131I-chTNT-mediated radioimmunotherapy for non-uptaking 131I pulmonary metastases from differentiated thyroid carcinoma (Postprint)

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Abstract

This study evaluated the safety and efficacy of 131I-labeled mouse/human chimeric monoclonal antibody (131I-chTNT)-mediated radioimmunotherapy in patients with non-iodine-avid pulmonary metastases from differentiated thyroid carcinoma (DTC). Sixteen patients received an intravenous injection of 131I-chTNT at a dose of 29.6$±$3.7 MBq · kg–1. Chest computed tomography was performed prior to treatment and at 28 and 70 days post-treatment. Treatment responses and safety were assessed. The results demonstrated that 131I-chTNT infusion was well tolerated, with 12.5% complete response, 18.8% partial response, 25.0% progressive disease, and 43.8% stable disease. Most treatment-related adverse effects were mild, transient, and reversible. 131I-chTNT shows promise for patients with non-iodine-avid pulmonary metastases from DTC.

Full Text

Preamble

131I-chTNT-Mediated Radioimmunotherapy for Non-Uptaking 131I Pulmonary Metastases from Differentiated Thyroid Carcinoma

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Abstract
This study evaluates the safety and efficacy of 131I-labeled mouse/human

chimeric monoclonal antibody (131I-chTNT)-mediated radioimmunotherapy in patients with non-uptaking 131I pulmonary metastases from differentiated thyroid carcinoma (DTC). Sixteen patients received intravenous injections of 29.6$\pm$3.7$MBq \cdot kg^{-1} 131I-chTNT. Chest computed tomography was performed before treatment and at 28 and 70 days post-treatment. Responses and safety were assessed throughout the treatment period. The results demonstrate that 131I-chTNT infusion was well tolerated, with a 12.5% complete response rate, 18.8% partial response rate, 25.0% progressive disease rate, and 43.8% stable disease rate. Most treatment-related adverse effects were mild, transient, and reversible. 131I-chTNT shows promise for patients with non-uptaking 131I pulmonary metastases from DTC.

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

Introduction

Pulmonary metastasis from differentiated thyroid carcinoma (DTC) represents an increasingly challenging issue in clinical oncology. Over the past years, high-dose 131I internal irradiation has been used for such metastases in clinical practice, but many pulmonary metastatic foci failed to uptake 131I, leading to DTC relapse [1]. Consequently, these patients require effective adjuvant therapeutic modalities.

Radioimmunotherapy (RIT) utilizing radiolabeled monoclonal antibodies to target specific tumor-associated antigens has emerged as a superior alternative to traditional chemotherapies due to its intrinsic low toxicity and high efficiency [2,3]. The iodine-131-labeled recombinant chimeric therapeutic monoclonal antibody (131I-chTNT) is a novel targeted antibody agent developed against tumor cell nucleus protein [4,5]. The 131I-chTNT has demonstrated potential against various tumors in vitro and in vivo, including lung cancer, lymphoma, glioblastoma, and colorectal carcinoma [6–9].

This study further investigates the effects of 131I-chTNT against DTC, applying this agent to pulmonary metastases patients from DTC for the first time. Chest computed tomography (CT) was performed before and after treatment, and responses and safety were assessed throughout the treatment period. The results show that 131I-chTNT infusion was well tolerated, with a 31.3% overall response rate, indicating that treatment-related adverse effects are mild, transient, and reversible.

2 Materials and Methods

This study was supported by the National Natural Science Foundation of China (NSFC) projects (No.81271606) and the Research Fund of Science and Technology Department. The protocol was approved by the institutional review boards

of China-Japan Union Hospital, Changchun, China. Written informed consent was obtained from all participants prior to study commencement.

2.1 Patient Eligibility

The study enrolled 16 patients (10 males and 6 females) with a mean age of 63.3$±7.9*yearsandbodyweightof*52±$10.8 kg, including 12 cases of papillary carcinoma and 4 cases of follicular carcinoma. All patients had non-uptaking 131I pulmonary metastases from DTC with no residual thyroid tissue confirmed by prior detections. No patient had undergone radiotherapy or chemotherapy within 30 days prior to study entry, and all had normal biochemical assays for hemogram, hepatic, and renal functions. Human anti-mouse antibody (HAMA) and iodine allergy tests were performed before agent administration.

2.2 Major Apparatuses and Drugs

The apparatuses used in this study included a 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 131I-chTNT, purchased from Shanghai Meien Biotechnology Corp., Ltd., was a clear primrose liquid with a radioactivity of 370 $\mathrm{MBq \cdot mL^{-1}}$, radiochemical purity $ $95%, specific antibody binding activity $ 50^{-1}$, and pH of 6.5-7.5.

2.3 Drug Administration

131I-chTNT (29.6$±3.7$MBq \cdot kg^{-1}$ in 5 mL) was administered via intravenous infusion, and the RM-905a counter was applied to determine its radioactivity.

2.4 Treatment Evaluation

CT was performed one day before treatment and at 28 and 70 days post-treatment. Treatment responses were evaluated using Response Evaluation Criteria in Solid Tumors (RECIST, version 1.1). Complete response (CR) was defined as disappearance of all target lesions for 28 days. Partial response (PR) required at least a 30% decrease in target lesion diameters for 28 days. Overall response (OR) represented the sum of CR and PR. Progressive disease (PD) was defined as at least a 20% increase in target lesion diameters. Stable disease (SD) indicated insufficient shrinkage or increase to qualify for PR or PD [10].

2.5 Safety Assessments

2.5.1 Conventional Examination To evaluate hepatic and renal function status, each patient underwent physical examination and comprehensive laboratory tests one day before treatment and at 14, 42, and 70 days post-treatment, in-

cluding radiologic studies, electrocardiogram (ECG), complete blood cell count (CBC), and biochemistry panels.

2.5.2 Micronucleus Assay and Karyotyping Peripheral blood samples were collected one day before treatment and at 7, 90, and 180 days post-treatment. Peripheral blood lymphocytes were isolated as previously described [11].

Human lymphocytes were seeded in RPMI1640 medium (Sigma) containing 5% heat-inactivated fetal bovine serum (FBS, Whittaker Bioproducts, Walkersville, MA), 100 U/mL penicillin (Sigma), and 100 g/mL streptomycin (Sigma). The cells were cultured in a 5% CO_2 incubator until cytochalasin-B was added 4 hours after the first cell division. After the second division, cultured cells (1 mL) were collected and centrifuged at 1000 rpm for 1 minute, discarding the supernatant. After washing three times with culture medium supplemented with 2% FBS, the cells were swollen for 15 minutes in a hypotonic solution (wash:distilled water = 1:4). Slides were prepared using a cytospin, with cells placed in a holder equipped with a filter and chamber and cytospun for 7 minutes. After recovery, slides were air-dried, fixed, and stained in Giemsa solution for microscopic analysis of micronuclei and chromosome mutations [12].

Micronuclei were scored in cells that completed nuclear division and were exposed to the agent. Two thousand metaphase cells were analyzed, with a micronucleus rate of less than 4‰ set as the normal cutoff. Metaphases were karyotyped according to ISCN 1995 classification [13]. After analyzing 1000 cells, chromosome changes and aberrations were determined to calculate chromosome mutation rates. A total mutation rate lower than 2.5% and a dicentromere plus centric ring rate lower than 0.05% were considered normal cutoffs.

3 Results

3.1 Clinical Efficacy

Among the 16 patients, 2 (12.5%) achieved complete response (CR) [Figure 1: see original paper], 3 (18.8%) achieved partial response (PR), 4 (25.0%) had progressive disease (PD), and 7 (43.8%) had stable disease (SD). The overall response (OR) rate was 31.3%.

3.2 Safety

All 16 patients well tolerated the 131I-chTNT infusion. During the study course, 7 patients (43.8%) reported treatment-related adverse events (AEs), including 1 case of nausea, 2 cases of leucopenia, and 2 cases of thrombocytopenia. Most treatment-related AEs were mild and transient. Hemogram status and hepatic and renal functions are shown in and .

**** The status of hemogram during treatment in all patients (mean±SD)

Parameter	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±			

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

**** The status of liver and renal functions during treatment (mean±SD)

Parameter	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±			

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

The lymphocyte micronucleus rate, chromosome aberration rate, and double centromere body plus centromere ring (DCB-CR) rate of all patients experienced a significant rise at 7 days post-treatment, returning to normal levels by 90 days and [Figure 2: see original paper].

**** Changes of lymphocyte micronucleus rate, chromosome aberration rate and DCB-CR rate during treatment (mean±SD)

Parameter	Day 1	Day 7	Day 90	Day 180
Lymphocyte micronucleus rate (‰)	1.00$±0.22\|4.40±0.73\|0.90±0.30\|1.00±0.75\|\|$Chromosomeaberrationrate$(±0.40|3.00±0.4			
	$CRrate(±0.01|1.30±0.55|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 pre-treatment and 7 days post-treatment (p<0.05) (1); and no significant differences between pre-treatment and 90 and 180 days post-treatment (p>0.05)(2).

[**Figure 1: see original paper**] Representative CT images of a patient with non-uptaking 131I pulmonary metastases from 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).

[**Figure 2: see original paper**] Status of lymphocyte micronucleus and chromosomes in peripheral blood at 7 days post-treatment ($×$40). Lymphocyte micronucleus appeared (a). Chromosome was normal before treatment (b),

and chromosome fragment appeared at 7 days post-treatment (c). Double centromere bodies (d) and centromere rings (e) appeared.

4 Discussion

As one of the most common malignancies worldwide, pulmonary metastases from DTC are associated with increased mortality. To date, mega-dose 131I therapy is considered the gold standard modality in clinical treatment. However, due to little or no uptake by pulmonary metastasis foci, approximately 20% of patients still have intractable residual lesions after several treatment rounds [14], and high-dose frequent administration can also induce radiation-associated pathological damage to normal tissues and organs, as evidenced by increasing micronucleus scores and chromosome mutation rates [15]. Thus, improved therapeutic regimens are urgently needed, such as tumor-specific molecular targeted radioimmunotherapy for pulmonary metastases.

Monoclonal antibodies represent an emerging strategy in clinical oncology that can directly kill target tumor cells and deliver cytotoxic toxins, drugs, and radionuclides to tumor microenvironments [16–19]. In recent years, several antibodies in the US and Europe have been approved for solid tumor treatment. The 131I-chTNT targets the DNA-histone complex exposed by dead and dying cells at the center of solid tumors. Its mechanism involves binding to dying tumor cells and delivering the radioactive payload to adjacent living tumor cells, essentially destroying the tumor from the inside out with minimal radiation exposure to healthy tissue [20–22]. The 131I-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 131I-chTNT in DTC patients with non-uptaking 131I pulmonary metastases.

This study shows that the 31.3% overall response rate is consistent with previously published works, revealing the characteristic efficacy of the TNT approach. The primary adverse event of 131I-chTNT was considered bone marrow-related toxicity. This study detected 2 cases of transient leucopenia and 2 cases of transient thrombocytopenia, which is lower than in patients receiving systemic chemotherapy. Another 2 patients experienced non-serious nausea and erythra that required no special treatment. These results demonstrate that 131I-chTNT is safe for clinical application.

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

Seven days after 131I radiotherapy, the aberration rates of lymphocyte micronucleus, chromosome, and DCB-CR experienced a significant rise, recovering after

90 days, indicating that the radiopathological effects were reversible and associated with the 131I dose but not disease category. Our results align with existing reports, confirming that micronucleus assay and karyotyping analysis can be used as biological radiation dosimeters to evaluate clinical outcomes of DTC patients treated with 131I.

As previously reported [23,24], transient chromosomal mutation of germ cells can be produced by damaging peripheral blood lymphocyte chromosomes. Therefore, long-term follow-up is necessary for young patients, and fertility should be considered only after chromosome recovery.

5 Conclusion

Non-uptaking 131I pulmonary metastases from DTC are difficult to treat in clinical oncology, but 131I-chTNT appears to offer a curative option. In this pilot clinical trial, 131I-chTNT-mediated radioimmunotherapy achieved a 31.3% overall response rate. Most treatment-related adverse events were mild, transient, and reversible. 131I-chTNT-mediated radioimmunotherapy is expected to become a new approach for non-uptaking 131I pulmonary metastases. Better clinical outcomes should be obtained by studying more patients and conducting long-term follow-ups.

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Note: Figure translations are in progress. See original paper for figures.