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Potential role of carbon nanoparticles in protection of parathyroid glands in patients with papillary thyroid cancer

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Abstract

As a novel type of lymphatic tracer, carbon nanoparticles (CNs) were reported not to stain parathyroid glands (PGs) into black, so it may have a clinical potential in protection of PGs during thyroidectomy. The purpose of this study was to investigate the clinical application and significance of CN in protection of PGs from surrounding tissues.

A total of 82 consecutive patients were enrolled into this study and were divided into CN group and control group. Parathyroid function (hypoparathyroidism and hypocalcemia) was evaluated.

The identification rates of PGs (≤2) and PGs (≥3) were 24.4% and 75.6% in the CN group and 46.3% and 53.7% in the control group, respectively. The difference in the identification rates between the 2 groups was statistically significant ($P=0.038$). Pathological results revealed 3 accidental PGs resection occurred in the CN group, whereas 9 accidental PGs removal occurred in the control group. The difference was statistically significant ($P=0.046$). Moreover, the incidence of the patients with hypoparathyroidism was statistically significant between the 2 groups (36.6% in CN group vs 53.7% in control group, $P=0.043$) at day 1, but not at day 7 ($P=0.424$).

CN may have a potential in protecting PGs clinically.

Abbreviations: CNs = carbon nanoparticles, PGs = parathyroid glands, PTC = papillary thyroid cancer.

Keywords: carbon nanoparticle, papillary, parathyroid glands, thyroid cancer

1. Introduction

Thyroid cancer is the most common endocrine malignancy. In 2014, it is estimated that 96% of all new endocrine organ cancers will originate from the thyroid gland, resulting in approximately 63,000 new cases.[1] Papillary thyroid cancer (PTC) is the most common subtype of all thyroid cancer, accounting for more than 90% of all thyroid cancer.[2] Cervical lymph node metastasis is quite common for PTC, which has been reported to occur in 12% to 81% of patients with PTC, and central neck is the most common site.[3] The most effective treatment for PTC is the complete removal of the primary tumor and the metastases in the regional lymph nodes, which is a prerequisite for adjuvant therapies.[4] A total or near-total thyroidectomy plus central neck dissection was usually adopted to treat PTC.[5] Inevitably, some complications associated with this surgery would occur. Apart from the common complication of the recurrent laryngeal nerve injury, postoperative hypoparathyroidism is another most common complication, with a reported incidence of from 1.6% to over 50%.[6,7] Hypocalcemia caused by postoperative hypoparathyroidism can cause neuromuscular symptoms (such as numbness, tingling sensations, and muscle cramps) and significantly affect the quality of life of patients.[8] The most common reasons for hypocalcemia could be mechanical damage, devascularization, or inadvertent removal of parathyroid glands (PGs) during operation.[6,7] Therefore, it is important for surgeons to know how to protect the PGs during operation and thus avoid hypocalcemia after operation. However, it is a great challenge for surgeons to do so. As a novel type of lymphatic tracer, carbon nanoparticles (CNs) were reported not to stain PGs into black, so it may have a clinical potential in protection of PGs during thyroidectomy.[9] The aim of the present study is to explore whether CN could help make PGs appear distinctively from surrounding tissues (lymph nodes, fat tissues, and thyroid glands), thereby protecting PGs during surgery and decreasing the occurrence of hypocalcemia after surgery.

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2. Materials and methods

2.1. Imaging agent for lymphatic vessels

CN (LUMMY Pharmaceutical Co., Chongqing, China) was injected as a suspension (50 mg suspended in 1 mL of 0.9% physiological saline).[10,11] This product is a stable suspension of carbon pellets of 150 nm in diameter. The diameter of carbon pellets is greater than the capillary endothelial cell gap (approximately 20–50 nm) and smaller than the lymphatic capillary endothelial cell gap (approximately 120–500 nm). A small amount of carbon particles was captured by macrophages, which then entered the lymphatic duct but not the blood circulation. No toxic side effects have been reported for CN in humans.[10,12]

2.2. Study patients

A total of 82 consecutive patients were enrolled into this study from August 2012 to June 2013. All the patients were diagnosed with PTC by preoperative aspiration pathology and underwent the first operation in our department. All the tumors found were located in 1 lobe of the thyroid gland in all PTC patients. The inclusion criteria were PTC (size of lesion between 1 and 4 cm), and all patients provided an informed consent. Exclusion criteria included nonthyroid cancer, previous thyroid or parathyroid surgery, preoperative hypoparathyroidism or hypocalcemia, pregnancy or lactation, suspicion of the presence of lateral neck lymph node metastasis, aged less than 18 years, and an inability to comply with the follow-up protocol. The patients were randomly divided into 2 groups: the CN group (n=41) and the control group (n=41). The randomization was done using computer-generated random number tables; patients were blinded to their group assignment. All the operations were performed by the same medical team, and all the patients underwent total or near-total thyroidectomy plus bilateral central neck dissection. Approval from the ethics committee of Beijing University Cancer Hospital was obtained before these procedures.

2.3. Surgical procedure

All patients underwent general anesthesia intubation and were placed supinely with necks hyperextended. A transverse incision of a length about 5 cm was placed in a skin crease 2 cm above the sternal notch. The skin, subcutaneous tissue, and platysma were cut layer by layer. Flap separation was performed under the platysma before making a longitudinal incision in the linea alba cervicalis. The strap muscles were retracted, and the front ipsilateral thyroid was revealed, and the side and rear parts of the lobe were kept intact to reduce damage to the lymphatic network around the thyroid. In the CN group, 0.1 mL per spot of CN was injected into tissues surrounding the tumor using a skin test syringe (1 mL). Two or 3 spots were injected for each tumor, and the total amount injected would be no more than 0.5 mL per lobe. Before being injected, the needle of the syringe with CN suspension was inserted deeply and aspirated to ensure that CN would not be injected into the blood vessels. The CN suspension was slowly pushed into the thyroid tissues. Upon completion, a gentle pressure was applied on the needle puncture site to prevent solution leakage.

After 5 minutes, the lymphatic ducts and the lymph nodes became black-stained (Fig. 1). Total or near-total thyroidectomy was then performed according to the meticulous capsular dissection technique.[4] Node clearance of the central neck compartment was performed cranially to both superior thyroid arteries and the pyramidal lobe.[13] When thyroidectomy was completed, the tumors in thyroid gland were sent for frozen pathology examination routinely to confirm the preoperative aspiration pathology. Patients in the control group underwent the standard total or near-total thyroidectomy and the central neck dissection without CN injection. All the thyroidectomy and neck dissection specimens were also sent for pathological examinations.

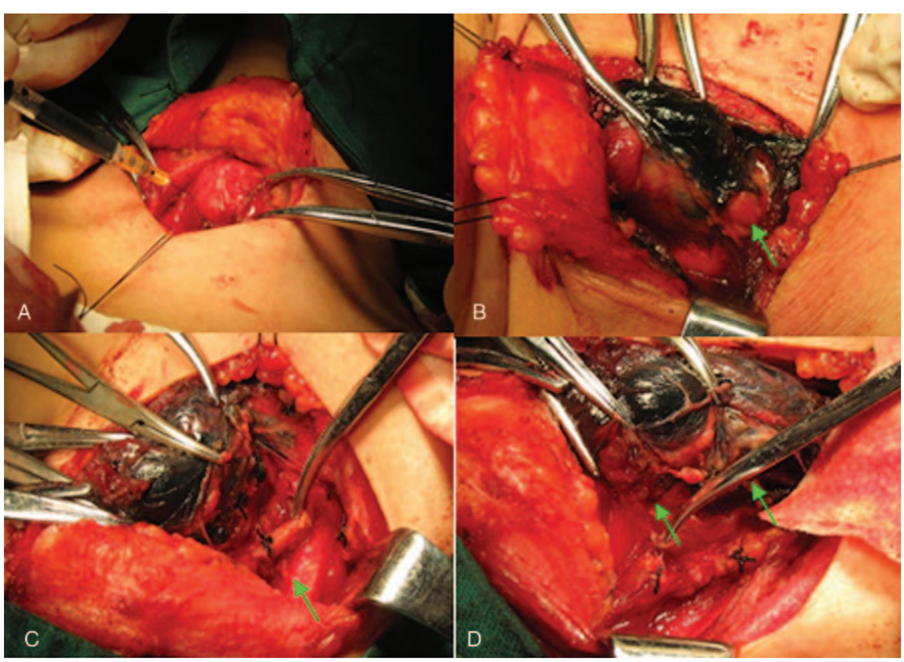

Figure 1. (A) Injection of carbon nanoparticle into thyroid gland. (B) and (C) Preserved superior parathyroid gland (PG) (arrow). (D) Superior and inferior PG (arrow).

During the operations, efforts were made to keep PGs in situ. We divided the inferior thyroid artery at its distal end close to the thyroid capsule to preserve the arterial branch; superior thyroid artery was ligated adjacent to the thyroid capsule[14]; the preserved thyroid arteries supplied the remaining PGs if a near-total thyroidectomy was performed. The superior glands adjacent to the cricothyroid joint lied roughly 1 cm above the inferior thyroid artery on the posterior surface of the thyroid gland, so they could be easily found. The most common location for the inferior PGs was 1 cm below the inferior thyroid artery, but they could be situated ectopically more inferiorly anywhere below the inferior thyroid artery, if no inferior PGs were found at the most common location. The close attention should be paid to suspicious nodules that were not stained black in central neck. Frozen pathology of the suspected nodule tissue would be performed during surgery for pathological confirmation. For the unintentionally removed PGs, frozen section of 1/4 to 1/3, biopsy was performed during surgery for pathological confirmation. When confirmed for PGs, the remaining tissue was transplanted into the sternocleidomastoid muscle.

2.4. Postoperative parathyroid function evaluation and follow-up

Postoperative hypocalcemia was defined as calcium levels of less than 2.0 mmol/L (reference range, 2.1–2.6 mmol/L), with or without clinical symptoms of hypocalcemia or subnormal serum calcium levels (2.0–2.1 mmol/L) with neuromuscular symptoms (such as numbness, tingling sensations, and muscle cramps). Postoperative hypoparathyroidism was defined as the level of parathyroid hormones (PTHs) less than normal range (16–65 pg/mL), and transient hypoparathyroidism was defined as the absence of hypocalcemic symptoms without medication within the 6-month period. We measured calcium and the level of PTH at 6 AM at days 1 and 7 after surgery.

Calcium supplementation was not routinely administered to patients, but calcium and vitamin D was prescribed to patients with symptomatic hypocalcemia routinely until the level of PTH recovered to the normal. Intravenous substitution of calcium was not a routine unless serious symptomatic hypocalcemia was present.

2.5. Statistical analysis

The *t* test and chi-square test were used for the continuous and category data, respectively. For all statistical analyses, SPSS 17.0 package was used; $P<0.05$ was considered to indicate a statistical significance.

3. Results

3.1. Characteristics of study patients

The clinical pathological characteristics of the patients in CN and control groups are summarized in Table 1. Total or near-total thyroidectomy plus bilateral central neck dissection were performed for all patients. PTC was confirmed both by perioperative and postoperative pathology for all patients. Nineteen cases were males and 63 cases were females, with a male-to-female ratio of 1:3.3. The ages of patients ranged from 18 to 75 years with a median age of 41.6 years. No significant differences were found in age ($P=0.501$), sex ($P=0.601$), primary tumor size ($P=0.553$), tumor multifocal ($P=0.625$), and extrathyroid extension ($P=0.647$) between the CN and the control groups.

Table 1

The clinical pathological characteristics of the patients in the CN and control groups.

Characteristics	CN group (n=70)	Control group (n=70)	P
Age (mean, y)	41.6±17.1	41.7±18.9	0.501
Sex			
Male	8	11	
Female	33	30	0.601
Size of primary tumor, cm	1.9±0.6	2.0±0.6	0.553
Extrathyroid extension, %	16 (39.0)	13 (31.7)	0.647
Tumor multifocal (≥2), %	7 (17.1)	10 (24.4)	0.625

CN = carbon nanoparticle.

3.2. Exposure and protection of PGs and its function

As shown in Fig. 1, the thyroid gland was black stained after CN injection. In contrast, the normal PGs were not stained after CN injection and were kept in situ. The symptomatic hypocalcemia would occur easily in the patients with identification of PGs less than 2.[7,8] We took the identification of 1 or 2 PGs as 1 group, 3 and 4 PGs as another group. The identification rates of PGs (≤2) and PGs (≥3) were 10 (24.4%) and 31 (75.6%) in the CN group and 19 (46.3%) and 22 (53.7%) in the control group, respectively. There was a significant difference between the identification rates in the 2 groups ($P=0.038$) (Table 2). Pathological results revealed the resection of 3 accidental PGs occurred in the CN group, whereas the removal of 9 accidental PGs occurred in the control group. The difference was statistically significant ($P=0.046$). Two accidentally resected PGs in the CN group were inferior ones; all 9 accidentally resected PGs in control group were inferior ones.

Hypoparathyroidism and hypocalcemia were evaluated at days 1 and 7 after surgery. There was a significant difference between the patients with hypoparathyroidism in the CN group (15, 36.6%) and the control group (22, 53.7%) ($P=0.043$) at day 1, but not at day 7 ($P=0.424$). The number of patients with hypocalcemia did not reach the significant difference between the 2 groups at day 1 ($P=0.058$) and at day 7 ($P=0.349$). Hypocalcemia symptoms occurred in 7 patients in the CN group and 15 patients in the control group. The difference between the 2 groups was statistically significant ($P=0.046$).

3.3. Central neck dissection in the CN and control groups

All patients underwent standard central neck dissection. Fifty-one cases were demonstrated with lymph node metastasis. Thirty patients (73.2%) were confirmed to have lymph node metastasis in CN group and 21 cases (51.2%) in control group. There was a statistically significant difference between these 2 groups ($P=0.040$). More lymph nodes were detected in CN group (391 lymph nodes) than those in the control group (187 lymph nodes). There is a statistically significant difference between these 2 groups ($P<0.001$).

3.4. Side effects and operative complications

No obvious systemic toxicity occurred in these patients during and after operations. For 2 patients before surgery, CN suspension leaked out of the thyroid gland and made surgical

Table 2

Clinical characteristics and function of PGs.

Characteristics	CN group (n=41)	Control group (n=41)	P
Number of PGs			
≤2	10 (24.4)	19 (46.3)	
≥3	31 (75.6)	22 (53.7)	**0.038**
Number of accidental parathyroid resection, %			
Yes	3 (7.3)	9 (22.0)	
No	38 (92.7)	32 (78.1)	0.061
Symptomatic patients, %			
Yes	7 (17.1)	15 (36.6)	
No	34 (82.9)	26 (63.4)	**0.046**
Patients with hypoparathyroidism, %			
Day 1			
Yes	15 (36.6)	22 (53.7)	
No	26 (63.4)	19 (46.3)	**0.043**
Day 7			
Yes	7 (17.1)	11 (26.8)	
No	34 (82.9)	30 (73.2)	0.424
Patients with hypocalcemia, %			
Day 1			
Yes	9 (22.0)	17 (41.5)	
No	32 (78.1)	24 (58.5)	0.058
Day 7			
Yes	4 (9.8)	8 (19.5)	
No	27 (90.2)	33 (20.5)	0.349

CN = carbon nanoparticle, PGs = parathyroid glands.

field (including recurrent laryngeal nerve and PGs) dark, which also made the operation process quite difficult. For these 2 cases, a gentle pressure was applied on the needle puncture site to prevent solution leakage for 5 minutes, and no further leakage occurred during the operation.

4. Discussion

Total or near-total thyroidectomy plus central neck dissection was usually adopted to treat PTC.[5] The most common complication of the recurrent laryngeal nerve injury has been reduced to a very low rate by meticulous surgical techniques and intraoperative nerve monitoring.[15] Postoperative hypoparathyroidism is another most common complication. The anatomic location of the superior PGs is relatively constant due to the close relationship between these glands and the thyroid gland. They typically reside on the dorsal aspect of the upper thyroid lobes at the level of the inferior border of the cricoid cartilage. The inferior PGs have a more variable location due to their embryologic relationship to the thymus.[16] For example, 58% inferior PGs are in extracapsular locations, 20% in intracapsular locations, and 22% in intrathyroidal (22%) locations.[17] Thus, it is not always possible to identify all 4 PGs by visual only. In addition, the blood of PGs were supplied by the superior and inferior thyroid arteries, and the blood supply for PGs would be bound to be affected by the operation of thyroid gland.[14] Thus, injury to PGs might be inevitable due to the anatomical location of the superior and inferior thyroid arteries as well as the highly variable anatomical locations of the inferior PGs when total thyroidectomy was performed, especially together with central neck dissection.[18] Injury to PGs would lead to hypocalcemia, which is often caused by the accidentally removing or destroying more than 1 of a patient's PGs or incidentally destroying the glandular blood supply.[19] Hypocalcemia would cause neuromuscular symptoms, leading to the patients at a higher risk of chronic renal impairment and basal ganglia calcification.[20]

How to keep the PG in situ and its blood supply intact to avoid the postoperative hypocalcemia is one of the difficulties in the field of thyroid surgery, especially for total thyroidectomy and bilateral central neck dissections.[19] Meticulous dissection for identifying parathyroids and their preservation, including the blood supply, was used routinely in thyroidectomy and central neck dissection to minimize parathyroid injury.[21] A variety of other techniques have been used to help localize and identify PGs. Hara et al[22] reported the subtraction technique for double-phase SPECT images to detect parathyroid lesions, with a sensitivity of 90.9% (40/44), specificity of 83.3%, and an accuracy of 92.8%, while these techniques were quite useful in parathyroid lesions but not normal PGs, particularly for the small weight and size of normal PGs. Although methylene blue has been used to detect PGs and parathyroid lesions,[23,24] the postoperative toxic metabolic encephalopathy has been reported.[24] In addition, several other techniques (methoxyisobutylisonitrile scintigraphy using a gamma probe, 5-aminolevulinic acid, and optical coherence tomography) have been reported without clinical effectiveness in protection of PGs.[25]

Recently, CN suspension has been found to have the potential role in helping identify PGs.[9,26] This product is a stable suspension of carbon pellets of 150 nm in diameter, which is greater than the capillary endothelial cell gap (approximately 20–50 nm) and smaller than the lymphatic capillary endothelial cell gap (approximately 120–500 nm). Rich lymphatics and lymphatic capillaries were found in the thyroid glands, while much less in PGs.[8,27] In addition, there are anatomically independent external capsules for the thyroid and PGs.[27] After injecting into the thyroid glands around the tumor, CNs were rapidly captured by macrophages, and their particles then entered the lymphatic vessels and accumulated in the lymph nodes, thus staining thyroid glands and lymph nodes black. The staining led to PGs and its surrounding blood supply appear different from black-stained lymph nodes. CNs have been applied to more accurately guide the dissection of lymph nodes during thyroidectomy and central neck dissection.[26] In this study, we found that CN may help identify and preserve PGs, thereby reducing the postoperative hypoparathyroidism and hypocalcemia. With the help of CN, more PGs were found and kept in situ in the CN group than in the control group, indicating that CN could easily distinguish the anatomical boundaries among lymphoid nodes, thyroid glands, and PGs. With carefully following and separating these boundaries, more PGs could be more easily found.

Some evidence has shown that the prevalence of hypocalcemia and hypoparathyroidism, protracted or permanent, were related to the number of PGs which remained in situ.[28,29] Furthermore, insufficient blood supply to PGs will seriously affect their function.[30] Since PGs are supplied by many very thin anastomosing branches of superior and inferior thyroid arteries,[31] we might not distinguish clearly each branch, and thus we usually leave PGs and the adjacent surrounding tissue as a whole to protect blood supply.[32] Because CN cannot stain blood vessels into black and can lead to the nonblack-stained tissues clear around PGs, including many tiny blood vessels. Thus, CN may help protect blood supply of PGs quite well.

As a result of mechanical damage, devascularization, or inadvertent removal of PGs during operation,[19,33] some PGs should be autotransplanted. While autotransplantation of PGs cannot completely restore the normal function, the more number of PGs transplanted, the more proportion of patients would develop permanent hypocalcemia.[34] In this study, pathological results revealed that the use of CN injection decreases the number of cases with the accidental removal of PGs, thereby decreasing the number of autotransplanted PGs. In addition, since the inferior PGs have a more variable location and are easier to be accidentally resected, the blood supply is also easier to be disturbed. In this study, 2 accidentally resected PGs in the CN group and all 9 accidentally resected PGs in the control were the inferior ones, indicating that CN is more useful for surgical technology in protection and dissection of inferior PGs.

In this study, we only found a significant difference between the patients with hypoparathyroidism in the CN group and the control group at day 1, but not at day 7. Furthermore, no significant difference on hypocalcemia was seen between the 2 groups both at days 1 and 7, while the number of the patients with hypocalcemia in CN group is much lower than that in control group. It could be partially due to the limited patient population enrolled in our study. However, Zhu et al[26] reported a study on the effect of CN on central lymph node dissection and found that the number of cases of postoperative hypocalcemia in the CN group was significantly less than that in the control group ($P=0.013$). Similar to other studies,[26,35] our study also found that CN could easily detect lymph nodes, and increased metastatic lymph nodes, and more lymph nodes were detected in the CN group than in the control group. Thus, CN might be used to achieve a more radical central neck dissection.

The use of CN is safe and simple during operation after injection with gentle pressing,[36] and no obvious systemic toxicity was observed in these patients during and after operations. Based on the results from this study, CN may help keep PGs in situ by staining thyroid and lymph nodes into black, but not PGs. CN may also help protect blood vessels of PGs by not staining blood vessels into black, leading to the nonblack-stained tissues around PGs including many tiny blood vessels. Therefore, CN may have a promising potential in clinical use for protection of PGs.

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