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In vivo biodistribution for tumor targeting of 5-fluorouracil (5-FU) loaded N-succinyl-chitosan (Suc-Chi) nanoparticles

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ABSTRACT

5-fluorouracil-loaded *N*-succinyl-chitosan nanoparticles (5-FU-Suc-Chi/NP) were prepared by an emulsification solvent diffusion. Biodistribution and tumor targeting were evaluated after i.v. administration of 5-Fu-Suc-Chi/NPs in Sarcoma 180-bearing mice. Also, pharmacokinetic profiles were evaluated after intravenous injection of 5-Fu-Suc-Chi/NP via the tail vein to rats. Our experimental results showed the 5-FU-Suc-Chi/NPs could be sustained at a high level in blood throughout a very long time, implying its long systemic retention in blood circulation. 5-FU-Suc-Chi/NPs were distributed mainly in tumor, liver and a small quantity of 5-FU-Suc-Chi-NPs was found in kidney and speen. 5-FU-Suc-Chi/NPs scarcely accumulated in the heart and lung, lowered the toxic effect of 5-FU to them. Pharmacokinetic analysis in plasma showed area under plasma concentration-time curve(AUC), elimination half-life ($t_{1/2}$) and residence time (MRT) were increased 2.5-fold, 10.98-fold and 10.8-fold for 5-FU-Suc-Chi/NP compared with that of free 5-FU, respectively. All the results indicates that longevity in blood circulation and tumor targeting of 5-FU-Suc-Chi/NPs should be achieved.

KEYWORDS

N-succinyl-chitosan;
Nanoparticles;
Biodistribution;
Pharmacokinetic;
Tumor targeting.

INTRODUCTION

Nanotechnology has advanced greatly in recent years and is becoming a promising approach for cancer treatment. Owing to their small size, prolonged circulation time, and sustained drug release profile, nanosized polymeric nanoparticles bearing anticancer drugs have received an increasing amount of attention for their ability to improve the efficacy of anticancer drugs[1–3]. the prolonged circulation time of polymeric nanoparticles allows them to extravasate and accumulate into tumor tissue, resulting in a disorganized and defective vascular architecture, which is referred to as the enhanced permeability and retention (EPR) effect in tumor tissue[4,5]. Therefore, tumor targeting of polymeric nanoparticles has been recognized as an effective strategy for passive tumor targeting in the body.

To reduce the toxicity and increase the therapeutic efficacy of anticancer drugs, Suc-Chi-NP have been developed by a emulsification solvent diffusion method

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previously reported by us[6]. In this study, the distribution of the particle size, the zeta potential, the drug loading content and the drug loading efficiency of the prepared nanoparticles, and their release profile were investigated in vitro. Also, the anti-tumor activity of 5FU-Suc-Chi-NP were evaluated by measuring the change in the tumor volume. The 5FU-Suc-Chi-NP showed good antitumour activities against Sarcoma 180 solid tumour and mild toxicity.

Following our previous work, further aim is to evaluate tissue distribution (blood, liver, spleen, kidney, lung, heart, and tumor) of 5-Fu-Suc-Chi/NP and their tumor selectivity by enhanced permeability and retention (EPR) effect in Sarcoma 180 -bearing mice. Also, pharmacokinetic profiles were evaluated after i.v. administration of 5-Fu-Suc-Chi/NP in rats.

EXPERIMENTAL

Materials *N*-Succinyl-chitosan sodium salt (Suc-Chi; MW 3×10^5; degree of *N*-succinylation per chitosan hexosamine unit 76%) was supplied by Shenyang Pharmaceutical University (Shen-yang, China). 5-FU was purchased from Jiqi Pharmaceutical Factory (Shen-yang, China). All other chemicals were obtained commercially as reagent-grade products.

Animals Male Balb/c mice weighing approximately 18–22g, and male Wistar rats weighing approximately 180–220g were provided by the Animal Experimental Center of Shenyang Pharmaceutical University. All studies were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication no.92–93, revised in 1985), and were approved by the Department of Laboratory Animal Research at Shenyang Pharmaceutical University. The procedures with animals were reviewed and approved by the Animal Ethical Committee at Shenyang Pharmaceutical University.

Tumours Sarcoma 180 cells (solid malignant tumors) were maintained by weekly transplantation of 1×10^7 cells suspended in Hanks' balanced solution(0.1ml) into the peritoneal cavity of each mouse. Sarcoma 180 cells (1×10^7) suspended in 0.1mL of Hanks' balanced solution, which were obtained from the above tumour-bearing mice, were inoculated subcutaneously into each mouse at the axillary region. The tumours were allowed to develope for 7 days.

Preparation of 5-FU-Suc-Chi/NP 5-FU-Suc-Chi/NPs were prepared by the previously reported method[6]. Briefly blank nanoparticles were obtained upon the addition of 10ml the Suc-Chi aqueous solution (2mg/ml) to 100ml of the Span-80 organic solution, which contains 20% ethanol (v/v) stirred at room temperature. water was then evaporated from the colloidal suspension with a magnetic at 40°C by a vacuum-pump. Nanoparticles suspension were centrifugated after ultrasonic treatments. The precipitate was washed and dispersed in 10ml of H_2O. Mannitol 0.1% (w/v) was added to the nanoparticle suspension and it was lyophilized. 5-FU-loaded nanoparticles were obtained according to the same procedure.

Pharmacokinetic analysis A single dose of free 5-FU or 5-FU-Suc-Chi/NP (30 mg/kg) was administered to rats. Blood samples were collected from rat veins at designated times after intravenous administration. 5-FU was extracted from plasma by mixing rat plasma with ethyl acetate and isopropyl alcohol (85/15, v/v). The samples were then dried with N_2 at 37°C and the dehydrated samples were dissolved in 400μl of mobile phase dilutent for subsequent HPLC. Pharmacokinetic(PK) parameters were calculated by noncompartment analysis based on statistical moment theory using microsoft excel software. The area under the plasma concentration–time curve up to the last time (t) (AUC_{0-t}), area under curve extrapolated to infinity ($AUC_{0-\infty}$) and area under the first moment curve extrapolated to infinity ($AUMC_{0-\infty}$) were calculated using the linear trapezoidal rule. The mean residence time (MRT) was calculated as AUMC/AUC.

Biodistribution of 5-FU-Suc-Chi/NP in tumor-bearing mice At 7 days after subcutaneous inoculation, A single dose of free 5-FU or 5-FU-Suc-Chi/NP (30mg/kg) were injected into the tail vein of the tumor-bearing mice. After a definite time period, blood samples were obtained using a capillary from the retro-orbital plexus. Animals were sacrificed and heart, kidney, liver, lung, spleen, and tumor were collected. The distributed amount of 5-FU in tissues and blood was estimated by HPLC. Each tissue taken from Sarcoma

180-bearing mice, was washed with phosphate-buffered saline (PBS, pH 7.4) and wiped with a filter paper, then PBS was added by a three-fold volume of the weight of the tissue, and the mixture was homogenized. After centrifugation of the homogenate, the concentrations of 5-FU in tissues samples were determined by HPLC. Consequently, the amount of 5-FU in each tissue was calculated from the concentration and tissue weight. relative tumor tissue exposures (Re)and the ratios of peak concentrations(Ce) were calculated according to the following formula[7,8]: 100% × $(AUC_{tumor})_{5\text{-Fu-Suc-Chi/NP}}/(AUC_{tumor})_{5\text{-FU}}$ and 100% × $(C_{max})_{5\text{-Fu-Suc-Chi/NP}}/(C_{max})_{5\text{-FU}}$.

HPLC assay Concentrations of 5-FU in tissues and plasma samples were determined by HPLC. The HPLC system consisted of a model LC-10AT pump (Shimadzu, Kyoto, Japan), and a model SPD-10A UV detector (Shimadzu, Kyoto, Japan). Separations were performed at 25°C using a 250mm × 4.6mm column (Diamonsil C18, Dikma, USA).The mobile phase was 0.01M KH_2PO4, which was filtered and delivered at a flow rate of 1.0mL min^{-1}. The column was maintained at a temperature of 25°C. The eluent was detected by UV detector at 266nm.

Statistic Analysis The results, obtained by in vivo studies, were statistically analyzed by using Student's *t*-test with a 95% confidence level ($p<0.05$) and were reported as mean ± standard deviation (SD).

RESULTS & DISCUSSION

5-fluorouracil-loaded *N*-succinyl-chitosan nanoparticles were prepared, using a modified emulsion solvent diffusion method[6]. The resulting nanoparticles had a mean diameter of 220–260nm, with a mean zeta potential of approximately -26mV. The formulation with an initial 5-FU concentration of 1000μg ml^{-1} provided the highest loading capacity (19%) and the highest extent of release (61% at 24 h)[6].

As shown in TABLE 1 and Figure 1, after i.v. administration of free 5-FU and 5-FU-Suc-Chi/NP suspensions in rats, area under plasma concentration-time curve(AUC), elimination half-life ($t_{1/2}$) and residence time (MRT) were increased 2.5-fold, 10.98-fold and 10.8-fold for 5-FU-Suc-Chi/NP compared with that of free 5-FU, respectively. This indicates that when 5-FU is loaded into nanoparticles, the 5-FU has sustained-release, prolonged half-life,and increased bioavailability.

TABLE 1 : Pharmacokinetic parameters of 5-FU-Suc-Chi/NPs and free 5-FU injection in mice after intravenous administration (n = 6) ** $p<0.01$; * $p<0.05$, compared to the corresponding parameters of free 5-FU.

Parameters	5-FU-Suc-Chi-NPs	5-FU injection
$T_{1/2}$ (h)	5.39±1.63 **	0.45±0.12
$AUC_{0-\infty}$ (μg·h/mL)	136.41±46.18 **	38.97±7.29
$AUMC_{0-\infty}$ (μg·h^2/mL)	1062.27±124.33	25.76±6.78
$MRT_{0-\infty}$ (h)	7.79±1.69 **	0.66±0.14
Ke(1/h)	0.13±0.02	1.51±0.34
CL(mL /h)	43.98±9.62	353.96±38.49
V(mL)	274.90±40.15	234.53±24.51

Biodistributions of the prepared nanoparticles in various organs in Sarcoma 180-bearing mice were evaluated at distinct durations after i.v. administration of 5-FU-Suc-Chi/NPs and 5-FU injection, as shown in Figure 2,3,4. At 1 hour, the amounts of 5-FU in the plasma did not show significant difference between 5-FU-Suc-Chi-NPs and 5-FU injection in the tumor-bearing mice. At 1day and 3 day, 5-FU injection couldn't be find in plasma and tissues samples. However, 5-FU-Suc-Chi/NPs could be sustained at a high level in blood throughout a very long time, implying its long systemic retention in blood circulation. The 5-FU were distributed mainly in tumor, liver and a small quantity of 5-FU was found in kidney and speen. The 5-FU scarcely accumulated in the heart and lung, lowered the toxic effect of 5-FU to them. The results indicates that longevity in blood circulation and tumor targeting of 5-FU-Suc-Chi/NPs should be achieved. The relative tumor tissue exposures (Re) and the ratios of peak concentrations(Ce) were 9.43±1.86 and 2.75±0.26 respectively. This showed that the tumor targeting. of 5-FU-Suc-Chi/NP was increased compared with 5-FU injection.

It is now generally known that the structure of solid tumors allows an enhanced permeation and retention (EPR) effect[9-11], as a result of which nanoparticles accumulate at the tumor site.

Our experimental results showed that 5-FU could be circulated in blood as a high level throughout the 3 days. The 5-FU were distributed mainly in kidney, tu-

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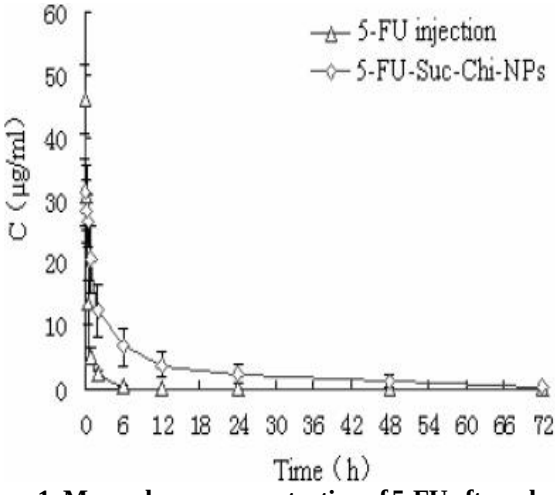

Figure 1: Mean plasma concentration of 5-FU after administration of free 5-FU and 5-FU-Suc-Chi/NPs. Data represent mean±SD (n = 6).

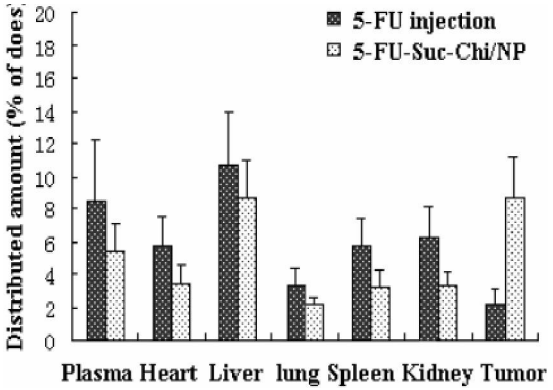

Figure 2: Biodistributions of of 5-FU at 1 hour after i.v. administration of 5-FU-Suc-Chi/NPs and 5-FU injection. At 7days after inoculation of Sarcoma 180 cells into the subcutaneous dorsa of mice, the 5-FU-Suc-Chi/NPs and 5-FU injection were injected into the tail vein of the tumor-bearing mice. Data represent mean±S.D. (n = 4).

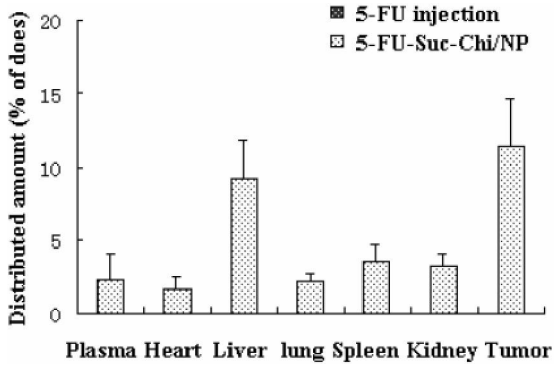

Figure 3 : Biodistributions of of 5-FU at 1day after i.v. administration of 5-FU-Suc-Chi/NPs and 5-FU injection. At 7days after inoculation of Sarcoma 180 cells into the subcutaneous dorsa of mice, the 5-FU-Suc-Chi/NPs and 5-FU injection were injected into the tail vein of the tumor-bearing mice. Data represent mean±S.D. (n = 4).

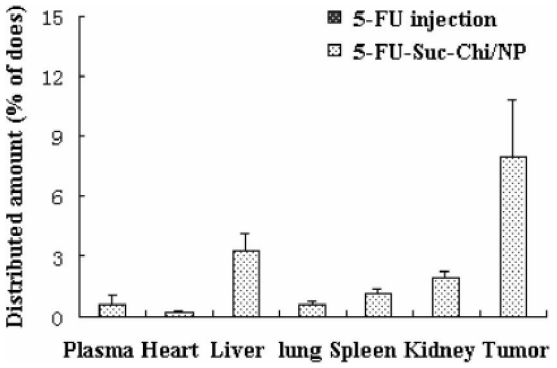

Figure 4: Biodistributions of of 5-FU at 3day after i.v. administration of 5-Fu-Suc-Chi/NPs and 5-FU injection. At 7days after inoculation of Sarcoma 180 cells into the subcutaneous dorsa of mice, the 5-FU-Suc-Chi/NPs and 5-FU injection were injected into the tail vein of the tumor-bearing mice. Data represent mean±S.D. (n = 4).

mor and a small quantity of 5-FU was found in liver and speen. This indicates that longevity in blood circulation of 5-FU-Suc-Chi/NP should be achieved, enabling a passive accumulation of 5-FU-Suc-Chi/NP into a solid tumor by EPR effect.

In general, the size of nanoparticles, developed for site-specific delivery of drugs must be controlled to avoid uptake by the reticuloendothelial system (RES)[12]. the optimal size should be less than 100 nm in diameter. It should also be pointed out that particles (240 nm in diameter)could accumulate passively in the tumor tissue, means that it should escape the reticuloendothelial cell system (RES) in spite of its large particle size. This can be suggested that surface properties generated by 5-FU-Suc-Chi/NP and /or the size change in blood by enzymatic degradation may partially be involved[13]. Consequently, 5-FU-Suc-Chi/NP were accumulated in the tumor tissue due to the EPR effect and its long systemic retention in blood circulation.

CONCLUSIONS

In the present study, the biodistribution of the 5-FU-Suc-Chi/NP in Sarcoma 180-bearing mice after i.v. injection was investigated. Our experimental results showed that 5-FU-Suc-Chi/NP could be circulated in blood as a high level throughout the 4 days and the accumulation amount of 5-FU-Suc-Chi/NP into the tumor site was increased as blood circulation time increased by the EPR effect. These findings indicate that

the nanoparticulate system with long circulation properties on the basis of Suc-Chi could be used as a carrier of anti-tumor drugs for tumor targeting.

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