Gene Delivery to the Rat Liver Using Cationic Lipid Emulsion/DNA Complex: Comparison between Intra-arterial, Intraportal and Intravenous Administration

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Objective: To compare the efficiency of intra-arterial, intraportal, and intravenous administration of cationic lipid emulsion/DNA complex, as used for gene transfer to rat liver.

Materials and Methods: DNA-carrier complex for the in-vivo experiment was prepared by mixing DNA and a cationic lipid emulsion. According to the administration route used (intra-arterial, intraportal, or intravenous), the animals were assigned to one of three groups. The heart, lung, liver, spleen and kidneys were removed and assayed for total protein and luciferase concentration.

Results: The cationic lipid emulsion/DNA complex used successfully transfected the various organs via the different administration routes employed. Luciferase activity in each organ of untreated animals was negligible. Liver luciferase values were significantly higher in the groups in which intra-arterial or intraportal administration was used.

Conclusion: The intra-arterial or intraportal administration of cationic lipid emulsion/DNA complex is superior to intravenous administration and allows selective gene transfer to the liver.

Index terms:
Animals
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Gene transfer into experimental animals or humans resulting in generalized or tissue-specific expression may allow precise *in-vivo* manipulation of biological processes to cure diseases and induce immune responses to pathogens (1, 2). The basic challenge in gene therapy is to develop approaches to the delivery of genetic material to appropriate cells in a way that is specific, efficient, and safe.

Continuous efforts have been applied to the development of gene delivery systems known as vectors, which encapsulate the gene and guide it to the target cell. Viruses are ideal vectors, being naturally suited to the highly efficient transfection of genetic material to cells. The use of viral vectors is, however, limited by safety concerns related to the immune and inflammatory responses they trigger and immune rejection phenomena arising due to repeated administration (1, 3, 4). As an alternative to viral vectors, various non-viral gene delivery systems, including cationic lipids, cationic polymers, and naked DNA, have been prepared for use in gene therapy.

In liver-directed gene therapy involving radiologists, approach routes may include direct percutaneous injection, and transcatheter intra-arterial or intraportal administration. In the case of viral vectors, it has been reported that intra-arterial administration is more efficient than intravenous administration (5), though cationic lipid vectors have not been compared in this way.

The purpose of this study was to compare the efficiency of intra-arterial, intraportal, and intravenous administration of cationic lipid emulsion/DNA complex, as used for

gene transfer to rat liver.

MATERIALS AND METHODS

Preparation of Cationic Lipid Emulsion

The emulsion we used contained 100 μL/mL oil (squalene) and lipid emulsifiers [1, 2-dioleyl-sn-glycro-3-trimethylammonium-propane (DOTAP), and 1, 2-dioleyl-sn-glycro-3-phosphoethanolamine (DOPE), combined in a ratio of 5:1 by weight], and was prepared as described previously (6). Briefly, lipid emulsifiers were weighed and dispersed in water, and the resulting mixture was sonicated in an ice/water bath using a probe type sonicator (high intensity ultrasonic processor, 600 W model; Sonic and Materials, Danbury, Conn., U.S.A.). The lipid solution was added to oil, and the mixture was sonicated further in an ice/water bath. Prior to use, the cationic lipid emulsion thus prepared was kept at 4℃, and its short-term stability was monitored by measuring the time-dependant absorbance changes occurring at 600 nm. The average size of emulsion particle was 164.5 nm.

Preparation of Plasmid DNA

As reporter genes, we used pCMV-Luc+ and pCMV-β. The latter, encoding *Escherichia coli* (E. coli) *l acZ* (β-galactosidase) gene expression plasmid driven by the human cytomegalovirus immediate-early promoter, was supplied by Clontech Laboratories (Palo Alto, Cal., U.S.A.), and the pCMV-Luc, consisting of the cytosolic form of *Phontinus pyralis* luciferase cDNA, was obtained from pGL3 (Promega, Madison, Wis., U.S.A.) using Xba I and Hind III restriction and was subcloned into the plasmid pcDNA3.1 (Invitrogen, Seoul, Korea). Both plasmids were amplified in the *E. coli* DH5-α strain and purified using a Qiagen mega-kit (Qiagen Inc., Chatsworth, Cal., U.S.A.), according to the manufacturer's instructions. The purity of the DNA used ($OD_{260}/OD_{280} \geq 1.8$) was determined by agarose gel electrophoresis and the measurement of optical density.

Animal Studies

To prepare DNA-carrier complexes for this experiment, 20 μg of pCMV-Luc+ and the carrier, the amount of which corresponded to the weight ratio between cationic lipid in the lipid formulation and DNA in the complex that showed the maximum transfection efficiency, were diluted with 0.5 mL of DMEM (Dulbecco's modified Eagle's medium) solution and mixed by inversion. The total volume of the mixture was 1 mL, and the time interval between mixing and infusion was minimized.

Twenty-four 8-week-old Sprague-Dawley rats, each weighing 200-300 gms, were used in this study; their housing and the procedure employed were in accordance with the National Institutes of Health guidelines. The animals were divided into three groups according to whether injection was performed intra-arterially (n=9), intraportally (n=8), or intravenously (n=7). Three other rats which did not undergo treatment were included as negative controls.

The animals were anesthetized in an ether jar prior to the injection of a 1:1 mixture of xylazine hydrochloride (Rompun; Bayer Korea, Seoul, Korea) and ketamine hydrochloride (Ketara; Yuhan Yanghang, Seoul, Korea) into the peritoneal cavity. Those in which administration was intra-arterial or intraportal were prepared for surgery, and a midline ventral incision was made. The liver was then retracted and connective tissues were cleared to visualize the gastroduodenal artery or mesenteric veins. For intra-arterial administration, the gastroduodenal artery was exposed and cannulated with a 28-G needle, and the DNA-carrier complex was slowly injected into the proper hepatic artery at a rate of 1 mL/min. The catheter was then removed and the gastroduodenal artery was ligated; the abdominal incision was sutured using the continuous interlocking method. For intraportal administration, a tributary of the superior mesenteric vein was selected, a 28-G needle was positioned in it and secured, and the DNA-carrier complex was delivered at the same rate as previously. The catheter was removed and the catheterized vein ligated, and the abdominal incision was closed in the same manner as before. For intravenous administration, the DNA-carrier complex was injected into the vein of the tail using a 24-G catheter, which was subsequently slowly flushed with saline and removed.

Luciferase Activity Assay

Two rats in Group II, in which administration was intraportal, expired prior to sacrifice. The remaining 22 rats survived and were sacrificed 24 hours after the procedure. The heart, lung, liver, spleen and kidneys were removed from each animal and underwent , separately, high-speed homogenization using T-25-Ultra-Turrax homogenizer (Janke & Kunkel GmbH, KG, Germany) and, for each collected organ, 5 μl/mg of pH 7.8 lysis buffer comprising 0.1 M Tris-HCl, 2 mM EDTA and 0.1% Triton X-100. After two freeze/thaw cycles, the homogenized organ lysates were centrifuged for 10 mins at 4℃ and at 12,000 rpm in an Eppendorf centrifuge. A portion of the supernatants was assayed for protein concentration using a Bio-Rad protein assay kit (Bio-Rad Laboratories, Hercules, Cal., U.S.A.). Luciferase activity in the lysates was quantified using a Promega kit (Madison, Wis., U.S.A.) with a luminometer (Turner Designs Luminometer, Model TD-20/20;

Promega); peak light emission was measured for 20 seconds at room temperature, and the luciferase count was calculated from relative light units using a standard curve obtained from purified firefly luciferase (Sigma). The luciferase count of each organ in each rat was tabulated, and intra-arterial, intraportal, and intravenous administration were compared in terms of the efficiency with which they facilitated gene transfer to the liver.

For all groups, mean values and standard deviations of luciferase enzyme activity were calculated, and using Wilcoxon's rank sum test, the statistical significance of differences in enzyme activity between the different routes of administration was determined.

RESULTS

The amount of luciferase enzyme in each organ, which varied according to the route of administration of the cationic lipid emulsion/DNA complex, is summarized in Table 1 and Figure 1. In groups I and II, luciferase activity was highest in the liver (22.94 and 115.21 pg/mg protein, respectively), but in group III, it was highest in the spleen (5.94 pg/mg protein). Luciferase activity in each organ of untreated animals was negligible. Luciferase values in the liver were significantly higher in groups I and II than in groups III (p = 0.0080 and 0.0034, respectively), though between groups I and II there was no significant difference (p = 1.0).

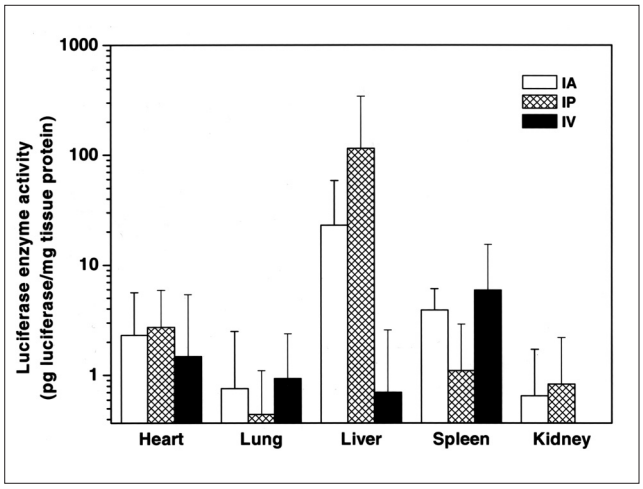

Fig.1. The graph illustrates average and standard deviation of the amount of luciferase enzyme in each organ of the three groups which underwent, respectively, intra-arterial (IA), intraportal (IP), and intravenous (IV) administration.

DISCUSSION

Since the liver is the site of many metabolic diseases and malignancies, it is an organ in which gene therapy may be particularly valuable. The potential indications for liver-directed gene therapy include inherited liver disorders such as familial hypercholesterolemia (7) and ornithine transcarbamylase deficiency (8); inherited systemic disorders including hemophilia (9); viral hepatitis, liver tumors, allograft or xenograft rejection, and ischemia/reperfusion injury (10), and are expanding continuously.

The availability of both the intravenous approach and local delivery routes is another important reason why the liver has been a model organ in the development of gene transfer technology. Except for recombinant adenoviruses and a few vectors with targeting moiety for hepatocytes, most viral and non-viral vectors are not liver specific. To achieve efficient delivery, these vectors have been injected into the liver or peritoneal cavity; infused into the portal vein or hepatic artery; or instilled into the bile duct. The purposes of local administration of vectors are to limit their transgenic expression in the liver and to enhance the efficiency of transfection. In an experimental study using a recombinant adenovirus encoding wild-type p53 or β-galactosidase, intra-arterial delivery increased the transgenic expression in tumor tissue and decreased systemic exposure in comparison with intravenous delivery (5). In murine liver metastases, tumors showed significantly higher transgenic expression after portal venous or intraperitoneal virus administration (11).

There are two kinds of cationic lipid-based gene delivery systems, namely liposome and emulsion. A cationic liposome is a closed double layer of cationic lipids filled with water. It is positively charged and interacts with negatively charged DNA molecules to form a stable complex. Cationic liposomes have been used widely in gene transfer both *in vitro* and *in vivo* (12). The oil-in-water emulsion

Table 1. Average and Standard Deviation of the Amount of Luciferase Enzyme (expressed in pg/mg protein) in Each Organ of the Three Groups of Rats [Intra-arterial (IA), Intraportal (IP), and Intravenous (IV) Administration]

Route of Administration	Heart	Lung	Liver	Spleen	Kidney
IA (Group I)	2.31 ±3.32	0.76 ±1.74	22.94 ±35.64	3.90 ±2.20	0.65 ±1.07
IP (Group II)	2.72 ±3.18	0.44 ±0.66	115.21 ±227.03	1.10 ±1.82	0.83 ±1.36
IV (Group III)	1.48 ±3.90	0.93 ±1.45	0.70 ±1.86	5.94 ±9.48	0.00 ±0.00
Untreated controls	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00	0.00 ±0.00

used consists of oil dispersed in the aqueous phase with a suitable emulsifying agent such as a phospholipid or nonionic surfactant. The use of cationic lipid emulsions as gene delivery vectors has not, however, been widely investigated.

Cationic lipid-mediated gene delivery is not yet as efficient as the use of a viral vector. Although intravenously-injected liposomes have an intrinsic affinity for the liver, the uptake of liposomes by this organ in the absence of a targeting mechanism is highly variable (13−15), and most liposomal carriers lose their ability to transfer DNA in the presence of ≥10% serum (12, 16, 17). Because of the physical instability of the liposome/DNA complex and the inactivation of complexes by negatively charged molecules in serum, its transfection efficiency has been found to be relatively low compared with that of viral vectors (18, 19). To enhance efficiency, a new formulation offering serum stability and a strategy for local administration is therefore needed.

To overcome the problems associated with liposomes, castor oil emulsions have been introduced as alternative gene transfer vectors (12); new cationic lipid emulsions use squalene or soybean oil as a core lipid (20). It has been shown that in the presence of serum, the use of a cationic lipid emulsion/DNA complex has led to efficient cell transfection (6). In *in-vitro* transfection assay of this complex, more than 60% of transfection efficiency was retained in the presence of up to 90% serum (6). Unlike liposomal carriers, cationic lipid emulsion retained its physical integrity while forming a complex with DNA. *In-vitro* DNA release tests have shown that binding of the emulsion/DNA complex was strong, and it was thus unaffected by the anionic polyion, poly-L-aspartic acid (16). *In-vivo*, this stable emulsion system delivered genes to endothelial cells in the mouse nasal cavity more efficiently than the commercially available liposomes, Lipofectamine and Lipofectin (4).

We conducted this animal experiment to determine whether local delivery of a cationic lipid emulsion/DNA complex through the hepatic artery or portal vein is advantageous, and our results clearly showed that intra-arterial or intraportal administration is superior to intravenous administration.

Several important issues in cationic lipid-mediated gene delivery await solution. For example, the distribution of liposomal vectors to various organs, and the efficiency with which this is achieved, are affected by factors which include liposome/DNA complex particle size (21−22). The use of a cationic lipid emulsion for gene transfection has not been thoroughly investigated, though it has been shown that after its intravenous administration, gene expression in the lung is usually higher than in the spleen and other organs (23). In our study, on the other hand, luciferase activity in the lung was quite low, probably because of the stability of the complex used in the experiment. Further investigation of the biodistribution of lipid emulsion in each specific organ is, however, required. In hepatic gene delivery, the optimal particle size of a liposome or emulsion, for example, may differ according to whether administration is intra-arterial or intraportal, or intravenous, and research into this is also needed. As a further example, the liver contains cell types which include hepatocytes, Kupffer cells, endothelial cells and various tumor cells, and in hepatic gene therapy, target cells can vary according to the disease being treated. In metabolic diseases, the primary target is hepatocytes, while in hepatic tumors, gene expression in tumor cells should be maximized and gene delivery to normal parenchyma minimized. In clinical or experimental studies using various different vectors, a clear understanding of exactly where transgenic expression should occur is therefore important. Because the majority of liposomes administered intravenously are endocytosed by the reticuloendothelial system (15, 17), the reduction of Kupffer cell uptake and enhancement of hepatocyte or tumor uptake are further challenges faced by research into liposome targeting.

To enhance targeted gene delivery, lipid carriers can be modified (24): polyethylene glycol conjugate or ligands for receptors, for example, can be incorporated into lipid carriers. An amphipathic polyethylene glycol such as Tween 80 can provide a steric barrier, as well as increasing the hydrophilicity of the liposome surface, both of which may reduce interaction between the liposome and plasma proteins and their recognition and uptake by macrophages of the reticuloendothelial system (25). Hepatocytes have glycosyl receptors on their surface, and the hepatic uptake of glycosylated liposome can be enhanced by receptor-mediated endocytosis. Other ligands for receptors under investigation include lactose, transferrin, and antibodies. A recent report described selective gene expression in hepatic VX2 carcinoma after intra-arterial delivery of the DNA/liposome/transferrin complex (26). The hemodynamic characteristics of this and other hepatic tumors are quite different from those of normal liver parenchyma: while normal hepatocytes, for example, receive blood primarily from the portal vein, the hepatic artery supplies nearly all the blood required by a hepatic malignancy. Because of these hemodynamic differences, the optimal conditions for lipid carriers may differ according to the target cells involved (hepatocytes or hepatic malignancies).

Although local delivery of a cationic lipid emulsion/DNA complex via the hepatic artery or portal vein increases hepatic transgenic expression, it may also maximize the he-

patic toxicity of the complex. Where the emulsion is used clinically, the hepatic toxicity of its therapeutic dose should therefore be investigated.

In conclusion, our findings show that in hepatic gene transfer, intra-arterial or intraportal delivery of a cationic lipid emulsion/DNA complex was superior to systemic delivery. Further investigation of the effect of surface modifications, the optimal conditions for the delivery route and target cells, and the hepatic toxicity of the emulsion is warranted.

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