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Mitoxantrone polybutyl cyanoacrylate nanoparticles as an anti-neoplastic targeting drug delivery system

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Abstract

Mitoxantrone polybutyl cyanoacrylate nanoparticles (PBCA-NP) for use as an anti-neoplastic targeting drug delivery system were prepared by the emulsion polymerization method. Their surface charge, drug loading, stability, morphology, size, release characteristics in vitro and distribution in animals were studied. The results revealed that their mean diameter was 55.11 nm, their drug load was 46.77% and their embedding ratio was 84.12%; their surface is negatively charged and their release characteristics in vitro obey two-phase kinetic laws. A colloidal solution of mitoxantrone PBCA-NP was sterilized by boiling for 30 min. After i.v. injection of ^{3}H-mitoxantrone PBCA-NP, the radioactivity was mainly concentrated in the liver, and the radioactivity in liver tumors was higher than in liver tissue. Fifteen minutes after the i.v. injection, mitoxantrone PBCA-NP were observed in the parenchymal cells. This method of preparation helps to increase the anti-liver-tumor efficacy and decrease the toxicity of mitoxantrone.

Keywords: Mitoxantrone; Polybutyl cyanoacrylate nanoparticles; Labeled mitoxantrone; Liver targeting

1. Introduction

Injectable, colloidal drug delivery systems, especially the nanoparticles have gained much interest during the last few years, as they improve the distribution of drugs in the body (Kreuter, 1979) because of their enhanced efficiency against tumors (Brasseur, 1980) and their reduced toxicity. However, commercial products have not been made from nanoparticles. The major problems with the clinical application of nanoparticles are that the drug load is too low to deliver an effective dose, preparation is tedious and preparations are not stable. The aim of this work was to solve these problems.

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Mitoxantrone (Mitoxantrone, DHAQ) (Zee-Cheng, 1978) used as a model drug for the preparation of nanoparticles is a new anthraquinone anti-tumor drug synthesized in the late 1970's. Its anti-tumor activity is higher than that of Adriacin, (ADR), and its toxicity is lower. DHAQ has good effects on breast-cancer, leukemia, Hodgkin's disease and primary hepatic cancers. About 45% of the DHAQ administered was concentrated in the liver after i.v. injection, but the concentrating rate was slow (about 6 h), so injections of DHAQ causes greater toxicity problems (Zheng and Fang, 1989; Fang and Zheng, 1990). The butylcyanoacrylate (BCA), used as carrier, is a non-toxic, biodegradable, and bioavailable, medical adhesive, and polybutyl cyanoacrylate nanoparticles (PBCA-NP) have been used as darodipin and adriamycin carriers (Kreuter, 1979; Brasseur, 1980).

The conditions and procedures for the preparation, of mitoxantrone-polybutyl cyanoacrylate-nanoparticles (DHAQ-PBCA-NP), and their morphology, size, size distribution, release characteristics, stability and distribution in animals were studied. Since the drug load in DHAQ-PBCA-NP is high, and the technological processes are simple, these particles may be manufactured for clinical use. The prescription, and the methods for preparing DHAQ-PBCA-NP freeze-dried injections prepared for intravenous administration are reported. The DHAQ-PBCA-NP prepared for i.v. injection was stable for 3 months.

2. Materials and methods

2.1. Materials

The DHAQ used in this study was kindly donated by Professor Peilin Xi (Chengdu, China), and its quality meets trial standards outlined by the Ministry of Public Health, China. The butylcyanoacrylate (BCA) monomer (Xian, 1980) was obtained from the Senzeng Nanguang Medicinal Colla Co-operation (Senzeng, China). Various additives including sodium chloride and sodium dithionite were purchased from the Chengdu Medicinal Co-operation (Chengdu, China), their quality meets the standards outlined in the Chinese Pharmacopoeia. Various chemicals including sodium hydroxide and hydrochloride were of an analytical grade. Dextran-70 was purchased from Pharmacia LKB (Uppsala, Sweden).

The following instruments were used: A 81-2 type constant temperature magnetic stirrer, for the preparation of DHAQ-PBCA-NP; a L8-80M freezing ultracentrifuge, for the separation of DHAQ-PBCA-NP from the colloidal solution; a 2040 freeze dryer, for the freeze-drying of the DHAQ-PBCA-NP colloidal solution; a DY-1 electrophoresis apparatus, for the determination of zeta potentials; a S-450 scanning electron microscope and a JEM-100SX transmission electron microscope (Japan), for the morphological analysis; a UV-250 spectrophotometer (Japan), for the determination of the DHAQ content; a FJ-2105 liquid scintillation counter (Xian, China), for studies on the distribution of DHAQ-PBCA-NP in vivo. A SHZ-88-1 desk constant temperature shaker was used to observe drug release from the nanoparticles.

2.2. Methods

The emulsion polymerization method was used to prepare DHAQ-PBCA-NP. An optimum procedure was established with the even design method (Zhang and Liao, 1994a). Briefly, the DHAQ, Dextran-70, and sodium dithionite were weighed, and dissolved in water. The pH of the solution was adjusted to 2.2 with a dilute hydrochloride solution, and transferred to a cone-flash container. BCA was slowly added to the solution which was being stirred, this was continued for 4 h at room temperature. Then, after the addition of sodium chloride, it was stirred for another 2 h. The solution became a milky blue colloidal solution. The pH of this colloidal solution was adjusted to between 5–7 with a dilute sodium hydroxide solution. The solution was filtered through a micro filter membrane (0.3 mm). After the additives had been added, it was boiled for 30 min. After cooling at room temperature, it was put into ampoules, freeze-dried and stored until use.

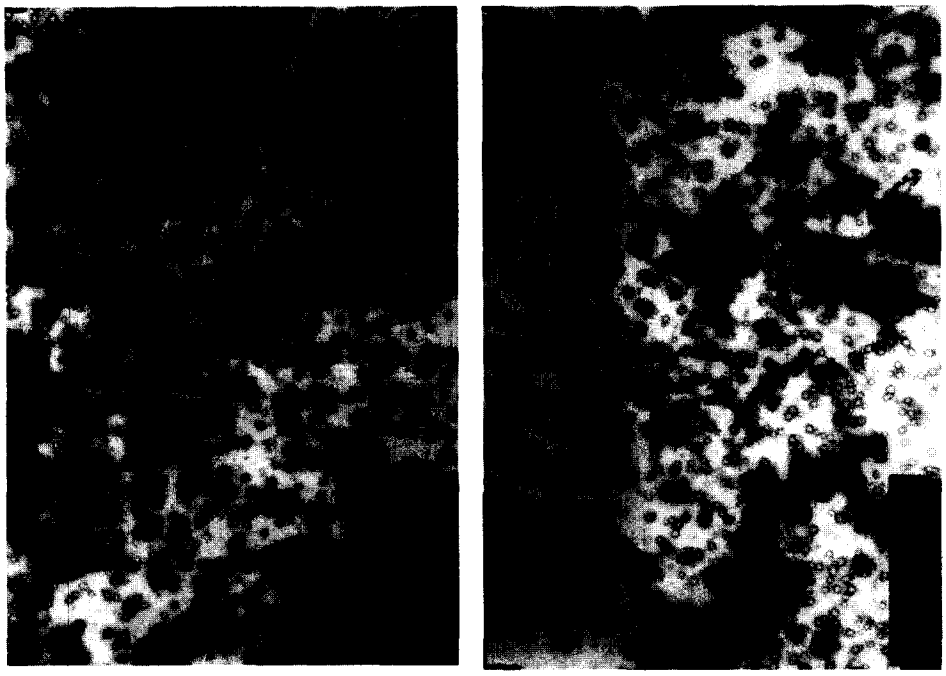

Fig. 1. Transmission electron micrographs of DHAQ-PBCA-NP before (a) and after (b) sterilization (× 15000).

Freeze-dried DHAQ-PBCA-NP powder was dispersed in physiological saline, and stained with 1.5% phosphotungstic acid. Micrographs were taken with a transmission electron microscope and a scanning electron microscope for the morphological analysis.

Spectrophotometry was used to determine the embedding ratio and the drug load at 610 nm (Zhang and Liao, 1994b). The standard curve equation was $A = 0.0074 + 0.4246C$ (C = mg/100 ml, $r = 0.99997$), where A is the absorbance, and C is the concentration. DHAQ added to the blank PBCA-NP was dissolved with ethyl acetate, and the DHAQ content was determined from the absorbance. The mean recovery was 99.61 ± 1.37% ($n = 9$) at high, intermediate and low concentrations. The freeze-dried DHAQ-PBCA-NP powder was weighed accurately, dispersed in quantitative physiological saline, followed by freeze ultracentrifugation, and then the absorbance (A_1) of the supernatant was measured. The sediments were dispersed in another fraction of physiological saline, followed by ultrasonic shaking for 1 min, and freeze ultracentrifugation, then the supernatant was separated, and the procedure was repeated twice as described above. The supernatant was mixed, and its absorbance was recorded as A_2. The sediments were weighed after drying and then dissolved with ethyl acetate, and their absorbances were recorded as A_3. The embedding ratio (ER%), drug load (DL%), surface drug load (SDL%) and inside drug load (IDL%) were calculated using A_1, A_2 and A_3. The definitions are as follows:

ER% = [DHAQ (mg) added — DHAQ (mg calculated from A_1)]/DHAQ(mg) added × 100%

DL% = [DHAQ (mg) added — DHAQ (mg calculated from A_1)]/PBCA-NP (mg) × 100%

SDL% = DHAQ (mg calculated from A_2)/PBCA-NP (mg) × 100%

IDL% = DHAQ (mg calculated from A_3)/PBCA-NP (mg) × 100%

The zeta potentials of the colloidal solutions of PBCA-NP and DHAQ-PBCA-NP were determined electrophoretically method (Rawlins, 1977), and both carried a negative charge, −65.8 mv and −2.42 mv, respectively. An advanced study showed that different agents could be used to change the zeta potential of PBCA-NP.

Table 1
The diameters of DHAQ-PBCA-NP from 5 different batches

Term	1	2	3	4	5	X
Count (n)	512	503	509	503	507	507
Diameter (nm)	55.12	55.56	57.33	49.64	57.92	55.11
S.D.	10.80	12.53	11.62	9.76	10.1	—

Table 2
Embedding ratio and drug loading of DHAQ-PBCA-NP

Batch No.	Embedding ratio (%)	Inside drug loading (%)[a]	Surface drug loading (%)[a]
9112021	83.26	46.80	3.49
9112022	81.73	45.17	3.87
9112023	85.59	47.82	4.01
9112024	85.89	47.27	4.47
Average	84.12	46.77	3.96

[a]mg DHAQ/100 mg PBCA-NP.

Table 3
Influence of additives on the zeta potential (zp), embedding ratio (ER%) and inside drug load (IDL%)

Term	Additives				
	$Na_2S_2O_4$ + NaCl	$Na_2S_2O_4$	$Na_2S_2O_4$	NaCl	KCl
ZP (mv)	−65.8	−50.5	−35.2	−30.4	−27.3
ER (%)	84.12	62.83	45.68	38.25	34.21
IDL (%)	46.77	3.01	17.23	12.72	9.28

The dynamic penetration method was used to observe drug release from DHAQ-PBCA-NP in vitro (Washington, 1990). Sufficient physiological saline was added to the DHAQ-PBCA-NP freeze-drying powder, followed by shaking and centrifugation. The sediments were dispersed in a small amount of physiological saline. The solution was transferred to a dialysis bag, which was suspended in a cone-flash container containing 50 ml of 1% vitamin C in a physiological saline solution. This container was sealed and shaken at 37 ± 1°C in a constant temperature water bath, and 2 ml samples of this solution were taken at regular time intervals, and their absorbance was determined at 610 nm. The standard curve equation was $A = 0.0361 + 0.3599C$ ($r = 0.9998$). The accumulative drug release percentage (Q) was calculated according to an equation.

^{3}H-DHAQ (labeled by the Institute of Atomic Energy Sciences, China; radiochemical purity being higher than 92%) was prepared by a method described previously (Zhang and Liao, 1993a).

3. Results

3.1. Morphology

The surface of the DHAQ-PBCA-NP was regular and non-adhesive (Fig. 1a). The maximum diameter of DHAQ-PBCA-NP was 125 nm, and the minimum was 21 nm. The average diameter of DHAQ-PBCA-NP was determined to be 55.11 nm. Table 1 shows the diameters of 5 batches of product. Statistical analysis showed that the diameter of the 5 batches of product were all abnormally distributed.

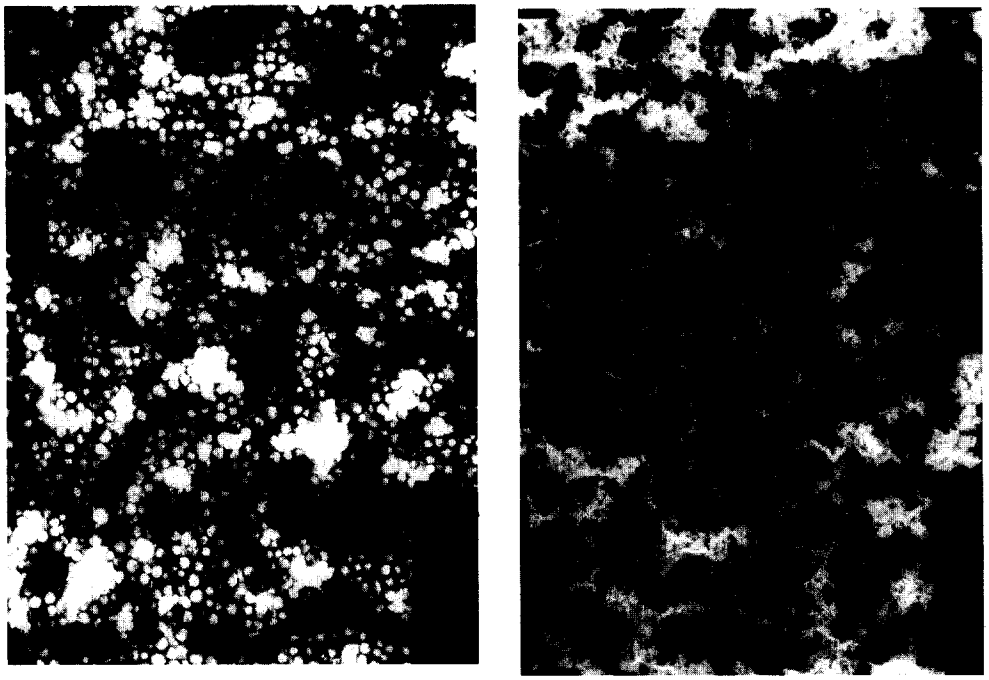

Fig. 2. Transmission electron micrographs of DHAQ-PBCA-NP kept at 37°C (RH = 75%) at time 0 and after 3 months (× 50 000).

Table 4
Diameters (nm), DHAQ contents (C) and Q* of DHAQ-PBCA-NP after 3 months

Batch No.		9112021		9112022		9112023	
Time (months)		0	3	0	3	0	3
3–5°C	(nm)	55.78	56.34	56.27	56.87	55.47	56.07
	C*	46.78	46.74	45.32	45.08	47.29	47.12
	Q*	0.8547 ± 0.0441	0.8509 ± 0.0388	0.8632 ± 0.0518	0.8607 ± 0.0483	0.8498 ± 0.0563	0.8465 ± 0.0578
20–25°C	(nm)	55.78	56.69	56.27	56.80	55.47	55.93
	C*	46.78	46.69	45.32	45.21	47.29	47.11
	Q*	0.8547 ± 0.0441	0.8513 ± 0.0423	0.8632 ± 0.0518	0.8601 ± 0.0504	0.8498 ± 0.0563	0.8437 ± 0.0565
37°C, RH 75%	(nm)	55.78	56.12	56.27	55.94	55.47	56.15
	C*	46.78	46.58	45.32	45.19	47.29	47.16
	Q*	0.8547 ± 0.0441	0.8515 ± 0.0418	0.8632 ± 0.0518	0.8592 ± 0.0537	0.8498 ± 0.0563	0.8429 ± 0.0583

*C = mg DHAQ/100 mg PBCA-NP.
*Q = DHAQ (mg) released from DHAQ-PBCA-NP in 312 h/DHAQ (mg).
DHAQ-PBCA-NP.

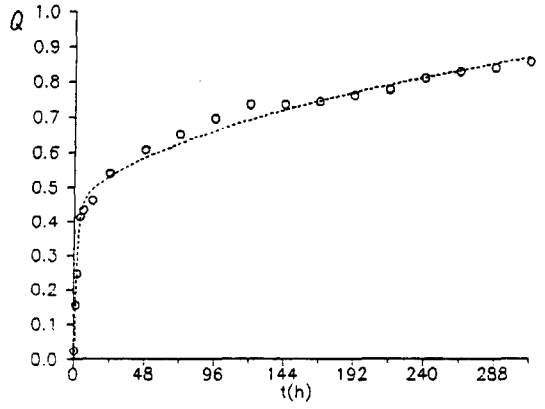

Fig. 3. Release profile of a DHAQ-PBCA-NP freeze-dried injection.

3.2. Drug loading characteristics.

Table 2 shows the embedding ratio, surface drug loading and inside drug loading of DHAQ-PBCA-NP. The carrying capacity of nanoparticles was found to be satisfactorily high.

Table 5
Release data (*Q) from a DHAQ-PBCA-NP freeze-dried injection and the calculated release data (Q), according to the regression equation

t (h)	*Q +RSD (n = 6)	Q
0.5	0.0227 + 0.0179	−0.0498
1.5	0.1534 + 0.0624	0.1924
2.0	0.2464 + 0.0980	0.2658
4.0	0.4117 + 0.0304	0.4079
6.0	0.4328 + 0.0253	0.4531
12	0.4614 + 0.0422	0.4918
24	0.5392 + 0.0221	0.5298
48	0.6063 + 0.0216	0.5836
72	0.6503 + 0.0212	0.6249
96	0.6947 + 0.0479	0.6597
120	0.6995 + 0.0378	0.6903
144	0.7337 + 0.0420	0.7181
168	0.7411 + 0.0503	0.7435
192	0.7584 + 0.0715	0.7673
216	0.7757 + 0.0370	0.7895
240	0.8093 + 0.0383	0.8106
264	0.8282 + 0.0503	0.8307
288	0.8375 + 0.0512	0.8498
312	0.8547 + 0.0441	0.8682

*Q = DHAQ (mg) released from DHAQ-PBCA-NP/DHAQ (mg) in DHAQ-PBCA-NP.

3.3. Relationship between the drug load and zeta potential

Table 3 shows the experimental results when 5 kinds of additives were used. Both the zeta potentials and the drug load were at a maximum when sodium dithionite and sodium chloride were used together. Regression analysis was performed on the zeta potential (Z) and drug load. The equation, DL% = −16.996 + 0.9756Z (r = 0.9996), showed a linear relationship between the zeta potential and the drug load.

3.4. Stability

Three batches of DHAQ-PBCA-NP freeze-dried powder were stored in a refrigerator at 3–5°C, room temperature (20–25°C) and 37°C, respectively (RH = 75%). There were no marked changes in their appearance, morphology, particles structure or DHAQ content at 0 and 3 months. Fig. 2 and Table 4 show these results.

3.5. Sterilization of DHAQ-PBCA-NP

The shape of the DHAQ-PBCA-NP colloidal solution, observed by transmission electron microscopy after ultracentrifugation, did not change after sterilization (boiling for 30 min at 100°C). Fig. 1b shows the morphological structure of the nanoparticles. After sterilization, the DHAQ-PBCA-NP colloidal solution was examined, according to a method outlined by the Chinese Pharmacopoeia, and was proven to be sterile.

3.6. Drug release characteristics

Table 5 shows the drug release data for DHAQ-PBCA-NP. Fig. 3 shows the drug release profile over 312 h.

The curve (Fig. 3) corresponds to the two-phases kinetics equation:

$$Q = 0.4000 + 0.0265\sqrt{t} - 0.6545\exp(-0.6686t)$$

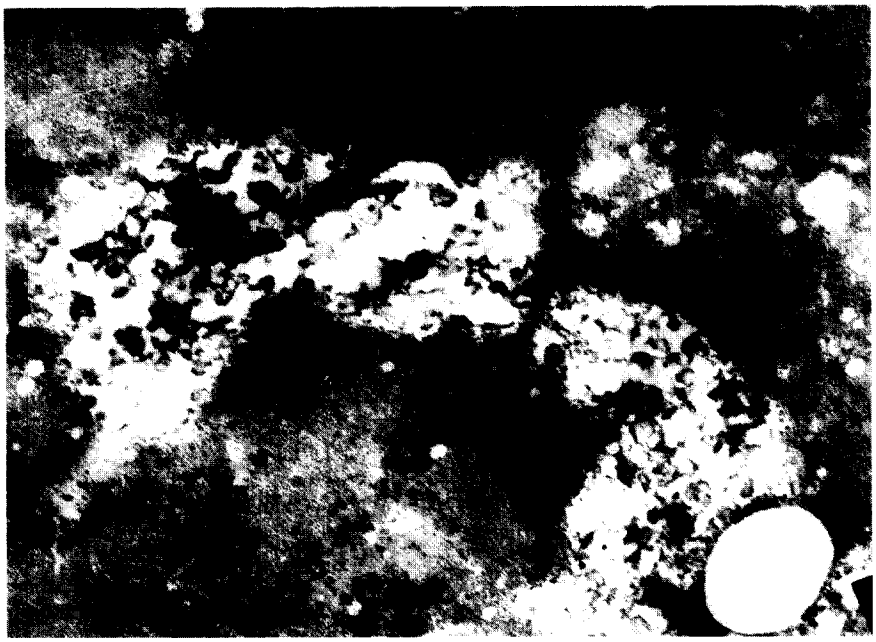

Fig. 4. Transmission electron micrograph of liver cells following a single i.v. injection of DHAQ-PBCA-NP.

3.7. Distribution of ^{3}H-DHAQ-PBCA-NP in animals

Fig. 4 shows the nanoparticles observed in parenchymal cell by TEM 15 min after their i.v. injection. These findings showed that ^{3}H-DHAQ-PBCA-NP targets liver and parenchymal cells.

4. Discussion

In the preparation of DHAQ-PBCA-NP, the result from the single factor test showed that at a constant pH, stirring rate and temperature, nanoparticles could be prepared using Tween 20, Tween 40, Tween 60, Tween 80, β-CD, pluronic F 68 or Dextran-70. Nanoparticles appeared to be adhesive to different degrees when Tween was used. The experiment also indicated that the temperature and stirring rate had little effect on PBCA-NP particle size and particle size distribution, but the influence of pH of the solution was obvious, so the key to the preparation of nanoparticles is to control the pH of the medium. In this study, the optimum quantitative equation was obtained by the progressive multiple linear regression of the experimental results by the even design method, so not only the results of the experiment could be predicted according to the recipe and conditions, but also the experimental conditions could be optimized within a given range according to the desired result, which is of some practical importance.

To develop a method to increase the PBCA-NP drug load, the influence of several kinds of additives upon the charge characteristics of PBCA-NP were studied. The findings showed that sodium dithionite and sodium chloride can significantly increased the negative PBCA-NP charge. The PBCA-NP drug load was increased to 46.77%, and the embedding ratio of PBCA-NP was increased to 84.12%. The drug load is a function of the zeta potential. This is thought to be because the nanoparticles carry negative charges, but mitoxantrone molecules carry a positive charge in an acidic solution, and mitoxantrone associates with nanoparticles by the adsorption of static electricity. Therefore, the negative charge of nanoparticles affects the amount of drug associated with the nanoparticles.

The anti-tumor effect of DHAQ-PBCA-NP were monitored by using a model of orthotopically transplanted human hepatocellular carcinoma (HHC) in nude mice. The findings showed that the inhibition of orthotopically transplanted HHC by DHAQ-PBCA-NP was 31.98% higher than that of DHAQ (Zhang and Liao, 1993b). The experimental result of DHAQ-PBCA-NP in mice showed that the acute toxicity of DHAQ-PBCA-NP i.v. injected was lower than that of on DHAQ injection (Zhang and Liao, 1995).

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