Radioimmunotherapy with ^{153}Sm-CEA monoclonal antibody in nude mice bearing human colon carcinoma: an experimental study

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Abstract: Objective Toobservethetherapeuticeffectof ^{153}Sm-labeledCEAmonodonalantibody(mAb)innudemicebearing humancoloncarcinoma. **Methods** Fifteennudemiceweresubjectedtosubcutaneousinoculationofhumancoloncarcinoma cells,and3dayslater,theyweredividedinto3groupswithequalnumberstoreceivesinglehigh-doseinjectionof11.1MBq 153Sm-CEAmAb(therapygroup),11.1MBq $^{153}SmCl_3$ (therapycontrolgroup), or 100 滋normalsaline(non-treatmentcontrol group). Thetumor-inhibitingeffectof ^{153}Sm-labeled CEA mAbwasevaluatedintermsofbodyweightchangesandtumor volumevariation4weeksafterthetreatment. Histologicalanalysisoftumorswere performed in allthe groups after theall otherobservationswerecompleted. **Results** ^{153}Sm-CEAmAbhadasignificantanti-tumoreffect,withatumorinhibitionrateof 74.29%at4weeksaftertreatment,whilefor $^{153}SmCl_3$, theinhibitionratewasonly15.90%.Rapidtumorgrowthwasobserved innon-treatment control group. No significant difference in the body weight changes was noted between the 3 groups. Histopathologicalexaminationrevealedtumornecrosisastheevidenceforradioactivedamageintherapygroup, which was not observedinnon-treatmentcontrolgroup. **Conclusions** ^{153}Sm-CEA mAb hasastrong selectiveinhibitoryeffect against coloncarcinomaandmaybepotentiallyusedasanagentinradioimmunotherapy.

Key words: radioimmunotherapy;Samariumradioisotopes;antibodies,monoclonal;Neoplasm,nudemice

The radionuclides now in use for radioimmunotherapy(RIT) haveexceeded10kinds, butnoonesingle 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 practicalapplication 咱1暂.Radioactivemetallicnuclidesuch asY-90wassubsequentlychosenforRIT.Problemswere notlessfatalwithY-90: the radiationinduceddamages to the bone marrow and liver rendered it risky to administer an effective radiation dose 咱2暂. It has been generally agreed that the selection of radionuclides for RITmustincorporatetheprimaryconsiderationofthose thatarecapableof 茁particle emission, especiallythose emitting intermediate-energy 茁particles and 酌rays (with 酌rayenergybelow300keV)suitableforimaging without excessive radiation to cause damage to normal tissues while at the same time, applicable in **in vitro** radioimmunoimaging(R域) studies.

Recently there has been increasing interest in utilizationof ^{153}Smlabelingfortherapeuticagents. ^{153}Sm labeled EDTMP has been used in human for the diagnosisandtreatmentofbonecarcinoma 咱3暂. In1989 Boniface咱4暂firstreportedtheradiolabelingofmonoclonal antibodies(mAb) with ^{153}Sm using bifunctional chelate cyclic DTPA anhydride (cDTPAa) in the study of imagingandbiodistributionina ratmodelsystem.This preliminary study indicated the feasibility of using radioimmunoscintigraphyincombinationwithradioimmunotherapyin a c linical setting. Until now, however, no similar report has been available in this country addressingtheapplicationof ^{153}Sminradioimmunoimaging and radioimmunotherapy. As a radiolanthanide, ^{153}Sm possesses excellent physical characteristics for radioimmunotherapy,capableofemitting 茁ray at E_{max}= 640 (30%), 710 (50%), and 810 (20%) keV with a half-life ($T_{1/2}$) of 1.95 days. Inaddition, italsoemits a 103-keV 酌ray that issuitable for 酌camera for target absorbeddose assessment. Produced bynuclearreactor with high yield and highly specific activity initiated by

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neutronactivation, ^{153}Smisaveryattractiveradioisotope forRIT[噎]. We thereforeselected ^{153}Smthatsuits nation-wide applicationinourinvestigationintotheradiolabe-ling of anti-CEA mAb with ^{153}Sm by cDTPAa, and observed the therapeutic effect of ^{153}Sm-CEA mAb in nudemicebearinghumancoloncarcinoma.

MATERIAL AND METHODS

Reagents

Anti-CEA mAb was obtained from Shanghai Institute of Immunology, and cyclic DTPA anhydride (cDTPAa) from Sigma ChemicalCo. (St. Louis, MO). $^{153}SmCl_3$ wassuppliedbyDepartmentofIsotopes,China Instituteof AtomicEnergy.SephadexG-50wasimported from PharmaciaCo. anddividedbyFactoryofShanghai Chemical Reagent. All the chemical reagents used in this study were of analytical grade, and preparedwith ion-depletedwater.

Tumor model

Balb/cnu/numice (female, bodyweightof20g) received subcutaneousxenograftinthethighwith5伊10^6 LoVocells. Thetumorsgrowingtoapproximately1cm in diameter was cut into tiny pieces and suspended in normal saline, aspiratedandinjected (approximately0.2 ml) subcutaneouslyintotheforelimbofBALB/Cnude mice (4 to 5 weeks old). The healing of the wound normallytook12h. After thetumors hadgrowntothe volumeof0.5to1.0cm^3, themicewereusedforsubse-quent study of pretargeting radioimmunoimaging and biodistribution. Thestudyofthetherapeuticeffectwas startedonthethirddayfollowingtumorinoculation.

Conjugation of anti-CEA mAb with DTPA

CouplingofcyclicanhydrideofDTPA (cDTPAa) with anti-CEA mAb was performed according to the methoddescribedbyHnatowich [噎]. Briefly, cDTPAa wassuspendedinchloroform (1mg/ml), analiquotof which was taken with a molar ratio of DTPA:mAb at 20:1andaddedintoanacid-washedvialforevaporation under a stream of high-puritydrynitrogen. Anti-CEA mAb(200 滋g) was then addedinto the vial, thoroughly shaken for 1 minand allowed at room temperature for 15-20minforreaction,whichwasterminatedbyacetic acid. Separation of the DTPA-CEA mAb conjugate fromfreeDTPAwasachievedbyamini-SephadexG50 chromatography. The immunoreactivityoftheDTPA-CEAmAbconjugatewasassessedusingindirectenzyme-linkedimmunosorbentassay(ELISA).

^{153}Sm labeling of anti-CEA mAb

$^{153}SmCl_3$ atadoseofapproximately40MBq(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 chromatographywascarriedoutwithXinhuaNo.1filter paper (30% ammonium nitrate-treated) asthesupporter andthemixtureoftributylphosphate, butanone, and aceticether(inaproportionof4:10:3)asthedeveloping agent, to determine the labeling efficiencyand radio-chemical purity. The immunoreactivityof the labeled mAbwastestedwithindirectELISA.

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 serumofthesamevolumeat37 益for 24h. Sampling ofthemixturewasperformedat12and24hrespectively for the determination of ^{153}Sm release ratefromlabeled mAbusingSephadexG-50chromatography.

Radioimmunotherapy

Treatment with a single high dose was adopted. Fifteen tumor-bearingmice wererandomlydividedinto 3 groups (5 in each group). The mice in group A received11.1MBqof ^{153}Sm-DTPA-CEA mAb (100 滋g) via intra-peritonealinjection, andmiceingroupBwas given ^{153}Sm-DB_2 atthedoseof11.1MBq (100 滋g) to serve as the therapeuticcontrol group. Intraperitoneal injectionwith100 滋l normalsalinewasadministeredin mice in Group C as the non-treatment control group. The length (a) and width (b) of tumors were measured withaslidingcaliperonce a weekforonemonth, and thetumorvolume (V) wascalculatedaccordingtothe formula: $V=1/6 \pi ab^2$. All the micewereweighedon the dayof injection and then once a week after it for one month. Theinhibitionrate (IR) oftumorgrowthwas

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 completed. 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).

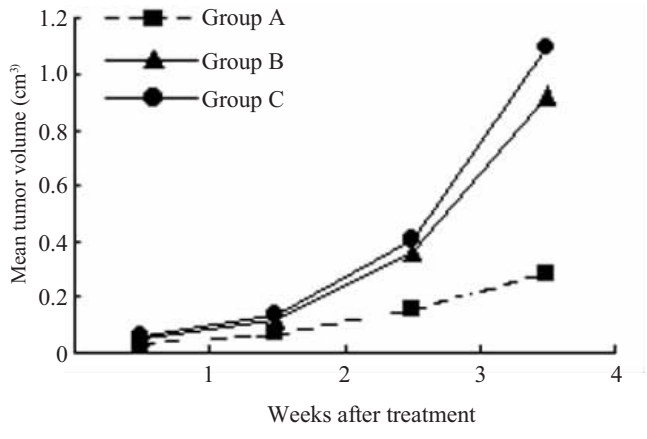

Fig.1

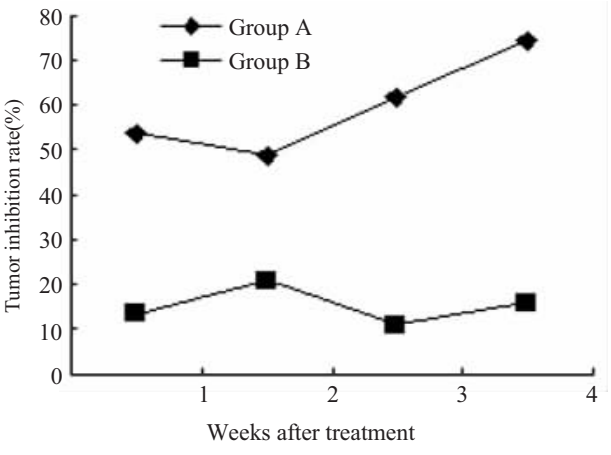

Fig.2

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. Histopathological 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 oftumorcells.

DISCUSSION

Radiolabeled mAb against tumor-associated antigens for tumor diagnosisandtherapy, ie. R域and RIT, has attractedmuchattentionfromresearchers specializedintumornuclearmedicine, withgreatachievements already made therein [1-3] owing much to the recent development of the mAbs with high affinity, improvementinlabelingand imagingtechniques, and optimized tumor microenvironment. The effect of targeting therapy depends heavily on the targeting ability of the carriers that delivers the irradiation damagebyradionuclidetothetarget.Inhundredsofthe anti-tumor mAb already developed, Anti-CEA mAb meritsspecial attention andhasalreadyenteredclinical applications, for instance, the determination ofCEA levels in serumandbodyfluid, immunohistochemical staining, tumorradioimmunoimaging, radioimmunoguided surgery and radioimmunotherapy. RII and RIT with CEA mAb are of important significance for early diagnosis oftumors (such as colorectal carcinoma), and are also useful in the detection and therapy of tumor recurrence and metastasis. In the United States, anti-CEAmAbisthefirstapprovedanti-tumorantibody for clinical application. Studies of experimental and clinical applicationofanti-CEAmAbinRII for various typesoftumorssuchascolorectalcarcinomahave been conductedinChina, andhaveverifiedthe value ofthe antibodies in the diagnoses and treatment of CEA-positivetumors[1-3].

This present paper, for the first time in China, described the radiolabeling of CEA mAb with ^{153}Sm usingthe cDTPAa,alongwiththeexperimentalstudyof 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 thetherapy, and atthefourth weekthetumorinhibition rate reached 74.29%. The mechanism underlying the strong selective inhibitory effect of ^{153}Sm-CEA mAb against colon carcinomamaybe that (1) 茁rayemitted byradionuclidemayresultinirreversibledamagetocell genetic materials and DNA by means of direct and indirectionizingradiation;(2)thelabeledantibodiesmay specificallybindthetumorcellsthroughdirectcontact, permeation, or localizationbygravitational forcebond; (3) 茁ray produced by ^{153}Sm with proper strength of penetration may kill those tumor cells that are at a distancefromthe targeting site, antigen-negativetumor cells in the neighborhood, and those hard to reach throughpermeationbymAbin RIT[1-3].

RIT is notideal, however, for treatingmassive solidtumors. Theinvestigationshaveshownanegative correlation between thecurative effect ofRITforsolid tumorsandthetumorbulk[1-3]. Theprincipalreasonslies in the fact that the antibodyuptake by the tumors may beaffectedbymanyfactorssuchasincreasedinterstitial pressurewithinthetumor, relativelydecreasednumber of thebloodcapillaries and possible necrosis presentin theincreasedtumorbulks. RIT istherefore considered particularly suited for treating sub-clinical microfocal recurrent tumors arising from previous tumor remnant or postoperative metastasis[1-3]. Atthesametime, the 茁 raysthat ^{153}Smemitswithintermediateorlow-energyis effective tokilltinyfocaltumors. Basedontheabove consideration, weconductedourexperimentwithRIT, which was designed to initiate on the third day followingtumorinoculation. Histopathological evidence revealed degeneration and necrosis of the tumor cells, which was rather extensive in some tumor tissues, suggestingtheefficacyof ^{153}Sm-CEAmAbontumorsin early stages of development, when less tumor cells, lower heterogeneityofthetumorantigenexpressionand higher sensitivitytoradioactivityarethemajorfeatures. In addition, $^{153}SmCl_3$ itself has, to a certain degree, tumor-inhibitingeffectatarateof15.90%atthefourth week of treatment, which may be attributed to unspecific radiation effect of this agent. But since $^{153}SmCl_3$ did not conjugate withmAb, 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, whichisparticularlysuitable for smalltumor or metastasisandcanbealsousefulinthepreventionof tumor recurrence. With its considerable value in the diagnosisandtherapyofcoloncarcinoma, ^{153}Sm-CEAmAb may become a new type of targeting therapy agent for RIT,alreadyshowingitspotentialinclinicalapplications.

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^{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

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摘要：目的 观察 ^{153}Sm 标记抗 CEA 单抗（^{153}Sm-CEAmAb）对肿瘤的抑制效应，探索 ^{153}Sm-CEAmAb 在荷人结肠癌移植瘤裸鼠模型中的放射免疫治疗作用。方法 体外培养的 LoVo 细胞株无菌接种于 15 只裸鼠前肢皮下，制备荷人结肠癌移植瘤裸鼠模型并分为 3 组，每组 5 只。在移植瘤接种后的第 3 天实施治疗，采用单次较大剂量治疗（1.1MBq/ 每鼠），治疗组注射 ^{153}Sm 标记（DTPA 环酐法）抗 CEA 单抗，单纯注射 $^{153}SmCl_3$ 的荷瘤裸鼠作为治疗对照组，非治疗对照组注射 100 滋 生理盐水。给药后定期测量裸鼠的体质量和肿瘤生长体积，并进行肿瘤的组织病理学观察。结果 ^{153}Sm-CEA mAb 治疗组对肿瘤有明显的抑制作用，治疗第 4 周肿瘤生长抑制率达到 74.29%，而非治疗对照组肿瘤体积增长迅速。治疗对照组第 4 周肿瘤生长抑制率为 15.90%，与治疗组相比有显著性差异（$P<0.05$）。治疗组与对照组相比，动物体质量没有显著性差异。组织病理学结果提示 ^{153}Sm-CEAmAb 治疗组肿瘤组织有坏死改变，而对照组肿瘤细胞呈典型的癌细胞形态。结论 ^{153}Sm-CEAmAb 对人结肠癌的荷瘤裸鼠具有抑制增长作用。^{153}Sm-CEAmAb 作为一种新型核素的导向治疗剂，具有定位诊断和导向治疗结肠癌的双重作用，具有良好的应用前景。

关键词： 放射免疫疗法；闪烁放射性同位素；抗体，单克隆；肿瘤，裸鼠