cells.

The mechanism of the HVJ-liposome technique that results in glomerulus-specific gene expression is unknown. A possible explanation is that the intrarenal hydrodynamic state facilitates the contact of HVJ-liposomes with glomerular cell membranes, and another is that the phagocytotic activity of glomerular mesangial cells facilitates the uptake of HVJ-liposomes (Tomita et al, 1992).

3. Cationic lipid/DNA complexes

Cationic lipid/DNA complexes have been used for kidney-targeted gene transfer via the renal artery.

3.1 Lipofectin

Lipofectin (Invitrogen, San Diego, CA) contains two lipid species, a cationic lipid, N[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), and a neutral phospholipid, dioleoylphosphatidyl ethanolamine (DOPE). After intrarenal-arterial injection of pCMV-lacZ-Lipofectin, lacZ was expressed mainly in the cytoplasm of renal tubular cells in the outer medulla with some staining in the cortex (Lai et al, 1997). It is not clear why there was no transgene expression in the inner medulla. The authors speculate that the high salt and urea content in the inner medulla might have affected the entry of the plasmid DNA-Lipofectin complex.

Table 1: Gene transfer via renal artery

Vector, driving force	Plasmid	Transgene	Animal	Targeted site	Observed length of expression	Incubation	Reference
<i>in vivo</i>							
HVJ	pAct	SV40 large T antigen	6-week-old male Wistar rat	Glomeruli	7 days		Tomita et al. 1992
HVJ	pAct	CAT	8-week-old SD rat	Glomeruli	7 days		Isaka et al. 1993
HVJ	pAct	Human TGF- β 1			7 days		
HVJ	pAct	Human PDGF-B			7 days		
HVJ	pSR α	Human CD59	Female canine	Glomeruli	7 days		Akami et al. 1994
HVJ	pUtkCAT	Renin (Ren1 ^d , Ren2 ^d)	40-day-old male DBA/2J mouse	Kidney	3 days		Yamada et al. 1995
HVJ	pCAGGS	Human renin	8-week-old SD rat	Glomeruli	3 days		Arai et al. 1995
HVJ	pAct	Human angiotensinogen					
Lipofectin	pCMV	β -gal	Normal male mouse (25-30 g)	Tubules	35 days	5 min	Lai et al. 1997
Naked	pCMV	β -gal		No expression			
DOGS	pCMV	<i>P. pyralis</i> Luc	8-week-old male SD rat	No expression			Boletta et al. 1997
AVE-type HVJ	pEBAct pAct	β -gal Luc Luc	6-week-old male SD rat	Glomeruli	4 days 56 days 7 days	10 min 10 min 10 min	Tsujie et al. 2001a
Lipofectamine DAC-Chol DMRIE	pBAG, pCMV pBAG, pCMV pBAG	β -gal, Human renin _{FURIN}	Male SD rat (250-350 g)	No expression		None or 10 min	Madry et al. 2001
25-kD PEI	pCMV	β -gal	8-week-old male SD rat	Proximal tubules	2 days 14 days	10 min 10 min	Boletta et al. 1997
Naked	pCMV	<i>P. pyralis</i> Luc β -gal		No expression			
25-kD PEI	pCMV	β -galNuc	8-week-old male SD rat	Proximal tubules	2 days	10 min	Foglieni et al. 2000
Electroporation	pCAGGS pEBAct pcDNA3 pcDNA3	β -gal Luc oriP, Luc EBNA-1, oriP, Luc	6-week-old male SD rat	Glomeruli	4 days 4 days 4 days 4 days		Tsujie et al. 2001b
Ultrasound-microbubble	pTRE pEFpurop-Tet-on	Mouse m2Smad7	Male SD rat UUO model (220-250 g)	Glomeruli, tubules, interstitium	7 days		Lan et al. 2003
<i>ex vivo</i>							
AVE-type HVJ	pRL-CMV pcDNA3 pCAGGS	<i>Renilla</i> Luc Human HSP70 Human bcl-2	Inbred male Lew (RT1 ^h) rat (250-300 g)	Tubules Tubules	2 days 2 days 2 days		Kita et al. 2003

HVJ, HVJ liposome; pAct, gene expression vector driven by the chicken β -actin promoter (Fregien and Davidson, 1986); CAT, chloramphenicol acetyltransferase; TGF- β 1, transforming growth factor- β 1; PDGF-B, platelet-derived growth factor-B; SD rat, Sprague-Dawley rat; pSR α , cDNA expression vector composed of the simian virus 40 early promoter and the R-U5' segment of human T-cell leukemia virus type 1 long terminal repeat (Tatebe et al, 1988); pUtkCAT, mammalian expression vector encoding CAT under the control of a truncated herpes simplex virus thymidine kinase (tk) promoter; pCAGGS, gene expression vector driven by the CAG (cytomegalovirus(CMV) immediate-early enhancer/chicken β -actin hybrid) promoter (Niwa et al, 1991); Lipofectin (Invitrogen), contains two lipid species, a cationic lipid, N[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride (DOTMA), and a neutral phospholipid, dioleoylphosphatidyl ethanolamine (DOPE); pCMV, gene expression vector driven by CMV promoter; β -gal, *Escherichia coli* β -galactosidase; DOGS, dioctadecylamidoglycyl spermine; *P. pyralis* Luc, firefly *Photinus pyralis* Luc; AVE-type HVJ, artificial viral envelope-type HVJ liposome; pEBAct, the EBV replication vector, which was constructed by cloning the Epstein-Barr virus nuclear antigen-1 (EBNA-1) and *oriP* sequences in the same plasmid; Luc, luciferase; Lipofectamine (Invitrogen), contains two lipid species, a cationic lipid, 2,3-dioleoyloxy-N-[2(spermincarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA), and a neutral phospholipid DOTMA; DAC-Chol, composed of 3 -[N-(N,N-dimethylaminoethane)carbamoyl] cholesterol and DOPE; DMRIE, 3-dimethyl-hydroxy-ethylammonium bromide; pBAG, β -gal-transducing vector driven by Moloney murine leukemia virus promoter (Price et al, 1987); Renin_{FURIN}, the human renin cDNA fragment is modified by insertion of a furin cleavage site at the end of the renin prosegment, rendering renin active following furin cleavage; 25-kD PEI, branched 25-kD cationic polymer polyethylenimine; β -galNuc, lacZ gene equipped with a nuclear localization signal; pcDNA3 (Invitrogen), expression vector driven by the cytomegalovirusimmediate-early enhancer/promoter; pTRE (Clontech), tetracycline-inducible vector driven by tetracyclin response element/minCMV promoter (CMV promoter deleted enhancer; pEFpurop-Tet-on (Clontech), improved pTet-on vector driven by the CMV promoter; m2Smad7, Smad7 cDNA with a flag tag (m2) at its NH₂ terminus; UUO model, unilateral ureteral obstruction model; pRL-CMV (Promega), *Renilla* luciferase expression vector driven by the cytomegalovirusimmediate-early enhancer/promoter; HSP70, heat shock protein 70. Note that in cases where no incubation time is given, this information was not reported in the cited study.

It is also unclear how the plasmid DNA-Lipofectin complex entered the tubular cells from the vasculature. The basement membranes do not preclude the transfection by plasmid DNA-Lipofectin of tubular epithelial cells via the vascular route (Lai et al, 1997). The major limitation of the liposomes tested so far for efficient *in vivo* gene transfer is their toxicity upon intrarenal arterial administration.

Lai et al, (1997) reported severe ischemic changes with necrosis and fibrosis at the time of mouse kidney harvest after the intrarenal arterial injection of Lipofectin liposomes. Thus, the high incidence of renal ischemic injury reported by Lai et al, (1997) indicates that there are serious safety concerns with this technique.

3.2. Others

Madry et al, (2001) reported that neither Lipofectamine (Invitrogen), which contains two lipid species, a cationic lipid, 2,3-dioleoyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA), and a neutral phospholipid, DOTMA, nor DAC-Chol liposomes, composed of 3-[*N,N,N*-dimethylaminoethane] carbamoyl] cholesterol (DAC-Chol), nor DOPE could deliver β -galactosidase or human renin_{FURIN} to the kidney. Cationic liposome-mediated gene transfer via the renal artery was accompanied by nephrotoxicity and did not result in marker gene expression (Madry et al, 2001). Neither the monocationic lipid DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N,N,N*-trimethylammonium methyl sulphate) (Boletta et al, 1997; Foglieni et al, 2000) nor the polycationic lipid dioctadecylamidoglycyl spermine (DOGS, Transfectam, Promega, Madison, WI) (Boletta et al, 1997) was effective for kidney-targeted gene transfer *in vivo*. When 3-dimethyl-hydroxy-ethylammonium bromide (DMRIE) liposomes or Lipofectamine liposomes were used, the treated kidneys showed pericapsular hematomas, hemorrhages, and necrotic areas consistent with infarction (Madry et al, 2001).

B. Cationic polymer/DNA complex

Cationic polymer/DNA complexes have been used as nonviral vectors for gene transfer.

1. 25-kD Polyethyleneimine (PEI)

Boletta et al, (1997) demonstrated that efficient reporter gene expression was achieved by injecting DNA complexed to the branched 25-kD cationic polymer polyethylenimine (PEI 25k) into the kidney. The product of the reporter gene, β -galactosidase, was localized almost exclusively to the proximal tubules. The transfection efficiency of the branched PEI 25k was significantly higher than that of the linear form of PEI 22k, PEI 800k. A critical parameter in nonviral DNA delivery is the overall charge of the DNA-containing particles. Whereas a net positive charge is desired for particle interaction with the cellular plasma membrane and its entry into the cell, an excessive positive charge might favor entrapment of the DNA complexes by the extracellular matrix (Boletta et al,

1997).

To investigate whether the specific transfection of the proximal tubules involves glomerular filtration of the DNA-containing particles, Foglieni et al (2000) prepared fluorescent PEI 25k polyplexes containing fluoresceinated poly-L-lysine (mean diameter, 93 nm). This allowed visualization of the route of the particles into the kidney. Foglieni et al (2000) demonstrated that polyplexes that can transfect proximal tubular cells have access to these cells through glomerular filtration. Conversely, fluorescent lipoplexes containing the cationic lipid DOTAP (mean diameter, 160 nm) were never observed in tubular cells. The size of the transfecting particles is a key parameter in this process, as expected by the constraints imposed by the glomerular filtration barrier. To date, PEI 25k has been the most useful polymer for kidney-targeted gene transfer. Cationic polymer/DNA complexes injected via the left renal artery are not confined to the left kidney and are also entrapped by other organs (Boletta et al, 1997).

C. Electroporation

Tsujie et al, (2001b) first demonstrated that *in vivo* electroporation provides an efficient approach for glomerulus-targeted gene transfer. Expression vectors in 0.5 ml of balanced salt solution were injected into the left renal artery via a catheter in a one-shot manner and the left renal vein was clamped immediately after the injection. The left kidney was then sandwiched between a pair of oval-shaped tweezer-type electrodes and electroporated. Four days after the transfection of pCAGGS-lacZ, β -galactosidase expression was observed in 75% of glomeruli from the injected kidney. Thus, *in vivo* electroporation with intrarenal-arterial DNA injection was more effective than the HVJ-liposome method.

For this study, Tsujie et al, (2001b) used a luciferase expression plasmid, pEBAct-Luc, and transferred it by electroporation with 25, 50, 75, or 100 V. Luciferase activities in glomeruli did not change significantly at voltages of 25 to 100 V. The authors observed few harmful effects on the treated kidneys, except for small burns on the surface of the kidney in contact with electrodes. No histologic damage was seen in the glomeruli or tubular epithelial cells. A possible explanation of the glomerulus-specific gene expression is that, as the glomerular capillaries have abundant fenestrations, a large volume of DNA solution could also enter the mesangial area through them (Tsujie et al, 2001b). Electroporation-mediated gene transfer can deliver naked plasmid DNA into the entire area in contact with the DNA within the electric field. Therefore, the exact mechanism of the glomerulus-specific gene expression requires further study. We speculate that a hydrodynamics-based transfection by the injection of a large volume of DNA solution in a one-shot manner could achieve the desired gene transfer.

D. Ultrasound-microbubble (Optison)

Lan et al, (2003) first demonstrated that the ultrasound-mediated disruption of gas-filled microbubbles

could be used effectively to transfer naked plasmid DNA into the kidney. The mechanism by which ultrasound microbubbles enhance transgene expression in all cell types within the kidney may largely be attributed to ultrasound-mediated microbubble cavitation. It is possible that the cavitation may largely increase the permeability of capillary and tubular basement membranes, which allows the locally released DNA to cross through these basement membranes and enter cells, including glomerular, interstitial, and tubular epithelial cells.

The ultrasound-Optison-mediated m2Smad7/Tet-on plasmid transfer resulted in transgene expression in more than 90% of glomerular, tubular, and interstitial cells. Gene transfer of inducible Smad7 using the ultrasound-microbubble system inhibited renal fibrosis in the rat unilateral ureteral obstruction model. The ultrasound treatment did not cause any abnormal histologic or functional changes, as evidenced by normal urinary protein excretion, normal glomerular and tubulointerstitial morphology, and the lack of cellular and interstitial edema and of local inflammation.

E. Naked plasmid DNA

It has not been possible to express transgenes in the kidney by injecting naked plasmid DNA via the renal artery (Lai et al, 1997, Boletta et al, 1997). Lai et al (1997) and Boletta et al (1997) performed intrarenal arterial injection of naked plasmid DNA while the renal arterial blood flow was interrupted but the renal venous blood flow was not. The hydrodynamics-based transfection mechanism did not work in either of these studies.

F. AVE-type HVJ-liposome *ex vivo*

The AVE-type HVJ-liposome method effectively induced the heat shock protein (HSP) 70 or the bcl-2 gene in kidney grafts. This was effective even when the HVJ-liposome vector was mixed with a cold-preservation solution (Ringer's lactated or University of Wisconsin solution) and infused into the renal artery just prior to storage of the kidney at 4°C for 24 to 48 h in the same preservation solution (Kita et al, 2003). The induction of the HSP70 or bcl-2 gene reduced the occurrence of primary non-function of grafted rat kidneys after long-term preservation. The transgene expression in the kidney was limited to the tubules. In contrast, Tsujie et al (2001a), as described above, observed expression exclusively in the glomeruli when they introduced plasmid DNA using the AVE-type HVJ-liposome by renal arterial injection. The discrepancy between the two studies may be due to the difference in plasmid vectors used, or for other, as yet unknown, reasons.

III. Kidney-targeted gene transfer via renal vein

Table 2 gives an overview of studies that have used kidney-targeted naked plasmid DNA transfer via the renal vein. Kidney-targeted gene transfer via the renal vein can

be achieved exclusively by hydrodynamics-based transfection

A. Hydrodynamics-based transfection

Maruyama et al, (2002a) have developed a technique for transferring naked plasmid DNA into the kidney of normal rats by hydrodynamics-based transfection by retrograde injection of the DNA into the renal vein. When this technique was performed using a lacZ expression plasmid as the reporter gene, lacZ expression was detected exclusively in the interstitial fibroblasts near the peritubular capillaries (PTC) of the injected kidney, as assessed by immunoelectron microscopic analysis. No nephrotoxicity attributable to gene transfer was apparent either by histological or functional examinations of the injected kidney. These authors also used this method to transfect rat kidney with erythropoietin (Epo) gene. Maximal Epo expression was obtained when the vector, pCAGGS-Epo, was injected in Ringer's solution within 5 sec, and with a volume of 1.0 ml. The transgene-derived Epo mRNA was detected by RT-PCR only in the targeted kidneys and without aberrant expression in nontarget organs. After an injection of 100 µg of pCAGGS-Epo, the serum Epo levels peaked at 208.3 ± 71.8 mU/ml at week 5, and gradually decreased to 116.2 ± 38.7 mU/ml at week 24. Transgene-derived Epo secretion resulted in significant erythropoiesis. The preparation of naked plasmid DNA is simple, compared with the preparation involved for other nonviral techniques. Moreover, the most long-term stable gene expression in the kidney has been obtained using this technique.

The mechanism underlying the transfer of the naked plasmid DNA into fibroblasts is unclear. The negatively charged (Dworkin et al, 2000) PTC could be refractory to the transfer of negatively charged naked plasmid DNA into the endothelium.

However, the PTC wall consists of an extremely thin endothelium, and fifty percent of the PTC endothelium is fenestrated, and thus highly permeable to water and small solutes (Lemley and Kriz, 1994). No incubation time is required for this technique; therefore, hydrostatic pressure (Liu et al, 1999) may be the mechanism underlying the transfer of the naked plasmid DNA. Efficient expression may depend on the elevated intravascular hydrostatic pressure caused by the rapid injection of a sufficient volume of fluid, which leads to the transfer of the naked plasmid DNA through the PTC endothelium, despite the negative charge. The sharp vascular resistance gradient between the efferent arteriole and the PTC (Lemley and Kriz, 1994) probably plays a major role in the blockade of the retrograde stream of injected naked plasmid DNA solution and in the dilation of the PTC, which are the most expandable sites in this route, resulting in transgene expression in the fibroblasts. Moreover, these cells may have the ability to take up the naked plasmid DNA.

Recently, Shimizu et al, (2003) demonstrated that the transfer of a kidney-targeted naked plasmid encoding 7ND (anti-monocyte chemoattractant protein-1) into the kidney interstitial cells using the technique of retrograde injection into the renal vein (Maruyama et al 2002a) attenuates the

Table 2. Gene transfer via renal vein

Driving force	Plasmid	Transgene	Animal	Targeted site	Observed length of expression	Incubation	Reference
Hydrodynamics-based transfection	pCAGGS	β -gal	8-week-old male Wistar rat	Interstitial fibroblasts	1 day	None	Maruyama et al. 2002
	pCAGGS	Epo			24 weeks	None	
Hydrodynamics-based transfection	pcDNA3	Anti-MCP-1 (7ND)	Male Lewis rat (200 g)	Interstitial cells	21 days	None	Shimizu et al. 2003

Epo, erythropoietin; MCP-1, Anti-monocyte chemoattractant

tubulointerstitial renal injury induced by protein-overload proteinuria.

IV. Kidney-targeted gene transfer via the urinary tract

Table 3 provides an overview of studies of kidney-targeted plasmid DNA transfer in a retrograde manner via the urinary tract. Two kinds of liposomes have been used in retrograde gene transfer via the pelvis or ureter. Using them, investigators can target tubules and other interstitial fibroblasts.

A. Lipofectin via the pelvis

After the intrarenal pelvic injection of pCMV- β -gal-Lipofectin, lacZ was expressed mainly in the cytoplasm of renal tubular cells in the outer medulla with some staining in the cortex (Lai et al, 1997). The transgene expression site accessed by this route was similar to that accessed by the intrarenal arterial route, as described above. Lai et al (1997) speculated that the difference in the microenvironments of the inner and outer medulla might affect the interactions between the DNA and the liposome, therefore preventing gene uptake by the papillary cells, and resulting in a lack of staining in the inner medulla. Alternatively, the transgene taken up by the papillary cells may not have been expressed properly, owing to factors such as limited promoter efficiency and specificity, early degradation of the DNA, and altered half-life of the mRNA and/or protein synthesized from transgene. Intrarenal pelvic injection is more feasible than intra-renal-arterial injection in mice, because it is difficult to inject an adequate amount of plasmid DNA-Lipofectin complex into the renal artery, and because intrarenal-arterial injection has a high incidence of renal ischemic injury (Lai et al, 1997).

Lai et al, (1998) also demonstrated a transient physiological effect of gene delivery into the kidney by the retrograde injection of a cationic liposome complexed with a carbonic anhydrase (CA) II chimeric gene, pCMV-CAII-Lipofectin into the renal pelvis of CAII-deficient mice. The CAII-deficiency was produced by introducing a point mutation into the CAII gene, and it manifested as renal tubular acidosis. The delivery of the CAII gene corrected the renal tubular acidosis of the CAII-deficient mouse model. Immunohistochemistry studies using anti-CAII

antibodies showed that CAII was expressed in the tubular cells of the outer medulla and corticomedullary junction. The gene therapy was not associated with nephrotoxicity, as assessed by evaluating the blood urea nitrogen levels and the renal histology. This is the first successful gene therapy of a genetic renal disease.

B. Naked plasmid DNA via pelvis

It has not been possible to express transgenes in the kidney by injecting naked plasmid DNA via the pelvis (Lai et al, 1997).

C. AVE-type HVJ-liposome via

To examine the pattern of transgene-expressing cells, Tsujie et al (2000) used the N-lacF fragment containing the SV40 nuclear transport signal at the N terminus of the lacZ gene, which limits the β -galactosidase protein localization to the nucleus of transfected cells and can be distinguished from endogenous β -galactosidase. pEBActN-lacF was introduced into the kidney of normal rats retrogradely via the ureter using the AVE-type HVJ-liposome method (Tsujie et al, 2000). Nuclear β -galactosidase activity was observed in interstitial fibroblasts for 2 weeks. After the introduction of the pEBActN-lacF expression vector into kidneys with an obstructed ureter, Tsujie et al, (2000) observed a greatly expanded interstitium with marked cell proliferation on days 7 and 14. In contrast, no pathological changes nor increased numbers of infiltrated cells were observed in the treated kidneys 1 day after the transfection, suggesting that there was no interstitial damage from the HVJ-liposome solution, but that the damage was caused by ureteral obstruction. It has been speculated that the AVE-type HVJ-liposome may reach interstitial fibroblasts by slipping between either tubular epithelial cells or papilla epithelial cells; gene transfer could then occur via the fusion activity of the HVJ-liposome.

Gene transfer targeting interstitial fibroblasts might be explained by the possibility that glycol-type sialic acids, the receptors for HVJ, are abundant in fibroblasts but rare in endothelial or epithelial cells (Tsujie et al, 2000). However, the precise mechanism remains to be elucidated.

Table 3 Gene transfer via urinary tract

Vector	Plasmid	Transgene	Animal	Targeted site	Observed length of expression	Incubation	Reference
Pelvis							
Lipofectin	pCMV	β -gal	Normal male mouse (25-30 g)	Tubules	35 days	5 min	Lai et al. 1997
Naked	pCMV	β -gal		No expression		5 min	Lai et al. 1997
Lipofectin	pCMV	Human CAII	CAII-deficient mouse	Tubules	32 days	5 min	Lai et al. 1998
Ureter							
AVE-type	HVI pEBAct	Nlac F	6-week-old male SD rat	Interstitial fibroblasts	14 days	10 min	Tsuje et al. 2000

CAII, carbonic anhydrase II; Nlac F, containing nuclear transport signal, which resulted in the specific and exclusive nuclear localization of the protein, was inserted into the plasmid at the N terminus of the LacZ gene

V. Direct injection

Intrarenal parenchymal injection of a plasmid DNA-Lipofectin complex did not result in gene transfer into the kidney (Lai et al, 1997). Direct injection caused focal infiltration of inflammatory cells. On the other hand, the intrarenal parenchymal injection of naked plasmid DNA led to gene expression in the tubules (Lai et al, 1997). The gene expression was limited to small areas near or deeper than the injection site. The transfection efficiency of naked plasmid DNA via intrarenal parenchymal injection is much less than that of liposome-mediated plasmid DNA transfer via intrarenal pelvic or intrarenal arterial injections.

VI. Systemic

Although the kidney can be targeted by a systemic intravenous route (tail vein injection), the gene transfer efficiency is not sufficient for the specific transfection of the kidney. The plasmid DNA:cationic liposome (DOTMA:DOPE) complex becomes trapped in the pulmonary tissue and vascular endothelial cells (Zhu et al, 1993), resulting in the highest expression in the lungs. Compared with plasmid DNA:cationic liposome 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) complexes, liposome-polycation-DNA complexes (LPD), such as DOTAP-protamine-plasmid DNA complexes, offer better protection of the plasmid DNA from enzymatic digestion and give higher gene expression in the mouse kidney following tail vein injection (Li and Huang, 1997). When Li and Huang (1997) used a luciferase reporter gene with this technique, they found gene expression in all the tissues examined, including lungs, heart, spleen, liver, and kidney, with the highest expression in the lungs.

These organs can also be transfected by hydrodynamics-based naked plasmid DNA transfection via tail vein injection. In this case, the highest level of transgene expression observed is in the liver (Liu et al, 1999; Zhang et al, 1999; Maruyama et al, 2002b; Higuchi et al, 2003).

Thus, the transgene expression that results from the systemic injection of a gene is not confined to the kidney.

VII. Future clinical applications

Although the precise mechanisms of the gene transfer into cells induced by the nonviral techniques has not yet been clarified, recent progress in kidney-targeted gene transfer is promising for future clinical applications. High transfection efficiency and long-term transgene expression are required for clinical applications. Nephrotoxicity attributable to gene transfer is obviously undesirable. An ischemic period is caused by the interruption of renal blood flow during the injection and the following incubation time, in the case of some techniques. The shortest ischemic duration possible is desirable because of safety concerns. For clinical uses, the nonviral techniques should confine transgene expression to the injected kidney, without aberrant expression in nontarget organs. Urinary tract infection attributable to gene transfer via the retrograde urinary tract route is undesirable.

A. *In vivo* catheter-mediated gene therapy

Putting the catheter technique to practical use, the above-mentioned gene transfer techniques via three different routes, renal artery anterogradely, renal vein retrogradely, the urinary tract (ureter or pelvis) retrogradely, can deliver therapeutic genes to the kidney less invasively than techniques that do not use catheters and therefore require an abdominal incision. Therefore, the development of catheters that are exclusively for gene transfer is key to the progress of catheter-mediated gene therapy. Similarly, ureterorenoscopes can be used to deliver therapeutic genes and specific adaptations to optimize their use should be undertaken (Lai et al, 1998).

B. *Ex vivo* gene therapy for kidney transplantation

To obtain successful outcomes following kidney transplantation, we need to overcome the common

problems of post-transplant ischemic injury and rejection. Some of the above-described techniques (Tsuje et al, 2001b; Maruyama et al, 2002a; Lan et al, 2003; Kita et al, 2003) could be useful for *ex vivo* plasmid DNA delivery during kidney transplantation. The kidney to be used for grafting is readily accessible for gene transfer. In addition, the transgene expression in the graft that is induced by *ex vivo* nonviral techniques is, because of the nature of *ex vivo* transfection, confined to the injected kidney.

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