^{131}I-chTNT-mediated radioimmunotherapy for non-uptaking ^{131}I pulmonary metastases from differentiated thyroid carcinoma

GAO Shi[1] JI Tiefeng[1] WEN Qiang[1] CHEN Bin[1] MA Qingjie[1]
CHEN Zuowei[2] LIU Lin[1,*]

[1]*China-Japan Union Hospital*, *Jilin University*, *Changchun 130033*, *China*

[2]*Department of Nuclear Medicine*, *People's Hospital of Shenzhen*, *Shenzhen 518020*, *China*

Abstract In this paper, the safety and efficacy of ^{131}I-labeled mouse/human chimeric monoclonal antibody (^{131}I-chTNT)-mediated radioimmunotherapy are evaluated because the patients have non-uptaking ^{131}I pulmonary metastases from differentiated thyroid carcinoma (DTC). The 16 patients were injected intravenously by 29.6±3.7 MBq·kg^{-1} using ^{131}I-chTNT. The chest computer tomography was performed before treatment, as well as 28 and 70 days after treatment. Responses and safety were assessed during the treatment. The results show that the ^{131}I-chTNT infusion was well tolerated with the 12.5% complete response, 18.8% partial response, 25.0% progressive disease, and the 43.8% stable disease, indicating that most treatment-related adverse effects are mild transient and reversible. The ^{131}I-chTNT is promising for patients with non-uptaking the ^{131}I pulmonary metastases from DTC.

Key words ^{131}I-chTNT, Pulmonary metastases, Differentiated thyroid carcinoma, Micronucleus

1 Introduction

The pulmonary metastasis treatment from differentiated thyroid carcinoma (DTC) is an increasingly challenging issue in clinic oncology. Over the past years, the large dose ^{131}I internal irradiation was used for such metastases in clinical practice, but many pulmonary metastatic foci failed to uptake ^{131}I due to leading to DTC relapse[1]. Thus, these patients need effective adjuvant therapeutic modalities.

In the past decade, the radioimmunotherapy (RIT) utilized the radiolabeled monoclonal antibodies (MABs) to target specific tumor-associated antigens, and was superior to the traditional chemotherapies due to its intrinsic low-toxicity and high-efficiency[2,3]. For immunotherapy against solid tumors, the iodine-131-labeled recombinant chimeric therapeutic monoclonal antibody (^{131}I-chTNT) is a new targeted antibody agent against tumor cell nucleus protein has been developed[4,5]. Also, the ^{131}I-chTNT was potential against various tumors *in vitro* and *in vivo*, including lung cancer, lymphoma, glioblastoma and colorectal carcinoma[6–9]. In this paper, the effects of ^{131}I-chTNT against DTC is further studied, this agent is apply to the pulmonary metastases patients from DTC for the first time, The chest computer tomography (CT) was performed before and after treatment. Responses and safety were assessed during the treatment. The results show that the ^{131}I-chTNT infusion was well tolerated with the 31.3% overall response, indicating that the most treatment-related adverse effects are mild transient and reversible.

2 Materials and methods

The protocol was approved by the institutional review board and independent ethic committees of China-Japan Union Hospital, Changchun, China. The written informed consent was obtained from all participants prior to the study commencement.

2.1 Patient eligibility

The 16 patients (10 males and 6 females) in the study have a mean age of 63.3±7.9 years and body weight of 52±10.8 kg, including 12 papillary carcinomas and 4

Supported by the National Natural Science Foundation of China (NSFC) projects (No.81271606) and the Research Fund of Science and Technology Department of Jilin Province (Nos. 201015185 and 201201041) and the Research Fund of Shenzhen Sci-tech Department of Guangdong Province (No.201102154).

* Corresponding author. *E-mail address*: liulin5413@126.com

Received date: 2013-01-20

follicular carcinomas. All patients were non-uptaking ^{131}I pulmonary metastases from DTC, and had no residual thyroid confirmed by the prior detections. No patient underwent radiotherapy or chemotherapy at least 30 days prior to the study entrance, and had abnormal findings in regular biological and biochemical assays for hemogram, hepatic and renal functions. The human anti-mouse antibody (HAMA) and the iodine allergy tests were performed before agent administrations.

2.2 Major apparatuses and drugs

The apparatuses in this study included SkyLight SPECT (single photon emission computerized tomography, 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). The ^{131}I-chTNT in a clear primrose liquid state, which was purchased from Shanghai Meien Biotechnology Corp., Ltd., had a 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 Drug administration

The ^{131}I-chTNT (29.6±3.7 MBq·kg^{-1} and 5 mL) was intravenously infused, and the RM-905a counter was applied to determine its radioactivity.

2.4 Treatment evaluation

CT was performed at 1 day before treatment as well as the 28 and 70 days after treatment. These treatments were evaluated by their response criteria in solid tumors (RECIST, version 1.1). The complete response (CR) shows that all target lesions disappeared for 28 days, the partial response (PR) meets that at least 30% diameters of target lesions decreased for 28 days, the overall response (OR) means the sum of both, progressive disease (PD) show that at least 20% diameters of target lesions increased, and the stable disease (SD) is no sufficient shrinkage or increase to qualify for PR or PD[10].

2.5 Safety evaluation

2.5.1 Conventional examination

To evaluate the status of haptic and renal functions, each patient underwent a physical examination and a batch of laboratory tests at 1 day before treatment as well as the 14, 42, and 70 days after treatment, including radiologic studies, electrocardiogram (ECG), and complete blood cell count (CBC), and biochemistry panels.

2.5.2 Micronucleus assay and karyotyping

Peripheral blood samples were collected at 1 day before treatment as well as the 7, 90, and 180 days after treatment. Peripheral blood lymphocytes were isolated as previously described[11].

Human lymphocytes are seeded in RPMI1640 medium (Sigma) containing the 5% heat-inactivated fetal bovine serum (FBS, Whittaker Bioproducts, Walkersville, MA), the 100 U/mL penicillin (Sigma), and the 100 g/mL streptomycin (Sigma). The cells were cultured in a 5% CO_2 incubator until adding Cytochalasin-B 4 h after the first cell division. After the second division, the cultured cells (1 mL) were collected and centrifuged at 1000 rpm for 1 min, thus discarding the supernatant. Washed 3 times by culture medium supplemented with 2% FBS, the cells were swollen for 15 min in an hypotonic solution (wash medium : distilled water=1:4). The slides were prepared by using a cytospin, and the cells were located in holder equipped with a filter and a chamber and cytospinned for 7 min. After the slides were recovered, air-dried, fixed and stained in a Giemsa solution, the stained interphase cells were microscopically used for analyzing their micronuclei and chromosome mutation[12].

The scored micronuclei in those cells were complete nuclear division, and exposure to the agent. The 2000 metaphases cells were analyzed, and micronucleus rate of less than 4‰ was set as the normal cutoff.

The metaphases were karyotyped according to ISCN 1995 classification[13]. After analyzing 1000 cells, the chromosome changes and aberrations were determined, calculating the chromosome mutation rates. A total mutation rate of lower than 2.5% and a

dicentromere plus centric ring rate of lower than 0.05% were considered as the normal cutoffs.

2.6 Statistical analysis

Data were expressed as mean ± SD, and analyzed by student's *t* test using SPSS Package 10.0.

3 Results

3.1 Clinical efficacy

Among 16 patients, 2 (12.5%) was CR (Fig.1); and 3 (18.8%), PR; and 4 (25.0%), PD; and 7 (43.8%), SD. The OR was 31.3%.

3.2 Safety

All 16 patients well tolerated the ^{131}I-chTNT infusion. During the study course, 7 patients (43.8%) reported treatment-related adverse events (AEs), including 1 nausea, 1 erythra, 2 leucopenias and 3 thrombocytopenias. Most treatment-related AEs were mild and transient. The hemogram status, haptic and renal functions were shown in Tables 1 and 2.

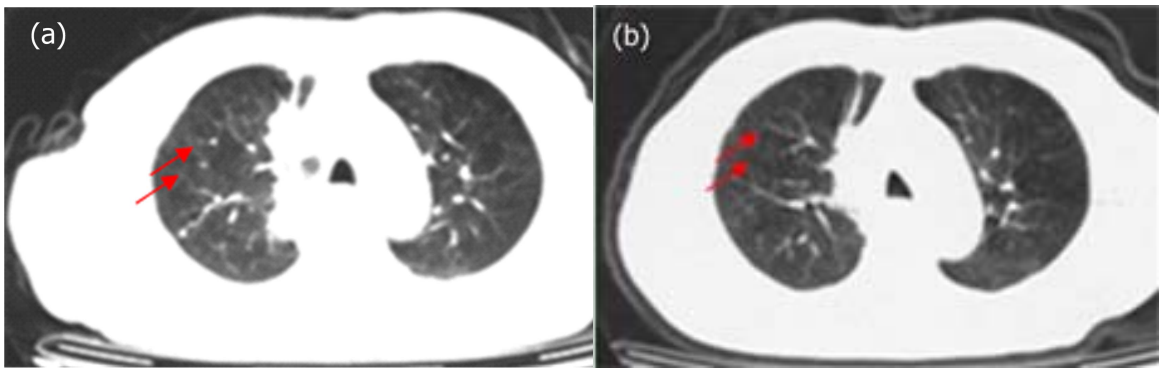

Fig.1 Representative CT images of a patient with the non-uptaking ^{131}I pulmonary metastases by DTC pre-treatment and post-treatment. Multiple pulmonary metastases appeared in the right upper lobe before treatment (a), most pulmonary metastases disappeared after treatment (b).

Table 1 The status of hemogram during treatment in all patients(χ±s)

	Day 1	Day 14	Day 42	Day 70
RBC ($\times10^{10}$/L)	405.0±27.2	396.7±28.2	390.0±33.3	393.3±35.6
Platelet ($\times10^{9}$/L)	201.3±42.6	203.1±42.6	187.3±30.2	192.5±34.5
WBC ($\times10^{9}$/L)	7.9±1.6	7.4±3.0	7.8±1.4	7.4±1.6
Neutrophils（$\times10^{9}$/L）	5.7±1.4	5.1±2.4	5.5±1.1	5.6±1.9

Note: 1. RBC: red blood cells, WBC: white blood cells. 2. There was no significant difference of hemogram between the pre-treatment and the 70 days post-treatment ($p>0.05$).

Table 2 The status of liver and renal functions during treatment(χ±s)

	Day - 1	Day 14	Day 42	Day 70
ALT (U/L)	17.0±10.0	17.9±11.9	16.1±9.5	17.1±9.9
AST (U/L)	22.5±11.1	22.7±15.1	21.5±6.4	20.9±10.9
BUN (mmol/L)	4.8±1.4	4.3±0.8	4.4±1.2	4.5±0.8

Note: 1. ALT: alanine aminotransferase, AST: aspartate aminotransferase, BUN: blood urea nitrogen. 2. There was no significant difference of liver and renal functions between the pre-treatment and the 70 days post-treatment ($p>0.05$).

Table 3 Changes of lymphocyte micronucleus rate, chromosome aberration rate and DCB-CR rate during treatment (χ±s)

	Day 1	Day 7	Day 90	Day 180
Lymphocyte micronucleus rate (‰)	1.00±0.22	4.40±0.73	1.35±0.75	1.00±0.75
Chromosome aberration rate (%)	1.10±0.40	3.00±0.44	1.30±0.55	1.10±0.43
DCB-CR rate (%)	0.03±0.01	0.90±0.30	0.02±0.01	0.02±0.01

Note: For lymphocyte micronucleus rates, chromosome aberration rates and DCB-CR rates, there were significant differences between the pre-treatment and the 7 days post-treatment ($p<0.05$) (1); and no significant, between pre-treatment, and the 90 and 180 days post-treatment ($p>0.05$)(2).

The lymphocyte micronucleus rate, the chromosome aberration rate and the double centromere body plus centromere ring (DCB-CR) rate of all patients experienced a significant rise 7 days post-treatment, returning to normal levels the 90 days apogee (Table 3 and Fig. 2).

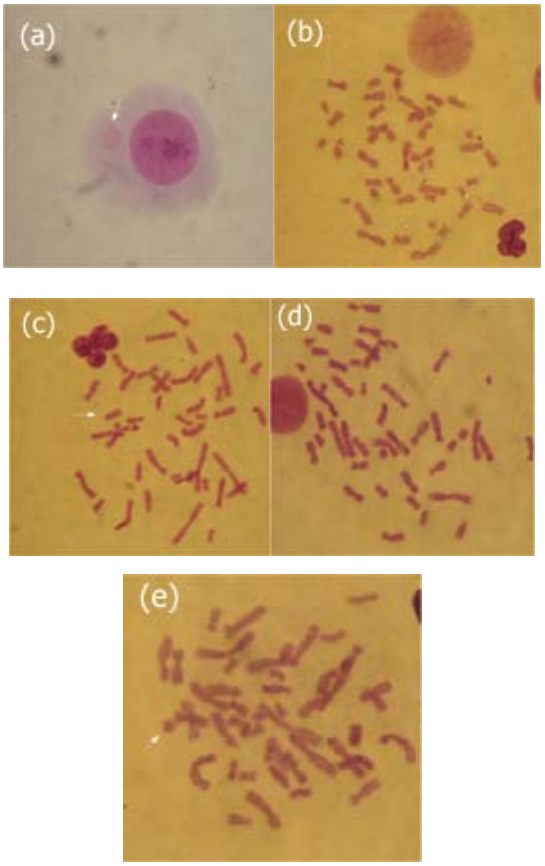

Fig.2 The status of lymphocyte micronucleus and chromosomes in peripheral blood at the 7 days post-treatment (×40). Lymphocyte micronucleus appeared(a). The chromosome was normal before treatment (b), and chromosome fragment appeared at the 7 days post-treatment(c). Double centromere bodies(d) and centromere rings(e) appeared.

4 Discussion

As one of the most common malignancies worldwide, the pulmonary metastases by DTC increase with mortality. To date, the mega-dose ^{131}I therapy is considered as a golden modality in clinical treatment. However, due to its little or non-intake by pulmonary metastasis foci, about 20% patients still had the intractable residual foci after treating several rounds[14], and its high-dose and frequent administration would also induce radiation associated pathological damages to normal tissues and organs, thus increasing micronucleus scores and chromosome mutation rates[15]. Thus, it is urgent to induce the improved therapeutic regimens, such as tumor specific molecular targeted radioimmunotherapy for treating the pulmonary metastases.

Monoclonal antibodies, which represent an emerging strategy in clinical oncology, can mostly kill target tumor cells directly, and deliver cytotoxic toxins, drugs and radionuclides to tumor microenvironments [16–19]. In recent years, several antibodies in US and Europe have been approved for solid tumor treatment. Exposed by dead and dying cells at the center of solid tumors, the ^{131}I-chTNT as target monoclonal antibody can design to bind the DNA histone complex. Its targeting mechanism is to bind the dying tumor cells and deliver the radioactive payload to the adjacent living tumor cells, then the tumor from the inside out are essentially destroyed by minimal radiation exposure to healthy tissue[20–22]. The ^{131}I-chTNT has been approved in China for radioimmunotherapy of advanced lung cancer or glioblastoma[8,20]. These findings prompted us to investigate the safety and efficacy of the ^{131}I-chTNT in the patients by DTC using the non-uptaking ^{131}I pulmonary metastases.

This study shows that the 31.3%OR was consistent with previously published works, revealing the characteristic of the TNT approach. The adverse event of ^{131}I-chTNT was considered as the bone marrow-related toxicity. Also, this study detected the 2 transient leucopenias and 3 transient thrombocytopenias, which is lower than patients receiving systemic chemotherapy. Another 2 patients, which experienced non-serious nausea and erythra, required no special treatment. These results show that ^{131}I-chTNT was safe for clinic application.

The ^{131}I-mediated radiotherapy for DTC with pulmonary metastases is currently the first therapeutic regimen, but it can damage normal tissues adjacent to tumor cells. Analyzing the radioactive risk of the treated patients was based on chromosomal mutation and micronucleus aberrations rates in peripheral blood lymphocytes, and associated with ^{131}I administration in the range of 0.25 to 5 Gy. For chromosomal mutation, the double centromere body and centromere ring was a sensitive criterion.

Seven days after the ^{131}I radiotherapy, the aberration rates of the lymphocyte micronucleus, chromosome and DCB-CR experienced a significant rise at the 7 days post-treatment, and recovered after 90 days, indicating that the radiopathological effects were reversible and associated with the ^{131}I dose but not disease category. Our results were in line with existing reports, confirming that the micronucleus assay and karyotyping analysis as biological radiation dosimeters can be used to evaluate the clinical outcomes of DTC patients by ^{131}I treatment. As reported[23,24], the transient chromosomal mutation of germ cells would be produced by damaging the peripheral blood lymphocyte chromosome. Therefore, a long-term follow-up is necessary for the young patients and the fertility should be considered only after recovering chromosome.

5 Conclusion

The non-uptaking ^{131}I pulmonary metastases from DTC are difficult to treat in clinic oncology, but the ^{131}I-chTNT appears to cure them. In this pilot clinical trial, the OR by ^{131}I-chTNT mediated radioimmunotherapy was 31.3%. Most treatment-related AEs were mild, transient and reversible. The ^{131}I-chTNT mediated radioimmunotherapy is expected as a new approach for the non-uptaking ^{131}I pulmonary metastases. Further, the better clinical outcomes should obtained by studying more patients and long-term follow-ups.

References

1 Pan Z Y. Clinical nuclear medicine. Beijing: Atomic Energy Press, 1994, 549–551.

2 Kwilas A R, Donahue R N, Bernstein M B, *et al*. Front Oncol, 2012, **2:** 104–106.

3 Sharkey R M, Goldenberg D M. J Nucl Med, 2005, **1:** 115–127.

4 Yu L, Ju D W, Chen W, *et al*. Cancer Biother Radiopharm, 2006, **21:** 5–14.

5 Bayés M, Rabasseda X, Prous J R. Methods Find Exp Clin Pharmacol, 2005, **27:** 711–738.

6 Like Y, Dian W J, Chen W P, *et al*. Cancer Biother Radio, 2006, **21:** 7–14.

7 Hilary H S, Michael L G, George A F, *et al*. Cancer Biother Radio, 2006, **21:** 243–256.

8 Alia H, Andrew S. Future Oncol, 2012, **8:** 659-669.

9 Pan H, Niu G Q, Pan J, *et al*. Acta Pharm Sinica, 2006, **41:** 506–512.

10 Eisenhauer E A, Therasse P, Bogaerts J, *et al*. Eur J Cancer, 2009, **45:** 228–247.

11 Kaczmarek L, Calabretta B, Baserga R. Proc Natl Acad Sci, 1985, **82:** 5375–5379.

12 Pelz A F, Müller G, Wieacker P. Cancer Genet Cytogenet, 2005, **157:** 157–159.

13 F. Mitelman ed. S. Karger, Basel. ISCN, 1995.

14 Ma Q J, Gao S, Zhao J. Nucl Sci Tech, 2008, **19:** 230–235.

15 Higashi T, Nishii R, Yamada S. J Nucl Med, 2011, **52:** 683–689.

16 Carter P. Nat Rev Cancer, 2001, **1:** 118–129.

17 Goldenberg D M. J Nucl Med, 2002, **43:** 693–713.

18 Milenic D E. Curr Pharm Des, 2002, **8:** 1749–1764.

19 Ross J S, Gray K, Gray G S, *et al*. Am J Clin Pathol, 2003, **119:** 472–485.

20 Chen S L, Yu L K, Jiang C Y, *et al*. J Clin Oncol, 2005, **23:** 1538–1547.

21 Patel S J, Shapiro W R, Laske D W, *et al*. Neurosurgery, 2005, **56:** 1243–1253.

22 William R S, Susan P C, Roberts K, *et al*. Expert Opin Biol Ther, 2006, **6:** 539–545.

23 Chang W P, Tsai M S, Hwang J S, *et al*. Mutat Res, 1999, 16, **428:** 99–105.

24 Hartford S A, Luo Y, Southard T L, *et al*. Proc Natl Acad Sci U S A, 2011, **108:** 17702–17707.