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A comparison between intravenous and peritoneal route on liver targeted uptake and expression of plasmid delivered by Glyco-poly-*L*-lysine

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Abstract

AIM To compare the effects of intravenous route and peritoneal route on liver targeted uptake and expression of plasmid delivered by galactose-t erminal glyco-poly-L-lysine (G-PLL).
METHODS The plasmid pTM/MMP-1 which could be expressed in eukaryotic cells was bound to G-PLL, and was then transferred into Wistar rats by intra venous and intraperitoneal injection. The expression and distribution of the plasmid were observed at different time periods by *in situ* hybridization and im munohistochemistry.
RESULTS The plasmid could be expressed significantly within 24 h a fter being transferred *in vivo* by both intravenous and intraperitoneal ro utes. One week later the expression began to decrease, and could still be observ ed three weeks later. Although both the intravenous and intraperitoneal route co uld target-specifically deliver the plasmid to the liver, the effect of the fo rmer was better as compared to that of the latter.
CONCLUSION Intravenous route is better for liver targeted uptake and expression of G-PLL-bound plasmids than the peritoneal route.

INTRODUCTION

The efficient transference and the high expression of exogenous genes in specifi c cells or tissues are critical steps for both *in vitro* and *in vivo* gene therapy[1-12]. Gene transference mediated by receptors is carried out by high affinity linkage between the ligands (binding to the foreign gene) and specific receptors on the surface of different kinds of cells, and then the fore ign gene can be delivered into the cells by phagocytosis[13,14]. There also exist some specific receptors on the surface of hepatocytes such as asialog lycoprotein receptors (ASGP-R)[15-19], which facilitate the researche rs to deliver exogenous genes into hepatocytes specifically using the ligand-re ceptor interaction. Galactose-terminal glyco-poly-L-lysine (G-PLL) cont ains the saccharide group of galactosan that can be specifically ligated to the asia loglycoprotein receptor (ASGP-R) on the surface of hepatocytes. At the same time, the cationic poly-L-lysine can bind to nucleotides with high affinity, so it can serve as a good carrier to deliver exogenous DNA to liver specifically and steadily[20-24].

Both peripheral veins and abdominal cavity can be used as the delivery route to target drug or nucleotide to liver[25-28], but the comparison of their effects on the targeted liver uptake has seldom been reported.

Using rats as the experimental animals, we compared *in vivo* the difference in distribution and expression of the plasmid given through intravenous or intrap eritoneal route.

MATERIALS AND METHODS

Preparation of the carriers

The original plasmid of rat interstitial collagenase was kindly provided by Prof. John J Jeffrey[29], and we reconstructed it with the plasmid of pT argetT (TM) (Promega Co., Madison, MI, USA), which could be expressed in eucaryo tic cells. We also inserted a segment of nucleotides (GAC TAC AAG GAC GAC GAT GA T AAG) before the terminator codon (TAA) of the rat interstitial collagenase. Th e 'Flag Domain' peptide (DYKDDDDK) encoded by the segment of nucleotide above, which was usually called 'Tag', could be fused in the rat interstitial colla genase[30] and could be specifically recognized by an M2 monoclonal anti body (Kodak, New Haven, CT, USA). This recombinant plasmid was named pTM/MMP-1. The plasmid of pTM/MMP-1 was extracted and purified using QIAGEN-Tip 500 kit (QIAGEN Inc., Valencia, CA, USA) according to the manufactures instructions. The plasmid was mixed with different amounts of galactose-terminal glyco-poly- L-lysine (kindly provided by Dr. Shou-Ming Wen of Air-Force General

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Hospi tal o f PLA, China. The mean molecular weight of this GPLL was 8500 and the ratio of glactose topoly-L-lysine was 15:28). The optimal proportion of the plasmid pTM/MMP-1 binding to galactose-terminal glyco-poly-L-lysine was determin ed by electrophoresis in 1% agarose gel.

Animal experiments

Eighteen male Wistar rats, with body weight of 130-150 g, were randomly divided into three groups of six rats each. Poly-L-lysine intravenous (PI) group: 50 μg plasmid pTM/MMP-1 bound to galactose-terminal glyco-poly-L-lysine wa s administered through cauda vein; poly-L-lysine intra-peritoneal (PP) g roup: each rat was given the same amount of pTM/MMP-1 bound to galactose-termin al glyco-poly-L-lysine intraperitoneally; normal group: control animals. Tw enty-four hours, 48 h, 72 h, 1 wk, 2 wk, 3 wk after the administrat ion of the plasmid, one rat from each group was randomly selected and anaestheti zed with 2% pentobarbital sodium intraperitoneally. Then 1 mL blood was obtained by cardiac puncture for the assay of alanine transaminase (ALT), aspartic trans aminase (AST), and creatinine (Cr) to observe the functions of important organs. After perfusion of the whole body with 20 mL phosphate-buffered saline and 40 mL 4% paraformaldehyde through ventricular injection, the tissues of liver, spl ee n, lungs, and kidneys were collected and fixed in 4% paraformaldehyde, encapsula t ed in paraffin and cut into sections 4-μm thick. This procedure above was approved by the Laboratory Animal Committee of Shanghai Medical University .

Immunohistochemistry

Immunohistochemistry was performed according to the literature[31,32]. The first antibody used was M2 monoclonal antibody which was specific for flag -domain tag (Kodak, New Haven, CT, USA) and the second antibody used was Horse anti-mouse IgG, labeled with biotin (Vecter, Burlingame, CA, USA). After the treatment with avidin and biotin (ABC kit, Vecter, Burlingame, CA, USA), color dev elopment was followed using dimethylaminoazobenzene (DAB) and counterstained with hematoxylin. Five fields were observed under high power from every immunostained section and the positive signals were counted.

In situ hybridization

The procedure of *in situ* hybridization was also described previously[2 5,26]. To state briefly, the oligonucleotide probe (5'-TGG TGT GAC TAC AAG GAC GAC GAT GAT AAG-3') was synthesized in Cell Biology Institute of Chinese Academy of Sciences (Shanghai), which could hybridize with the (mRNA) of the fl ag-domain tag in the plasmid pTM/MMP-1, and the 5' end of the probe was labeled with biotin. After the hybridization of the target mRNA with the probe, the rest of the procedure was similar to that followed in immunohistochemistry excluding the step of hematoxylin counterstaining.

Other biochemical assays

ALT, AST, and Cr were assayed using the 7170A Automatic Analyzer (HITACHI, Ja pan) to observe the changes in the important organs' function.

Data analysis

All of the data were analyzed by the software SPSS 7. 0 for windows (one-way ANOVA).

RESULTS

Ratio of G-PLL to plasmid

According to the electrophoresis results, we found that 0.3 μg of g alacto se-terminal glyco-poly-L-lysine could throughly bind to 1 μg of the plasmid , which meant that about 72 molecules of G-PLL could carry one molecule of the plasmid pTM/MMP-1 (Figure 1).

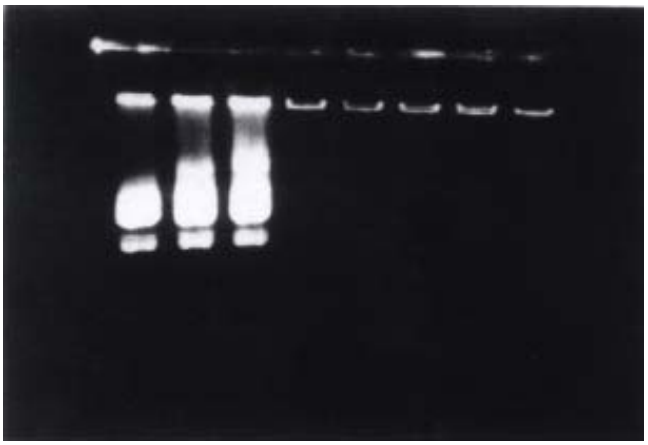

Figure 1 Determination of optimal proportion of G-PLL bound to plasmid by 1% agar ose electrophoresis. Lane 1-8 are respectively 0.05 μg, 0.1 μg, 0.2 μg, 0.3 μg, 0.4 μg, 0.5 μg, 1.0 μg, and 1.5 μg G-PLL mixed with 1 μg pTM / MMP- 1 p lasmid. pTM / MMP-1 1 μg plasmid could only be bound completely by more than 0. 4 μg G-PLL.

The changes in the functioning of important organs

Compared with the normal group, there was no obvious elevation of the ALT, AST, and Cr levels in the PI and PP groups.

The distribution and expression of the plasmid in liver, spleen, lung and kidney

The results of the immunohistochemistry and *in situ* hybridization showed tha t the plasmid binding to G-PLL could be expressed *in vivo*, regardless of th e introducing route and the results of immunohistochemistry were more sensitive a nd stable. In addition, the protein product of the plasmid could be secreted ext racellularly (Figures 2 and 3), similar to the expression of interstiti al collag enase in the physiological state[30,33]. We found that both intravenous route and intraperitoneal route could make liver as the major distribution organ of the plasmid bound to G-PLL.

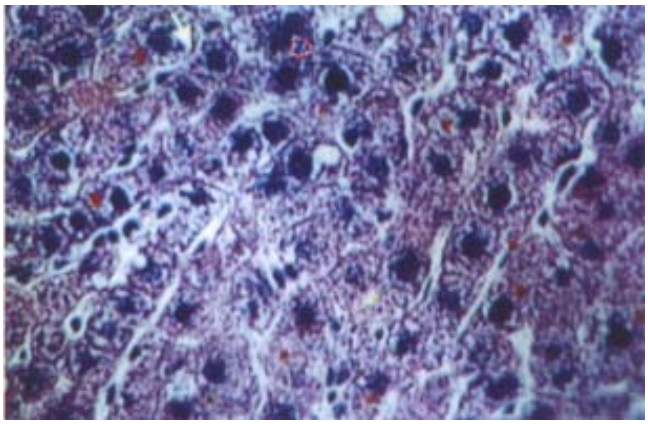

Figure 2 Immunostaining of flag-domain tag in the liver all hours after admin istration of the plasmid bound to G-PLL (galactose-terminal glyco-poly-L-lysine) via cauda vein. × 200

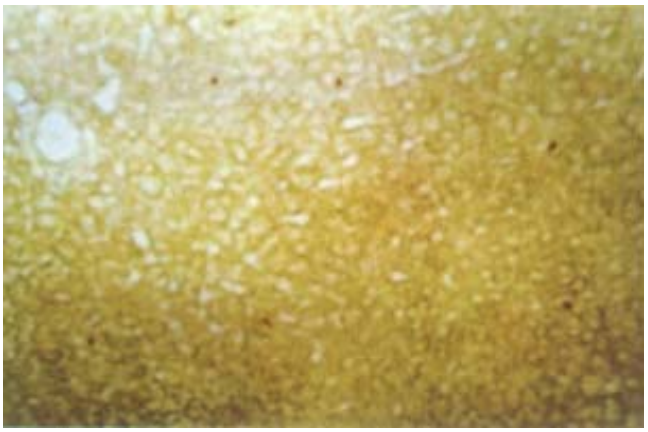

Figure 3 *In situ* hybridization with biotin labeled oligonucleotide probe in th e liver 3 wk after the administration of the plasmid bound to G-PLL (galacto se-terminal glyco-poly-L-lysine) via abdominal cavity. × 100

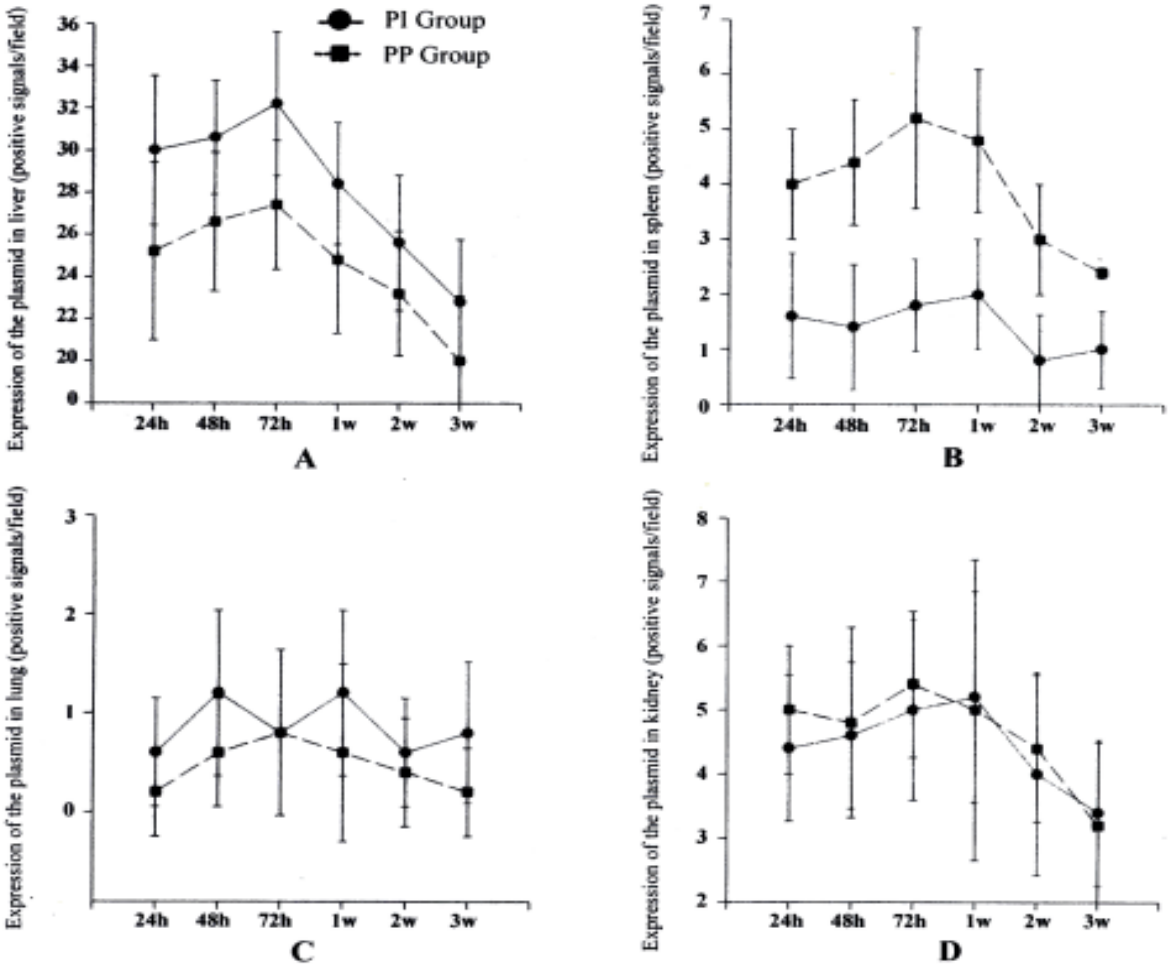

Figure 4 The distribution and expression of the plasmid bound to G-PLL (galactosetermina l glyco-poly-L-lysine) in different tissues and at different time period and administered intraperitoneally or intravenously. A: liver, B: spleen, C: lung, D: kidney. PI: plasmid bound to G-PLL introduced intravenously, PP: plasmid bound to G-PLL introduced intraperitoneally.

The obvious expression of the plasmid could be observed 24 h after the administration and began to decrease one week later, although it could still be observed weakly even two or three weeks later. Among the two groups, we

observed that the expression and distribution of the plasmid in the liver of the PI group was significantly higher than that of the PP group. Besides the liver, the plasmid in PI group could also be expressed in lung at a lower level, and almost could not be expressed in spleen and kidney. As for the PP group, most of the distribution and expression was located in the liver and a relatively higher level could be seen in the spleen and kidney, whereas low expression could be observed in the lungs (Figure 4).

DISCUSSION

Regarding the gene therapy of hepatic diseases, efficient delivery of the exogenous genes to the liver and its high expression there could increase its local accumulation and minimize the side-effects on other tissues and organs as well[34-36].

For the gene therapy of hepatic diseases in the animal experiments, exogenous ge nes were usually delivered to the liver through the portal vein, bile duct injec tion, or even by direct liver injection[37-41]. From the viewpoint of clinical application these methods have limitations concerning its invasive trauma and possible risk. If the gene transference to liver could be accomplished by a peripheral vein or abdominal cavity, the limitations could t hen be avoided or decreased and it would also be easily accepted by the patients . So we observed the efficacy of liver targeted gene delivery using intravenous and intraperitoneal routes.

Receptor mediated gene transfer was carried out by high affinity linkage between the ligands (binding to the foreign gene) and specific receptors on the surface of different kinds of cells, such that the foreign gene could be delivered into the cells by phagocytosis[24,42]. It has been reported that the ratio of the targeted uptake by the liver delivered by G-PLL could reach up to 70%-90% *in vivo*[43,44]. In our experiments we found that in additio n to its main location in the liver, the plasmid binding to G-PLL could be also expressed in kidneys, spleen, and lungs. It might be due to the existence of AS GP-R in the other extrahepatic tissues[45,46].

Recently Zhang *et al*[42]found that intravenous injection was an us eful method for delivery to liver by a target carrier. We found that the peripheral vein route was better than abdominal cavity in the targeted delivery of the plasmid bound to G-PLL. We conjectured that although the majority of the DNA/ ca rrier complex would reach the liver through portal vein after the absorption by the abundant capillary bed of peritoneum, quite a lot of the complex would possibly be absorbed or degraded by other organs in the abdominal cavity, thus leadin g to the decreased distribution and expression of the plasmid in the liver. In the same way, it might be one of the reasons that its distribution in the spleen reached to a relatively high level. In this experiment, we also observed whether G-PLL binding to the plasmid would induce some toxicity to the body. We did not find any detrimental effect on the functioning of important organs such as liver, heart, and kidney, and this further indicated that G-PLL could be used safely *in vivo* as the delivery carriers for drug or nucleotides.

In conclusion, we found that the plasmid bound to GPLL could be delivered to the liver efficiently and safely, and the intravenous transference was better than peritoneal transference for the targeted uptake by the liver when G-PLL was used as a carrier. But whether G-PLL can be used to deliver drugs or nucleotides for the treatment of liver diseases in human beings by intravenous route deserves further investigations.

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