Preparation of magnetic polybutylcyanoacrylate nanospheres encapsulated with aclacinomycin A and its effect on gastric tumor

Hong Gao, Ji-Yao Wang, Xi-Zhong Shen, Yong-Hui Deng, Wei Zhang

INTRODUCTION

Cytotoxic medicine has extensively been employed in cancer chemotherapy. However, the usage of these drugs has been limited by the non-targeting towards cancer and serious toxicity to normal cells in the body. To enhance the therapeutic efficacy of anticancer drugs and reduce the toxicity to normal tissue of the body, targeted drug delivery systems at solid tumors have been developed.

Magnetic targeted drugs are the fourth generation of targeted reagents. The advantage of the magnetic targeted drug delivery systems over other drug targeting techniques is their ability to minimize the uptake by reticuloendothelial system (RES)[3]. Some investigators have reported successful tumor remission in animal experiments upon the use of magnetically responsive anticancer drug carriers under magnetic fields, but in previous studies the majority of magnetically responsive drug carriers, which included magnetic albumin microspheres and magnetic liposomes had been administered intra-arterially to obtain highly efficient localized targeting during the first circulation passing through a strong magnetic field[2][3]. However intra-arterial administration of these carriers is not considered to be suitable for the treatment of multiple systemic lesions and is inconvenient to apply. Accordingly, the treatment of solid tumors requires the development of magnetically responsive carriers that can be effectively delivered to any systemic site via intravenous administration. Yet the therapeutic efficacy of intravenously administered magnetically responsive carriers has not been established maturely to date.

Polyalkylcyanoacrylates (PACAs) were not employed as polymers until the early 1980s. However the corresponding monomers, alkylcyanoacrylates, have been extensively used as tissue adhesives for the closure of skin wounds[4]. More recently, one application of the polymers is the use of PACAs as nanoparticulate drug carriers[5][6]. This very exciting area of research has gained increasing interest in therapeutics, especially for cancer treatments. Other molecules of interest, including poorly stable compounds such as peptides and nucleic acids, have been combined with PACAs nanoparticles for targeting purposes[7][8]. Today, PACAs nanoparticles are considered the most promising polymer colloidal for drug delivery system and are already in clinical development for cancer therapy[9][10]. The main attraction of PACAs nanoparticles is their ability of tissue targeting and enhancing intracellular penetration of drugs[11]. Among the species, polybutylcyanoacrylate (PBCA), as a polymer with medium-
length alkyl side chain, is of lower toxicity, proper degradation time. PBCA carrying drugs could increase the antibacterial efficacy\cite{22}, elevate the anti-cancer effect\cite{23}, enhance the relative bioavailability of insulin\cite{24} etc. So PBCA has recently been regarded as a kind of widely used, biocompatible, degradable, low-toxic drug carrier.

Employing supermagnetic iron oxide as the ferromagnetic material, aclacinomycin A (ACM) as the targeted fat soluble model drug and PBCA as the carrier, a kind of magnetically targeted polymer encapsulated with an anticancer drug, magnetic PBCA nanoparticles encapsulated with ACM were designed and successfully prepared. The anticancer efficacy of the magnetically targeted system was investigated on gastric cancer in vivo and in vitro.

MATERIALS AND METHODS

Materials

Butylcyanoacrylate was supplied by Zhejiang Golden Roc. Chemicals Co. Inc. ACM was obtained from Shenzhen Main Luck Pharmaceuticals Inc. Human gastric cancer MKN-45 cell line and BALB/c nude mice (SPF, female) were kindly supplied by Shanghai Cancer Institute. Column NdFeB permanent magnets (surface field strength 2.5 T, diameter 1 mm, length 10 mm) were supplied by Shanghai Jieling Magnetic & Device Co. Ltd. Semi-solid methylcellulose M3545 and Iscove’s Modified Dulbecco’s Medium (IMDM) were purchased from Stemcell Company, Canada.

Methods

Synthesis of magnetic colloidal nanoparticles

Magnetic colloidal iron oxide nanoparticles were prepared with the method of coprecipitation as described before\cite{25}. Briefly, 10 mol/L sodium hydroxide was added into the mixture of solution of FeCl$_3$ and FeCl$_2$ (Fe$^{3+}$/Fe$^{2+}$ molar ratio 1/2) in a nitrogen atmosphere. The solution was stirred for 1 h at 20 $^\circ$C, and heated at 90 $^\circ$C for 1 h. The obtained iron oxide suspension was then stirred for 30 min at 90 $^\circ$C with the addition of 100 mL of 2% Na$_2$SO$_4$ solution (0.3 mol/L). Subsequently, the iron oxide dispersion was cooled down to room temperature with continuous stir. The magnetic particles were washed with double distilled deionized water and collected with the help of a magnet. Finally, the ultrafine magnetic particles were redispersed in water and the suspensions were adjusted to 2.0% (w/v) for further use.

Preparation of ACM encapsulated magnetic PBCA nanoparticles (MPNS-ACM)

Interfacial polymerization was applied to synthesize MPNS-ACM based on the methods used before\cite{23,24}. Briefly, 20 mg ACM dispersed in diluted hydrochloric acid and 2 mL magnetic fluid were mingled, then the mixture was added to 100 g hexane including 2 g Span 80 and 0.6 g polysorbate 80 which was stirred at 600 r/min. The fluid was stirred for 0.5-1 h to make it uniform and emulsive. Then 2 mL butylcyanoacrylate was added dropwise with constant stir at room temperature. After 6 h polymerization the particles were separated with the help of a magnet and washed with methanol for several times. Then the particles were washed with deionized water for several times. The particles were lyophilized and 60Co irradiation (15 Kgy) was performed to sterilize them. The preparation of magnetic PBCA nanoparticles (MPNS) is similar to the synthesis of MPNS-ACM except no ACM in HCl solution.

Characterization and measurements

Transmission electron microscopy (TEM) was performed for MPNS (HitachiHU-11B). Scanning electron microscopy (SEM, Philips XL30) was used to determine the size and morphology of MPNS. Dynamic light scattering (DLS, Malvern 4700) was used to measure the hydrodynamic diameter of nanoparticles. The particles were treated with ammonia and then ACM was extracted with ethyl acetate. ACM concentration was determined with UV spectrophotometer at 259 nm. And then drug contents were calculated.

Human gastric carcinoma model of nude mice

Human gastric carcinoma MKN-45 cells during exponential growth phase were adjusted to 5×10^5/mL with RPMI 1640. Then 0.2 mL suspension of MKN-45 cells was inoculated subcutaneously near right foot in female BALB/c nude mice. Two weeks later, the solid tumors were taken out from the mice in which the tumors were well growing without necrosis. Tumor masses were cut into small pieces with the diameter of about 2 mm. One tiny piece was implanted into one mouse subcutaneously near right foot with a needle. The models were successfully produced after about 2 wk when the tumor grew up to 1 cm in diameter.

Tumor inhibition rate in vivo

Thirty nude mice models were randomly divided into 5 groups of six each: ACM group (8 mg/kg bm), high dose of MPNS-ACM group (equivalent to ACM 8 mg/kg bm), low dose of MPNS-ACM group (equivalent to ACM 1.6 mg/kg bm), MPNS carrier group (equivalent to MPNS-ACM loaded with ACM 8 mg/kg bm) and control group (normal saline). Magnets (with surfacial field strength 2.5 T) were implanted into the center of the tumors one day before the administration. Above-mentioned agents were administrated intravenously on the first day and sixth day. The largest diameter (LD) and its vertical diameter (VD) of the tumors were measured with calipers every two days after the beginning of administration. The volume of tumor was equal to LD×VD/2. The mice were sacrificed on the eleventh day after treatment. The tumors were taken out, weighed and the tumor inhibition rate (TIR) was calculated with the following formula: TIR(%)=(1-average tumor weight of experimental group/average tumor weight of control group)×100%.

Stem cells colony-forming unit assay of bone marrow was performed. White blood cell, serum alanine aminotransferase (ALT) and creatine of the mice were examined.

Assay of granulocyte-macrophage colony-forming unit (CFU-GM) of bone marrow

The assay of CFU-GM was performed with semi-solid methylcellulose culture. The femorals of the mice were taken out under sterile condition. Both extremities of them were cut and the bones were immersed into Iscove’s modified Dulbecco’s medium (IMDM). The bone marrow was washed out and the concentration of the cells with nuclei was adjusted with IMDM to 2×10^5/mL. Cell suspension 0.2 mL was added to 2 mL M3534 semi-solid culture. The mixture was added to a 12-well cell culture plate. The cells were cultured for 7 d at 37 $^\circ$C with 50 mL/L CO$_2$ in air and >95% humidity. The number of colonies (>30 cells) were counted under inverted microscope\cite{26}.

Anticarcinoma effect on gastric cancer cell line in vitro

Gastric cancer cell line MKN-45 cells were trypsinized and suspended in RPMI 1640 with the concentration of 2×10^5/mL. The cells were seeded onto 96-well culture plates with 190 µL per well and then were cultured at 37 $^\circ$C with 50 mL/L CO$_2$ in air and >95% humidity for 24 h. Different concentrations of ACM, MPNS and MPNS-ACM in RPMI 1640 were added to the wells and the final concentrations were 0.001, 0.01, 0.1, 1.0, 10 and 100 µg/mL, respectively. The cells were cultured for another 48 h. Then, 10 µL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT) was added to each well and the cells were cultured for 4 h at 37 $^\circ$C. Then the culture medium was discarded, and 150 µL dimethylsulfoxide was added to each well and the absorbance at 570 nm (A$_{570}$) was measured with microplate reader.

Inhibition rate=(1-A$_{570}$ in treatment group/A$_{570}$ in control group)×100%.
Statistical analysis
The data were presented as mean±SD. One-Way ANOVA analysis was used to perform single factor multiple comparison in animal tests. The level of significance was set at P<0.05. Logistic regression was applied to analyze the inhibition rate of ACM, MPNS and MPNS-ACM in the in vitro study.

RESULTS
Characteristics of MPNS-ACM
The average particle size was 210 nm and the size distribution range was 100-400 nm with the most frequent size around 210 nm (Figure 1). A typical core-shell structure is shown under TEM (Figure 2), it indicated black Fe₃O₄ was surrounded by white polymer. SEM photograph shows uniform sphere. The drug content of MPNS-ACM was 12.0%.

Figure 1 Size distribution of MPNS–ACM.

Tumor mass and volume were significantly decreased in both ACM group and MPNS-ACM group than control group (P<0.05). The tumor weight of low dosage of MPNS-ACM group was lower than that of control group (P<0.05). The TIR of ACM was 22.63%. The tumor mass of ACM group on the eleventh day (1.06±0.27 g) was much lower than that of control group (1.37±0.21 g) (P<0.05).

The TIR of high dosage of MPNS-ACM was 52.55%. The tumor mass on the eleventh day (0.65±0.26 g) was much lower than those of the same dosage of ACM group, low dosage of MPNS-ACM group (0.95±0.15 g), MPNS carrier group (1.23±0.25 g) and control group.

The TIR of low dosage of MPNS-ACM (1.6 mg/kg bm) group was 30.66%, which was higher than that of ACM group (8 mg/kg bm), but there was no statistical difference as to the tumor mass between the two groups (P>0.05). The tumor weight of low dosage of MPNS-ACM group was lower than those of MPNS group, and control group (P<0.05).

The TIR of MPNS carrier was 10.22%. The tumor weight (1.23±0.25 g) was similar to that of control group (1.37±0.21, P>0.05).

Side effects of the agents
The white blood cell counts, serum ALT and creatine of the mice in all the groups were similar (P>0.05) (Table 2). The number of CFU-GM of ACM group was much lower than those of the other four groups (P<0.001). The white blood cell counts in ACM group was lower than that in control group, yet the ones in MNPS carrier and MNPS-ACM groups were similar to that in control group (P>0.05).

Anti-tumor effect on gastric cancer cell line in vitro
The IC50 of ACM, MPNS and MPNS-ACM was 0.09, 97.78 and 1.07 (Table 3).

DISCUSSION
Selective targeting agents are the trend of antineoplastic chemotherapy. However the production of the biodegradable agents of proper size, high targeting ability and good

Table 1 Tumor mass on the 11th day and TIR of the five groups (n=6, mean±SD)

| Group           | Tumor mass (g) | TIR (%) |
|-----------------|----------------|---------|
| ACM             | 1.06±0.27a     | 22.63   |
| High dosage of MPNS-ACM | 0.65±0.26ag   | 52.55   |
| Low dosage of MPNS-ACM | 0.95±0.15aa   | 30.66   |
| MPNS            | 1.23±0.20      | 10.22   |
| Control         | 1.37±0.21      | /       |

aP<0.05 vs control group; agP<0.05 vs ACM group; aaP<0.05 vs group of low dosage MPNS-ACM; cP<0.05 vs MPNS group.

Table 2 White blood cells, marrow CFU-GM, serum ALT and creatine(n=6, mean±SD)

| Group                  | White blood cells (×10⁹/L) | CFU-GM number (/ 10⁶) | ALT (U/ L) | Creatine (µmol/ L) |
|------------------------|---------------------------|----------------------|------------|--------------------|
| ACM                    | 76.67±17.32               | 74.75±21.91b         | 30.67±16.51| 12.17±1.17         |
| High dosage of MPNS-ACM| 70.00±8.74                | 107.83±14.75         | 29.17±14.36| 12.50±1.05         |
| Low dosage of MPNS-ACM | 74.67±9.71                | 115.25±12.53         | 23.83±3.55 | 13.00±2.19         |
| MPNS                   | 79.00±11.23               | 117.50±12.75         | 20.83±4.71 | 12.00±1.55         |
| Control                | 74.83±6.08                | 117.00±12.48         | 37.83±35.13| 12.67±1.51         |

bP<0.001 vs control group.

Table 3 Inhibition concentration of the drugs (µg/ mL)

| Group        | IC10 | IC50 | IC90 |
|--------------|------|------|------|
| ACM          | 0.006| 0.09 | 1.53 |
| MPNS         | 10.34| 97.8 | 925  |
| MPNS-ACM     | 0.19 | 1.07 | 6.03 |

Figure 2 TEM photograph of MPNS-ACM.
bioconsistency is an ongoing challenge. Small-sized magnetic particles (<400 nm) can be extravasated into the tumor interstitium and retained there, owing to the enhanced permeability and retention effect of the tumor\(^{[24]}\). Polymer carriers encapsulated with magnetite are difficult to prepare because of the different solubility of magnetite and polymers. Here, a kind of modified superparamagnetic iron oxide particles was introduced to prepare the magnetic targeting agents and the particle size could be controlled to 210 nm also, which was very important for the tolerance and efficacy of the agents.

There were two steps for preparation of MPNS-ACM: the preparation and modification of superparamagnetic iron oxide nanoparticles and the synthesis of magnetic polymer loaded with drug. Chemical coprecipitation was applied to synthesize the iron oxide nanoparticles. After modification with acid, well-suspended and stable magnetic fluid was successfully made. It can be stored as suspension for over one year at room temperature. The magnetite can be localized under magnetic field and dispersed when the magnetic force disappears. Interfacial polymerization was applied to the second step where the biodegradable macromolecular material butylcyanoacrylate reacted at the interface between oil and water. Magnetic nanoparticles and fat soluble ACM were encapsulated during the polymerization. The encapsulated ACM was more stable than the one by attachment. The lyophilization agent can be stored long-term at room temperature. After complete unaspiration, the nanoparticles intravenously administered could locate at the tumor by magnetic force. ACM was slowly released to produce high efficacy and low toxicity with the degradation of polymer.

The results showed that the anti-tumor effect of MPNS-ACM in vitro without magnetic field was similar to that of MPNS carrier group (considering the drug content was 12% approximately ), yet the anti-tumor test in vivo showed higher inhibitory efficacy of the magnetic carrier encapsulated with ACM on the gastric cancer model under magnetic field, which was based on the high targeting capacity of the system. TIR of targeted agent was higher than that of five-fold dosage of non-targeted drug. On the other hand, no toxicity to marrow, liver function and kidney function was found from targeted agents. The results show the high therapeutic efficacy on the tumor and the low toxicity to other organs of the magnetic targeted drug delivery system. It is a kind of safe chemotherapeutic agent.

Due to the difference between fat soluble drugs and water soluble drugs, different methods have been applied to encapsulate the drugs to the biopolymer carrier system with or without magnetite. The attempt to load ACM in to the carrier benefits the studies on other drugs including fat soluble drugs and water soluble drugs.

In conclusion, the magnetic targeted chemotherapy using MPNS-ACM has better tumor targeting, therapeutic efficacy and lower toxicity.

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REFERENCES

1 Gupta PK, Hung CT. Magnetically controlled targeted microcarrier systems. Life Sci 1989; 44: 175-186
2 Widder KJ, Morris RM, Poore GA, Howard DP, Senyei AE. Selective targeting of magnetic albumin microparticles containing low-dose doxorubicin: total remission in Yoshida sarcoma-bearing rats. Eur J Cancer Clin Oncol 1983; 19: 135-139
3 Rudge S, Peterson C, Vessely C, Koda J, Stevens S, Catterall L. Adsorption and desorption of chemotherapeutic drugs from a magnetically targeted carrier (MTC). J Control Release 2001; 74: 335-340
4 King ME, Kinney AY. Tissue adhesives: a new method of wound repair. Nurse Pract 1999; 24: 66-74
5 de Verdiere AC, Dubernet C, Nemat F, Soma E, Appel M, Fertej, Bernard S, Puisieux F, Couvreur P. Reversion of multidrug resistance with polyalkylycyanoacrylate nanoparticles:towards a mechanism of action. Br J Cancer 1997; 76: 198-205
6 Zhang ZR, He Q. Study on liver targeting and hepatocytes permeable valacidovir polybutylcyanoacrylate nanoparticles. World J Gastroenterol 1999; 5: 330-333
7 Ravi Kumar MN. Nano and microparticles as controlled drug delivery devices. J Pharm Pharm Sci 2000; 3: 234-258
8 Soppimuth KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. J Control Release 2001; 70: 1-20
9 Couvreur P, Barratt G, Fattal E, Legrand P, Vauthier C. Nanocapsole technology: a review. Crit Rev Ther Drug Carrier Syst 2002; 19: 99-134
10 Kattan J, Droz JP, Couvreur P, Marino JP, Boutan-Laroze A, Rouger P, Brault P, Vranckx H, Groquet JM, Morge X, Sanchez-Garnier H. Phase I clinical trial and pharmacokinetic evaluation of doxorubicin carried by polyisohexylocyanoacrylate nanoparticles. Invest. New Drugs 1992; 10: 191-199
11 Steila B, Arpicco S, Peracchia MT, Desmaeule D, Hoebeke J, Renoir M, D’Angelo J, Cattel L, Couvreur P. Design of folic acid-conjugated nanoparticles for drug targeting. J Pharm Sci 2000; 89: 1452-1464
12 Brigger I, Chaminade P, Marsaud V, Appel M, Besnard M, Gurny R, Renoir M, Couvreur P. Tamoxifen encapsulation within polyethylene glycol-coated nanospheres. A new antioestrogen formulation. J Pharm Pharmacol 2001; 24: 71-82
13 Calvo P, Gourtin B, Chauon H, Desmaeule D, D’Angelo J, Nol JP, Georgin D, Fattal E, Andreux JP, Couvreur P. Long-circulating PEGylated polycyanoacrylate nanoparticles as new drug carrier for brain delivery. Pharm Res 2001; 18: 1157-1166
14 Li YY, Pei YY, Zhou ZH, Zhang XY, Gu ZH, Ding J, Zhou JJ, Gao XJ, Zhu JH. Stealth polycyanoacrylate nanoparticles as tumor necrosis factor-alpha carriers: pharmacokinetics and anti-tumor effects. Biol Pharm Bull 2001; 24: 662-665
15 Vauthier C, Dubernet C, Fattal E, Pinto-Alandary H, Couvreur P. Polyalkylocyanoacrylates) as biodegradable materials for biomedical applications. Adv Drug Deliv Rev 2003; 55: 519-540
16 Skidan IN, Ge‘ perina SE, Severin SE, Guliava AE. Enhanced activity of rifampicin loaded with polybutyl cyanoacrylate nanoparticles in relation to intracellularly localized bacteria. Antibiot Khimioter 2003; 48: 23-26
17 Zhang ZR, He Q, Liao GT, Bai SH. Study on the anticarcinogenic effect and acute toxicity of liver-targeting mitoxantrone nanoparticles. World J Gastroenterol 1999; 5: 511-514
18 Zimmer S, Gao H, Zhu JH. Stealth polycyanoacrylate nanoparticles as new drug delivery devices. J Adjuvant Ther 1999; 19: 145-150
19 Ferte J, Bernard S, Puisieux F, Couvreur P. Reversion of multidrug resistance with polyalkylycyanoacrylate nanoparticles: mechanism of action. Br J Cancer 1997; 76: 198-205
20 Kreuter J. Evaluation of nanoparticles as drug-delivery systems I: preparation methods. Pharm Acta Helv 1983; 58: 196-209
21 Sommefeld P, Schroeder U, Sabel BA. Long-term stability of PBCA nanoparticle suspensions suggests clinical usefulness. Int J Pharm 1997; 155: 201-207
22 Kuwata T, Wang IM, Tamura T, Ponnamperuma RM, Levine R, Holmes KL, Morse HC III, De Luca LM, Ozato K, Vitamin A deficiency in mice causes a systemic expansion of myoid cells. Blood 2000; 95: 3349-3356
23 Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: the polyalkylocyanoacrylate nanoparticles. Cancer Res 1986; 46(12 Pt 1): 5637-5642
24 Yuan F, Delmian M, Fukumura D, Leunig M, Berk DA, Torchilin VP, Jain RK. Vascular permeability in a human tumor xenograft: molecular size dependence and cutoff size. Cancer Res 1995; 55: 3752-3756

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