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Therapeutic safety evaluation of sodium glycididazole combined with ^{131}I radiotherapy for differentiated thyroid carcinoma

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Abstract To evaluate safety and therapeutic efficacy of sodium glycididazole (CMNa) combined with ^{131}I radiotherapy for differentiated thyroid carcinoma (DTC), the 60 patients of DTC therapeutic protocols were selected and divided into 3 groups of the DTC 4.44 GBq ^{131}I, DTC 3.70 GBq ^{131}I, and combination of DTC 3.70 GBq ^{131}I with CMNa, and the 20 patients of Graves' Disease were selected as the control group. Peripheral blood was sampled at ^{131}I pre-treatment of 1 day, and ^{131}I post-treatment of 7, 91, and 182 days, thus analyzing lymphocyte micronucleus scores and karyotyping profiles. Compared with the control group, the lymphocyte micronucleus and chromosome mutation rates in ^{131}I treated DTC increased after post-treatment of 7 days, recovered after post-treatment of 91 days, and did not bounce after post-treatment of 182 days. The micronucleus and chromosome mutation rates in the combination of DTC 3.70 GBq ^{131}I with CMNa showed less significant variation than other treated DTC groups. Our results demonstrate that micronucleus assay and karyotyping analysis are favorable to evaluate ^{131}I radiotherapy for DTC. The combination of the CMNa with ^{131}I radiotherapy was safe for DTC patients without affecting the long-term therapeutic outcomes.

Key words Radioactive nuclide, Iodine, Thyroid, Radiosensitizer, Metaphase

1 Introduction

Differentiated thyroid carcinoma (DTC) is a common endocrinological malignancy with increased incidence [1]. Thyroid follicular cell-derived papilloma and adenoma account for most of DTC cases in pathological classification[2]. Up to date, thyroidectomy combined with post-operational ^{131}I therapy is considered as a golden modality in clinical treatment. Still, a portion of DTC patients undergoing such therapeutic strategy show the residual tumor or metastasis to adjacent cervical lymph nodes. These require to administrate a second ^{131}I of high dose and frequency[3], causing radiation-associated pathological damages to normal tissues and organs of the patients, with increased micronucleus scores and chromosome mutation rates[4]. Thus, a novel protocol will benefit DTC patients by enhancing ^{131}I therapeutic efficacy and reducing the side effects.

Radiosensitizers have been used for over 30 years for brachytherapy of solid tumors in the hypoxic regions, to enhance the susceptibilities and radiosensitivities, and facilitate therapeutic efficacy[5–7]. Sodium glycididazole ($C_{18}H_{22}N_7NaO\cdot 3H_2O$, CMNa), the first radiosensitizer of nitroimidazole compound developed in China, bears radiation-enhancing effects on solid tumors *in vitro* and *in vivo*, and multiple phase I in clinical trials[8,9]. Nevertheless, it is unknown whether the CMNa could be applied to ^{131}I internal radiotherapy for DTC, and how is its radiopathological profile in the therapy.

In this study, combined CMNa amd ^{131}I radiotherapy was used for DTC patients to evaluate the efficacy of the CMNa enhancing radiotherapeutic effects and the safety profiles. The results show that

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the CMNa was a favorable and safe radiosensitizer facilitating the ^{131}I internal radiotherapy and micronucleus score from brachytherapy. The karyotyping analysis was used to evaluate the personalized CMNa associated with radiopathological profiles and therapeutic processes.

2 Materials and methods

2.1 Patients and groups

From May 2008 tp July 2009, sixty patients (13 male and 47 female patients, aged from 21 to 62 with a median age of 42.6±7.5) after thyroidectomy or ^{131}I radiotherapy at China-Japan Union Hospital were confirmed pathologically as DTC with metastasis to cervical lymph nodes, including 51 cases of thyroid papillary carcinomas and 9 cases of thyroid follicular adenomas. All the patients with adjacent metastasis to cervical lymph nodes and without distal metastasis were confirmed by color Doppler ultrasonic, chromatographic, and the ^{131}I radioactive imaging.

The 60 patients were divided into three groups of 4.44 GBq ^{131}I (Group I, 18 cases), 3.70 GBq ^{131}I (Group II, 20 cases), and 3.70 GBq ^{131}I combined with CMNa (Group III, 22 cases). The 20 Graves' diseases (10 males and 10 females) were used as control hyperthyroidism without previous chemotherapy or radiotherapy. All patients with thyroid stimulating hormone over 30 mIU/L and ^{131}I uptake rate over 1% were strictly restrained from thyroid hormone drugs under iodine free diet. For patients with a thyroid radiotherapy history, an interval of ^{131}I treatment was over 182 days.

The written informed consents for patients were obtained, and the research protocol was approved by the Institutional Review Board for Human Protection in our hospital.

2.2 Drugs and reagents

Na^{131}I solution was purchased from Zhong He Gao Tong Isotope Company, Chengdu, China. The CMNa injection solution was from Green Leaf Company, Shandong, China. Colchicine, phytohaemagglutinin, heparin, Giemsa solution, and potassium chloride powder were purchased from Sigma, St. Louis, MO, USA.

2.3 Drug administration

To achieve indicated total doses, the DTC patients were orally administrated with ^{131}I quaque die for 4 consecutive days. The CMNa was intravenously administrated at body surface area (BSA) of 800 mg/m^2 within 30 min, and the ^{131}I was orally taken orally after 60 min of CMNa injection. The BSA (m^2) was calculated by BSA = 0.0061body height (cm) + 0.0128 body weight (kg) – 0.1529. The patients of control group were administrated to the total doses of 111–296 MBq.

2.4 Sample collection

Peripheral blood was sampled one day before the ^{131}I treatment (referred as Day–1), and 7, 91, and 182 days after the ^{131}I post-treatment (referred as Day 7, 91 and 182), and the peripheral blood mononuclear cells (PBMC) were isolated as previously described[10].

2.5 Micronucleus assay and karyotyping

Human lymphocytes were seeded in RPMI1640 medium (Sigma) with 5% heat-inactivated fetal bovine serum (FBS) (Whittaker Bioproducts, Walkersville, MA), 100 U/mL penicillin (Sigma), and 100 μg/mL streptomycin (Sigma), and were cultured in a 5% CO_2 incubator until the Cytochalasin-B was added 4 hours after the first cell division. At the end of the second cell division, 1-mL culture solution from each sample was collected and centrifuged at 1000 rpm for 1 min to discard the supernatant. Washed three times by culture medium supplemented with 2% FBS, the cells were swollen for 15 min in a hypotonic solution of the wash medium and distilled water (v:v, 1:4), were suspended on holder equipped with a filter and a chamber, and cytospinned for 7 min. After the slides were recovered, air-dried, fixed and stained by a Giemsa solution, the presence of micronuclei and chromosome mutation were microscopically analyzed using the harvested and stained interphase cells[11].

Micronucleus score was carried out by the cells with the complete nuclear division after exposure to the test item. The metaphases of 2000 cells were analyzed, and micronucleus rate under 4‰ was set as the normal cutoff.

The metaphases were karyotyped with ISCN 2005 classification[12], and chromosome change and aberration were determined by analyzing 1000 cells to calculate its mutation rate. A total mutation rate of lower than 2.5% and a dicentromere and centric ring rate of lower than 0.05% were considered as the normal cutoffs.

2.6 Statistic analysis

The data expressed in mean ± SD were analyzed by student's *t* test by SPSS Package 10.0. P<0.05 was considered as a statistically significant variance between the indicated groups.

3 Results

3.1 Micronucleus scores at ^{131}I pre- and post-treatment

The micronucleus scores were normal for the three patient groups before the ^{131}I treatment, and the control after ^{131}I treatment. However, the micronucleus scores for the three patient groups on Day 7 increased obviously (Fig.1). They returned to normal for 58 patients on Day 91, and the ^{131}I-induced micronucleus score did not bounce 182 days after the ^{131}I treatment (Fig. 2). Notably, a male DTC patient in Group I (4.44 GBq) had 4.5‰ micronucleus scores on Day 91, and another in Group II (3.70 GBq) was (4.0‰) on Day 182, but both were recovered to the normal ranges 365 days after the treatment.

3.2 Karyotyping profiles pre- and post-treatment

The control group was of normal karyotyping profiles. And so were the patients before the ^{131}I treatment, but their chromosome mutation rates increased significantly on Day 7 (Fig.3), and returned to normal on Day 91 for 58 of the patients. The ^{131}I induced chromosome mutation did not elapse 182 days after the treatment (Fig.4). Further, as described in in Section 3.1, the two male patients were up to 3% chromosome mutation rates on Day 91; to 3‰ on Day 182; and to the normal 365 days after the treament.

The rates of centromere and centric ring for control group patients did not vary statistically on Day 7, 91 and 182 (Fig.5). But the rates of centromere and centric ring for all the patients, which were in normal ranges before the treatment, increased significantly on Day 7, and returned to normal on Day 91 (Fig.6).

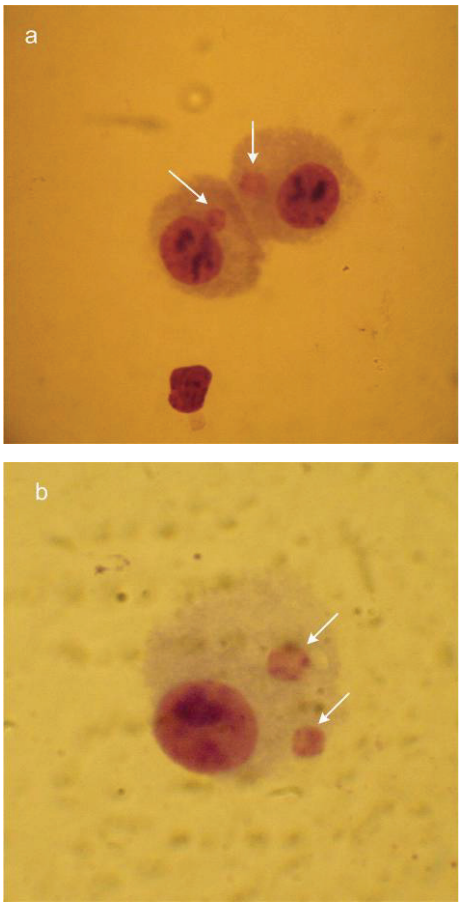

Fig.1 Mono-micronucleus (a) and bi-micronuclei (b) in lymphocytes 7 days after ^{131}I treatment.

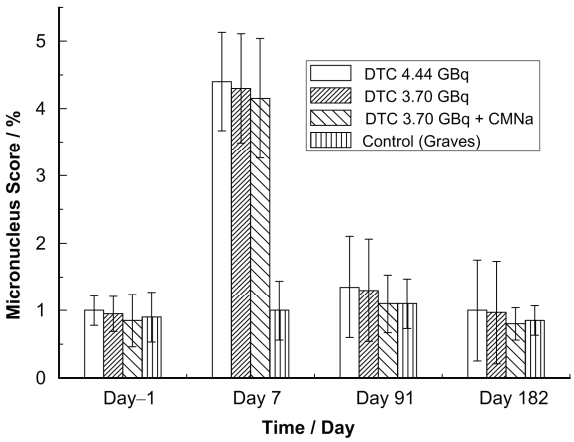

Fig.2 Micronucleus scores in patient lymphocytes on different days. The data on Day 7 were the highest for all the groups (P<0.05).

4 Discussion

Combined with CMNa, radiopathological effects of ^{131}I radiotherapy on DTC were evaluated by micronucleus assay and karyotyping analysis.

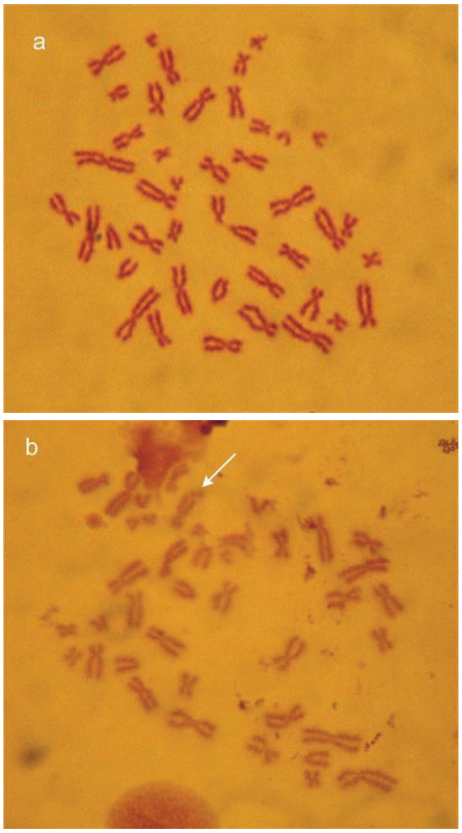

Fig.3 Normal karyotype in DTC patient lymphocytes (a) and karyotype with chromosome fragments on Day 7 (b).

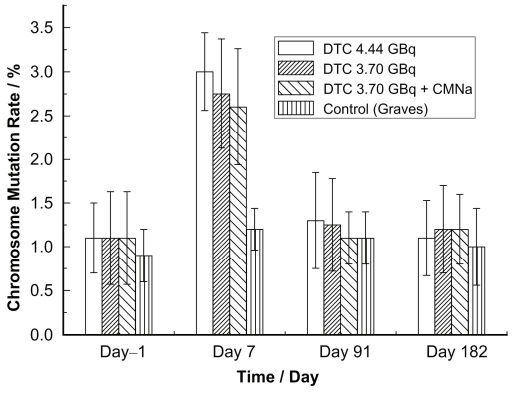

Fig.4 Chromosome mutation rates in patient lymphocytes on different days. The Day 7 data are the highest (P<0.05).

Currently, ^{131}I radiotherapy with an effective rate of over 92% is the first therapeutic regimen for the DTC to metastasize to cervical lymph nodes because of limitation of surgical process[1]. DTC cells adjacent to normal tissues can be damaged by radiation-induced cell apoptosis in the ^{131}I treatment, resulting in severe side effects due to inappropriate ^{131}I doses[13]. Therapeutic efficiency of the ^{131}I radio-pathological profiles and side effects need be evaluated by quantitative or semi-quantitative strategies.

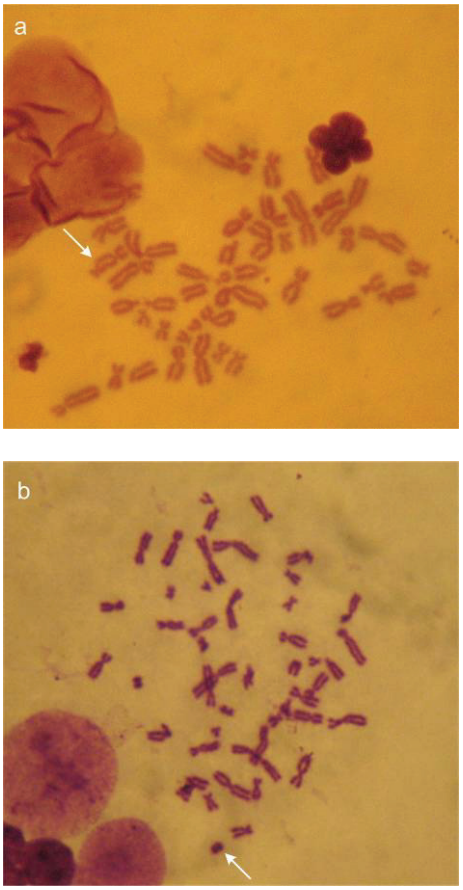

Fig.5 The dicentromere (a) and a centric ring (b) in DTC lymphocyte at 7 days after ^{131}I post-treatment.

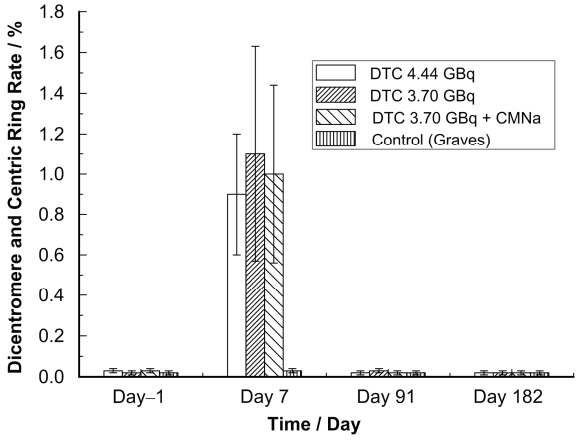

Fig.6 Rates of dicentromere and centric ring in patient lymphocytes on different days. The Day 7 data are the highest for all groups (P<0.05).

Compared with control group, the rates of micronucleus and chromosome mutation 7 days after the ^{131}I radiotherapy increased, and recovered on Day 91, indicating the association of lymphocyte micronucleus generation and chromosome mutation with the ^{131}I treatment but not disease category. The micronucleus assay and karyotyping analysis as biological

radiation dosimeters show that a period of 7 days is long enough to evaluate radiopathological damage profiles in lymphocytes of DTC patients[14,15].

Because the hypoxia-induced low radiosensitivity and radioactive susceptibility in solid tumor region might hamper therapeutic efficiency of ^{131}I treatment, a higher ^{131}I dose was conventionally adopted, resulting in the longer term, more radioactive damage to normal tissues[16,17]. The CMNa as a radiosensitizer preferentially aided to conquer the hypoxic barrier in DTC tumor cells. But the radiopathological effects of 4.44 and 3.70 GBq ^{131}I treatment on DTC showed no significant variation because the current clinic doses of 3.70 GBq ^{131}I was higher than the therapeutic threshold, though it was are optimal dose for individual patient. This should be further clarified by more cases.

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5 Conclusions

Micronucleus assay and karyotype analysis on peripheral blood lymphocytes are favorable to evaluate the therapeutic efficacy and radiopathological process of ^{131}I radiotherapy for the DTC patients. The combination of CMNa with the ^{131}I radiotherapy showed safe profiles in DTC patients, and reduced radioactive dose of the ^{131}I radiotherapy, thus achieving the equal output with the higher dose ^{131}I without affecting the long-term therapeutic outcomes. This may benefit the patients and the medical practitioners.

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