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Sustained-release hydroxycamptothecin polybutylcyanoacrylate nanoparticles as a liver targeting drug delivery system

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Received August 1, 2003, accepted December 30, 2003

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Pharmazie 59: 791–794 (2004)

Sustained release hydroxycamptothecin polybutylcyanoacrylate nanoparticles (HCPT-PBCA-NP) associated with polybutylpyrolidone (PVP) for liver targeting were prepared by the adsorption-entrapping method. The morphology, sizes, drug loading efficiencies, release characteristics *in vitro*, distribution and pharmacokinetic parameters *in vivo* of the HCPT-PBCA-NP associated with PVP were studied. The median diameter of the particles was 81 nm and the drug loading was 1.2%. The release characteristics *in vitro* were in accordance with the Higuchi equation: $Q = 0.0615 + 0.0940\sqrt{t}$. 64.5% of the HCPT were concentrated in the liver at 15 min after i.v. administration of HCPT-PBCA-NP associated with PVP. The plasma drug concentration-time curve of the HCPT in rabbits was fitted to a two-compartment open model. The Vc, $T_{1/2}$ and CL were 3.5L; 147h; 0.18L · h^{-1}, respectively. The method of preparation presented in this paper seems to be an alternative for the preparation of PBCA-NP of poorly soluble drugs both in water and in lipid.

1. Introduction

Hydroxycamptothecin (HCPT) is an anti-tumor drug isolated from *Camptotheca acuminata* Decne. in the 1960's. Its toxicity is lower than that of camptothecin (Xu and Yang 1985). HCPT has good effects on hydroperitonia hepatic cancer, head and neck tumor, gastric carcinoma and bladder carcinoma (Ling and Xu 1993). It belongs to the type of cell cycle specific agents and its releasing time significantly influences treatment efficacy. Since the $t_{1/2}$ value of HCPT is only 5 min and it is metabolized rapidly in vivo, we are trying to slow down its releasing rate from the formulation and improve its therapeutic effect.

Nanoparticles have been shown to accumulate in the RES (Kreuter et al. 1979). Furthermore, many studies revealed that nanoparticle drug delivery systems have good liver targeting and sustained release characteristics, especially for the anti-neoplastic therapy with time-dependent and fast-metabolized drugs (Couvreur et al. 1995). In the present work, nanoparticles were selected for the development as HCPT sustained-released vehicles. However, since HCPT is poorly soluble both in water and in lipid solvents, the preparation is rather difficult. The aim of this work was to establish an efficient method of preparing sustained-release HCPT nanoparticles with liver targeting properties and to provide a reference for preparing nanoparticles of other anti-neoplastic drugs with similar properties. The preparation conditions and procedures of the hydroxycamptothecin polybutylcyanoacrylate nanoparticles (HCPT-PBCA-NP) were investigated. Furthermore, their morphology, size, drug loading, release characteristics *in vitro*, distribution in animals and pharmacokinetic parameters were also studied.

2. Investigations, results and discussion

2.1. Preparation of hydroxycamptothecin polybutylcyanoacrylate nanoparticles (HCPT-PBCA-NP)

Polybutylcyanoacrylate (PBCA) nanoparticles were prepared by the emulsion polymerization method. The average diameter of the particles was 55.7 nm. Drugs may be added to PBCA nanoparticles either by incorporating the drug in the polymerization medium or by adsorbing it to previously polymerized nanoparticles (Couvreur and Vauthier 1991; Tian and Groves 1999). In the present work, an optimum adsorption-entrapment procedure was established to prepare HCPT-PBCA-NP associated with PVP by the even design method (Zhang et al. 1996). The resulting nanoparticles had an average diameter of 78 nm. Freeze-dried HCPT-PBCA-NP associated with PVP powder was dispersed in physiological saline. A micrograph taken with a transmission electron microscope (TEM) is shown in Fig. 1. The median particle diameter in the redispersed lyophilized HCPT-PBCA-NP suspension was 81 nm, which was obviously larger than that of PBCA-NP without adsorption of HCPT.

The loading efficiency and the drug loading of the HCPT-PBCA-NP associated with PVP prepared were 39% and 1.2% respectively. The data of the drug loading fitted a Langmuir isotherm (Fig. 2). Since PBCA nanoparticles were prone to carry negative charges, its zeta potential was negative. As the positive zeta potential of the drug was higher, the drug loading and the loading efficiency were increased accordingly (Zhang et al. 1996). We found that the adsorption ability of HCPT on the nanoparticles was lower in basic solution while it was enhanced when the pH of the solution was declined to near neutral. This

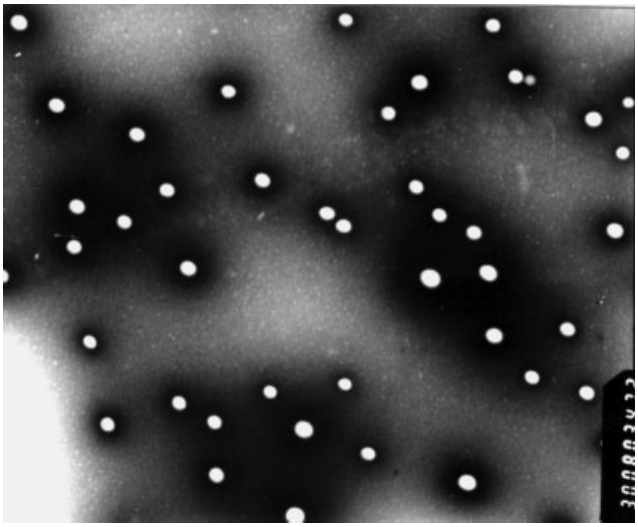

Fig. 1: Transmission electron microscope (TME) micrograph of re-dispersed lyophilized HCPT-PBCA-NP (× 30000)

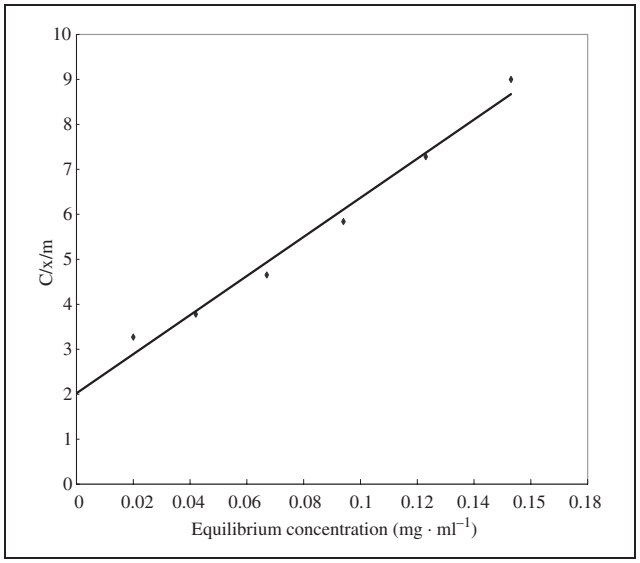

Fig. 2: Langmuir isotherm of adsorption of HCPT by PBCA nanoparticles ($r = 0.9913$). (C = equilibrium concentration (mg ml^{-1}); x = amount (mg) of HCPT adsorbed; m = mass (mg) of PBCA nanoparticles)

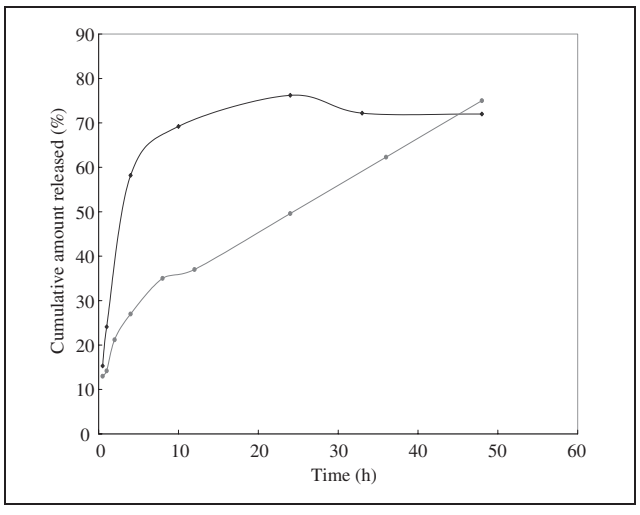

Fig. 3: Release of HCPT into physiological saline. (◆)HCPT-PBCA-NP, (●)HCPT-PBCA-NP associated with PVP

was presumably because HCPT was mainly present as anion in the basic solution and was therefore rejected by the PBCA-NP carrying negative charges. When the pH of the solution decreased, the fraction of the molecular HCPT increased and the adsorption ratio was enhanced. Since the solubility of HCPT decreased when the pH of the solution declined, we screened a series of solution systems and selected an appropriate one. The selected solution system met the requirements that HCPT could be adsorbed to the largest extent and the solubility of the drug was not too low to be dissolved.

2.2. In vitro release

HCPT was rapidly released from HCPT-BPCA-NP prepared by the method of adsorption. Fig. 3 shows the curves of release of HCPT from the nanoparticles. It revealed that almost 70% of HCPT was released within the first 10 h, which may be due to the desorption effect of HCPT from the PBCA-NP. When HCPT-PBCA-NP was intravenously injected, it may be even much easier for the drug to be desorbed under the action of blood circulation. This, however, is favorable for the long-term effect and the targeting function of nanoparticles. To solve this problem, a hydrophilic macromolecular compound (PVP) was added to prepare HCPT-PBCA-NP associated with PVP and as a result, the release rate was significantly decreased. It was shown that only 35% of HCPT was released from HCPT-PBCA-NP associated with PVP during the first 10 h and 75% during 48 h (Fig. 3). The release data of the HCPT-PBCA-NP associated with PVP fitted the Higuchi equation: $Q = 0.0615 + 0.0940\sqrt{t}$, where Q is the cumulative amount released (%).

2.3. In vivo distribution

Intravenously injected particulate substances or drug carriers with an average size below 7 μm are usually taken up by the macrophages of the RES, particularly by the Kupffer-cells of the liver (Löbenberg et al. 1998; Waser et al. 1987). This was also apparent in the current study for HCPT adsorbed to PBCA nanoparticles. Table 1 shows the tissue distribution (%) of HCPT-PBCA-NP associated with PVP and HCPT injection (control solution) in mouse after i.v. injection. Fifteen minutes after injection of HCPT-PBCA-NP associated with PVP, about 68% of the dose was found in the liver, whereas only about 19% of the dose was found there after injecting the control solution. This indicates that HCPT-PBCA-NP associated with PVP has a liver-targeting property, suggesting that nanoparticles adsorbed to hydroxycamptothecin could constitute a good carrier of the drug and are able to target at the liver.

Table 1: Tissue distribution (%) of HCPT-PBCA-NP associated with PVP (A) and HCPT injection (B) in mice 15 min after intravenous injection

Drug	A	B
Liver	68.2 ± 13.5	18.9 ± 8.3
Heart	–	2.6 ± 0.92
Blood	26.9 ± 3.25	64.5 ± 15.8
Spleen	–	2.0 ± 0.84
Lung	–	3.8 ± 1.59
Kidney	–	4.1 ± 2.34
Total	95.1	95.9

(Mean ± SD, n = 8)

Table 2: Plasma levels of HCPT injection and HCPT-PBCA-NP associated with PVP in rabbits after 15 min i.V. administration

Time (h)	HCPT injection ($\mu g \cdot ml^{-1}$, n = 5)	HCPT-PBCA-NP ($\mu g \cdot ml^{-1}$, n = 5)
0.083	14	1.37
0.25	3.6	0.638
0.5	1.92	0.457
1	0.32	0.476
2	0.075	0.12
4	0.082	0.114
8	–	0.13
12	–	0.109
24	–	0.116
36	–	0.11

2.4. Pharmacokinetic study

In the pharmacokinetic study, HCPT-PBCA-NP associated with PVP and HCPT were administered intravenously to rabbits. HCPT concentrations in plasma and the plasma concentration-time profiles after the administration are shown in Table 2. Since the drug was metabolized very rapidly, it was not detectable in the plasma after 4 h. Nevertheless, after injection of HCPT-PBCA-NP associated with PVP, the concentration of the drug decreased rapidly in the first 2 h and then went up slightly afterwards and maintained the same level for 30 h, suggesting a sustained release of HCPT from the PVP associated PBCA nanoparticles. The possible reason was that HCPT-PBCA-NP associated with PVP was taken up by the RES organs such as liver and spleen, which resulted in a rapid decrease in blood concentration. And then HCPT was slowly released from the PVP-associated nanoparticles in the liver or spleen so that the blood concentration rose slightly. When the release rate and the eliminating rate of HCPT reached a dynamic balance, the plasma concentration remained constant. Since data obtained from the HCPT injection group was limited, only the data of HCPT-PBCA-NP associated with PVP was used for the fitting of the two-compartments open model. Table 3 shows the pharmacokinetic parameters of HCPT in rabbits after intravenous administration of HCPT-PBCA-NP associated with PVP. It was found that the volume of distribution of the central compartment (Vc), half-life ($T_{1/2}$) and total systemic clearance ($CL_{(s)}$) were 3.5 L; 147 h; $0.18\ L \cdot h^{-1}$, respectively. The equation of the plasma concentration-time curve was $C = 1.28e^{-4.87t} + 0.130e^{-0.00472t}$. It fitted a two-compartment model.

Table 3: Pharmacokinetic parameters of HCPT-PBCA-NP associated with PVP in rabbits after intravenous administration

Parameter	Value	Standard error
A ($mg \cdot L^{-1}$)	1.279	4.57E-02
α (h^{-1})	4.776	2.59E-01
B ($mg \cdot L^{-1}$)	0.130	7.41E-03
β (h^{-1})	0.005	3.38E-03
V(c) (L)	3.548	
$T_{1/2\alpha}$ (h)	0.145	
$T_{1/2\beta}$ (h)	147.0	
K_{21} (h^{-1})	0.445	
K_{10} (h^{-1})	0.050	
K_{12} (h^{-1})	4.286	
AUC ($mg \cdot h \cdot L^{-1}$)	27.801	
CL(s) ($L \cdot h^{-1}$)	0.179	

Abbreviations: A and B, intercepts of HCPT plasma concentration (at zero point) of compartment A and compartment B, respectively; α and β, the corresponding elimination rate constant. $t_{1/2}$, half-life; AUC, area under the concentration curve; $CL_{(s)}$, total systemic clearance; Vc, volume of distribution of the central compartment.

Based on the pharmacokinetic study and the body distribution pattern, drug-loaded PBCA-NP associated with PVP may be advantageous for targeting of anti-neoplastic drugs when effective drug concentrations in RES organs should be maintained for a longer time.

3. Experimental

3.1. Chemicals

Hydroxycamptothecin (Reference Standard) was obtained from the Institute for Drug Control of Hubei Province (Wuhan, China); hydroxycamptothecin from Hubei Huangshi Feiyun Pharmaceutical Ltd. Company; α-butyl-cyanoacrylate (BCA) from the Senzheng Nanguang Medicinal Colla Co-operation (Shenzhen, China). Dextran-70, PVP and other chemicals are for drug use, and their quality met the standards outlined in the Chinese Pharmacopoeia. All the chemicals were of analytical grade.

3.2. Preparation of hydroxycamptothecin polybutylcyanoacrylate nanoparticles (HCPT-PBCA-NP)

The nanoparticles were prepared by the emulsion polymerization method. An optimum procedure was established with the even design method (Zhang et al. 1996). Briefly, Dextran-70 and sodium dithionite were weighed and dissolved in water. The solution was adjusted to pH 2.2 with dilute hydrochloric acid. BCA was slowly added to the solution under magnetic stirring for 3 h. Then, after the addition of sodium sulphate, it was stirred for another 1 h. The above solution was adjusted to a pH between 5 and 7 with a dilute sodium hydroxide solution and was filtered through a 0.45 μm membrane. A milky colloidal solution of PBCA-NP was obtained.

Then, an accurately weighed quantity of HCPT was dissolved in a certain volume of 0.1M solution of sodium hydroxide to obtain a solution having a HCPT concentration of 1 mg per ml. An accurately measured volume of this solution was transferred to a 25 ml volumetric flask. Afterwards, a certain volume of PBCA-NP colloidal solution was added and the pH was adjusted to 6.0. The solution was diluted with a 0.1M solution of sodium hydroxide to volume and mixed, followed by ultrasonic shaking for 5 min, and then the solution was incubated overnight at room temperature. HCPT-PBCA-NP colloidal solution with adsorption balance was obtained. HCPT-loaded nanoparticles were removed by centrifugation at 35000 rpm for 90 min at 4 °C with a freezing ultracentrifuge (Beckman L8–80M, America). The supernatant recovered for HCPT was assayed by a spectrophotometer at 413 nm. The amount of loaded HCPT was calculated from the difference between results from loaded media and from the initial solution and expressed as milligram (mg) HCPT/milligram (mg) PBCA-NP nanoparticles. The loading efficiency was defined as $(1\text{-}C_f/C_0) \times 100\%$, where C_f and C_0 were the concentration of HCPT in the final loading media and the initial solutions, respectively.

3.3. Preparation of HCPT-PBCA-NP associated with PVP colloidal solution and freeze-dried formulation

An aqueous solution of PVP was added to the HCPT-PBCA-NP solution and a colloidal solution containing 4% PVP was obtained. The solution was sonicated for 5 min and shaken for another 12 h at room temperature. Then a solution of HCPT-PBCA-NP associated with PVP was obtained. A cryoprotector (lactose) was dissolved in the HCPT-PBCA-NP associated with PVP colloidal solution and the solution was divided into vials. The solution was lyophilized by the freeze-dried method (Wang et al. 2002). Freeze-dried HCPT-PBCA-NP powder dispersed in physiological saline. Micrographs were taken with a transmission electron microscope (JEM-100SX, Japan). Nanoparticle size and homogeneity were determined using a laser particle analyzer (Master 2000, Malvern).

3.4. In vitro release

In vitro release was evaluated using a dialysis bag diffusion technique (Wei and Zhang 2001). The HCPT-PBCA-NP associated with PVP colloidal solution and HCPT-PBCA-NP solution (5 ml) were placed in dialysis bags, which were suspended in a cone-flash container containing 100 ml of 0.9% physiological saline solution. This container was sealed and shaken at 37 ± 1 °C in a constant temperature water bath. Samples of this solution 2 ml were taken at regular time intervals, and their absorbance was determined at 413 nm. The standard curve equation was $A = 8.57 \times 10^{-4} + 0.0562\,C$ ($r = 0.9999$). The accumulative drug release percentage (Q) was calculated.

3.5. Body distribution of HCPT-PBCA-NP associated with PVP in mice

HCPT-PBCA-NP freeze-dried powders were suspended in double distilled water to obtain an HCPT-PBCA-NP associated with PVP suspension. Sixteen Kuming mice (weighing 18–22 g, provided by the Laboratory Animal Center of Sichuan University) were divided into two groups (eight for each group) and they were kept under standard conditions with free access to water and food. HCPT-PBCA-NP associated with PVP suspension and HCPT-solutions were injected intravenously into the tail vein with a single dose of 10 mg HCPT kg^{-1} body weight. At 15 min after injection, blood samples were collected from the ocular artery after eyeball removal and the animals were dissected to obtain each tested organ including heart, liver, spleen, lung, and kidney. Every organ sample was accurately weighed and homogenized. The concentrations of HCPT were assayed based on the following reversed-phase HPLC methods.

HPLC analysis was performed with an LC-10A HPLC system (Shimazhu, Japan). The liquid chromatogragh was equipped with a 382 nm detector and a CLC-ODS column (5 μm, 150 mm × 4.6 mm, I.D., Shimpack) operated at 35 °C. Mobile phase was a mixture of methanol, 5 M phosphate buffer solution (pH 6.5) and 0.3 M tetrabutylammonium phosphate aqueous solution (pH6.5) (500 : 500 : 15). The flow rate was 1 ml/min. 0.1 ml of all samples including plasma and homogenized tissues were transferred to 10 ml tubes, 0.4 ml methanol was added and vortex-mixed for 30 min, followed by ultrasonic shaking for 5 min. The samples were then centrifuged at 15000 rpm for 10 min. Aliquots of 20 μl clear supernatant were injected into the chromatograph. A standard curve was prepared according to the chromatographic conditions. The recoveries of HCPT in plasma and mouse tissue homogenates were between 99.8% and 107.0% and the relative standard deviations were below 6%.

3.6. Pharmacokinetic study of HCPT-PBCA-NP associated with PVP in rabbits

Ten white Japanese rabbits (provided by the Laboratory Animal Center of Sichuan University) were divided into two groups (five for each group) and used for the pharmacokinetic study. HCPT-PBCA-NP associated with PVP suspension and HCPT-injection were injected intravenously into the ear vein of the rabbit with a single dose of 10 mg HCPT kg^{-1} body weight. Blood 2 ml was collected from another ear vein at predetermined times after drug administration, and the HCPT plasma concentration was assayed by reversed-phase HPLC. The chromatographic conditions of the HPLC method were the same as that of the distribution study except for the mobile phase which was changed to a mixture of methanol and 10 M phosphate buffered solution (60 : 40, pH 4.0). The pretreatment method was changed as follows in order to improve the sensitivity of the assay method. Full blood 2 ml was centrifuged at 600×g (2000–3000 rpm) for 10 min. Then 0.5 ml of plasma was transferred to a tube and the pH was adjusted to 2.0 with 85% phosphoric acid. Afterwards, 0.5 ml acetonitrile was added and mixed, followed by ultrasonic shaking for 5 min and centrifuging at 15000×g (15000 rpm) for 10 min. The supernatant was extracted with 0.5 ml of ethyl acetate saturated by 0.5 M HCl for two times. The extracted solution was mixed and evaporated to dryness under nitrogen atmosphere in a 55 °C water bath. The residue was dissolved in 150 μl methanol and aliquots of 50 μl clear supernatant was injected into the chromatograph after centrifugation. The detection limit of HCPT was 50 ng/ml. The average recovery was 103% and the relative standard deviation was 3%.

3.7. Pharmacokinetic data analysis

The pharmacokinetic data of PVP-HCPT-PBCA-NP were processed using a 3P87 programme (Practical pharmacokinetic program, edited by the Chinese Society of Mathematical Pharmacology, Beijing, China).

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