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ScienceDirect
Nuclear Science and Techniques 19 (2008) 230–235
NUCLEAR SCIENCE AND TECHNIQUES

Biological distribution of ^{131}I-labeled anti-nucleus antigen monoclonal antibody chTNT in patients with pulmonary metastases from differentiated thyroid carcinoma

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Abstract This work is to study the *in vivo* biological distribution of ^{131}I-labeled mouse/human chimeric monoclonal antibody (^{131}I-chTNT) in patients with pulmonary metastases from differentiated thyroid carcinoma. Ten patients with differentiated thyroid carcinoma were injected intravenously with a single dose of ^{131}I-chTNT (5 MBq·kg^{-1} body weight). Radioactivity of blood and urine samples was measured at different time points. The *in vivo* stability and the metabolic status of ^{131}I-chTNT were detected with supersaturated trichloroacetic acid. Continuous imaging was performed to outline the region of interest (ROI) and estimate the intake level on the whole body, major organs and tumor lesions at different time points. The serum time-radioactivity curve of ^{131}I-chTNT accorded with the two-compartment model after a single intravenous injection: $T_{1/2}$(h)=65.28±14.83, $AUC_{0\text{-}t}$(MBq·h·mL^{-1})=8.93±1.32, $AUC_{0\text{-}\infty}$(MBq·h·mL^{-1})=10.58±2.19, and CL(mL·min^{-1}·kg^{-1})=1635±359. The time-radioactivity percentage curve of ^{131}I-chTNT urine excretion accorded with the one-compartment model after a single intravenous injection: $T_{1/2}$(h)=99±10, and accumulative (31±9) % radioactivity of the injected dose was excreted in urine in one week. The percentages of serum ^{131}I-chTNT in radioactive components at 24, 48 and 72 h were over 95% and it was still (88±7) % at 168 h. As for chemical composition of radioactive substances in urine, radioactivity in urine samples originated from free ^{131}I by 100%. Radioactivity of ^{131}I-chTNT after intravenous administration was mainly concentrated in the lung and liver, least in the brain. Radioactivity of tumor tissues reached the maximum at 24 h and the tumor/normal tissue (*T*/*N*) ratio reached the maximum (1.28~3.83) during 3~7 d. The characteristics of *in vivo* biological distribution of ^{131}I-chTNT in patients with pulmonary metastases from differentiated thyroid carcinoma are favorable for its therapeutic application for the metastasis tumors.

Key words Radionuclide, chTNT, Biological distribution, Pulmonary metastasis

CLC number R817.9

1 Introduction

Currently, lung metastases from differentiated thyroid carcinoma are mainly treated with large dose ^{131}I internal irradiation in clinical medicine. However, pulmonary metastatic foci in some patients do not ingest ^{131}I, causing failure of the treatment[1]. In this article, we study the *in vivo* biological distribution of ^{131}I-labeled mouse/human chimeric monoclonal antibody (^{131}I-chTNT) in patients with pulmonary metastases from differentiated thyroid carcinoma, in hopes of providing scientific evidence for further exploring its value of clinical application in treating lung metastases from thyroid carcinoma.

2 Materials and methods

2.1 Subjects

Ten patients (6 males and 4 females) with differentiated thyroid carcinoma were included in the

Supported by Nature Science Foundation of Jilin Province (No.200705277)

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Received date: 2008-04-23

study. They had a mean age of 41.3 years (16~78 years) and mean weight of 59±11.5 kg. Six of them had papillary carcinoma, and the others had follicular carcinoma. General conditions of the patients were moderate, with their expected survival time over three months. Having not been treated in at least one month before the study with radiotherapy, chemotherapy, radionuclide imaging or other treatments, they had thier hemogram, hepatic and renal functions within the normal range. Results of their tests for human anti-mouse antibody (HAMA) responses and iodine allergy testing were negative. The whole body ^{131}I imaging, ultrasonic and CT scan were employed to determine that there were no residual thyroid tissues, recurrent or distal metastasis foci in other sites apart from the existence of pulmonary metastasis foci that did not incept ^{131}I.

2.2 Major apparatuses and drugs

The apparatuses include SkyLight SPECT (single photon emission computerized tomograph, Philips), RM-905a radioactivity counter (China Metrology Development Corp. Group), GC-2016 radioimmunoassay (RIA) γ counter (Xi'an Zhongjia Co) and TDL-5Z centrifuge (Toshiba). ^{131}I-chTNT was purchased from the Shanghai Meien Biotechnology Corp., Ltd. It was clear primrose liquid with radioactivity of 370 MBq·mL^{-1}, radiochemical purity of ≥ 95%, specific antibody binding activity of ≥ 50%, bacterial endotoxin of <10 EU·mL^{-1}, and pH of 6.5~7.5.

2.3 Methods

2.3.1 Standard ^{131}I-chTNT curve and the detection limit

Radioactive activity of 1 mL ^{131}I-chTNT solution (diluted already) was 7.15 MBq, as determined by the RM-905a counter. A share of 20 μL solution, which was diluted in ratios of 1:2, 1:4, 1:8, 1:16, 1:32 and 1:64, was measured at each concentration. GC-2016 RIA γ counter was used to determine the counts per minute (cpm), then the linear regression equation for specific radioactivity of solution (kBq) vs. the cpm data as derived and the standard curve was plotted.

2.3.2 The administration dose

The intravenous infusion of ^{131}I-chTNT was performed at 5 MBq·kg^{-1} and 5 mL volume. RM-905a counter was applied to determine the radioactivity of the ^{131}I-chTNT injector before (full injector) and after (empty injector) the injection and the difference of the two measurements was the administration dose of ^{131}I-chTNT.

2.3.3 Collecting and processing the serum samples

One milliliter of the intravenous blood was collected at 0.5, 4, 24, 48, 60, 72, 144 and 168 h after ^{131}I-chTNT administration, respectively. The serum (~500 μL) was isolated by centrifugation. From each sample, 20 μL serum was measured (in cpm) using the RIA γ counter. After correction, time-radioactivity curve of the serum samples was plotted and the pharmacokinetic parameters were calculated. Protein was all precipitated from 400 μL of each serum sample by 1.2 mL supersaturated trichloroacetic acid (TCA). The radioactivity (in cpm) in the precipitate was measured by the RIA γ counter to determine the *in vivo* stability of ^{131}I-chTNT.

2.3.4 Collecting and processing of urine samples

Daily urine output (24 h urine) of all the patients was collected for one week after ^{131}I-chTNT administration. The radioactivity was measured, analyzed against the standard curve and the percentage of daily urine radioactivity among the total injected doses was calculated to plot time-radioactivity curve of the urine. Urinary metabolite of ^{131}I-chTNT was analyzed by TCA precipitation and radioactivity measurements of 400 μL urine sample.

2.3.5 *In vivo* imaging

The SPECT equipped with a high-energy collimator was used to collect the anterior and posterior images of the whole body at 0.5, 24, 48, 72, 120 and 168 h, at the 364 keV peak with a 25% window width, 1024×256 matrix and 10 cm·min^{-1} velocity.

2.3.6 Image analyzing

Three experienced physicians specialized in nuclear medicine were recruited to read the films collectively, to observe if there were abnormal foci of radioactive concentration in the lung or not. The ROI

(region of interest) of radioactive concentration with the same size was plotted at the same site at different time points in correspondence with the pulmonary foci on the CT film and taking the internal radioactivity count as T and radioactivity count adjacent to the normal pulmonary tissue as N, the radioactivity count ratios of the tumor to the normal tissues (T/N) at different time points were calculated. When imaging different organs, the same method will be repeated as for lung tissue. The radioactivity-intake percentages of different organs at different time points were calculated, and compared with the anterior and posterior total counts at 0.5 h after administration[2].

2.4 Statistical analysis

Data were expressed in $\overline{X}$±s and the SPSS 10.0 software was used for statistical analysis.

3 Results

3.1 ^{131}I-chTNT standard curve and detection limit

A linear regression curve was plotted of the radioactive activity y (kBq) vs. x (cpm data) of the samples. The radioactivity of 2.2~71.5 kBq was in a favorable linear relationship with the cpm data being analyzed by regression equation y=0.0003x+0.3437 with r=0.9997.

3.2 Pharmacokinetics

The serum time-radioactivity curve after a single intravenous injection of ^{131}I-chTNT was shown in Fig.1, which agreed with the two-compartment model as analyzed with the SPSS10.0 code. The pharmacokinetic results were given in Table 1. The time-radioactivity percentage curve of urine excretion after a single intravenous injection of ^{131}I-chTNT was shown in Fig.2. It is in accordance with the one-compartment model as analyzed with the SPSS10.0 software. The semi-excretion time for urine radioactivity was 99±10 h. The accumulative radioactivity (31±9)% of the injected dose was excreted from urine within one week.

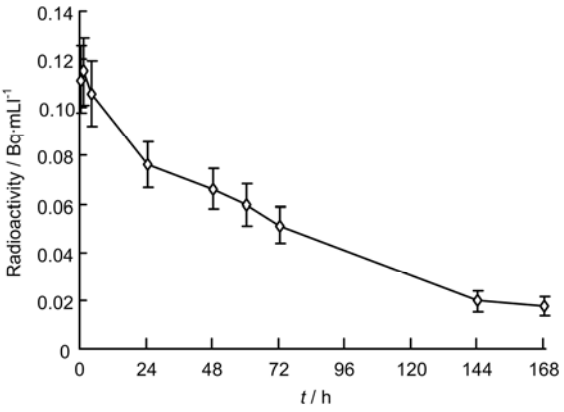

Fig.1 Serum time-radioactivity curve after ^{131}I-chTNT injection in patients with pulmonary metastases from differentiated thyroid carcinoma.

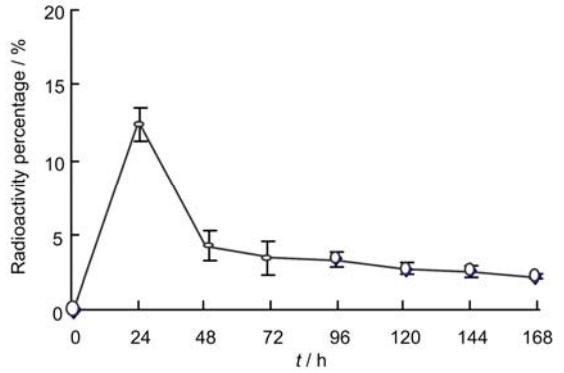

Fig.2 Urine excretion time-radioactivity percentage curve after ^{131}I-chTNT injection in patients with pulmonary metastases from differentiated thyroid carcinoma.

Table 1 Pharmacokinetic parameters after ^{131}I-chTNT injection in patients with pulmonary metastases from differentiated thyroid carcinoma

Pharmacokinetic parameters	$\overline{X}$±s
$T_{1/2}$ / h	65.28±14.83
k_e / h^{-1}	0.011±0.001
AUC_{0-t} / MBq·h·mL^{-1}	8.93±1.32
$AUC_{0-\infty}$ / MBq·h·mL^{-1}	10.58±2.19
CL / mL·min^{-1}·kg^{-1}	1635±359
V_d / L·kg^{-2}	8945±1013
C_{max} / MBq·mL^{-1}	0.12±0.01

3.3 *In vivo* stability and metabolite analyzing

After ^{131}I-chTNT injection, the percentages of non-protein bound radioactivity in serum were all over 95% at 24, 48 and 72 h, respectively, and it was still (88±7) % at 168 h. As to chemical composition of the radioactive substances in urine, the

radioactivity of urine samples originated from free ^{131}I by 100% from 24 to 168 h.

3.4 *In vivo* distribution

The radioactivity of ^{131}I-chTNT after intravenous injection was mainly distributed in the lung and liver, and the least in the brain. As shown in Fig.3, the images of tumor tissues taken on different days became gradually clearer, though the 0.5 h image was not clear. The radioactivity of tumor tissues reached the maximum at 24 h and the *T*/*N* ratio reached the maximum (1.28~3.83) from the third to seventh day. Total counting rate of the tumor, whole body and major organs, and the anterior and posterior (0.5 h after administration) whole body was estimated with continuous planar imaging. By the background reduction, the intake percentages at different time points were listed in Table 2.

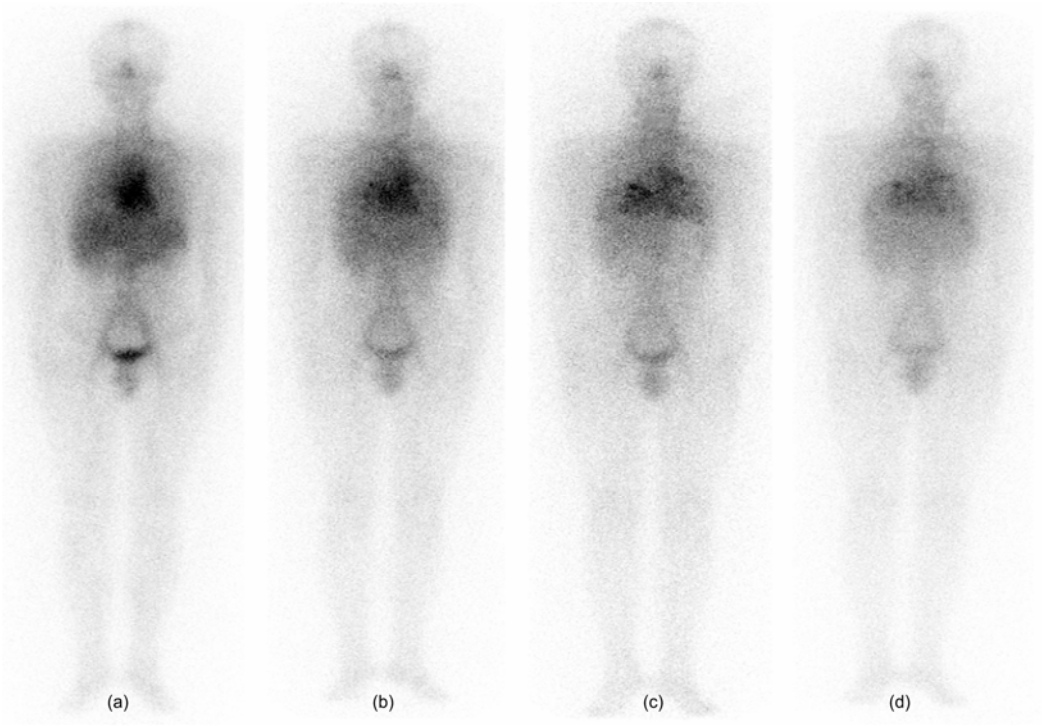

Fig.3 Whole body SPECT image at different time points after ^{131}I-chTNT injection in patients with pulmonary metastases from differentiated thyroid carcinoma, 0.5 h (a), 24 h (b), 48 h (c) and 168 h (d).

Table 2 The ^{131}I intake percentages (%) of the tumor, whole body and major organs at different time points after ^{131}I-chTNT injection in patients with pulmonary metastases from differentiated thyroid carcinoma

Site	Time / h					
	0.5	24	48	72	120	168
The whole body	100	88.00±5.35	78.00±5.12	67.00±4.34	51.00±5.67	38.00±5.54
Tumor	0	2.94±1.93	2.71±1.72	2.11±1.44	1.41±0.95	0.93±0.56
Liver	9.31±1.52	7.42±1.41	6.33±1.78	5.22±1.24	3.81±0.99	3.21±0.74
Lung	8.51±1.96	6.94±1.66	5.73±1.32	4.62±1.03	3.21±0.7	2.13±0.53
Heart	6.53±1.72	5.92±1.40	4.74±1.04	3.61±1.04	2.12±0.63	1.73±0.32
Kidney	2.53±0.82	2.36±0.55	1.73±0.44	1.36±0.32	1.15±0.26	0.72±0.36
Spleen	2.12±0.53	1.92±0.43	1.41±0.55	1.12±0.44	1.04±0.44	0.63±0.27
Brain	1.12±0.34	0.95±0.28	0.81±0.17	0.71±0.18	0.52±0.21	0.39±0.18

4 Discussion

chTNT is a kind of new drugs targeting at degenerated or necrotic nuclei in the tumor necrosis zone, and it has a broad spectrum of anti-solid tumor effects[3-5]. It has been approved in China for radio- and chemotherapy of advanced lung cancer with limited response to conventional therapy or high recurrence rate. Due to little or no intake of ^{131}I by pulmonary metastasis foci after several rounds of treatment, therapeutic effects of some patients with pulmonary metastases from differentiated thyroid carcinoma are not remarkable. In the present study, we investigated the *in vivo* distribution, the metabolic process and the structural integrity of ^{131}I-chTNT in patients with pulmonary metastases from differentiated thyroid carcinoma. The results provided scientific evidence for further exploring the effect of ^{131}I-chTNT in treating lung metastases from thyroid carcinoma and its security in clinical application.

The results for pharmacokinetics in this study showed that the percentages of ^{131}I-chTNT in serum radioactive components were over 95% within 72 h and still remained (88±7)% at 168 h ($T_{1/2}$ = 65.28±14.83 h), which were close to what had been reported previously[6]. Blood radioactivity was eliminated through urine continuously. Accumulative radioactivity (31±9)% of the injected dose was excreted from urine within one week. Basic experiments showed that the *in vivo* radioactive chemical composition included ^{131}I-chTNT and ^{131}I after ^{131}I-chTNT injection[6]. ^{131}I-chTNT is a biological macromolecule that cannot penetrate the normal renal basilar membranes, hence no original drugs in urine. Urine metabolite analysis demonstrated that chemical ingredients of radioactive substances in urine were all free ^{131}I. This pharmacokinetic characteristic is beneficial for ^{131}I-chTNT to concentrate on the target tissues and to accelerate the removal of metabolites, which not only improves the radiation dose at the target zone but also reduces the injury of normal tissues.

Results of the *in vivo* distribution showed that ^{131}I-chTNT was distributed to the whole body immediately after intravenous injection. The *in vivo* ^{131}I-chTNT radioactivity observed from the image of the whole body tomography decreased in a time-dependent manner, which was mainly due to the continuous excretion of metabolites containing radioactive substances through urine. After entering the body, ^{131}I-chTNT gradually penetrated the capillary wall to reach necrotic tumor lesions and was bound to the tumor cell nuclear antigen specifically with a trend of accumulation. At 24 h after injection, the radioactivity of tumor tissues reached the maximum and the *T*/*N* ratio reached the maximum during 3~7 d; the time and degree of concentration were correlated with blood supply and antigen-antibody affinity. The lung and liver incepted radioactive tracers at high level, and maintained the radioactivity for prolonged time. At 120 h after ^{131}I-chTNT administration, there was still 7.1% of the injected radioactivity in the lung and liver, which may be related to the rich pulmonary blood supply and the metabolic activity of liver. The very low intracerebral radioactivity indicated that ^{131}I-chTNT or its metabolites could not penetrate blood-brain barrier. Because there were no residual thyroid tissues or thyroid carcinoma due to recurrence and metastasis incepting ^{131}I in the patients, the radioactivity distributions in this study were not affected by these factors.

In summary, the characteristics of biological distribution of ^{131}I-chTNT *in vivo* in patients with pulmonary metastases from differentiated thyroid carcinoma are favorable for its therapeutic application in the metastatic tumor. However, the therapeutic effects of this method and the security of its clinical applications still need more systematic studies. It is encouraging to further explore the possibility of ^{131}I-chTNT as a new approach to sequential therapy for pulmonary metastasis foci which do not intake ^{131}I due to significant necrosis after treatment with other means.

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