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ORIGINAL PAPER

Antitumor efficacy of Poly(dimer acid-dodecanedioic acid) copolymer in mice bearing Sarcoma-180 tumor

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Abstract The drug release profiles of poly(dimer acid-dodecanedioic acid) P(DA-DDDA) copolymer containing 5% adriamycin hydrochloride (ADM) in vitro were evaluated. The biocompatibility of P(DA-DDDA) under mice skin was also evaluated, macroscopic observation and microscopic analysis demonstrated that the copolymer is biocompatible and well tolerated in vivo. Antitumor efficacy of P(DA-DDDA) copolymers containing 5% adriamycin hydrochloride (ADM) implanted subcutaneously in mice bearing Sarcoma-180 tumor exhibited increased volume doubling time (VDT) (31 ± 1.5 days) compared to plain subcutaneous injection of ADM (7 ± 0.9 days). The studies suggest that P(DA-DDDA) copolymer as an effective carrier for antineoplastic drug like adriamycin hydrochloride has a very good prospect in the treatment of noumenon tumors.

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Introduction

Polyanhydrides [1–3] have been wildly used in drug controlled release because of their desired physicochemical properties. They often containing short carbon chain unit, such as sebacic acid (pH 3.88, 10 °C in water) which has used in some occasions and showed good biocompatibility [4, 5], but they might lead to inflammation reactions potentially in the implanted tissue because of high acidity. Zhou ZB introduced that poly(dimer acid-sebacic acid) copolymer, a type of polyanhydride which containing sebacic acid, showed minimal edema observed in rabbits brain, else essentially equivalent to the absorbable gelatin sponge [6]. Dodecanedioic acid (pH 4.41, 10 °C in water) might be better candidate, because it has longer carbon chain and lower acidity than sebacic acid and might be more biocompatible than sebacic acid. Poly(dimer acid-dodecanedioic acid) P(DA-DDDA) copolymer has been prepared previously [7]. In vitro studies showed that all the P(DA-DDDA) copolymers are degradable in phosphate buffer at 37 °C and the release profiles of a hydrophilic model drug, ciprofloxacin hydrochloride, follow first-order kinetics. The degradation and drug release rate were adjustable by rectify the monomer unit ratio in relative polymers. The in vitro studies suggested that the P(DA-DDDA) copolymer might be potentially used as drug delivery devices.

In this work, the drug release profiles of poly(dimer acid-dodecanedioic acid) P(DA-DDDA) copolymer containing 5% adriamycin hydrochloride (ADM) in vitro, biocompatibility of the copolymer under mice skin and antitumor efficacy of P(DA-DDDA) containing 5% adriamycin hydrochloride (ADM) in mice bearing Sarcoma-180 tumor were evaluated.

Material and methods

Materials

Adriamycin hydrochloride(ADM) was purchased from Tianjing Central Pharmaceutical Corp., Ltd. (Tianjing, China). Poly(dimer acid-dodecanedioic acid) P(DA-DDDA) copolymers were homemade as follows [7]. The copolymers of DA with DDDA were synthesized by melt polycondensation of the corresponding prepolymers and the prepolymers were synthesized by refluxing the purified DA or DDDA with acetic anhydride (50 g in 300 ml) for 20–25 min. In a typical copolymerization, 5 g DA prepolymer and 5 g DDDA prepolymer were added to a glass tube (2 × 20 cm) with two side arms with magnetic stirring and polymerized at l80 °C under high vacuum conditions (about 20–50 Pa) for 1 h. During the polymerization, a strong nitrogen sweep with vigorous agitation of the melt was performed for 30 s every 15 min. The crude polymer was dissolved in CH_2Cl_2 and precipitated by filtering into vigorously stirring dry petroleum ether (60–90 °C). The precipitate was filtered off, washed with petroleum ether, and dried at 40 °C under vacuum for 48 h.

Animals

For tumor studies, six to eight weeks old KunMing mice weighing 20–25 g were purchased from Hubei experimental animal center. Animal's sex was in random. The animals were given sterile food and water per day under controlled conditions of temperature (23 ± 2 °C), humidity (50 ± 5%).

Drug release file in vitro

The drug-loading cylinder (5% Adriamycin hydrochloride) in a cylindrical shape (4 mm in diameter, 10 mm in long, about 150 mg) was produced by sieving the copolymer and the drug powder to 100–150 μm and pressed in a homemade polytetrafluoroethylene (PTFE) mould under 20 atm pressure. After this the mould was dismantled and the device removed. The drug-loading cylinder in 50 ml of 0.1 M phosphate buffer solutions (pH7.4) was performed in an incubator shaker (Model HQ45A, Factory of Scientific Instrument, Wuhan, CN) agitated at 60 rpm with a constant temperature of 37 ± 1 °C in air bath. The phosphate buffer solutions were daily changed to maintain sink conditions throughout, and the device was carefully freeze-dried. The amount of drug released was detected by the absorbance of the release medium at 480 nm using a Perkin Elmer Uv/Vis spectrometer, Lambda Bio 40. All measurements were carried out in triplicate by analyzing three separate devices.

All the detected data were processed using Microcal Origin 6.1 software on a PC computer.

Biocompatibility study

The biocompatibility of P(DA-DDDA) copolymer was evaluated in mice skin as compared the clinically used biopolymer, absorbable gelatin sponge. Briefly the tested device and the absorbable gelatin sponge (3 mm in diameter, 1 mm in thickness) were subcutaneously implanted in two sides of the mice abdomen, respectively. The evaluation was to last for 1 month. At the predetermined time, mice were sacrificed and their skins were removed and the microscopic histopathology was assessed by Hematoxylin-eosin stained analysis.

Tumor propagation

The tumor Sarcoma-180 cells from the Center of Collection, Wuhan University, was propagated in adult Kunming mice by serial transplantation of 10^6 viable tumor cells into the intraperitoneal cavity of female mice. Solid tumors were produced by intradermal injection of 5×10^5 viable cells on the alar skin after dilution with Dolbecco's Modified Eagle's Medium so as to get 10^6 cells in 100 μl [8]. The tumor volume of mice was daily timing measured with a caliper after the mice have been narcosised with aether. The tumor growth was monitored by the tumor volume.

The tumor volume (V) was calculated using the formula:

$$V = \pi \cdot D_1 \cdot D_2 \cdot D_3/6$$

where $D_1 \cdot D_2 \cdot D_3$ are the diameters of each tumor in three perpendicular planes.

The monitoring was started when the tumor volume reached 100 ± 10 mm^3 and the time taken to reach 200 ± 10 mm^3 was considered as the volume doubling time and was calculated as follows

$$\mathrm{VDT} = \log 2 \times (T_1 - T_0)/(\log V_1 - \log V_0)$$

where V_0 is the volume of the tumor at time T_0 and V_1 is the volume at time T_1 [9].

Volume doubling time and animal survival

The time required to double the treatment volume (VDT) from 100 mm^3 to 200 ± 10 mm^3 was taken as

criterion to assess the antitumor efficacy. The mice were divided into different groups, each group consisting of ten mice. The first group, control group, was injected PBS (pH 7.4), the second group placebo blank devices, the third group injected of plain adriamycin hydrochloride (ADM) in PBS pH 7.4 (5 mg/kg body weight), the fourth group implanted of P(DA-DDDA) [weight ratio W_{DA}:W_{DDDA} = 50:50] containing 5% ADM (10 mg/kg body weight) beside tumor. The tumor volume was monitored till it reached 200 ± 10 mm^3. The animal survival was monitored at different time intervals.

Statistical analysis

In vivo tumor response results were analyzed using log-rank test. Only P values less than 0.05 were considered statistically significant.

Results

In vitro drug release

The drug release characteristics from P(DA-DDDA) [weight ratio W_{DA}:W_{DDDA} = 50:50] were determined using 5% adriamycin hydrochloride loading devices. The plots of cumulative release percent of the device versus release time is shown in Fig. 1. As shown in Fig. 1, P(DA-DDDA)-ADM device showed the steady drug release rate relatively and the drug release rate profile of P(DA-DDDA)-ADM device closed to its erosion rate profile, but didn't follow first-order kinetics which was the drug release profiles of P(DA-DDDA)-ciprofloxacin hydrochloride device [7].

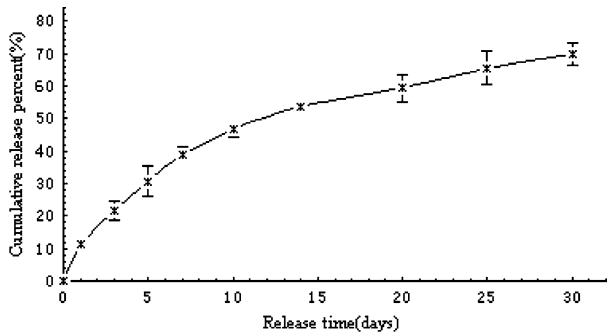

Fig. 1 The drug release profile of 5% drug loading ADM-P(DA-DDDA) [weight ratio W_{DA}:W_{DDDA} = 50:50] device at 37 °C in 0.1 mol/L pH 7.4 PBS

Preliminary biocompatibility

Macroscopically, the results that all the experimental mice survived healthily and actively to the date of their sacrifice indicated that the devices were well tolerated. The implanted sites were clean and the remainder devices were easily retrieved, no obvious damage to the locally implanted tissue was observed. Figure 2 A and B shows the photomicrography of mice skin at day 2(acute period) after P(DA-DDDA)[weight ratio W_{DA}:W_{DDDA} = 50:50] and absorbable gelatin sponge implantation, respectively. The shape of P(DA-DDDA) material was steadiness on the whole but became a little smallish showed the characteristic of surface erosion and the volume of absorbable gelatin sponge became a little increscent and has cranny on it showed the characteristic of bulk erosion obviously. The tissue showed the characteristics of the middling of acute inflammation tissue in both P(DA-DDDA) material and absorbable gelatin sponge. Have a little bleeding and edema arounded the implanted materials and the reaction of gelatin sponge showed severity relatively. The hyperplasias of the fibrous tissues and capillary vessels were not observed. Figure 3 A and B shows the photomicrography of mice skin at day 12(sub-acute period) after P(DA-DDDA)[weight ratio W_{DA}:W_{DDDA} = 50:50] and absorbable gelatin sponge implantation, respectively. The volume of P(DA-DDDA) material became small unceasingly and the cranny enlarged on absorbable gelatin sponge in which have some inflammation cell, lymphocyte and foam cells and there was some multinuclear giant cells surrounded. Bleeding and edema vanished basically. The responses of cells and tissue to the implanted polymer were slightly than those of the absorbable gelatin sponge. Figure 4 A and B shows the photomicrography of mice skin at day 30(chronic period) after P(DA-DDDA)[weight ratio W_{DA}:W_{DDDA} = 50:50] and absorbable gelatin sponge implantation, respectively. The most part of P(DA-DDDA) material had been absorbed, and the absorbable gelatin sponge has been smashed. The tissue showed the characteristics of the convalescence of chronic inflammation tissue. The hyperplasias of the fibrous tissues and capillary vessels were observed and many foam cells and multinuclear giant cells surrounded and phagocytosed the foreign body simultaneity. There was a clear verge between the local cell response region and the normal tissue region. Microscopically histopathologic observations showed that the responses of cells and tissue to the implanted polymer had not significant difference with those of the absorbable gelatin sponge.

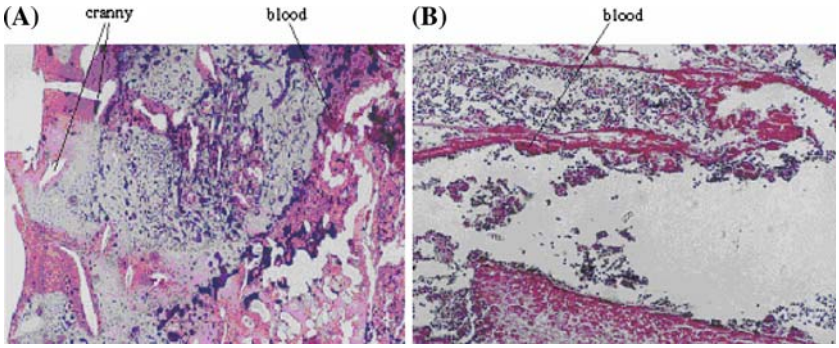

Fig. 2 Hematoxylin-eosin stained photomicrograph(10×) of mice skin at day 2 after absorbable gelatin sponge implantation (**A**) and P(DA-DDDA)(W_{DA}:W_{DDDA} = 50:50) implantation (**B**)

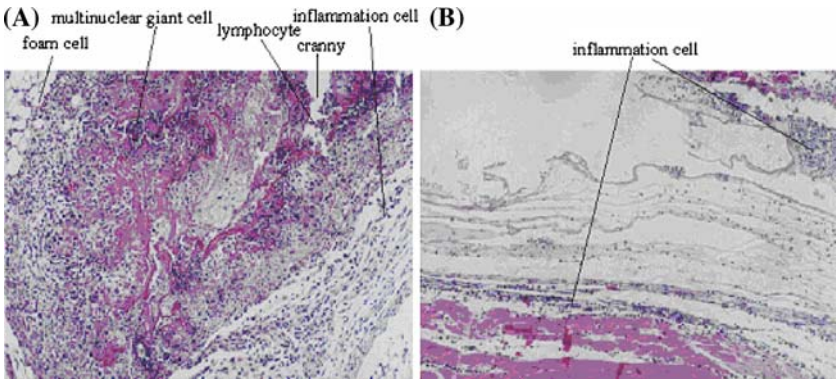

Fig. 3 Hematoxylin-eosin stained photomicrograph(10×) of mice skin at day 12 after absorbable gelatin sponge implantation (**A**) and P(DA-DDDA)(W_{DA}:W_{DDDA} = 50:50) implantation (**B**)

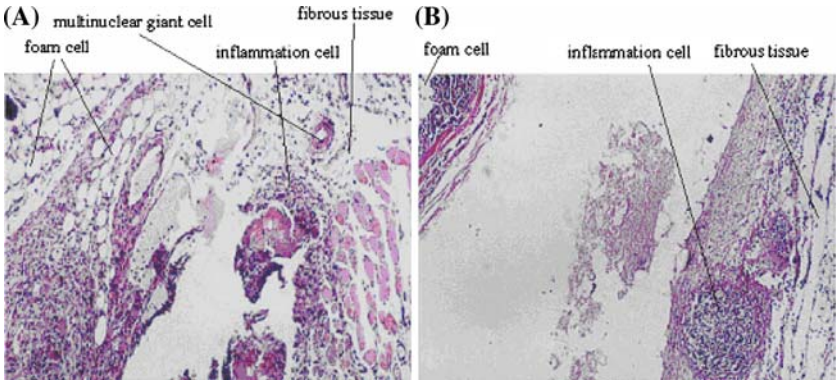

Fig. 4 Hematoxylin-eosin stained photomicrograph(10×) of mice skin at day 30 after absorbable gelatin sponge implantation (**A**) and P(DA-DDDA)(W_{DA}:W_{DDDA} = 50:50) implantation (**B**)

The results presented here suggest that P(DA-DDDA) copolymer are well tolerated by the skin tissue of mice.

Antitumor efficacy

As shown in Table 1, the fourth group of drug controlled release preparation of P(DA-DDDA) [weight ratio W_{DA}:W_{DDDA} = 50:50] containing 5% ADM exhibited an increased survival time(30% survival at 120 days) compared with third group of injection of plain ADM (10% survival at 90 days). The difference is statistically significant ($P < 0.025$). As shown in Fig. 5, the fourth group exhibited an increased cytotoxic effect as revealed by increased VDT (31 ± 1.5 days). About the fourth group tumor

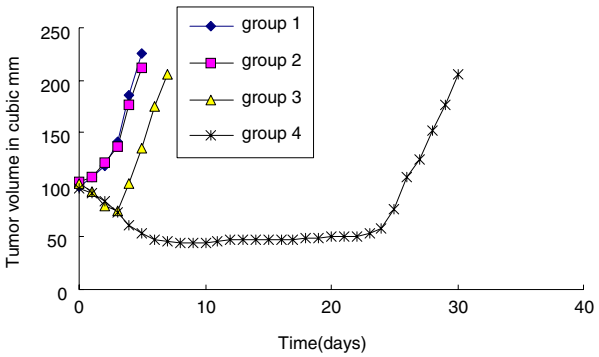

Fig. 5 The sarcoma-180 tumor volume growth in Kunming mice on subcutaneous administration of plain and adriamycin hydrochloride (ADM) loaded drug controlled release preparation of P(DA-DDDA)(DA:DDDA = 50:50, w/w). Group 1: control group, was injected PBS pH 7.4; Group 2: placebo blank devices; Group 3: injected of plain adriamycin hydrochloride (ADM) in PBS pH 7.4 (5 mg/kg body weight); Group 4:injected of P(DA-DDDA)(DA:DDDA = 50:50, w/w) containing 5% ADM (10 mg/kg body weight) beside tumor

Table 1 Survival time of mice implanted Sarcoma-180 tumor under skin

Observed time (days)	Cumulative survival ratio of mice		
	Group 2	Group 4	Group 3
0	1.0	1.0	1.0
16	1.0	1.0	1.0
17	0.9	1.0	1.0
18	0.7	1.0	1.0
19	0.6	1.0	0.9
20	0.5	1.0	0.9
21	0.3	1.0	0.8
24	0.3	1.0	0.8
25	0.2	0.9	0.7
27	0.2	0.9	0.7
28	0.1	0.8	0.6
40	0.1	0.8	0.6
45	0.0	0.7	0.4
60	0.0	0.6	0.3
70	0.0	0.6	0.3
80	0.0	0.5	0.2
90	0.0	0.4	0.1
120	0.0	0.3	0.0

volume decreased considerably and the cytotoxic activity was sustained for 24 days, after which the tumor volume increased sharply. Since the initial release of the drug from the preparation was faster, therefore reduction in tumor volume during the initial stages was also faster. Injection of plain ADM was not effective in substantially decreasing the tumor volume, although reduction in tumor volume during the first few days was higher compared to drug controlled release preparation of P(DA-DDDA). The second group showed similar tumor growth curves with the control group. The animal survival at the end of 40, 80 and 120 days for the control group was 10, 0, 0%, for placebo blank device was 10%, 0%, 0%, for plain ADM, 60, 20, 0%, for drug controlled release preparation of P(DA-DDDA)-ADM it was 80, 50, 30%, respectively. The drug controlled release preparation of P(DA-DDDA) obviously prolonged the survival time of the mice. The experiment results showed the nice Antitumor efficacy of P(DA-DDDA)-ADM device.

Discussion

Generally, the polyanhydride-drug delivery devices were produced by mixing the carrier materials with drugs under melt condition and implanted in the diseased tissue after surgery in the treatment of noumenon tumors [3, 4]. P(DA-DDDA)-ADM device cannot use this method for preparation because adriamycin hydrochloride cannot bear heating but this can use pressing method, so the equality was in problem and the drug release profiles of P(BA-PA)-ADM device were departure to first-order kinetics. This can explain the drug release profiles of P(DA-DDDA)-ADM device differed from P(DA-DDDA)-ciprofloxacin hydrochloride showed in Fig. 1. However, the drug release rate profile of P(DA-DDDA)-ADM device showed the steady drug release rate relatively and may be use in vivo potentially. In vivo biocompatibility experiments of P(DA-DDDA) under mice skin compared with the currently clinically used biopolymer, absorbable gelatin sponge showed that P(DA-DDDA) has better biocompatibility and demonstrated that the copolymer may be used as materials suitable for implantation. Polyanhydrides used in drug controlled release for the local controlled chemotherapeutics are visible, such as low side-effect, long effect, high drug concentration in focus and have been wildly used in the treatment of noumenon tumors [10], then the Antitumor efficacy of drug-polymer controlled release device is very important. Generally the drug-polymer controlled release devices have the better antitumor efficacy than by using chemotherapeutic drugs solely [11]. As shown in Table 1 and Fig. 5, the drug controlled release preparation of P(DA-DDDA) obviously prolonged the survival time of the mice. The experiment results showed the nice Antitumor efficacy of P(DA-DDDA)-ADM device. With a view to its good biocompatibility, the P(DA-DDDA) copolymer as an effective carrier for antineoplastic drug like adriamycin hydrochloride has a very good prospect in the treatment of noumenon tumors.

Conclusions

In vitro studies showed that the drug release rate of poly(dimer acid-dodecanedioic acid) P(DA-DDDA) copolymer containing 5% adriamycin hydrochloride (ADM) were relatively steady. Biocompatibility of P(DA-DDDA) in mice skin showed that this copolymer might be potential candidates for the delivery of hydrophilic drugs locally in body. Antitumor efficacy of P(DA-DDDA) containing 5% adriamycin hydrochloride (ADM) in the mice bearing Sarcoma-180 tumor was obviously better than that of injection of plain ADM.

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