Vol. 186, No. 1, 1992 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1992 Pages 129-134

DIRECT IN VIVO GENE INTRODUCTION INTO RAT KIDNEY

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Received April 3, 1992

We established a simple and highly efficient method for in vivo gene transfer using HVJ (Sendai virus) and liposomes. Plasmid DNA and high mobility group 1 (HMG1) protein were co-encapsulated in liposomes by agitation and sonication and were co-introduced into cells by HVJ-mediated membrane fusion. pACT SVT DNA, as a reporter gene, was introduced into the kidney of intact rats through a cannula in the renal artery, and SV40 large T antigen was detected by enzyme immunohistochemistry in glomerular cells 4 days after its introduction. This newly developed kidney-directed gene transfer method should be useful not only in basic research but also in potential gene therapeutics of renal diseases.

Gene therapy now, seems a promising strategy for therapy of various diseases including renal disease (1). The applicability of this strategy to the cardiovascular system has been demonstrated by Nabel et al. (2), who showed expression of a transfected lac-Z gene (β-galactosidase) in implanted endothelial cells, and by Lim et al. (3), who reported direct in vivo transfer of the luciferase gene into the vasculature of intact dogs. However, for success in in vivo gene introduction, a highly efficient gene transfer method is required to make plasmid DNA pass through the cell membrane and to carry it into the nuclei of the target cells. Recently, we established a simple, highly efficient method for in vivo gene introduction which involved the entrapment of DNA and nonhistone chromosomal protein within liposomes and the use of Sendai virus to enhance fusion of liposomes to cell membranes. This delivery system

0006-291X/92 $4.00

results in transient gene expression in liver cells of adult rats (4,5). Using this technique, in this study we first succeeded in introducing a reporter gene into the kidneys of intact adult rats.

Materials and Methods

Construction of Plasmid. pAct-c-myb (a gift from Dr. Ishii, The Institute of Physical and Chemical Research), contains the 5'-promoter region (370 base pairs) and the first intron (900 base pairs) of the chicken β-actin gene (6). The Kpn I/Bam HI fragment of the SV40 genome, containing the SV40 large T antigen gene, was cloned into pUC 18. The Stu I site of the fragment was changed to the Nco I site by linker ligation. The Nco I/Bam HI fragment encoding the SV40 large T antigen was isolated and cloned into the Nco I/Bam HI site of pAct-c-myb to generate pAct-SVT.

Preparation of HVJ-liposomes. Liposomes containing plasmid DNA and HMG1 (high mobility group 1) were constituted as we described previously (4,5). Briefly, dried lipid (phosphatidylserine, phosphatidylcholine and cholesterol), and plasmid DNA, previously incubated with HMG1, were shaken vigorously and sonicated to form liposomes. Then the liposomes and HVJ, inactivated by ultraviolet irradiation ($11 J \cdot m^{-2} \cdot s^{-1}$) for 3 minutes just before use, were mixed to form HVJ-liposomes by fusion. About 200 μg of plasmid DNA was entrapped in 4ml of liposome suspension (10mg of lipids).

Introduction of DNA-HMG1 complex into adult rat kidney. Six-week-old male Wistar rats were anesthetized by intraperitoneal injection of pentobarbital (50mg/kg), and both kidneys and the abdominal aorta near the renal arteries were exposed. A catheter was inserted via the left common carotid artery and the thoracic aorta, placing its tip proximally to the right renal artery. The abdominal aorta was clipped distally beneath the left renal artery. The vascular lumen and kidneys were flushed with 1 ml of 0.9% saline, and then 2ml of HVJ-liposomes suspension was injected. The catheter and clip were then removed. Two, four or seven days after this procedure, the rats were decapitated, and their kidneys were fixed by perfusing a 4% paraformaldehyde solution and then removed for detection of SV40 large T antigen.

Detection of SV40 large T antigen. SV40 large T antigen in 6-μm-thick kidney cryostat sections was detected with a commercial enzyme immunohistochemical kit (Histostain-SP kit, Zymed Lab. Inc., South San Francisco, CA) with a mouse monoclonal antibody against SV40 large T antigen (Oncogene Science, Manhasset, NY).

Results

Four days after injection of HVJ-liposome, SV40 large T antigen was detected immunohistochemically in 15% of the glomerular cells (mesangial cells and/or capillary cells) in the kidney (Fig 1). Thereafter, expression of the SV40 large T antigen gene decreased. SV40 large T antigen was not expressed at all in other tissues such as tubules or vascular cells (Fig 2). The blood urea

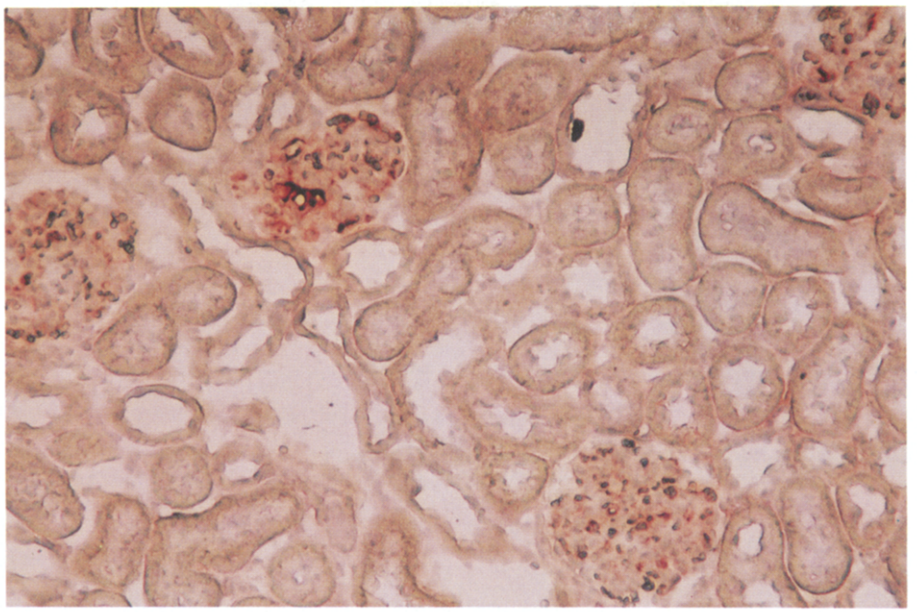

Fig.1. Expression of SV40 large T antigen in the glomerulus of the kidney of the rat. Four days after injection of HVJ-liposomes containing pAct SVT, the rat kidney was perfused with a 4% paraformaldehyde solution. After fixation, the tissue was sectioned at 6 μm thickness in a cryostat and stained. SV40 large T antigen was stained red by enzyme immunohistochemically (X100 optical magnification).

nitrogen [control (pre-transfer);17±2 mg/dl, 4 days after transfer; 17±1 mg/dl] and plasma creatinine concentrations [control (pre-transfer); 0.40±0.01 mg/dl, 4 days after transfer; 0.42±0.02 mg/dl] did not change significantly after the procedure. Proteinuria was not observed in the rats treated with HVJ-liposomes.

Discussion

Successful in vivo gene transfer depends mainly on the method used for introduction of the gene into the nuclei of target cells. Several researchers have reported their preliminary successes in expression of foreign genes after direct injection into target cells by use of retroviruses or liposomes (1). However, the use of a retrovirus has the disadvantage that it is potentially pathogenic to humans, causing infection or oncogenesis. Moreover, the construction of a virus gene containing a foreign gene is complex. Liposomes are safe and easy to prepare, and several kinds of liposomes are being developed

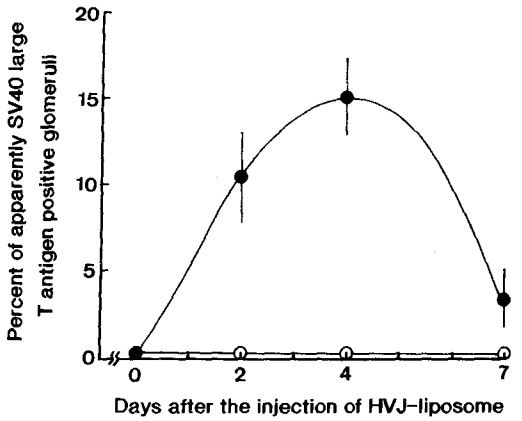

Fig.2. Kinetics of the expression of SV 40 large T antigen as shown in percent of glomeruli which were apparently SV 40 large T antigen positive. Closed circles show the glomeruli and open circles indicate the other cells such as tubular and vascular cells. Each circle indicates mean±SE from 5 to 6 animals.

(7). In the present study, we used liposomes with inactivated HVJ spikes on their surfaces, because HVJ can fuse numerous types of cells efficiently. The contents of liposomes can be introduced into the cytoplasm 100-10,000 times more efficiently by HVJ-liposomes than by liposomes without HVJ (8). Furthermore, transfer of DNA into the nucleus is 3-10 times higher on its co-introduction with HMG1 than DNA only (4,5). When the method was applied to cultured mouse Ltk$^-$ cells, the foreign DNA was transported into the nuclei and expressed in more than 95% of the cells (9,10). Confirmed efficiency of the integration of foreign gene into genome by our HVJ-liposome is at most 1% in cultured cells (10). When we used this method in the adult rat liver for introduction and expression of the human insulin gene and the hepatitis B virus surface antigen gene, the plasmid DNA existed extra-chromosomally, not integrated into genome (4,5).

In the present study, we first showed that the foreign gene can be introduced in vivo into the intact adult rat kidney. In the kidney, expression of the SV40 large T antigen was detected only in glomerular cells (glomerular mesangial cells and/or glomerular capillary cells). The reason for this glomerulus-specific gene expression is unknown. A possible explanation is that

the intra-renal hydrodynamic state facilitates the contact of HVJ-liposomes with glomerular cell membranes, and another is that the phagocytotic activity of glomerular mesangial cells facilitates uptake of HVJ-liposomes, but the exact mechanism requires further study. Whatever the mechanism involved, however, selective gene introduction into glomerular cells is advantageous because the glomeruli play key roles in various physiological and pathological states.

In the present study, we only succeded in transient gene expression because transferred foreign gene by our method was not integrated into the host genome. However, the transient expression of foreign genes offers an advantage, such as, low risk of untoward permanent excessive gene expression. Such a gene therapeutics as our present method can be best utilized as a new drug delivery system. As a requisite for future gene therapy, longer expression of exogenous DNA is needed. A method for integration of exogenous DNA into chromosomal DNA using homologous recombination or autonomous replication of plasmid DNA *in vivo* should be developed.

We conclude that our simple and efficient gene introduction method is applicable to the kidney, and can be a useful tool in research and a candidate for gene therapeutics of various renal diseases.

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