Drug Development and Industrial Pharmacy, 2011; 37(12): 1473–1480

ISSN 0363-9045 print/ISSN 1520-5762 online
DOI: 10.3109/03639045.2011.587429

informa
healthcare

RESEARCH ARTICLE

Preparation of amifostine polylactide-co-glycolide microspheres and its irradiation protective to mouse through oral administration

Ting-Li Lu[1], Wei-guang Sun[2], Wen Zhao[1], and Tao Chen[1,3]

[1]*Key Laboratory for Space Biosciences & Biotechnology, Faculty of Life Science, Northwestern Polytechnical University, Xi'an, China,* [2]*Hospital of Northwestern Polytechnical University, Xi'an, China, and* [3]*Xi'an Libang Pharmaceutical Co., Ltd, Liposome Research Center of Shaanxi Province, Xi'an, China*

Abstract

The objectives of this study were to prepare the amifostine polylactide-co-glycolide (PLGA) microsphere and investigate its irradiation protective to mouse through oral administration. Amifostine-loaded PLGA microsphere was formulated using a modified double emulsion-solvent evaporation technique. The microsphere particle was spherical with a mean diameter of 2.8 ± 0.1 μm. Release data of amifostine PLGA microsphere was tested in phosphate-buffered saline at 37°C using a dialysis method and its release profiles was biphasic, showing a relatively large burst effect (50%) over the first 6 h, followed by a slower release phase, which sustained with 80% amifostine released in 48 h and almost 100% release till 6 days (144 h). A diffusion-controlled release model (Higuchi equation, $R^2 = 0.9725$) was obtained for amifostine releasing from PLGA microsphere. The radiation experiment was performed by applied cobalt-60 γ-radiation source. One hour before γ-radiation exposure, the mouse was orally given free amifostine and PLGA microsphere, respectively. The irradiation effects, such as blood cell concentration, superoxidase dismutase (SOD) activity and malondialdehyde (MDA) level were monitored. The results indicated that amifostine PLGA microsphere was more irradiation protective to mouse than that of free amifostine under the same oral administration route.

Keywords: Amifostine; PLGA microsphere; irradiation; oral administration; SOD

Introduction

Amifostine is an organic thiophosphate prodrug that serves as a cytoprotective agent in cancer chemotherapy and radiotherapy[1]. After dephosphorylation by alkaline phosphatase, amifostine forms an activated free thiol metabolite WR1065, which has been shown to prevent both radiation-induced cell death and mutagenesis while facilitating the normal cell repair[2]. So it exerts a protector to the healthy tissues through free-radical scavenging, hydrogen donation, and direct binding to the active species of alkylating and platinum agents[3–7]. Moreover, amifostine can induce antioxidant enzymatic activities in normal tissues. So it can offer an additional potential for overall radiation protection[8]. For these reasons, amifostine has been approved by the US Food and Drug Administration and acts as a protector for the normal tissue against the damaging effects of ionizing radiation and chemotherapy.

Although amifostine is used to improve the quality of life of cancer patients, it is associated with various adverse effects, which lead to discontinuation of amifostine treatment and sometimes delays of radiotherapy[9–11]. Amifostine in its present available formulation is not effective when administered orally, so it must be administered intravenously[12,13]. Unfortunately, amifostine does not cross the blood-brain barrier even when administered systemically[14] and will be rapidly cleared from the body with a very short distribution half-life of 15-min intravenous (IV) infusion in serum[15,16]. Furthermore, amifostine has dose-limiting toxicities including hypotension, nausea, and vomiting, which are significantly augmented upon IV route of administration[17,18]. All of the above

Address for Correspondence: Ting-Li Lu, Faculty of Life Science, Northwestern Polytechnical University, Box 707, No. 127 Youyi West Road, Xi'an, 710072 China. E-mail: lutinglixinxin@nwpu.edu.cn

(Received 01 February 2011; revised 27 April 2011; accepted 05 May 2011)

mentioned barriers are likely to remain severely limited to amifostine clinical use and might be overcome by utilizing alternative route of administration or formulation strategies based on drug delivery system (DDS).

In an attempt to search for an alternative route of administration, Cassatt and his coworker investigated the effect of different amifostine administration route to prevent xerostomia in patients receiving radiotherapy for head-and-neck cancer[19]. They found the effect of amifostine treated by subcutaneous (SC) administration was at least as effective as that by IV administration, whereas some others found that SC administration provided a more effect than that of IV administration[16,20–22].

In addition to finding the different route for amifostine administration, attempts had also been made to develop various formulations of amifostine including microencapsulation using biodegradable polymers, such as the aliphatic polyesters copolymer (polylactide-co-glycolide [PLGA]) based on lactic acid and glycolic acid. Pamujula et al. used solvent evaporation technique to prepare PLGA microcapsules of amifostine and found 24–96 h sustained release of amifostine from microcapsules[23–25]. They thought that this slow release would provide long-term amifostine concentration to the specific organs. They also prepared amifostine-loaded hybrid microcapsules using PLGA and chitosan[26]. For the incorporation of chitosan into inner of hybrid microcapsules, the drug release increased significantly and it was found that 45% amifostine released in 24 h and almost 100% released in 11 days. Mandal prepared amifostine PLGA (50:50) microcapsules by solvent evaporation technique and found approximately 50% amifostine was released during the first 6 h *in vitro* release study[23,24]. All of the above research work hoped to obtain the orally active controlled-release formulation of amifostine, but they did not investigate the oral activity of amifostine microcapsules *in vivo*. Srinivasan prepared biodegradable pellet of amifostine by a patented procedure involving incorporation of amifostine in a proprietary matrix and evaluated the use of a subcutaneously implantable for the radioprotector in mouse exposed to cobalt-60 γ-radiation[14]. Compared with the conventional SC administration, SC implantation was effective for administration of amifostine as a radioprotector.

In this paper, we aim to develop the microsphere formulation of amifostine and investigate its irradiation protective in mouse exposed to cobalt-60 γ-radiation through oral administration. The irradiation protective of oral PLGA microsphere in mouse was recorded by comparing its effects with those of free amifostine via oral route and intraperitoneal administration, respectively. The results might answer whether oral administration of PLGA microsphere is as effective in making the drug available as giving the drug via intraperitoneal injection or it had more irradiation protective in mouse than that of free amifostine through the same oral administration.

Materials and methods

Materials

Amifostine was obtained from Sigma Aldrich (St Louis, MO). The copolymer poly(DL-lactic/glycolic acid) (PLGA 75:50; inherent viscosity 0.4 dL/g, MW 50,000, polydispersity 2.0) was obtained from Sichuan Zhuoxin Biomaterials Co. (Chengdu, China). Egg phosphatidylcholine (EPC) with the purity of 98% was supplied by Xi'an Libang Pharmaceutical Technology Company (Xi'an, China). Dichloromethane (DCM) and polyvinyl alcohol (PVA, MW 30,000–70,000, 98–99% hydrolyzed) were obtained from Tianjin Kemiou Chemical Regent Co. (Tianjin, China). Fluorescamine was obtained from Beijing Aoboxing Biotechnology Co. Acetonitrile was high performance liquid chromatography (HPLC) grade and purchased from Sigma Corp. All other general chemicals and reagents were obtained commercially at analytical grade. Male Chinese Kunming mouse, 3-month-old and weighing approximately 20 ± 2 g, were supplied from the Experimental Animal Centre of Fourth Military Medical University (Xi'an, China).

Preparation of PLGA microspheres

Amifostine was formulated using a modified double emulsion-solvent evaporation technique (w/o/w solvent extraction process) as previously reported[27] (Figure 1). Briefly, 20 mg amifostine powder was dissolved in 0.5 mL deionized water and formed the internal water phase (w_1). 100 mg PLGA was dissolved in 3 mL DCM solution and formed oil phase (o). In addition, the oil phase contained 0.1% EPC, which acted as an emulsifier. Then, 0.5 mL amifostine solution (w_1) was fed into 3.0 mL PLGA solution (o) and emulsified by ultrasonic homogenization to form the primary emulsion (w_1/o). 2% PVA aqueous phase was used as the external water phase (w_2). The primary emulsion (w_1/o) was injected using a glass syringe with a 21.5 G needle into PVA aqueous solution (w_2) to produce a double (w_1/o/w_2) emulsion. This emulsion was homogenized for 30 min using the ultrasonic at constant room temperature. Then, DCM was evaporated at room temperature for 6 h under a stirring with a rate of 1000 rpm. After that, the microsphere solution was centrifuged at a rate of 6000 rpm for 30 min at 4°C. The precipitated microsphere was washed three times with deionized water. Then, the microspheres were vacuum-dried overnight and stored at 4°C. For control, the blank microsphere without amifostine contained was also prepared using the above similar methods.

Particle size and morphology

The size of microsphere was determined by Malvern Zeta Sizer Nano2s (MS 2000, UK). Samples were prepared by diluting the microsphere suspension with deionized water and sonicated for 30 s to ensure the homogeneous. Three measurements were carried out for each sample and the average values were recorded.

The surface morphology of microspheres was observed by Scanning Fiber-optic Microscope (FEI Quanta 200, USA). Microspheres were mounted onto metal stubs using double-sided adhesive tape. After being vacuum-coated with a thin layer (100~150) of gold, the microspheres were examined by SEM at high vaccum 10-6 Torr, 3.0 nm at 30 kV.

Amifostine content in microsphere

For each formulation, 10 mg sample was dissolved in 1 mL DCM. Ten milliliter of 0.15% Tween 80 was added to the solution and the resultant precipitate was removed by ultracentrifugation (30,000 rpm at 8°C) for 1 h. The supernatant fluid was imbibed and filtered through 0.22 μm microporous film. The amifostine concentration was determined by HPLC.

HPLC analysis of amifostine

The amount of amifostine contained in microsphere was determined by pre-column derivatization HPLC using Water ALLIANCE 2695 series HPLC system with a fluorescence detector (U-3310).The excitation and emission wavelengths were 395 and 480 nm, respectively. The Agilent HC-C_{18} (250 mm × 4.6 mm, Agilent Technologies Inc., California) column was used at ambient temperature. The derivatization reagent was prepared by dissolving 1.0 mg fluorescamine in 10.0 mL acetonitrile, adjusted to pH 9.0 by 0.2 M borate saline buffer. The solution was stored in brown glass bottles and kept at 4°C. The mobile phase was an aqueous mixture solution of acetonitrile, H_2O, and 10% H_3PO_4 aqueous solution (25:75:1, v:v:v). The flow rate was 1.0 mL/min and 10 μL of sample was injected. Standard calibration curves ($r^2 > 0.99$) for amifostine in the range 0.125–8.000 μg/mL concentrations were prepared. The concentration of amifostine in each sample was determined by intrapolating the peak height to the amifostine standard curve. Each sample was analyzed in triplicate. The chromatographic control system, data acquisition, and analysis were performed using Water empower software.

Measurement of stability of microsphere

Stability studies of PLGA microspheres were performed by measuring the surface morphology change through SEM at a predetermined sampling time of 0, 7, 14, 21 days. The 50 mg PLGA microsphere was incubated into phosphate-buffered saline (PBS) medium and kept at 37°C. The surface morphology of microsphere was observed at time intervals.

In vitro release kinetics of amifostine

The *in vitro* release of amifostine from microspheres was determined by incubating 50 mg amifostine-loaded microspheres (after separation of unencapsulated amifostine) in 50 mL PBS buffer containing 0.02% Tween 80, 0.02% Tween 20, and 0.01% sodium azide. The PBS buffer was in a glass beaker and put in a shaking (constant, 150 oscillations/min) water bath at 37°C. Then the samples were hold in dialysis, both ends were fastened, and put into PBS buffer. The sample was analyzed in triplicate. At time intervals of 0, 2, 4, 8, 12, 24, 48, and 96 h, 5 mL of releasing PBS buffer was withdrawn and the same amount of fresh PBS buffer was replaced and it was made sure the releasing PBS buffer volume was 50 mL during the whole experiment period. The amount of amifostine released from microsphere was determined by HPLC.

Animals

All the animal studies were conducted in accordance with the internationally accepted "Guide for the Care

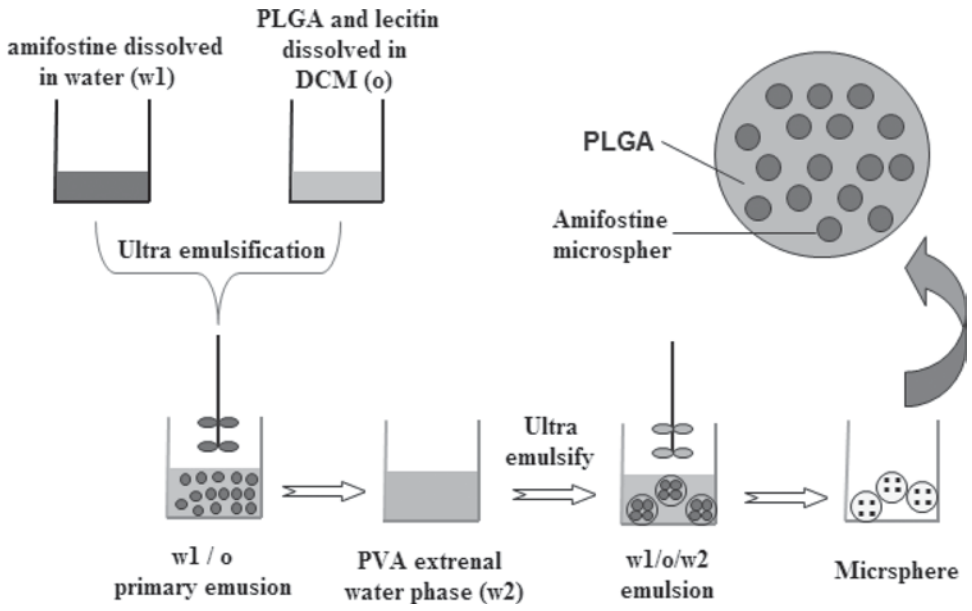

Figure 1. The processing to form the microsphere formulation of amifostine.

and Use of Laboratory Animals" published by the US National Institutes of Health (NIH Publication No. 80–23, revised 1996) and approved by the local ethics committees for animal experimentation. Forty male Chinese Kunming mice were randomized by body weight to five groups and each group had eight mice. Five groups were different depending on different amifostine administration route used. The mouse in the normal control (NC) group had free access to food without radiation exposure and drug given. In the radiation control (RC) group, the mouse was exposed to radiation but without any drug given. The mouse in intraperitoneal amifostine (IPA) group was given amifostine aqueous solution intraperitoneally. The mice in oral amifostine (OA) group and oral amifostine microsphere (OMA) group were orally given free amifostine and amifostine microsphere, respectively. The drug dosage of mouse in every group was 500 mg/kg and was given 1 h before exposure to radiation.

Radiation experiment

The cobalt-60 (^{60}Co) γ-irradiation was supported by Northwestern Nuclear Technique Institute (Xi'an, China) and a radiation dose level of 6.0 Gy, which was far lower than the lethal dose (8.5 Gy) for mouse, was selected. Mouse was kept in a well-ventilated perspex covered box and exposed to whole body γ-irradiation at a dose rate of 1.0 Gy/min at a source-to-animal distance of 80 cm. After the irradiation exposure, the mouse exposed to 6.0 Gy was killed 3 days later. Eyeball blood was collected and the red blood cell (RBC), white blood cell (WBC), platelet, and lymphocyte counts were monitored. The spleen and thymus were removed and weighed using the electronic balance (MP6001, quantity sensitive: 0.1 g, China) after absorbing water and tissue fluid on the surface of tissue using drying paper. The spleen and thymus indices were the ratio of organ weight to body weight. The activity of superoxidase dismutase (SOD) and the level of malondialdehyde (MDA) in testicular tissue were also determined following the previous reports[28]. Testicular tissue was removed and washed three times in cold isotonic saline (0.9%). Tissues were homogenized with cold PBS buffer (pH 7.4) and 10% homogenate obtained (w/v), which was centrifuged (4000 rpm, 4°C) for 15 min. The clear supernatant was obtained to determine SOD activity and MDA content.

Statistical analysis

Data were analyzed using the SPSS statistical package (V.10; Chicago, IL) by one-way analysis of variance (ANOVA). Statistically significant differences ($p < 0.05$) were found between or within the experimental groups by ANOVA; individual differences were assessed by post hoc analyses (Tukey's test). Data are expressed as mean ± standard deviation (SD) in the text and tables and as mean ± standard error of the mean (SEM) in the figures.

Results and discussions

Microsphere characteristics

Particle size and morphology

The particles were between 0.8 and 10.0 μm, with a mean diameter of 2.8 ± 0.1 μm (Figure 2). Surface SEM photo showed that PLGA microsphere was spherical and some accumulation of smaller microcapsules on the surface of the larger particles (Figure 3). Still there was some small pitting on few of the particle surfaces, which might be due to the evaporation of the trapped organic solvent during the drying process.

Stability of microsphere

The stability of PLGA microspheres was tested over a 21-day period at 25°C and the particle surface SEM morphology was monitored at weekly intervals (Figure 4). We could see the intact spherical structure of PLGA microsphere at the beginning (a, 0 day). After 7 days storage, a number of pores were seen scattered over the surface of microspheres (b, 7 days). The pore size increased obviously with the elapsed time and some of the fragments could be observed (c, 14 days). During the following 7 days, only porous remnants of microspheres remained (d, 28 days). The increased permeability of PLGA microsphere with elapsed time would be the good reason for the surface morphology change of PLGA microsphere.

In vitro release kinetics

Release data of amifostine from PLGA microsphere was shown as cumulative percent released over 144-h study periods (Figure 5). The results showed that the release

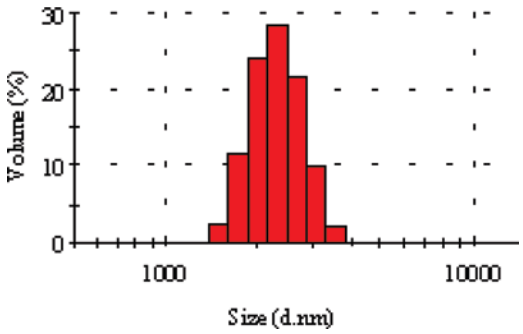

Figure 2. Particle size distribution of amifostine polylactide-co-glycolide (PLGA) microsphere.

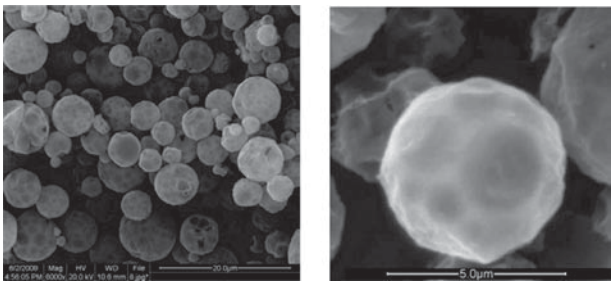

Figure 3. Scanning electron photomicrographs of surface of amifostine polylactide-co-glycolide (PLGA) microsphere.

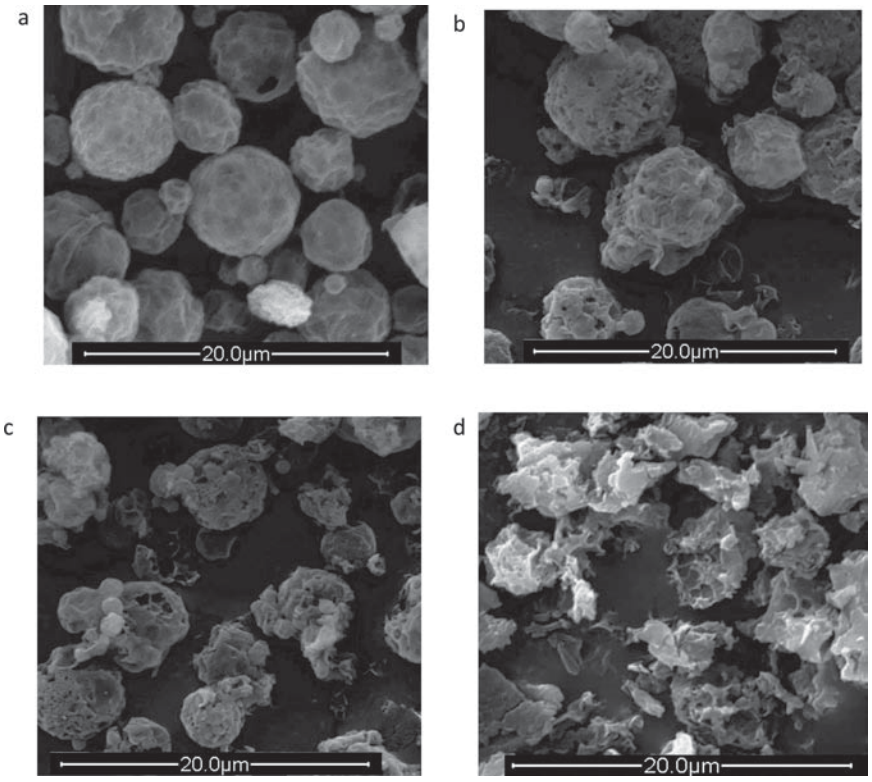

Figure 4. Surface standard error of the mean (SEM) morphology of polylactide-co-glycolide (PLGA) microspheres at different time.

profile of amifostine microsphere was biphasic, showing a relatively large burst effect over the first 6 h and almost 50% diffusion, followed by a slower release phase, which reached 80% after 48 h. Then the release of amifostine from microsphere was slow and there was almost 100% diffusion till 144 h.

In terms of drug release model, three of the most common kinetic profiles, zero-order, first-order, and Higuchi equations, were applied and the correlation coefficient R^2 was used as the mark to determine the releasing mechanism of amifostine from microsphere. Higher correlation coefficients ($R^2 = 0.9725$) were obtained for the Higuchi equation, indicating a diffusion-controlled release model of amifostine PLGA microsphere.

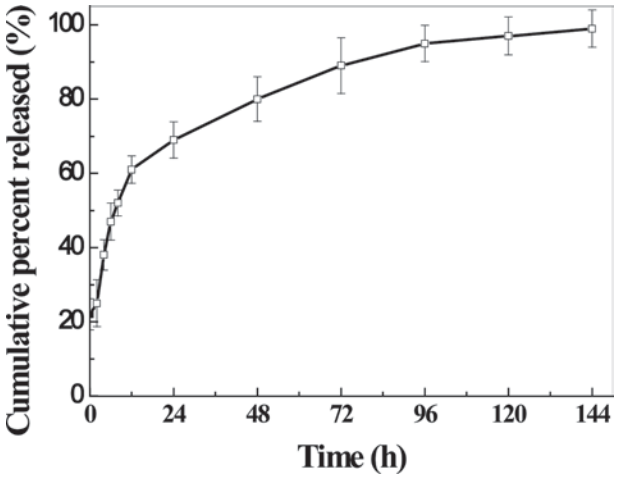

Figure 5. Amifostine release profile of polylactide-co-glycolide (PLGA) microsphere in PBS.

Irradiation protective of PLGA microsphere to mouse

Blood cells concentration

The radiation dose of 6.0 Gy was far below the lethal dose (8.5 Gy) and could monitor the radiation effects in mice. Compared with the mice in NC group, the mice in four radiation groups (RC, IPA, OA, OMA) all had the decreasing concentration of blood cells, such as RBC, WBC, and lymphocytes, and platelet, which indicated radiation did have some effects on the hematological system in mouse (Figure 6). The platelet concentration had the minimal decline for its insensitive response to irradiation. When compared with mice in RC group, the mice in three amifostine administration groups (IPA, OA, OMA) had the improved condition and the results were different with different amifostine administration route. The mice in OMA and IPA groups all had significant increase in RBC and lymphocyte concentration and there was no statistical significant difference between them. The mice in OMA group all had the higher blood cell concentration than that in OA group.

Tissue level

The mice in NC group had an average testicular SOD activity of 80 U/mgprot, whereas the mice in four radiation groups (RC, IPA, OA, OMA) all had low SOD activity value (Figure 7). Compared with mice in RC group, the mice in IPA group had an improved SOD activity, which was almost the same value as that in NC group. Moreover, the mice with the amifostine microsphere orally given

made increased SOD activity and reached almost 70 U/mgprot, whereas SOD activity had almost no change in OA group with oral free amifostine given.

For MDA level, mice in all radiation groups (RC, IPA, OA, OMA) had the higher MDA concentration than that in RC group, which indicated the product of lipid peroxidation resulted from irradiation exposure. The mice in OA group expressed the highest MDA level, which showed free amifostine given orally had no inhibition to MDA level. While comparing with RC group, the mice in OMA and IPA groups all had lower value of MDA level.

Spleen and thymus were the important immunity and hematopoietic organs, so the spleen index and thymic index could reflect the radiation damage to the immune system. Similarly, the mice in all radiation groups had significant decrease in spleen and thymic indices (Figure 7). Free amifostine given orally had no effect on spleen index or thymic index. Still, amifostine PLGA microsphere

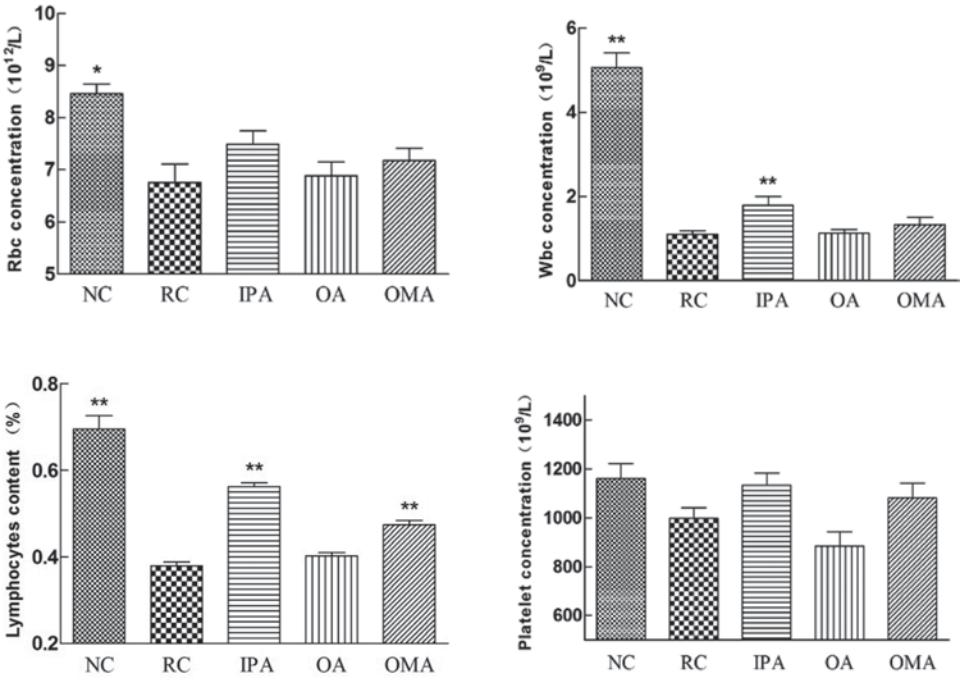

Figure 6. The blood cell concentration of mouse exposed to 6.0 Gy radiation dose.

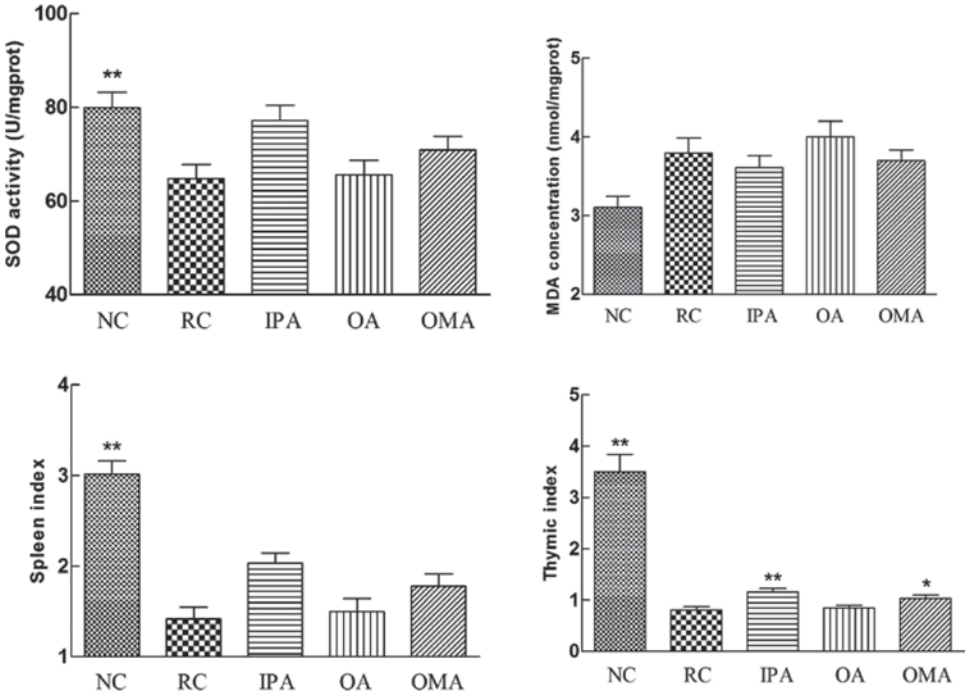

Figure 7. Tissue level of rat exposure to 6.0 Gy radiation dose.

orally or free amifostine via IP injection all showed significant improvement in these two indices.

Oral administration was a convenient drug administration route. The goal of our study was to form the sustain-released amifostine PLGA microsphere and confirm its radiation protective to mouse via oral administration and eventually to determine whether oral administration of amifostine PLGA microsphere is as effective as free amofistine given via IP injection.

Exposure to the radiation dose (6.0 Gy) had some damage to the hematological system of mouse, which was the decrease in blood cells concentration, such as RBC, WBC, and lymphocytes, and platelet. It also did damage to organs and made some tissue-level change, such as low testicular SOD activity and high MDA level. The irradiation damage to immune system expressed with the low spleen index and thymic index. Amifostine administrated 1 h before irradiation exposure could reduce the damage to some degree, which depended on the administration route of amifostine. In general, the amifostine PLGA microsphere orally resulted in increase in blood cells concentration, high testicular SOD activity and spleen and thymic indices but low MDA level. The same protective results could be obtained with amifostine IP injection but could not be obtained with free amifostine given orally. So our research indicated that amifostine PLGA microsphere orally was (i) almost as effective as giving free amifostine via intraperitoneal injection and (ii) more radiation protective to mouse than that of oral free amifostine.

It was well known that microspheres ensure a physical barrier between the internal and the external compartments and thus protect encapsulated drug molecules, which would provide reasonable assurance that most amifostine will be absorbed through the gastrointestinal tract. In addition, from the release protocol, the amifostine microsphere was able to provide sustained release of amifostine, as a result there would be more constant blood levels as compared with free amifostine when given by the same route. Moreover, the steroid structure of PLGA would protect amifostine and prolong its effect to irradiation. We thought all of the above reasons made amifostine PLGA microsphere have better results than that of free amifostine, when using the same oral route.

Conclusion

Amifostine PLGA microsphere was successfully prepared by a modified double emulsion-solvent evaporation technique. Amifostine release behavior *in vitro* displayed that this formulation could realize a sustained release and showed a biphasic release profile, a relatively large burst effect over the first 6 h, followed by a slower release phase. The irradiation protective to mouse was investigated through oral administration 1 h before exposure to ^{60}Co γ-radiation. The animal experiment showed that amifostine in biodegradable PLGA microsphere orally was effective, which would be convenient and promising as an oral administration formulation to protect the normal tissue against the damaging effects of ionizing radiation and chemotherapy. However, it was only a preliminary study for the application of PLGA microspheres to amifostine oral delivery. The *in vivo* amifostine absorption mechanism would be investigated in detail in the future work.

Declaration of interest

This work was financially supported by the Natural Science Foundation of China (NSFC, No. 20872119) and NPU Foundation for Fundamental Research (No. NPU-FFR- WO18104).

References

1. Anné PR, Curran WJ Jr. (2002). A phase II trial of subcutaneous amifostine and radiation therapy in patients with head and neck cancer. Semin Radiat Oncol, 12:18–19.
2. Tarnuzzer RW, Colon J, Patil S, Seal S. (2005). Vacancy engineered ceria nanostructures for protection from radiation-induced cellular damage. Nano Lett, 5:2573–2577.
3. Mabro M, Faivre S, Raymond E. (1999). A risk-benefit assessment of amifostine in cytoprotection. Drug Saf, 21:367–387.
4. Sriswasdi C, Jootar S, Giles FJ. (2000). Amifostine and hematologic effects. J Med Assoc Thai, 83:374–382.
5. Lindegaard JC, Grau C. (2000). Has the outlook improved for amifostine as a clinical radioprotector? Radiother Oncol, 57:113–118.
6. Barutca S, Meydan N, Akar H, Yavasoglu I, Kadikoylu G, Bolaman Z. (2004). Efficacy and tolerability of amifostine in elderly cancer patients. Current Therapeutic Res, 65:113–124.
7. Lialiaris TS, Kotsiou E, Pouliliou S, Kareli D, Makrinou H, Kouskoukis A et al. (2009). Cytoprotective activity of amifostine on cultured human lymphocytes exposed to irinotecan. Food Chem Toxicol, 47:2445–2449.
8. Grdina DJ, Murley JS, Kataoka Y, Baker KL, Kunnavakkam R, Coleman MC et al. (2009). Amifostine induces antioxidant enzymatic activities in normal tissues and a transplantable tumor that can affect radiation response. Int J Radiat Oncol Biol Phys, 73:886–896.
9. McDonald S, Meyerowitz C, Smudzin T, Rubin P. (1994). Preliminary results of a pilot study using WR-2721 before fractionated irradiation of the head and neck to reduce salivary gland dysfunction. Int J Radiat Oncol Biol Phys, 29:747–754.
10. Antonadou D, Pepelassi M, Synodinou M, Puglisi M, Throuvalas N. (2002). Prophylactic use of amifostine to prevent radiochemotherapy-induced mucositis and xerostomia in head-and-neck cancer. Int J Radiat Oncol Biol Phys, 52:739–747.
11. Rades D, Fehlauer F, Bajrovic A, Mahlmann B, Richter E, Alberti W. (2004). Serious adverse effects of amifostine during radiotherapy in head and neck cancer patients. Radiother Oncol, 70:261–264.
12. Schuchter LM. (1996). Guidelines for the administration of amifostine. Semin Oncol, 23:40–43.
13. Capizzi RL. (1999). Clinical status and optimal use of amifostine. Oncology (Williston Park, NY), 13:47–59; discussion 63, 67.
14. Srinivasan V, Pendergrass JA Jr, Kumar KS, Landauer MR, Seed TM. (2002). Radioprotection, pharmacokinetic and behavioural studies in mouse implanted with biodegradable drug (amifostine) pellets. Int J Radiat Biol, 78:535–543.
15. Bonner HS, Shaw LM. (2002). New dosing regimens for amifostine: a pilot study to compare the relative bioavailability of oral and subcutaneous administration with intravenous infusion. J Clin Pharmacol, 42:166–174.

16. Cassatt DR, Fazenbaker CA, Bachy CM, Hanson MS. (2002). Preclinical modeling of improved amifostine (Ethyol) use in radiation therapy. Semin Radiat Oncol, 12:97–102.
17. Komaki R, Lee JS, Kaplan B, Allen P, Kelly JF, Liao Z et al. (2002). Randomized phase III study of chemoradiation with or without amifostine for patients with favorable performance status inoperable stage II-III non-small cell lung cancer: preliminary results. Semin Radiat Oncol, 12:46–49.
18. Schuchter LM, Hensley ML, Meropol NJ, Winer EP; American Society of Clinical Oncology Chemotherapy and Radiotherapy Expert Panel. (2002). 2002 update of recommendations for the use of chemotherapy and radiotherapy protectants: clinical practice guidelines of the American Society of Clinical Oncology. J Clin Oncol, 20:2895–2903.
19. Cassatt DR, Fazenbaker CA, Kifle G, Bachy CM. (2003). Subcutaneous administration of amifostine (ethyol) is equivalent to intravenous administration in a rat mucositis model. Int J Radiat Oncol Biol Phys, 57:794–802.
20. Godette KD, Thomas CR Jr. (2001). Clarification on the potential of subcutaneous ethyol as a radioprotective agent. J Clin Oncol, 19:1582–1583.
21. Koukourakis MI, Romanidis K, Froudarakis M, Kyrgias G, Koukourakis GV, Retalis G et al. (2002). Concurrent administration of Docetaxel and Stealth liposomal doxorubicin with radiotherapy in non-small cell lung cancer: excellent tolerance using subcutaneous amifostine for cytoprotection. Br J Cancer, 87:385–392.
22. de Castro G Jr, Federico MH. (2006). Evaluation, prevention and management of radiotherapy-induced xerostomia in head and neck cancer patients. Curr Opin Oncol, 18:266–270.
23. Mandal TK, Bostanian LA, Graves RA, Chapman SR. (2002). Poly(D,L-lactide-co-glycolide) encapsulated poly(vinyl alcohol) hydrogel as a drug delivery system. Pharm Res, 19:1713–1719.
24. Mandal TK, Bostanian LA, Graves RA, Chapman SR, Womack I. (2002). Development of biodegradable microcapsules as carrier for oral controlled delivery of amifostine. Drug Dev Ind Pharm, 28:339–344.
25. Pamujula S, Graves RA, Kishore V, Mandal TK. (2004). Preparation and *in vitro* characterization of amifostine biodegradable microcapsules. Eur J Pharm Biopharm, 57:213–218.
26. Pamujula S, Graves RA, Moiseyev R, Bostanian LA, Kishore V, Mandal TK. (2008). Preparation of polylactide-co-glycolide and chitosan hybrid microcapsules of amifostine using coaxial ultrasonic atomizer with solvent evaporation. J Pharm Pharmacol, 60:283–289.
27. Cun D, Foged C, Yang M, Frøkjaer S, Nielsen HM. (2010). Preparation and characterization of poly(DL-lactide-co-glycolide) nanoparticles for siRNA delivery. Int J Pharm, 390:70–75.
28. Dillioglugil MO, Kir HM, Demir C, Ilbay G, Sahin D, Dillioglugil O et al. (2010). Effect of pentylenetetrazole and sound stimulation induced single and repeated convulsive seizures on the MDA, GSH and NO levels, and SOD activities in rat liver and kidney tissues. Brain Res Bull, 83:356–359.