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Imaging and treatment of malignant metastatic tumors by using radiation-sensitive, immunolabeled liquid-core microcapsules

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ABSTRACT

In this study, two types of microcapsules were designed: (1) computed tomography (CT)-detectable anti-αvβ3 (E[c(RGDfK)]$_2$) microcapsules, containing P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1), for the observation of metastases through αvβ3-antigen–antibody accumulation; and (2) metastasis-targeting microcapsules that upon irradiation release anticancer drugs with high affinity for P-selectin. These microcapsules were tested on C_3He/N mice with MM48 tumors undergoing two radiotherapy sessions.

The injected anti-αvβ3 microcapsules accumulated in the vascular endothelium of metastatic tissues and could be detected by CT. These microcapsules released P-selectin in response to the first irradiation, which was also induced in the endothelium of vessels in the case of metastasis. The microcapsules used for radiosensitizing metastases were injected 6 h after the first radiotherapy session. Their accumulation reached a maximum at 3 h after injection. After the second radiotherapy session, the microcapsules released carboplatin, which reduced the number of metastases; however, this reduction was not significant.

1. Introduction

One of the major goals in the design and development of cancer treatment regimens is to improve the survival rate of cancer patients, which is strongly influenced by the success rate of controlling cancer metastasis [1]. Furthermore, effective control of metastasis is largely dependent on its early detection and treatment [2]. Thus, increased efforts to develop methods for the early detection and treatment of metastasis have been driven by the need to improve the survival rate of cancer patients. However, early detection is often difficult; it is currently challenging to detect metastatic cells because of their extremely small size. The treatment of invisible metastases is also difficult and often interrupts early initiation of cancer chemotherapy, which enhances severe adverse effects and prevents the accurate delivery of radiation during radiotherapy.

We have previously developed radiosensitive liquid-core microcapsules that release their core contents in response to radiation using alginate polymerized with Fe^{2+} and hyaluronic acid [3,4]. Radiation causes the conversion of hyaluronic acid into acetyl-glucosamine, while alginate-Fe^{2+} is depolymerized and exchanges Fe^{2+} for Fe^{3+} [3]. This technique permits the encapsulation of computed tomography (CT) contrast medium, P-selectin antigen, and carboplatin (a platinum [Pt]-containing anticancer drug), and enables the labeling of capsules with the αvβ3 antibody.

Recent reports have shown that the αvβ3 antibody is produced in the vascular endothelium of metastatic tissues of mammary tumors [5,6]. By labeling the radiosensitive micro capsules with αvβ3, which contains either CT contrast medium or an anticancer drug, the intravenously injected microcapsules may then accumulate in incipient metastatic foci through an antigen–antibody reaction with the αvβ3 antibody. These microcapsules within metastatic foci are then detected by CT, facilitating more precise diagnosis of cancer. The anticancer drug released from these microcapsules might lead to better treatment of metastatic cancer.

Carboplatin is a Pt-containing anticancer drug that binds to one or both DNA strands in tumor cells to interrupt DNA replication or repair in response to irradiation damage, thus resulting in the death of tumor cells or enhancement of the antitumor effect of radiation.

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In this study, 2 types of microcapsules were designed: (1) CT-detectable anti-αvβ3 ($E[c(RGDfK)]_2$) microcapsules containing P-selectin and P-selectin glycoprotein ligand-1 (PSGL-1) for the observation of malignant tumors through αvβ3-antigen–antibody accumulation; and (2) microcapsules for malignant tumor treatment, which release anticancer drugs upon irradiation with high affinity for P-selectin. To test the suitability of these microcapsules for imaging and treating malignant tumors, we subjected C3He/N mice with MM48 tumors to two radiotherapy sessions.

2. Materials and methods

2.1. Production of microcapsules

Approximately 20 mL of olive oil was overlaid onto 50 mL of 1.2 mol/L $FeCl_2$ and 0.6 mol/L $CaCl_2$ supplemented with antibody for labeling microcapsules, and the mixture was then mixed using an ultrasound vibrator (Sakura VF-5, 100 w). A 10 mL aqueous solution containing chemicals for encapsulation, i.e., 0.2% (weight/volume) alginate and 0.1% hyaluronic acid, was sprayed into the vibrating solution using an ultrasound disintegrator (Branson SONIFIIER 150) with 9 w of output power. Polymerization of alginate by $FeCl_2$ and $CaCl_2$ was completed within 5 min, generating the microcapsules. These microcapsules were then purified using a Nalgene disposable filter (8-0301-84 DP591) and resuspended in 0.1 mol/L Tris (hydroxymethyl) aminomethane buffer (Tris, pH 7.4). The capsule concentration was adjusted to 1.0×10^{10}/mL in 10 mL of Tris.

The diameter of the microcapsules was measured by analyzing photos of the microcapsules on a hemocytometer, which was observed under an optical microscope. The Image J software package (freeware developed by National Institute of Health, USA) was used to measure the diameters of the microcapsules. Calibration was performed based on the 5-μm squares of the hemocytometer.

To detect metastatic cells with the microcapsules, the P-selectin antibody was encapsulated, and the αvβ3($E[c(RGDfK)]_2$) antibody was used for labeling. To treat metastatic cells with microcapsules, the encapsulated chemical employed was carboplatin (1 mg/1.0×10^{12} microcapsules), and the antibodies used for labeling were P-selectin (Mouse Anti-human P-selectin monoclonal antibody, Milipore cat # MAB 2154, 1:10 dilution) and P-selectin glycoprotein ligand-1 (PSGL-1, Mouse Anti-P-Selectin Glycoprotein Ligand-1 monoclonal antibody, Milipore cat # MAB4092, 1:10 dilution).

2.2. Preparation of mouse tumor models

Approximately 1×10^6 MM48 tumor cells, which are derived from a mouse mammary cancer [7] (supplied by Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer Tohoku University) were intramuscularly injected into the left hind legs of 6-week-old male C3He/N mice (purchased from Nippon Clea Inc., Shizuoka, Japan), and 87.6% of mice were successfully inoculated. Treatment was started when the tumors had attained a diameter of 8 mm. Eight mice were used for each experiment. The animal experiments were performed according to the guidelines of the institutional animal care and use committee of Iwate Medical University (No. 4513).

2.3. Imaging of metastasis using microcapsules

A 0.1 mL aliquot of a solution containing 1×10^9 microcapsules loaded with P-selectin antibody and labeled with αvβ3 antibody was intravenously injected through the tail vein of the mice. The microcapsules were allowed to interact with the αvβ3 antigen from 5.8 to 6.2 h after the first irradiation. Whole-body scanning using CT was then performed to check for pulmonary metastasis [8], which was the most popular metastatic site. Eight mice were used in this experiment.

2.4. First radiation session

Immediately after the CT scan, 10 or 20 Gy doses of 140 keV X-rays (Softex M150 WE) were administered to the lungs and tumors at a dose rate of 0.315 Gy/minute [9]. During irradiation, the irradiated total doses were monitored using a radiation dose meter (RAMTEC Smart, TOYO MEDIC Co. Ltd.). When the dose reached 10 or 20 Gy, the irradiation was stopped automatically. There was slight fluttering of the dose rate and irradiation time because of fluttering of the electric power voltage supplied to the irradiator. For 10 Gy irradiation, the irradiation time ranged from 1902 to 1906 s, and for 20 Gy irradiation, the irradiation time ranged from 3804 to 3812 s.

Some mice were sacrificed using a CO_2 chamber, and their lungs were excised and each lung was cut into 2 pieces. One piece was kept in the freezer at −80 °C and was later used for determining the number of accumulated microcapsules. The second piece was used for immunohistochemical analysis of P-selectin by *in situ* hybridization (ISH). Before radiation, some mice were taken as controls. Eight mice were used in each of the 10 or 20 Gy radiation and control groups.

2.5. Second radiation session

Approximately 6 h (range, 5.8–6.1 h) after the first irradiation, a 0.1 mL aliquot of a solution containing 1×10^9 metastasis-targeting microcapsules containing carboplatin and labeled with P-selectin antibody and PSGL-1, was intravenously injected via the tail vein of the mice. The injected solution was allowed to interact with the P-selectin antigen that was released from the microcapsules and induced in the endothelium of metastatic tissues during the first radiation session. After incubation, the second radiation treatment was then administered using the same procedure as described above.

In order to evaluate the influence of carboplatin encapsulation, 0.1 ml of 1 μg unencapsulated carboplatin (equivalent to 1×10^9 of metastasis-targeting microcapsules) was injected into mice via the tail vein and two radiation sessions were administered as described above. In order to evaluate the effectiveness of the radiation, a non-irradiated group of mice was injected with either encapsulated or unenecapsulated carboplatin. Eight mice were included in each treatment group.

2.6. Lung excision after the second radiation session

Immediately after the second radiation session, the remaining mice were sacrificed and their lungs were excised and kept frozen at −80 °C. These tissues were used for micro PIXE camera analysis of the release of the anticancer drug and quantitative PIXE measurement of the amount of anticancer drug released.

2.7. Preparation of the PIXE samples for micro PIXE camera imaging and analysis

The lungs were covered with dry ice powder and cut into 3 μm-thick slices using a microtome; the slices were subsequently placed onto a 1 μm-thick Mylar film held in a plastic PIXE sample holder with a 3-cm diameter. The sections were then vacuum dried (1×10^{-3} Torr) for 72 h and subjected to micro PIXE analysis. Five PIXE samples were prepared from 1 piece of lung. Eight mice were used for these experiments.

Each PIXE sample was irradiated with a 3 MeV proton beam (diameter, 2 μm), and the X-rays induced were recorded using a