^{153}Sm-CEA 单抗对结肠癌移植瘤裸鼠模型放射免疫治疗的实验研究

The radionuclides now in use for radioimmuno- therapy (RIT) have exceeded 10 kinds, but no one single agent has merited general recognition as the best for RIT. In earlier studies iodine-131 was often adopted, but its unreliable physical property and rapid in vivo deiodination of the radiolabelod antibodies limited its practical application [1]. Radioactive metallic nuclide such as Y-90 was subsequently chosen for RIT. Problems were not less fatal with Y-90: the radiationinduced damages to the bone marrow and liver rendered it risky to administer an effective radiation dose[2]. It has been generally agreed that the selection of radionuclides for RIT must incorporate the primary consideration of those that are capable of β particle emission, especially those emitting intermediate-energy β-particles and γ-rays (with γ ray energy below 300 keV) suitable for imaging without excessive radiation to cause damage to normal tissues while at the same time, applicable in in vitro radioimmunoimaging (RⅡ) studies.

Recently there has been increasing interest in utilization of ^{153}Sm labeling for therapeutic agents. ^{153}Sm labeled EDTMP has been used in human for the diagnosis and treatment of bone carcinoma[3]. In 1989 Boniface[4]first reported the radiolabeling of monoclonal antibodies(mAb) with ^{153}Sm using bifunctional chelate cyclic DTPA anhydride (cDTPAa) in the study of imaging and biodistribution in a rat model system. This preliminary study indicated the feasibility of using radioimmunoscintigraphy in combination with radioim- munotherapy in a clinical setting. Until now, however, no similar report has been available in this country addressing the application of ^{153}Sm in radioimmunoimag- ing and radioimmunotherapy. As a radiolanthanide, ^{153}Sm possesses excellent physical characteristics for radioimmunotherapy, capable of emitting β ray at E_{max}= 640 (30%), 710 (50%), and 810 (20%) keV with a half-life ($T_{1/2}$) of 1.95 days. In addition, it also emits a 103-keV γ ray that is suitable for γ camera for target absorbed dose assessment. Produced by nuclear reactor with high yield and highly specific activity initiated by neutron activation, ^{153}Sm is a very attractive radioisotope for RIT[5]. We therefore selected ^{153}Sm that suits nation- wide application in our investigation into the radiolabe- ling of anti-CEA mAb with ^{153}Sm by cDTPAa, and observed the therapeutic effect of ^{153}Sm-CEA mAb in nude mice bearing human colon carcinoma.

MATERIAL AND METHODS

Reagents

Anti-CEA mAb was obtained from Shanghai Institute of Immunology, and cyclic DTPA anhydride (cDTPAa) from Sigma Chemical Co. (St. Louis, MO). $^{153}SmCl_3$ was supplied by Department of Isotopes, China Institute of Atomic Energy. Sephadex G-50 was imported from Pharmacia Co. and divided by Factory of Shanghai Chemical Reagent. All the chemical reagents used in this study were of analytical grade, and prepared with ion-depleted water.

Tumor model

Balb/c nu/nu mice (female, body weight of 20 g) received subcutaneous xenograft in the thigh with 5×10^6 LoVo cells. The tumors growing to approximately 1 cm in diameter was cut into tiny pieces and suspended in normal saline, aspirated and injected (approximately 0.2 ml) subcutaneously into the forelimb of BALB/C nude mice (4 to 5 weeks old). The healing of the wound normally took 12 h. After the tumors had grown to the volume of 0.5 to 1.0 cm^3, the mice were used for subse- quent study of pretargeting radioimmunoimaging and biodistribution. The study of the therapeutic effect was started on the third day following tumor inoculation.

Conjugation of anti-CEA mAb with DTPA

Coupling of cyclic anhydride of DTPA (cDTPAa) with anti-CEA mAb was performed according to the method described by Hnatowich[6]. Briefly, cDTPAa was suspended in chloroform (1 mg/ml), an aliquot of which was taken with a molar ratio of DTPA:mAb at 20:1 and added into an acid-washed vial for evaporation under a stream of high-purity dry nitrogen. Anti-CEA mAb (200 μg) was then added into the vial, thoroughly shaken for 1 min and allowed at room temperature for 15-20 min for reaction, which was terminated by acetic acid. Separation of the DTPA-CEA mAb conjugate from free DTPA was achieved by a mini-Sephadex G50 chromatography. The immunoreactivity of the DTPA- CEA mAb conjugate was assessed using indirect enzyme- linked immunosorbent assay (ELISA).

^{153}Sm labeling of anti-CEA mAb

$^{153}SmCl_3$ at a dose of approximately 40 MBq (with specific activity of 22.2 GBq/ml) was mixed with purified CEA mAb-DTPA conjugate (0.1 ml), and incubated at room temperature for 20 min. Paper chromatography was carried out with Xinhua No.1 filter paper (30% ammonium nitrate-treated) as the supporter and the mixture of tributyl phosphate, butanone, and acetic ether (in a proportion of 4:10:3) as the developing agent, to determine the labeling efficiency and radio- chemical purity. The immunoreactivity of the labeled mAb was tested with indirect ELISA.

In vitro stability of ^{153}Sm-DTPA-CEA mAb

Following coupling and purification, a 0.3 ml aliquot of the labeled mAb was mixed with mouse serum of the same volume at 37 ℃ for 24 h. Sampling of the mixture was performed at 12 and 24 h respectively for the determination of ^{153}Sm release rate from labeled mAb using Sephadex G-50 chromatography.

Radioimmunotherapy

Treatment with a single high dose was adopted. Fifteen tumor-bearing mice were randomly divided into 3 groups (5 in each group). The mice in group A received 11.1 MBq of ^{153}Sm-DTPA-CEA mAb (100 μl) via intra-peritoneal injection, and mice in group B was given ^{153}Sm-DB_2 at the dose of 11.1 MBq (100 μl) to serve as the therapeutic control group. Intraperitoneal injection with 100 μl normal saline was administered in mice in Group C as

the non-treatment control group. The length (a) and width (b) of tumors were measured with a sliding caliper once a week for one month, and the tumor volume (V) was calculated according to the formula: $V=1/6\pi ab^2$. All the mice were weighed on the day of injection and then once a week after it for one month. The inhibition rate (IR) of tumor growth was calculated according to the formula: IR=(Mean tumor volume of non-treatment control group - Mean tumor volume of therapeutic group)/ Mean tumor volume of non- treatment control × 100%.

Histological examination

Pathological examination was also performed in these mice after all the above observations were com- pleted. The mice were sacrificed, their organs isolated and weighed, and then fixed in 10% formol solution and embedded in paraffin before sections 4 μm in thickness (stained with hemalum-eosin-safran) were prepared for routine histological examination.

RESULTS

Quality control and in vitro stability of the labeled compounds

The labeling efficiency of ^{153}Sm-DTPA- CEA mAb was 56%, with a specific activity of 15.54 GBq/mol, a radiochemical purity above 95% and immunoreactivity of approximately 50%. After mixed with mouse plasma for 12 and 24 h at room temperature, the labeled mAb showed a ^{153}Sm-release rate of 5.47±2.64 % and 9.13±0.29 %, respectively.

Changes in body weight of tumor-bearing nude mice

Before treatment, the body weight of the nude mice in Group C (non-treatment control) and Group B (therapeutic control) were 19.24±1.49 g and 17.10±1.40 g respectively, which increased to 24.60±2.89 g and 19.84±1.12 g respectively 4 weeks after the treatment. The body weight of the mice in Group A (therapy group) measured at the same 2 time points were 19.24±1.58 g and 22.14±1.23 g respectively, showing no significant body weight loss in comparison with the other 2 groups.

Dynamic observation of the tumor volume

In the first week after treatment, the tumor volume showed little difference between the groups, while in the second week slower tumor growth rate in Group A was noted. Till the fourth week, the tumor volume was significantly smaller in group A than in the other 2 groups ($P<0.001$, Fig 1). When the tumor inhibition rate in Group C was considered to be zero, the tumor inhibition rate at 4 weeks after the therapy was as high as 74.29% in Group A, while only 15.90% in Group B, with significant difference between the latter 2 groups ($P<0.01$, Fig 2).

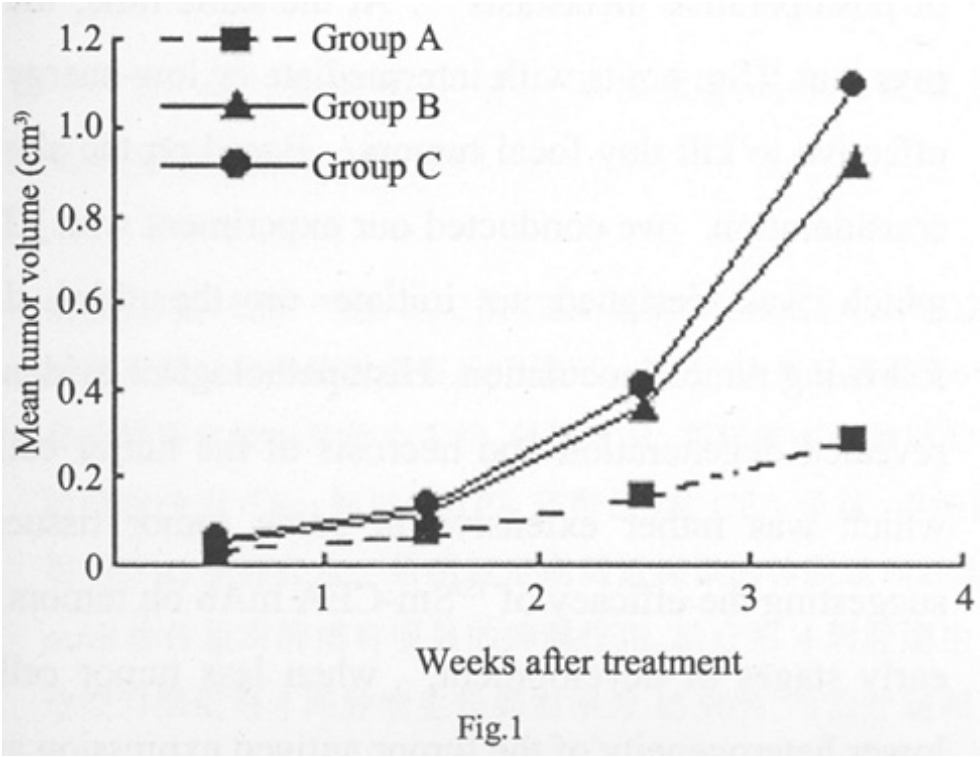

Fig.1 Growth curves of the implant tumors in the 3 groups

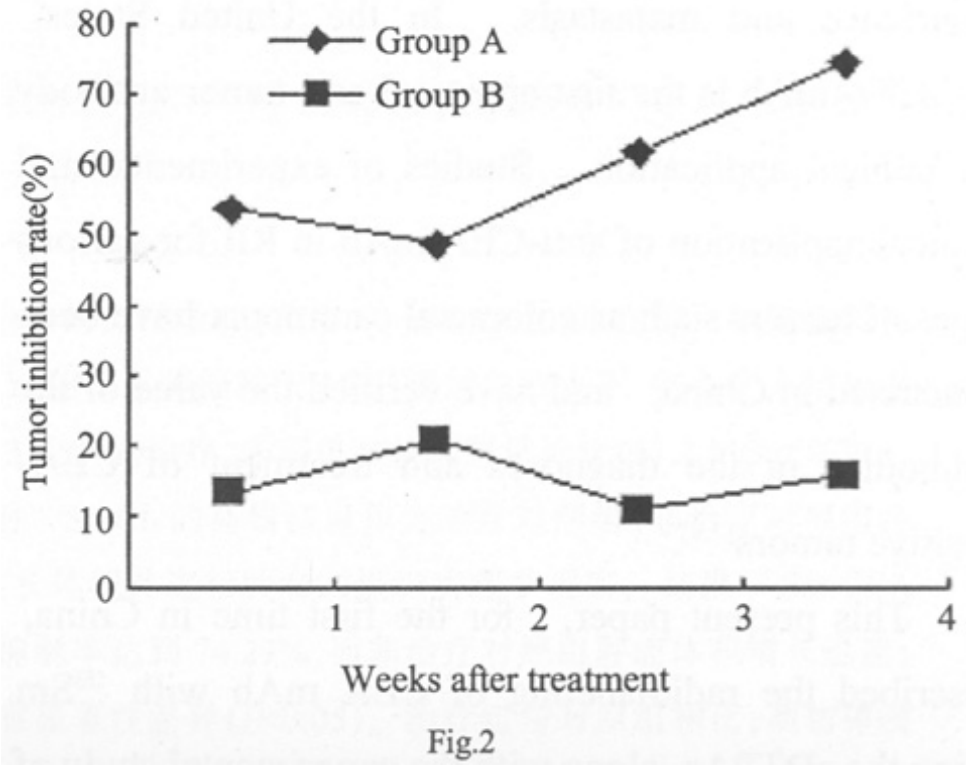

Fig.2 Curves for tumor growth inhibition in the 2 therapy groups

Histological examination

Samples of the tumor tissues were obtained from Group A at the end of the observation. Histopatholo- gical examination revealed evidences for degeneration and necrosis of the tumor cells such as nucleus pycnosis and fragmentation. Some tumor tissues exhibited complete necrosis and lysis of the tumor cells to form liquefied cisternae. While the tumor cells in Group C were characterized by absence of necrosis, with morphology typical of

tumor cells.

DISCUSSION

Radiolabeled mAb against tumor-associated antigens for tumor diagnosis and therapy, ie. RⅡand RIT, has attracted much attention from researchers spe- cialized in tumor nuclear medicine, with great achieve- ments already made therein[2][3] owing much to the recent development of the mAbs with high affinity, improvement in labeling and imaging techniques, and optimized tumor microenvironment. The effect of targeting therapy depends heavily on the targeting ability of the carriers that delivers the irradiation damage by radionuclide to the target. In hundreds of the anti-tumor mAb already developed, Anti-CEA mAb merits special attention and has already entered clinical applications, for instance, the determination of CEA levels in serum and body fluid, immunohistochemical staining, tumor radioimmunoimaging, radioimmuno- guided surgery and radioimmunotherapy. RII and RIT with CEA mAb are of important significance for early diagnosis of tumors (such as colorectal carcinoma), and are also useful in the detection and therapy of tumor recurrence and metastasis. In the United States, anti-CEA mAb is the first approved anti-tumor antibody for clinical application. Studies of experimental and clinical application of anti-CEA mAb in RII for various types of tumors such as colorectal carcinoma have been conducted in China, and have verified the value of the antibodies in the diagnoses and treatment of CEA- positive tumors[7][8].

This present paper, for the first time in China, described the radiolabeling of CEA mAb with ^{153}Sm using the cDTPAa, along with the experimental study of radioimmuno- therapy in nude mice bearing human colon carcinoma. The results showed that ^{153}Sm-CEA mAb at the dose of 11.1 MBq had obvious inhibitory effect on colon carcinoma xenografted in nude mice. Tumor growth inhibition was observed 2 weeks after the therapy, and at the fourth week the tumor inhibition rate reached 74.29%. The mechanism underlying the strong selective inhibitory effect of ^{153}Sm-CEA mAb against colon carcinoma may be that (1) β-ray emitted by radionuclide may result in irreversible damage to cell genetic materials and DNA by means of direct and indirect ionizing radiation; (2) the labeled antibodies may specifically bind the tumor cells through direct contact, permeation, or localization by gravitational force bond; (3) β-ray produced by ^{153}Sm with proper strength of penetration may kill those tumor cells that are at a distance from the targeting site, antigen-negative tumor cells in the neighborhood, and those hard to reach through permeation by mAb in RIT[9].

RIT is not ideal, however, for treating massive solid tumors. The investigations have shown a negative correlation between the curative effect of RIT for solid tumors and the tumor bulk[10]. The principal reasons lies in the fact that the antibody uptake by the tumors may be affected by many factors such as increased interstitial pressure within the tumor, relatively decreased number of the blood capillaries and possible necrosis present in the increased tumor bulks. RIT is therefore considered particularly suited for treating sub-clinical microfocal recurrent tumors arising from previous tumor remnant or postoperative metastasis[11]. At the same time, the β rays that ^{153}Sm emits with intermediate or low-energy is effective to kill tiny focal tumors. Based on the above

consideration, we conducted our experiment with RIT, which was designed to initiate on the third day following tumor inoculation. Histopathological evidence revealed degeneration and necrosis of the tumor cells, which was rather extensive in some tumor tissues, suggesting the efficacy of ^{153}Sm-CEA mAb on tumors in early stages of development, when less tumor cells, lower heterogeneity of the tumor antigen expression and higher sensitivity to radioactivity are the major features. In addition, $^{153}SmCl_3$ itself has, to a certain degree, tumor-inhibiting effect at a rate of 15.90% at the fourth week of treatment, which may be attributed to unspecific radiation effect of this agent. But since $^{153}SmCl_3$ did not conjugate with mAb, effective in vivo localization of tumor cells would not take place, and this unspecific radiation effect was understandably limited.

Based on literature review and the results of this investigation, we conclude that RIT with ^{153}Sm-CEA mAb may serve as an auxiliary method for tumor treatment, which is particularly suitable for small tumor or metastasis and can be also useful in the prevention of tumor recurrence. With its considerable value in the diagnosis and therapy of colon carcinoma, ^{153}Sm-CEA mAb may become a new type of targeting therapy agent for RIT, already showing its potential in clinical applications.

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