Therapeutic effectiveness of slow-release PLGA-oxaliplatin microsphere on human colorectal tumor-bearing mice

Jing-Quan Li[a], Shi-Liang Wang[c], Fei Xu[a], Zhao-Yang Liu[b] and Rong Li[a]

The aim was to develop a slow-release poly-lactic-co-glycolic acid (PLGA)-oxaliplatin microsphere and to assess the therapeutic effectiveness and safety of this preparation on colorectal tumor *in vivo*. The PLGA-oxaliplatin microsphere was prepared based on a spray-drying method, and the drug loading and in-vitro oxaliplatin release profile were carried out using high performance liquid chromatography. The inhibiting effect on tumor growth was examined using in-vivo subcutaneously inoculated colorectal tumor models of nude mice. The size of the microsphere was less than 100 μm, drug loading was 18–22% and drug release time lasted as long as 30 days. PLGA-oxaliplatin microspheres significantly restrained tumor growth and this effect correlated with decreased expression of proliferating cell nuclear antigen and increased expression of terminal deoxynucleotidyl transferase dUTP nick end labeling in tumor cells. Body weight measurement and blood analysis did not suggest significant adverse effects on the mice during the study. The PLGA-oxaliplatin microsphere developed here was suitable for regional use; it appears safe and effective in controlling the tumor growth. This preparation shows promise in reducing local recurrence of colorectal cancer after resection, but needs further investigation.
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Introduction

Colorectal cancer is the third most common cancer in both men and women, accounting for 9% of all cancer deaths [1]. In patients with colorectal cancer who undergo radical surgery, 4–33% may develop local relapse [2]. Incomplete resection with positive margins and micrometastasis in regional lymph nodes are the two main factors related to tumor recurrence, which in turn leads to poor prognosis [3,4].

Adjuvant chemotherapy aims to control the local metastasis and tumor remnant after surgery has been performed with limited success. Oxaliplatin, a third-generation platinum-based agent, is among one of the most active agents against colorectal cancer; however, the efficacy obtained is suboptimal [5,6]. To improve the efficacy of oxaliplatin, a novel delivery system is required. Slow-release systems are capable of delivering drugs directly into the tumor site (as well as into the tumor interstitium, tumor bed and regional lymph drainage area) to act for a long period to control the tumor remnant and regional lymph node metastasis. These kinds of locally acting preparations have minimal side effects and some have obtained satisfactory results [7,8].

Poly-lactic-co-glycolic acid (PLGA) is a copolymer and has been approved by the Food and Drug Administration for use in some therapeutic devices. It is biodegradable and biocompatible and is well suited as a carrier of slow-release preparations. This study was designed to develop a novel slow-release system of PLGA-oxaliplatin microsphere based on a spray-drying method and to assess whether this slow-release system works effectively in inhibiting the growth of heteroplastic colorectal tumor inoculated in nude mice.

Materials and methods

Reagents

PLGA was provided by Sino-Implant Technology Co., Ltd. (Wuhu, China); oxaliplatin and carboxymethylcellulose were commercially purchased from FuRuiKangZe Pharmaceutical Co., Ltd. (Beijing, China).

Tumor line and animals

Human colorectal carcinoma cells (HCT-116) were provided by Professor Zhang Wei (The Academy of Medical Sciences, Beijing, China). These cells were cultured in McCoy's 5A medium containing 1.5 mg/l glutamine and 2200 mg/l sodium bicarbonate supplemented with 10% fetal bovine serum, 100 units/ml streptomycin, and 100 units/ml penicillin at 37.0°C in an atmosphere of 5% CO_2. Female Balb/c-nu strain mice at 6–8 weeks of age were supplied by the Institute of Laboratory Animal Sciences (The Academy of Medical

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Sciences, Beijing, China). All the animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the NIH.

Microsphere preparation

The PLGA-oxaliplatin microspheres were prepared based on a spray-drying method. Twenty micrograms of oxaliplatin was crushed and mixed with 80 mg of PLGA (molecular proportion of lactic acid and glycolic acid was 72 : 25, molecular weight was 35 000, melting range was 68–74°C). The mixture in solid form was then dissolved in 100 ml of methylene chloride. The organic solvent was removed through a vacuum-dehydration procedure, which was then pulverized and sprayed at a temperature of −5°C (freeze-drying). Using a screen-sizing technique, suitable PLGA-oxaliplatin microspheres (oxaliplatin was encapsulated within the PLGA) were obtained. PLGA microspheres were prepared using the same method. Finally, the particles were sterilized by β-irradiation.

Before this research, materialogical study of PLGA showed that PLGA is unstable under hard light and high temperature; PLGA is degradable and it degrades slowly when in contact with the water. The stability study of PLGA-5-fluorouracil microspheres showed that the microspheres were stable within 24 months when sealed and kept in a dark place at a temperature of −10°C. PLGA-oxaliplatin microspheres developed in this study were also stored in the same conditions as mentioned above to maintain stability.

Drug loading and drug release detection

High performance liquid chromatography was used for drug loading and drug release detection. A 515 high performance liquid chromatography pump (Waters), UV228 ultraviolet–visible detector and C18 (4.6 × 25 mm, 5 μm Hyoersil OD2) were purchased from Dalian Elite Analytical Instruments Co., Ltd. (Dalian, Liaoning, China). The mobile phase was methanol–water (10 : 90) and the detection wavelength was 250 nm. Limit of quantification of the system was 1.2 μg/ml, mean recovery was 98.32% (relative standard deviation: 1.02%, $n = 5$), within-day precision was 1.07% ($n = 15$), and day-to-day precision was 1.34% ($n = 5$).

Standard curve fitting

A suitable amount of standard oxaliplatin was dissolved in distilled water, with concentrations of 1, 2, 2.4, 4.8, 9.6, 14.4, and 19.2 μg/ml. Twenty microlitres of each dilution were put into the liquid chromatograph, the chromatogram was then recorded, the peak area measured and a linear regression was fitted between the peak area and concentration. The standard curve of linear regression was: $A = 3.165 \times 103\mathrm{C} + 2.469 \times 10^2$, in which A = peak area; C = concentration; $r = 0.9997$; linear range: 1–19.2 μg/ml, $n = 6$.

Drug loading detection

A suitable amount of PLGA-oxaliplatin microspheres (containing about 10 mg of oxaliplatin, $n = 6$) was placed into the pestle, crushed and mixed with two to three drops of distilled water. After initial grinding, 2 ml of distilled water was added until it was thoroughly ground. The solvent was then placed in a 25 ml measuring flask. Fifteen microliters of distilled water was added to the remaining oxaliplatin paste, which was then put into the measuring flask. Thereafter, distilled water was added to scale up to 25 ml; this was then stirred and filtered. The primary filtrate was discarded and 20 μl of the successive filtrate was put into the liquid chromatograph. The chromatogram was then recorded, the peak area measured and the drug loading calculated according to the linear regression equation.

Drug release detection

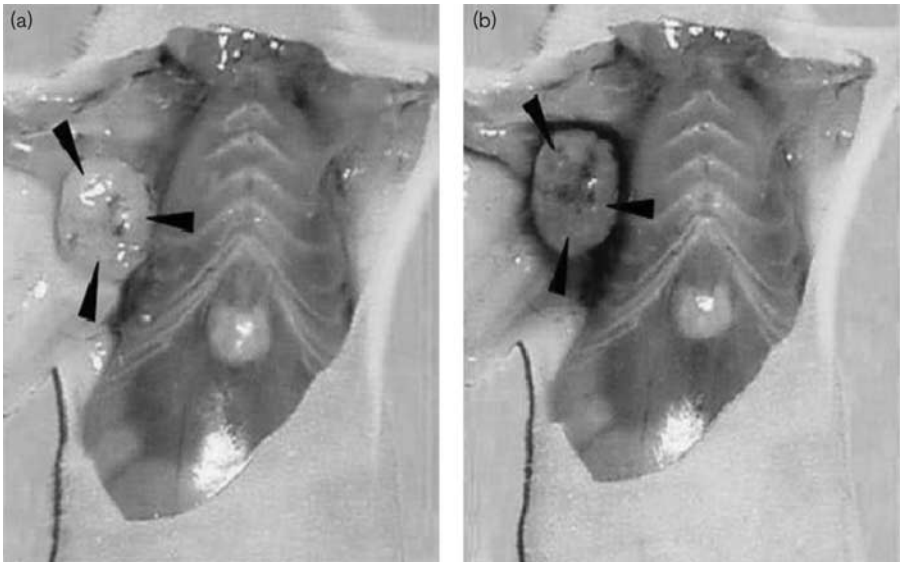
The oxaliplatin release profile was determined *in vitro*. PLGA-oxaliplatin microspheres (10 mg of oxaliplatin, $n = 6$) were weighed and placed in a dialysis bag, and the molecular weight cutoff of the dialysis membrane was 25 000. The dialysis bag was then placed in a 10 ml tube, which was placed in distilled water (37°C) for incubation. Small volumes were then removed from the solution at different times, adding the same volume of distilled water. Twenty microlitres of the filtrate were put into the liquid chromatograph, the chromatogram was then recorded, the peak area measured and the drug release rate calculated according to the linear regression equation.

Therapy for colorectal tumor *in vivo*

An in-vivo experiment to evaluate the therapeutic effect of PLGA-oxaliplatin microspheres was carried out using colorectal tumor models of nude mice. HCT-116 cells (1×10^6 cells suspended in 50 μl of Dulbecco's modified Eagle's medium) were subcutaneously (s.c.) inoculated in the right thoracal lateral region of 6 week-old Balb/c-nu mice. Mice were randomly separated into six groups as follows with 10 mice per group: group I, no treatment; group II, treatment with 32 mg of PLGA microspheres (the amount of PLGA microspheres was corresponding to the treatment for group VI, with a maximum PLGA microsphere amount compared with other groups); group III, treatment with 4 mg/kg of oxaliplatin; group IV, treatment with 8 mg/kg of oxaliplatin; group V, treatment with 20 mg/kg of PLGA-oxaliplatin microspheres (4 mg/kg of oxaliplatin); group VI, treatment with 40 mg/kg of PLGA-oxaliplatin microspheres (8 mg/kg of oxaliplatin). Treatment was commenced 7 days after inoculation when tumors developed (Fig. 1a).

All drugs (PLGA-oxaliplatin microsphere, PLGA microsphere, oxaliplatin) were dissolved in 0.02 ml of 1% carboxymethylcellulose sodium and were injected into

Fig. 1



(a) Arrows indicate the colorectal tumor in subcutaneously inoculated human colorectal carcinoma cells in the right thoracic lateral region of nude mice at day 7. (b) Arrows indicate where poly-lactic-co-glycolic acid-oxaliplatin microspheres were injected into the tumor; here a small quantity of methylene blue was added to the solution as a color agent.

the tumor only once. In group I, 0.02 ml of carboxymethylcellulose sodium was injected into the subcutaneous tissue. After the injection of PLGA-oxaliplatin microspheres, the drug diffused within the tumor (Fig. 1b).

The diameter of the tumor was measured with calipers and the volume of the tumor was calculated according to the formula: tumor volume $= 0.52 \times ab^2$, in which a is the longest diameter, and b is the shortest diameter of the tumor.

Immunohistological study

The animals were killed by cervical dislocation 9 days after therapy, and the tumors were removed and fixed in 10% neutral buffered formalin. Tissue specimens were then embedded in paraffin and sliced into 5 μm-thick sections. To detect proliferating cells in the tumor, cross-sections were incubated with a 1/200 dilution of antiproliferating cell nuclear antigen (PCNA) antibody (PC-10; Invitrogen, USA) at 4°C overnight. The following procedure was performed according to the protocol provided in the kit (PV-6000-G, Zhongshan, China) To detect apoptotic cells in the tumor, cross-sections were stained by terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method (In Situ Apoptosis Detection Kit; Roche, USA) according to the manufacturer's instructions. All sections were visualized by instillation with 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, Saint Louis, Missouri, USA) for 2 min. Counter staining was performed with haematoxylin.

Proliferating cells and apoptotic cells in the tumor were quantified in 10 different, randomly selected fields (0.135 mm^2 fields at $\times$ 200 magnification) with a computer-assisted morphometric image system (Image-Pro Plus, version 6.0, Media Cybernetics, Inc., Bethesda, Maryland, USA). The results were expressed as the positive cell numbers per field.

Toxicity assessment

To study the toxicity accompanied with therapy on bone marrow, liver, and kidney, blood samples of nude mice were examined on the ninth day (9 days after drug administration) for white blood cell count, alanine, aspartate, urea nitrogen, and creatinine. All nude mice were weighed using a scale at days 0, 3, 6, and 9.

Statistical analysis

Data were shown as mean ± SD. Student's t-test was used to examine the differences in mean tumor volume, white blood cell count, the value of alanine, aspartate, urea nitrogen, and creatinine, and the differences of weight. The differences in immunohistochemical staining between the case and control groups were evaluated with the analysis of variance test. Differences were considered significant only when the P value of comparison was less than 0.05. All statistical analysis was performed with SAS software (version 8.2; SAS Institute, Inc., Cary, North Carolina, USA).

Results

Microsphere preparation

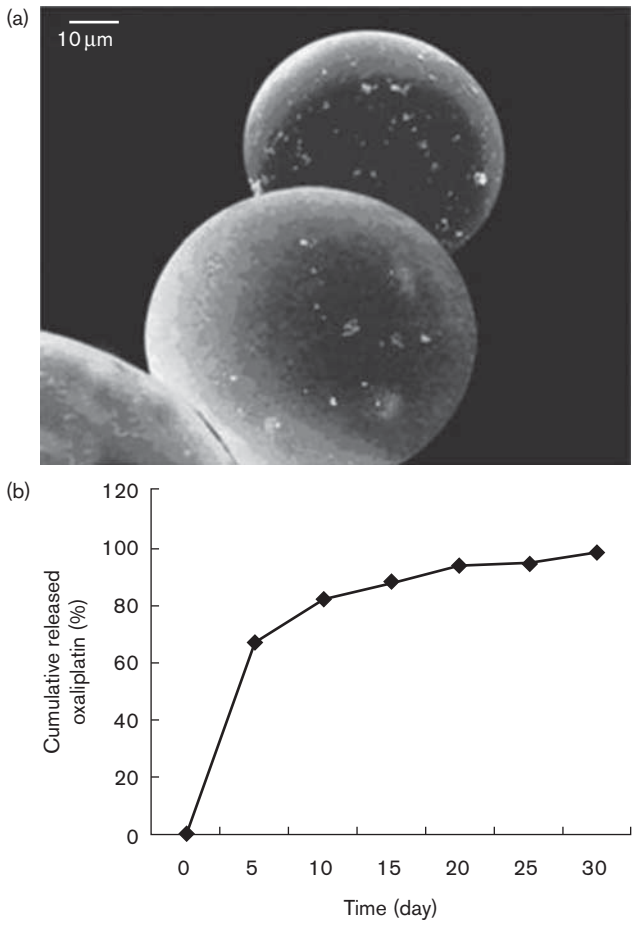
The physicochemical characterization of the PLGA-oxaliplatin microspheres was show in Table 1.

Table 1 The physico-chemical characterization of PLGA-oxaliplatin microsphere

Molecular weight of PLGA	Proportion of lactic acid and glycolic acid in PLGA	Configuration of the microsphere	Size range of the microsphere	Drug loading of the microsphere
35 000	72 : 25	Smooth surface, spherical or elliptical in shape	<100 μm	18–22%

PLGA, poly-lactic-co-glycolic acid.

Fig. 2



(a) Scanning electron photomicrograph of poly-lactic-co-glycolic acid-oxaliplatin microspheres. (b) Release profile of oxaliplatin *in vitro*.

Most of the microspheres were spherical, with only a small amount of microspheres elliptical in shape (Fig. 2a). The drug loading was between 18 and 22%. The drug was released initially as a 'burst' pattern at 1 h and the release rate was 19.1%; the drug was released steadily thereafter. 60% of oxaliplatin was released at about 96 h; the overall release time lasted as long as 30 days (Fig. 2b).

To test the release profile in 1% carboxymethylcellulose sodium, we replaced distilled water with 1% carboxymethylcellulose sodium in the tube for dialysis. The results showed that the drug release profile in 1% carboxymethylcellulose sodium was in accordance with that in distilled water.

In-vivo antitumor activity

The time course of tumor volume after initiation of therapy and the appearance of heteroplastic colorectal tumor at day 9 are shown in Fig. 3a and b. The tumors in groups I (blank control) and II (PLGA microspheres) grew rapidly with time. In groups III and IV (oxaliplatin injection groups, 4 and 8 mg/kg, respectively), tumors also grew rapidly in the same manner as those in groups I and II, indicating that oxaliplatin injection could not inhibit the tumor growth.

In contrast, in groups V and VI (PLGA-oxaliplatin microsphere injection, containing 4 and 8 mg/kg of oxaliplatin, respectively), a significant reduction of tumor volume was observed as compared with groups I, II, III, and IV at days 6 and 9. In group IV, the tumor volume at day 9 was smaller than at the day of drug administration.

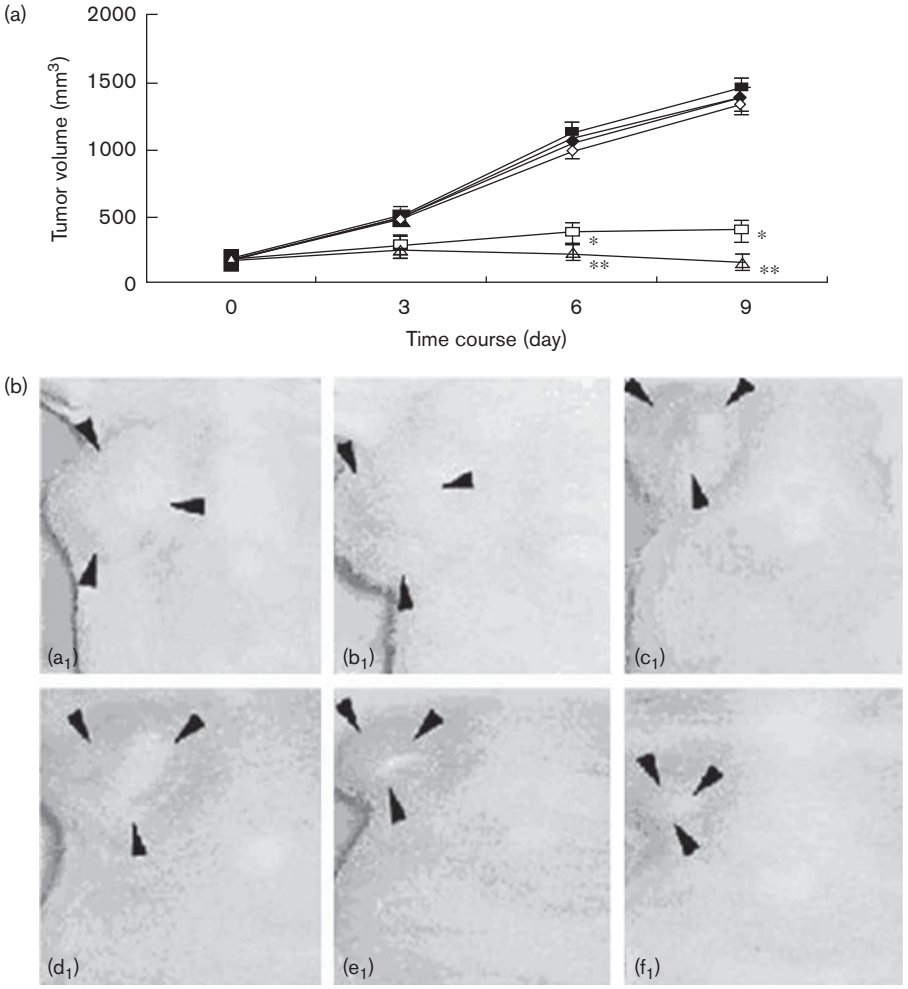
To test the inhibiting activity of PLGA-oxaliplatin microspheres on a different cell model, SW-480 (provided by Professor Zhang Wei, The Academy of Medical Sciences, Beijing, China), a HCT cell line was used. The cells were stored and cultured according to manufacture's recommendation; the animal, grouping, drug administrating strategies and other study conditions were the same as above. SW-480 cells (1×10^6 cells suspended in 50 μl of Dulbecco's modified Eagle's medium) were s.c. inoculated in the right thoracal lateral region of 6-week-old Balb/c-nu mice. Treatment was commenced 7 days after inoculation when tumors developed.

In this study the observation time was prolonged to 12 days and the results on the whole correlated with the study on HCT-116. In groups I (blank control), II (PLGA microspheres), III and IV (oxaliplatin injection groups, 4 and 8 mg/kg, respectively), the tumors grew rapidly with time in the same manner as in the study of HCT-116. In group V and VI (PLGA-oxaliplatin microsphere injection groups, containing 4 and 8 mg/kg of oxaliplatin, respectively) a significant reduction in colorectal tumor volume was observed as compared with groups I, II, III, and IV at days 6, 9, and 12. After day 9, the tumor volume had no obvious change in group V; however, the tumor volume continued to decrease in group VI (Fig. 4).

Immunohistological study

PCNA and TUNEL (apoptosis) staining were conducted at day 9 after the treatment (Fig. 5a). The percentage of PCNA-positive (proliferating) cells was significantly lower in tumors treated with PLGA-oxaliplatin microspheres

Fig. 3



(a) Effects of therapy on colorectal tumor subcutaneously inoculated in nude mice (human colorectal carcinoma cells): group I (blank control, ◆), group II [poly-lactic-co-glycolic acid (PLGA) microsphere, ■], group III (oxaliplatin 4 mg/kg, ▲), group IV (oxaliplatin 8 mg/kg, ◇), group V (PLGA-oxaliplatin microsphere, 4 mg/kg of oxaliplatin, □), group VI (PLGA-oxaliplatin microsphere, 8 mg/kg of oxaliplatin, △). Data are shown as mean ± SD (10 mice/each group), group V compared with groups I, II, III, and IV at days 6 and 9 (*$P<0.05$); group VI compared with groups I, II, III, and IV at days 6 and 9 (**$P<0.05$). (b) Appearance of heteroplastic human colorectal tumor in nude mice at day 9 (arrowheads): group I (a_1); group II (b_1); group III (c_1); group IV (d_1); group V (e_1) and group VI (f_1).

(groups V and VI) compared with tumors of the other groups. In contrast, the percentage of TUNEL-positive (apoptotic) tumor cells increased to 30 and 38% in group V and group VI, respectively, which was more than five times higher than those of the other groups (Fig. 5b).

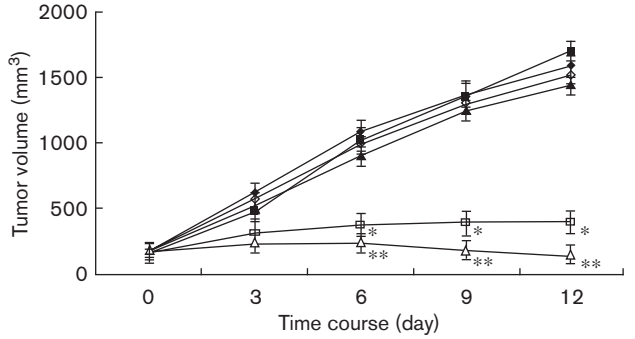
Toxicity assessments

Blood analysis and body weight measurement of nude mice were conducted to evaluate the side effects of the drugs. In all groups, no mice died of these therapies within the observation period (9 days). Body weight loss was statistically significant in groups III and IV at day 3 compared with groups I and II. Though the same trends were found in groups V and VI, the differences were not found between groups I and II. Weight increased with time, and at day 9 no differences were found among all the groups (Fig. 6a). Blood analysis indicated little difference in the data of the bone marrow (Fig. 6b), liver (Fig. 6c), and kidney (Fig. 6d) functions between each group, and all laboratory values were within the normal range.

Discussion

Systemic chemotherapy is used in advanced stage colorectal cancer to control tumor remnant and local metastatic focus after resection for the purpose of preventing the tumor recurrence. On account of poor blood supply, effective drug concentration is difficult to achieve in the tumor site and together with dose dependent toxicity, the effect of conventional systemic chemotherapy is unsatisfactory. Oxaliplatin, unlike other platinum-based agents, has activity and is among the most effective

Fig. 4



Effects of therapy on colorectal tumor subcutaneously inoculated in nude mice (SW-480): group I (blank control, ◆), group II [poly-lactic-co-glycolic acid (PLGA) microsphere, ■], group III (oxaliplatin 4 mg/kg, ▲), group IV (oxaliplatin 8 mg/kg, ◇), group V (PLGA-oxaliplatin microsphere, 4 mg/kg of oxaliplatin, □), group VI (PLGA-oxaliplatin microsphere, 8 mg/kg of oxaliplatin, △). Data are shown as mean ± SD (10 mice/each group), group V compared with groups I, II, III, and IV at days 6, 9, and 12 (*$P<0.05$); group VI compared with groups I, II, III, and IV at days 6, 9, and 12 (**$P<0.05$).

agents for colorectal cancer. However, the systemic chemotherapy of oxaliplatin alone has obtained limited results [5]. Even in combined projects, complete responses cannot be achieved [6]. Therefore, a novel administration strategy is required to improve the prognosis of the patients who have been surgically treated.

As early as in the 1980s, slow-release drug preparations were introduced with the aim to enhance the effects of the drugs meanwhile reducing the systemic toxicity. The drugs released from the preparations and diffused to local areas, forming high local concentrations and acting as 'regional chemotherapy'. Pioneering work on this kind of chemotherapy has earlier studied the intracerebral injection of liposome entrapped bleomycin in rats, and the results showed that bleomycin when enwrapped within the liposome and injected intracerebrally was of low toxicity to normal cerebral tissue, and was cleared more slowly from the injection site than when the free drug was injected [6]. The results obtained prompted the study of various slow-release systems and some have acquired clinical success. Gladel wafer (containing carmustine) is a successful slow-release preparation approved for regional chemotherapy in patients with malignant glioma after resection, with studies confirming that implanting 1,3-bis(2-chloroethyl)-1-nitroso-urea wafers after resection could prevent tumor recurrence and prolong the survival time [7]. Other slow-release preparations have also been studied in recent years and some have acquired promising results. The local recurrence rate of osteosarcoma after limb-sparing surgery in dogs and humans has been reported up to *28%*. When a biodegradable cisplatin-containing implant (OPLA-Pt) was inserted into the lesions, dogs in the OPLA-Pt group have been shown to be 53.5% less likely to develop local recurrence than dogs in the control group [9]. In an earlier study [10], photocured gelatin gel immobilized with gemcitabine was injected into the pancreatic tumor in nude mice; the result suggested that the slow-release system could significantly reduce the tumor volume compared with the gemcitabine injection. The preparation was promising in reducing or inhibiting local recurrence in pancreatic cancer patients who have undergone resection.

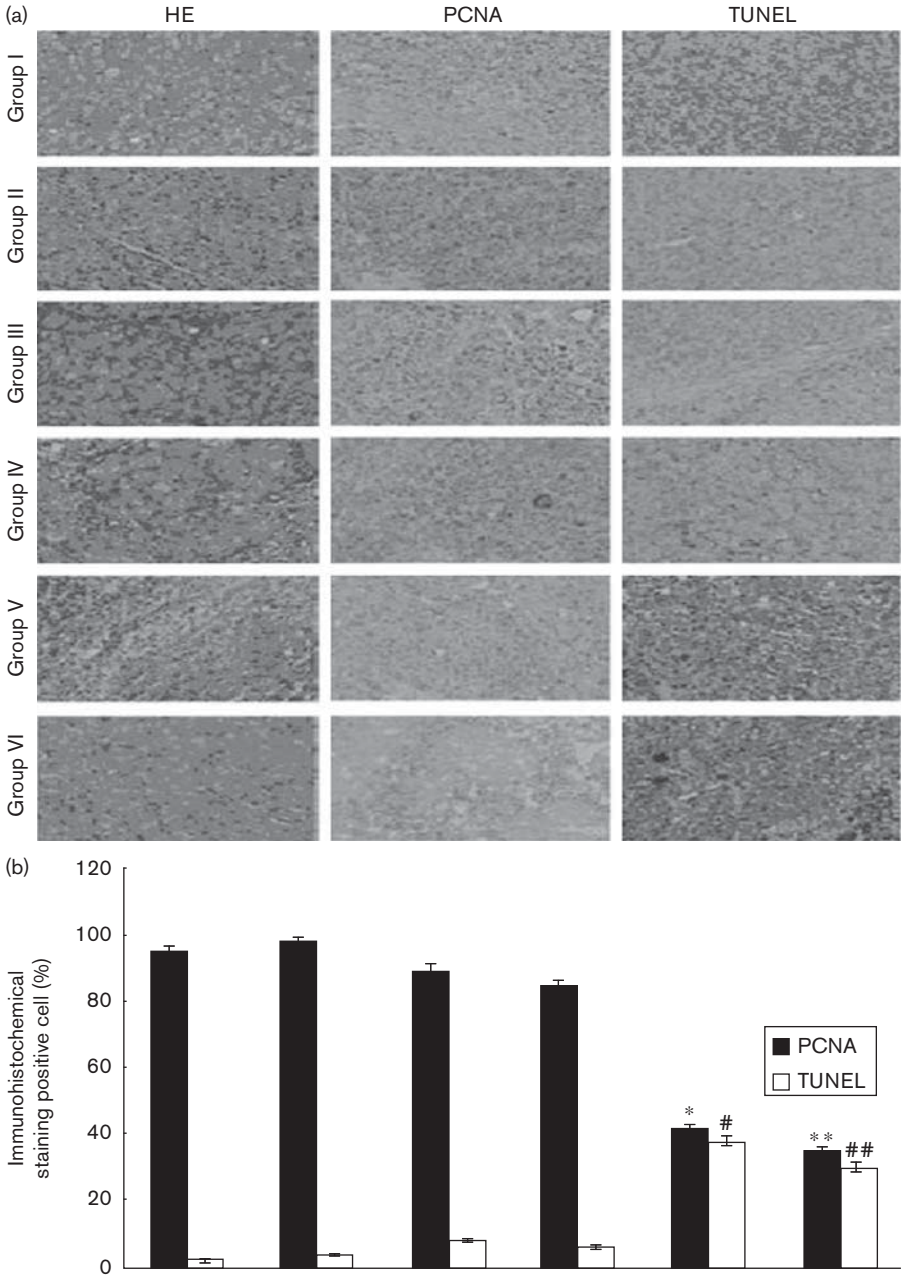
PLGA is a copolymer and has been approved by the Food and Drug Administration for use in some therapeutic devices. PLGA is of biodegradability and biocompatibility; it undergoes hydrolysis in the body to produce the original monomers of lactic acid and glycolic acid. These two monomers are the by-products of various metabolic pathways in the body. As the body effectively deals with the two monomers, there is minimal systemic toxicity associated with using PLGA for drug delivery or biomaterial applications [11]. Through controlling the molecular weight and the ratio of lactide and glycolide used for the polymerization, the degradation rate can be controlled. This results in the slow release of the drug enwrapped in the microsphere.

Our strategy was to develop slow-release PLGA-oxaliplatin microspheres through a spray-drying synthetic process, which is expected to release oxaliplatin steadily for a prolonged time, and thereby increase the bioavailability of the chemotherapeutic agent and minimize harmful systemic side effects. Such preparations used immediately after operations might form a very effective strategy to prevent tumor recurrence.

In this study, the size of the PLGA-oxaliplatin microspheres synthesized was less than 100 μm with a smooth surface that was spherical or elliptical in shape, which make the microspheres ideal for injection. Though the initial drug release was of a 'burst' pattern at 1 h, its release pattern was steady afterwards and the overall release time lasted up to 30 days (Fig. 2). This indicated that the microsphere is suited to prolonged local therapy.

The results obtained from two different cell lines of colorectal tumor showed that intratumoral injection of PLGA-oxaliplatin microspheres was significantly effective in inhibiting the growth of colorectal tumors s.c. inoculated in nude mice, and the growth inhibitory effect was dose dependent. When the dose was increased, the inhibiting effect was improved. However, oxaliplatin injection did not inhibit the colorectal tumor growth (Figs 3 and 4). Immunohistochemical study showed that the therapeutic success was correlated with increased cell apoptosis and decreased cell proliferation, which was supported by the results from TUNEL and PCNA staining (Fig. 5), which were in accordance with the therapeutic effects in the different groups.

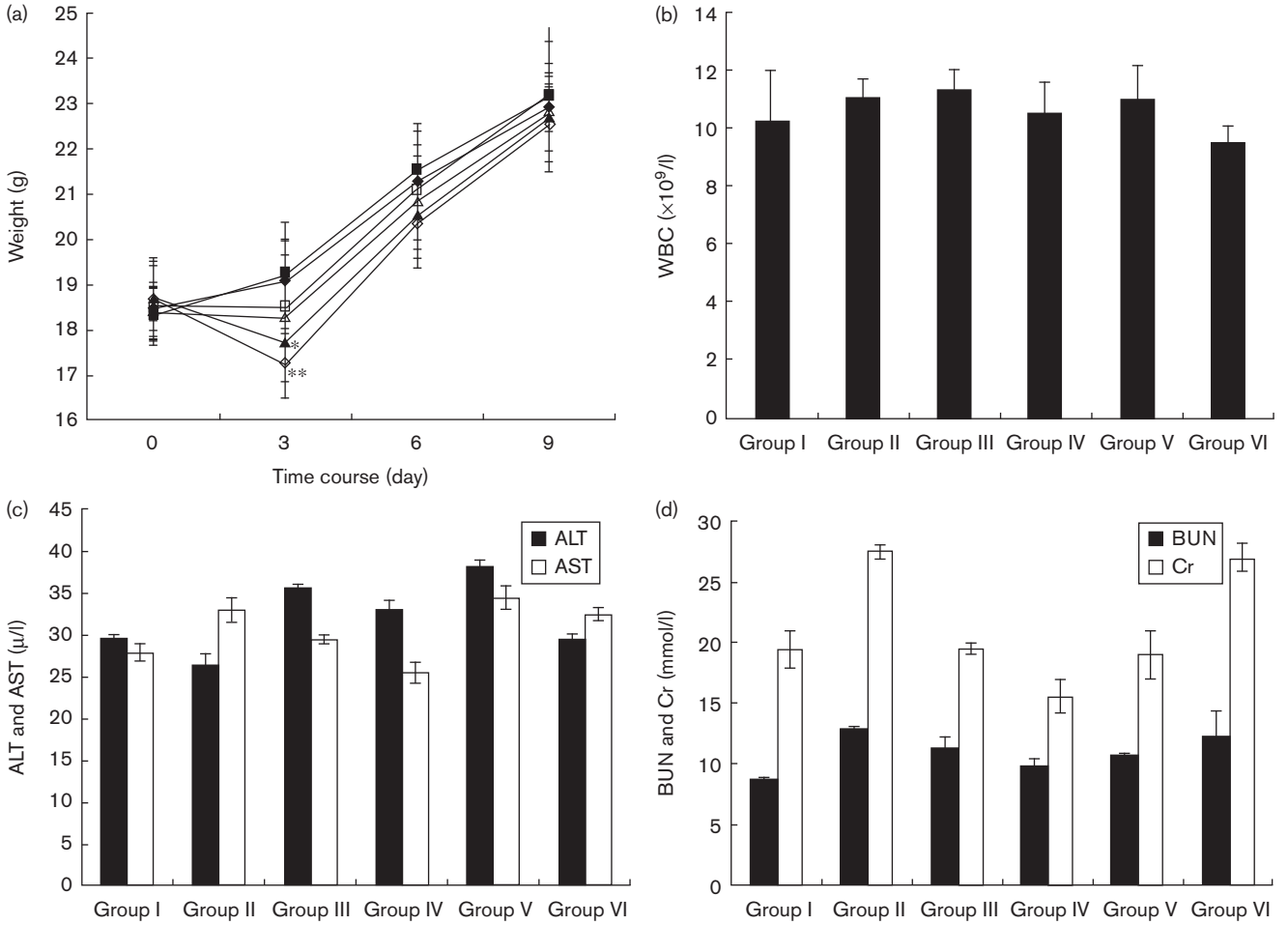
Fig. 5



(a) Immunohistochemical staining of colorectal tumor (subcutaneous inoculation of human colorectal carcinoma cells in nude mice) for hematoxylin and eosin (HE), proliferating cell nuclear antigen (PCNA proliferation) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining at day 9 after therapy. Representative pictures at $\times 200$ magnification. (b) Quantification of PCNA staining and TUNEL staining positive cells in colorectal tumor. Data are shown as mean ± SD [10 random high-power fields (0.135 mm^2)]: group V compared with groups I, II, III, and IV (*,$^{\#}P<0.05$); group VI compared with groups I, II, III, and IV (**,$^{\#\#}P<0.05$).

Residual tumor and micrometastasis in regional lymph nodes are the two main factors related to tumor recurrence after the operation [3,4]. For this reason, the requirement for effective chemotherapy is an anticancer drug permeating in all of the targeted tumor cells to achieve a lethal concentration, and this can be achieved only if the drug concentration is sustained and sufficient [12]. Studies on gemcitabine in the treatment of pancreatic cancer showed that the increase of gemcitabine exposure time from 2 to 24 h resulted in the increase of cytotoxicity up to 75-fold in IC_{50}, which indicated that the duration of drug exposure seems to be an important parameter for the anticancer effect of gemcitabine [13]. In an earlier research, we studied the effect

Fig. 6



(a) Body weight of nude mice after inoculation of human colorectal carcinoma cells: group I (◆); group II (■); group III (▲); group IV (◇); group V (□); and group VI (△). Data are shown as mean ± SD (10 mice/each group): group III compared with groups I and II at day 3 (*$P<0.05$); group IV compared with groups I and II at day 3 (**$P<0.05$). Blood analysis (day 9) of nude mice: (b) bone marrow (c) liver (d) kidney. Data are shown as mean ± SD (five mice each group). ALT, alanine aminotransferase; AST, aspartate transaminase; BUN, blood urea nitrogen; Cr, creatinine; WBC, white blood cell count.

of slow-release PLGA-gemcitabine microspheres on pancreatic tumor in nude mice, and the results showed that intratumoral injection of PLGA-gemcitabine microspheres could maintain high drug concentrations above 0.34 μg within the tumor and last as long as 40 days; however, in intraperitoneal gemcitabine administration, the gemcitabine concentration within the tumor quickly decreased to 0.30 μg in 30 min, and it continually decreased and was lower than 0.09 μg at day 10 [14]. Comparison of PLGA-oxaliplatin microspheres and oxaliplatin in this study also showed that effective drug concentration and sufficient acting time were crucial to chemotherapeutic efficacy. Even for oxaliplatin, to which the colorectal tumor cells are highly sensitive [15,16], as soon as the drug was injected into the tumor, it was cleared quickly in case of systemic chemotherapy and is rapidly converted to inactive metabolites, resulting in limited therapeutic efficacy to tumor cells. On the contrary, sustained release of oxaliplatin enabled the drug to be in contact with the tumor cells for a prolonged time, resulting in effective site-specific anti-cancer activity.

The major toxicity of oxaliplatin is the development of peripheral sensory neuropathy of two distinct types, an acute neurotoxicity that includes cold-related dysesthesia and may last days after administration [17]; oxaliplatin also causes a sensory neuropathy with increasing the cumulative dose [18]. These toxicities limit the use of oxaliplatin. This study showed that weight decreased significantly in oxaliplatin injection groups, and though the same tendency was found in PLGA-oxaliplatin microsphere groups, there were no differences compared with the blank control and PLGA-microsphere groups (Fig. 6a). In addition, blood examination of the bone marrow, liver and kidney were within the normal range in two PLGA-oxaliplatin microsphere groups (Fig. 6b–d),

which suggests that PLGA-oxaliplatin microspheres can decrease the risk of systemic side effects and its use is safe.

These in-vivo data support the strategy of immediate regional use (as well as in the tumor interstitium, tumor bed and regional lymph drainage area) of slow-release PLGA-oxaliplatin microspheres after a colorectal cancer operation to exert antitumor activity while reducing the probability of adverse events occurrence. Besides, the drug could also be administered intraperitoneally in patients with peritoneal carcinomatosis or interventionally administrated in patients with liver metastatis. Although it is too early to judge the long-term efficacy of this treatment, the results obtained in this study showed that the approach is worth further investigation to assess whether it inhibits local recurrence and improves the prognosis of colorectal cancer patients in clinical practice.

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References

1 American Cancer Society. *Cancer facts & figures 2008*. Atlanta: American Cancer Society; 2008.
2 Moriya Y. Treatment strategy for locally recurrent rectal cancer. *Jpn J Clin Oncol* 2006; **36**:127–131.
3 Gosens MJ, Klaassen RA, Tan-Go I, Rutten HJ, Martijn H, van den Brule AJ, *et al.* Circumferential margin involvement is the crucial prognostic factor after multimodality treatment in patients with locally advanced rectal carcinoma. *Clin Cancer Res* 2007; **13** (**22 Pt 1**):6617–6623.
4 Bosch Roig CE, Roselló-Sastre E, Alonso Hernández S, Almenar Cubells D, Grau Cardona E, Camarasa Lillo N, *et al.* Prognostic value of the detection of lymph node micrometastases in colon cancer. *Clin Transl Oncol* 2008; **10**:572–578.
5 Zori Comba A, Blajman C, Richardet E, Bella S, Vilanova M, Cóppola F, *et al.* A randomised phase II study of oxaliplatin alone versus oxaliplatin combined with 5-fluorouracil and folic acid (Mayo Clinic regimen) in previously untreated metastatic colorectal cancer patients. *Eur J Cancer* 2001; **37**:1006–1013.
6 Hind D, Tappenden P, Tumur I, Eggington S, Sutcliffe P, Ryan A. The use of irinotecan, oxaliplatin and raltitrexed for the treatment of advanced colorectal cancer: systematic review and economic evaluation. *Health Technol Assess* 2008; **12**:iii–iix. xi-162.
7 Firth G, Oliver AS, McKeran RO. Studies on the intracerebral injection of bleomycin free and entrapped within liposomes in the rat. *J Neurol Neurosurg Psychiatry* 1984; **47**:585–589.
8 Westphal M, Ram Z, Riddle V, Hilt D, Bortey E. Gliadel wafer in initial surgery for malignant glioma: long-term follow-up of a multicenter controlled trial. *Acta Neurochir (Wien)* 2006; **148**:269–275.
9 Withrow SJ, Liptak JM, Straw RC, Dernell WS, Jameson VJ, Powers BE, *et al.* Biodegradable cisplatin polymer in limb-sparing surgery for canine osteosarcoma. *Ann Surg Oncol* 2004; **11**:705–713.
10 Okino H, Maeyama R, Manabe T, Matsuda T, Tanaka M. Trans-tissue, sustained release of gemcitabine from photocured gelatin gel inhibits the growth of heterotopic human pancreatic tumor in nude mice. *Clin Cancer Res* 2003; **9**:5786–5793.
11 Astete CE, Sabliov CM. Synthesis and characterization of PLGA nanoparticles. *J Biomaterials Sci – Polymer Edition* 2006; **17**:247–289.
12 Gutman RL, Peacock G, Lu DR. Targeted drug delivery for brain cancer treatment. *J Control Release* 2000; **65**:31–41.
13 Kornmann M, Butzer U, Blatter J, Beger HG, Link KH. Pre-clinical evaluation of the activity of gemcitabine as a basis for regional chemotherapy of pancreatic and colorectal cancer. *Eur J Surg Oncol* 2000; **26**:583–587.
14 Li J-Q, Zhao P, Wang S-L, Tang Z-Q, Yang M, Liu Z-Y, *et al.* Phamacokinetical study of interstitial chemotherapy with PLGA-gemcitabine microsphere. *Oncology Progress* 2008; **6**:191–196.
15 Howells LM, Mitra A, Manson MM. Comparison of oxaliplatin- and curcumin-mediated antiproliferative effects in colorectal cell lines. *Int J Cancer* 2007; **121**:175–183.
16 Shen Y, Wang J, Yang T, Li Y, Jiang W, Guan Z, *et al.* Platinums sensitize human epithelial tumor cells to lymphotoxin alpha by inhibiting NFkappaB-dependent transcription. *Cancer Biol Ther* 2008; **7**:1407–1414.
17 Gamelin L, Boisdron-Celle M, Delva R, Guérin-Meyer V, Ifrah N, Morel A, *et al.* Prevention of oxaliplatin related neurotoxicity by calcium and magnesium infusions: a retrospective study of 161 patients receiving oxaliplatin combined with 5-fluorouracil and leucovorin for advanced colorectal cancer. *Clin Cancer Res* 2004; **10** (**12 Pt 1**):4055–4061.
18 André T, Boni C, Mounedji-Boudiaf L, Navarro M, Tabernero J, Hickish T, *et al.* Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. *N Engl J Med* 2004; **350**:2343–2351.