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Clinical application of carbon nanoparticles in curative resection for colorectal carcinoma

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Purpose: To explore the potential of carbon nanoparticles (CNs) for the intraoperative detection of positive and negative lymph nodes in the treatment of colorectal cancer.

Patients and methods: The clinical data of 470 patients undergoing surgical procedures for colorectal cancer from June 2010 to February 2013 were analyzed retrospectively. The patients were divided into the CN group (183 males and 161 females; mean age, 58.6±12.4 years), who were given a CN suspension, and the control group (78 males and 48 females; mean age, 59.1±12.2 years), who were not given a CN suspension. The operative time, blood loss, number of lymph nodes detected/positive lymph nodes, and prevalence of postoperative complications were compared between the two groups. Three years after surgery, 444 cases (327 cases in the CN group and 117 cases in the control group) were interviewed, with the remaining 26 cases lost to follow-up. With regard to tumor, node, metastasis staging, the survival and prevalence of recurrence in each group at 3 years were analyzed.

Results: The number of positive lymph nodes was higher and the prevalence of blood loss was lower in the CN group than in the control group ($p<0.05$). There were no significant differences in the operative time, number of lymph nodes detected, or the prevalence of postoperative complications, survival, metastasis, or recurrence between the two groups at 3 years ($p>0.05$).

Conclusion: The application of CNs is convenient for the detection of lymph nodes to reduce blood loss and increase the probability of detecting positive lymph nodes accurately and rapidly.

Keywords: colorectal neoplasms, curative resection, positive lymph nodes, prognosis

Introduction

Surgery is the primary treatment for colorectal cancer.[1,2] Colorectal cancer often metastasizes to lymph nodes, so lymphadenectomy is the key factor influencing the prognosis.[3–7]

Carbon nanoparticles (CNs) are approximately 150 nm in diameter. This diameter is intermediate between those of capillaries and lymphatic vessels. Hence, CNs can enter and stain only the lymphatic vessels but not the capillaries after injection into the intestinal mucosa peripheral to tumor tissues.[8] This feature grants CN specificity toward lymph nodes.

CNs are widely used as tracers in radical gastrectomy, excision of breast tumors, thyroidectomy, and resection of colorectal cancer.[9–13] The safety of CNs has been studied by various researchers, and the suspensions of CNs for injection have been shown to be safe.[14–16]

Besides their function in biopsies, CNs have also been used as a delivery system for cancer treatment.[17] Several clinical studies have shown CNs to be beneficial for lymphadenectomy and reduction in blood loss.[7,12,17–19] Nevertheless, the relation

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between CNs and the prognosis is not clear. Few studies have focused on the prognosis of colorectal tumors.[15,19] In this study, we attempted to ascertain whether CNs, when used as a tracer in colorectal cancer, can influence lymphadenectomy and the prognosis of patients.

Patients and methods

Ethical approval of the study protocol

The study protocol was approved by the ethics committee of Tongji Medical College (Wuhan, China). Written informed consent was obtained from all the participants.

Imaging agents for lymph nodes

A suspension of CNs for injection (H20073246; Lummy, Chongqing, China) consisting of stable suspensions of diameter 150 nm was used.[20] The diameter of these CNs is smaller than the gap between the lymphatic endothelial cells (approximately 120–500 nm) but larger than the gap between the capillary endothelial cells (approximately 20–50 nm). Hence, these CNs can enter the lymphatic circulation before the blood circulation.

Study cohort

A total of 470 patients with colorectal cancer (excluding cases of adenoma, neuroendocrine carcinoma, leiomyosarcoma, and distant metastases of primary cancers of the liver, lung, and other organs) who underwent surgery from June 2010 to February 2013 at Wuhan Union Hospital (Wuhan, China) were enrolled in the study.

They were divided into the CN group (n=344) and control group (n=126). The CN group comprised 183 males and 161 females (mean age, 58.6±12.4 years). The control group comprised 78 males and 48 females (mean age, 59.1±12.2 years).

Depending on the histological type of the tumor, patients were subdivided into four groups: adenocarcinoma, mucinous adenocarcinoma, signet-ring cell carcinoma, and malignant melanoma. There were no significant differences in the tumor, node, and metastasis (TNM) staging of these tumors ($p>0.05$). No patients underwent preoperative chemotherapy or radiotherapy. There was no significant difference in baseline conditions between the CN group and control group ($p>0.05$; Table 1).

Table 1 Baseline characteristics of the study cohort

Characteristics	CN group (n=344)	Control group (n=126)	Statistics	*p*-value
Age (mean, years)	58.6±12.4	59.1±12.2	t=0.045[§]	0.964
Sex				
Male	183	78	χ^2=2.738	0.098
Female	161	48		
Diameter of primary tumor (cm)	3.96±1.73	4.24±1.66	t=0.383[§]	0.702
Differentiation			χ^2=0.440	0.803
Good	15	6		
Moderate	235	82		
Poor	94	38		
Histology			χ^2=0.985	0.321[#]
Adenocarcinoma	256	88		
Mucinous adenocarcinoma	80	33		
Signet-ring cell cancer	5	3		
Melanoma	3	2		
TNM staging			χ^2=2.433	0.486
I	34	14		
II	135	50		
III	159	52		
IV	16	10		

Notes: [#]The *p*-value was calculated based on adenocarcinoma and the total prevalence of mucinous adenocarcinoma, signet-ring cell carcinoma, and melanoma. Data are mean ± SD for continuous variables and numbers for categorical variables. [§]Student's *t*-test was used.

Abbreviations: CN, carbon nanoparticle; TNM, tumor, node, metastasis.

Surgical procedure

All patients underwent general anesthesia with intubation. In the CN group, after the abdominal cavity had been opened and the focus exposed clearly, the CN suspension was injected into the submucosa of the normal intestinal wall <1 cm from the tumor center. Four to six points were chosen for injection, and 0.2 mL of the suspension was used for each point. The standard procedures of radical resection of colorectal cancer were undertaken without tumor remnants, and regional lymph nodes were removed simultaneously.[20] The control group underwent an identical procedure to that of the CN group excluding the injection of the CN suspension.

Postoperative evaluation and follow-up

The extent of intraoperative blood loss, operative time, and prevalence of postoperative complications were compared between the CN group and control group. According to the postoperative pathology results, the number of lymph nodes detected and prevalence of detection of positive lymph nodes in the two groups were compared.

A total of 444 cases were tracked for 3 years after surgery (327 cases in the CN group and 117 cases in the control group), but 26 cases were lost to follow-up. According to the TNM staging, there were 31 cases of stage I cancer in the CN group and 13 in the control group. There were 132 cases and 49 cases of stage II cancer in the CN group and control group, respectively. There were 153 cases of stage III cancer

in the CN group and 50 in the control group. There were 11 cases of stage IV cancer in the CN group and 5 in the control group. The 3-year survival and the prevalence of metastasis and recurrence within 3 years after surgery were compared according to TNM staging.

Statistical analyses

Data were analyzed using SPSS v19.0 (IBM Corporation, Armonk, NY, USA). The chi-square test was used for the qualitative and quantitative data. A value of $p<0.05$ was considered to be significant.

Results

Comparison of intraoperative factors

No significant differences in the operative time or number of lymph nodes were detected between the two groups ($p>0.05$). There was a significantly lower prevalence of hemorrhage in the CN group compared to the control group ($p=0.02$; Table 2). A total of 574 and 166 positive lymph nodes were detected in the CN group and control group, respectively. Meanwhile, a total of 2,569 and 954 negative lymph nodes were detected in the CN group and control group, respectively. The number of positive lymph nodes in the CN group was significantly higher than that in the control group ($\chi^2=6.817$, $p=0.009$; Table 3).

Postoperative complications

In the CN group (n=344), two cases had an abdominal mass and one case had anastomotic bleeding; these complications did not affect any of the patients in the control group (n=126). There were no significant differences in the prevalence of postoperative complications and all complications ($p>0.05$) between the two groups (Table 4).

Survival

The 3-year survival of patients with stage I disease (no metastasis or recurrence within 3 years) was 100% in the two groups. There were no significant differences in the prevalence of stage II or III disease in the two groups ($p>0.05$; Tables 5 and 6).

There were 11 cases of stage IV disease in the CN group, and seven of these cases survived for at least 3 years. There were five cases of stage IV disease in the control group, and four of these cases survived for at least 3 years. There was no significant difference in the 3-year survival among patients with stage IV disease between the two groups ($p>0.05$).

Table 2 Intraoperative characteristics of the study cohort

Characteristics	CN group (n=344)	Control group (n=126)	$t^{\S}$	*p*-value
Operative time (min)	115.71±1.01	123.33±41.65	1.76	0.81
Blood loss (mL)*	50.68±28.09	63.67±49.40	2.29	0.02
Number of lymph nodes detected	9.13±6.30	8.89±6.11	0.39	0.70

Notes: *$p<0.05$. §Student's *t*-test was used.
Abbreviation: CN, carbon nanoparticle.

Table 3 Number of positive and negative lymph nodes

Group	Positive	Negative	Total
Control	166	954	1,120
CN	574	2,569	3,143
Total	740	3,523	4,263

Note: $\chi^2=6.817$, $p=0.009$.
Abbreviation: CN, carbon nanoparticle.

Discussion

Morl[21] reported on the use of biological dyes as tracers for lymph nodes in the treatment of breast cancer in the 1950s. Since then, there has been considerable progress in the use of such "lymphatic tracers" but, in general, these tracers exhibit light staining, poor diffusion, and nonuniform particles, which can lead to misjudgments by surgeons.[15,22] Moreover, the injection time of pigments or lymphatic tracing methods is not easy to control.

In the current study, the baseline information for the CN group and control group was comparable. The surgical data revealed that CN use did not greatly increase the number of lymph nodes needed to be dissected or shorten the operative time. The mean operative time for the CN group was reduced compared to that of the control group, but this had very little impact on the overall operative time. This observation is contrary to the belief held by some researchers that CN use can reduce the operative time considerably.[7]

Table 4 Postoperative complications

Parameter	CN group, n (%)	Control group, n (%)
Postoperative fever of unknown origin	6 (1.74)	3 (2.38)
Wound infection/suppuration	10 (2.91)	2 (1.59)
Anal/stoma infection	8 (2.33)	2 (1.59)
Flatulence/stomach ache	16 (4.65)	10 (7.94)
Reduction/disappearance of flatulence	6 (1.74)	3 (2.38)
Abnormal abdominal mass	2 (0.58)	0 (0)
Anastomotic stoma fistula	13 (3.78)	2 (1.59)
Anastomotic bleeding	1 (0.29)	0 (0)
Gastric retention	7 (2.03)	2 (1.59)
Total	69 (20.06)	24 (19.05)

Abbreviation: CN, carbon nanoparticle.

Table 5 Three-year follow-up for stage II disease

Items	Recurrence		Metastasis		Death	
	CN group (n=132)	Control group (n=49)	CN group (n=132)	Control group (n=49)	CN group (n=132)	Control group (n=49)
Yes	1	3	11	3	34	8
No	131	46	121	46	98	41
χ^2	2.600		0.033		1.784	
p	0.107		0.856		0.182	

Abbreviation: CN, carbon nanoparticle.

Complete staining of intermediate and central lymph nodes can occur 15–20 min after CN injection. If a surgical procedure is conducted immediately after CN injection, distal lymph nodes may not be stained and could therefore be neglected. Our research team has found that staining may be poor for distal/deep lymph nodes or due to an insufficient amount of the tracer. However, additional injections of CNs can be administered locally for poorly stained small lymph nodes. This strategy may permit the visualization of small lymph nodes. Too fast injections should be avoided to reduce tracer leakage and staining of surrounding tissues, which will interfere with identification of the lymph nodes. Hence, CN use can benefit the identification of small lymph nodes, and help reduce the risk of missing such small lymph nodes.

The number of lymph nodes detected by CNs in patients did not change significantly compared with that using a traditional detection method. However, the number of positive lymph nodes detected by CN treatment was markedly higher than that detected using the traditional method. This observation suggests that CN treatment could enhance the sensitivity of detection of lymph node metastasis. The reason for this discrepancy is not clear but could be due to the retention effects of CNs.

In curative resection for colorectal carcinoma, lymphadenectomy usually involves the removal of paracolic lymph nodes as well as the intermediate and central lymph nodes located in the distribution area of the branches. The length of the intestinal canal to be resected and extent of resection of the mesentery are determined by the surgeons, so the resection margin of the mesentery can differ between surgeons. CNs used as tracers can be used to visualize local lymph nodes, thereby providing guidance for the determination of the resection margin in the mesentery. However, the extent of resection remains a challenge. Excessive lymphadenectomy will lower surgical safety and increase the risk of postoperative complications, whereas incomplete lymphadenectomy will lower the chance of curative treatment. We recommend thorough intraoperative examination, especially of the central lymph nodes and lymph nodes at the roots of the vessels. Decisions can be made based on frozen pathology sections. Good communication is needed with the pathologist.

Table 6 Three-year follow-up for stage III disease

Items	Recurrence		Metastasis		Death	
	CN group (n=153)	Control group (n=50)	CN group (n=153)	Control group (n=50)	CN group (n=153)	Control group (n=50)
Yes	4	3	11	2	24	4
No	149	47	142	48	129	46
χ^2	0.480		0.218		1.872	
p	0.489		0.640		0.171	

Abbreviation: CN, carbon nanoparticle.

The current study suggests that CN use can aid in the detection of positive lymph nodes and contribute to the accuracy of lymphadenectomy. There was considerable reduction in intraoperative blood loss in the CN group that could be attributed to precise determination of the extent of lymphadenectomy using CNs.

Conclusion

The use of CNs did not improve the prognosis significantly after curative resection of colorectal carcinoma, reduce the prevalence of complications, or improve the 3-year survival or metastasis. The prognosis of patients with colorectal carcinoma after curative resection is still determined by the TNM staging. This may be due to hematogenous metastasis that occurs at an early stage of colorectal carcinoma. Lymph node staining with CNs can permit the precise determination of the extent of lymphadenectomy needed, but the benefits in terms of reducing the prevalence of postoperative complications or prolonging survival are limited. While recognizing the importance of CNs in the surgical treatment of cancer, we should make efforts to develop more effective techniques in the resection of colorectal carcinoma.

Disclosure

The authors report no conflicts of interest in this work.

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