cecum carcinoma in the literature. The patient reported underwent surgical resection following chemotherapy and is surviving recurrence free. Generally, lymph node recurrence after colorectal cancer surgery is regarded as systemic disease, and in such cases, chemotherapy, radiotherapy or a combination of both, rather than surgery, is selected. With regard to isolated lymph node recurrence such as this case, there are some reports of resection, but the significance of surgical treatment remains unclear (9–13). Of the previous cases, one patient survived 19 months disease free, one patient survived 36 months although the patient developed hepatic metastasis and was successfully resected, and the other patient died after 18 months as a result of peritoneal dissemination without lymph node recurrence. (9, 11, 12). If there is no finding of recurrence in other regions and surgery is not difficult to perform, then it may be necessary to consider surgery.

An interesting aspect about this particular case is the lymphatic pathway the cecum carcinoma followed to metastasize to the lymph node in the right external iliac region. Most lymphatic pathways run along arteries and it is generally considered that the lymphatic system from cecum carcinoma usually extends to the root of the superior mesenteric artery along the ileocolic artery (14). Lymphatic pathways running to the right external iliac region have not been reported to date. In this case, although obvious tumor invasion into the abdominal wall was not detected histopathologically in the primary lesion, tumor invasion into the abdominal wall was suspected macroscopically at the time of the first operation. One possibility is that the tumor invaded part of the abdominal wall microscopically and then metastasized to the lymph node in the region of the right external iliac artery through a lymphatic pathway along the right inferior epigastric artery.

Isolated lymph node recurrence rarely occurs in colorectal cancer and there is no agreement regarding surgical indication for this condition. However, in surgical treatment for liver and pulmonary metastases, the minimum requirement is local control (1–8). In our case, favorable local control was achieved by initial surgery and, therefore, surgical resection was indicated for recurrent lesion, because of the possibility of achieving long-term prognosis. With regard to *en bloc* resection of blood vessels, it goes without saying that there is a fear of increased risk of complications. However, from the oncological viewpoint, even if the tumor does not invade blood vessels through the capsule of the lymph node, the risk of tumor cell spillage is increased if the dissection maneuver cuts into the lymph node capsule, even to a slight degree. It should of course be avoided. In patients with lateral pelvic lymph node metastasis from rectal carcinoma at our institution, we have reported the favorable effect of lateral lymph node dissection with *en bloc* resection of the internal iliac vessels on local control (15). However, *en bloc* resection of the external iliac vessels requires revascularization and if the range of resection is wide, artificial vessels become necessary. For lymph node recurrence near blood vessels, *en bloc* resection of the vessels may be preferable from the viewpoint of local control, but should be considered only if it can be justified after considering the risks associated with surgery.

CONCLUSION

We encountered a case of right external iliac lymph node recurrence after radical resection for cecum carcinoma, successfully treated by surgical resection. For isolated lymph node recurrence of colorectal carcinoma, surgical resection should be considered, if favorable local control has been achieved. However, further cases need to be accumulated with regard to treatment outcome.

Conflict of interest statement
None declared.

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Diseases of the Colon & Rectum

Minute Depressed-Type Submucosal Invasive Cancer–5 mm in Diameter with Intermediate Lymph-Node Metastasis: Report of a Case

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We report a rare case of colon cancer in which a depressed-type tumor only 5 mm in diameter invaded the submucosal layer and produced intermediate lymph node metastasis. A 47-year-old male received a total colonoscopy for a depressed-type lesion with marginal elevation in the sigmoid colon. The lesion measured 5 mm in diameter. On chromoendoscopic examination, the depression was clearly demarcated and an irregular pit pattern was identified in the demarcated area by magnification suggesting invasion of the submucosal layer requiring surgery. Laparoscopic-assisted sigmoidectomy was performed and the resected specimen demonstrated well-differentiated adenocarcinoma. The depth of invasion was only 900 μm. There was no lymphovascular invasion although not only paracolic, but also intermediate lymph node metastasis was detected. There have been some reports about small depressed-type colorectal cancer invading the submucosal layer; however, intermediate LN metastasis is very rare in submucosal colorectal cancer. In this case, there were two noteworthy points: 1) despite the small size, submucosal invasion could be estimated preoperatively, therefore, a successful lymph node dissection was performed by laparoscopic surgery; and 2) although this depressed-type cancer invaded the submucosal layer only 900 μm and there was no lymphovascular invasion, intermediate lymph node metastasis was detected. [Key words: Early colorectal cancer; Chromoendoscopy; Magnifying colonoscopy]

Colorectal polypoid-type adenoma is considered the precursor in the majority of colorectal cancer (CRC) cases. The early detection and treatment of these lesions is thought to reduce CRC mortality. Morson[1] estimated that up to two-thirds of CRC develop from adenomatous polyps. Recently, improved endoscopic imaging and advancements in diagnostic technology have led to a higher rate of detection of superficial and small colorectal tumors,[2,3] It has been reported that lesions <10 mm in diameter, whether polypoid or nonpolypoid, were unlikely to be advanced cancer[4]; however, Japanese researchers have reported the existence of advanced cancer lesions <10 mm in diameter.[5,6] Several reports, mostly from Japan, have suggested that some CRC also can develop *de novo* from normal mucosa.[7–9] An alternative explanation is that some carcinomas have an especially aggressive growth pattern that quickly destroys the adjacent adenomatous tissue.

With regard to the pathology, the method of measuring the depth of submucosal (sm) invasion remains controversial. A relative classification system

Reprints are not available.

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Figure 1. Conventional colonoscopic views showing a reddish lesion approximately 5 mm in diameter located in the sigmoid colon.

has been used to evaluate sm depth of invasion as follows: sm1, infiltration into the upper third of the submucosal layer; sm2, middle third; and sm3, lower third. Several studies have demonstrated the usefulness of this method for predicting lymph node metastasis[10]; however, this method has not been useful for specimens obtained endoscopically because these specimens do not include the muscularis propria. An absolute classification system, therefore, has become generally accepted to evaluate endoscopic resected specimens. This method classifies the vertical depth of sm invasion from the lowest edge of the muscularis mucosae as follows: sm superficially,

Figure 2. Slightly reddish and depressed lesion detected with marginal elevation (IIa + IIc).

Figure 3. Chromoendoscopy after indigo carmine dye spraying more clearly showed the demarcated depressed area with the center of the depressed area slightly elevated.

<1 mm; and sm deep, ≥1 mm. A standardized method of measuring sm depth has not been established yet. We report a case of small Dukes C colon cancer in which a 5-mm depressed-type tumor was diagnosed endoscopically.

REPORT OF A CASE

A 47-year-old male was referred to our institution for further treatment of a colonic lesion in September 2004. Neither the patient nor the patient's family had a past medical history of cancer. The patient had consulted the previous hospital because of a positive fecal-occult-blood-test. At that time, a total colonoscopy identified a small, depressed lesion in the sigmoid colon. Conventional colonoscopic examination showed a slightly reddish depressed-type lesion with a marginal elevation (IIa+IIc) in the sigmoid colon (Figs. 1 and 2). This lesion measured only 5 mm in diameter and there were no other lesions in the colorectum. After 0.2 percent indigo carmine dye spraying, chromoendoscopic examination showed a clearly demarcated depression (Fig. 3) and crystal violet staining with magnified view (Olympus CF Q 240ZI; Olympus, Tokyo, Japan) identified an irregular pit pattern in the demarcated area corresponding to an invasive pattern (Fig. 4).[11,12] This tumor, therefore, was diagnosed as having invaded the sm layer, resulting in a contraindication for endoscopic mucosal resection (EMR). A biopsy sample demonstrated well-differentiated adenocarcinoma, and there was

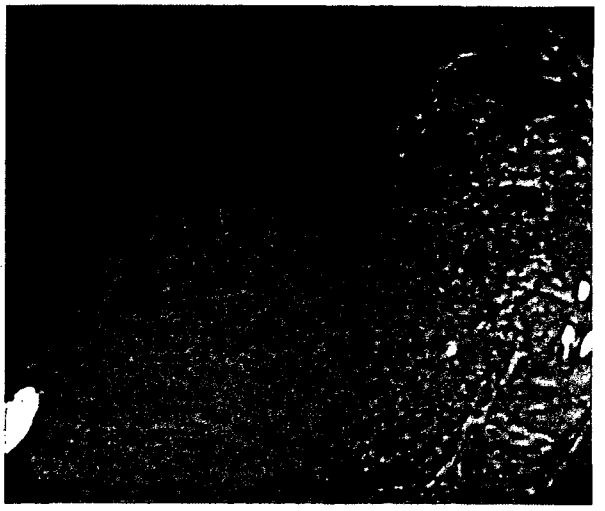

Figure 4. Magnified view with crystal violet staining of the surface of the central depression with an irregular pit pattern identified in the demarcated area.

Figure 6. High-power magnification showing the surface glands of the depressed area were destroyed was consistent with the magnifying colonoscopy findings.

no evidence of metastasis found on computed tomography. A laparoscopic-assisted sigmoidectomy was performed without complication and the resected specimen also demonstrated well-differentiated adenocarcinoma. The depth of invasion was 900 μm and there was no lymphovascular invasion found (Figs. 5 and 6). A deeper cut was performed to evaluate for lymphatic invasion, but there were no findings suggesting such invasion. As for lymph-node metastasis, 14 lymph nodes were examined: two were positive and one of these was intermediate LN (lymph node) metastasis (Fig. 7).

DISCUSSION

There have been some reports about small, depressed-type CRC invading the sm layer; however, intermediate LN metastasis was very rare.[13] In this case, there were two noteworthy points as follows: 1) despite the small size of the lesion, endoscopic findings, including magnifying chromoendoscopy were able to diagnose sm invasion before treatment; and 2) although this depressed-type cancer invaded the sm layer only 900 μm and there was no lymphovascular invasion, intermediate LN metastasis

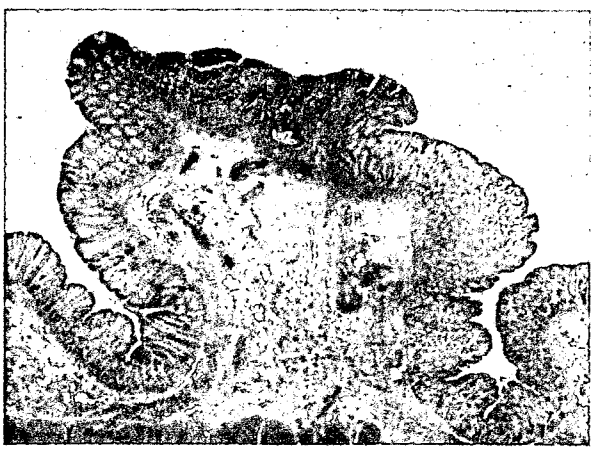

Figure 5. Histologic views showing a central depressed area with a well-differentiated adenocarcinoma invading the submucosa (900 μm). Although there was no lymphovascular invasion, the muscularis mucosa was completely destroyed.

Figure 7. High-power magnification of the cut section of the intermediate lymph node showed focal well-differentiated adenocarcinoma suggesting metastasis.

was detected. After the preoperative diagnosis, successful LN dissection was performed by laparoscopic surgery and this lesion was definitively diagnosed as Dukes C colon cancer.

The adenoma-carcinoma sequence is thought to be the main pathway of CRC carcinogenesis in which carcinoma develops from adenoma.[1] Various oncogenes and tumor suppressor genes, including the APC, K-*ras*, p53, and DCC genes, have been reported to be involved in the carcinogenesis of CRC.[14] In addition, the existence of some depressed-type CRC has been reported, particularly from Japan, raising the possibility that some cancers may develop *de novo* following a different pathway from the adenoma-carcinoma sequence. In fact, a lower frequency of K-*ras* gene mutations is more likely to be found in these lesions, showing a much higher rate of sm invasion despite their small size in contrast to protruding-type lesions.[15,16] Endoscopists, therefore, should pay particular attention to depressed lesions given their higher malignancy potential.

Recently, endoscopic resection has become a generally accepted procedure for superficial or small CRC where the probability of lymph-node metastasis is low and depth of sm invasion is considered an important predictive factor for lymph-node metastasis. According to the Paris workshop guidelines, superficial-type CRC with a depth of invasion <1,000 μm has a very low risk of lymph-node metastasis.[17] The incidence of lymph node metastasis is reported to be approximately 10 percent for CRC with sm invasion, 2 to 3 percent for CRC superficially invading the sm, and 8 to 12 percent for CRC deeply invading the sm.[18] In a recent collaborative Japanese study of nonpedunculated sm invasive CRC, the rate of lymph-node metastasis was 0 percent when the sm depth of invasion was <1,000 μm.[19] In that analysis, 1) an undifferentiated-type tumor, 2) existence of lymphatic or venous infiltration, and 3) a depth of invasion ≥ 1,000 μm from the muscularis mucosae (mm) were independent risk factors for LN metastasis based on multivariate analysis, whereas univariate analysis identified the destruction of the mm as an additional risk factor. Another study conducted at our institution recently[20] used multivariate analysis to show that lymphatic invasion and high-grade focal dedifferentiation at the submucosal invasive front were independent factors predicting lymph-node metastasis.

In this case, only destruction of the mm met these criteria and there were no other risk factors for LN metastasis. The depth of invasion was only 900 μm, and there was no lymphovascular invasion or poorly differentiated component. According to our institution's recent study,[20] univariate analysis showed that the status of the remaining muscularis mucosa had a significant connection with lymph-node metastasis. Physicians should be careful when encountering this type of lesion, because the complete destruction of the mm may be one of the risk factors for LN metastasis. After the preoperative diagnosis, a lymphadectomy was successfully performed by laparoscopic surgery, and there has been no apparent recurrence detected 12 months after surgery.

CONCLUSIONS

We report a rare case of CRC invading the sm layer and showing metastasis to not only the paracolic but also intermediate LN despite the small size of the lesion. For a depressed-type cancer, it is necessary to carefully examine the lesion to establish an accurate diagnosis and perform suitable treatment.

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Impact of Upward Lymph Node Dissection on Survival Rates in Advanced Lower Rectal Carcinoma

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Key Words
Lymph node dissection · Upward lymph node dissection · Lower rectal carcinoma

Abstract
Background/Aims: This study investigated appropriate level of upward lymph node (LN) dissection in advanced lower rectal carcinoma. ***Methods:*** A total of 285 consecutive patients with stage II/III lower rectal carcinoma were analyzed. LN dissection was classified as follows: division of the root of the superior rectal artery (UD2), division of the root of the inferior mesenteric artery (UD3) and UD3 with para-aortic LN dissection (UD4). ***Results:*** LN metastases at the root of the inferior mesenteric artery were found in 4 patients. Their prognoses were worse than those of the other stage III patients ($p = 0.011$). On the other hand, LN metastases along the superior rectal artery were discovered in 14 patients, whose 5-year overall survival rate was 61.2%. By removing the LNs either UD2 or UD3/4, a similar survival rate was achieved in stage III patients with LN metastases along the superior rectal artery. ***Conclusion:*** Survival of a minority with metastatic LNs at the root of the inferior mesenteric artery was poor. Additionally, survival is no worse in patients with positive LN along the superior rectal artery as long as these positive nodes are resected by either UD2 or UD3/4. Low ligation is adequate for advanced lower rectal carcinoma.

Introduction

It is well known that lower rectal carcinoma has two routes of lymphatic spread, i.e. upward and lateral spread. There have been many reports that discuss the significance of lateral pelvic lymph node dissection for advanced lower rectal carcinoma [1–4]. However, there have not been any definitive conclusions and various opinions have been expressed around the world. On the other hand, the impact of upward lymph node dissection for sigmoid colon or upper rectal carcinoma has been discussed in several reports [5–7], and yet few studies have focused on this issue in advanced lower rectal carcinoma. Although Pezim et al. [8] reported that high ligation of the inferior mesenteric artery had no survival advantage for rectal carcinoma patients, no counterarguments have been published and it remains difficult to generalize about the impact of upward lymph node dissection. The appropriate extent of upward lymph node dissection for advanced lower rectal carcinoma remains an unsolved issue and guidelines need to be established.

This study presents a detailed estimation of how the level of upward lymph node dissection affects survival rates following curative resection in advanced lower rectal carcinoma.

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Patients and Methods

Between 1990 and 2002, a series of 303 consecutive patients at the National Cancer Center Hospital, Tokyo, underwent curative surgery for stage II or III lower rectal carcinoma. Lower rectal carcinoma was defined as a tumor with a distal margin 7 cm or less from the dentate line by digital examination and/or proctoscopy. Five patients with a history of malignancy (sigmoid colon carcinoma in 3 and bladder carcinoma in 2), who previously underwent lymph node dissection along the inferior mesenteric artery or in the lateral pelvis, were excluded, because the routes of lymphatic spread seemed to be changed in these cases. Two patients with synchronous advanced rectosigmoid carcinoma were excluded. Three stage II patients and 8 stage III patients did not undergo lymph node dissection along the inferior mesenteric artery but only in the mesorectum (UD1), because of preoperative underestimation. These 11 patients were also excluded. Consequently, 285 patients were eligible for this study. The mean (SD) distance from the dentate line of the tumor was 2.4 (1.0) (range 0.0–7.0) cm. No patients received preoperative radiotherapy and/or chemotherapy. All patients were evaluated before surgery by total colonoscopy, barium enema and computed tomography. To evaluate comorbid conditions, cardiopulmonary function and renal function tests were performed. In our study, lateral pelvic lymph nodes were regarded as regional lymph nodes according to the Japanese classification of colorectal carcinoma [9], although lateral pelvic lymph node metastases are regarded as distant metastases in the TNM classification system [10]. Clinical stage II or III middle or lower rectal carcinoma, located at or below the peritoneal reflection, is an indication for lateral pelvic lymph node dissection in our hospital [2, 3]. Postoperative adjuvant chemotherapy using oral or intravenous fluoropyrimidines was administered for 6 months to 27 stage III patients. Two stage III patients received postoperative radiotherapy and another underwent concomitant chemoradiotherapy.

The incidence of upward lymph node metastases based on histopathological data from the resected specimen, recurrence sites and survival rate were retrospectively analyzed and the appropriate extent of upward lymph node dissection for advanced lower rectal carcinoma was evaluated.

Classification of the Level of Upward Lymph Node Dissection

Standard surgical procedures at our institution were previously reported in detail [11, 12]. The extent of upward lymph node dissection was classified as follows: UD1 is defined as resection of the mesorectum, UD2 as division of the root of the superior rectal artery with lymph node dissection below that level, UD3 as division of the root of the inferior mesenteric artery with lymph node dissection below that level and UD4 as UD3 with the addition of para-aortic lymph node dissection (fig. 1) [12]. The level of upward lymph node dissection was determined by preoperative and intraoperative findings. When a patient was diagnosed as stage I, UD1 to UD2 lymph node dissection was performed. UD2 to UD4 lymph node dissection was performed for patients with stage II or III tumor. UD4 was performed until the first half of the 1990s, but has not been performed thereafter because of excessive operative time, blood loss and a high incidence of postoperative sexual dysfunction, especially in males [11, 13, 14].

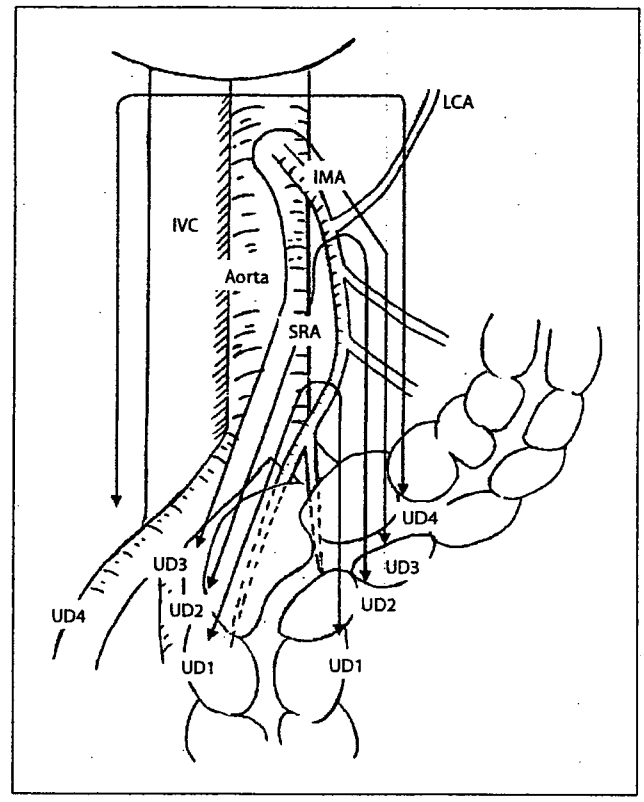

Fig. 1. Classification of the level of upward lymph node dissection. UD1 is defined as resection of the mesorectum; UD2 as division of the root of the superior rectal artery (SRA) and lymph node dissection below this level; UD3 as division of the root of the inferior mesenteric artery (IMA) and lymph node dissection below this level; and UD4 as UD3 with para-aortic lymph node dissection. IVC = Inferior vena cava; LCA = left colic artery.

Statistical Analysis

Survival curves were traced using the Kaplan-Meier method. The differences between curves were tested using the log-rank test. Comparisons between groups were performed using χ^2 test. $p < 0.05$ was considered significant. All statistical calculations were made using SPSS computer software (SPSS 11.0, SPSS Inc., Chicago, Ill., USA).

Results

The characteristics of 285 patients according to the UD classification are shown in table 1. There were 78 (27.4%), 133 (46.7%) and 74 (26.0%) patients who underwent UD2, UD3 and UD4, respectively. All patients were followed up until death or for at least 3 years with a mean follow-up period of 66 months. The rate of sphincter-pre-

Table 1. Patient characteristics according to the UD classification

	Total (n = 285)	UD2 (n = 78)	UD3 (n = 133)	UD4 (n = 74)
Age, years (mean)	58.2	58.1	58.2	58.4
Sex ratio (male:female)	191:94	53:25	90:43	48:26
Follow-up period (mean)	66	59	57	88[a, c]
Surgical procedure				
Sphincter-preserving surgery	143 (50.2)	53 (67.9)	64 (48.1)	26 (35.1)[a, b]
Non-sphincter-preserving surgery	142 (49.8)	25 (23.1)	69 (51.9)	48 (64.9)
Lateral LNs dissection				
No	68 (23.9)	32 (41.0)	31 (23.3)	5 (6.8)[d]
Yes	217 (76.1)	46 (59.0)	102 (76.7)	69 (93.2)
Evaluated LN, n (mean)	42	31	39	57[d]
Metastatic LN, n (mean)	3	2	3	3
TNM classification				
Stage II	94 (33.0)	29 (37.2)	38 (28.6)	27 (36.5)
Stage III	191 (67.0)	49 (62.8)	95 (71.4)	47 (63.5)

Values in parentheses are percentages.
[a] $p < 0.05$ UD2 vs. UD3, [b] $p < 0.05$ UD2 vs. UD4, [c] $p < 0.05$ UD3 vs. UD4, [d] $p < 0.05$ between each UD classification.

serving surgery was higher in UD2 patients than in those who underwent UD3 or UD4. The rate of undergoing lateral lymph node dissection and the number of evaluated lymph nodes increased significantly with the extension of upward lymph node dissection. However, there were no significant differences in the number of metastatic lymph nodes and the ratio of stage II to III among UD classifications.

In each TNM stage, the overall survival curves in relation to the extent of upward lymph node dissection were evaluated and there were no significant differences according to the extent of upward lymph node dissection (fig. 2). Recurrence sites after curative resection are demonstrated in table 2. In both groups with or without lymph node dissection at the root of the inferior mesenteric artery, the lung was the most common site of recurrence followed by the liver. Recurrence sites did not significantly differ between the groups, including para-aortic or mediastinal lymph node metastases.

Table 3 summarizes the characteristics and outcomes of 4 patients with lymph node metastases at the root of the inferior mesenteric artery. They accounted for 1.9% of the 207 patients who underwent UD3 or UD4. Recurrences developed in all cases and their prognoses were significantly worse than those of the other stage III patients who underwent UD3 or UD4 ($p = 0.011$) (fig. 3). None of 4 patients survived for 5 years.

Table 2. Recurrent sites after curative resection

Recurrent site	UD2 (n = 78)	UD3/UD4 (n = 207)	p value
Lung	16 (20.5)	36 (17.4)	0.543
Liver	6 (7.7)	19 (9.2)	0.692
Pelvic cavity	7 (9.0)	15 (7.2)	0.626
Para-aortic or mediastinal LNs	3 (3.8)	4 (1.9)	0.352

Values in parentheses are percentages.

On the other hand, lymph node metastases along the superior rectal artery were discovered in 14 patients, excluding 3 patients with metastatic lymph nodes at the root of the inferior mesenteric artery, and table 4 shows their characteristics. They accounted for 4.9% of all patients. Ten patients developed recurrence and the lung was the most common site (6 patients), followed by the liver (2 patients). The 5-year overall survival rate was 61.2% in this group and there were no significant differences in overall survival among the patients with and without lymph node metastases along the superior rectal artery ($p = 0.338$) (fig. 4a). In addition, there were no significant differences in survival of the patients with lymph node metastases along the superior rectal artery according to the extension of upward lymph node dissection performed (UD2 or UD3/4) ($p = 0.642$) (fig. 4b).

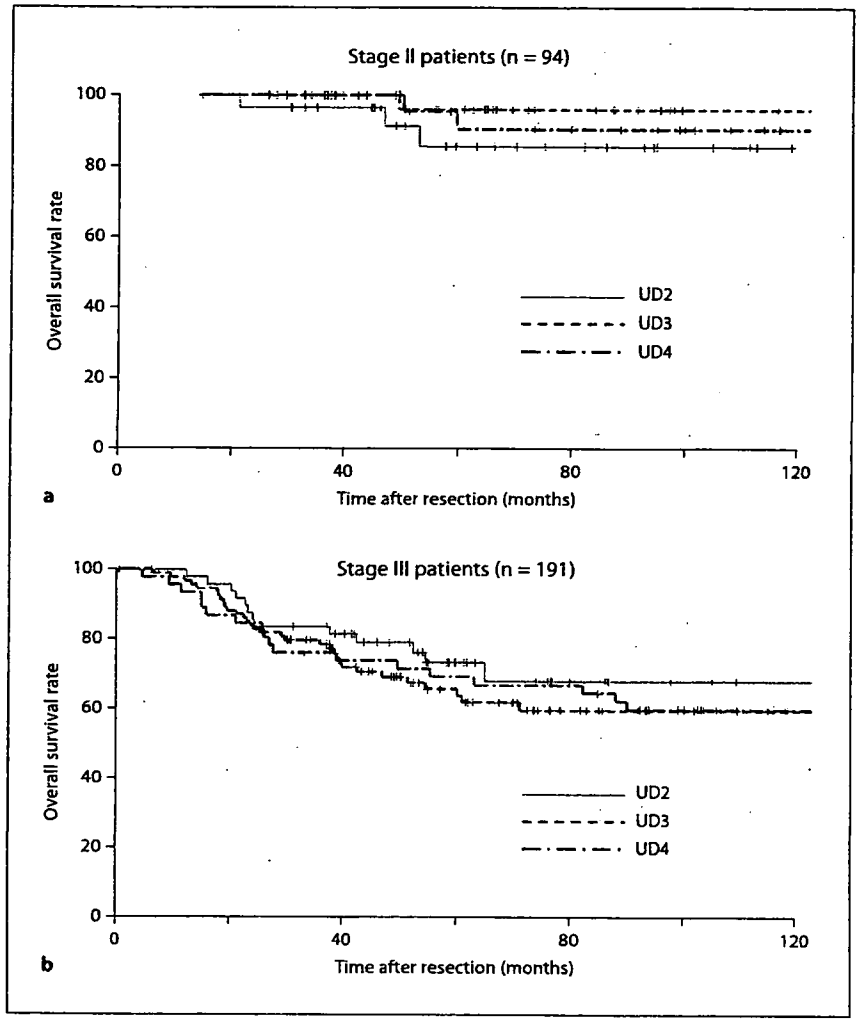

Fig. 2. Overall survival curves in relation to the extent of upward lymph node dissection at each stage: (**a**) stage II and (**b**) stage III. There were no significant differences in each stage.

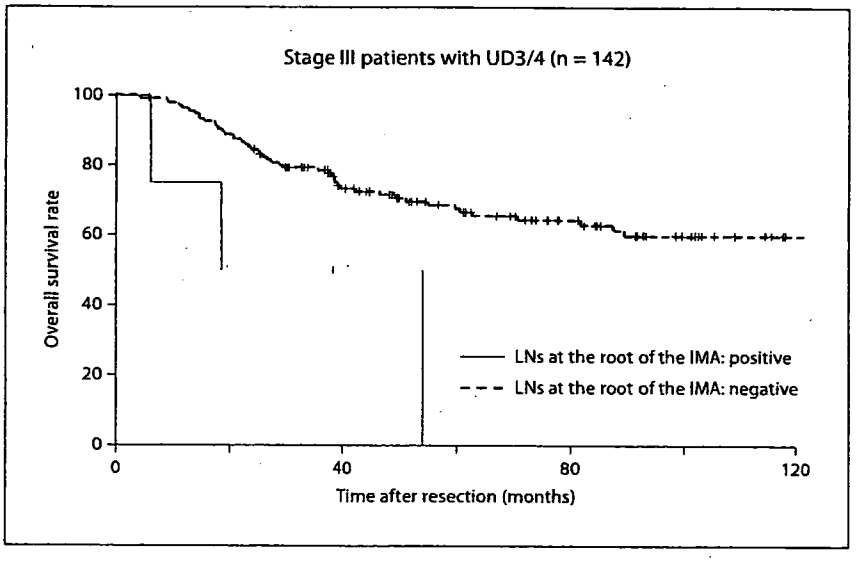

Fig. 3. Overall survival curves for the stage III patients with or without metastatic lymph nodes at the root of the inferior mesenteric artery (IMA). The former was significantly worse than the latter ($p = 0.011$).

Table 3. Characteristics of the patients with metastatic LNs at the root of the inferior mesenteric artery

Age	Sex	UD	Histology	pT	Metastatic LNs, n	Recurrent site	Disease-free time, months	Outcome months
33	F	3	well-differentiated adenocarcinoma	pT3	3	lung, bone	25	died (54)
64	F	3	moderately differentiated adeno-carcinoma	pT3	4	lung	22	alive with recurrent tumor (39)
51	M	3	poorly differentiated adenocarcinoma	pT3	25	pelvic cavity	11	died (19)
57	M	3	poorly differentiated adenocarcinoma	pT3	16	pelvic cavity, peritonium	4	died (6)

Discussion

Surgical decisions regarding upward lymph node dissection for advanced lower rectal carcinoma remain controversial. In our study, patients with metastatic lymph nodes at the root of the inferior mesenteric artery comprised a small minority (4 patients, 1.9%) and their prognoses were very poor. Their prognoses seemed to be almost equal to those of patients who underwent UD4 dissection and were pathologically proven to have metastatic para-aortic lymph node, although such patients are classified as stage IV in TNM classification and were excluded from this study. Furthermore, we could not demonstrate an effect of prophylactic lymph node dissection at the root of the inferior mesenteric artery in patients with any stage of disease. Moreover, lymph node dissection without the root of the inferior mesenteric artery did not result in increased para-aortic or mediastinal lymph node metastases, which we had thought might be caused by failing to perform lymph node dissection. We conclude that lymph node dissection at the root of the inferior mesenteric artery does not provide any survival advantage for patients with advanced lower rectal carcinoma and metastatic lymph nodes at this level have systematic disease.

Likewise, there were also a small number of patients with metastatic lymph nodes along the superior rectal artery (14 patients, 4.9%) and the positive rate was far below the rate of lateral lymph nodes (55 of 217 patients who underwent lateral lymph node dissection, 25.3%) in this series. However, the 5-year overall survival rate in this group was 61.2% and there were no significant differences among stage III patients with and without lymph node metastases along the superior rectal artery. In addition, survival is no worse in patients with positive lymph node along the superior rectal artery as long as these positive nodes are resected by either UD2 or UD3/4. We conclude that UD2 lymph node dissection is adequate even for stage III patients with lymph node metastases along the superior rectal artery.

Table 4. Characteristics of the patients with metastatic LNs along the SRA (exception for three with metastatic LNs at the root of the IMA)

Patients		14
Age, years (mean)		58.8
Sex ratio (male:female)		12:2
Upward LNs dissection	UD2	4
	UD3	6
	UD4	4
Lateral LNs dissection	no	5
	unilateral pelvic	2
	bilateral pelvic	7
pT category in TNM classification	pT1	2
	pT2	2
	pT3	7
	pT4	3
pN category in TNM classification	pN1	7
	pN2	7
Recurrence	yes	10
	no	4

SRA = Superior rectal artery; IMA = inferior mesenteric artery.

There are some problems with the existing classifications of rectal carcinoma. TNM classification considers lymph nodes at the root of the inferior mesenteric artery as regional lymph nodes for colorectal carcinoma without regard to the location of the tumors, as well as lymph nodes along the superior rectal artery [10]. Under this classification, patients with metastatic regional lymph nodes are regarded as stage III and are subcategorized into three groups by the depth of tumor invasion and number of metastatic lymph nodes, not by the location of metastatic lymph nodes. The problem with this classification is that we cannot distinguish whether stage III patients have lymph node metastases at the root of the inferior mesenteric artery.

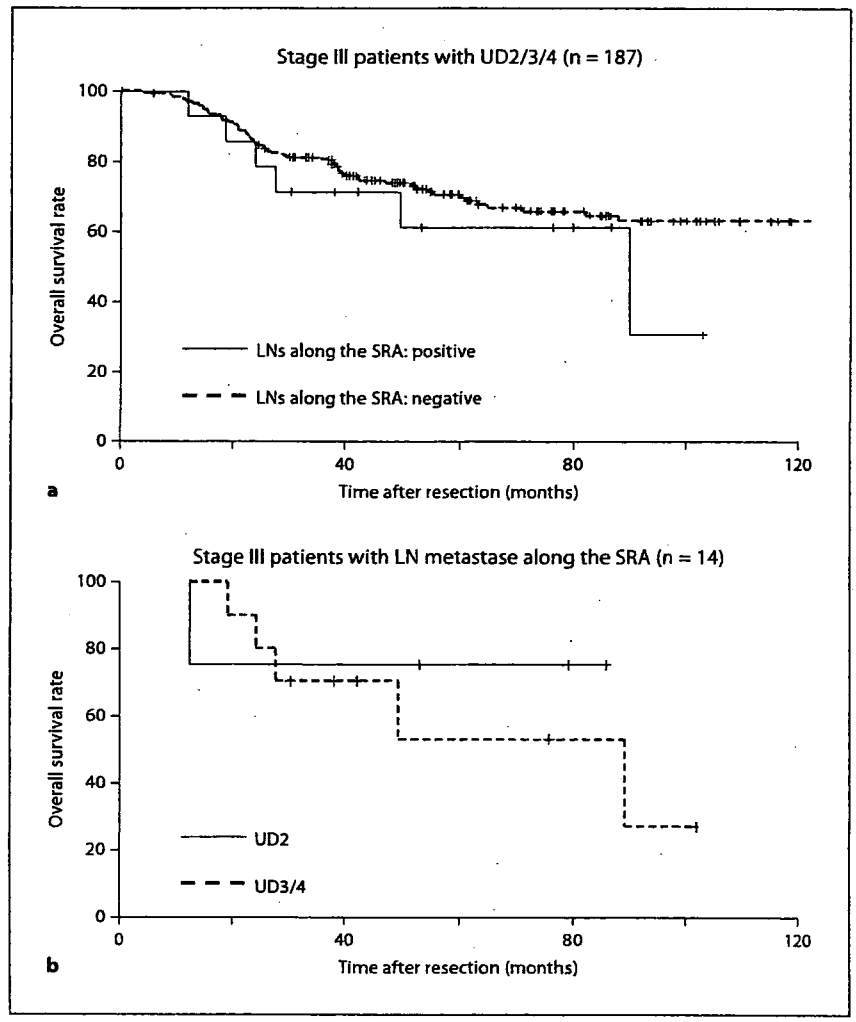

Fig. 4. a Overall survival curves for stage III patients with or without metastatic lymph nodes along the superior rectal artery, excluding 4 patients with lymph node metastases at the root of the inferior mesenteric artery. There were no significant differences in overall survival between both groups ($p = 0.338$). **b** Overall survival curves in relation to the extent of upward lymph node dissection for stage III patients with metastatic lymph nodes along the superior rectal artery, excluding 3 patients with lymph node metastases at the root of the inferior mesenteric artery. There were no significant differences in survival of the patients with lymph node metastases along the superior rectal artery according to the extension of upward lymph node dissection performed (UD2 or UD3/4) ($p = 0.642$).

In comparison, the Japanese classification of colorectal carcinoma [9] treats regional lymph nodes in rectal carcinoma as follows: pararectal lymph nodes are defined as group 1, lymph nodes along the superior rectal artery as intermediate lymph nodes (group 2) and lymph nodes at the root of the inferior mesenteric artery as the main lymph nodes (group 3). However, this classification defines patients with metastatic lymph nodes in group 2 and/or group 3 as same stage (stage IIIb). Based on the results of this study, these criteria should be reevaluated.

In recent years, sphincter-preserving surgery has been increasingly adopted in patients with lower rectal carcinoma [15, 16]. The most important postoperative complication in this procedure is anastomotic leakage. To avoid this complication, all colorectal surgeons pay attention to blood flow in the remnant colon, together with the tension of the anastomosis. Therefore, Western surgeons perform mobilization of the splenic flexure for most patients [17], but the position of the splenic flexure in Japanese is usually very deep in the left upper subphrenic area and it is sometimes rather difficult to mobilize the left side colon. However, Japanese patients usually have a long sigmoid colon, and if the surgeon preserves 1 or 2 arcades of marginal vessels of the sigmoid colon by dividing the sigmoid artery between the superior rectal artery and these marginal vessels, mobilization of the splenic flexure becomes unnecessary. In this situation, arterial blood flow is not being compensated. Preservation of the blood flow of the left colic artery is one solution to this problem,

because the appropriate extent of upward lymph node dissection for lower rectal carcinoma is considered to be UD2. When the length of the vascular pedicle for lower anastomosis is short, we can cut the periphery of the left colic artery. Some surgeons choose left colic artery-preserving lymph node dissection at the root of the inferior mesenteric artery, but this increases the risk of damaging the lumbar splanchnic nerve.

Another problem encountered with lymph node dissection for lower rectal surgery is lateral lymph node dissection. Some reports mainly from Japan have supported the effectiveness of lateral pelvic lymph node dissection, and it is well established as the standard procedure in leading hospitals in Japan. However, in Western countries, the survival benefits of lateral pelvic lymph node dissection are regarded as doubtful. Instead, preoperative chemoradiotherapy is widely performed [18, 19]. To resolve this disparity, a multicentric randomized clinical trial that compares lateral pelvic lymph node dissection with autonomic nerve preservation to total mesenteric excision (JCOG-0212) is underway in Japan and data regarding this issue will become available in the near future [20].

In conclusion, survival of a minority with metastatic lymph nodes at the root of the inferior mesenteric artery was very poor. In addition, survival is no worse in patients with positive lymph node along the superior rectal artery as long as these positive nodes are resected by either UD2 or UD3/4. Surgeons should take these data into consideration and recognize that low ligation is adequate for advanced lower rectal carcinoma.

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ORIGINAL ARTICLE

Randomized, Multicenter Trial of Antibiotic Prophylaxis in Elective Colorectal Surgery

Single Dose vs 3 Doses of a Second-Generation Cephalosporin Without Metronidazole and Oral Antibiotics

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Hypothesis: Use of prophylactic antibiotics in elective colorectal surgery is essential. Although single-dose prophylactic antibiotics are recommended, the efficacy of single-dose cephalosporin without metronidazole and oral antibiotics is not fully proven. We conducted a multicenter, randomized trial of a single dose vs 3 doses of the second-generation cephalosporin cefmetazole.

Design: A prospective, randomized, multicenter trial in patients undergoing elective colorectal surgery.

Setting: Seven major hospitals in Japan that offer cancer treatment.

Patients: Patients with colorectal cancer treated from May 6, 2004, to April 25, 2005.

Interventions: Patients were randomized to 1 of 2 groups: a single-dose group given a single dose of cefmetazole just before skin incision and a 3-dose group given 2 additional doses of cefmetazole every 8 hours after the first dose just before skin incision.

Main Outcome Measures: Incidences of incisional surgical site infection (SSI), organ or space SSI, and all other infectious complications within 30 days after surgery.

Results: A total of 384 patients were enrolled. Seven patients were excluded because of additional surgery or the inability to tolerate mechanical preparation. The incidence of incisional SSI was higher in the single-dose group (27/190 or 14.2%) than in the 3-dose group (8/187 or 4.3%) ($P=.009$). Incidences of organ or space SSI and other postoperative infectious diseases did not differ significantly between the 2 groups. In multivariate analysis, antibiotic dose was the only significant factor related to the incidence of incisional SSI.

Conclusion: Three-dose cefmetazole administration is significantly more effective for prevention of incisional SSI than single-dose antibiotic administration.

Trial Registration: clinicaltrials.gov Identifier: NCT00292708

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PROPHYLACTIC ANTIBIOTICS have become a standard treatment for patients undergoing colorectal surgery,[1,2] but controversy still persists concerning the administration route for antibiotics (oral, intravenous, or both) and the number of administrations.[3] A recent meta-analysis[1] and a literature review[4] have suggested that oral administration of antibiotics is of no added value

when appropriate parenteral antibiotics are administered. Moreover, preoperative oral antibiotics increase the incidence of *Clostridium difficile* colitis[5] and gastrointestinal symptoms, including nausea, vomiting, and abdominal pain.[6] A single dose of antibiotics has been shown to be as effective as multiple doses in many trials that have compared a single-dose regimen with a multiple-dose regimen.[1] Although the 1999 Hospital Infection Control Practices Advisory Committee guidelines for prevention of surgical site infection (SSI)[2] recommend cefoxitin or some other second-generation cephalosporin in the distal intestinal tract, the efficacy of a single-dose regimen of cephalosporin without metronidazole and oral antibiotics is not clear, because combination regimens, such as cephalosporin and metronidazole or cephalosporin and oral antibiotics, have been used in most studies of antibiotics dose.[1] In fact, in trials without metroni-

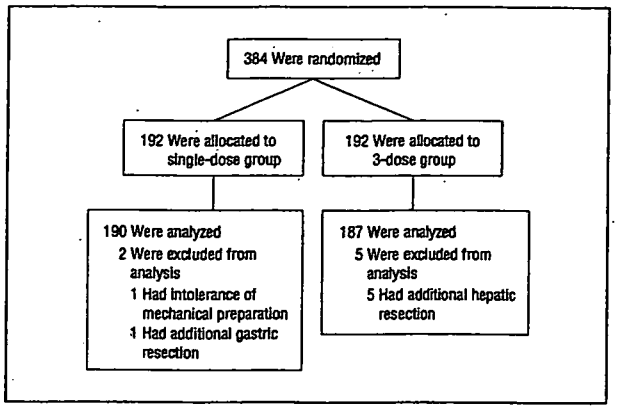

Figure. Flowchart of the trial.

dazole, the number of enrolled patients was small and the difference in the incidence of incisional SSI between a single-dose and a multiple-dose regimen was unclear.[7] Moreover, a single-dose regimen of cephalosporin without metronidazole was associated with a slightly higher incidence of incisional SSI than a regimen of metronidazole alone.[7-9] Therefore, we conducted a multicenter, randomized trial of single-dose vs 3 doses of the second-generation cephalosporin cefmetazole without metronidazole and oral antibiotics.

METHODS

This randomized multicenter trial was conducted at 7 major hospitals in Japan that offered cancer treatment from May 6, 2004, to April 25, 2005. The protocol was approved by the institutional review board at each hospital, and written informed consent was obtained from all of the patients who participated.

Patients aged 20 through 80 years scheduled to undergo elective colorectal surgery were eligible for enrollment in the study. Exclusion criteria included emergency operations, obstruction of the small bowel, stomal surgery or bypass surgery, preoperative infectious diseases, penicillin or cephalosporin allergy, antibiotic administration before hospitalization, inflammatory bowel diseases, angina or myocardial infarction, mild or severe renal dysfunction, mild or severe diabetes mellitus, and steroid administration before surgery.

Patients underwent mechanical bowel preparation with 2 L of polyethylene glycol–electrolyte solution (Niflec, Ajinomoto Pharma, Tokyo, Japan) 1 day before surgery. On the basis of a block-randomized, computer-generated list balancing tumor site, the patients were randomized by a study secretary into 1 of 2 groups: a single-dose group given a single intravenous dose of 1 g of cefmetazole just before skin incision and a 3-dose group given an intravenous dose of 1 g of cefmetazole just before skin incision and 2 postoperative 1-g doses at 8 and 16 hours after the first administration. Although additional doses during surgery and every 3 or 4 hours were recommended in the 1999 guidelines for prevention of SSI,[2] no additional dose was given, even for operations that lasted more than 3 hours. The surgeon was notified of the allocation after the randomization. To ensure that the trial results were applicable generally, specific instructions on surgical techniques and on postoperative management were not included in the protocol.

The primary end point was incidence of incisonal SSI. Secondary end points were incidences of organ or space SSI and other infectious diseases, including urinary tract infection, pneumonia, septicemia, infective diarrhea, and intravenous line sepsis. Other postoperative complications and postoperative hospital stay were also examined. Demographic data, including sex, age, operative procedure, operative time, and operative blood loss, were collected for all patients. Incisional SSI, organ or space SSI, and other infectious diseases were checked for daily by an attending surgeon until hospital discharge and checked for again at the first postoperative hospital visit.

This trial was designed as a noninferiority test to detect a 5% difference in the incidence of incisional SSI between the 2 groups, with a confidence interval of 95% and a power of 90%, assuming that the incidence of incisional SSI in the 3-dose group would be 5%. Therefore, a sample size of 238 was required in both arms. After 1 year of enrollment, interim analysis was performed. Because a significant difference in the incidence of incisional SSI was seen between the groups, enrollment was stopped. The χ^2 test or Fisher exact test, which was used when the variables were lower than 5, was used to analyze categorized variables. The *t* test was used to analyze continuous variables. In multivariate analysis, logistic regression analysis was used. Significance was defined as $P<.05$.

Table 1. Patient Characteristics[a]

Characteristic	Single-Dose Group (n = 190)	3-Dose Group (n = 187)	*P* Value
Age, mean ± SD, y	59.4 ± 11.1	62.1 ± 9.8	.01
Sex			.07
Male	126	107	
Female	64	80	
Tumor site			.91
Colon	122	119	
Rectum	68	68	
Type of surgery			.50
Conventional	129	133	
Colectomy	73	77	
Anterior resection	56	53	
Abdominoperineal resection	0	3	
Laparoscopic	61	54	
Colectomy	49	42	
Anterior resection	12	12	
Operative time, mean ± SD, min	178.8 ± 69.4	170.0 ± 77.5	.25
Blood loss, mean ± SD, mL	164.3 ± 272.6	162.7 ± 397.9	.96

[a]Data are presented as number of patients unless otherwise indicated.

RESULTS

PATIENT CHARACTERISTICS

A total of 384 patients were enrolled in this study (**Figure**). Seven patients were excluded because of additional surgery (gastric and hepatic resection) or the inability to tolerate mechanical preparation. Therefore, 377 patients were examined. All of the enrolled patients had colon or rectal cancer. The numbers of patients in the single-dose and 3-dose groups were 190 and 187, respectively. Patient characteristics are given in **Table 1**. Although patient age was significantly higher in the 3-dose

Table 2. Incisional SSI, Organ or Space SSI, and Other Postoperative Infectious Diseases

Complication	No. (%) of Patients: Single-Dose Group (n = 190)	3-Dose Group (n = 187)	P Value
Incisional SSI	27 (14.2)	8 (4.3)	.009
Organ or space SSI	5 (2.6)	9 (4.8)	.26
Other	12 (6.3)	9 (4.8)	.52
Total	40 (21.1)	24 (12.8)	.03

Abbreviation: SSI, surgical site infection.

group than in the single-dose group, other patient characteristics, including sex, tumor site (colon or rectum), type of surgery (conventional or laparoscopic), operative time, and operative blood loss, were identical.

SSI AND OTHER POSTOPERATIVE INFECTIOUS DISEASES AND COMPLICATIONS

The incidence of incisional SSI was significantly higher in the single-dose group (27/190 or 14.2%) than in the 3-dose group (8/187 or 4.3%) ($P=.009$; **Table 2**). The total incidence of incisional and organ or space SSI and other postoperative infectious diseases in the single-dose group (40/190 or 21.1%) was also significantly higher than in the 3-dose group (24/187 or 12.8%) ($P=.03$). Among these postoperative infectious diseases, the incidences of organ or space SSI and other infectious diseases were similar between the groups, and only the incidence of incisional SSI differed significantly. The incidence of other postoperative complications, mainly small-bowel obstruction, was 9.5% (18/190) in the single-dose group and 9.6% (18/187) in the 3-dose group. The mean±SD postoperative hospital stay was 12.5±7.4 days in the single-dose group and 12.2±5.6 days in the 3-dose group. Postoperative complications and hospital stay were identical between the groups, with no statistically significant differences ($P=.96$ and .66, respectively). However, the mean±SD postoperative stay of the patients with incisional SSI (n=35) was significantly longer than that of patients without incisional SSI (n=342) (14.6±9.3 and 12.1±6.2 days, respectively; $P=.03$).

INCISIONAL SSI IN SUBSETS

Because only the incidence of incisional SSI differed significantly between the groups, this variable was examined in subset analysis. In every subset except for laparoscopic surgery, the incidence of incisional SSI was significantly higher in the single-dose group than in the 3-dose group (**Table 3**). Although the difference in the incidence of incisional SSI was not significant in the laparoscopic surgery subset, a large difference was found between the groups: 9.8% in the single-dose group and 1.9% in the 3-dose group. A multivariate analysis that examined age, sex, tumor site, operative time, operative blood loss, and type of surgery showed that antibiotic dose was the only significant factor associated with incisional SSI ($P=.002$).

Table 3. Incisional Surgical Site Infections in Patient Subsets

Characteristic	No. (%) of Patients: Single-Dose Group (n = 190)	3-Dose Group (n = 187)	P Value
Age, y			
≤60	14/95 (14.7)	4/80 (5.0)	.045
>60	13/95 (13.7)	4/107 (3.7)	.02
Sex			
Male	18/126 (14.3)	6/107 (5.6)	.03
Female	9/64 (14.1)	2/80 (2.5)	.01
Tumor site			
Colon	15/122 (12.3)	6/119 (5.0)	.046
Rectum	12/68 (17.6)	2/68 (2.9)	.009
Type of surgery			
Conventional	21/129 (16.3)	7/133 (5.3)	.04
Laparoscopic	6/61 (9.8)	1/54 (1.9)	.12
Operative time			
≤3 h	14/104 (13.5)	6/123 (4.9)	.02
>3 h	13/86 (15.1)	2/64 (3.1)	.02
Operative blood loss			
≤200 mL	19/152 (12.5)	7/152 (4.6)	.01
>200 mL	8/38 (21.1)	1/35 (2.9)	.03

COMMENT

Many studies[1,2,10] have shown that prophylactic antibiotics are essential for patients undergoing elective colorectal surgery. If prophylactic antibiotics are not used in colorectal surgery patients, the reported incidence of incisional SSI is 30% to 50%.[11-16] After the efficacy of oral antibiotics was initially proven,[11] oral, intravenous, or oral plus intravenous antibiotics were adopted, and the incidence of incisional SSI improved to approximately 5% to 20%. A meta-analysis[1] confirmed that the use of prophylactic antibiotics is effective for prevention of incisional SSI after colorectal surgery. In this analysis, there were no significant differences between single-dose and multiple-dose regimens, and oral antibiotics gave no added value when appropriate parenteral antibiotics were administered. However, in many single-dose vs multiple-dose trials, metronidazole was used, and the incidence of incisional SSI for the single-dose regimen was the same as that for the multiple-dose regimen.[7,17-19] In trials without metronidazole, the number of trials was limited, and the difference between the incidence of SSI for the single-dose regimen and that for the multiple-dose regimen was unclear.[7,20,21] Moreover, a single-dose regimen of cephalosporin without metronidazole was associated with a higher incidence of incisional SSI than a metronidazole regimen.[7-9] The incidence of incisional SSI for a single-dose regimen of cephalosporin without metronidazole was 10% to 15% and that for a single-dose regimen of cephalosporin with metronidazole was 5% to 10%. Therefore, single-dose cephalosporin without metronidazole has not been proved to be an ideal prophylaxis for patients undergoing colorectal surgery. These findings prompted us to perform the present trial.

Our study clearly showed that 3-dose administration of the second-generation cephalosporin cefmetazole was sig-

nificantly more effective for prevention of incisional SSI than single-dose administration. This phenomenon was observed in every subset, including age (≤60 years or >60 years), sex (male or female), tumor site (colon or rectum), type of surgery (conventional or laparoscopic), operative time (≤3 hours or >3 hours), and operative blood loss (≤200 mL or >200 mL). Therefore, our finding was not considered to be the result of chance. Because the incidence of incisional SSI in the 3-dose group was 4.3%, which was compatible with the incidence for the single-dose regimen of cephalosporin with metronidazole,[4,7,17-19] 3-dose administration of cephalosporin without metronidazole should be considered as one of the options for prevention for SSI in patients undergoing colorectal surgery.

This trial was not a double-blind study. Although double-blinding is ideal, placebo is expensive and was not used in this study. Because of this, every surgeon in charge was easily aware of the allocation and therefore could not be a blinded observer. One solution is to prepare a blinded observer to document the occurrence of infectious diseases. Because the blinded observer needs to be a physician or a nurse who is unaware of the nature of the trial, which was difficult in the present multicenter trial setting, an attending surgeon was asked to examine patients for infectious diseases in this trial.

The disadvantage of oral antibiotics is their adverse effects. Preoperative oral antibiotics increase the incidences of gastrointestinal symptoms, including nausea, vomiting, and abdominal pain,[6] and of *C difficile* colitis.[5] The latter is a well-known complication of colorectal surgery and is thought to be caused by mechanical cathartic agents and oral antibiotics, which diminish the variety of intraluminal bacteria and predispose the colon to *C difficile* colonization. A previous study[5] found that the incidences of *C difficile* colitis in colorectal surgery patients who received oral antibiotics and in those who did not were 7.4% and 2.6%, respectively, the difference in incidence being significant ($P = .03$). In our study, *C difficile* colitis occurred in only 2 patients in the 3-dose group (1.1%) and in none of the patients in the single-dose group. A randomized comparative study[22] of 137 patients undergoing elective colorectal surgery for carcinoma and receiving oral, systemic, and intraluminal antibiotics found no significant differences in the incidence of incisional SSI among the 3 groups, and the oral antibiotic regimen induced a greater change in the intestinal flora and was associated with more frequent postoperative diarrhea. Recently, the role of mechanical bowel preparation in lowering the incidence of postoperative infectious complications has been questioned by several randomized trials.[23] These data also indicated that use of oral antibiotics may not offer additional advantages over parenteral antibiotics. In fact, a recent survey of members of the American Society of Colon and Rectal Surgeons indicated that more than 50% of the respondents were skeptical about the usefulness of oral antibiotics.[3]

To be effective against SSI, the level of antibiotics in the tissue around the surgical site should be sufficient at the time of bacterial contamination.[2,24] Cephalosporin exhibits a time-dependent antibacterial action, and the therapeutic effect is maintained when the level of the antibiotic exceeds the minimum inhibitory concentration for the target pathogen. A pharmacokinetic study[25] of cefmetazole showed that additional doses were unnecessary for surgery that lasted less than 3 hours from the time of initial administration, because the tissue concentration at wound closure exceeded the minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli*. Therefore, we analyzed the relationship between incisional SSI and operation time. In our study, the incidence of incisional SSI in the 3-dose group was lower even in patients whose surgery lasted 3 hours or less than that in the single-dose group. This result indicated that postoperative administration of cephalosporin was important even for short operations.

Because the length of surgery is reported to be an important factor in SSI,[26-28] an additional dose of antibiotics is recommended during operations that exceed the time during which the therapeutic level of antibiotics is lower than the minimum inhibitory concentration.[2] Logically, maintaining the therapeutic level of antibiotics during surgery is important for prevention of SSI.[2] In this study, the incidence of incisional SSI did not change in patients whose operations lasted more than 3 hours, even in the single-dose group. Therefore, the efficacy of an additional dose of antibiotics in patients undergoing colorectal surgery that lasted more than 3 hours should be examined in a randomized study.

Because the incision in laparoscopic surgery is shorter than that in conventional open surgery, the former is considered to have a lower incidence of incisional SSI.[29] In our study, the incidences of incisional SSI in patients undergoing laparoscopic surgery were 9.8% and 1.9% and those in patients undergoing open surgery were 16.3% and 5.3% in the single-dose group and 3-dose group, respectively. The incidence of incisional SSI associated with laparoscopic surgery was thus lower than that in conventional surgery, although the difference was not statistically significant.

Other patient characteristics, including age, sex, and operative blood loss, did not affect the incidence of incisional SSI. Among these factors, blood loss is considered to be related to SSI, because blood loss reduces the concentration of antibiotics. In fact, the incidence of incisional SSI in patients who lost more than 200 mL of blood was 21.1% in the single-dose group, which was higher than that in patients who lost 200 mL of blood or less. However, no such difference was seen in the 3-dose group, and the finding was not statistically significant.

Incisional SSI is generally associated with a prolonged hospital stay. Although the period of hospitalization did not differ between the single-dose and 3-dose groups, that for patients with incisional SSI was significantly longer than for patients without. Therefore, prevention of incisional SSI is important for reducing the period of hospitalization and thus cost. Our results indicate that a single dose of prophylactic antibiotics does not always save costs.

In conclusion, if oral antibiotics and metronidazole are not used for prophylaxis in patients undergoing colorectal surgery, administration of the 3-dose second-generation cephalosporin cefmetazole is significantly more effective for prevention of incisional SSI than single-dose antibiotic administration regardless of patient age,

sex, tumor site, type of surgery, operative time, or operative blood loss.

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Quantification of CD10 mRNA in Colorectal Cancer and Relationship between mRNA Expression and Liver Metastasis

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Abstract. *CD10 mRNA expression in colorectal cancer and its relationship with cancer progression and prognosis were investigated. Patients and Methods: CD10 mRNA was quantified in 167 colorectal cancer and matched normal tissue samples using real-time polymerase chain reaction (RT-PCR). The tumor to normal tissue (T/N) CD10 mRNA ratio was compared with clinicopathological factors and prognosis. Results: CD10 mRNA was overexpressed in 138 of the 167 tumors in comparison with the matched normal tissues. T/N was higher in colon, pN1/pN2, stage III and IV, and well- or moderately-differentiated adenocarcinoma than in rectum, pN0, stage I and II, and poorly-differentiated or mucinous adenocarcinoma, respectively. However, these differences were not significant. T/N was not associated with prognosis. Conclusion: CD10 mRNA showed significantly higher expression in tumor tissues than in matched normal tissues. Although CD10 mRNA was associated with invasion depth, lymph node status and TNM stage, it was not associated with prognosis.*

CD10 is a 100 kDa cell surface zinc metalloendopeptidase that was initially identified as the common acute lymphoblastic leukemia antigen. Although CD10 is commonly expressed on hematopoieteic cells and tumors, it is also expressed in a variety of normal and tumor tissues. Recently, several studies have shown an association between CD10 expression and progression of various kinds of tumors including gastric cancer (1-5), colorectal cancer (6-10), pancreatic endocrine tumor (11), ovarian cancer (12), cervical carcinoma (13), renal cell carcinoma (14, 15), prostate cancer (16), breast cancer (17), non-small cell lung cancer (18), melanoma (19, 20), nasopharyngeal carcinoma (21), oral cavity squamous cell carcinoma (22) and B-cell lymphoma (23). Therefore, CD10 is considered to play an important role in both normal and tumor tissues. We recently demonstrated that CD10 protein expression in colorectal cancer was significantly associated with liver metastasis (10). This result prompted us to examine the association between CD10 mRNA expression and liver metastasis. In the present study, CD10 mRNA in colorectal cancer tissues was quantified by real-time PCR in comparison with matched normal tissues, and the relationship between CD10 mRNA expression and clinicopathological characteristics was examined.

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Key Words: Colorectal cancer, CD10, liver metastasis, prognosis.

Patients and Methods

Patients and tissues. Tumor tissue and adjacent normal tissues (10 cm away from the tumor) were obtained from 175 patients with colorectal cancer between January 1995 and September 1996 at the National Cancer Center Hospital, Tokyo, Japan, after informed consent had been obtained. Among these, a total of 167 samples in which CD10 expression was examined using the avidin-biotin-peroxidase method with mouse monoclonal antibody 56C6 (Novocastra, Newcastle, UK) in our previous study (10) were investigated for CD10 mRNA quantification. Although in our previous study >5% staining of tumor cells had been judged as positive, in the present study we considered staining of >5% of tumor and/or stromal cells as positive, because CD10 is also expressed in stromal cells (7). Tissues had been obtained immediately after surgery and stored frozen in liquid nitrogen until RNA extraction. All surviving patients had been followed up for more than 5 years, initially at 3-month intervals for 2 years and then at 6-month intervals thereafter. Median follow-up time was 7.9 years, and no adjuvant chemotherapy was given in this period.

RNA extraction and relative mRNA quantification. Total RNA was extracted from the frozen tissues according to the procedure described by Chomczyski and Sacchi (24). Randomly primed cDNA was synthesized from 1 μg of total RNA using a High-Capacity cDNA Archive Kit in accordance with the manufacturer's instructions (Applied Biosystems, CA, USA). CD10 mRNA expression was quantified using TaqMan gene expression assay and

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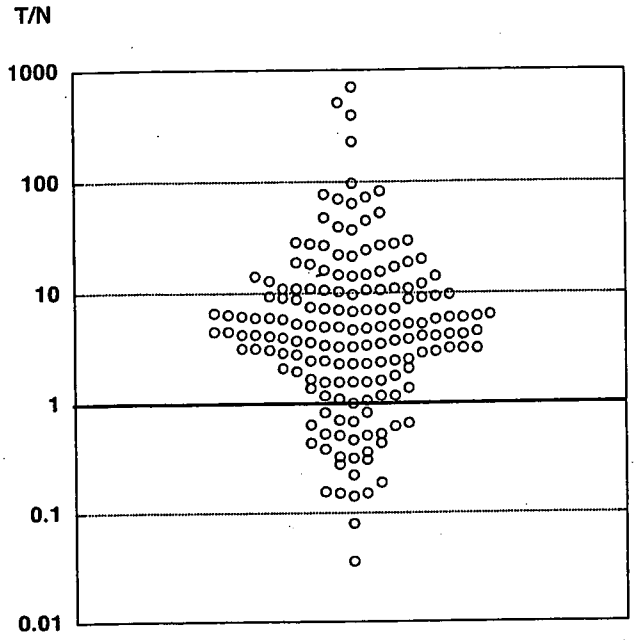

Figure 1. *Distribution of CD10 mRNA T/N. Mean T/N±standard deviation was 20.89±75.80, range was 0.036 to 709.176.*

a 7300 Real-Time PCR System (Applied Biosystems) in accordance with the manufacturer's instructions. The CD10 mRNA level in the tumor was compared with that in matched normal mucosa after standardization against 18S rRNA as an internal control gene (25). The CD10 mRNA level was calculated using the formula: $-\Delta\Delta$ Ct (cycle threshold) (Δ Ct of tumor (CD10 Ct - 18S rRNA Ct) – Δ Ct of matched normal tissue (CD10 Ct – 18S rRNA Ct)) to the power two ($2^{-\Delta\Delta Ct}$). This value is the ratio of CD10 mRNA in the tumor relative to that in matched normal tissue (T/N).

Statistical analysis. T/N was compared statistically using Mann-Whitney *U*-test. Survival rates were calculated by the Kaplan-Meier method and survival curves were compared by the log-rank test. Data differences between groups were considered statistically significant at $p<0.05$.

Results

Patient characteristics and CD10 mRNA. The distribution of CD10 mRNA T/N is shown in Figure 1. Mean T/N±standard deviation was 20.89±75.80, and the range was 0.036 to 709.176. In 138 (83%) of the 167 tumors, T/N was more than one, which meant that CD10 was overexpressed in the tumor tissue compared with the matched normal tissue. Patient characteristics and T/N are shown in Table I. T/N was higher in colon, pN1/pN2, stage III and IV, and well or moderately-differentiated adenocarcinoma than in rectum, pN0, stage I and II, and poorly-differentiated or mucinous adenocarcinoma, respectively. However, these differences were not significant. Because CD10 protein expression had been examined in our previous study (10), T/N was compared with CD10 protein expression, and was found to be significantly associated.

Table I. *Patient clinicopathological characteristics and CD10 mRNA (T/N).*

Characteristic	No. (n=167)	CD10 mRNA (T/N±S.D.)	*P*
Age (yr)			
≤ 60	71	25.39±79.97	0.645
60 <	96	17.57±72.81	
Gender			
Male	99	19.77±64.88	0.881
Female	68	22.52±89.88	
Tumor site			
Colon	100	29.31±96.56	0.156
Rectum	67	8.33±14.04	
Depth of invasion (pT)			
pT1/pT2	1/27	11.98±17.37	0.333
pT3/pT4	105/34	22.68±82.66	
Lymph node status			
pN0	76	9.81±14.88	0.847
pN1/ pN2	54/37	30.14±101.11	
Stage			
I/II	19/49	9.60±15.16	0.996
III/IV	67/32	28.64±97.09	
Tumor differentiation			
Well/Moderate	70/85	22.21±78.53	0.063
Poor/Mucinous	9/3	3.86±4.30	
Lymphatic invasion			
Negative	60	19.03±66.30	0.643
Positive	107	21.94±80.92	
Venous invasion			
Negative	80	18.55±62.41	0.859
Positive	87	23.05±86.62	
CD10 protein expression			
Negative	83	5.33±5.65	0.003
Positive	84	36.64±105.35	

Relationship between CD10 mRNA and liver metastasis. Among the 167 patients, 32 had synchronous metastasis: liver metastasis in 22 cases, peritoneal dissemination in 4, lung metastasis in one, and distant lymph node metastasis in 5. The remaining 135 patients who had no synchronous metastasis underwent curative resection. Among these patients, 41 suffered cancer recurrence, 20 of them developing liver metastasis. The relationship between CD10 mRNA and metastasis is shown in Table II. There was no significant relationship between CD10 mRNA and metastasis including liver metastasis. Because the median T/N was 4.55, the survival curves of patients with T/N ≥5 and of patients with T/N <5 were analyzed (Figure 2), but