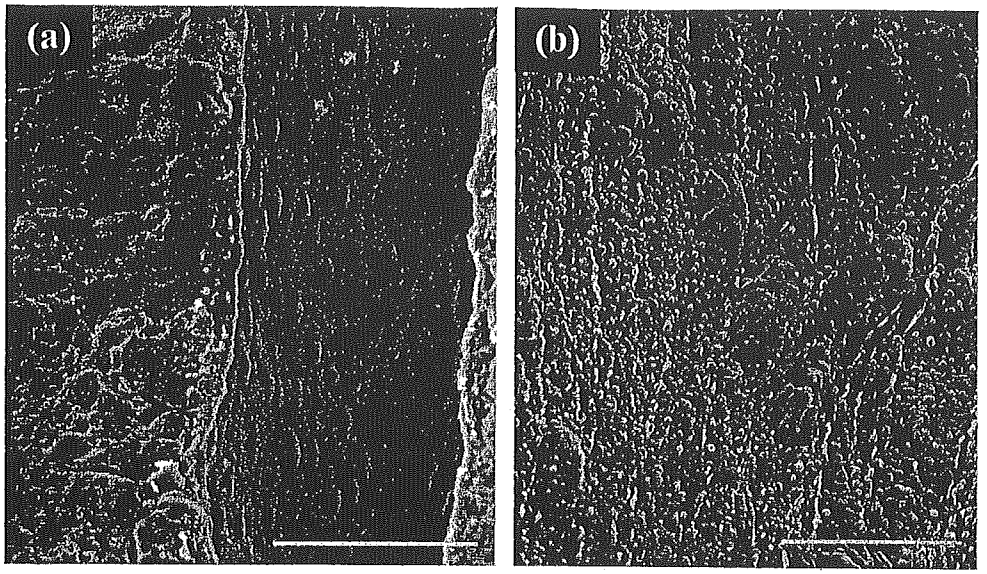

Fig. 1. Hepatic vascular endothelium of untreated mice. **a** Original magnification, ×500. The bar represents 60 μm. **b** Original magnification, ×2,000. The bar represents 15 μm.

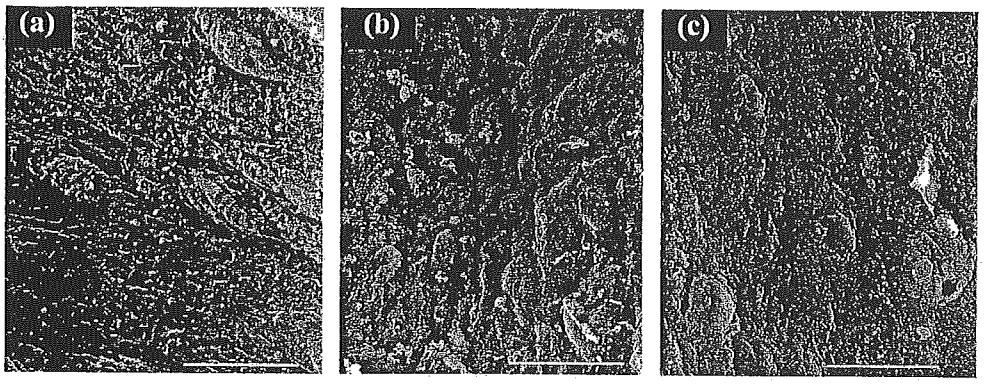

Fig. 2. In the CO_2 pneumoperitoneum group, there were marked changes in the hepatic vascular endothelium. Dilatation of intercellular clefts, irregular arrangement of endothelial cells, and a rough surface were observed. Original magnification, ×2,000. The bar represents 15 μm. **a** Immediately after CO_2 pneumoperitoneum. **b** Day 1. **c** Day 3.

establishing the pneumoperitoneum has been described previously [16]. A 22-gauge intravenous cannula was inserted into the left lower quadrant and used as an insufflation needle. A 20-gauge intravenous cannula was inserted into the right lower quadrant and used to measure intraperitoneal pressure. A disposable syringe was used to inject the gas. A syringe pump was used to maintain continuous insufflation, and intraperitoneal pressure was measured as the distance between the right and left water levels in the U-shaped tube.

In the laparotomy group (n = 9), a 3-cm abdominal midline incision was made, and the laparotomy conditions were maintained for 60 min. The mice were killled after either 0 h (n = 3), 24 h (n = 3), or 72 h (n = 3).

The control group (n = 9) underwent only diethyl ether anesthesia for 60 min. The mice were killed after either 0 h (n = 3), 24 h (n = 3), or 72 h (n = 3).

After each procedure, the left lobe of the murine liver was excised, and four samples from each liver were prepared for examination with an SEM. These samples were put into fixative solution composed of 2% formaldehyde and 2.5% glutaraldehyde in 0.05 M cacodylate buffer solution. They were then placed into 1% osmium tetroxide for 60 min, dehydrated stepwise in alcohol, and dried by mens of a critical points apparatus. The dried specimens were mounted on aluminium stubs, spatter-coated with gold, and examined with an SEM (Hitachi S800, Ibaragi, Japan). Photographs of the five areas selected at random in each sample were analyzed.

None of the animals died at any time during this experiment.

Analysis

Following the same procedure used by Suematsu et al. [17], the photographs were evaluated by five independent observers (one histologist, one pathologist, and three surgeons) who were not informed of the procedures used to quantify the results. The following characteristics of the hepatic vascular endothelium were observed: (a) dilatation of intercellular clefts, (b) derangement of the endothelial cells, and (c) a rough surface. We then compared these characteristics with those of normal hepatic vascular endothelium of untreated mice and graded the changes as none or slight (−), moderate (+), or marked (+ +). If over half of the observers were in agreement, the results were adopted. In cases where the observers disagreed about the results, the lower grade was adopted.

Results

Figure 1 shows the normal hepatic vascular endothelium of untreated mice. Normal liver endothelium is characterized by a smooth surface, a regular arrangement of the endothelial cells that corresponds with the direction of the blood flow, and no intercellular clefts. In the CO_2 pneumoperitoneum group (Fig. 2), we observed both derangement of the hepatic vascular endothelial cells and intercellular clefts on day 1. These changes were recognized immediately after creation of the CO_2 pneumoperitoneum and persisted 3 days. However, in the air pneumoperitoneum group, the changes were not as marked on day 1 (Fig. 3a). Also, on day 1 after helium pneumoperitoneum, the changes were unremarkable (Fig. 3b). There were also no remarkable changes at any time in the control group or the laparotomy group

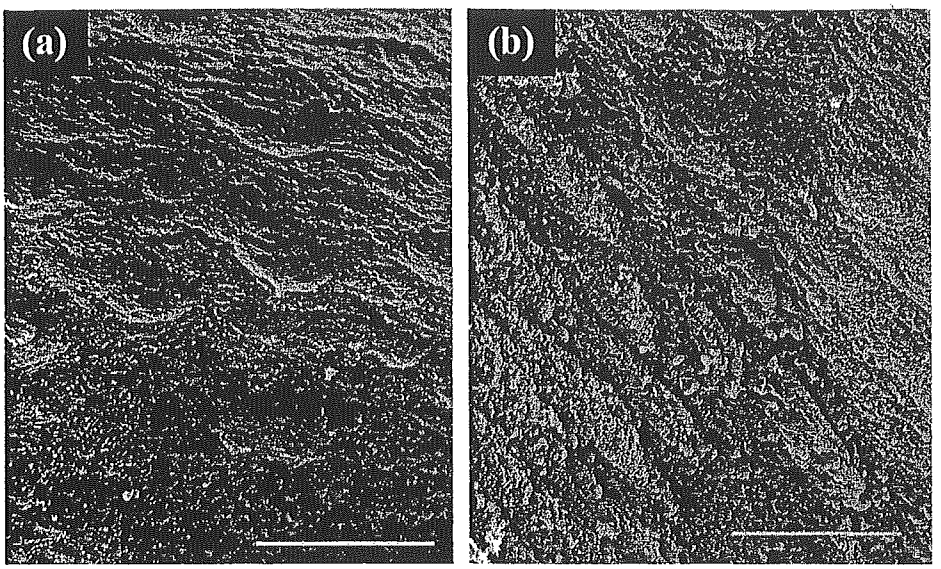

Fig. 3. In both the air (**a**) and helium (**b**) pneumoperitoneum groups, changes to the hepatic vascular endothelium were slight as compared with the CO_2 pneumoperitoneum group on day 1. Original magnification, ×2,000. The bar represents 15 μm.

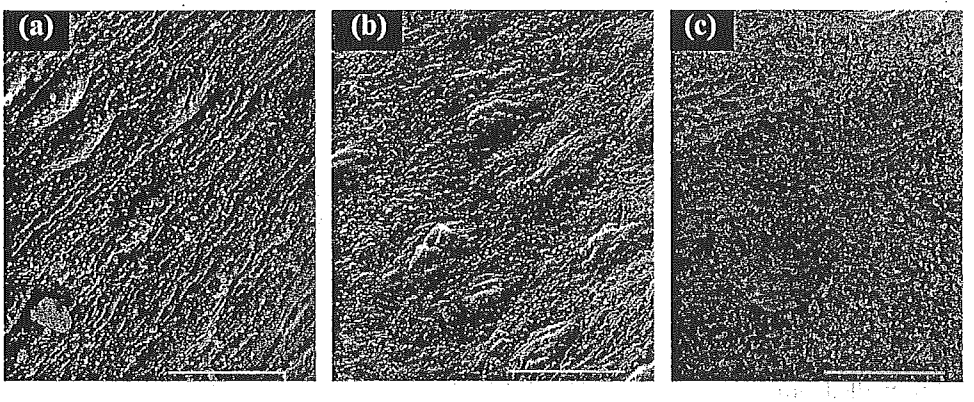

Fig. 4. In the control group, there were only minimal changes to the hepatic vascular endothelium. Original magnification, ×2,000. **a** Immediately after ether anesthesia. **b** Day 1. **c** Day 3. The bar represents 15 μm.

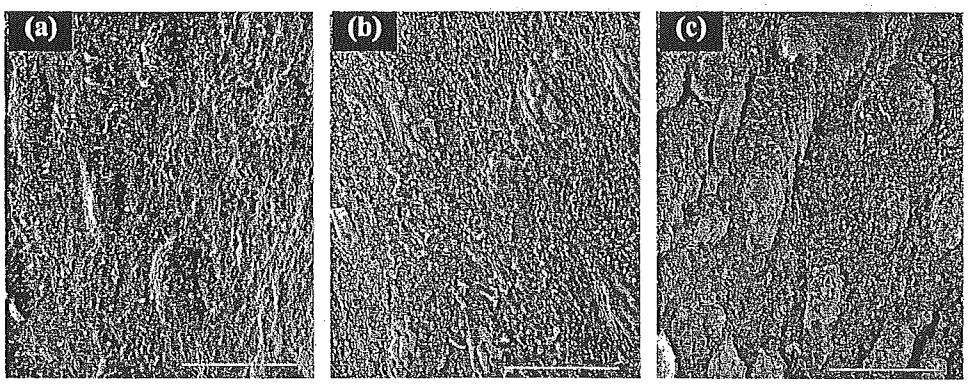

Fig. 5. In the laparotomy group, there were only minimal changes to the hepatic vascular endothelium. Original magnification, ×2,000. **a** Immediately after laparotomy. **b** Day 1. **c** Day 3. The bar represents 15 μm.

(Figs. 4 and 5). In the laparotomy group, only the rough surface of endothelium was observed on day 3, but this change was slight as compared with the CO_2 pneumoperitoneum group.

Table 1 summarizes the morphological changes in each group. There were no wide disparities among the five observers in grading the results.

Discussion

Laparoscopic surgery has been adopted for colorectal cancer because it is less invasive and yields a better cosmetic result. However, the effects of the pneumoperitoneum on liver metastasis, which is the most important prognostic factor for colorectal cancer, remain unclear. In the present study, we used SEM to investigated the morphologic changes to hepatic vascular endothelial cells that occur after CO_2 pneumoperitoneum. Other studies have used SEM to investigate morphological changes to the peritoneum after CO_2 pneumoperitoneum [17, 20], but this is the first study to clarify changes to the hepatic vascular endotheliem after CO_2 pneumoperitoneum.

The first step in liver metastasis is the adherence of tumor cells to the hepatic vascular endothelium. Thus,

Table 1. Summary of morphological changes of hepatic vascular endothelium after carbon dioxide (CO_2), helium, or air pneumoperitoneum, laparotomy, or anesthesia alone

	Dilatation of intercellular clefts	Irregular arrangement of endothelial cells	Rough surface
Immediately after each procedure			
CO_2	−	+	−
Laparotomy	−	−	−
Anesthesia	−	−	−
24 h after each procedure			
CO_2	++	++	++
Air	−	−	+
Helium	−	−	+
Laparotomy	−	−	−
Anesthesia	−	−	−
72 h after each procedure			
CO_2	+	+	+
Laparotomy	−	−	+
Anesthesia	−	−	−

−, none or slight; +, moderate; ++, marked

injury to the hepatic vascular endothelium may be associated with an increase in the incidence of liver metastasis. In hepatic ischemia-reperfusion injury, the damage to the ischemic lobe creates a favorable condition for liver metastasis or intrahepatic tumor growth [2], and the expression of adhesion molecules promotes the establishment of liver metastasis.

Several studies have previously shown that the intraabdominal insufflation of CO_2 causes a marked and rapid decrease (35% to 84%) in portal blood flow [8, 12, 14]. This reduction correlates with the degree of intraabdominal pressure, and may be caused by either mechanical compression of the thin-walled portal vein or hypercapnia-induced vasoconstriction; by contrast, the hepatic arterial flow appears to be less compromised. In this study, intraabdominal pressure was kept at 8–10 mmHg after insufflation in the pneumoperitoneum group. This high level of intraabdominal pressure must influence portal blood flow, and it may serve to induce hepatic ischemia after creation of the pneumoperitoneum.

In the CO_2 pneumoperitoneum group, we observed distinct morphological changes to the hepatic vascular endothelium, including (a) dilatation of intercellular clefts, (b) irregular arrangement of the endothelial cells, and (c) a rough surface. On day 1 after CO_2 pneumoperitoneum, these changes were remarkable. However, on day 1 after air and helium pneumoperitoneum, the changes to the hepatic vascular endothelium were comparatively slight. Our results suggest that these morphological changes are caused not only by the reduction in portal blood flow induced by intraabdominal high pressure but also by the CO_2 itself.

Shuto et al. [15] have shown that metabolic acidosis occurs after both helium and CO_2 pneumoperitoneum, but hypercapnia occurs only after CO_2 pneumoperitoneum. Furthermore, several studies have shown that whereas helium pneumoperitoneum does not cause either hypercapnia or acidic changes, CO_2 pneumoperitoneum induces both hypercapnia and acidosis[1, 3, 9–11]. Therefore, morphological changes to the hepatic vascular endothelium may be a result of a combination of CO_2 absorption and the hepatic ischemia induced by the CO_2 pneumoperitoneum.

In conclusion, morphological changes to the hepatic vascular endothelium occur after CO_2 pneumoperitoneum. Clinical studies are needed to investigate whether these changes are associated with the enhancement of liver metastasis after laparoscopic colorectal surgery.

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Clinical Trial Note

Randomized Controlled Trial to Evaluate Laparoscopic Surgery for Colorectal Cancer: Japan Clinical Oncology Group Study JCOG 0404

Seigo Kitano[1], Masafumi Inomata[1], Akihiro Sato[2], Kenichi Yoshimura[3] and Yoshihiro Moriya[4] for the Colorectal Cancer Study Group (CCSG) of Japan Clinical Oncology Group

[1]Department of Gastroenterological Surgery, Oita University Faculty of Medicine, Oita, [2]Medical Oncology Division, [4]Colorectal Surgery Division, National Cancer Center Hospital and [3]Statistics and Cancer Control Division, National Cancer Center Research Center for Cancer Prevention and Screening, Tokyo, Japan

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A randomized controlled trial was started in Japan to evaluate whether laparoscopic surgery is the optimal treatment for colorectal cancer. Patients with T3 or deeper carcinoma in the colorectum without transverse and descending colons are pre-operatively randomized to either open or laparoscopic colorectal resection. Surgeons in 24 specialized institutions will recruit 818 patients. The primary end-point is overall survival. Secondary end-points are relapse-free survival, short-term clinical outcome, adverse events, the proportion of conversion from laparoscopic surgery to open surgery, and the proportion of completion of laparoscopic surgery.

Key words: colorectal cancer – laparoscopic surgery – randomized controlled trial

INTRODUCTION

The benefits of laparoscopic surgery (LAP) in comparison with open surgery (OPEN) have been suggested with respect to decreased morbidity, decreased pain, faster recovery and shorter hospital stay (1–4). However, the long-term survival of LAP for colorectal cancer is still unclear, especially for advanced colorectal cancer requiring extended lymphadenectomy. Thus, we designed a study which investigates whether LAP is suitable for advanced colorectal cancer with respect to survival and post-operative morbidity. The Clinical Trial Review Committee of the Japan Clinical Oncology Group (JCOG) approved the protocol in September 2004, and the study was activated in October 2004.

PROTOCOL DIGEST OF THE JCOG 0404

PURPOSE

To evaluate LAP in comparison with OPEN for T3 and T4 colorectal cancer.

Seigo Kitano, study chair; Masafumi Inomata, study coordinator; Akihiro Sato, protocol coordinator; Kenichi Yoshimura, study statistician; Yoshihiro Moriya, group chair
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STUDY SETTING

A multi-institutional (24 specialized centers), randomized controlled trial.

RESOURCES

Health and Labour Sciences Research Grants for Clinical Research for Evidenced Based Medicine, Clinical Cancer Research and Grants-in-Aid for Cancer Research (14S-4, 17S-5), from the Ministry of Health, Labour and Welfare, Japan.

END-POINTS

The primary end-point is overall survival. Secondary end-points are relapse-free survival, short-term clinical outcomes, adverse events, the proportion of conversion from LAP to OPEN and the proportion of completion of LAP. All LAP cases which require skin incision >8 cm are counted as a conversion to OPEN, except for those in which retrieval of the resected specimen alone requires this length of incision. The completion of LAP is defined as completion of the curative operation without conversion to OPEN. The short-term clinical outcomes are proportion of use of analgesics, duration from operation to flatus, highest body temperature during hospitalization and highest body temperature during the 3 days after the operation.

Eligibility Criteria

Tumors are staged according to the TNM classification system.

Inclusion criteria. For inclusion in the study, patients must fulfill the following requirements pre-operatively: (i) histologically proven colorectal carcinoma; (ii) tumor located in the cecum, ascending colon, sigmoid colon or rectosigmoid colon; (iii) T3 or deeper lesion without involvement of other organs; (iv) without multiple lesions other than carcinoma *in situ*; (v) cancer classified as N0–2 and M0, according to the TNM classification system; (vi) tumor size ≤8 cm; (vii) no bowel obstruction; (viii) age ≥20 and <75 years; (ix) sufficient organ function; (x) no history of gastrointestinal surgery; (xi) no history of chemotherapy or radiotherapy; and (xii) provide written informed consent.

Exclusion criteria. Exclusion criteria are as follows: (i) synchronous or metachronous (within 5 years) malignancy other than carcinoma *in situ*; (ii) severe pulmonary emphysema, interstitial pneumonitis or ischemic heart disease; (iii) pregnant or lactating women; (iv) severe mental disease; and (v) continuous systemic steroid therapy.

Randomization

By telephone or fax to the JCOG Data Center after confirmation of the inclusion/exclusion criteria, the patients are randomized by the minimization method of balancing the arm according to the location of tumor and institution.

Quality Control of Surgery

To control the quality of the operation, we limit the operator to accredited surgeons and perform central review of the surgical procedure by photograph in all patients and by videotape in arbitrarily selected patients in both the LAP and OPEN arms. All operations are done or directly supervised by certified surgeons. Surgeons who have experience of at least 30 cases of open surgery in the OPEN arm, and experience of at least 30 case of both open and laparoscopic surgeries in the LAP arm are certified by the study chair.

Treatment Method

In both arms, resection of the colon or rectum with D3 lymphadenectomy is performed according to the Japanese Classification of Colorectal Carcinoma (5). In the LAP arm, pneumoperitoneal and intracorporeal approaches are used to explore the abdomen, mobilize the colon, identify critical structures and ligate the vascular pedicle. Mobilization of the colon and identification of critical structures are performed by the pneumoperitoneal approach only. Resection of the colon, ligation of the vascular pedicle and reconstruction are performed by the pneumoperitoneal approach or the intracorporeal approach via a small incision (≤8 cm). Hand-assisted laparoscopic surgery is permitted, but sliding window and moving window methods are not permitted.

Additional Treatment

In the case of pathological stage III colorectal carcinoma, three cycles of adjuvant chemotherapy with fluorouracil (500 mg/m^2 by bolus infusion on days 1, 8, 15, 22, 39 and 36) and l-leucovorin (250 mg/m^2 by 2 h drip infusion on days 1, 8, 15, 22, 39 and 36) are administered.

Follow-up

Patients are observed by their surgeon every 4 months for the first 2 years and then every 6 months for 3 years after operation. Blood tests, abdominal computed tomography and plain chest X-ray are carried out at each visit.

Study Design and Statistical Method

This trial is designed to evaluate the non-inferiority of LAP to standard OPEN in terms of overall survival. If the overall survival is equivalent, LAP will be the preferred treatment. The null hypothesis to be tested is that the hazard ratio for the primary end-point with the LAP technique, as compared with the OPEN technique, was 1.366. The planned sample size is 818, 409 cases per arm, with 5 years of follow-up after 3 years of accrual. This provides 80% power to reject the null hypothesis when the survival is equivalent. This calculation assumed that there was a 5-year survival of 75% among patients treated with the OPEN technique.

Interim Analysis and Monitoring

Interim analysis is planned to take place twice, taking multiplicity into account by the Lan-Demets method with O'Brien and Fleming type boundaries. The Data and Safety Monitoring Committee (DSMC) of the JCOG will independently review the interim analysis report and consider stopping the trial early. In-house interim monitoring will be performed by the Data Center to ensure data submission and study progress. The monitoring reports will be submitted to and reviewed by the CCSG every 6 months.

Participating Institutions (from North to South)

Jichi Medical School Omiya Medical Center, National Cancer Center Hospital East, Juntendo University Urayasu Hospital, Toho University School of Medicine Sakura Hospital, National Cancer Center Hospital, Kyorin University School of Medicine, Keio University Hospital, Tokyo Medical and Dental University, Toranomon Hospital, Toho University School of Medicine Ohashi Hospital, Kitasato University East Hospital, Teikyo University school of Medicine Mizonokuchi Hospital, Ishikawa Prefectural Central Hospital, Showa University Northern Yokohama Hospital, Nagano Municipal Hospital, Shizuoka Cancer Center, Fujita Health University, Osaka University Graduate School of Medicine Faculty of Medicine, Osaka City General Hospital, Osaka Medical College, Hiroshima University

Faculty of Medicine, Shikoku Cancer Center, Kurume University School of Medicine, Oita University Faculty of Medicine.

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Endoluminal surgery

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Usefulness of transanal endoscopic surgery for carcinoid tumor in the upper and middle rectum

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Abstract
Background: This study evaluated the indications and outcome for transanal endoscopic surgery (TES) used to manage rectal carcinoid tumor as compared with those of conventional transanal local resection (TAR).
Methods: The retrospective study subjects were 28 patients with rectal carcinoid tumor treated by TES (n = 17) or TAR (n = 11) between January 1995 and December 2001. Patient and tumor characteristics, operative results, and postoperative outcomes were compared between the two groups.
Results: The distance from the anal verge to the distal tumor margin in the TES group (range, 4–12 cm; median, 6.8 cm) was significantly greater than in the TAR group (range, 3–6 cm; median, 4.5 cm) (p = 0.001). The median tumor diameter was 5.5 mm (range, 3–11 mm) in the TES group and 5.0 mm (range, 3–8 mm) in the TAR group, showing no statistical difference. Microscopically, resected specimens in both groups were typical carcinoid tumors restricted to the submucosal layer. No recurrence was noted in either group.
Conclusion: Whereas TES is useful for patients with small rectal carcinoid tumor of typical histology within the submucosal layer in the upper and middle rectum, TAR is effective for accessing the lower rectum.

Key words: Rectal carcinoid — Local resection — TEM — TES

Although carcinoid tumor of the rectum is rare, increasing numbers of rectal carcinoid tumor are being reported [11, 18]. Among the gastrointestinal carcinoid tumors, rectal carcinoid is the second most frequent lesion in the United States and Europe (27.4%) [11] and the most frequent lesion in Japan (36.2%) [18]. The recent marked increases in the number of rectal carcinoids may be attributable to improved endoscopic diagnosis and increased awareness of carcinoid tumors [11].

Local rectal excision is recommended as a curative and minimally invasive treatment for most rectal carcinoid tumors smaller than 2 cm in diameter and restricted to the submucosal layer [6, 20]. Conventional transanal local resection (TAR) often is used to remove a lesion in the lower rectum [14], but other procedures are been required for removal of a lesion in the middle or upper rectum. Thus, transanal endoscopic microsurgery (TEM) was developed by Buess et al. [3] in 1984. Since then, several modified TEMs, such as insufflation video endoscopic TEM (insufflation VTEM) [21] and gasless VTEM [2, 12], have been developed. One Japanese group of TEM researchers has applied the generic term "transanal endoscopic surgery (TES)" to both the original and modified TEM procedure [7]. Recently, TES has been performed worldwide. However, TES for rectal carcinoid tumor has not been evaluated in detail with respect to postoperative results.

We conducted a study aimed at clarifying the usefulness of TES for the treatment of rectal carcinoid tumor. Our approach was to compare TES indications and results with those of TAR for this rectal tumor.

Patients and methods

Between January 1995 and December 2001, 28 patients (11 men and 17 women; average age, 54.4 years; range, 36–70 years) with rectal carcinoid tumor underwent transanal local treatment. Whereas 17 of these patients underwent TES, 11 patients underwent conventional TAR. Their clinical records were reviewed for age, sex, tumor diameter and location, tumor distance from the anal verge to the distal margin of the tumor, central umbilication, operation time, blood loss, postoperative complications, recurrence, and histologic findings.

Treatment in the 28 cases followed the schema shown in Fig. 1. During the study period, no open surgery for this disease was performed. The patients in the TES group had surgery under gen-

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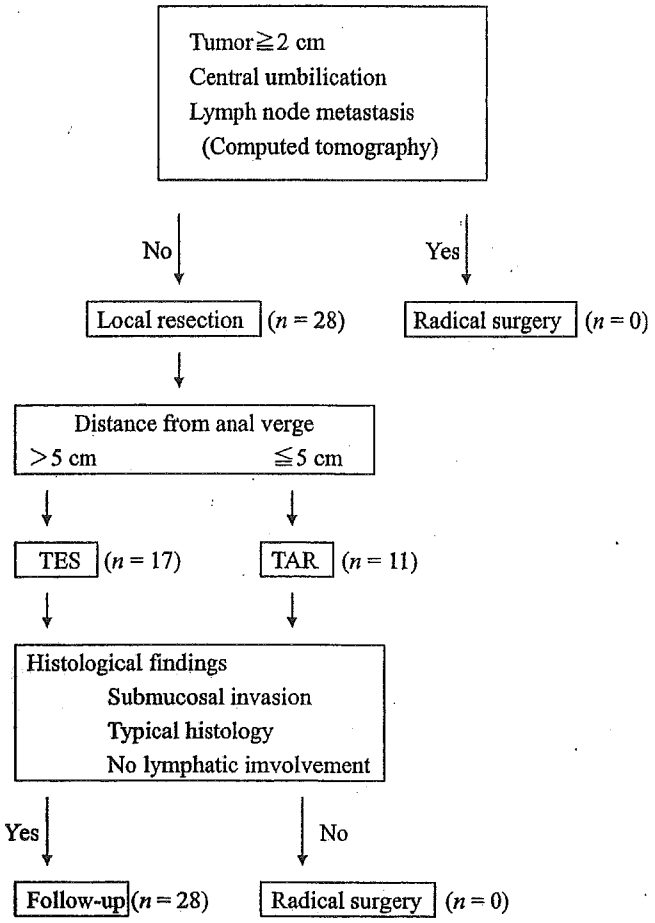

Fig. 1. Strategy for the treatment of rectal carcinoid tumor at the authors' institution as applied to the study patients. TES, transanal endoscopic surgery; TAR, transanal local resection.

eral anesthesia. The TES procedures were performed as described elsewhere [2, 7, 12, 21]. In short, TES is transanal surgical procedure using a rectal tube with a laparoscope. Depending on the tumor location, the lithotomy, prone, or lateral position of the patient was used. Gentle rectal dilation was performed, and a rectoscope tube (length, 12 or 20 cm long; width, 40 mm) was inserted. The scope position was adjusted so that the lesion occupied the center of the field of view. Then the standard 10-mm laparoscope was connected to a video system. The binocular stereoscopic eyepiece system was not used. Carbon dioxide (CO_2) insufflation was used until a needle-tip knife was used to outline a 5-mm margin of normal mucosa circumferentially. Adrenaline 1:100,000 solution was injected into the submucosal layer for lifting of the lesion and hemostasis. The carcinoid tumor was resected in a partial thickness under laparoscope.

The first 3 patients were treated with CO_2 insufflation during the operation (insufflation VTEM [21]), and the 14 subsequent patients were treated without CO_2 insufflation after circumferential marking (gasless VTEM [2, 12]). In the TAR group, the conventional Parks approach [14] was performed with the patients under spinal anesthesia. Park's anorectal retractor was inserted into the rectum and opened to expose the tumor. The carcinoid tumor was resected in a partial thickness, as visualized by the eye. No additional radical surgery was performed in either group.

The patients were examined every 3 months for 2 years after the operation, and every 6 months thereafter. The mean follow-up period was 47.1 months (range, 12–96 months) for the TES group and 23.8 months (range, 6–49 months) for the TAR group. Differences between the groups were analyzed using the Mann–Whitney *U* test or Fisher's exact test as appropriate. A p value less than 0.05 was considered statistically significant.

Results

The two groups were comparable in terms of age, sex ratio, and tumor characteristics including size, location, and gross appearance (Table 1). The median tumor diameter was 5.5 mm (range, 3–11 mm) in the TES group and 5.0 mm (range, 3–8 mm) in the TAR group, showing no statistical difference. The distance from the anal verge to the distal tumor margin in the TES group was significantly greater than in the TAR group (Table 1). The distribution of this distance is shown in Fig. 2. In the TES group, the tumors were located 4 to 12 cm from the anal verge, whereas in the TAR group, the tumors were 3 to 6 cm from the anal verge (median, 6.8 vs 4.5 cm).

The operative findings and postoperative courses are shown in Table 2. The mean operation time was greater in the TES group than in the TAR group (86 vs 35 min). Blood loss was minimal for all the patients. The postoperative course was satisfactory except for one patient treated by insufflation TEM in whom retroperitoneal emphysema developed because of a full-thickness excision. However, this complication improved conservatively within 9 days. Three patients in the TES group experienced transient soiling because of the rectoscope tube width (40 mm), but recovered completely within 1 week. The mean follow-up period for all the patients was 38 months (range, 6–96 months). No recurrence or tumor-related mortality was noted.

The pathologic findings for the carcinoid tumors resected by transanal local resection are shown in Table 3. In both groups, microscopic examination showed that all resected specimens were typical carcinoid tumors restricted to the submucosal layer. Carcinoid cells were not seen in the lateral or vertical margin of any specimen. Lymphatic involvement was not observed in any patient. Vascular involvement was noted in only one patient in the TAR group. Ulceration was not observed in any patient.

Discussion

Our results showed TES to be superior to TAR in terms of tumor distance from the anal verge, but inferior to TAR in terms of operation time. The findings showed TES be safe because there was no tumor-related death and no persistent complication. In addition, recurrence was absent. Thus, as with TAR used for the lower rectum, TES is useful for the middle or upper rectum of patients with rectal carcinoid tumor of typical histology confined to the submucosa.

The prognostic factors for rectal carcinoid are reported to be tumor size [6, 10, 16], infiltration of the muscularis propria [10, 16], central umbilication or ulceration of the tumor [6], atypical histology [8, 17], and lymphatic invasion [5]. The incidences of distant metastasis for rectal carcinoid tumors with diameters less than 1 cm, 1 to 2 cm, and exceeding 2 cm are 0% to 3%, 0.4% to 27%, and 45% to 75%, respectively [6, 8, 10, 20]. It has been suggested that a tumor larger than 2 cm

Table 1. Patient and tumor characteristics in the two study groups

	TES (n = 17)	TAR (n = 11)	p Value
Patients			
Age (years)[a]	55 (38–70)	54 (36–69)	NS[b]
Sex ratio (M:F)	8:9	3:8	NS
Tumor			
Size(mm)[a]	5.5 (3–11)	5.0 (3–8)	NS[b]
Location (A/P)	10/7	8/3	NS
Distance from anal verge (cm)[a]	6.8 (4–12)	4.5 (3–6)	0.001[b]
Central umbilication (yes/no)	0/17	0/11	NS

TES, transanal endoscopic surgery; TAR, transanal local resection; A, anterior wall; P, posterior wall; NS, not significant, Fisher's exact test
[a] Values are median (range)
[b] Mann-Whitney U test

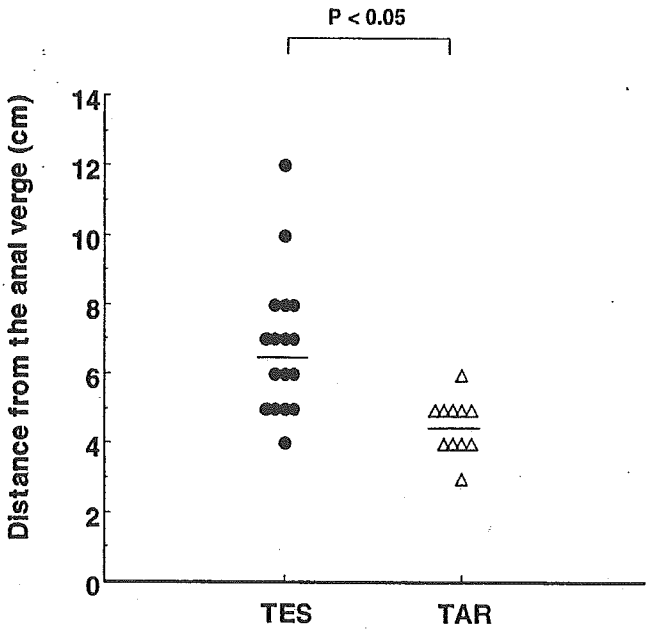

Fig. 2. Distance of rectal carcinoids from the anal verge in the two study groups (solid lines mark the mean distance). TES, transanal endoscopic surgery; TAR, transanal local resection.

in diameter can be regarded as malignant. Previous studies have suggested that complete local resection of a carcinoid tumor is associated with a low rate of local recurrence [6, 15], whereas residual macroscopic disease is associated with a poor outcome [1].

Application of endoscopic resection is not common. Endoscopic mucosal resection is reserved for complete resection of carcinoid tumors because these tumors are sessile and develop from the deep mucosal layer toward the submucosa [9, 13]. However, Stinner et al. [20] reported that radical resection for rectal carcinoid should be performed only after accurate histologic study of a local resected specimen. These authors also indicated that when hematogenous metastasis is absent, radical surgery should be performed for patients with a lesion diameter larger than 2 cm [20]. To the contrary, several authors suggest that radical surgery is inappropriate even if the lesion diameter exceeds 2 cm [8, 15, 16].

Currency, complete local excision is widely accepted for small rectal carcinoids. All the tumors in our study were relatively small (≤ 11 mm) without central umbilication. Postoperative histologic study pointed to a good prognosis for all the patients, and no additional resection was required. To date, there has been no recurrence in any case, and we consider transanal local excision to have been sufficient in all cases. Nevertheless, there have been several reports of malignant potential of tumors smaller than 1 cm [5, 8, 15, 16, 19]. Therefore, even where local excision is indicated, the possibility of metastasis should be taken into account.

Conventional TAR is a useful, minimally invasive procedure for local resection of rectal tumor [14]. However, obtaining sufficient surgical field is sometimes difficult. In addition, TEM can be a useful and safe procedure for rectal tumor, unreachable via the conventional TAR approach. As compared with conventional TAR, TEM provides a superior intraluminal operation field in the middle and upper rectum because the surgical field is broadened by CO_2 insufflation [4]. Although gasless VTEM is slightly inferior to the original TEM with CO_2 insufflation for obtaining a good intraluminal operative field [12], we used gasless VTEM after encountering a patient treated by insufflation VTEM in whom retroperitoneal emphysema developed.

Most rectal carcinoid tumors are located in the middle portion of the rectum [6, 19], and TES is superior to TAR for treating them. Thus, selection of the appropriate treatment depends on the distance from the anal verge to the distal tumor margin: TAR is performed when the distance is 5 cm or less, and TES is performed when it is greater than 5 cm. Digital examination remains the simplest means of determining that distance. The indication for local rectal excision is benign rectal carcinoid tumor that is small (diameter, < 20 mm) and localized within the submucosal layer. Tumors 20 mm in diameter or smaller can be treated effectively using the gasless technique.

In conclusion, TES is safe and useful for patients with a histologically typical small rectal carcinoid tumor that lies within the submucosal layer of the upper or middle rectum.

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Table 2. Operative details and postoperative course in the two study groups

	TES (n = 17)	TAR (n = 11)	p Value
Operative details			
Operation time (min)[a]	86 (45–140)	35 (20–55)	0.001[b]
Blood loss	Minimum	Minimum	NS
Postoperative course			
Complications			
Bleeding	0	0	NS
Stenosis	0	0	NS
Retroperitoneal emphysema	1	0	NS
Recurrence	0	0	NS

TES, transanal endoscopic surgery; TAR, transanal local resection; NS, not significant (Fisher's exact test)
[a] Values are median (range)
[b] Mann-Whitney U test

Table 3. Histologic findings for the two study groups

	TES (n = 17)	TAR (n = 11)	p Value
Histologic type			
Typical/atypical	17/0	11/0	NS
Depth of invasion			
Submucosa/muscularis propria	17/0	11/0	NS
Lateral margin			
Positive/negative	0/17	0/11	NS
Vertical margin			
Positive/negative	0/17	0/11	NS
Lymphatic involvement			
Yes/no	0/17	0/11	NS
Vascular involvement			
Yes/no	0/17	1/10	NS
Ulceration			
Yes/no	0/17	0/11	NS

TES, transanal endoscopic surgery; TAR, transanal local resection; NS, not significant (Fisher's exact test)

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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Liver metastasis and ICAM-1 mRNA expression in the liver after carbon dioxide pneumoperitoneum in a murine model

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Abstract

Background: Liver metastasis of colorectal malignancies is an important prognostic factor. Several studies have demonstrated that carbon dioxide (CO_2) pneumoperitoneum enhances liver metastasis in animal models. Little is known about intercellular adhesion molecule-1 (ICAM-1) and tumor necrosis factor-alpha (TNF-(α) mRNA expression in the liver after CO_2 pneumoperitoneum.

Methods: Forty-five male BALB/c mice were randomly divided into three groups after intra-splenic tumor cell (colon 26) inoculation and the following procedures were performed: CO_2 pneumoperitoneum (n = 15), open laparotomy (n = 15), and anesthesia alone (n = 15). On day 7 after each procedure, the livers were excised and the number and diameter of the tumor nodules and the cancer index score were determined. Another 90 male BALB/c mice were randomly divided into three groups as described above, and they underwent each procedure (n = 30 each). After each procedure, the livers were excised on days 0, 1, 3, and ICAM-1 and TNF-α mRNA expression were examined by real-time RT-PCR using SYBR Green I.

Results: The number of tumor nodules and the cancer index score were larger in the CO_2 pneumoperitoneum group than in the control group ($p < 0.05$). The mean diameter of the tumor nodules was not different among the three groups. The expression of ICAM-1 in the CO_2 pneumoperitoneum group was higher than that in the other groups on day 1 ($p < 0.05$), and the TNF-α mRNA was higher than that in the control group on day 1 ($p < 0.05$).

Conclusions: CO_2 pneumoperitoneum enhances liver metastasis compared with anesthesia alone, and ICAM-1 expression in the liver after the pneumoperitoneum plays an important role in establishing liver metastasis in a murine model.

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Key words: pneumoperitoneum — Liver metastasis — Adhesion molecules — Murine model — Real-time RT-PCR — ICAM-1

The liver is the most frequent site of tumor metastasis in colorectal carcinoma, and liver metastasis is the most important prognostic factor in patients with primary colorectal cancer. Recently, the use of laparoscopic colorectal surgery has increased because it has become less invasive and because early recovery has become possible. Several randomized controlled trials (RCTs) showed better early short-term outcomes of laparoscopic colectomy [2, 26], but few RCT have been performed with regard to long-term outcomes [16, 17, 24], and the influence of CO_2 pneumoperitoneum on cancer progression is still controversial. In experimental studies, Ishida et al. and Gutt et al. have demonstrated that CO_2 pneurnoperitoneum enhances liver metastasis, and these researchers concluded that hepatic ischemia by CO_2 insufflations may be one of the causes of this phenomenon [7, 8, 10]. Furthermore, previous studies have demonstrated that CO_2 pneumoperitoneum reduces portal blood flow [11, 20, 21].

An important first step in establishing liver metastasis is for free tumor cells to adhere to the hepatic vascular endothelial surface. Yadav et al. have shown that ICAM-1 mediates reperfusion injury in the warm ischemic mouse liver [27]. Alexiou et al. have demonstrated that the serum level of ICAM-1 may reflect tumor progression and metastasis in colorectal cancer patients [1]. However, the expression of ICAM-1 and TNF-α mRNA in the liver after CO_2 pneumoperitoneum has not been clearly established.

In the present study, we investigated the effect of CO_2 pneumoperitoneum and the role of local ICAM–1 expression in establishing liver metastasis in an animal model.

Materials and methods

Animals

All animals were kept under standard laboratory conditions (temperature 20–24°C, relative humidity 50–60%, 12-h light/dark cycle) and were given a standard laboratory diet with free access to water *ad libitum* before and after surgery. All experiments were performed according to the guidelines for animal experimentation of Oita University. This study was performed using a murine pneumoperitoneum model [22]. A total of 135 male BALB/c mice, preserving T- and B-cell immunity, aged 6–8 weeks and weighting 20–24 g, were used. All surgical procedures were performed under ether anesthesia.

Tumor cell line

A mouse colon carcinoma cell line, colon 26 [13, 25], was maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum and penicillin-streptomycin at 1000 IU/ml and incubated in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. For the establishment of liver metastases, tumor cell suspension of 1×10^6 cells/0.1 ml in PBS was used.

Operative procedure

All surgical procedures was done under general anesthesia induced by diethyl ether inhalation. A total of 135 BALB/c mice (including both experiments 1 and 2) were divided into three operative groups. In the pneumoperitoneum group ($n = 45$), mice were treated with CO_2 pneumoperitoneum at 8–10 mmHg for 60 min as previously reported [10]. Pneumoperitoneum condition was created by following procedure.

(a) A 22-gauge intravenous cannula was inserted into the left lower quadrant and used as an insufflation needle.
(b) A 20-gauge intravenous cannula was inserted into the right lower quadrant and used to measure intraperitoneal pressure.
(c) A disposable syringe to inject the gas was used as the insufflator. A syringe pump was used for continuous insufflation, and intraperitoneal pressure was measured as the distance between the right and left water levels in the U-shaped tube. In the laparotomy group ($n = 45$), a 3-cm abdominal midline incision was made, and the laparotomy condition was maintained for 60 min. In the control group ($n = 45$), only diethyl ether anesthesia was performed for 60 min.

Experiment 1 Induction of liver metastasis using a murine intra-splenic tumor cell inoculation model.

Forty-five mice were used in this experiment. A 5-mm skin incision was made at the left back side, and the spleen was pulled out gently. Then, we injected intra-splenically 1×10^6 tumor cells/0.1 ml in PBS using a 30-gauage needle. At 2 min after the tumor-cell injection, the spleen was excised, and the skin was then closed in layers using nonabsorbable interrupted sutures. Immediately after this procedure, the mice were divided into three groups. In the pneumoperitoneum group ($n = 15$), we administered CO_2 pneumoperitoneum at 10 mmHg for 60 min. In the laparotomy group (n = 15), a 3-cm midline laparotomy was performed and maintained for 60 min. The skin incision was closed by interrupted sutures using 4-0 nylon. In the control group ($n = 15$), we administered general ether anesthesia for 60 min. All mice were killed on day 7 after each procedure, and we evaluated the numbers, diameter, and cancer index score [7] of metastatic nodules. Each cancer nodule on the liver surface was scored using the cancer index as shown in Table 1, and the total cancer index for each mouse was calculated as the sum of the cancer indices of each nodule.

Experiment 2 Expressions of ICAM-1 and TNF-α mRNA in the liver

Ninety mice were randomized and divided into three groups: the pneumoperitoneum group, the open laparotomy group, and the control group (n = 30 each). Each operative procedure was performed by the same methods used in Experiment 1. After each procedure, the animals' livers were excised on days 0, 1, and 3, snap-frozen in liquid nitrogen, and stored at −80°C until total RNA was extracted. Total mRNA was isolated from the liver by the acid guanidinium thiocyanate–phenol–chloroform extraction procedure [3]. The cDNA was synthesized by reverse transcription from 2.5 μg of total RNA. The cDNA specific for ICAM-1, TNF-α, was measured by PCR. The mRNA of β-actin was measured as the internal control. All PCR reactions were measured by a real-time PCR method using the Light Cycler System (Roche Diagnostics, Mannheim, Germany), and the detection was performed by measuring the binding of the fluorescent dye SYBR Green I to double-stranded DNA. The PCR reactions were set up in microcapillary tubes in a total volume of 20 μl. A master mix of the following reaction components for ICAM-1 and β-actin was prepared to the indicated final concentration: 8.6 μl water, 2.4 μl $MgCl_2$, 1 μl forward and reverse primers, and 2 μl Light Cycler Fast Start DNA Master SYBR Green I (Roche, Mannheim, Germany). A master-mix of the following reaction components for TNF-α was prepared to the indicated final concentration: 9.4 μl water, 1.6 μl $MgCl_2$, 1μl forward and reverse primers, and 2 μl Light Cycler Fast Start DNA Master SYBR Green I. Table 2 presents an overview of primer sequences and factor-specific amplification conditions with the single fluorescence measurement were used in this study. The following general real-time PCR protocol was used: a denaturation program (95 °C for 10 min), followed by an amplification program that was repeated 40 times (Table 2), a melting curve program (60–99°C with a heating rate of 0.1°C /sec and continuous fluorescence measurements), and finally a cooling program down to 40°C. The PCR product sizes for ICAM-1, TNF-α, and β-actin were 326 bp, 349 bp, and 189 bp, respectively. The relative fluorescence of each mRNA was normalized to that of β-actin for semiquantification.

Table 1. Cancer index scoring dependent on the diameter

Cancer index (score)	Diameter of nodule (mm)
1	< 5
2	5–10
3	> 10

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Differences between the mean of the control group and those of the treatment group were evaluated by analysis of variance (ANOVA) followed by the Tukey HSD multiple comparison test. The differences between the groups were regarded as significant when $p < 0.05$. All statistical calculations were performed using the Dr. SPSS (version 11.01) program for Windows computers.

Results

Experiment 1

The number of metastatic nodules was greater in the CO_2 pneumoperitoneum group than in the control group (15.82 ± 5.69 vs 8.80 ± 6.80, $p < 0.05$) (Fig. 1). However, the mean diameter of the tumor nodules was not significantly different among all groups (Fig. 2). The total cancer index in the CO_2 pneumoperitoneum group was higher than that in the control group (26.00 ± 9.76 vs 13.70 ± 11.26, $p < 0.05$) (Fig. 3). Both the number of metastatic nodules and the total cancer index were not significantly different between the CO_2 pneumoperitoneum group and the laparotomy group.

Table 2. Sequences of primers used for RT-PCR and amplification conditions with a single fluorescence measurement

Molecule		Primer sequence (5′–3′)	Real-time PCR cycling conditions (sec/°C)		
			Denaturation	Annealing	Elongation
β-actin	Sense	TGG-AAT-CCT-GTG-GCA-TCC-ATG-AAA-C	15/95	10/55	14/72
	Antisense	TAA-AAC-GCA-GCT-CAG-TAA-CAG-TCC-G			
ICAM-1	Sense	TGC-GTT-TTG-GAG-CTA-GCG-GAC-CA	15/95	10/60	13/72
	Antisense	CGA-GGA-CCA-TAC-AGC-ACG-TGC-CAG			
TNF-α	Sense	CCA-CGT-CGT-AGC-AAA-CCA-C	10/95	10/60	7/72
	Antisense	TGG-GTG-AGG-AGC-ACG-TAG-T			

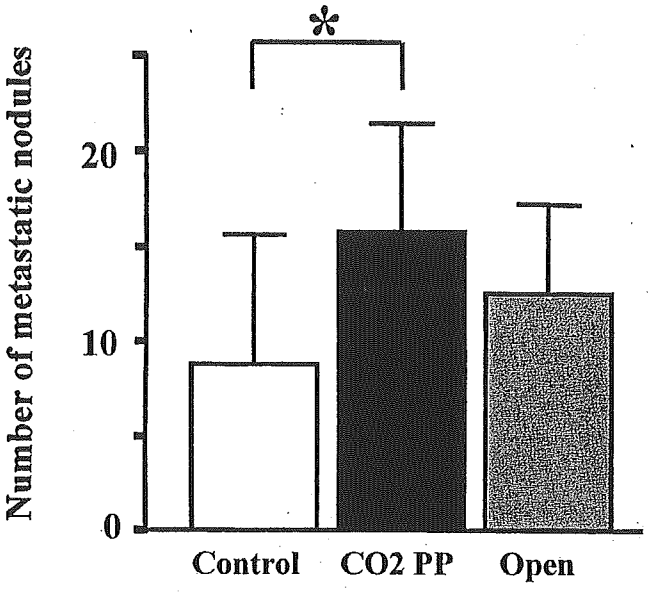

Fig. 1. The number of metastatic nodules on the liver surface was significantly greater in the CO_2 pneumoperitoneum group than in the control group. PP, pneumoperitoneum (*$p < 0.05$).

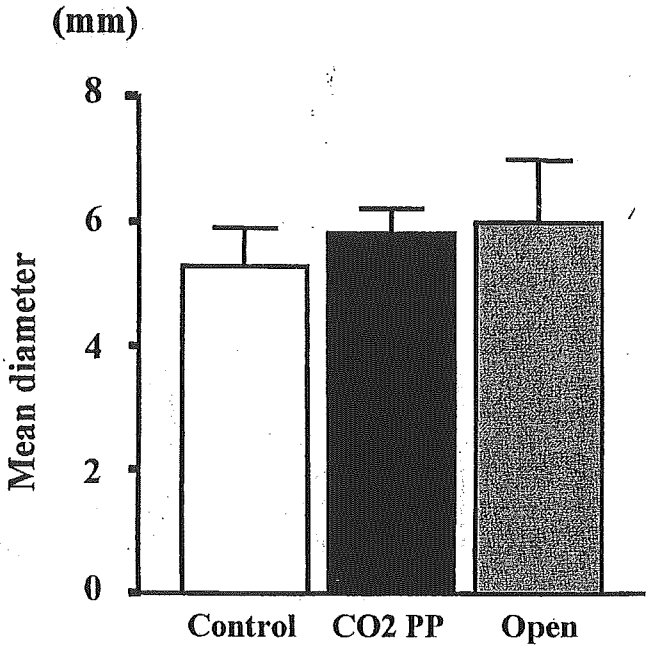

Fig. 2. The mean diameter of metastatic nodules was not significantly different among any of the groups.

Experiment 2

The expression of ICAM-1 mRNA in this study is shown in Fig. 4a. On day 0 (immediately after each procedure), the expression of ICAM-1 mRNA was not significantly different among the groups. On day 1, the expression of ICAM-1 mRNA was higher in the CO_2 pneumoperitoneum group than in the control and the open group (1.86 ± 0.56 vs 0.59 ± 0.42, 1.14 ± 0.40, $p < 0.05$). On day 3, the expression of ICAM-1 mRNA was higher in the CO_2 pneumoperitoneum and laparotomy groups than in the control group (2.03 ± 0.79, 1.62 ± 0.71 vs 0.74 ± 0.35, $p < 0.05$).

The expression of TNF-α mRNA in the CO_2 pneumoperitoneum group was higher than that in the control group on day 1 (0.177 ± 0.078 vs 0.025 ± 0.031, $p < 0.05$) (Fig. 4b). On days 0 and 3, there were no significant differences among any of the groups.

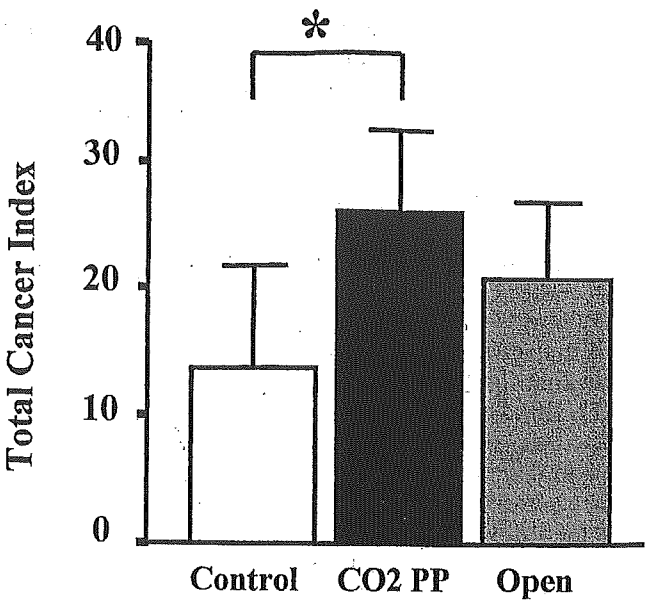

Fig. 3. The total cancer index score was significantly greater in the CO_2 pneumoperitoneum group than in the control group (*$p < 0.05$).

Discussion

In the present study, we examined the effect of CO_2 pneumoperitoneum on liver metastasis from the view-

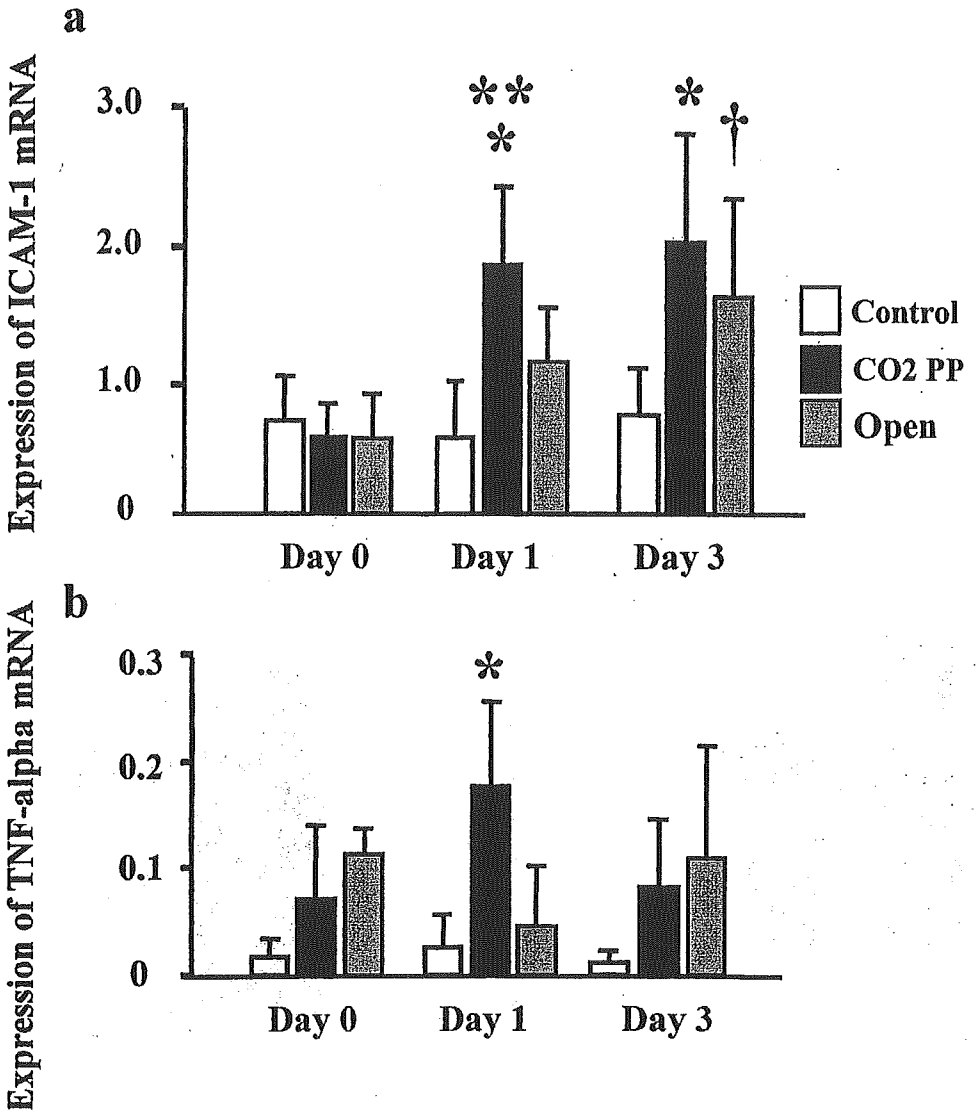

Fig. 4. **a** Expression of ICAM-1 mRNA and **b** TNF-α-mRNA in the liver measured by real-time RT-PCR. The relative expression of each mRNA is normalized to the expression of β-actin for semi-quantification. $p < 0.05$ CO_2 pneumoperitoneum versus control group, $^{**}p < 0.05$ CO_2 pneumoperitoneum versus open group, $\dagger p < 0.05$ laparotomy versus control group.)

points of intrahepatic adhesion molecule expression using a murine liver metastasis model. Our results showed that both the number of tumor nodules on the liver surface and the cancer index score were higher in the CO_2 pneumoperitoneum group than in the control group. The intrahepatic expression of ICAM-1 was higher in the CO_2 pneumoperitoneum group than in the other groups. Thus, in a murine model, CO_2 pneumoperitoneum enhanced liver metastasis, and the induction of ICAM-1 after CO_2 pneumoperitoneum may play an important role in the establishment of liver metastasis.

The first step in the establishment of liver metastasis is the adherence of free tumor cells to the hepatic vascular endothelium. Several studies have previously demonstrated that intrabdominal insufflation of CO_2 causes a marked and rapid decrease (35% to 84%) in portal blood flow [11, 20, 21]. In this study, portal blood flow may decrease because of the high pressure of CO_2 pneumoperitoneum, which was used in the previous study [10]. Doi et al. demonstrated that the condition of the ischemic lobe is favorable for liver metastasis [5], and the expression of adhesion molecules located in the vascular endothelium may play a crucial role in the establishment of liver metastasis. Our results showed an enhancement of liver metastasis and an increase of ICAM-1 and TNF-α after CO_2 pneumoperitoneum. It is possible that CO_2 pneumoperitoneum causes damage to the hepatic vascular endothelium by inducing liver ischemia.

ICAM-1 is a member of the immunoglobulin supergene family of adhesion molecules. Previous studies have demonstrated that ICAM-1 mediates hepatic reperfusion injury in the ischemic mouse liver [15, 27, 28]. Taketomi et al. demonstrated that the enhancement of inflammation in the liver is related to intrahepatic recurrence through ICAM-1 in patients with hepatocellular carcinoma [23]. The expression of ICAM-1 can be upregulated by inflammatory cytokines such as TNF-α and interleukin-1 [4, 18]. TNF-α is one of the most effective cytokines for inducing the expression of ICAM-1 on the endothelial cells [14, 19]. Gulubova et al. concluded that the enhanced expression of adhesion molecules in the liver sinusoids could direct the adhesion of new circulating tumor cells to the sinusoidal endothelium [6]. Kamei et al. demonstrated that TNF-α mRNA expression in the liver is higher 3-24 h after air pneumoperitoneum than after anesthesia alone [12]. In the

present study, we demonstrated the increases of ICAM-1 and TNF-α mRNA expression in the liver after CO_2 pneumoperitoneum. Also, the peak of TNF-α mRNA expression appeared earlier than that of ICAM-1 after CO_2 pneumoperitoneum. These results suggested that CO_2 pneumoperitoneum caused liver ischemia, and enhanced the expression of ICAM-1 induced by inflammatory cytokines such as TNF-α on the hepatic endothelium. Furthermore, the possibility that new circulating tumor cells adhered to the sinusoidal endothelium via ICAM-1 was shown.

Recently, in a clinical setting, randomized controlled trials regarding the long-term outcome after laparoscopic colorectal cancer surgery were reported [16, 17, 24]. A Spanish trial showed that the cancer-related survival rate in patients with stage III tumors was higher in the laparoscopic group than in the open group [16]. On the other hand, trials in the United States and Hong Kong showed that there were no significant differences in the survival rate between these two groups [17, 24]. In this experimental study, there were no significant differences in the incidence of liver metastasis between the CO_2 pneumoperitoneum group and the laparotomy group. However, we demonstrated that CO_2 pneumoperitoneum enhanced liver metastasis in comparison with the control group, and also that this effect might be associated with the induction of ICAM-1 and TNF-α in establishing liver metastasis. For the inhibition of liver metastasis after CO_2 pneumoperitoneum, it may be necessary to prevent portal blood flow depression by means of a gasless procedure or lower insufflation pressure [9, 10].

In conclusion, in a murine model, CO_2 pneumoperitoneum increased the expression of ICAM-1 and TNF-α in the liver and enhanced liver metastasis compared with anesthesia alone. Further investigation is necessary to clarify the mechanism and established a prevention method of liver metastasis after CO_2 pneumoperitoneum.

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へるす出版

3. 胃癌の外科治療

(1) 腹腔鏡下手術と縮小手術

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はじめに

診断技術の向上や検診制度の普及により，いまや胃癌の約50～60％が早期胃癌として発見される。早期胃癌のリンパ節転移率は低く，m癌2～5％，sm癌15～20％であり，転移があったとしても，ほとんどが胃周囲のリンパ節に限局している。このような早期胃癌に対し，無意味な拡大リンパ節郭清を避け，郭清を必要十分な範囲にとどめる縮小手術が実践されている。

一方，低侵襲手術，すなわち患者にやさしい手術として胃癌治療に導入された腹腔鏡下手術は，『胃癌治療ガイドライン』では臨床研究として位置づけられており，今後の普及・発展が期待されている。

本稿では，腹腔鏡下手術の中でもリンパ節郭清を伴う胃切除術を中心にその適応・手技・現状と問題点について述べる。

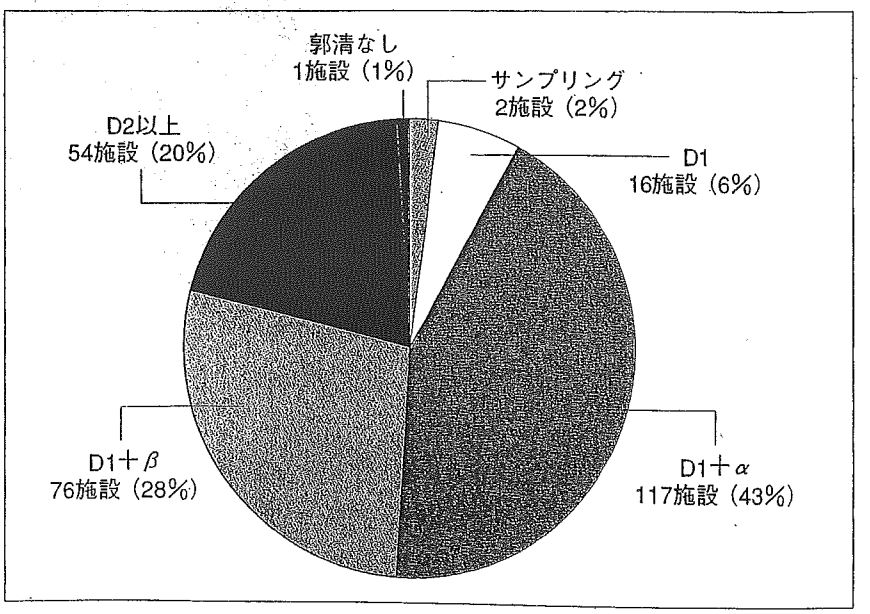
適　応

2004年4月に改訂された日本胃癌学会による『胃癌治療ガイドライン』では，腹腔鏡下手術は縮小手術と位置づけられ，日常診療の適応として早期胃癌に限っている(表1)[1]。これは，手技の安全性や長期成績がまだ十分示されていないためと思われる。日本内視鏡外科学会(JSES)の第7回アンケート調査結果によると(図1)，約79％の施設でD1＋βまでの郭清範囲にとどめており，D2リンパ節郭清を行っている施設は約20％であった[2]。しかしながら，手技の安定化に伴って適応が拡大する傾向にあり，前述のガイドラインでも臨床研究とし

表1　早期胃癌に対する治療

〔文献1）より引用〕

T1（M）	N0	N1
	ⅠA EMR（一括切除） （分化型，2.0cm以下， 陥凹型ではUL（－））	ⅠB 縮小手術B （2.0cm以下） 定型手術 （2.1cm以上）
T1（SM）	縮小手術A（上記以外）	
	ⅠA 縮小手術A （分化型，1.5cm以下） 縮小手術B（上記以外）	



〔文献2）より引用〕

図1　リンパ節郭清度

て進行癌T2N0，T2N1までを適応としている。

手　技

リンパ節郭清を伴う腹腔鏡補助下幽門側胃切除術(LADG)は1991年に始まった[3]。そのLADG(D1＋α)の一般的な手順は，腹腔鏡下操作にて①大網・胃結腸間膜を脾下極まで切開しつつ左胃大網動静脈を切離，②胃結腸間膜を切開し膵頭部を露出，③右胃大網静脈を根部にて切離(No.6リンパ節郭清，図2)，④胃十二指腸動脈を同定し右胃大網動脈を根部で切離，⑤十二指腸球部上方の無血管野からのアプローチにて右胃動静脈根部を同定し切離(No.5リンパ節郭清，図3)，⑥小網を切離し，胃膵間膜を切開，⑦左胃動静脈を同定し切離(No.7リンパ節郭清，図4)，⑧No.1，3リンパ節郭清，⑨腹腔鏡下操作が終了した後，剣状突起下(肥満例などではやや右側)に約5cmの小開腹，⑩小開腹創からの操作にて，直視下に，幽門側胃切除，⑪通常Billroth-Ⅰ法による再建，⑫再気腹の後，止血確認・洗浄，である。

LADGは急速に普及し，現在では噴門側胃切除術，胃全摘術などが行われるようになってきた。また，リンパ節郭清もD1＋αからD1＋βやD2へとより根治性を求める方向に向かっている。さらに腹腔内吻合，Roux-en Y吻合など，種々の手技の工夫が図られている。

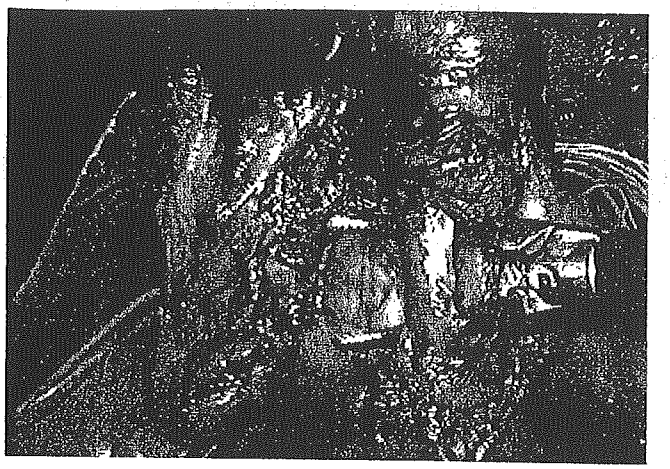


図2

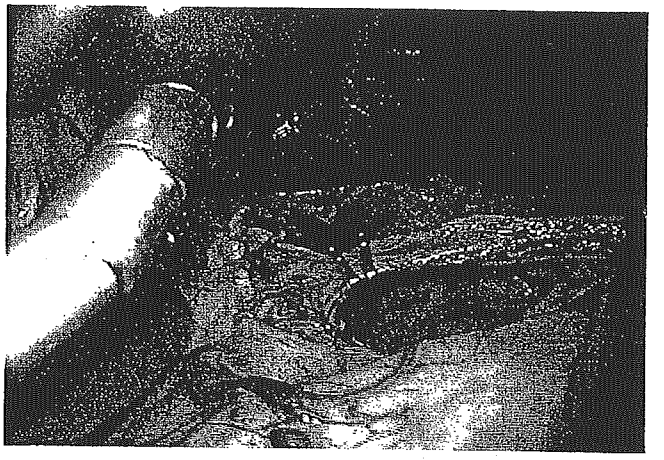


図3

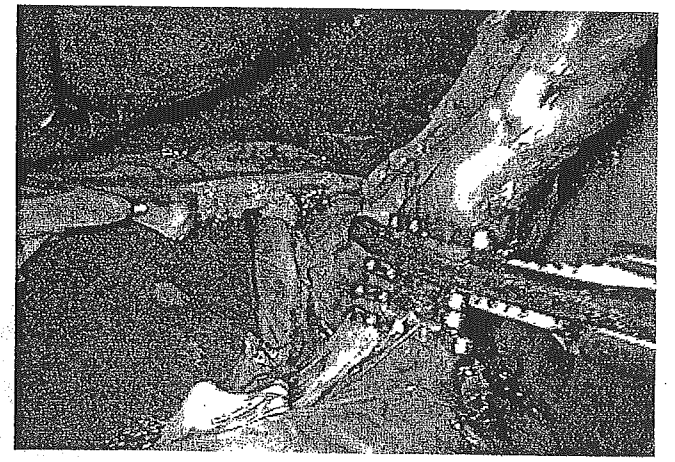


図4

現状と問題点

A. 現状

アンケート調査結果によると，1991年から2003年12月までに約7,800例の胃癌症例に腹腔鏡下手術が行われている(図5)。その中で，リンパ節郭清を伴うLADG症例がもっとも多く，次いでリンパ節郭清を伴わない胃局所切除術である。前述のごとく，LADGのリンパ節郭清範囲は，約43％の施設でD1＋α，約28％の施設でD1＋βの郭清が行われており，D2リンパ節郭清は約20％の限られた施設で行われているのが現状である。厚生労働省がん研究助成金研究班(北野班)の調査結果から，No.6と2群リ