J Mol Med (1995) 73:289–297

REVIEW

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Increased expression of DNA cointroduced with nuclear protein in adult rat liver

Received: 7 April 1995

Key words HVJ · Liposome · Nuclear protein · Gene therapy

Abbreviations *ACE* Angiotensin-converting enzyme · *AGN* Angiotensinogen · *BSA* Bovine serum albumin · *CAT* Chloramphenicol acetyltransferase · *FITC* Fluoroscein isothiocyanate · *HMG* High-mobility group · *HSV-TK* Herpes simplex virus thymidine kinase · *HVJ* Hemagglutinating virus of Japan · *ID* Inhibiting dose · *Ltk*⁻ L cells deficient in the TK gene · *ODN* Oligonucleotide · *pActCAT* Chicken β-actin promoter controlled chloramphenicol acetyltransferase · *pActHIN* Chicken β-actin promoter controlled human insulin vesicle complex · *pActSVT* Chicken β-actin promoter controlled SV40 large T antigen · *RBC* Red blood cell · *TK* Thymidine kinase · *VSMC* Vascular smooth muscle cell [*CE2*]

Introduction

Gene therapy has recently attracted attention as a novel therapeutic strategy, and vectors for in vivo gene transfer have been developed. Retroviral vectors, adenoviral vectors, and liposomes are the current major delivery systems for gene therapy, and each has advantages and limitations. There are several problems in transferring DNA into cells and obtaining the efficient expression of gene, one of which that of introducing DNA directly into the cytoplasm without degradation. Molecules that have been endocytosed tend to be degraded in the endosome or lysosome. In this regard we have focused our efforts on the direct introduction of macromolecules into the cytoplasm by the Sendai virus (hemagglutinating virus of Japan, HVJ, mediated cell fusion. DNA encapsulated in liposomes has been successfully introduced into cells by using the fusion activity of HVJ [1]. Another problem is enhancing the expression of introduced genes in nondividing cells. This includes several events, including nuclear targeting of DNA, long-term retention of DNA in the nucleus, and activation of the transcription. We have found that cointroduction of DNA with DNA-binding nuclear protein in animal organs facilitates nuclear migration of the DNA and enhances its expression [2]. This contribution describes our gene delivery system, the effects of nuclear proteins on in vivo gene transfer, and its application for experimental gene therapy.

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The development of a novel vector for cointroduction of DNA with protein

We have developed vesicle complexes to cointroduce DNA with protein [2]. The vesicle complex consists of DNA-loaded liposomes, erythrocyte ghost containing proteins, and HVJ (Fig. 1).

HVJ belongs to the paramyxovirus family, which has HN and F glycoproteins on its envelope [3]. HN binds with glycol-type sialic acid and degrades the receptor by its own neuraminidase activity. F glycoprotein is cleaved to generate hydrophobic fusion peptide by some proteases, and the activated F can interact directly with the lipid bilayer and induce fusion. The receptors for HVJ are distributed universally, so that the virus can fuse with almost all the cells but peripheral lymphocytes. As regards safety, HVJ is not pathogenic to humans and is completely inactivated by appropriate UV irradiation without losing the fusion activity [3].

Liposomes containing DNA were prepared by the reverse-phase evaporation method, which traps about 30% of the plasmid DNA in liposomes [1]. Proteins cannot be incorporated because of the use of organic solvents. About 50 μg plasmid DNA was entrapped in 2 ml lipo-

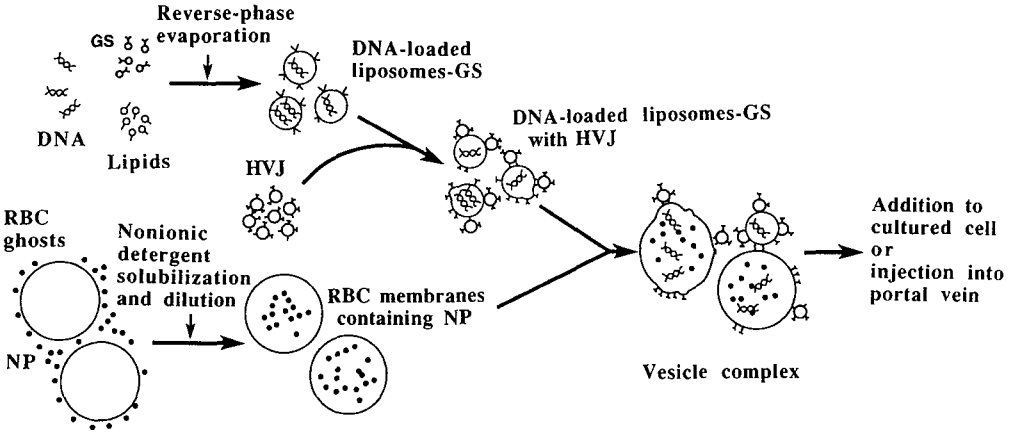

Fig. 1 The procedure for simultaneous introduction of plasmid DNA and nuclear proteins into cells or adult rat liver, using the vesicle complex. *GS*, Gangliosides; *NP*, nuclear protein. This procedure consists of three steps. The first is the interaction of DNA-loaded liposomes with HVJ. The second is the construction of RBC ghosts containing nuclear proteins. The final step is the formation of vesicle complexes by mixing DNA-loaded liposomes with RBC ghosts containing nuclear proteins

some suspension (10 mg lipids). Liposomes suspended in 2 ml balanced salt solution were mixed with 2 ml HVJ (about 15000 hemaglutinating units per 0.5 ml) and 1 mM $CaCl_2$ and incubated at 4° C for 20 min and then at 37° C for 40 min. Next, 600 µl packed volume of human red blood cell (RBC) membranes (RBC ghosts), prepared as described [4], was mixed with 300 µl proteins. RBC membrane vesicles containing proteins were then prepared by the detergent-solubilization and dilution method [4]. These vesicles (about 320 µl) were incubated with 4 ml HVJ liposomes at 4° C for 20 min and then at 37° C for 3.5 h with shaking. The resulting vesicle complex (about 2×10^8 vesicles/ml) was incubated with cultured cells or injected into portal vein of 6 to 8-week old rats. To examine the cointroduction of DNA with protein using the vesicle complex, the vesicle complex was formed by mixing HVJ liposomes containing a thymidine kinase (TK) gene and RBC ghost containing the fluorescent protein, phycoerythrin. When these complexes were added to cultured mouse cells deficient in the Tk gene (Ltk^- cells) more than 95% of the cells showed red fluorescence in their cytoplasm and incorporated [^{3}H]thymidine into their nucleus as a result of the expression of the herpes simplex virus thymidine kinase (HSV-Tk) gene (Fig. 2).

Cointroduction of DNA with nuclear protein

The DNA introduced into the cytoplasm cannot be expressed unless the DNA reaches the nucleus. Although the nuclear transport of nuclear protein has been well characterized, the mechanism of transport of exogenous DNA into the nucleus remains to be elucidated, especially in nondividing tissue cells. Therefore we attempted to cointroduce DNA with DNA-binding nuclear protein.

Extracts of *Xenopus laevis* oocytes were applied to a DNA cellulose column, and the column was washed with 0.1 M NaCl. DNA-binding proteins were then eluted with a step gradient of NaCl from 0.15 to 1.05 M. The HSV-Tk gene was cointroduced into mouse Ltk^- cells with the DNA-binding proteins eluted with various concentrations of NaCl. The rapid and enhanced expression of the TK gene was often observed when 0.35 M NaCl eluates were cointroduced. From the result we searched the responsible protein to enhance gene expression of

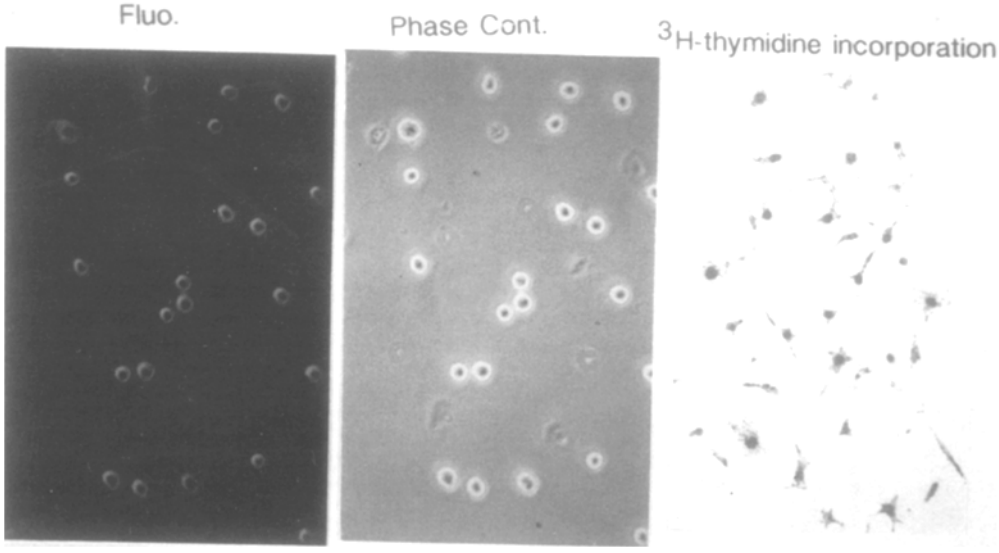

Fig. 2 Cointroduction of DNA with protein by vesicle complexes. The HSV-TK gene was cointroduced with the fluorecent protein phycoerythrin into cultured mouse Ltk^- cells. Red fluorescence was observed in the cytoplasm in all the Ltk^- cells, and TK gene expression was visualized by the incorporation of [^{3}H] thymidine in the nucleus of more than 95% of Ltk^- cells

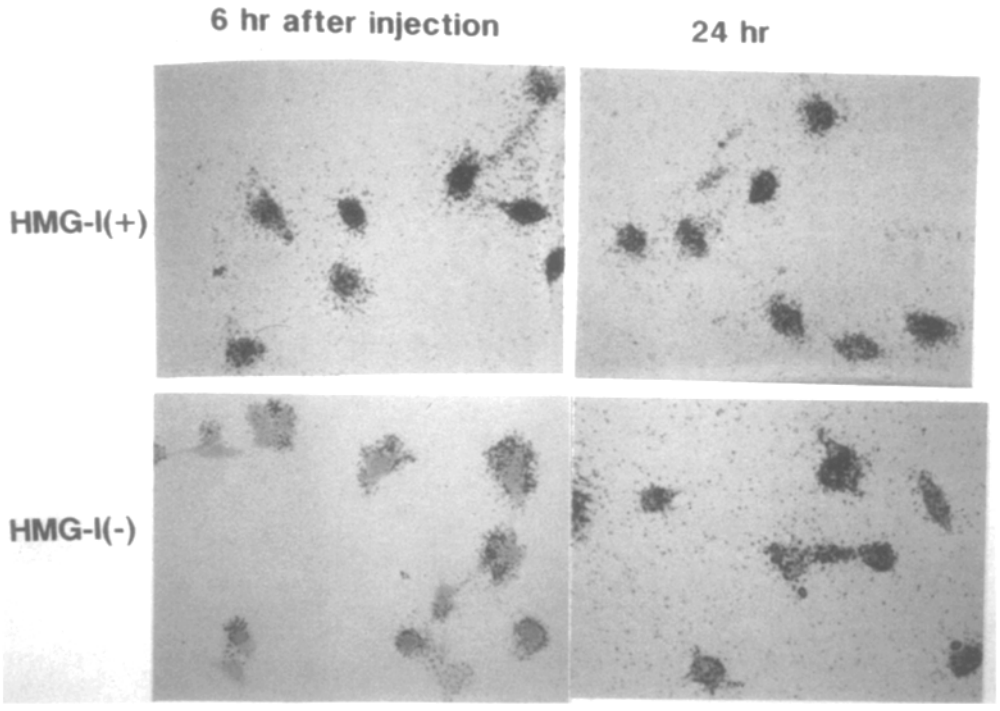

Fig. 3 Localization of DNA introduced into mouse L cells. Using vesicle complexes, plasmid DNA was cointroduced with HMG-1 or BSA into mouse Ltk⁻ cells, and the localization of the DNA was examined by in situ hybridization using ^{35}S-labeled plasmid DNA. The DNA was concentrated in the nuclei within 6 h after cointroduction with HMG-1. In contrast, the DNA was present exclusively in the cytoplasm 6 h after being cointroduced with BSA [*HMG 1*(−)], and it took more than 24 h for the DNA to enter the nuclei

transgene and finally marked high-mobility group 1 (HMG-1), a 28-kDa non-histone chromosomal protein, rich in the fraction. Although HMG-1 is localized both in the cytoplasm and in the nucleus [5], the protein migrates into the nucleus when microinjected into the cytoplasm [6]. In the nucleus HMG-1 is known to localize at the linker region between the nucleosomes [5]. The study using a monoclonal antibody against HMG-1 showed that HMG-1 had two distinct domains, one for nuclear targeting and another for DNA binding [7]. However, the functions of HMG-1 are not yet clearly elucidated, although several reports have suggested that HMG-1 regulates DNA recombination [8], is involved in chromosome architecture [9], or facilitates transcription mediated by RNA polymerase II [10]. The vesicle complex was used to cointroduce pBR-SV40 DNA with either HMG-1 or bovine serum albumin (BSA) into mouse Ltk⁻ cells. In this system approximately 6 μg DNA and 150 μg HMG-1 were cointroduced into 5×10^6 cells, and the localization of the DNA was examined by in situ hybridization 6 or 24 h after the transfer [2]. Within 6 h of treating the cells with the vesicle complex containing DNA and HMG-1, the DNA was concentrated in the cell nuclei (Fig. 3). In contrast, when BSA was employed instead of HMG-1, the grains were located mainly in the cytoplasm after 6 h and were seen in both the cytoplasm and the nucleus after 24 h. In the vesicle complex without HVJ no specific grains were detected in the cells. This suggests that HMG-1 facilitates the nuclear migration of DNA introduced into the cytoplasm.

Chloramphenicol acetyltransferase (CAT) activity reached a maximum within 6 h when chicken β-actin promoter controlled CAT (pAct-CAT) was introduced with HMG-1 into mouse Ltk⁻ cells and remained at this level for at least 24 h after introduction of the gene (Fig.

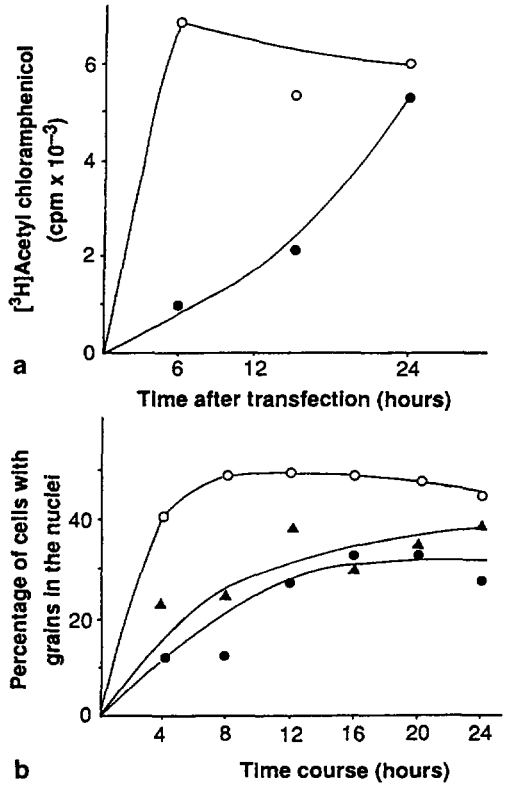

Fig. 4 Effect of HMG-1 on rapid expression of the CAT gene (**a**) and TK gene (**b**) in mouse Ltk⁻ cells. **a** pAct-CAT was introduced into mouse L cells by vesicle complexes containing HMG-1 (*open circle*) or nonimmune rabbit IgG (*closed circle*). CAT activity was expressed as the radioactivity of [^{3}H]acetyl-chloramphenicol formed from [^{3}H]acetyl-coenzyme A. **b** pTK4 (HSV-Tk gene) was cointroduced with HMG-1 (*open circle*), nonimmune rabbit IgG (*open triangle*), or no protein (*closed circle*) into mouse Ltk⁻ cells. Cells were labeled with [^{3}H]thymidine for h, and the number of the nuclei with silver grains were counted after autoradiography

4a). On the other hand, when nonimmune IgG was used instead of HMG-1, CAT gene expression was not significant at 6 h and gradually increased to almost the same level as that with HMG-1. A similar experiment was carried out, as shown in Fig. 4b. Ltk$^-$ cells have a generation time of 16–18 h as measured by counting cell number when grown in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% fetal bovine serum at 37° C. TK activity in these cells was measured as the incorporation of [^{3}H]thymidine into DNA. This reached a plateau within 8 h when pTK4 DNA was cointroduced with HMG-1 but took more than 20 h when nonimmune IgG was used instead of HMG-1. Similar results were obtained when a mixture of nucleoplasmin of frog nuclear protein and histones was used instead of HMG-1 (data not shown). When nonimmune IgG was cointroduced, the maximum expression was about 80% of that obtained with nuclear proteins. These results indicate that when DNA is introduced into cells with DNA-binding nuclear proteins, it can reach the nuclei of interphase cells before mitosis, and suggest that DNA may be inserted into the nuclei of nondividing tissue cells to enhance gene expression in vivo.

In vivo gene transfer using vesicle complexes

Vesicle complexes containing chicken β-actin promotor controlled SV40 large T antigen (pAct-SVT) DNA and HMG-1 or BSA into the portal veins of adult rats. Four days after injection the synthesis of protein directed by introduced SV40 DNA was examined in rat liver sections by means of indirect radioimmunostaining with a monoclonal antibody to SV40 large T antigen. About 10% of the cells contained labeled nuclei; more than half of these were identified as hepatocytes (Fig. 5a). When BSA was used as the cointroduced protein, 1–3% of total nuclei were immunoreactive, and the number of grains per nucleus was much smaller than that observed with HMG-1 (Fig. 5b).

Next we introduced human insulin DNA with HMG-1 into rat liver. The vesicle complex containing the human insulin gene under the control of the chicken β-actin promotor (pAc-HIN) was injected into the portal vein of adult rats. DNA from isolated nuclei of the livers of rats killed 3 days after injection was analyzed by electrophoresis. Injection of vesicle complexes resulted in the appearance of DNA bands that hybridized to a DNA probe for T antigen (Fig. 6a). No bands were detected after injection of vesicle complexes that lacks either HVJ or DNA. On the basis of the density ratio of hybridized bands in each lane, DNA was carried into the nucleus three to seven times as efficiently by HMG-1 as by the nonnuclear protein BSA. The amount of DNA introduced into nuclei with HMG-1 remained unchanged for at least 7 days and then decreased rapidly between 7 and 12 days. We next carried out northern blot analysis of polyadenylated [poly(A)$^+$] RNA extracted from the livers of rats that had been injected with vesicle complexes containing pAct-HIN and HMG-1 or BSA (Fig. 6b). The amount of poly(A)$^+$ RNA encoding human insulin was more than ten times as great when HMG-1 was the added protein as observed when BSA was used.

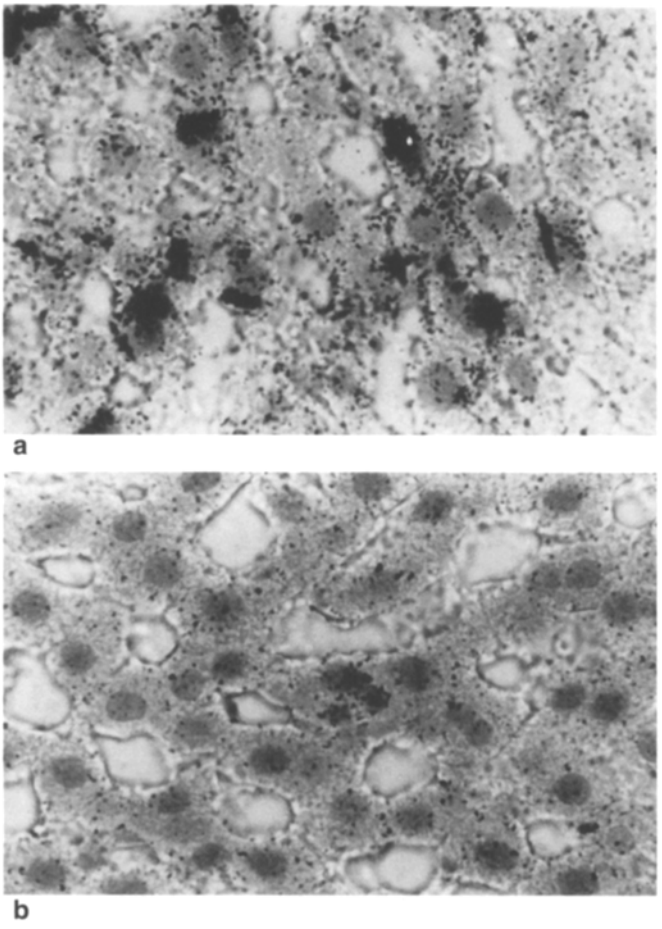

Fig. 5 Expression of SV40 large T antigen DNA in adult rat liver on day 4 after cointroduction with HMG-1 (**a**) or BSA (**b**) by vesicle complexes. The expression of the DNA was detected on frozen liver sections by radioimmunostaining using ^{3}H-labeled second antibody

The change in level of human insulin secreted into rat serum was examined. For this, blood was collected from a tail vein every day after the injection, and the amount of human insulin in rat serum was determined by radioimmunoassay (Fig. 1). The plasmid was injected at two different concentrations using the vesicle complex containing HMG-1. In both cases human insulin could be detected in the rat serum for about 10 days and showed the same profile of daily increase to a peak on day 7 or 8 and then a rapid decrease. The peak level of human insulin in rat serum corresponded to almost the normal concentration in human serum, being 20–30 and 10–25 μU/ml after injection of 30 and 10 μg DNA, respectively. When pAct-HIN was cointroduced with BSA, the level of human insulin in rat serum was much lower and did not show the clear peak seen after its cointroduction with HMG-1.

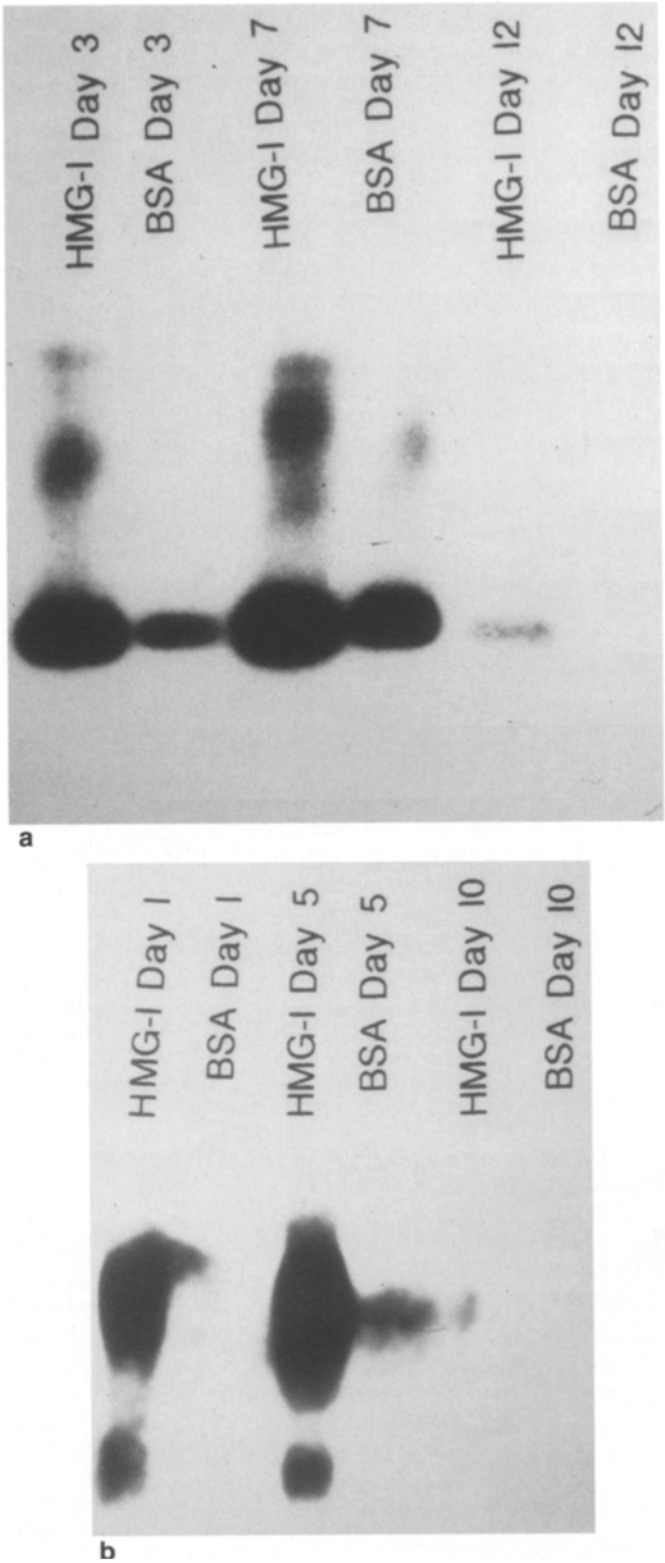

Fig. 6 Southern blot of pAct-HIN (**a**) and northern blot analysis of poly(A)$^+$ RNA of human insulin in rat liver (**b**). **a** Total genomic DNAs were isolated from rat liver nuclei on various days after injection of the vesicle complex containing pAct-HIN and HMG-1 or BSA. Undigested DNA (30 μg/lane) was electrophoresed and hybridized with ^{32}P-labeled human insulin DNA. **b** Poly(A)$^+$ RNA (20 μg/lane) was hybridized with the same probe as in for **a**. Rat albumin probe was used for internal control (data not shown)

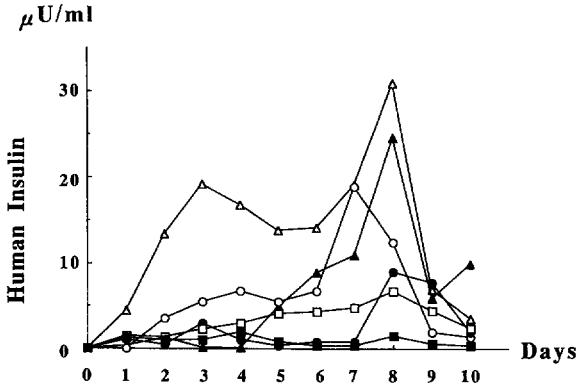

Fig. 7 Kinetics of changes of human insulin in rat serum. Blood was collected from the rats after injection of the vesicle complex containing pAct-HIN and HMG-1 (*circles*, *triangles*) or BSA (*squares*), and the human insulin concentration in the sera was measured by a specific radioimmunoassay for human insulin. About 30 μg DNA (*open circles*, *triangles*, *squares*) or 10 μg DNA (*closed circles*, *triangles*) of pAct-HIN plasmid/rat was injected

These in vivo experiments indicate that DNA-binding nuclear proteins can facilitate the migration of foreign DNA into the nucleus and consequently enhance its transcription and translation in nondividing cells. Thus, cointroduction of plasmid DNA with HMG-1 enhances the gene expression of the introduced DNA, and this system may be applicable for gene therapy of various diseases.

Improvement of in vivo gene delivery system

Vesicle complexes were useful for the cointroduction of DNA with proteins, but the preparation of vector was complicated because three different particles, liposomes, HVJ, and RBC ghosts, were required. We simplified the delivery system by simultaneous trapment of DNA and protein liposome [12]. Phosphatidylcholine, phosphatidylserine, and cholesterol were mixed at the weight ratio of 4.8:1:2 in tetrahydrofuran, and and 10 mg lipid mixture was dried by a rotary evaporator. Then DNA (200 μg) preincubated with HMG-1 (65 μg) was added to dried lipids, and liposome-trapping DNA and protein were constructed by the vortexing, sonication, and annealing method. The trapping efficiency of DNA and HMG-1 was about 20% and 50%, respectively. The resulting liposome was incubated with UV-inactivated HVJ to form HVJ liposome, and the HVJ liposome was isolated by sucrose gradient centrifugation. The HVJ liposome fuses with cells at the surface and introduces, the DNA and protein directly into the cytoplasm (Fig. 8).

Using this delivery system, pAct-HIN was also introduced into the portal vein of 8-week-old BALB/c mouse, and the daily change of human insulin in mouse plasma was detected by the same method described in Fig. 7 (Fig. 9) [13]. Human insulin was detected in mouse plasma for about 14 days, with a profile that showed a daily increase to a peak on day 8 followed by a rapid decrease.

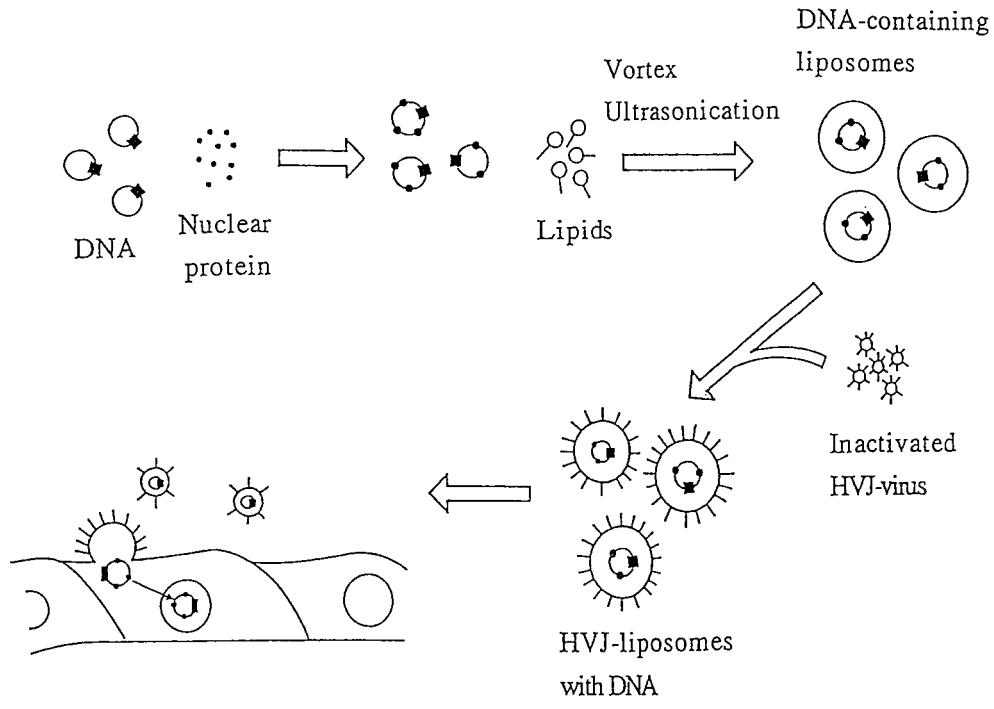

Fig. 8 The procedure of HVJ liposomes. DNA and nuclear protein (HMG-1) are enclosed in liposomes by vortexing-sonication, and the liposomes are treated with HVJ. The resulting HVJ liposomes deliver the DNA – HMG-1 complex into the cytoplasm, and the complex migrates rapidly into the nucleus

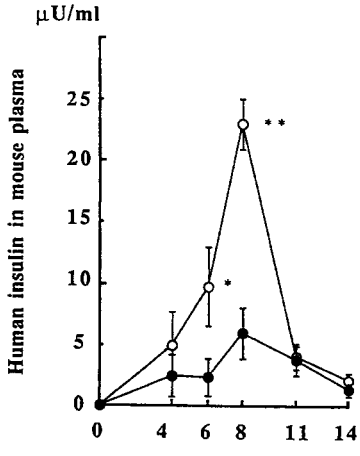

Fig. 9 Changes in human insulin in mouse plasma. *Open circles*, human insulin levels in mice (n=6) that received HVJ liposomes containing the human insulin gene with HMG-1; *closed circles*, human insulin levels in mice (n=6) that received HVJ liposomes containing human insulin DNA and BSA. * $P<0.05$; ** $P<0.01$ vs. human plasma insulin level in mice that received injections of HVJ liposomes with BSA, by analysis of variance followed by multiple comparison

The peak level of human insulin in mouse plasma corresponded to almost the normal concentration in human plasma, 20–30 μU/ml. However, when cointroduced with BSA, the amounts of the expressed human insulin gene in mouse plasma were much lower than when cointroduced with HMG-1. The peak level of the expressed human insulin gene was seen on day 8 in both cases. No human insulin in the plasma was detected when HVJ liposomes without the DNA were injected. There was no deterioration in liver function or other blood parameters after the injection of HVJ liposomes into the portal vein (Table 1).

Advantages and limitations of HVJ liposomes

The advantages of HVJ liposomes are as follows. (a) The HVJ liposome delivery system requires a shorter incubation time (10–30 min) than cationic liposome mediated gene transfer (5–24 h), and the efficiency of gene transfer and expression is generally compatible with cationic liposome method in cultured cells, and much higher in in vivo gene transfer than lipofection. (b) The HVJ liposome does not require cell division for DNA transfer. (c)

Table 1 Murine liver and renal function before and after injection of HVJ liposomes

	Days		
	0	5	10
Asparate aminotransferase (IU/l)	44.9±3.7	43.5±2.4	46.3±3.8
Alanine aminotransferase (IU/l)	28.1±1.3	29.6±2.7	29.3±3.4
Total protein (g/dl)	5.2±0.2	4.9±0.4	4.8±1.1
Total cholesterol (mg/dl)	112 ±9.8	110 ±8.2	108 ±7.2
Blood urea nitrogen (mg/dl)	21.4±3.5	23.2±2.2	22.8±3.1
Creatinine concentration (mg/dl)	0.4±0.1	0.6±0.2	0.5±0.2

Table 2 Results of in vivo gene transfer by HVJ liposomes (*HBsAg*) hepatitis B virus surface antigen, *TGFβ* transforming growth factor beta, *PDGF* platelet-derived growth factor, *n.d.* not determined

Organ (animal)	Gene	Duration	Effect of gene expression
Liver (rat)	Insulin	10–14 days	Decrease in blood glucose level
	HBsAg	7 days	Liver cell degeneration and infiltration
	Renin	7 days	High blood pressure
Kidney (rat)	TGFβ, PDGF	10 days	Glomerulosclerosis
Heart (rat)	TGFβ	>2 weeks	Cardiac hypertrophy
Heart (dog)	CAT	>2 weeks	No change
Skeletal muscle (mouse)	Dystrophin	2 weeks	Dystrophin expression in mdx mouse muscle
Artery (rat)	Renin, ACE	>2 weeks	Hyperplasia and hypertrophy of VSMC
	Antisense ODN	>2 weeks	Inhibition of neointima formation
Lung (rat)	TGFβ	>2 weeks	Fibrosis
Joint space	SVT	n.d.	Gene expression at synovia and cartilage
Testis	CAT	>8 months	no change
Brain	Antisense ODN	n.d.	n.d.
Bone marrow	β-galactosidase	n.d.	n.d.

CAT: chloramphenicol acetyl transferase; VSMC: vascular smooth muscle cell; n.d.: not determined; ACE: angiotensin converting enzyme
SVT: SV40 large T antigen

The HVJ liposome can trap DNA of at least 50 kbp and can cointroduce DNA with protein. (d) The method results in a significant increase in stability and effectiveness of antisense oligonucleotides. (e) The vector does not induce apparent cytotoxic effects in small animals. (f) The in vivo injection of HVJ liposome generates antibodies against HVJ, but the antibody does not neutralize successive treatments of the vector.

The limitations of the system are as follows. (a) Gene expression is transient. (b) Targeting the delivery of the gene is impossible. (c) Preparation of the vector is complex, and the vector is less stable after the preparation. Although the HVJ liposome gene delivery system does have such limitations, successful results of gene delivery and expression in many organs have been reported using this system (Table 2).

Efficient delivery of oligonucleotides using HVJ liposome

Antisense RNA has been reported as an attractive method to inhibit expression of a specified gene by the complementary sequence for the gene. However, it is very hard to inhibit the translation of mRNA by introducing plasmid with the reversed sequense of the target gene. Synthesized oligonucleotides (ODNs) have begun to be employed to block the gene expression, and the ODNs can be stabilized in medium or in blood by substituting sulfate or methyl-residue for phosphate residue of ODNs. Nevertheless, antisense ODNs do not always work well due to ineffective delivery systems. ODNs have been introduced into cells, probably by endocytosis, after coincubation with cells, and through the endocytic pathways most of the ODNs are degraded in endosome or lysosome before reaching the cytoplasm.

Therefore, we attempted to directly introduce ODNs into the cytoplasm by the HVJ liposome delivery system. Fluorescein isothiocyanate (FITC) labeled ODNs were introduced into cultured vascular smooth muscle cells (VSMCs) by HVJ liposomes (Fig. 10). Fluorescence was accumulated in the nucleus of all the cells 5 min after the transfer and remained there for at least 3 days. When naked FITC-ODNs were coincubated with cells, fluorescence was scattered in the cytoplasm, not in the nucleus, and had disappeared 24 h after the transfer. Various

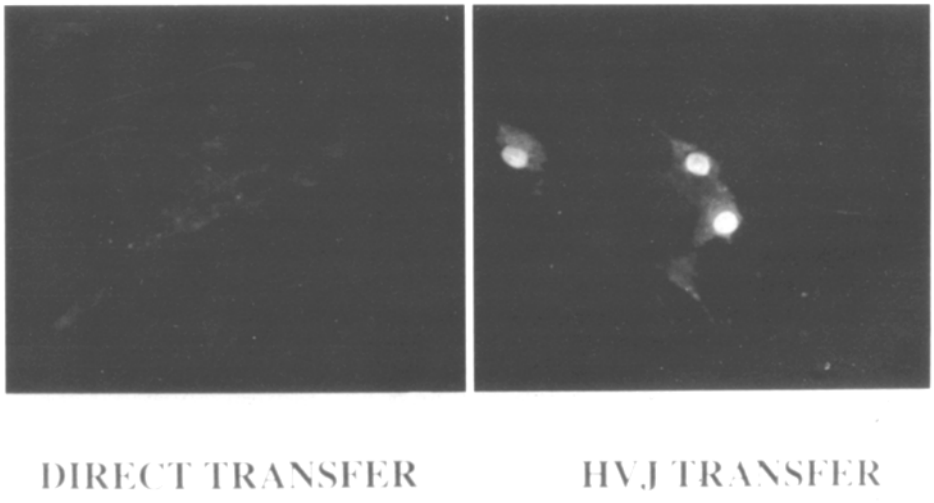

Fig. 10 Localization of FITC-labeled ODNs in VSMCs. Direct transfer: FITC-labeled ODN 30 μM was added to cultured VSMCs, and the cells were incubated for 5 min at 4° C and for 30 min at 37° C. HVJ transfer: HVJ liposomes with FITC labeled ODN (3 μM) was added to cultured VSMCs, and the cells were treated as described above. Then, 5 min after changing to fresh medium with 5% calf serum the cells were fixed and observed by fluorescent microscopy

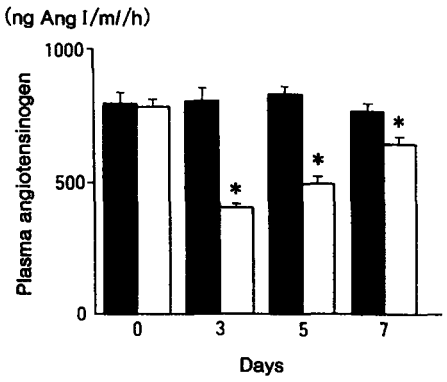

Fig. 11 AGN concentration in rat plasma. Antisense or sense ODN against rat AGN was introduced into rat portal vein by HVJ liposomes, and the concentration or plasma AGN was measured by radioimmunoassay on days 3, 5, and 7 after the transfer. ■ Sense; □ antisense; * $P<0.05$

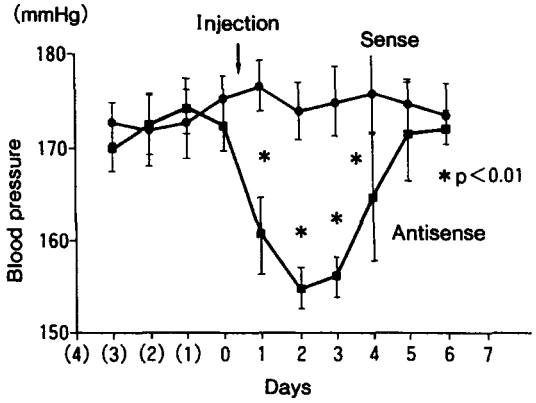

Fig. 13 Effect of antisense ODN against rat AGN on blood pressure. HVJ liposomes containing antisense or sense ODN against rat AGN was injected into portal vein of spontaneous hypertensive rats. Blood pressure was measured by tail-cuff method every day

methods (naked ODNs alone, cationic liposome, and HVJ liposome) were employed to introduce antisense ODNs against basic fibroblast growth factor into VSMCs and effective doses of ODNs sufficient to inhibit DNA synthesis by 25% (ID_{25}) were compared among the methods. The ID_{25} for HVJ liposome was 0.1 μM, while that for cationic liposome was 10 μM and that of naked ODNs 20 μM. This result shows that HVJ liposomes are about 50 times and 100 times more efficient in the transfer of antisense ODNs than cationic liposome and naked ODNs alone, respectively.

In vivo introduction of antisense ODNs toward the therapy of postnatal disorders

Based on the results described above we applied the antisense strategy to the therapy of hypertension [14]. Angiotensinogen (AGN) produced in liver is one of the factors for hypertension, and this can therefore be one of the targets for gene therapy of hypertension. Several antisense ODNs against rat angiotensinogen were synthesized and introduced into rat primary hepatocytes by the HVJ method. One of the antisense ODNs (20 mer) covering the first exon-intron boundary was found to be effective for reducing rat angiotensinogen secreted in the cultured medium. Then, 15 μM antisense ODNs against AGN was introduced into rat portal vein using HVJ liposomes. On day 3 after the transfer the AGN in rat serum was reduced to about 50% of that of normal rats (Fig. 11). The inhibitory effect was observed for about 1 week after the transfer. Northern blot analysis revealed that the AGN level of mRNA by the transfer of antisense ODNs was about half of that by the transfer of sense ODNs (Fig. 12). HVJ liposomes containing the antisense ODNs were then injected into the portal vein of spontaneous hypertensive rats, and the daily change of the blood pressure was followed using tail-cuff method. As shown in Fig. 13, the blood pressure was changed from about 180 to 155 mm Hg, and the blood pressure returned to the initial level on day 7 after the transfer.

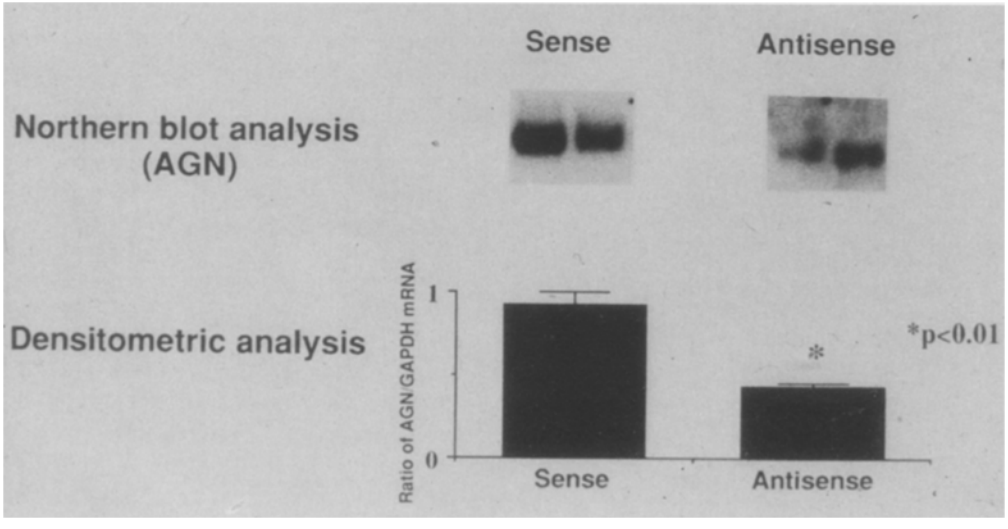

Fig. 12 Northern blot analysis of rat AGN poly(A)$^+$ RNA in rat liver. Antisense or sense ODN against rat AGN was introduced into rat portal vein by HVJ liposomes, and the poly(A)$^+$ RNA for rat AGN was detected by northern blot analysis on day 3 after the transfer. The density of hybridized band was measured using a densitometer, and the relative amount of rat AGN poly(A)$^+$ RNA was expressed as the ratio to the density of poly(A)$^+$ RNA for rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH)

Conclusion

We have developed an efficient in vivo gene transfer system based on liposomes, HVJ, and nuclear proteins. In this system DNA is delivered directly into the cytoplasm using virus-cell fusion, and the DNA is most likely inserted into the nucleus and stabilized there to result in efficient gene expression by the cointroduced nuclear protein. HMG-1 is so far the most successful nuclear proteins in enhancing gene expression. Moreover, our delivery system results in a significant increase in the stability and effectiveness of antisense ODNs. Although there still exist limitations in the delivery system (e.g., transient gene expression, inability to target), the HVJ liposome or vesicle complex will be one of the most practical gene delivery systems not only for the treatment of postnatal disorders but also for the analysis of molecular aspects of diseases.

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