and damage to normal tissue, PDT has the advantage over radiation because the photosensitizing agent used in PDT specifically accumulates in cancer cells as opposed to normal cells. PDT has become increasingly accepted as a treatment option for early lung cancers, gastric cancer, esophageal cancer, and others (12-14), and has also been clinically applied in poor surgical candidates with advanced cancers (15-17). Because laser energy penetrates to a depth of only 2 mm in tissues (18,19), the cytotoxicity of PDT is limited to the treatment of superficial lesions. Therefore, disseminated small nodules on the peritoneal surface could potentially be suitable targets for PDT.

Talaporfin, a second-generation photosensitizer, has several advantages over the first-generation photosensitizers, including a lack of prolonged photosensitization and a shorter interval (4-6 h) required between drug administration and laser light exposure (20,21) as compared to photofirin, a first-generation drug that requires 48-72 h. Talaporfin-mediated PDT has been examined in the treatment of several solid tumors (22,23). However, thus far, no experimental or clinical studies have used talaporfin-mediated PDT for the treatment of peritoneally disseminated gastric cancer. In this study, we examined the efficacy of talaporfin-mediated PDT in the treatment of peritoneal gastric cancer micrometastases using a mouse model.

Materials and methods

Cell culture. MKN-28 and MKN-45 EGFP (which was made by transfecting pEGFP-N1 into the MKN-45 cell line) cells were provided by the Department of Gastroenterological Surgery, Graduate School of Medicine, Osaka University, Osaka, Japan (24). MKN-7 cells were purchased from the Health Science Research Resources Bank (Osaka, Japan). The cells were cultured in RPMI-1640 medium with 10% FBS and antibiotics.

Photosensitizer and laser light delivery system. We used talaporfin sodium provided by Meiji Seika Kaisha Ltd., Tokyo, Japan. It was reconstituted in normal saline to a final concentration of 25 mg/ml and stored in the dark at -20˚C until used. The laser we used was a diode laser system (Diode laser, Panasonic Shikoku Electronics Co., Ltd., Yokohama, Japan). The treatment wavelengths used were 664 nm and 150 mW/cm^2.

Spectrophotometric analysis device. We examined the uptake and accumulation of talaporfin in peritoneal metastatic nodules, liver and small intestine using a semiconductor laser with a VLD-M1 spectrometer (M&M Co., Ltd., Tokyo, Japan) that exposed a laser light with a peak wavelength of 405±1 nm and a light output of 140 mW. The spectrometer and its accessory software (BW-Spec V3.24; B&W TEK, Inc., Newark, Del., USA) were used to analyze the spectrum waveform (25) and revealed an amplitude peak (relative fluorescent intensity) at a wavelength of 508 nm for MKN-45 EGFP, at 505 nm for autofluorescence and at 678 nm for talaporfin. The relative intensity of the talaporfin solution (with concentrations ranging from 0 to 50 μg/ml), which was measured by the spectrometer, was observed to have a linear correlation with the talaporfin concentration. To reduce measurement error, we compared the relative fluorescent intensity ratio of talaporfin in the target tissue, which was calculated by dividing the relative fluorescent intensity by that of GFP, or the known autofluorescence.

An in vivo mouse model of peritoneal metastasis. Five-week-old BALB/c male nude mice (CLEA Japan Inc., Osaka, Japan) were used in this study. We created a peritoneal dissemination model using the method described below. An aliquot of 1x10^6 MKN-45 EGFP cells was injected into the peritoneal cavity of nude mice. Seven days after injection of the cells, mice were subjected to fluorescent stereomicroscopic observation, and several GFP fluorescent nodules, which were <1 mm in diameter, were detected on the omentum and mesenterium. The animal experiments were carried out according to the Institutional ethics guidelines.

Cytotoxicity of talaporfin-mediated PDT on cell lines. The cytotoxic effect of PDT was measured using the CellTiter 96 Aqueous One Solution cell proliferation assay (Promega, Madison, WI). MKN-45 EGFP cells were suspended in RPMI-1640 with 10% FBS and subsequently placed into 96-well plates at a concentration of 2x10^3 cells/well. MKN-7 and MKN-28 cells were plated at a concentration of 4x10^3 cells/well. The cells were incubated at 37˚C for 24 h. The indicated concentration of talaporfin (0-30 μg/ml) was then added and cells were incubated for another 24 h. After incubation, the cells were washed twice with PBS(-), resuspended in fresh medium, and treated with laser light at one of the following doses: 2, 5, 10, 30, or 50 J/cm^2. After the laser treatment, the cells were incubated for 48 h at 37˚C. The CellTiter 96 Aqueous One Solution was then added to the wells, and the absorbency was measured 3 h later at a wavelength of 490 nm on an automatic microplate reader (Benchmark; Bio-Rad, Hercules, CA). The survival ratio of the treated cells was compared to that of untreated control cells that did not receive PDT.

Evaluation of talaporfin concentration in an in vivo mouse model of peritoneal metastasis. Talaporfin sodium (5 or 10 mg/kg) was administered intraperitoneally to the peritoneal dissemination model mice 14 days after initial cell injection. The mice were laparotomized at 2, 4 and 8 h after talaporfin administration, and peritoneal metastatic nodules were excised and washed with normal saline. Next, we measured the relative fluorescent intensity ratio of talaporfin using a spectrometer. We also measured the same ratio in liver and intestine using the same method at 2, 4 and 8 h after intraperitoneal administration of 10 mg/kg of talaporfin.

Evaluation of the pathological response of PDT. We treated the target nodules using 2, 5, and 10 J/cm^2 doses of laser light using a special probe that adjusted the photo-radiated field to 5 mm in diameter and shaded the region outside of the target to minimize damage to normal tissues. We then performed a second laparotomy on PDT day 3, excised the tissue in the treatment field, and preserved it in formalin.

The removed tissues were fixed in 10% formaldehyde and sliced into 0.5 mm serial longitudinal sections. The pathological

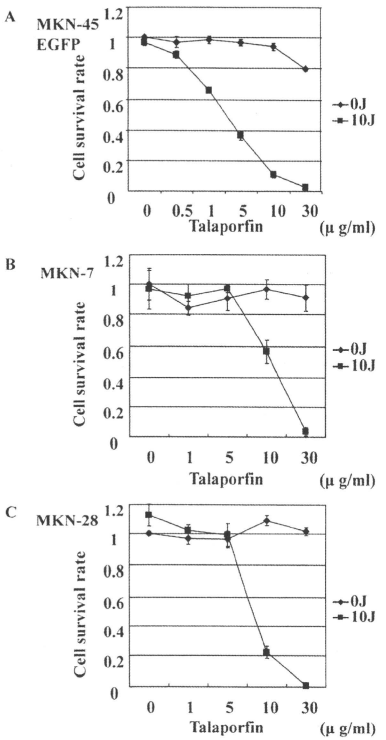

Figure 1. The gastric cancer cell lines (A) MKN-45 EGFP, (B) MKN-7, and (C) MKN-28 were incubated with a variable concentration of talaporfin (range 0-30 μg/ml) for 24 h, and were then treated with laser light at a dose of 0 J/cm^2 (controls) or 10 J/cm^2 (cases). In order to cause cell death in >50% cell deaths using this laser dose, at least 5 and 10 μg/ml of talaporfin were necessary for MKN-45 EGFP cells (A) and for MKN-7 (B) and MKN-28 cells (C), respectively.

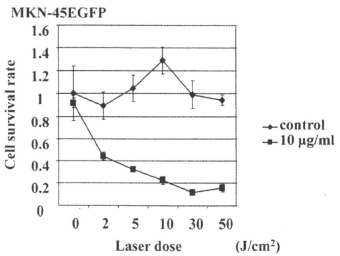

Figure 2. After MKN-45 EGFP cells were incubated with 10 (treatment group) or 0 μg/ml (control group) of talaporfin for 24 h, they were treated with the indicated dose of laser light (0-50 J/cm^2). More than 50% cell death was obtained at a dose of 2 J/cm^2, and the survival rate of MKN-45 EGFP cells gradually decreased and reached a plateau at a dose of 30 J/cm^2.

response of PDT was evaluated by comparing the proportion of viable cancer cells with that of all cancer cells in the tissue in hematoxylin and eosin-stained sections of the surgical specimens. Pathologic response was classified as follows: grade 1, a minor effect, with viable cancer cells accounting for more than two-thirds of the tumor tissue; grade 2, a moderate effect, with viable cancer cells accounting for more than one-third but less than two-thirds of the tumor tissue; and grade 3, a pathological response in which viable cancer cells account for less than one-third of the tumor tissue. Grade 3 also includes specimens that have no residual viable cancer cells.

Statistical analysis. Associations between the pathological effects in two groups were analyzed using the Fisher's exact test. In all analyses, P-values <0.05 were considered statistically significant. All statistical analyses were performed using the StatView software package, version 5.0 (Abacus Concepts, Inc., Berkeley, CA).

Results

The effects of PDT on cultured cancer cell lines. The *in vitro* anti-cancer effects of talaporfin-mediated PDT were evaluated using three gastric cancer cell lines: MKN-45 EGFP, MKN-7 and MKN-28. After each cell line was incubated with one of the indicated concentrations of talaporfin (0-30 μg/ml) for 24 h, they were treated with laser light at a dose of 10 J/cm^2. The cell survival rate was estimated by the MTS assay. Talaporfin showed strong anti-tumor effects in all three cell lines in a dose-dependent manner in cells that were treated with PDT, whereas in the control cell lines that did not undergo PDT, talaporfin did not have any significant anti-tumor effects. To obtain a cell death rate of >50% using a 10 J/cm^2 dose, at least 5 μg/ml of talaporfin was necessary in MKN-45 EGFP cells (Fig. 1A) and 10 μg/ml of talaporfin was necessary for MKN-7 (Fig. 1B) and MKN-28 cells (Fig. 1C). Next, we examined the dose-dependent effects of laser energy on survival of MKN-45 EGFP cells. After MKN-45 EGFP cells were incubated with 10 μg/ml of talaporfin for 24 h, they were treated with the indicated dose of laser light (0-50 J/cm^2). A cell death rate higher than 50% was obtained at a 2 J/cm^2 dose, and the survival rate of MKN-45 EGFP cells gradually decreased, reaching a plateau at 30 J/cm^2 (Fig. 2).

Uptake and accumulation of talaporfin in peritoneal metastatic nodules. Before starting *in vivo* experiments, we first examined whether talaporfin accumulated in peritoneal metastatic nodules. Fourteen days after the i.p. injection of 1x10^6 of MKN-45 EGFP cells into the abdominal cavity of mice (by which time the peritoneal metastases should reach 1-2 mm in diameter according to our preliminary studies), 10 mg/kg of talaporfin was intraperitoneally administered. The mice were sacrificed 2, 4 and 8 h later, and their micrometastatic nodules (tagged with GFP) were excised and subjected to spectrometric analysis (VLD-M1 M&M Co. Ltd., Osaka, Japan) to determine the talaporfin concentration in the metastatic foci. Samples of liver and small intestine were

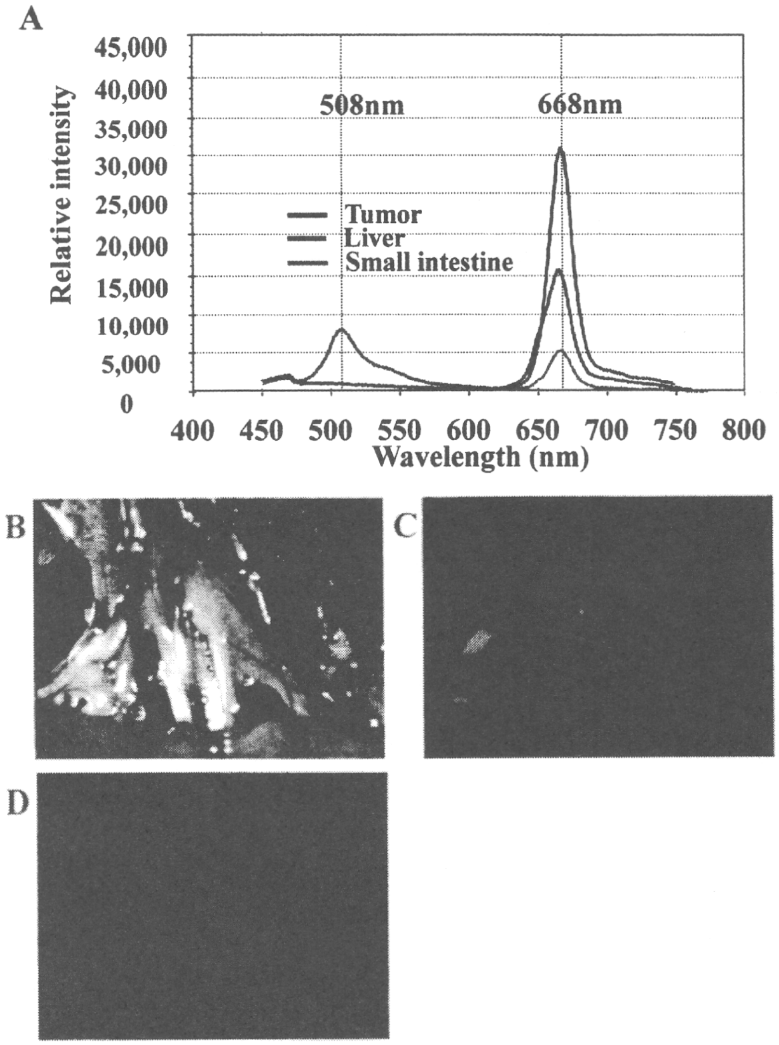

Figure 3. (A) Ten mg/kg of talaporfin was intraperitoneally administered to mice that had been inoculated with MKN-45 EGFP to create an *in vivo* model of peritoneal metastasis. Four hours after talaporfin administration, the spectrum waveform of the peritoneal metastatic nodules, liver and small intestine were analyzed by a VLD-M1 spectrometer. Peritoneal metastatic nodules showed two peaks of fluorescence emission spectra by spectrophotometric analysis, one at 508 nm, corresponding to GFP, and another at 668 nm, corresponding to talaporfin. The small intestine also showed a weak but significant level of fluorescence at 668 nm. Fluoromicroscopic findings of the nodules in mice that were intraperitoneally injected with MKN-45 EGFP cells followed by 10 mg/kg of i.p. talaporfin. The peritoneal micrometastatic nodules were observed by normal white light microscopy (B). Green fluorescence (indicating GFP) (C), and red fluorescence (indicating talaporfin) (D) were observed using fluorescence microscopy.

also removed and subjected to spectrophotometric analysis.

Peritoneal metastatic nodules showed two peaks of fluorescence emission spectra by spectrophotometric analysis: one at 508 nm, corresponding to GFP, and another at 678 nm, corresponding to talaporfin, while liver and small intestine also showed a weak but notable level of fluorescence at 678 nm (Fig. 3A). Fig. 3B shows the fluoromicroscopic findings of the peritoneum of mice that were intraperitoneally injected with MKN-45 EGFP cells and subsequently treated with talaporfin. Micrometastatic nodules on the peritoneal surface had a red fluorescence pattern and were easily detectable by fluoromicroscopy, suggesting that the talaporfin had accumulated in the cancer cells. Furthermore, we also examined the uptake and accumulation of intravenously injected talaporfin into peritoneal metastatic nodules. Compared with i.p. administration, intravenous (i.v.) administration of talaporfin resulted in less fluorescent intensity in the metastatic nodules as analyzed by spectrometry (Fig. 4B) and poorer visualization by fluoromicroscopy (data not shown). Therefore, for the subsequent experiments, talaporfin was administered via an i.p. route.

Optimal dose of injected talaporfin and optimal time-point of laser treatment in an in vivo mouse model of peritoneal metastasis. To determine the optimal talaporfin dose, we measured the relative fluorescence intensity ratio in peritoneal metastatic nodules after i.p. administration of various doses of talaporfin (Fig. 4A). The relative fluorescent intensity ratio of metastastic nodules was calculated by dividing the relative fluorescent intensity of these tissues by that of GFP. Mice treated with 10 mg/kg of talaporfin had a relative intensity ratio that was about 3-fold higher than that in mice treated with 5 mg/kg of talaporfin. Next, to determine the optimal time-point of laser treatment after talaporfin administration - at which cytotoxic effects on normal tissues are reduced while cytotoxic effects on tumor cells are maintained - we

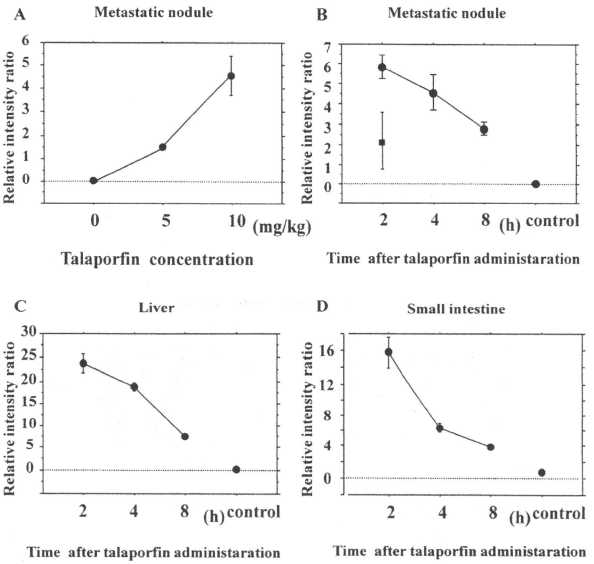

Figure 4. (A) Four hours after intraperitoneal injection of talaporfin, the mice treated with a 10 mg/kg dose of talaporfin showed a relative intensity ratio that was about 3-fold higher than that observed in mice treated with a 5 mg/kg dose (B). After intraperitoneal injection of 10 mg/kg of talaporfin, the relative fluorescent intensity ratio in the peritoneal tumors gradually decreased over time compared to the ratio observed at 2 h, with 78% present at 4 h and 48% present at 8 h (●). The relative fluorescent intensity ratio 2 h after intravenous administration (■) of talaporfin was lower than that obtained via intraperitoneal injection. Relative fluorescent ratios in liver (C) and small intestine (D) were decreased (as compared to the 2 h value) to 79% of the 2 h value at 4 h, 31% at 8 h, 36% at 4 h and 24% at 8 h, respectively.

measured talaporfin concentration over time in mouse peritoneal metastatic nodules, liver and small intestines using spectrometry. Relative fluorescent intensity ratios of the liver and the small intestine were calculated by dividing the relative fluorescent intensity of these tissues by the known autofluorescent intensity. The relative fluorescent intensity ratios in peritoneal tumors gradually decreased with time as compared with the relative fluorescent intensity ratio present 2 h after talaporfin administration (78% was present at 4 h and 48% was present at 8 h) (Fig. 4B). In liver and small intestine, relative fluorescent intensity ratios were decreased to 79% at 4 h and 31% at 8 h, and 36% at 4 h and 24% at 8 h, respectively, as compared with the ratio at 2 h (Fig. 4C and D).

Pathological response in peritoneal cancer nodules by PDT. We evaluated the effects of talaporfin-mediated PDT on peritoneal metastases using an *in vivo* mouse model of peritoneal metastasis. Seven days after i.p. injection of $1x10^6$ of MKN-45 EGFP cells, mice were given 10 mg/kg of talaporfin intraperitoneally. Mice were laparotomized 2 h or 4 h later, and then treated with varying doses of laser light.

Twenty-four hours after the laser treatment, the mice were re-laparotomized to assess the effects of PDT. We found edematous changes and few or no adhesions on the laser-treated tissues macroscopically. However, a white color change was frequently noted on the liver, which was found to be coagulative necrosis on microscopic analysis. The average size of targeted omental nodules was 696±474 μm.

In mice that underwent PDT 2 h after talaporfin administration, a grade 3 response was seen in 52.5% of cancer nodules at a laser dose of 2 J/cm^2, in 43.2% at a dose of 5 J/cm^2, and in 64.4% at a dose of 10 J/cm^2, respectively. In mice that underwent PDT 4 h after talaporfin administration, the rates of a grade 3 response were 20.8% at a dose of 2 J/cm^2, 25.5% at a dose of 5 J/cm^2, and 26.2% at a dose of 10 J/cm^2, respectively. Anti-tumor effects were significantly dependent on the time interval between the laser treatment and talaporfin administration at all laser doses (2 J/cm^2, $P<0.0001$; 5 J/cm^2: $P=0.022$; 10 J/cm^2, $P<0.0001$), but it was independent of the laser dose at all time intervals (Fig. 5).

To determine PDT conditions that would be tolerable in terms of toxicity, we treated the small intestine and mesenterium 1-6 cm proximal to the ileocecal junction with various laser doses. In mice that were treated with a 2 h interval between talaporfin administration and laser treatment, substantial edematous changes were seen in the ileum at a dose of 2 J/cm^2, and remarkable edematous and ischemic changes were seen at doses of 5 and 10 J/cm^2 1 day after PDT. The mice that were treated with a laser dose of 2 J/cm^2 with a 2 h interval (n=5) all died of intestinal perforation

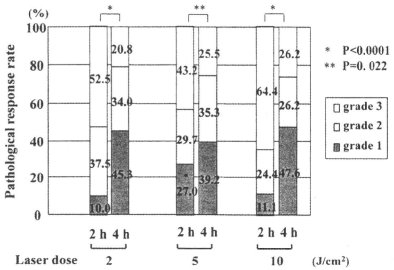

Figure 5. Anti-tumor pathological effects were significantly dependent on the time interval between laser treatment and talaporfin administration at all laser doses (2 J/cm^2, P<0.0001; 5 J/cm^2, P=0.022; 10 J/cm^2, P<0.0001), but they were independent of the laser dose at both times.

within 3 days after PDT treatment. On the other hand, in mice treated with a 4 h interval, remarkable edema was noted at a dose of 10 J/cm^2, but only slight edematous changes were noted at a dose of 5 J/cm^2 and no changes were noted at a dose of 2 J/cm^2. All the mice that were treated with a laser dose of 2 J/cm^2 with a 4 h interval (n=5) survived without any complications until 30 days after PDT treatment. Thus, the optimal treatment conditions for talaporfin-mediated PDT are considered to be a laser dose of 2 J/cm^2 and a 4 h interval between talaporfin administration and laser treatment (Table I).

Discussion

PDT has proved to be a promising new therapeutic modality in the treatment of cancer. Porphyrin-based photosensitizers such as hematoporphyrin derivative and porfimer sodium are most often used clinically for the treatment of cancer. But recently, promising results using new generation photosensitizers, such as talaporfin and 5-aminolevulinic acids (ALA), are frequently reported to shorten the *in vivo* retention that increases the risk of phototoxicity and the interval required between drug administration and laser treatment. This study is the first to show that intraperitoneally injected talaporfin accumulates in peritoneal metastatic nodules from gastric cancer and that talaporfin-mediated PDT exerts substantial anti-tumor effect *in vivo* (using a mouse model of peritoneal metastasis).

With regard to 5-aminolevulinic acids (ALA), numerous studies have reported that 5-ALA is useful for photodynamic diagnosis (PDD) of various malignant tumors (26-28). Several reports have shown promising preliminary results of 5-ALA-mediated photodynamic therapy (PDT) in the treatment of malignant tumors in the abdominal cavity (29,30). However, there are no reports examining 5-ALA-mediated PDT for peritoneal metastasis in gastrointestinal cancers, probably because of its low anti-tumor effects (31,32).

On the other hand, porphyrin-mediated PDT was reported to show survival benefit in animal models of peritoneal metastasis. Also, clinical trials examining debulking surgery followed by intraoperative photofirin-mediated PDT for peritoneal or pleural dissemination showed good local control rates with acceptable toxicity (33-35). The anti-tumor effects of photofirin-mediated PDT were more potent than those of 5-ALA-mediated PDT, but the toxicity rates were higher. Surgical debulking and i.p. PDT using photofirin for peritoneal dissemination was associated with a risk of capillary leak syndrome, which is a significant inflammatory response syndrome after surgery and necessitates massive fluid resuscitation, careful ICU monitoring, and, frequently, prolonged ventilator support (36). Capillary leak syndrome is essentially considered to be burn of an extensive surface area of the peritoneal cavity. If PDT is to become a clinically useful therapy, it must prevent the occurrence of capillary leak syndrome.

In our study examining talaporfin-mediated PDT, no lethal side effects, such as capillary leak syndrome, were noted. Talaporfin-mediated PDT has the advantage of a lack of prolonged photosensitization because the drug washes out early in normal tissue. Talaporfin-mediated PDT may therefore be less toxic than photofirin-mediated PDT and may reduce the risk of capillary leak syndrome, although these findings need to be confirmed in clinical trials.

Initially, we employed an i.v. route for talaporfin administration because this method had been used previously in mouse experiments (18,37). However, our preliminary study showed that, as compared with i.v. administration, i.p. administration resulted in a higher accumulation of talaporfin in the metastatic nodules as well as a more complete pathological effect (data not shown).

Gormer *et al* (20) showed that talaporfin concentration in subcutaneous tumors and other organs did not significantly differ according to the mode of drug administration (i.v. versus i.p), the tumor size or the time of laser treatment (4 h and 24 h after talaporfin administration). The difference between Gormer's results and ours may be explained by the difference of targeted tumor sites (subcutaneous tumors vs. peritoneal tumors). Particularly in the case of peritoneal

Table I. Toxicity: optical findings of the ileum 1 day after PDT and mortality.

Interval between drug injection and laser treatment	Laser dose (J/cm^2)			Died/number of mice (day after treated at a laser dose of 2 J/cm^2)
	2	5	10	
2 h	(+)	(++)[a]	(++)[a]	5/5 (2 at day 2, 3 at day 3)
4 h	(-)	(+)	(++)	0/5

metastatic nodules, which have poor tumor blood supply, cancer cells seem to be more directly exposed to intraperitoneally administered talaporfin than to intravenously administered talaporfin. As shown in this study, the relative intensity ratio of peritoneal tumors 2 h after talaporfin administration was higher in animals administered talaporfin by the i.p. route than in those treated with i.v. talaporfin (Fig. 4B). In the photofirin study, Perry *et al* showed no difference in the intestinal uptake of photofirin according to the route of administration, but did demonstrate an increased photofirin elimination half-time in peritoneal tumors treated via the i.p. route (38). Drug uptake studies demonstrated an increase in ^{14}C-labeled mTHPC (meta-tetrahydroxyphenylchrorin) in peritoneally disseminated tumors after i.p. administration of the drug as compared with i.v. administration (39). Taking into consideration the results of the above reports and ours, as compared with the i.v. administration route of talaporfin, the i.p. administration route increases the intratumoral concentration of talaporfin in peritoneal tumors, without increasing the concentration in other organs. Therefore, we think that the i.p. route should be the first choice for talaporfin-mediated PDT in the treatment of peritoneal tumors.

The anti-cancer effects of PDT have been theorized to be associated with not only direct singlet oxygen cytotoxicity on cancer cells but also with damage to tumor vasculature. It has been reported that responsiveness to PDT after i.v. talaporfin administration correlates with the time interval between drug administration and laser treatment, laser dose and photosensitizer levels in the plasma, but does not correlate with the photosensitizer concentration in tumor tissue, which suggests that damage to tumor blood vessels may be a primary target in talaporfin-mediated PDT (20,37). Interestingly, however, there was no significant correlation between the pathological response and laser dose in i.p. talaporfin-mediated PDT in our study. In our study, the target lesions were peritoneal metastatic nodules on the omentum, where tumor blood supply is extremely poor. Previous mechanistic investigations have shown that talaporfin enters the cells via endocytosis (21). Therefore, in the case of peritoneal tumors, talaporfin might enter the tumor cells directly by endocytosis rather than by the surrounding tumor vessels. If this is true, the anti-tumor effects of i.p. talaporfin-mediated PDT might be mediated by direct singlet oxygen cytotoxicity rather than by damage to tumor vasculature.

In our *in vitro* study, high rates of cytotoxicity were found at a treatment dose of 2 J/cm^2, and there were no significant differences in cell survival rates between doses ranging from 2 to 10 J/cm^2. Thus, a laser dose of 2 J/cm^2 was considered to be enough energy to consume the absorbed talaporfin in the micrometastatic nodules. In our *in vivo* study, we showed that a laser dose of 2 J/cm^2 was adequate to treat small nodules (<1 mm in diameter) in i.p. talaporfin-mediated PDT.

Our study demonstrated that talaporfin accumulated in small peritoneal metastatic nodules by i.p. drug administration and that i.p. talaporfin-mediated PDT did not require a high laser dose. The pathological response rate was about 20% under optimal conditions, which is comparable to that of chemotherapy. We think that the biggest benefit of talaporfin-mediated PDT is the short interval required between drug administration and light exposure. This advantage makes it possible to perform PDT immediately after surgery for gastric cancer in confirmed cases of serosa-positive cancer or in cases with positive cytology from peritoneal washings. Thus, intraperitoneally delivered talaporfin-mediated PDT seems to be a promising treatment modality for peritoneal recurrence of gastric cancer and may have a role in the prevention of peritoneal dissemination after surgery.

Acknowledgments

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References

1. Verdecchia A, Corazziari I, Gatta G, Lisi D, Faivre J and Forman D: Explaining gastric cancer survival differences among European countries. Int J Cancer 109: 737-741, 2004.
2. Maruyama K, Kaminishi M, Hayashi K, *et al*: Gastric cancer treated in 1991 in Japan: data analysis of nationwide registry. Gastric Cancer 9: 51-66, 2006.
3. Sun P, Xiang JB and Chen ZY: Meta-analysis of adjuvant chemotherapy after radical surgery for advanced gastric cancer. Br J Surg (In press).
4. Liu TS, Wang Y, Chen SY and Sun YH: An updated meta-analysis of adjuvant chemotherapy after curative resection for gastric cancer. Eur J Surg Oncol 34: 1208-1216, 2008.
5. Zhao SL and Fang JY: The role of postoperative adjuvant chemotherapy following curative resection for gastric cancer: a meta-analysis. Cancer Invest 26: 317-325, 2008.
6. Panzini I, Gianni L, Fattori PP, *et al*: Adjuvant chemotherapy in gastric cancer: a meta-analysis of randomized trials and a comparison with previous meta-analyses. Tumori 88: 21-27, 2002.
7. Mari E, Floriani I, Tinazzi A, *et al*: Efficacy of adjuvant chemotherapy after curative resection for gastric cancer: a meta-analysis of published randomised trials. A study of the GISCAD (Gruppo Italiano per lo Studio dei Carcinomi dell'Apparato Digerente). Ann Oncol 11: 837-843, 2000.
8. Earle CC and Maroun JA: Adjuvant chemotherapy after curative resection for gastric cancer in non-Asian patients: revisiting a meta-analysis of randomised trials. Eur J Cancer 35: 1059-1064, 1999.
9. Yan TD, Black D, Sugarbaker PH, *et al*: A systematic review and meta-analysis of the randomized controlled trials on adjuvant intraperitoneal chemotherapy for resectable gastric cancer. Ann Surg Oncol 14: 2702-2713, 2007.
10. Baeza MR, Giannini TO, Rivera SR, *et al*: Adjuvant radiochemotherapy in the treatment of completely resected, locally advanced gastric cancer. Int J Radiat Oncol Biol Phys 50: 645-650, 2001.
11. Petit T, Velten M, d'Hombres A, *et al*: Long-term survival of 106 stage IIIc ovarian cancer patients with minimal residual disease after second-look laparotomy and consolidation radiotherapy. Gynecol Oncol 104: 104-108, 2007.
12. Furuse K, Fukuoka M, Kato H, *et al*: A prospective phase II study on photodynamic therapy with photofrin II for centrally located early-stage lung cancer. The Japan Lung Cancer Photodynamic Therapy Study Group. J Clin Oncol 11: 1852-1857, 1993.
13. Mimura S, Ito Y, Nagayo T, *et al*: Cooperative clinical trial of photodynamic therapy with photofrin II and excimer dye laser for early gastric cancer. Lasers Surg Med 19: 168-172, 1996.
14. Prosst RL, Wolfsen HC and Gahlen J: Photodynamic therapy for esophageal diseases: a clinical update. Endoscopy 35: 1059-1068, 2003.
15. Ortner ME, Caca K, Berr F, *et al*: Successful photodynamic therapy for nonresectable cholangiocarcinoma: a randomized prospective study. Gastroenterology 125: 1355-1363, 2003.
16. Takita H, Mang TS, Loewen GM, *et al*: Operation and intracavitary photodynamic therapy for malignant pleural mesothelioma: a phase II study. Ann Thorac Surg 58: 995-998, 1994.

17. Barr H, Krasner N, Boulos PB, Chatlani P and Bown SG: Photodynamic therapy for colorectal cancer: a quantitative pilot study. Br J Surg 77: 93-96, 1990.
18. Kobayashi W, Liu Q, Nakagawa H, *et al*: Photodynamic therapy with mono-L-aspartyl chlorin e6 can cause necrosis of squamous cell carcinoma of tongue: experimental study on an animal model of nude mouse. Oral Oncol 42: 46-50, 2006.
19. Grant WE, Speight PM, Hopper C and Bown SG: Photodynamic therapy: an effective, but non-selective treatment for superficial cancers of the oral cavity. Int J Cancer 71: 937-942, 1997.
20. Gomer CJ and Ferrario A: Tissue distribution and photosensitizing properties of mono-L-aspartyl chlorin e6 in a mouse tumor model. Cancer Res 50: 3985-3990, 1990.
21. Roberts WG, Smith KM, McCullough JL and Berns MW: Skin photosensitivity and photodestruction of several potential photodynamic sensitizers. Photochem Photobiol 49: 431-438, 1989.
22. Kujundzic M, Vogl TJ, Stimac D, *et al*: A Phase II safety and effect on time to tumor progression study of intratumoral light infusion technology using talaporfin sodium in patients with metastatic colorectal cancer. J Surg Oncol 96: 518-524, 2007.
23. Kato H, Furukawa K, Sato M, *et al*: Phase II clinical study of photodynamic therapy using mono-L-aspartyl chlorin e6 and diode laser for early superficial squamous cell carcinoma of the lung. Lung Cancer 42: 103-111, 2003.
24. Kaneko K, Yano M, Tsujinaka T, *et al*: Establishment of a visible peritoneal micrometastatic model from a gastric adenocarcinoma cell line by green fluorescent protein. Int J Oncol 16: 893-898, 2000.
25. Utsuki S, Oka H, Miyajima Y, Shimizu S, Suzuki S and Fujii K: Auditory alert system for fluorescence-guided resection of gliomas. Neurol Med Chir (Tokyo) 48: 95-98, 2008.
26. Loning M, Diddens H, Kupker W, Diedrich K and Huttmann G: Laparoscopic fluorescence detection of ovarian carcinoma metastases using 5-aminolevulinic acid-induced protoporphyrin IX. Cancer 100: 1650-1656, 2004.
27. Till H, Bergmann F, Metzger R, *et al*: Videoscopic fluorescence diagnosis of peritoneal and thoracic metastases from human hepatoblastoma in nude rats. Surg Endosc 19: 1483-1486, 2005.
28. Collinet P, Sabban F, Cosson M, *et al*: Laparoscopic photodynamic diagnosis of ovarian cancer peritoneal micro metastasis: an experimental study. Photochem Photobiol 83: 647-651, 2007.
29. Major AL, Rose GS, Chapman CF, *et al*: *In vivo* fluorescence detection of ovarian cancer in the NuTu-19 epithelial ovarian cancer animal model using 5-aminolevulinic acid (ALA). Gynecol Oncol 66: 122-132, 1997.
30. Bergmann F, Stepp H, Metzger R, Rolle U, Johansson A and Till H: *In vitro* and *in vivo* evaluation of photodynamic techniques for the experimental treatment of human hepatoblastoma and neuroblastoma: preliminary results. Pediatr Surg Int 24: 1331-1333, 2008.
31. Tan WC, Fulljames C, Stone N, *et al*: Photodynamic therapy using 5-aminolaevulinic acid for oesophageal adenocarcinoma associated with Barrett's metaplasia. J Photochem Photobiol B 53: 75-80, 1999.
32. Mlkvy P, Messmann H, Regula J, *et al*: Photodynamic therapy for gastrointestinal tumors using three photosensitizers - ALA induced PPIX, Photofrin and MTHPC. A pilot study. Neoplasma 45: 157-161, 1998.
33. Hendren SK, Hahn SM, Spitz FR, *et al*: Phase II trial of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumors. Ann Surg Oncol 8: 65-71, 2001.
34. Friedberg JS, Mick R, Stevenson JP, *et al*: Phase II trial of pleural photodynamic therapy and surgery for patients with non-small-cell lung cancer with pleural spread. J Clin Oncol 22: 2192-2201, 2004.
35. De Laney TF, Sindelar WF, Tochner Z, *et al*: Phase I study of debulking surgery and photodynamic therapy for disseminated intraperitoneal tumors. Int J Radiat Oncol Biol Phys 25: 445-457, 1993.
36. Canter RJ, Mick R, Kesmodel SB, *et al*: Intraperitoneal photodynamic therapy causes a capillary-leak syndrome. Ann Surg Oncol 10: 514-524, 2003.
37. Ferrario A, Kessel D and Gomer CJ: Metabolic properties and photosensitizing responsiveness of mono-L-aspartyl chlorin e6 in a mouse tumor model. Cancer Res 52: 2890-2893, 1992.
38. Perry RR, Smith PD, Evans S and Pass HI: Intravenous vs intraperitoneal sensitizer: implications for intraperitoneal photodynamic therapy. Photochem Photobiol 53: 335-340, 1991.
39. Veenhuizen RB, Ruevekamp MC, Oppelaar H, Helmerhorst TJ, Kenemans P and Stewart FA: Foscan-mediated photodynamic therapy for a peritoneal-cancer model: drug distribution and efficacy studies. Int J Cancer 73: 230-235, 1997.

症 例

放射線化学療法奏効後，根治切除を施行した高度進行食道胃接合部癌の5例

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Five Cases of Advanced Gastroesophageal Junction Adenocarcinoma Successfully Treated with Chemoradiotherapy Followed by Curative Resection: Masanori Takahashi, Keisuke Koeda, Hisataka Fujiwara, Takehiro Chiba, Akira Sasaki and Go Wakabayashi (*Dept. of Surgery, Iwate Medical University*)

Summary

We reviewed five patients with advanced gastroesophageal cancer who were successfully treated with chemoradiotherapy followed by a curative resection. Patients with histologically-documented adenocarcinoma of the gastroesophageal junction were eligible. Direct tumor extension into the stomach (cT3 or cT4), and involvement of lymph nodes were observed. The patients stopped receiving orally administered carcinostatic drugs due to digestive stenosis or tumor bleeding. They received 25 mg/m^2 of cisplatin and 60 mg/m^2 of paclitaxel once a week on days 1, 8, 15 and 22. Radiation was administered concurrently at a total dose of 45 Gy in 1. 8 Gy fractions for over 25 treatments. Effectiveness of the therapy was evaluated 4 weeks after the chemoradiotherapy. All patients with clinical partial responses underwent gastrectomy (n=4) or esophagogastrectomy (n=1). Curative resection was performed in 5 patients (resection A/B 4/1), and no patient suffered from major postoperative complications. Four patients were downstaged according to the pathological findings. The histologically effective responses of all patients were Grade 2.

The obvious chemotherapeutic efficacy of the present regimen suggested that it may be a good treatment option for advanced gastroesophageal cancers. Further studies including randomized controlled trials are needed to evaluate the significance of preoperative chemoradiotherapy. **Key words**: Gastroesophageal cancer, Chemoradiotherapy (*Received Feb. 10, 2010/Accepted May 17, 2010*)

要旨 高度進行食道胃接合部癌は極めて予後不良であり，根治切除困難症例も多く認められる。今回，高度進行食道胃接合部癌に対し放射線化学療法を施行した後，根治切除を施行した5例を経験したので報告する。

放射線化学療法の適応は，多数個の集塊をなすリンパ節転移を伴う高度進行食道胃接合部癌で，出血や狭窄により内服での抗癌剤投与継続が困難な症例とした。レジメンは，cisplatin (CDDP 25 mg/m^2)/paclitaxel (PTX 60 mg/m^2) を週1回で4週間投与し，照射線量計45 Gyを併せて施行した。治療終了後から4週間後に効果判定を行い，手術を施行した。加療後の効果判定では，全例PRで手術の根治度は根治度A/Bが4/1であった。病理所見においてdown stagingを4症例で認め，組織学的効果判定は全例Grade 2であった。また，術後の縫合不全などの合併症は認めなかった。比較的限局した進行胃癌に対し，放射線化学療法の有効性は期待でき，今後さらに検討されるべき治療法の一つと思われる。

はじめに

食道胃接合部癌全体の5年生存率は欧米では38%，わが国では52%と報告されており，他の占拠部位の胃癌に比べ予後は不良である[1,2]。また，多変量解析ではリンパ節転移の有無（N因子），根治度（R因子）が独立した予後因子と報告されている[2,3]。よって高度リンパ節転移を伴い手術により根治度が得られない胃切除後の予後延長は，期待度が低く治療方針の検討が必要と考えられる。今回われわれは，高度進行食道胃接合部癌に対し放射線化学療法で奏効が認められた後に根治切除を施行した5症例を経験したので報告する。

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Ⅰ. 対　象

食道胃接合部腺癌への放射線化学療法適応基準として，① PS<2（ECOG scale）で前治療歴なし，② cT3 もしくは cT4 であり，かつ画像診断で多数個の集塊をなすリンパ節転移が認められる［cN（+），cH0，cP0，cM0］[4]，③腫瘍による出血や狭窄にて経口抗癌剤の継続内服困難，④心機能や呼吸機能で開胸手術の高リスク症例を対象とした。放射線療法は前後対向2門照射で行い，総線量を 45 Gy とし，1回線量 1.8 Gy を 25 回に分割し週5回照射した。同時期に化学療法は cisplatin（CDDP）25 mg/m^2 と paclitaxel（PTX）60 mg/m^2 の経静脈投与を day 1，8，15，22 に計4回施行した（図1）。2003 年～2008 年 12 月までに上記の適応基準を満たした5症例に施行し，治療終了4週間後に効果判定を行い治療効果が認められ，根治手術可能と診断した症例に対し手術を行った。5例（男性/女性：5/0）の組織型は，mucinous adenocarcinoma/moderately differentiated tubular adenocarcinoma/solid type poorly differentiated adenocarcinoma がそれぞれ 1/1/3 例であった。

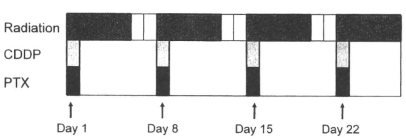

図 1　放射線化学療法
Radiation: 1.8 Gy/day×25 times, total 45 Gy
CDDP 25 mg/m^2/PTX 60 mg/m^2: day 1, 8, 15, 22

Ⅱ. 結　果

全例で原病巣は縮小し，測定可能病変は効果判定にて治療後 30% 以上縮小しており partial response（PR）と診断した[5]（図 2a～d）。手術では，根治度 A/B がそれぞれ 4/1 であり全症例で根治手術が施行可能であった。術後の縫合不全や肺炎などの重篤な合併症は認めなかった。病理分類では，臨床分類と比較し down staging を認めた症例は4例（4/5）で，組織学的効果判定は，全症例で Grade 2 であった[5]。grade 3 の有害事象は1例で悪心を認め，grade 2 が好中球減少と食欲不振をそれぞれ1例ずつ認めるのみであり，全例治療の完遂が可能であった[6]。

1例は術後 47 か月で腹膜播種再発を確認し，50 か月目で原病死となった。他の4症例は現在も無再発健存中である（表1）。

Ⅲ. 考　察

欧米では胃癌に対して放射線治療は手術，化学療法とならび標準治療として用いられており，INT-0116，MAGIC trial をはじめ放射線化学療法の有用性が示されている[7,8]。アジアにおいても Kim らによる phase Ⅱ study にて胃癌術後に放射線化学療法を施行することによって5年無再発生存期間，全生存期間の延長を認めた[9]。本邦では手術による局所制御率の高さを理由に，放射線の意義は手術に比較して極めて低く有用性が否定的であるという意見が多いが，胃癌の Stage Ⅳを主体と

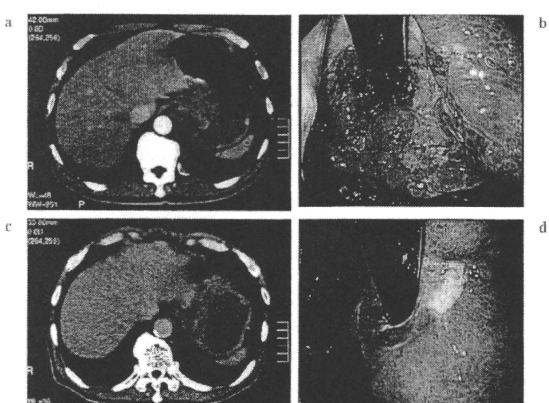

図 2　症例3の腹部 CT と内視鏡所見
a, b: 治療前。壁在リンパ節と一塊となった漿膜浸潤した腫瘍。
c, d: 治療後。腫瘍による壁肥厚の軽減と周堤の平低化を認める。

表1 症例背景

症例	性別/年齢	組織型*	縮小率	術式	Clinical classification	Pathological classification	根治度	組織学的効果判定	無再発期間
1	M/63	muc	31%/PR	左開胸開腹下部食道，胃全摘，横隔膜，胆嚢合併切除，D3郭清	T4(SI-横隔膜), N2, H0, P0, M0, Stage Ⅳ	T4(SI-横隔膜), N2, H0, P0, M0, Stage Ⅳ	Cur B	Grade 2	47か月(腹膜再発)
2	M/74	tub 2	75%/PR	開腹胃全摘 D2郭清	T3 (SE), N2, H0, P0, M0, Stage ⅢB	T2 (MP), N1, H0, P0, M0, Stage Ⅱ	Cur A	Grade 2	28か月(無再発)
3	M/65	por 1	80%/PR	開腹胃全摘 D2+16a2lat 郭清	T3 (SE), N3, H0, P0, M0, Stage Ⅳ	T2 (SS), N0, H0, P0, M0, Stage ⅠB	Cur A	Grade 2	15か月(無再発)
4	M/69	por 1	36%/PR	開腹胃全摘 D2郭清	T3 (SE), N2, H0, P0, M0, Stage ⅢB	T1 (SM), N1, H0, P0, M0, Stage ⅠB	Cur A	Grade 2	13か月(無再発)
5	M/62	por 1	90%/PR	開腹胃全摘 D2郭清	T3 (SE), N1, H0, P0, M0, Stage ⅢA	T2 (MP), N0, H0, P0, M0, Stage ⅠB	Cur A	Grade 2	9か月(無再発)

*muc: mucinous adenocarcinoma/tub 2: moderately differentiated tubular adenocarcinoma/por 1: solid type poorly differentiated adenocarcinoma

した手術不能症例へのS-1+CDDP+放射線療法の有用性[10]や，手術不能胃癌症例と局所再発へのPTX+CDDP+放射線療法のphase I studyの報告[11]もみられる。

進行食道胃接合部癌は，pT3/4ではそれぞれ85～90/90～100%と高率にリンパ節転移がみられ，その予後も不良であり[12]，そのような症例では強力な補助療法を考慮する必要がある。Safranらによる食道胃接合部腺癌に対する放射線化学療法では治療後complete response (CR)が24.1%と高い奏効を認めたという報告[13]から本治療では同レジメンを使用したところ，同様に高い効果が認められた。治療による局所制御により手術の根治率が向上したと考察される。また，Patelらは胃癌に放射線化学療法を施行した症例では治療前よりも術後の病理学的診断後の進行度が予後に強い影響を与えると報告している[14]。以上より，本治療による奏効例においては長期的な予後の改善が期待できると考えられた。

文 献

1) Feith M, Stein HJ and Siewert JR: Adenocarcinoma of the esophagogastric junction: surgical therapy based on 1602 consecutive resected patients. *Surg Oncol Clin N Am* **15**(4): 751-764, 2006.
2) Nakamura T, Ide H, Eguchi R, *et al*: Adenocarcinoma of the esophagogastric junction: a summary of responses to a questionnaire on adenocarcinoma of the esophagus and the esophagogastric junction in Japan. *Dis Esophagus* **15**(3): 219-225, 2002.
3) Yonemura Y, Kojima N, Kawamura T, *et al*: Treatment results of adenocarcinoma of the gastroesophageal junction. *Hepatogastroenterology* **55**(82-83): 475-481, 2008.
4) Japanese Gastric Cancer Association: Japanese Classification of Gastric Carcinoma (The 13th Edition): 1999.
5) Therasse P, Arbuck SG, Eisenhauer EA, *et al*: New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. *J Natl Cancer Inst* **92**(3): 205-216, 2000.
6) Instructions and Guidelines for Japanese CTCAE v3.0 by JCOG and JSCO: CTCAE v3.0日本語訳 JCOG/JSCO盤（解説と指針 v1.0): 2004.
7) Macdonald JS, Smalley SR, Benedetti J, *et al*: Chemoradiotherapy after surgery compared with surgery alone for adenocarcinoma of the stomach or gastroesophageal junction. *N Engl J Med* **345**(10): 725-730, 2001.
8) Cunningham D, Allum WH, Stenning SP, *et al*: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. *N Engl J Med* **355**(1): 11-20, 2006.
9) Kim S, Lim DH, Lee J, *et al*: An observational study suggesting clinical benefit for adjuvant postoperative chemoradiation in a population of over 500 cases after gastric resection with D2 nodal dissection for adenocarcinoma of the stomach. *Int J Radiat Oncol Biol Phys* **63**(5): 1279-1285, 2005.
10) Saikawa Y, Kubota T, Takahashi T, *et al*: Is chemoradiation effective or harmful for stage Ⅵ gastric cancer patients? *Oncol Rep* **13**(5): 865-870, 2005.
11) Yoshikawa T, Tsuburaya A, Hirabayashi N, *et al*: A phase I study of palliative chemoradiation therapy with paclitaxel and cisplatin for local symptoms due to an unresectable primary advanced or locally recurrent gastric adenocarcinoma. *Cancer Chemother Pharmacol* **64**(6): 1071-1077, 2009.
12) 田中浩明，大平雅一，久保尚士・他: 食道胃接合部癌．日本臨床 別冊消化管症候群（上): 480-483, 2009.
13) Safran H, Gaissert H, Akerman P, *et al*: Paclitaxel, cisplatin, and concurrent radiation for esophageal cancer. *Cancer Invest* **19**(1): 1-7, 2001.
14) Patel PR, Mansfield PF, Crane CH, *et al*: Clinical stage after preoperative chemoradiation is a better predictor of patient outcome than the baseline stage for localized gastric cancer. *Cancer* **110**(5): 989-995, 2007.