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Study on in vivo distribution of liver-targeting nanopaticles encapsulating thymidine kinase gene (TK gene) in mice

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Abstract Nanoparticles formulated from polylactic-co-glycolic acid (PLGA) polymer loading a new recombinant plasmid pEGFP-TKAFB (TK-PLGA-NPs) were prepared by a double-emulsion evaporation technique. Both in vitro and in vivo release behaviors of TK-PLGA-NPs (with particle diameter ranged from 50 to 100 nm) were investigated, using ethidium bromide (EB) staining and gamma scintigraphy, respectively. The results indicated that the in vitro release rate of DNA (pEGFP-TKAFB plasmid) in TK-PLGA-NPs showed good fit into the Higuichi Equation and dependence in the molecular weight of PLGA polymer. 0.5 h after injection of nanoparticles containing ^{32}P labeled pEGFP-TKAFB plasmid (^{32}P-TK-PLGA-NP) via caudal vein of the mice, the ratio of radioactivity intensity in the liver to total intensity was above 70%, which showed a 1.4-fold increase over that by injection of ^{32}P labeled pEGFP-TKAFB plasmid (^{32}pEGFP-TKAFB plasmid, ^{32}P-TK). Similarly, 2 h after hypodermic injection of ^{32}P-TK-PLGA-NPs in mice, the ratio of radioactivity in the liver against total radioactivity was more than 70%, which was 1.6-fold compared with naked ^{32}P-TK. All these data showed that the TK-PLGA-NPs has the potential for liver-targeting and delayed drug release.

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Introduction

In recent years, nanoparticles (NPs) have attracted extensive attention as an efficient and promising drug delivery system. Several studies [1, 2] have shown that as a drug delivery carrier, nanoparticles possess several advantages: alteration of biodistribution of the drugs, low immunogenicity, low toxicity, good stability as well as simple and convenient preparation procedures; besides, nanoparticles, when modified on the surface, will develop some specific characters, for instance, targetibility [3]. Moreover, nanoparticles have the suitability to scale up and industrialized production and can be stably stored using the technique of lyophilization. Such properties suggest that nanoparticles be prospective novel drug carriers in clinical applications.

Among all present gene therapy protocols, combination of administration of GCV and transfection of thymidine kinase gene of *Herpes simplex virus* (HSV-TK) into tumor cells is perhaps the most practical and potentially useful way in intratumoral gene therapy. In the tumor cells, the expression product of the TK gene can catalyze the phosphorylation of non-toxic GCV to form a cytotoxic agent, which can cause the suicide of cells. This protocol presents good potential in intratumoral gene therapy [4, 5]. However, common TK genes (naked genes) aren't capable to target to specific organs and tissues, and thus can be harmful to the normal cells and tissues. In addition, they are easily degraded by nucleases in vivo.

To solve the problems mentioned above, we constructed a recombinant plasmid (pEGFP-TKAFB) composed of fragment of TK gene, enhanced green fluorescent protein (EGFP) gene and α-fetoprotein (AFP) promoter. The AFP promoter has specific activity in cells containing α-fetoprotein, for instance, hepatoma cells. Therefore, under non-pathological conditions, the recombinant plasmid could be

expressed in liver-tumor specifically. Such a tumor-selective plasmid was encapsulated into the non-toxic and biodegradable PLGA polymer to form TK-PLGA-NPs, which could protect the plasmid DNA from digestion bynuclease, effectually.

The biodistribution behavior of nanoparticles was important and significant in evaluation of a targeting drug delivery system. Previous studies [6, 7] indicated that nanoparticles were immediately phagocytosized by the macrophage cells after entering the blood circulation and then were delivered to the organ located with high density of macrophage cells, liver in particular. In our current study, in order to evaluate the liver-targetibility of the TK-PLGA-NPs, which size ranged from 50 to 100 nm, gamma scintigraphy was used to investigate the in vivo distribution of TK-PLGA-NPs in mice.

Materials and methods

Instruments and materials

Instruments

FJ-2015 Liquid Scintillation Counter (Xi'an 206 Factory, China); TGL-16 Table-top Ultracentrifuge (The 6th Factory of Shanghai Medical Appliance, China); 1712 Electronic Balance (Startorius, Germany); JY92-II Probe Ultrasonic Processor (Ningbo Scientz Biotechnology Co.,Ltd., China); ModulyoD Freeze Dryer (ThermoSavant, U.S.A.); RF-5301 Spectrofluorophotometer (Shimadzu, Japan); 85–2 Constant Temperature Magnetic Stirrer (ShangHai SiLe equipment factory, China); Hoefer^{TM} Horizontal Gel Electrophoresis System and SX100 Gel Imaging System (Amersham, U.S.A.)

Materials

pNGVL-TK and pUC-AFPLB plasmid were kind gifts from Dr. Jin Huang (Shanghai first people's hospital), pEGFP-N1 plasmid were stored in our laboratroy, DNase I was purchased from Chengdu Huamei Biochemicals Cooperation (Sichuan Province, China), T4 Polynucletide Kinase (10 U/mL), Mung Bean Nuclease, [γ-32P]dATP, Hind III, BamH I were purchased from Promega Co.Ltd., Calf intestinal alkaline phosphatase (CIAP) were from Promega Co.Ltd.. Poly(D,L-lactic-co-glycolic acid) (PLGA; lactic-glycolic acid ratio: 75:25, Mr = 30 000, batch number: 020112) was purchased from Chengdu Institute of Organic Chemistry, Chinese Academy of Science. Kunming mice, weighted 18–22 g, were provided by Laboratory Animal Center of Sichuan University.

Methods

Construction of recombinant plasmid (pEGFP-TKAFB)

pEGFP-N1 gene fragment, TK gene fragment (1125 bp), AFP-Alb promoter fragment (800 bp, containing promoter and enhancer) were attained by digesting the pEGFP-N1, pNGVL-TK and pUC-AFPLB plasmid with restriction enzyme separately. TK fragment was ligated with the pEGFP-N1 fragment at the sticky 5′ ends cut by restriction enzyme Sma I and the AFP-Alb fragment ligated at the sticky 3′ ends cut by restriction enzyme Hind III. Then, the sticky-ends of the combined long fragment were cut into blunt-end by Mung Bean Nuclease and the recombinant plasmid was obtained by ligating the two blunt-ends. Figure 1. shows the construction of recombinant expression vector pEGFP-TKAFB). The recombinant plasmids were transformed into

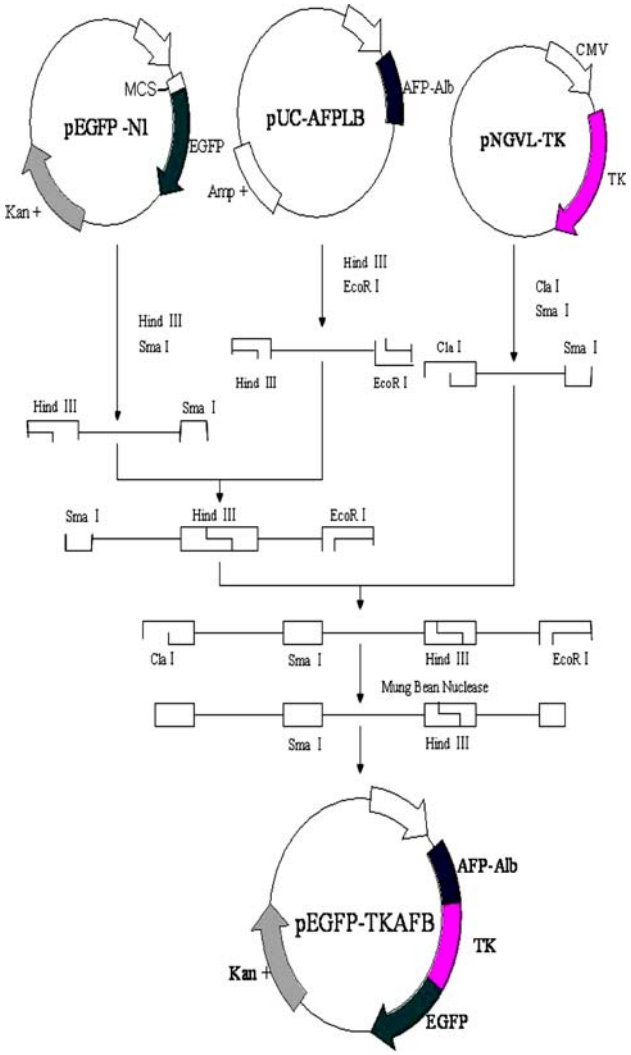

Fig. 1 The construction of recombinant expression vector pEGFP-TKAFB

the *Ecoli DH-5α*. The positive transformant clones were selected by kalamycin and the plasmids purified from the positive clone were analyzed by the 1% agarose gel electrophoresis of restriction enzyme analysis map, using the horizontal gel electrophoresis system and gel imaging system. (See "Identification of recombinant plasmid (pEGFP-TKAFB)" section.

Preparation of TK-PLGA-NPs [8]

TK-PLGA-NPs were prepared by w/o/w double-emulsion evaporation technique. Briefly, 100 μl pEGFP-TKAFB (2 mg/mL) was added in dichlormethane in a penicillin glass bottle, emulsified by Ultrasonic Processor (5 s, 40 w), then 2 mL 1% PVA solution was added into the primary emulsion, emulsified by the Ultrasonic Processor using the same parameters mentioned above. Then, the emulsion was added with 8 mL 1% PVA solution, and the mixture was agitated under magnetic stirring at room temperature for 3 h to evaporate the organic solvent, with the aid of the constant temperature magnetic stirrer. The colloid solution of ^{32}P-TK-PLGA-NPs was finally obtained, which could be lyophilized with freeze dryer, adding 1% lactose (w/v) as cryoprotectant for long-term storage.

The release rate of TK-PLGA-NPs in vitro

Lyophilized TK-PLGA-NPs were separately prepared with PLGA in different ratios, by the method mentioned above. Each lyophilized preparation was redissolved with 10 mL saline, and the colloid solution was incubated in 37 °C water-bath, oscillating. Sampling TK-PLGA-NPs solutions according to the sampling scheduler (See Fig. 4), each nanoparticle sample (10 mL) was centrifuged by table-top ultracentrifuge at 40,000 rpm/min for 1 h, at 4 °C, respectively. The supernatant was assessed by florescence spectrometry after stained with ethidium bromide (EB) to determine the concentration of released DNA and the sediment was dispersed with 10 mL saline again and continued incubation in 37 °C water-bath. The release rate (F) was estimated as the ratio of the accumulated of concentration DNA in supernatant at each schedualed time interval to the total concentration contained in each nanoparticle solution. The release curve of lyophilized TK-PLGA-NPs was illustrated in Fig. 4.

The body distribution study of the ^{32}P-TK-PLGA-NPs in mice [9]

End labeling the recombinant plasmid with 32P [10[t1]]

Linearization of pEGFP-TKAFB. The purified recombinant plasmid was linearized with the restriction enzyme Hind III, and the product was analyzed by 1% agarose gel electrophoresis with the horizontal gel electrophoresis system, to investigate whether the digest reaction was completed. The product was then extracted by hydroxybenzene-chloroform and deposited with ethanol.

Dephosphorylation of the 5′ end. 100 μg (30 μl) linearized pEGFP-TKAFB plasmid, 10 μl 10 × CIAP buffer, 50 U (1 U/μl) CIAP and water (adding the reaction volume to 100 μl) were mixed together, reacting at 37 °C for 0.5 h, then 50 U (1 U/μl) CIAP was added, incubating at 37 °C for 0.5 h, then at 65 °C for 1 h., The product was extracted by hydroxybenzene-chloroform method and was deposited with ethanol.

End labeling the dephosphorylated-5′ end plasmid with ^{32}P. 100 μg linear dephosphorylated-5′ end pEGFP-TKAFB, 5 μl 10 × T^{4}Polynucleotide Kinase buffer, 50 pmol [γ-^{32}P]dATP(specific activity was 3,000 Ci/mmmol 10 uCi/μl,10–20 U T^4 Polynucleotide Kinase, and water (being added to the reaction volume to 50 μl) were incubated together at 37 °C for 0.5 h, then 2 μl EDTA (0.5 mol/L pH 8.0) was added in, the product was extracted by hydroxybenzene-chloroform once and deposited with ethanol. And the raw product was deposited with ethanol twice to separate the ^{32}P-DNA from the residuary [γ-^{32}P]dATP. The Radiochemical purity of the purified ^{32}P-DNA (^{32}P-TK or ^{32}pEGFP-TKAFB) was 97.22%.

Sample preparation for liquid scintigraphy detection

Each tissue sample was digested with 0.2 mL 60% oxydol and 0.01 mL *n*-octyl alcohol in 80 °C waterbath for 30 min. 0.1 mL digested juice and 10 mL double-distilled water was added into the detecting utensil, and then was assessed with the liquid scintillation counter to obtain the cpm value of each sample.

The in vivo distribution after caudal-vein injection

100 Kunming mice, weighing 18–22 g, male or female, were randomly divided into ten test groups and ten control groups, five in each group. ^{32}P-TK-PLGA-NPs were administrated in the test group via caudal-vein injection, at a dose of 0.1 mL/10 g. Similarly, each mouse in the control group received the ^{32}P-TK (^{32}pEGFP-TKAFB) plasmid at the same dose via caudal intravenous injection. At predetermined time intervals, mice in the test and control groups were sacrificed. Blood, hearts, livers, spleens, lungs, and kidneys were collected and removed from each mouse, respectively. Each organ was weighed (the blood weight was thought to share 8% of body weight) and then digested following the method mentioned above (See 2.3). The radioactivity intensity of each organ was detected by liquid scintillation counter. The cpm of each organ (cpmi) was the ratio of scintillation count value to the weight of the organ, and the cmpt of a mouse was the total value of cmpi in each organ. The ratio of cmpi/cmpt × 100% represented the distributed content of DNA in each viscera and blood.

The in vivo distribution after hypodermic injection

100 Kunming mice, weighing 18–22 g, male or female were randomly divided into 10 test groups and 10 control groups, five in each group. The ^{32}P-TK-PLGA-NPs were administrated in test group via hypodermic injection at a dose of 0.1 mL/10 g. Similarly, each mouse in the control group received the ^{32}P-TK (^{32}pEGFP-TKAFB) plasmid at the equal dose of 0.1 mL/10 g via hypodermic injection. All mice were treated with the same method as the caudal-vein injection groups. The ratio of cmpi/cmpt × 100% was calculated using the same method mentioned above.

Results

Identification of recombinant plasmid (pEGFP-TKAFB)

After incubation with endonucleases BamH I, the recombinant plasmid (pEGFP-TKAFB) was cleaved into two fragments: a 5.1 kb fragment and a 1,530 bp fragment, which indicated that the exogenous fragments (TK fragment and AFP-Alb fragment) containing the targeting site for BamH I, had successfully inserted into the host plasmid (pEGFP-N1) without specific site for BamH I.

About of 4,700 bp fragment and 1,930 bp fragment were obtained after digestion of the recombinant plasmids by Sma I and Hind III simultaneously, which substantiate the insertion of exogenous fragments into the host plasmid in the correct orientation (Fig. 2).

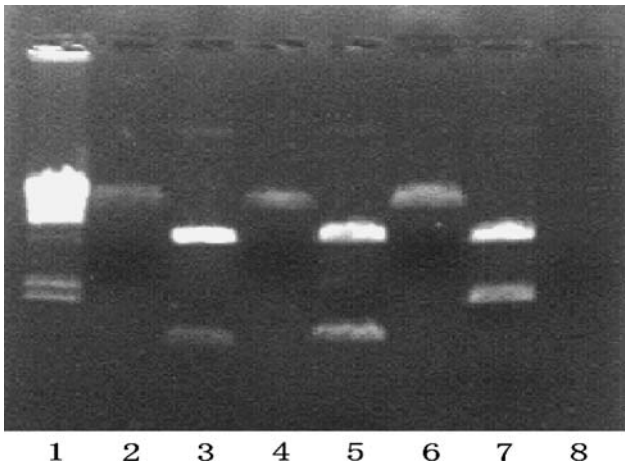

Fig. 2 Agarose gel electrophoresis of restricted endonucliotide enzyme analysis map of recombinant expression vector ((1) λDNA/HindIII maker, (2) pEGFP-TKAFB(11) plasmid, (3) pEGFP-TKAFB(11) plasmid/ SmaI+HindIII, (4) pEGFP-TKAFB(18) plasmid, (5) pEGFP-TKAFB(18) plasmid/ SmaI+HindIII, (6) pEGFP-TKAFB(18) plasmid, (7) pEGFP-TKAFB(18) plasmid/ BamHI

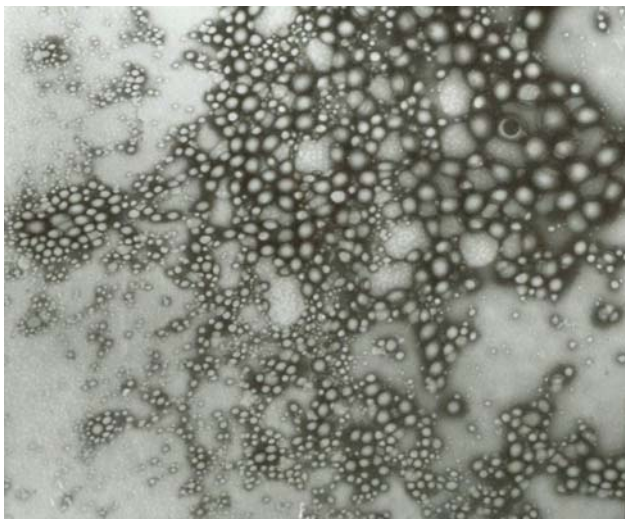

Fig. 3 The TEM image of the colloid solution of TK-PLGA-NPs

Size, morphology and entrapment efficiency

The TK-PLGA-NPs prepared by w/o/w double-emulsion evaporation technique showed regular spherical morphology and a narrow size distribution with an average size of 72 ± 12 nm detected by the laser diffusion method. The TEM image of the colloid solution of TK-PLGA-NPs was displayed in Fig. 3. The entrapment efficiency was 91.25% analyzed by fluorescence spectrophotometry.

The in vitro release kinetics

From the release curve (Fig. 4), it was confirmed that the release rate increased as the GA/PLGA ratio increased, and at the same GA/PLGA ratio, the release rate increased as the viscosity of PLGA reduced.

The five release curves of TK-PLGA-NPs prepared by different PLGA were fit into Higuichi equation, First-order Kinetic equation, and two-biexponentical equation. The

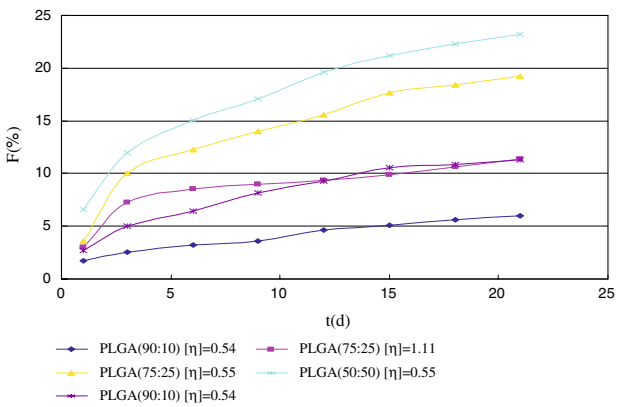

Fig. 4 The release curves of lyophilized DNA-PLGA-NPs

result indicated that the in vitro release kinetics of TK-PLGA-NPs could be well chatacterized by Higuichi Equation.

The in vivo distribution of ^{32}P-TK after caudal-vein injection

0.5 h after the caudal-vein injection of 32P-TK-PLGA-NPs, the radioactivity ratio in the mice livers achieved 73.37% while the radioactivity ratio in the livers administrated with ^{32}P-TK was 52.59%, which exhibited significant difference. ($p < 0.01$) (Table 1and 2) The ^{32}pEGFP-TKAFB plasmid (^{32}P-TK) loaded in TK-PLGA-NPs showed higher specificity to liver, resulting in the higher concentration of the pEGFP-TKAFB in liver than in other organs.

The in vivo distribution of ^{32}P-TK in mice after hypodermic injection

2 h after hypodermic injection with ^{32}P-TK-PLGA-NP in mice, the radioactivity ratio against the mice livers against total radioactivity exceeded 70%, which was 1.6-fold of that after injection with ^{32}P-TK, (See Table 3 and 4), the difference was significant ($p < 0.01$). But the radioactivity ratio reduced quickly. All these results indicated that TK-PLGA-NPs were a potential liver-targeting and delayed-release carrier. Moreover, our study revealed that the radioactivity ratios in each organ after hypodermic injection were much lower than that after caudal-vein injection.

Discussion

In general, it is indeed difficult for exogenous plasmid DNA to reach the nucleus of host cell, due to the following reasons such as the charge-repulsion between the negatively-charged DNA, the negatively-charged cell membrane and the nuclease digestion in cytoplasm, etc. At present, endocytosis is considered to be the major route by which the host cells uptake DNA [11]. Cationic liposomes are frequently used as gene delivery carriers, however, they have severe cytotoxicity especially at high concentrations and their delivery action is drastically deterred by some unknown components in cytoplasm, which strongly restricts its application in gene delivery [12]. In contrast, nanoparticles are an ideal gene carrier to effectively protect

Table 1 The body distribution of ^{32}P-TK in mice after ^{32}P-TK plasmid after i.v. administration(via caudal intravenous injection, $n = 5$)

Time	Heart	Liver	Spleen	Lung	Kidney	Blood
5 min	3.14 ± 0.60	36.67 ± 2.65	3.56 ± 1.58	16.32 ± 0.79	23.52 ± 1.48	16.79 ± 1.62
15 min	3.15 ± 0.97	41.26 ± 2.89	5.25 ± 0.86	14.16 ± 0.99	20.61 ± 1.62	15.57 ± 1.58
30 min	6.94 ± 1.57	52.59 ± 4.02	10.46 ± 0.75	7.95 ± 0.58	13.10 ± 0.85	2.95 ± 0.65
1 h	6.11 ± 1.95	54.62 ± 3.12	14.85 ± 1.23	7.82 ± 0.67	13.50 ± 0.94	3.10 ± 0.78
2 h	4.56 ± 1.01	51.34 ± 2.88	14.93 ± 0.68	9.65 ± 1.41	15.03 ± 1.34	4.49 ± 0.28
6 h	5.12 ± 0.79	48.66 ± 1.87	13.15 ± 0.84	9.66 ± 1.27	18.26 ± 1.58	5.15 ± 1.67
12 h	5.48 ± 0.99	44.52 ± 2.02	11.32 ± 1.33	11.36 ± 1.34	21.83 ± 2.54	5.49 ± 0.58
24 h	3.93 ± 0.65	43.31 ± 1.67	11.48 ± 1.11	10.12 ± 0.64	25.01 ± 3.12	6.16 ± 0.94
48 h	3.43 ± 0.84	36.75 ± 1.65	11.56 ± 0.82	13.36 ± 1.60	28.52 ± 2.15	6.38 ± 0.83
72 h	4.52 ± 0.77	34.48 ± 1.85	12.03 ± 1.73	13.88 ± 1.63	28.31 ± 3.01	6.78 ± 0.91

Table 2 The body distribution of ^{32}p-TK in mice after ^{32}P-TK-PLGA-NPs after i.v. administration(via caudal intravenous injection, $n = 5$)

Time	Heart	Liver	Spleen	Lung	Kidney	Blood
5 min	2.22 ± 0.51	57.32 ± 2.36	2.96 ± 0.69	8.29 ± 1.33	16.58 ± 2.02	12.63 ± 1.24
15 min	3.52 ± 0.64	69.69 ± 3.32	3.19 ± 0.58	5.77 ± 0.55	13.87 ± 0.99	3.96 ± 0.36
30 min	2.38 ± 0.67	73.37 ± 3.62	7.55 ± 1.03	3.64 ± 0.41	11.28 ± 1.28	1.78 ± 0.65
1 h	2.08 ± 0.54	80.14 ± 4.56	3.37 ± 0.67	3.08 ± 0.62	9.34 ± 0.68	1.99 ± 0.54
2 h	2.59 ± 0.28	78.45 ± 4.02	3.73 ± 0.76	5.35 ± 0.58	8.36 ± 0.94	1.47 ± 0.23
6 h	3.18 ± 0.60	75.03 ± 3.69	7.38 ± 1.03	3.74 ± 0.39	9.61 ± 0.96	1.06 ± 0.24
12 h	2.21 ± 0.51	69.34 ± 3.96	6.17 ± 1.24	7.88 ± 0.64	10.17 ± 1.04	4.22 ± 0.32
24 h	1.94 ± 0.32	65.59 ± 3.25	6.46 ± 1.04	7.95 ± 0.57	12.10 ± 1.23	5.96 ± 0.59
48 h	2.21 ± 0.29	61.18 ± 2.69	7.01 ± 0.48	9.27 ± 0.71	12.87 ± 0.86	6.46 ± 0.86
72 h	2.51 ± 0.37	60.56 ± 2.98	6.45 ± 0.69	9.98 ± 0.65	13.54 ± 1.11	6.96 ± 0.75

Table 3 The body distribution of ^{32}P-TK in mice after ^{32}P-TK plasmid after s.c. administration(via subcutaneous injection, $n = 5$)

Time	Heart	Liver	Spleen	Lung	Kidney	Blood
5 min	4.91 ± 0.59	25.63 ± 2.51	4.11 ± 0.35	9.85 ± 0.77	30.76 ± 2.69	24.74 ± 2.02
15 min	5.89 ± 0.53	34.86 ± 2.02	3.32 ± 0.34	7.25 ± 0.64	26.35 ± 1.58	22.33 ± 1.36
30 min	5.90 ± 0.39	40.75 ± 1.68	4.84 ± 0.42	6.82 ± 0.68	23.21 ± 1.84	18.48 ± 1.64
1 h	9.16 ± 1.25	42.80 ± 1.95	9.17 ± 0.68	9.20 ± 0.58	20.13 ± 2.34	9.54 ± 0.69
2 h	5.97 ± 0.51	49.09 ± 0.96	10.67 ± 1.66	7.30 ± 0.47	18.06 ± 1.47	8.91 ± 0.54
6 h	6.74 ± 0.46	52.91 ± 3.02	11.13 ± 2.31	8.42 ± 0.63	13.09 ± 1.63	7.71 ± 0.44
12 h	7.91 ± 0.48	53.33 ± 3.69	11.14 ± 1.38	9.38 ± 1.06	10.76 ± 1.21	7.48 ± 0.52
24 h	7.74 ± 0.68	46.74 ± 3.58	11.92 ± 1.19	10.31 ± 0.67	16.45 ± 0.67	6.84 ± 0.42
48 h	7.83 ± 0.44	44.47 ± 4.01	9.47 ± 0.47	11.91 ± 0.76	19.75 ± 1.25	6.57 ± 0.35
72 h	7.52 ± 0.15	35.48 ± 2.34	12.03 ± 1.02	13.88 ± 1.28	25.31 ± 2.21	5.78 ± 0.51

Table 4 The distribution of ^{32}P-TK in mice after ^{32}P-TK-PLGA-NPs after s.c. administration (via subcutaneous injection, $n = 5$)

Time	Heart	Liver	Spleen	Lung	Kidney	Blood
5 min	4.65 ± 0.63	37.19 ± 2.31	3.26 ± 0.21	8.22 ± 0.36	27.13 ± 1.84	19.55 ± 0.56
15 min	3.72 ± 0.26	46.21 ± 2.54	4.35 ± 0.25	6.96 ± 0.48	25.02 ± 2.07	13.74 ± 0.69
30 min	5.65 ± 0.44	52.49 ± 3.01	6.14 ± 0.32	5.53 ± 0.25	20.83 ± 1.58	9.36 ± 0.57
1 h	4.13 ± 0.41	59.52 ± 3.20	7.38 ± 0.23	4.95 ± 0.34	15.59 ± 0.98	8.43 ± 0.21
2 h	2.96 ± 0.35	77.80 ± 3.54	4.95 ± 0.19	3.81 ± 0.16	10.41 ± 0.38	6.07 ± 0.14
6 h	3.43 ± 0.36	78.40 ± 3.11	3.69 ± 0.30	2.81 ± 0.13	6.61 ± 0.44	5.06 ± 0.11
12 h	4.29 ± 0.51	68.25 ± 2.57	8.17 ± 0.34	5.87 ± 0.21	9.41 ± 0.51	4.01 ± 0.17
24 h	2.94 ± 0.28	65.59 ± 2.74	7.46 ± 0.24	7.95 ± 0.28	12.10 ± 0.47	3.96 ± 0.24
48 h	4.47 ± 0.19	62.76 ± 2.68	9.58 ± 0.41	6.51 ± 0.14	12.77 ± 0.71	3.91 ± 0.20
72 h	7.29 ± 0.24	57.25 ± 2.74	9.46 ± 0.57	8.64 ± 0.32	13.85 ± 0.22	3.51 ± 0.13

DNA from nuclease digestion and carry several kinds of plasmids simultaneously. In addition, nanoparticles could remain stable for a relatively long time after lyophilization, their surface can be modified easily, plasmid DNA encapsulated in nanoparticles can obtain some desirable properties, such as controlled release, and more importantly, low cytotoxicity.

In the release study of TK-PLGA-NPs, it was found that the release rate increased with the ratio of GA in PLGA increased or the viscosity of PLGA was reduced. The reduced viscosity resulted in the descension of phase-transition temperature which lead to the faster release of nanoparticle [13]. As the viscosity is closely associated with the molecular weight, nanoparticles with control release character could be prepared using PLGA with proper molecular weight.

The release kinetics of DNA in TK-PLGA-NPs followed the Higuichi equation very well, which indicate that TK-PLGA-NPs exhibited a release pattern without "burst effect" and the DNA was completely encapsulated in the nanoparticle.

The in vivo distribution study of DNA was rarely reported. However, with the rapid development of gene therapy, the in vivo distribution of therapeutic genes will become more and more important in the study of the target gene delivery systems.

The isotope tracer technique has many advantages in the in vivo distribution study such as high sensitivity, easy detection and less interference to the metabolism of test animals. And in isotope tracer technique, the selection of radioactive label should be based on the half-life and the radiability of the substance. In our study, ^{32}P was considered to be an ideal label material for the body distribution assays, because its half-life is 14 days while the investigation just lasted 3 days and its ray was a kind of high-energy ß ray which was easily tested, in addition, ^{32}P is one of the most commonly used probes to label DNA.

Conclusion

In this paper, nanoparticle gene delivery system—TK-PLGA-NPs were prepared by w/o/w double-emulsion evaporation technique. Such nanoparticles have an ideal in vitro release pattern that could be easily controlled by changing the ratio of GA in PLGA and the molecular

weight of PLGA. And the in vivo distribution studies suggested TK-PLGA-NPs characterized by liver-targeting and delayed-release properties could be a prospective drug carrier in liver cancer gene therapy.

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