

Figure 1. Iron depletion suppressed tumor growth *in vivo*. (a) An iron-deficient diet was started 3 weeks before inoculating A549 cells on the backs of mice. The iron-deficient diet was continuously fed until the end of the study. Tumor volume was measured as a cube (length \times width \times height) and was tracked up to 6 weeks. Tumor growth was suppressed in the iron-deficient diet group. Tumor growth was expressed as mean tumor volume \pm SD. Statistical significance (*) was determined as $p < 0.01$. (b) Prussian blue and Ki-67 staining showed the positive spot area and proliferating cells were reduced in the iron-depleted condition. Scale bars: 50 μ m. (c) Ki-67 labeling index was decreased in iron-depleted condition. Data are means \pm SD. Statistical significance (**) was determined as $p < 0.05$.

Results

Iron-deficient diet produced iron-depleted mice

We first confirmed that the iron-deficient diet resulted in an iron-depleted mouse model. The 6-week-old male BALB/c nu/nu mice were randomized into two groups of eight mice each. Blood sampling was performed after 3 weeks. The iron-deficient diet reduced serum iron levels (Table 2). To confirm iron deficiency histologically, Prussian blue staining was done using surgically resected murine spleens. Although the positive blue spots were diffusely recognized in the normal diet group, no positive blue spot was recognized in the iron-deficient diet group (Supporting Information Fig. 1). A reduction of iron in the reticuloendothelial system proves that the iron-deficient diet reduced serum iron levels.

Iron depletion suppressed tumor growth

We investigated the tumor growth under an iron-depleted condition. A549 subcutaneous xenografts were produced on

the backs of mice after 3 weeks of an iron-deficient diet and tumor size was measured once a week. Tumor growth was suppressed in the iron-deficient diet group (tumor volume: normal diet vs. iron-deficient diet = $1,375.9 \pm 688.4$ vs. 497.0 ± 192.2 mm³; $p = 0.0037$) (Fig. 1a). There were no mice that died and no significant side effects were observed during the experiment. Moreover, diet intake and body weight were not significantly different between normal diet group and iron-deficient diet group (Supporting Information Fig. S2).

Iron depletion reduced iron levels in tumor tissue and suppressed cancer cell proliferation

To identify the differences in tumor progression in an iron-depleted condition, we performed histological and immunohistological examinations (Fig. 1b). As shown above, an iron-deficient diet reduced the serum and tumor tissue iron levels. Interestingly, there was only difference of positive spot area in stroma tissue of the tumor. Proliferating cells (G1, S, G2 and M cycling stages of cell division) were stained

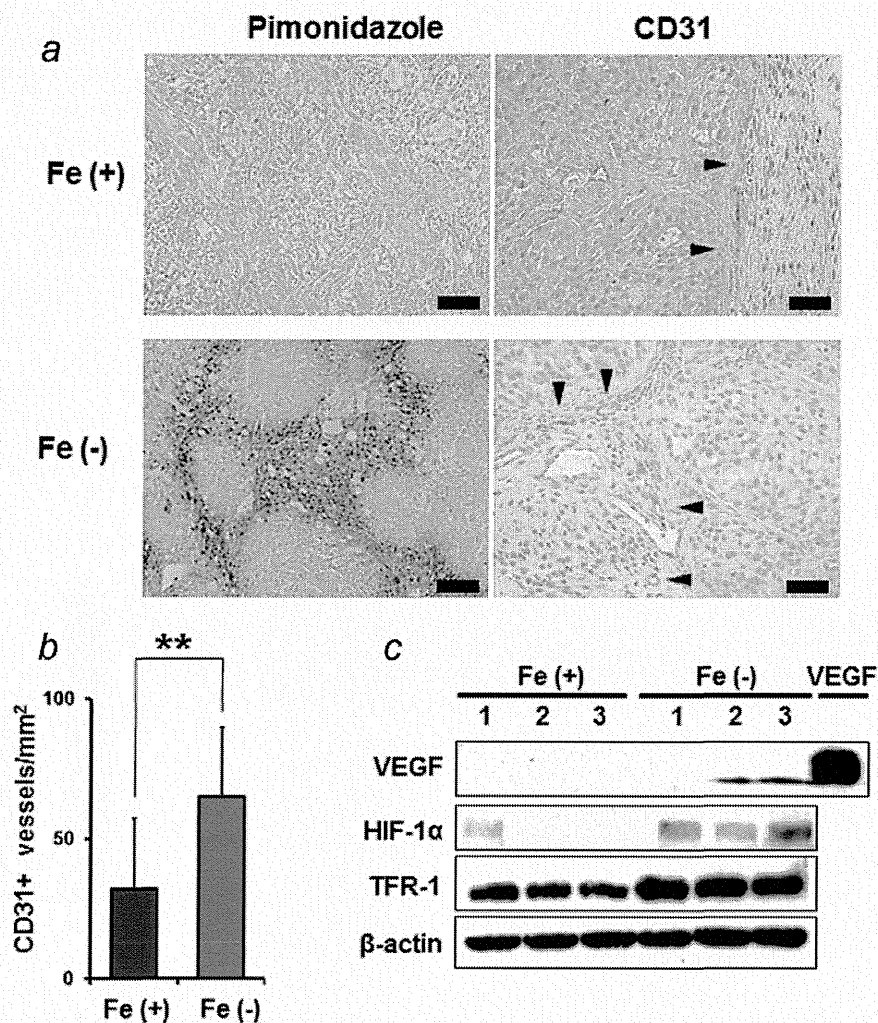


Figure 2. Iron depletion reciprocally induced angiogenesis *via* upregulation of HIF-1α. (a) Tumor tissues obtained as described in the legend to Figure 1 were analyzed for angiogenesis. Pimonidazole and CD-31 staining showed that positive spot area and positive stained vessels were increased in the iron-depleted condition. Scale bars: 50 mm. (b) MVD is increased in the iron-depleted condition. Data are means ± SD. Statistical significance (**) was determined as $p < 0.05$. (c) Western blot analysis of tumor-homogenized samples showed that VEGF and Tfr-1 were upregulated in the iron-depleted condition. Similarly, HIF-1α was also upregulated at the nucleus protein level. Each tumor sample was obtained from three individual mice.

positive; G0 cycling stage cells were excluded. The Ki-67 labeling index revealed that an iron-deficient diet suppressed cancer cell proliferation compared to the normal diet group (Ki-67 labeling index: normal diet *vs.* iron-deficient diet = 0.211 ± 0.035 *vs.* 0.133 ± 0.032 ; $p = 0.0459$). Prussian blue staining was almost negative in the tissue in iron-deficient group.

Iron depletion followed by hypoxia and angiogenesis

We hypothesized that iron depletion induced a reduction of serum hemoglobin and tissue hypoxia. Iron depletion also upregulates angiogenesis in the tumor. To test these hypotheses, we investigated pimonidazole and CD-31 staining (Fig. 2a). Tumor hypoxia was increased in the group fed an iron-deficient diet. CD-31 staining was performed to investigate whether iron depletion induced angiogenesis. Microvessel

density (MVD) was calculated to count CD-31-positive vessels (Fig. 2b). CD-31-positive vessels were increased in an iron-depleted condition. The MVD of the iron-deficient diet group was higher than that of the normal diet group (MVD: normal diet *vs.* iron-deficient diet = 32.02 ± 25.24 *vs.* 64.96 ± 24.71 ; $p = 0.045439$). This result suggested that iron depletion induced angiogenesis *via* hypoxia.

Angiogenesis was induced by iron depletion *via* HIF-1α upregulation

To identify the mechanism by which iron depletion induced angiogenesis *via* hypoxia, a Western blot analysis was performed using homogenized tissue samples. The expression of transferrin receptor 1 (Tfr-1) was determined to confirm the effect of iron depletion in tumor tissue samples. As Tfr-1

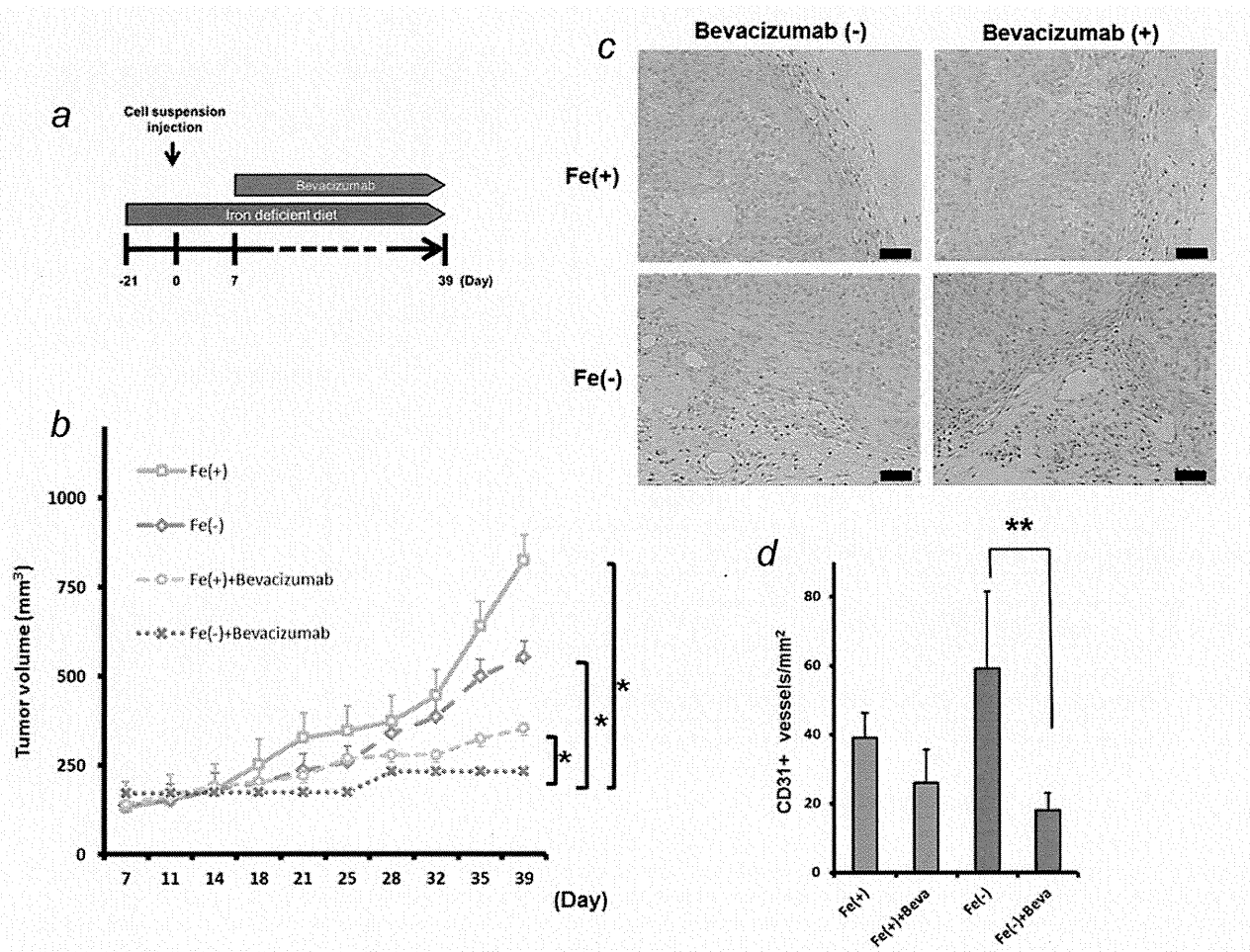


Figure 3. Bevacizumab synergistically suppressed tumor growth in an iron-depleted condition. (a) An iron-deficient diet was started 3 weeks before inoculating A549 cells on the backs of mice (day 21). The iron-deficient diet was continuously fed until the end of the study. (b) Bevacizumab (5 mg/kg) or saline as a control was administered ip twice/week. Tumor volume was measured as a cube (length \times width \times height) and was tracked up to 39 days. Five mice were used for each group. Tumor growth was expressed as mean tumor volume \pm SD. Statistical significance (*) was determined as $p < 0.01$. Tumor growth was significantly inhibited in the combination group as compared to the control, bevacizumab or Fe (-) groups. (c) CD-31 staining revealed that positively stained vessels were increased in the iron-depleted condition. Scale bars: 50 μ m. (d) Bevacizumab decreased MVD in both normal diet group and iron-deficient diet group.

expression changes based on the serum iron level, a decrease in serum iron levels increases TfR-1 expression.¹⁹ Whole-cell lysate from homogenized tissue samples showed TfR-1 was upregulated in the iron-deficient diet group (Fig. 2c). Subsequently, the expression status of HIF-1 α in nuclei was determined. HIF-1 α is known to play a critical role in angiogenesis *via* hypoxia.^{2,20} Western blot analysis of an extraction from nuclei showed the expression of HIF-1 α in the iron-deficient diet group was higher than that of the normal group (Fig. 2c). This result suggested that iron depletion induced hypoxia *via* HIF-1 α , which caused angiogenesis.

Bevacizumab synergistically suppressed tumor growth by inhibiting upregulated angiogenesis

As shown above, we found that iron depletion was followed by hypoxia and angiogenesis. Thus, an antiangiogenic thera-

peutic agent (bevacizumab) was predicted to have a synergistic effect on suppressing tumor growth in an iron-depleted condition. Bevacizumab was administrated ip 5 mg/kg twice a week to mice with subcutaneous tumors fed either an iron-deficient or a normal diet. This dose and schedule were cited in the previous reports.^{21,22} Bevacizumab had a synergistic effect on inhibiting tumor growth on Day 39 (tumor volume: normal diet [857.6 \pm 129.0 cm³], iron-deficient diet [401.8 \pm 126.6 cm³], normal diet + bevacizumab [221.6 \pm 63.8 cm³], iron-deficient diet + bevacizumab [61.0 \pm 27.5 cm³]) (Figs. 3a and 3b). To calculate MVD, CD-31 staining was performed (Fig. 3c). We could confirmed that bevacizumab inhibited angiogenesis in spite of induction by iron depletion condition (MVD: normal diet [39 \pm 7.3], iron-deficient diet [59 \pm 22.4], normal diet + bevacizumab [26 \pm 9.7], iron-

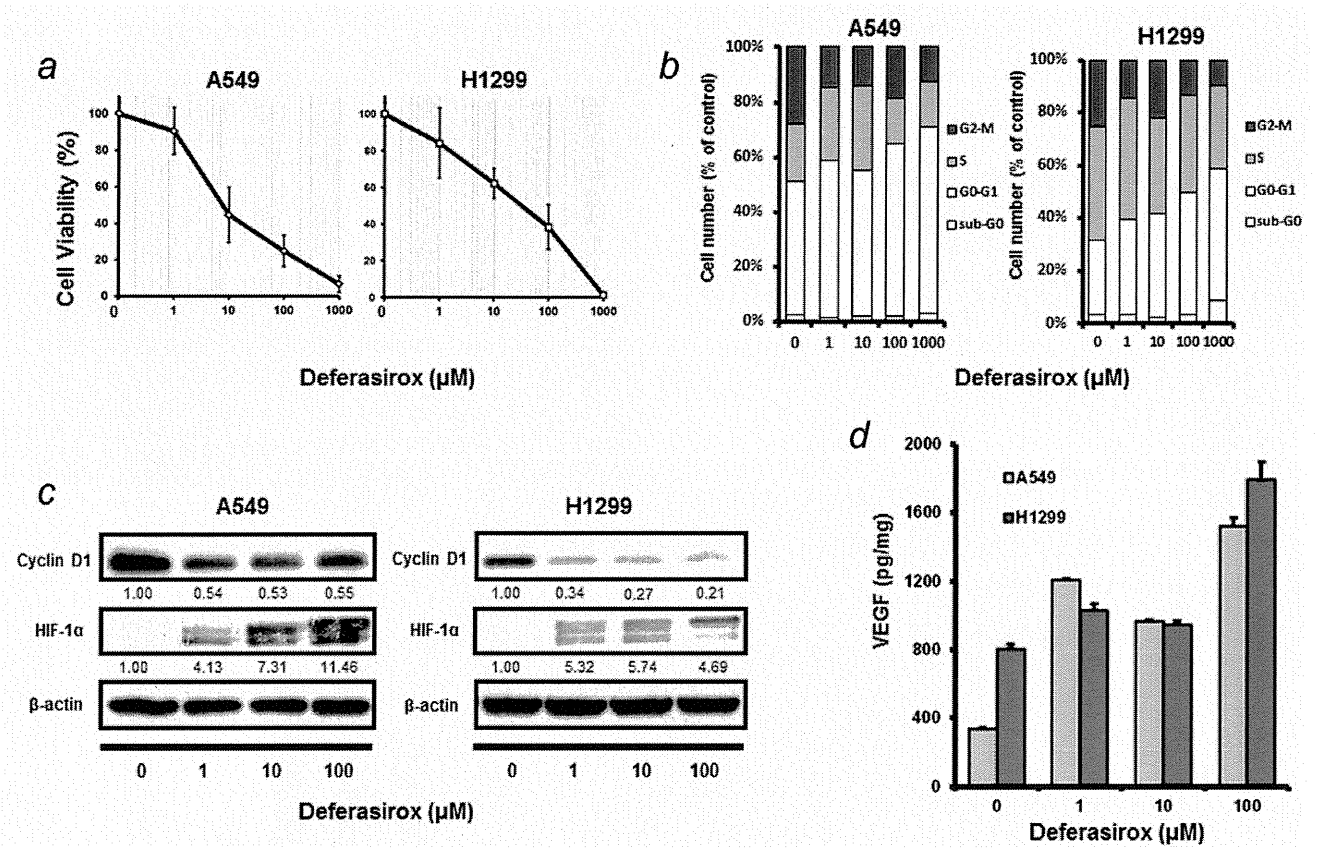


Figure 4. Iron depletion inhibited cell proliferation *via* cell-cycle arrest and induced VEGF secretion *in vitro*. (a) Cultured A549 cells and H1299 cells were treated with the indicated concentrations of deferasirox for 24 h and the cell viability was measured by the WST-1 method. (b) The cells were treated with different concentrations of deferasirox for 24 h and the cell-cycle distribution was analyzed by flow cytometry. Each histogram consists of the following four cell cycle populations; sub-G0 (black), G0-G1 (white), S (light gray) and G2-M (dark gray). (c) Whole-cell lysates and nuclear protein of these cells treated with the indicated concentrations of deferasirox were used for Western blot to determine its inhibitory effects on cell cycle and upregulation effects on HIF-1α. The expression level of each protein was calculated relative to its expression in mock-treated cells, whose expression level was designated as 1. (d) Supernatant treated with the indicated concentrations of deferasirox was harvested and VEGF secretion examined by ELISA.

deficient diet + bevacizumab [18 ± 5.1]) (Fig. 3d). None of the mice died owing to drug-induced toxicity and no other significant adverse events were observed.

Iron depletion inhibited cell proliferation via cell-cycle arrest in vitro

To reproduce an iron-depleted condition *in vitro*, an iron chelator was used (deferasirox, Exjade™). Deferasirox suppressed cancer cell proliferation in A549 and H1299 cells in a dose-dependent manner (Fig. 4a). To identify the mechanism of inhibition, a flow cytometry analysis for cell-cycle distribution was performed (Fig. 4b). Deferasirox increased the population of the G0-G1 phase in the A549 and H1299 cell lines. To confirm the results of the flow cytometry analysis, Western blot analysis was performed using a total protein extraction of the A549 and H1299 cells. The Western blot showed that deferasirox decreased cyclin D1 in a dose-dependent manner (Fig. 4c). Taken together, these results sug-

gest that iron depletion inhibited cancer cell proliferation *via* cell-cycle arrest *in vitro*.

Iron depletion induced VEGF secretion in A549 and H1299 supernatant via upregulation of HIF-1α

To investigate whether iron depletion induces angiogenesis *via* HIF-1α, a Western blot analysis and ELISA assay were performed. Deferasirox treatment induced nuclear HIF-1α expression (Fig. 4c) and significantly increased VEGF secretion from A549 and H1299 cells, both in a dose-dependent manner (Fig. 4d). These results suggested that iron deficiency induced angiogenesis *via* HIF-1α and VEGF signaling. However, *in vitro* angiogenesis assay (tube formation assay) did not show the increase by deferasirox administration (Supporting Information Fig. S3). The reason was that deferasirox did not affect HUVEC in proliferation and VEGF secretion in the absence of tumor cells *in vitro*. Thus, the combination

effect of iron depletion with antiangiogenic therapy is prominent only *in vivo* with tumor cells.

Discussion

Cancer cells have the ability to survive under severe conditions. One of the mechanisms for survival under hypoxic conditions is the activation of VEGF signaling *via* HIF-1 α .²³ This mechanism also allows cancer cells to, sometimes, be able to resist chemotherapy.^{24,25} Therefore, the effectiveness of chemotherapy can be compromised or attenuated. These experiences indicate that chemotherapy could be more effective under conditions where cancer cells would have difficulty resisting. Antiangiogenic drugs, especially bevacizumab, are known to have an excellent antitumor effect in the clinic³⁻⁵ and are commonly used concurrently with antitumor drugs. When multiple antitumor and antiangiogenic drugs are prescribed, the financial burden on the patient is increased.^{26,27} Therefore, a new strategy that would enhance the effect of antiangiogenic therapy would be beneficial. As iron depletion is known to reduce serum hemoglobin and oxygen supply to tissue in humans,^{10,11} we hypothesized that iron depletion with antiangiogenic therapy could have a novel therapeutic effect for the treatment of cancer. Moreover, iron-controlled treatment has some advantages such as having its own antitumor effect, being easily controllable in daily living, and being inexpensive.

First, we examined the effect of iron depletion on the mice and tumor growth. The iron-depleted mice revealed low serum levels of hemoglobin, iron, red blood cells and ferritin compared to the mice fed a normal diet. Tumor growth was suppressed in the iron-depleted mice. Tumor tissue was extracted and examined by histology and immunohistology. Iron deposits in the stroma of the tumors were reduced in the iron-depleted mice. This phenomenon suggested that the iron condition of the tumor influenced not only the cancer cell but also stroma cells. Pimonidazole (Hypoxyprobe-1 kit) and CD-31 staining showed that iron depletion induced hypoxia and angiogenesis. To confirm hypoxia in the tumor and analyze the differences in cell signaling, we further examined the expression of HIF-1 α . HIF-1 α was predictably upregulated in iron-depleted tumors at the protein level. Taken together, these results suggested that iron depletion suppressed tumor growth and reciprocally induced angiogenesis *via* hypoxia. To the best of our knowledge, there is no previous report of this reciprocal phenomenon.

We next investigated the mechanism of iron deficiency and reciprocally induced angiogenesis *in vitro*. We used an iron chelator (deferiasirox) to stimulate iron depletion owing to its usefulness *in vitro* and the expectation to apply it clinically. Deferiasirox is the first orally bioavailable iron chelator with an indication for the treatment of iron overload in transfusion-dependent anemias. Deferiasirox is the most useful iron chelator in the clinical setting and has been

reported to have an antiproliferative effect in leukemia and hepatoma cells.^{28,29} In our study, deferiasirox inhibited the cancer cell proliferation in lung cancer cell lines A549 and H1299. We also examined apoptosis using the tunnel assay and found no significant difference in the deferiasirox treatment group (data not shown). An ELISA assay and Western blot analysis showed that deferiasirox treatment significantly increased VEGF secretion *via* upregulation of HIF-1 α . Similar to the *in vivo* results, our *in vitro* study showed that iron depletion inhibited cancer cell proliferation and reciprocally induced angiogenesis. These results may suggest that the cancer cells overexpressed VEGF to escape the iron-depleted condition.

We then hypothesized that iron depletion increased the antitumor effect of an antiangiogenic drug. Bevacizumab, an antibody against VEGF, is the most common antiangiogenic drug used clinically and is approved for the treatment of many kinds of cancer. Bevacizumab is usually used as a combination with other antitumor drug. The reason is that bevacizumab is targeting only VEGF instead of the cancer cell. This is the first study of bevacizumab combination therapy with controlled internal iron condition. Bevacizumab had a dramatic synergistic antitumor effect with iron depletion in our *in vivo* study, indicating an inexpensive method to enhance the effectiveness of chemotherapy. Additionally, these findings may lead to the changes in the daily diet recommendations for patients being treated with an antiangiogenic drug. Iron depletion condition induced antiproliferative effect and angiogenesis. As a result, bevacizumab inhibited angiogenesis and provided strong antitumor effect. Of course, the mechanism is not completely explained and further *in vivo* studies are necessary. For example, *in vivo* live imaging of the tumor growth as well as angiogenesis may be extremely useful.³⁰⁻³⁴

Bevacizumab is an established antiangiogenic drug with clinical benefits for many kinds of cancer.^{35,36} However, there is no reliable biomarker or method by which curative effect can be predicted.^{37,38} Our study showed that bevacizumab combined with iron depletion was very effective; therefore, we hypothesized that serum iron level could be a novel bevacizumab biomarker. We previously conducted a retrospective study to investigate the correlation between serum hemoglobin level and bevacizumab response rate in 34 patients with colorectal cancer in our facility assessed by RECIST criteria between September 2007 and July 2010.³⁹ Patient characteristics are summarized in Supporting Information Table 1. The response rate of the low-Hb patient group was higher than that of high-Hb group (41.2 vs. 17.6%). This result combined with the results of our present study suggests that a prospective study of bevacizumab with iron control therapy in patients with cancer is warranted.

Conclusions

In conclusion, iron depletion inhibited the cancer cell proliferation and reciprocally induced angiogenesis *in vitro* and *in*

vivo. Bevacizumab had a dramatic synergistic antitumor effect with iron depletion. Treatment to create an iron-depleted condition could induce a novel therapeutic effect with antian-giogenic drugs in the treatment of cancer.

References

- Kim KJ, Li B, Winer J, et al. Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. *Nature* 1993;362:841–4.
- Forsythe JA, Jiang BH, Iyer NV, et al. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996;16:4604–13.
- Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–42.
- Kabbinnar FF, Hambleton J, Mass RD, et al. Combined analysis of efficacy: the addition of bevacizumab to fluorouracil/leucovorin improves survival for patients with metastatic colorectal cancer. *J Clin Oncol* 2005;23:3706–12.
- Saltz LB, Clarke S, Diaz-Rubio E, et al. Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. *J Clin Oncol* 2008;26:2013–19.
- Richmond HG. Induction of sarcoma in the rat by iron-dextran complex. *Br Med J* 1959;1:947–9.
- Okada S, Hamazaki S, Toyokuni S, et al. Induction of mesothelioma by intraperitoneal injections of ferric saccharate in male Wistar rats. *Br J Cancer* 1989;60:708–11.
- Kato J, Miyamishi K, Kobune M, et al. Long-term phlebotomy with low-iron diet therapy lowers risk of development of hepatocellular carcinoma from chronic hepatitis C. *J Gastroenterol* 2007;42:830–6.
- Hann HW, Stahlhut MW, Blumberg BS. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. *Cancer Res* 1988;48:4168–70.
- Becker A, Stadler P, Lavey RS, et al. Severe anemia is associated with poor tumor oxygenation in head and neck squamous cell carcinomas. *Int J Radiat Oncol Biol Phys* 2000;46:459–66.
- Vaupel P, Mayer A, Briest S, et al. Oxygenation gain factor: a novel parameter characterizing the association between hemoglobin level and the oxygenation status of breast cancers. *Cancer Res* 2003;63:7634–7.
- Shirakawa Y, Naomoto Y, Kimura M, et al. Topological analysis of p21WAF1/CIP1 expression in esophageal squamous dysplasia. *Clin Cancer Res* 2000;6:541–50.
- Chang YS, Adnane J, Trail PA, et al. Sorafenib (BAY 43-9006) inhibits tumor growth and vascularization and induces tumor apoptosis and hypoxia in RCC xenograft models. *Cancer Chemother Pharmacol* 2007;59:561–74.
- Schmeding M, Rademacher S, Boas-Knoop S, et al. rHuEPO reduces ischemia-reperfusion injury and improves survival after transplantation of fatty livers in rats. *Transplantation* 2010;89:161–8.
- Fei J, Hong A, Dobbins TA, et al. Prognostic significance of vascular endothelial growth factor in squamous cell carcinomas of the tonsil in relation to human papillomavirus status and epidermal growth factor receptor. *Ann Surg Oncol* 2009;16:2908–17.
- Myoung H, Hong SD, Kim YY, et al. Evaluation of the anti-tumor and anti-angiogenic effect of paclitaxel and thalidomide on the xenotransplanted oral squamous cell carcinoma. *Cancer Lett* 2001;163:191–200.
- Hashimoto Y, Watanabe Y, Shirakiya Y, et al. Establishment of biological and pharmacokinetic assays of telomerase-specific replication-selective adenovirus. *Cancer Sci* 2008;99:385–90.
- Ohara T, Takaoka M, Toyooka S, et al. Inhibition of mTOR by temsirolimus contributes to prolonged survival of mice with pleural dissemination of non-small-cell lung cancer cells. *Cancer Sci* 2011;102:1344–9.
- Kohgo Y, Niitsu Y, Kondo H, et al. Serum transferrin receptor as a new index of erythropoiesis. *Blood* 1987;70:1955–8.
- Ke Q, Costa M. Hypoxia-inducible factor-1 (HIF-1). *Mol Pharmacol* 2006;70:1469–80.
- Schicher N, Paulitschke V, Swoboda A, et al. Erlotinib and bevacizumab have synergistic activity against melanoma. *Clin Cancer Res* 2009;15:3495–3502.
- Argov M, Kashi R, Peer D, et al. Treatment of resistant human colon cancer xenografts by a fluoxetine-doxorubicin combination enhances therapeutic responses comparable to an aggressive bevacizumab regimen. *Cancer Lett* 2009;274:118–25.
- Shima DT, Deutsch U, D'Amore PA. Hypoxic induction of vascular endothelial growth factor (VEGF) in human epithelial cells is mediated by increases in mRNA stability. *FEBS Lett* 1995;370:203–8.
- Guminski AD, Harnett PR, deFazio A. Scientists and clinicians test their metal-back to the future with platinum compounds. *Lancet Oncol* 2002;3:312–8.
- Chang A. Chemotherapy, chemoresistance and the changing treatment landscape for NSCLC. *Lung Cancer* 2011;71:3–10.
- Tappenden P, Jones R, Paisley S, et al. The cost-effectiveness of bevacizumab in the first-line treatment of metastatic colorectal cancer in England and Wales. *Eur J Cancer* 2007;43:2487–94.
- Montero AJ, Avancha K, Gluck S, et al. A cost-benefit analysis of bevacizumab in combination with paclitaxel in the first-line treatment of patients with metastatic breast cancer. *Breast Cancer Res Treat* 2012;132:747–51.
- Ohyashiki JH, Kobayashi C, Hamamura R, et al. The oral iron chelator deferasirox represses signaling through the mTOR in myeloid leukemia cells by enhancing expression of REDD1. *Cancer Sci* 2009;100:970–77.
- Lescoat G, Chantrel-Groussard K, Pasdeloup N, et al. Antiproliferative and apoptotic effects in rat and human hepatoma cell cultures of the orally active iron chelator ICL670 compared to CP20: a possible relationship with polyamine metabolism. *Cell Prolif* 2007;40:755–67.
- Hoffman, RM. Orthotopic metastatic mouse models for anticancer drug discovery and evaluation: a bridge to the clinic. *Invest New Drugs* 1999;17:343–59.
- Hoffman, RM. The multiple uses of fluorescent proteins to visualize cancer in vivo. *Nat Rev Cancer* 2005;5:796–806.
- Hoffman RM, Yang M. Color-coded fluorescence imaging of tumor-host interactions. *Nat Protoc* 2006;1:928–35.
- Amoh Y, Yang M, Li L, et al. Nestin-linked green fluorescent protein transgenic nude mouse for imaging human tumor angiogenesis. *Cancer Res* 2005;65:5352–7.
- Amoh Y, Li L, Tsuji K, et al. Dual-color imaging of nascent blood vessels vascularizing pancreatic cancer in an orthotopic model demonstrates antiangiogenesis efficacy of gemcitabine. *J Surg Res* 2006;132:164–9.
- Shih T, Lindley C. Bevacizumab: an angiogenesis inhibitor for the treatment of solid malignancies. *Clin Ther* 2006;28:1779–1802.
- Wheatley-Price P, Shepherd FA. Targeting angiogenesis in the treatment of lung cancer. *J Thorac Oncol* 2008;3:1173–84.
- Jubb AM, Harris AL. Biomarkers to predict the clinical efficacy of bevacizumab in cancer. *Lancet Oncol* 2010;11:1172–83.
- Mancuso A, Sternberg CN. Colorectal cancer and antiangiogenic therapy: what can be expected in clinical practice? *Crit Rev Oncol Hematol* 2005;55:67–81.
- Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 2009;45:228–47.

Multicenter phase II study of S-1 and docetaxel combination chemotherapy for advanced or recurrent gastric cancer patients with peritoneal dissemination

Kunitoshi Shigeyasu, Shunsuke Kagawa, Futoshi Uno, Masahiko Nishizaki, Hiroyuki Kishimoto, Akira Gochi, Toshikazu Kimura, et al.

Cancer Chemotherapy and
Pharmacology

ISSN 0344-5704

Cancer Chemother Pharmacol
DOI 10.1007/s00280-013-2086-0

ONLINE
FIRST

Cancer Chemotherapy and Pharmacology

REVIEW ARTICLE

The anti-cancer activities of jasmonates
Z. Raviv · S. Cohen · D. Reischer-Peled · 275

ORIGINAL ARTICLES

Comparison of pharmacokinetics and safety profiles of two
capecitabine tablet formulations in patients with colon,
colorectal or breast cancer
S. Chachad · S. Purnanandare · G. Malhotra · R. Naidu · 287

Effect of topical thymidine on immunohistochemical levels of IL-1β
and TNF-α in 5-fluorouracil-induced oral mucositis in hamsters
M. Correa · M.A. Martins · L.S. Louren · A.C.A. Pelliccioli ·
M. Sant'Ana Filho · Y.C.S. Pavesi · V.C. Carand · W.D. Martins · 293

CYP3A4 phenotype indicates for optimized antiestrogen efficacy
associates with outcome in early breast cancer patients
A. Trojan · A. Vargopoulos · U. Breitenstein · C. Tausch · B. Seifert ·
W. Jochke · 301

Sunitinib combined with paclitaxel and docetaxel results of a phase I
dose-escalation and pharmacokinetic study in patients with advanced
solid malignancies, with an expanded cohort in non-small cell lung
cancer and mesothelioma
D.R. Comridge · H. Bials · D.J. Jorke · D. Soulières · R.C. Doebele ·
A. Ruiz-García · A. Thill · K. Zhang · S.A. Laurie · R.C. Chao ·
L.O. Chow · 307

A phase I trial of imatinib in combination with mFOLFIRI-bevacizumab
in patients with advanced colorectal cancer
M. Michael · J. Zalcberg · P. Gilroy · L. Lister · M. Gouillou · M. Jefford ·
G. McArthur · M. Copeman · K. Lynch · N.C. Tebbutt · 321

In vitro cytotoxicity, pharmacokinetics, tissue distribution, and metabolism
of small molecule protein kinase D inhibitors, Jh-16142-70 and Jh-16185-09,
in mice bearing human cancer xenografts
J. Guo · D.M. Clausen · J.H. Beumer · R.A. Parise · M.J. Egorin ·
K. Bravo-Alamirano · P. Wipf · E.R. Sharlow · Q.J. Wang ·
J.L. Eisenman · 331

Phase II study of nedaplatin and irinotecan in patients with extensive
small-cell lung cancer
F. Oshita · M. Sugiyama · S. Murakami · T. Kondo · H. Saito · K. Yamada · 345

Pharmacokinetic study and effectiveness evaluation of slow-release
PLGA-5-fluorouracil microspheres
J. Li · Y. Pu · S. Wang · M. Ding · D. Chen · M. Zhu · 351

Gender-specific elimination of continuous-infusional 5-fluorouracil
in patients with gastrointestinal malignancies: results from a prospective
population pharmacokinetic study
F. Mueller · B. Büchel · D. Rübe · S. Schürch · B. Pfister ·
St. Krähenbühl · T.K. Friedlrich · C.R. Largiadèr · M. Joeger · 361

Triple combination with paclitaxel, docetaxel and 5-FU is effective
in metastatic and/or recurrent nasopharyngeal carcinoma
C. Chen · F. Wang · X. An · H. Luo · Z. Wang · Y. Liang · L. Zhang · Y. Li · 371

In vitro and in vivo studies of pharmacokinetics and antitumor efficacy
of DQ7001-F4, an oral gemcitabine formulation
W.-H. Hsu · J.-J. Wang · S.-P. Hsueh · P.-J. Hsu · L.-C. Chang · C.-S. Hou ·
K.-Y. Hsu · 379

Dose-finding study of hepatic arterial infusion of oxaliplatin-based
treatment in patients with advanced solid tumors metastatic
to the liver
A.M. Eisenberg · M.B. Leick · J. Lin · S. Fu · J. Wheeler · S.A. Pihl-Paul ·
D. Hong · G.S. Falchook · A. Nain · J.M. Subbiah · A. Fortner ·
R. Avritscher · R. Kurzrock · 389

Phase II study of docetaxel and oral VP16 in patients with refractory or
relapsed Ewing sarcoma
C. Owens · V. Lawrence · L. Benboukhar · A. S. Defachellier ·
D. Cupissol · H. Rubie · H. Brisse · A. Rey · L. Olivier · D. Couanet ·
C. Baumin · C.M. Ari-Oukhatir · O. Oberlin · 399

Association between bevacizumab-related hypertension and vascular
endothelial growth factor (VEGF) gene polymorphisms in Japanese
patients with metastatic colorectal cancer
S. Morita · K. Uehara · G. Nakayama · I. Shibata · T. Oguri · M. Inoue ·
T. Shimokata · H. Sugihito · A. Mitsuma · Y. Ando · 405

Contents continued on the back cover

Submit manuscripts at: <http://ccp.manuscriptcentral.com>

 Springer

Your article is protected by copyright and all rights are held exclusively by Springer-Verlag Berlin Heidelberg. This e-offprint is for personal use only and shall not be self-archived in electronic repositories. If you wish to self-archive your work, please use the accepted author's version for posting to your own website or your institution's repository. You may further deposit the accepted author's version on a funder's repository at a funder's request, provided it is not made publicly available until 12 months after publication.

Multicenter phase II study of S-1 and docetaxel combination chemotherapy for advanced or recurrent gastric cancer patients with peritoneal dissemination

Kunitoshi Shigeyasu · Shunsuke Kagawa · Futoshi Uno · Masahiko Nishizaki · Hiroyuki Kishimoto · Akira Gochi · Toshikazu Kimura · Takaomi Takahata · Yasuyuki Nonaka · Motoki Ninomiya · Toshiyoshi Fujiwara

Received: 30 August 2012 / Accepted: 10 January 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract

Purpose Peritoneal dissemination is the most frequent and life-threatening mode of metastasis and recurrence in patients with gastric cancer. A multicenter phase II study was designed to evaluate the efficacy and tolerability of S-1 and docetaxel combination chemotherapy regimen for the treatment of advanced or recurrent gastric cancer patients with peritoneal dissemination.

Methods Nineteen patients with histologically confirmed unresectable or recurrent gastric cancer with peritoneal dissemination were enrolled. Oral S-1 at 80 mg/m²/day was administered twice daily for 2 weeks, followed by 1 drug-free week. Docetaxel infusion at 40 mg/m² was performed on day 1, simultaneous with S-1 administration. The primary endpoints were overall survival (OS) and time to progression (TTP). The secondary endpoints were the response rates and safety status.

Results Patients received a median of 4 cycles of the S-1 and docetaxel regimen (range 1–43). The disease control rate was 73.7 % (14/19). Median overall survival was 459 days (15.3 months), while median time to progression was 212 days (7.1 months). Neutropenia was the most common type of toxicity ($n = 7$, 36.8 %).

Conclusions Combination chemotherapy with S-1 and docetaxel is a tolerable and effective treatment for advanced or recurrent gastric cancer patients with peritoneal dissemination.

Keywords Gastric cancer · Peritoneal dissemination · S-1 · Docetaxel · Phase II study

Introduction

Gastric cancer is the second most common cause of cancer-related deaths, with 700,000 worldwide mortalities reported annually [1]. In Japan, the proportion of patients with gastric cancer has recently decreased; however, it remains one of the most refractory cancers [2]. The prognosis for patients with unresectable or recurrent gastric cancer is extremely poor. The reported median survival time of patients with untreated gastric cancer is 3–5 months [3, 4]. In particular, peritoneal dissemination is the most frequent and life-threatening form of metastasis and recurrence in patients with gastric cancer, and it is often refractory to chemotherapy [5–7]. Randomized clinical trials using combination chemotherapies such as docetaxel, cisplatin and fluorouracil (DCF) or epirubicin, cisplatin and fluorouracil (ECF) were carried out on non-curable gastric cancer patients; however, the median survival time (MST) was only 8.9 and 9.2 months, respectively [8, 9]. These

K. Shigeyasu · S. Kagawa · F. Uno · M. Nishizaki · H. Kishimoto · A. Gochi · T. Fujiwara (✉)
Department of Gastroenterological Surgery,
Okayama University Graduate School of Medicine,
Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho,
Kita-ku, Okayama 700-8558, Japan
e-mail: toshi_f@md.okayama-u.ac.jp

T. Kimura · T. Takahata
Department of Surgery, Okayama Saiseikai General Hospital,
Okayama, Japan

Y. Nonaka
Department of Surgery, Tsuyama Chuo Hospital,
Tsuyama, Japan

M. Ninomiya
Department of General Surgery, Hiroshima City Hospital,
Hiroshima, Japan

outcomes were considered unsatisfactory and, therefore, the search for more effective regimens is warranted.

The anticancer drug S-1 is an orally active combination of tegafur (a pro-drug converted to fluorouracil), gimeracil (a dihydropyrimidine dehydrogenase inhibitor that degrades fluorouracil) and oteracil (a fluorouracil phosphorylation inhibitor in the gastrointestinal tract that reduces fluorouracil toxicity) in a molar ratio of 1:0.4:1 [10, 11]. S-1 has been evaluated in two randomized controlled trials conducted in Japan: the JCOG9912 trial and the S-1 plus cisplatin versus S-1 randomized controlled trial for the treatment for gastric cancer (SPIRITS) [12, 13]. The former demonstrated that S-1 is superior to continuous infusion of 5-fluorouracil with respect to overall survival (OS). The latter demonstrated that S-1 plus cisplatin (CDDP) is superior to S-1 alone with respect to OS and that this combination could be an effective standard chemotherapy regimen for advanced or recurrent gastric cancer.

The novel drug docetaxel has also shown promise for the treatment of advanced gastric cancer, both as monotherapy [14] and in combination with other agents [15–17]. Two previous phase II studies evaluated the effects of S-1 and docetaxel combination chemotherapy and reported overall response rates of 56.3 and 57.8 %, and median OS of 14.3 and 15.3 months, respectively [18, 19]. These studies demonstrated that the combination of S-1 and docetaxel is a promising therapy for advanced gastric cancer; however, the specific effect of this regimen on gastric cancer patients with peritoneal dissemination remains unclear as both measurable and non-measurable lesions were included in previous studies. Paclitaxel as well as docetaxel is expected to be effective for peritoneal dissemination because of their high affinity for the peritoneum [20].

In this study, we evaluated an S-1 and docetaxel combination regimen for the treatment of advanced or recurrent gastric cancer patients with peritoneal dissemination, which has not been defined as a measurable lesion in conventional phase II studies.

Methods

Patient eligibility

Patients who met the following criteria were included: histologically proven unresectable or recurrent gastric adenocarcinomas; presence of positive peritoneal cytology or macroscopic peritoneal dissemination diagnosed by imaging or staging laparoscopy; no prior therapy within the previous 28 days; adequate hepatic, cardiac, renal and bone marrow function (hemoglobin ≥ 8 g/dl; white blood cell count, 3,500–12,000/mm³; neutrophil

count $\geq 1,500$ /mm³; platelet count $\geq 100,000$ /mm³; total bilirubin ≤ 1.5 mg/dl; and aspartate aminotransferase and alanine aminotransferase $\leq 2.5 \times$ the upper limit of the institution); Eastern Cooperative Oncology Group (ECOG) performance status of 0–1 estimated life expectancy >3 months; between 20 and 75 years old; ability to take medications orally; no abnormal electrocardiography findings; and written informed consent. Patients were not excluded, even if they had other metastatic sites (such as distant lymph nodes, liver, lung or bone). This study was approved by the Institutional Review Board of the participating institutions.

Treatment strategy

The trial was a multicenter, open-labeled, non-randomized and non-controlled phase II study of S-1 and docetaxel. S-1 was orally administered twice daily at 80 mg/m² for 2 weeks, followed by a drug-free interval of 1 week (one cycle). Docetaxel was intravenously administered at 40 mg/m² on day 1, simultaneous with S-1 administration. Dexamethasone was infused before docetaxel to prevent an allergic response. Granulocyte colony-stimulating factor (G-CSF) was used therapeutically in the event of grade 4 febrile neutropenia. The primary end points of this study were the overall survival (OS) and time to progression (TTP). The secondary end points were the response rates and safety. Treatment continued until evidence of tumor progression, unacceptable toxicity, patient refusal or the physician's decision to stop treatment. Toxicity was graded according to CTCAE v3.0. S-1 was reduced to 50 mg/m² in cases of grade 4 leukopenia or neutropenia, \geq grade 3 thrombocytopenia or \geq grade 3 non-hematologic toxicity except anorexia, nausea and vomiting occurred. Docetaxel was maintained at 40 mg/m². Treatment was resumed after recovery from toxic events.

Evaluation of the disease

Tumor response of measurable lesions was assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [21]. Tumor markers, including carcinoembryonic antigen (CEA) and carbohydrate antigen 19–9 (CA19–9), were monitored monthly. Non-measurable lesions were assessed by upper gastrointestinal endoscopy, ultrasonography, computed tomography, upper gastrointestinal radiography and cytology. In addition to objective tumor response based on RECIST guidelines, the amount of malignant ascites was assessed by CT according to the Japanese Classification of Gastric Carcinoma—2nd English Edition [22]. All those evaluations were taken into account to calculate tumor response rate of measurable and non-measurable lesions.

Statistical analysis

The required number of patients was calculated according to the Southwest Oncology Group One Arm Survival program [23]. The JCOG 9603 study reported that the median survival time was 155 days (95 % confidence interval (CI), 131–225 days) in the sequential treatment with Methotrexate and 5-FU in patients with peritoneal dissemination which is cytologically confirmed malignant ascites [24]. Tahara et al. [25] reported that the median survival time was 259 days in a retrospective study of 95 patients with peritoneal dissemination of gastric cancer treated with sequential MTX/5-FU. Based on these evidences, assuming a null hypothesis of 150 days and an alternative hypothesis of 259 days with one-sided type-I error of 0.05 and power of 0.8, with an accrual time of 2 years and follow-up of 1 year after closure of recruitment, it was necessary to enroll 23 assessable patients. TTP and OS were calculated using the Kaplan–Meier method. TTP was calculated as the interval between chemotherapy initiation and disease progression. OS was measured as the interval between chemotherapy initiation and death. Disease control rate (DCR) was defined by complete and partial responses and stable disease.

Results

Patient characteristics

A total of 19 patients with histologically proven unresectable or recurrent gastric cancer with peritoneal dissemination were enrolled onto this clinical trial from October 2005 to September 2010 (Table 1). The patients comprised 13 males and 6 females, with a median age of 54.3 years (range 21–73 years). The ECOG performance status for all patients was 0 or 1. Most patients had undifferentiated adenocarcinoma (17/19, 89.5 %). Fourteen patients (73.7 %) had no prior therapy. Of the 5 patients (26.3 %) who received prior therapies, 2 patients underwent gastrectomy (10.5 %) and 3 patients had gastrectomy followed by S-1 therapy (5.3 %), S-1 plus CDDP followed by gastrectomy (5.3 %) and S-1 plus CDDP followed by S-1 monotherapy (5.3 %). The lesions were measurable in 2 patients (10.5 %) and non-measurable in 17 patients (89.5 %).

Clinical outcome

The median number of courses per patient was 4 (average = 6.9), and the range was 1–43 courses (Table 2). All patients were evaluated for clinical response by upper gastrointestinal endoscopy, ultrasonography, computed

Table 1 Patient characteristics ($n = 19$)

Characteristic	Number of patients (%)
Gender	
Male	13 (68.4)
Female	6 (31.6)
Age	
Median	54.3
Range	24–73
Performance status by ECOG	
0	16 (84.2)
1	3 (15.8)
2	0 (0)
3	0 (0)
Disease status	
Advanced	15 (78.9)
Recurrent	4 (21.1)
Histological type	
Differentiated	2 (10.5)
Undifferentiated	17 (89.5)
Prior therapy	
None	14 (73.7)
Gastrectomy	2 (10.5)
Gastrectomy, S-1	1 (5.3)
S-1 + CDDP, Gastrectomy	1 (5.3)
S-1 + CDDP, S-1	1 (5.3)
Measurable lesion	
Yes	2 (10.5)
No	17 (89.5)

ECOG eastern cooperative oncology group; CDDP cisplatin

tomography, upper gastrointestinal radiography, cytology and clinical findings. Objective responses consisted of a partial response in 2 patients (10.5 %), stable disease in 12 patients (63.2 %) and progressive disease in 5 patients (26.3 %); the disease control rate (DCR) was 73.7 % (14/19) (Table 3). Overall survival analysis by Kaplan–Meier was 57.9 % at 1 year and 25.2 % at 2 years (Fig. 1). The median survival time (MST) was 459 days (15.3 months). The median TTP was 212 days (7.1 months), with a 1-year progression-free rate of 26.3 % (Fig. 2).

Adverse events

Toxicities and adverse events (AE) that occurred in all patients are presented in Table 4. Among the grade 3 and 4 hematologic toxicities, neutropenia was the most common (7/19, 36.8 %), followed by febrile neutropenia (2/19, 10.5 %). All adverse events were manageable with G-CSF. Non-hematologic toxicity was limited, with three incidences of grade 3 anorexia (5.3 %), nausea (5.3 %) and allergic reaction (5.3 %). All non-hematologic toxicities

Table 2 Treatment courses

Number of cycles	
Median	4
Range	1–43
Mean	6.9

Table 3 Response assessment ($n = 19$)

Number of patients (%)	
Complete response	0 (0)
Partial response	2 (10.5)
Stable disease	12 (63.2)
Progressive disease	5 (26.3)

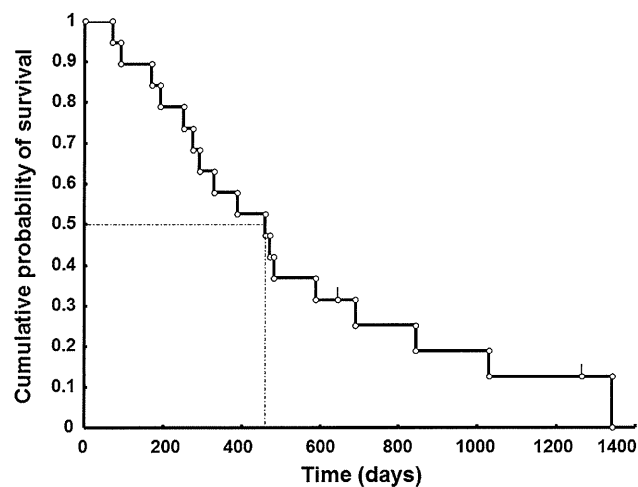


Fig. 1 Kaplan-Meier plot for overall survival time of all 19 patients. The MST was 459 days (15.3 months), with a 1-year survival of 57.9 % and a 2-year survival of 25.2 %

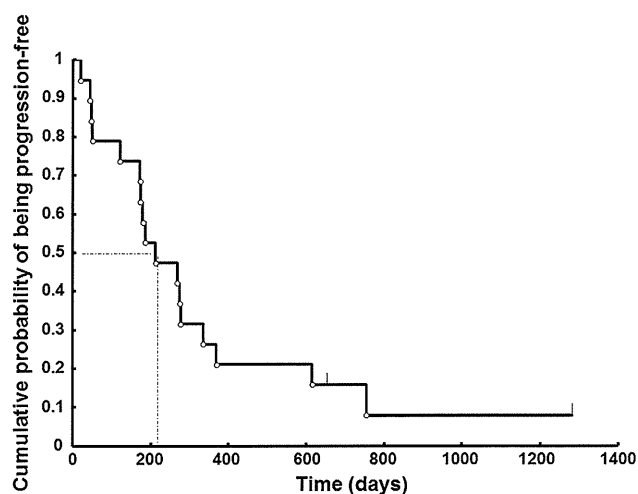


Fig. 2 The TTP of all 19 patients. The median time to progression was 212 days (7.1 months), with a 1-year progression-free rate of 26.3 %

were manageable with optimal treatment. All patients recovered with conservative care, and there were no treatment-related deaths.

Discussion

Peritoneal dissemination is the most common and significant cause of mortality in patients with advanced or recurrent gastric cancer. To improve the overall prognosis of gastric cancer patients, the treatment outcome of patients with peritoneal dissemination must be improved. Various treatment regimens have been developed for advanced or recurrent gastric cancer; however, the efficacy is still less than ideal [5–7] and, therefore, a safe, effective and tolerable regimen is required. In the present study, we conducted a prospective multicenter phase II study to assess the efficacy and safety of oral S-1 plus systemic docetaxel in gastric cancer patients with peritoneal dissemination.

The oral anticancer drug S-1 inhibits the phosphorylation of fluorouracil in the gastrointestinal tract and reduces gastrointestinal toxicities [10, 11]. It has been reported that S-1 monotherapy is associated with a response rate of 45 % and a 2-year survival rate of 17 % in advanced gastric cancer [26, 27]. These findings led us to consider that S-1 in combination with other chemotherapeutic drugs might

Table 4 Toxicity profile

Toxicity	Grade (number of patients)				3 or 4 (%)
	1	2	3	4	
Hematologic					
Leukopenia	2	8	1	5.3	36.8
Neutropenia	1	3	5	2	
Anemia	2	3			
Thrombocytopenia	2				
Febrile neutropenia	2	10.5			
Nonhematologic					
Alopecia	3	8			
Anorexia	7	1	1	5.3	
General fatigue	2				
Nausea	3	2	1	5.3	
Vomiting	1	1			
Diarrhea	4				
Constipation	2				
Allergic reaction	1	1	5.3		
Sensory neuropath	1	2			
Dysguesia	3				
Hyperpigmentation	3				
Stomatitis	1	1			

Toxicity was graded according to Common Terminology Criteria for Adverse Events (CTCAE) v3.0

be more effective than S-1 alone. Potential partners for S-1 include cisplatin, irinotecan (CPT-11) and taxane such as paclitaxel and docetaxel. The SPIRITS trial showed a survival benefit of S-1 plus cisplatin over S-1, with response rates of 31–54 % and OS of 11–13 months, resulting in the establishment of a standard care for advanced gastric cancer in Japan [13]. In contrast, the GC0301/TOP-002 trial failed to prove the advantages of S-1 and irinotecan therapy over S-1 monotherapy [28]. Recently, the combination of S-1 and paclitaxel was reported to be an effective regimen for the treatment of advanced gastric cancer with peritoneal dissemination [29]. Paclitaxel was administered intravenously at 50 mg/m² and intraperitoneally at 20 mg/m² on days 1 and 8, respectively. S-1 was administered at 80 mg/m²/day for 14 consecutive days, followed by 7 days rest. Although the outcome—a 1-year OS rate of 78 % and overall response rate of 56 %—appears encouraging, patients nevertheless have to be implanted with ports by mini-laparotomy under local anesthesia.

Docetaxel has synergistic antitumor activity in vivo with S-1 and is not associated with any overlapping toxicities [30]. In addition, docetaxel exhibits high antitumor activity in poorly differentiated adenocarcinoma [31]. The S-1 with docetaxel regimen has been evaluated in two previous phase II studies. Yoshida et al. [18] have reported the activity and tolerability of docetaxel and S-1 combination therapy for advanced or recurrent gastric cancer. Patients were treated with intravenous docetaxel (40 mg/m²) on day 1 and oral S-1 (80 mg/m²/day) on days 1–14. The overall response rate was 56.3 %, while the tumor control rate was 93.8 %. The median OS was 14.3 months, and the median TTP was 7.3 months. Kunisaki et al. [19] reported a phase II study of biweekly docetaxel and S-1 combination chemotherapy as a first-line treatment for advanced gastric cancer. Patients with histologically proven unresectable or recurrent gastric cancer were eligible for inclusion. Patients received oral S-1 twice daily for 1 week, followed by a drug-free interval of 1 week. Docetaxel (40 mg/m²) was administered intravenously on days 1 and 15. The overall response rate was 57.8 %, the median OS was 15.3 months, the median TTP was 6.9 months and the median response duration was 8 months.

On the basis of these results, we assessed the efficacy of the S-1 and docetaxel regimen for the treatment of advanced gastric cancer with peritoneal dissemination. We focused on the most treatment-refractory patient population having a wide dispersion of cancer cells in the abdominal cavity. Our phase II study demonstrated that S-1 and docetaxel combination therapy to be a highly effective for the treatment of histologically proven unresectable or recurrent gastric cancer patients with peritoneal dissemination, with an acceptable and manageable toxicity profile.

The combination achieved promising results in terms of median TTP (7.1 months) and median OS (15.3 months). Although the present study was originally planned as a study involving 23 patients, patient enrollment had been delayed and finally terminated before the projected number of patients was achieved. This was caused by the eligibility criteria for this study. Most patients with peritoneal metastasis tend to have relatively poor performance status and impaired organ function. In addition, it was also difficult for them to be fully informed and consent to join the study. They were considered to be critical issues delaying patient enrollment. Even with the actual sample size of 19 patients, the median survival time of 459 days (95 % confidence interval: 274–645) was observed, which was longer than initially anticipated. Non-hematologic toxicities were generally mild, and grade 3 non-hematologic toxicities, including anorexia, nausea and allergic reaction, occurred in only 5.3 % patients. The predominant toxicity was myelo-suppression, and grade 3–4 neutropenia occurred in 36.8 % patients; however, both hematologic and non-hematologic toxicities were manageable by conservative treatments. Recently, Kim et al. reported the results of a randomized phase III study of S-1 and docetaxel for unresectable or recurrent gastric cancer (JACCRO GC03 study: START trial), in which the combination had no significant survival benefit over S-1 alone; however, in patients with non-measurable disease, the OS and TTP of the combination group were significantly superior to those treated with S-1 alone [32], which is consistent with our results. Our study represents the first multicenter, prospective phase II study of S-1 plus docetaxel targeting only patients with peritoneal dissemination.

In conclusion, the present study demonstrates that S-1 and docetaxel combination chemotherapy is tolerable and effective for the treatment of advanced or recurrent gastric cancer patients with peritoneal dissemination.

Conflict of interest All authors declare no potential conflict of interest.

References

1. Kamangar F, Dores GM, Anderson WF (2006) Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 24:2137–2150
2. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, Feuer EJ, Thun MJ (2005) Cancer statistics, 2005. *CA Cancer J Clin* 55:10–30
3. Murad AM, Santiago FF, Petroianu A, Rocha PR, Rodrigues MA, Rausch M (1993) Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. *Cancer* 72:37–41
4. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M (1995) Randomised comparison of fluorouracil, epidoxorubicin and

- methotrexate (FEMTX) plus supportive care with supportive care alone in patients with non-resectable gastric cancer. *Br J Cancer* 71:587–591
5. MacDonald JS, Schein PS, Woolley PV, Smythe T, Ueno W, Hoth D, Smith F, Boiron M, Gisselbrecht C, Brunet R, Lagarde C (1980) 5-Fluorouracil, doxorubicin, and mitomycin (FAM) combination chemotherapy for advanced gastric cancer. *Ann Intern Med* 93:533–536
6. Findlay M, Cunningham D, Norman A, Mansi J, Nicolson M, Hickish T, Nicolson V, Nash A, Sacks N, Ford H et al (1994) A phase II study in advanced gastro-esophageal cancer using epirubicin and cisplatin in combination with continuous infusion 5-fluorouracil (ECF). *Ann Oncol* 5:609–616
7. Ohtsu A, Shimada Y, Shirao K, Boku N, Hyodo I, Saito H, Yamamichi N, Miyata Y, Ikeda N, Yamamoto S, Fukuda H, Yoshida S (2003) Randomized phase III trial of fluorouracil alone versus fluorouracil plus cisplatin versus uracil and tegafur plus mitomycin in patients with unresectable, advanced gastric cancer: the Japan Clinical Oncology Group Study (JCOG9205). *J Clin Oncol* 21:54–59
8. Van Cutsem E, Moiseyenko VM, Tjulandin S, Majlis A, Constenla M, Boni C, Rodrigues A, Fodor M, Chao Y, Voznyi E, Risse ML, Ajani JA (2006) Phase III study of docetaxel and cisplatin plus fluorouracil compared with cisplatin and fluorouracil as first-line therapy for advanced gastric cancer: a report of the V325 Study Group. *J Clin Oncol* 24:4991–4997
9. Webb A, Cunningham D, Scarffe JH, Harper P, Norman A, Joffe JK, Hughes M, Mansi J, Findlay M, Hill A, Oates J, Nicolson M, Hickish T, O'Brien M, Iveson T, Watson M, Underhill C, Wardley A, Meehan M (1997) Randomized trial comparing epirubicin, cisplatin, and fluorouracil versus fluorouracil, doxorubicin, and methotrexate in advanced esophagogastric cancer. *J Clin Oncol* 15:261–267
10. Shirasaka T, Shimamoto Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K, Fukushima M (1996) Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators. *Anticancer Drugs* 7:548–557
11. Diasio RB (1999) Clinical implications of dihydropyrimidine dehydrogenase inhibition. *Oncology (Williston Park)* 13:17–21
12. Boku N, Yamamoto S, Fukuda H, Shirao K, Doi T, Sawaki A, Koizumi W, Saito H, Yamaguchi K, Takiuchi H, Nasu J, Ohtsu A (2009) Fluorouracil versus combination of irinotecan plus cisplatin versus S-1 in metastatic gastric cancer: a randomised phase 3 study. *Lancet Oncol* 10:1063–1069
13. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W, Toh Y, Nagaie T, Takagi S, Yamamura Y, Yanaoka K, Orita H, Takeuchi M (2008) S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. *Lancet Oncol* 9:215–221
14. Einzig AI, Neuberg D, Remick SC, Karp DD, O'Dwyer PJ, Stewart JA, Benson AB 3rd (1996) Phase II trial of docetaxel (Taxotere) in patients with adenocarcinoma of the upper gastrointestinal tract previously untreated with cytotoxic chemotherapy: the Eastern Cooperative Oncology Group (ECOG) results of protocol E1293. *Med Oncol* 13:87–93
15. Thuss-Patience PC, Kretschmar A, Reichardt P (2006) Docetaxel in the treatment of gastric cancer. *Future Oncol* 2:603–620
16. Fushida S, Fujimura T, Oyama K, Yagi Y, Kinoshita J, Ohta T (2009) Feasibility and efficacy of preoperative chemotherapy with docetaxel, cisplatin and S-1 in gastric cancer patients with para-aortic lymph node metastases. *Anticancer Drugs* 20:752–756
17. Zang DY, Yang DH, Kim MJ, Jang KM, Hwang SW, Yoo KS, Han T, Kim HY, Kim HJ, Kwon JH, Song HH, Park S, Jung JY, Kim HS, Kim JH (2009) Dose-finding study of docetaxel, oxaliplatin, and S-1 for patients with advanced gastric cancer. *Cancer Chemother Pharmacol* 64:877–883
18. Yoshida K, Ninomiya M, Takakura N, Hirabayashi N, Takiyama W, Sato Y, Todo S, Terashima M, Gotoh M, Sakamoto J, Nishiyama M (2006) Phase II study of docetaxel and S-1 combination therapy for advanced or recurrent gastric cancer. *Clin Cancer Res* 12:3402–3407
19. Kunisaki C, Takahashi M, Makino H, Oshima T, Fujii S, Takagawa R, Kimura J, Kosaka T, Ono HA, Akiyama H, Kameda K, Kito F, Morita S, Endo I (2011) Phase II study of biweekly docetaxel and S-1 combination chemotherapy as first-line treatment for advanced gastric cancer. *Cancer Chemother Pharmacol* 67:1363–1368
20. Kobayashi M, Sakamoto J, Namikawa T, Okamoto K, Okabayashi T, Ichikawa K, Araki K (2006) Pharmacokinetic study of paclitaxel in malignant ascites from advanced gastric cancer patients. *World J Gastroenterol* 12:1412–1415
21. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, Rubinstein L, Shankar L, Dodd L, Kaplan R, Lacombe D, Verweij J (2009) New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer* 45:228–247
22. Japanese Gastric Cancer Association (2001) Japanese classification of gastric carcinoma—2nd English edition—response assessment of chemotherapy and radiotherapy for gastric carcinoma: clinical criteria. *Gastric Cancer* 4:1–8
23. Lawless J (1982) Statistical models and methods for lifetime data. Wiley, New York
24. Yamao T, Shimada Y, Shirao K, Ohtsu A, Ikeda N, Hyodo I, Saito H, Iwase H, Tsuji Y, Tamura T, Yamamoto S, Yoshida S (2004) Phase II study of sequential methotrexate and 5-fluorouracil chemotherapy against peritoneally disseminated gastric cancer with malignant ascites: a report from the Gastrointestinal Oncology Study Group of the Japan Clinical Oncology Group, JCOG 9603 Trial. *Jpn J Clin Oncol* 34:316–322
25. Tahara M, Ohtsu A, Boku N, Nagashima F, Muto M, Sano Y, Yoshida M, Mera K, Hironaka S, Tajiri H, Yoshida S (2001) Sequential methotrexate and 5-fluorouracil therapy for gastric cancer patients with peritoneal dissemination: a retrospective study. *Gastric Cancer* 4:212–218
26. Koizumi W, Kurihara M, Nakano S, Hasegawa K (2000) Phase II study of S-1, a novel oral derivative of 5-fluorouracil, in advanced gastric cancer. For the S-1 Cooperative Gastric Cancer Study Group. *Oncology* 58:191–197
27. Sakata Y, Ohtsu A, Horikoshi N, Sugimachi K, Mitachi Y, Taguchi T (1998) Late phase II study of novel oral fluoropyrimidine anticancer drug S-1 (1 M tegafur-0.4 M gimestat-1 M otastat potassium) in advanced gastric cancer patients. *Eur J Cancer* 34:1715–1720
28. Narahara H, Iishi H, Imamura H, Tsuburaya A, Chin K, Imamoto H, Esaki T, Furukawa H, Hamada C, Sakata Y (2011) Randomized phase III study comparing the efficacy and safety of irinotecan plus S-1 with S-1 alone as first-line treatment for advanced gastric cancer (study GC0301/TOP-002). *Gastric Cancer* 14:72–80
29. Ishigami H, Kitayama J, Kaisaki S, Yamaguchi H, Yamashita H, Emoto S, Nagawa H (2010) Phase I study of biweekly intravenous paclitaxel plus intraperitoneal cisplatin and paclitaxel for gastric cancer with peritoneal metastasis. *Oncology* 79:269–272
30. Van Den Neste E, de Valeriola D, Kerger J, Bleiberg H, Kusenda Z, Brassinne C, Bartholomeus S, Selleslags J, Hennebert P, Wythouck H, Cazenave I, Lefresne-Soulas F, Piccart M (2000) A phase I and pharmacokinetic study of docetaxel administered in combination with continuous intravenous infusion of 5-fluorouracil in patients with advanced solid tumors. *Clin Cancer Res* 6:64–71

31. Taguchi T, Sakata Y, Kanamaru R, Kurihara M, Suminaga M, Ota J, Hirabayashi N (1998) Late phase II clinical study of RP56976 (docetaxel) in patients with advanced/recurrent gastric cancer: a Japanese Cooperative Study Group trial (group A). *Gan To Kagaku Ryoho* 25:1915–1924
32. Fujii M (2008) Chemotherapy for advanced gastric cancer: ongoing phase III study of S-1 alone versus S-1 and docetaxel combination (JACCRO GC03 study). *Int J Clin Oncol* 13:201–205

The effects of the Kampo medicine (Japanese herbal medicine) “Daikenchuto” on the surgical inflammatory response following laparoscopic colorectal resection

Kozo Yoshikawa · Mitsuo Shimada · Masanori Nishioka · Nobuhiro Kurita · Takashi Iwata · Shinya Morimoto · Tomohiko Miyatani · Masato Komatsu · Hideya Kashihara · Chie Mikami

Received: 17 January 2011 / Accepted: 27 May 2011 / Published online: 28 December 2011
© Springer 2011

Abstract

Purposes The inflammatory response after surgery is associated with various postoperative complications. The aim of the present prospective study was to evaluate the effects of Daikenchuto (DKT) (a Japanese herbal medicine) on the inflammatory response in patients following laparoscopic colorectal resection.

Methods Thirty patients who underwent laparoscopic colectomy for colorectal carcinoma were divided into two groups: a DKT intake group (D group, $n = 15$) and a control group (C group, $n = 15$). The D group took 7.5 g/day of DKT from the day after surgery until the 7th postoperative day. The body temperature, heart rate, WBC count, lymphocyte count, C-reactive protein (CRP) level, β -D-glucan level and Candida index were compared between the two groups.

Results The patients' mean age in the D group was significantly younger than that in the C group. D3 lymph node dissection was performed more often in the D group. The time until first flatus was significantly shorter in the D group (1.8 ± 0.5 days) than in the C group (2.7 ± 0.5 days). The CRP level was significantly lower in the D group (4.6 ± 0.6 mg/dl) than in the C group (8.3 ± 1.1 mg/dl) on the 3rd postoperative day.

Conclusions Postoperative DKT administration significantly suppressed the CRP level and shortened the time until first flatus. DKT administration also significantly

suppressed postoperative inflammation following surgery for colorectal cancer.

Introduction

The inflammatory response after surgery is associated with various postoperative complications. For example, an increase in inflammatory cytokines is responsible for a significant portion of the loss of the epithelial barrier function [1]. Impaired integrity of the intestine causes bacterial translocation (BT) which is a particularly severe complication [2]. BT is known to be a key factor leading to conditions causing high morbidity, such as sepsis and multiple organ failure [3]. A reduction of postoperative inflammation is associated with fewer postoperative complications. To suppress the postoperative inflammatory response, less invasive surgery, such as laparoscopic-assisted surgery, has become increasingly common, and various drugs have also been used to reduce surgical stress. Laparoscopic colectomy has been carried out at many institutions and is considered to be a less invasive therapy. Preventing the release of inflammatory cytokines at an early stage is very important for preventing sepsis and bacterial translocation.

Daikenchuto (DKT) has been used to treat adhesive bowel obstruction and a feeling of coldness in the abdomen [4]. Several studies have shown that DKT accelerates gastrointestinal transit [5–11]. Recently, other effects of DKT have been reported; it induces increases in intestinal blood flow [12], reduces the blood ammonia level after hepatectomy [13], and has anti-inflammatory effects [14]. We previously reported that DKT prevented BT by maintaining the intestinal barrier integrity through its anti-inflammatory and anti-apoptotic effects in a rat BT model [15]. We hypothesized that DKT may also prevent the inflammatory response in

K. Yoshikawa · M. Shimada (✉) · M. Nishioka · N. Kurita · T. Iwata · S. Morimoto · T. Miyatani · M. Komatsu · H. Kashihara · C. Mikami
Department of Surgery, Institute of Health Biosciences,
The University of Tokushima Graduate School, Japan,
3-18-15 Kuramoto, Tokushima, Tokushima 770-8503, Japan
e-mail: mshimada@clin.med.tokushima-u.ac.jp

postoperative patients. The aim of the present prospective randomized trial was to evaluate the effects of DKT on the inflammatory response in patients following colorectal resection. To judge the effects of DKT in a controlled fashion and diminish the effects of individual differences in surgical stress, we focused on laparoscopic resection.

Methods

Participants

Thirty patients who underwent laparoscopic colectomy for colorectal carcinoma in the Department of Digestive Surgery of Tokushima University from July 2007 to June 2008 were enrolled in this study. The study was performed as an alternate study. The patients were divided into two groups: a DKT-treated group (D group, $n = 15$) and a control group (C group, $n = 15$). The patients in C group did not take any drugs or a placebo. The inclusion criteria were as follows: histologically confirmed adenocarcinoma of the colorectum, no involvement of other organs, a performance status of 0 or 1, no prior abdominal surgery, and no prior chemotherapy or radiotherapy for any malignancy. The exclusion criteria were as follows: unstable anemia, myocardial infarction within 6 months before registration, uncontrolled hypertension or diabetes mellitus, severe respiratory disease, and

intestinal leakage or obstruction. The patients' mean age was 62 ± 12 years in D group and 70 ± 5 years in C group. The clinical stages of colorectal carcinoma according to the *Japanese Classification of Colorectal Carcinoma* were stage 0, I, II, IIIA, IIIB and IV in 0, 6, 5, 3, 0 and 1 patient in D group and 4, 3, 3, 1, 2 and 2 in C group, respectively. The factor that led to patients being classified to have stage IV disease was liver metastasis. None of the patients had any significant associated diseases, such as cirrhosis, renal failure, pulmonary diseases, or cardiac diseases. The patients' mean body mass index (BMI) was 21 ± 3.7 in D group and 24 ± 4.2 in C group. There were no significant differences between the two groups in terms of these patient characteristics except for age (Table 1).

Study design

D group took 7.5 g/day of DKT from the day after surgery to the 7th postoperative day, while C group did not take DKT. This amount of DKT was half of the recommended dose, because some patients had difficulty in taking 15 g after surgery in a previous study. Both groups drank water from the day after surgery and began eating from the 5th postoperative day. The patients' body temperature, heart rate, WBC count, lymphocyte count, C-reactive protein (CRP) level, β -D-glucan level and Candida index were checked preoperatively and on the 1st, 3rd, 5th, and 7th

Table 1 Patient characteristics

	D group ($n = 15$)	C group ($n = 15$)	<i>P</i> value
Gender (male/female)	8/7	8/7	0.99
Age (years)			
Mean \pm SE	62 ± 12	70 ± 5	0.04
Range	41–80	61–86	
Tumor location			
Cecum	2	1	
Ascending colon	1	4	
Transverse colon	0	2	
Descending colon	1	0	
Sigmoid colon	4	2	
Rectosigmoid	1	1	
Rectum	6	5	0.43
Clinical staging, n (%)			
0	0	4	
I	6	3	
II	5	3	
IIIA	3	1	
IIIB	0	2	
IV	1 (liver meta)	2 (liver meta)	0.08
BMI			
Mean \pm SE	21 ± 3.7	24 ± 4.2	0.32
Range	17–28	20–34	

postoperative days. The time until the first flatus after the operation, the duration of the postoperative hospital stay, and the postoperative complications were also compared between the two groups. Any possible medications that might have affected gastrointestinal motility and functions, including prokinetics, antacids, and parasympathetic nerve blockers, were prohibited for 2 weeks before and during the study. All patients underwent a mechanical bowel preparation with magnesium citrate solution. During the operation, the incision was washed under high pressure while closing the abdomen. Only Loxoprofen was administered for postoperative pain, and the frequency of use was checked. This study was performed under the authors’ institutional guidelines and after permission was obtained from the local institutional review board. Written informed consent was obtained from every patient.

DKT

DKT, from Tsumura & Co., Tokyo, Japan, is a traditional Japanese herbal medicine, containing 1.25 g of DKT extract powder, 10 g of malt sugar and 3.75 g of vehicle in a total amount of 15 g. DKT extract powder is a mixture of dried ginger root, ginseng and zanthoxylum fruit at a ratio of 5:3:2, respectively. D group took 7.5 g/day of DKT.

Statistical analysis

The results are expressed as the means ± SE, and an analysis of variance (ANOVA) was used to evaluate the results. The statistical analyses were performed using the Newman–Keuls method for multiple comparisons. Statistical significance was set at *P* < 0.05.

Results

Clinical findings

The surgical findings are shown in Table 2. Although there were no significant differences in the procedure performed, the inclusion of lymph node dissection was significantly different, with D3 dissection performed more frequently in D group. The intraoperative blood loss and length of the operation were not significantly different between D group (20 ± 6 ml; 204 ± 11 min) and C group (33 ± 12 ml; 224 ± 17 min). The frequency of the Loxoprofen use during this study was not different between the two groups (D group: 2.8 ± 0.7, C group 3.1 ± 0.6).

The postoperative outcomes are listed in Table 3. One patient in C group developed a complication (wound

Table 2 Operative data

	D group (<i>n</i> = 15)	C group (<i>n</i> = 15)	<i>P</i> value
Performed operation, <i>n</i> (%)			
Ileocecal resection	2	4	
Ascending colectomy	1	1	
Transverse colectomy	0	2	
Descending colectomy	1	0	
Sigmoid colectomy	4	2	
High anterior resection	1	1	
Low anterior resection	6	5	0.62
Lymph node dissection, <i>n</i> (%)			
D1	0	5	
D2	4	3	
D3	11	7	0.05
Intraoperative blood loss (mL), mean ± SE	20 ± 6	33 ± 12	0.37
Operation time (min), mean ± SE	204 ± 11	224 ± 17	0.51

Table 3 Postoperative outcomes

	D group (<i>n</i> = 15)	C group (<i>n</i> = 15)	<i>P</i> value
Wound infection, <i>n</i> (%)	0	1 (7)	
Ileus, <i>n</i> (%)	0 (0)	0 (0)	
Mortality, <i>n</i> (%)	0 (0)	0 (0)	
Frequency of NSAID	2.8 ± 0.7	3.1 ± 0.6	0.45
Time until start of flatus (day), mean ± SE	1.8 ± 0.5	2.7 ± 0.5	0.02
Duration of postoperative hospital stay (day), mean ± SE	12.2 ± 0.6	12.8 ± 0.9	0.75

infection). The incidence of ileus and the mortality were not significantly different between the two groups. The time until the first flatus was significantly shorter in D group (1.8 ± 0.5 days) than in C group (2.7 ± 0.5 days). However, the duration of the postoperative hospital stay was not significantly different between D group (12.2 ± 0.6 days) and C group (12.8 ± 0.9 days). The body temperature on the 3rd postoperative day was significantly lower in D group ($36.2 \pm 0.4^\circ\text{C}$) than in C group ($36.9 \pm 0.6^\circ\text{C}$). The heart rate was not significantly different between the two groups on any postoperative day (Fig. 1a, b).

Laboratory data analysis

The results of all of the laboratory tests are presented in Fig. 2a–c. The CRP levels of both groups reached a peak on the 3rd postoperative day and then gradually decreased. However, the CRP level was significantly lower in D group (4.6 ± 0.6 mg/dl) than in C group (8.3 ± 1.1 mg/dl) on the 3rd postoperative day. The WBC and lymphocyte counts were not significantly different between the two groups. β -D-glucan was positive in one patient in each group, and the Candida index was positive in 1 patient in D group and 2 patients in C group.

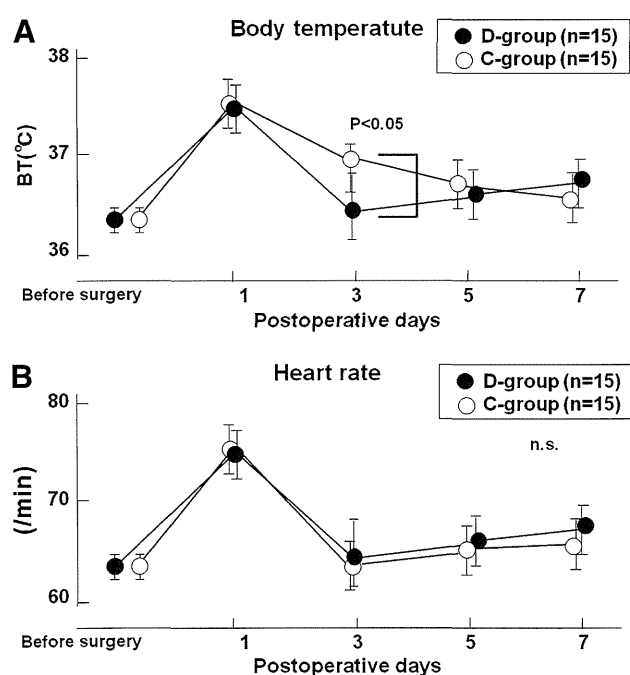


Fig. 1 A comparison of the body temperature (a) and heart rate (b) between the two groups. The Mann–Whitney *U* test was used for the analysis

Comments

In this study, we focused on patients undergoing laparoscopic surgery. This is the first report showing that DKT has anti-inflammatory effects following less invasive surgery. DKT is increasingly being used in the treatment of a variety of gastrointestinal diseases [13, 16]. Recent studies have been gradually clarifying the mechanism by which DKT stimulates the intestinal tract. One study suggested that DKT stimulates the gastrointestinal tract through cholinergic and non-cholinergic pathways [17–19]. Acceleration of the motility in the small intestine prevents endoluminal bacterial overgrowth [20]. An increase in the intestinal blood flow is also produced by one of the active components of DKT in a dose-dependent manner [10]. These phenomena were observed when the clinical symptom of feelings coldness in the abdomen improved after

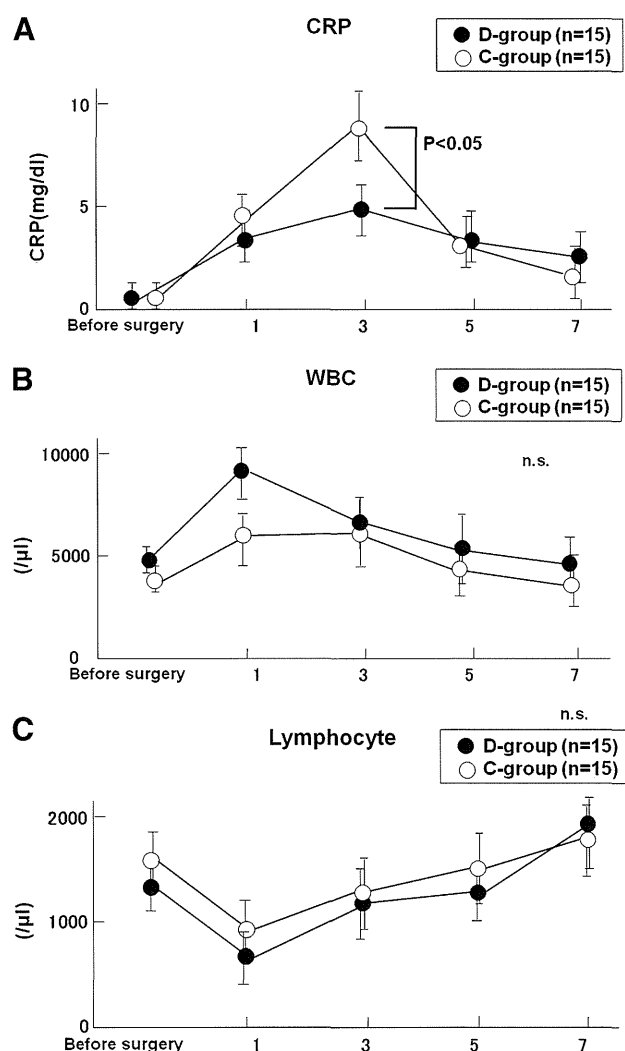


Fig. 2 A comparison of the CRP level (a), WBC count (b), and lymphocyte count (c) between the two groups. The Mann–Whitney *U* test was used for the analysis

DKT administration [13]. In previous studies, DKT induced acetylcholine release from intrinsic cholinergic nerves, mainly as a result of the zanthoxylum fruit [6, 21], and increased the intestinal blood flow as a result of the ginger rhizome, which may enhance intestinal motility [10, 13]. We have already reported that DKT exerted an anti-inflammatory effect by inhibiting the production of inflammatory cytokines, including IFN- γ , IL-6 and TNF- α , and significantly alleviated intestinal epithelial apoptosis in a rat model [15].

Furthermore, it is of clinical interest that a significant suppression of the postoperative elevation of body temperature on postoperative day 3 was found in D group. As the body temperature is one factor involved in the systemic inflammatory response syndrome (SIRS), DKT may have the potential to prevent SIRS and subsequent multiple organ failure (MOF). Compared to the control group, D group had decreased inflammation 2 days after the first DKT intake. Compared with D group, there were more elderly patients and less invasive surgery (D1 lymph node dissection) performed in C group. Although elderly patients and less invasive surgery are likely to be associated with a decreased inflammatory response, D group showed a better inflammation status after the surgery. As a result, it was concluded that DKT had anti-inflammatory effects even in patients with severe conditions. This result suggests that preoperative DKT intake may have an anti-inflammatory effect during the early postoperative period. Further investigations are needed to determine the most appropriate DKT regimen, including the starting day and the duration of treatment, in order to best control surgical stress.

Previous reports have indicated that DKT maintains the integrity of the intestinal epithelia and increases intestinal blood flow [12]. By maintaining epithelial integrity, lipopolysaccharide release may be suppressed. This mechanism also relates to the anti-inflammatory effects in postoperative patients. Although the WBC count was not significantly different between the two groups, the CRP level was lower in D group than in C group. This discrepancy is likely due to the fact that the CRP level is influenced by the presence of lipopolysaccharide.

In summary, the anti-inflammatory effects of DKT were evaluated in colorectal cancer patients following laparoscopic colectomy. This prospective, randomized, controlled study demonstrated that DKT accelerates intestinal motility, suppresses the CRP level and suppresses the body temperature postoperatively. Therefore, DKT has anti-inflammatory effects and this herbal medicine may help patients recover better following surgery.

Conflict of interest Drs. Kozo Yoshikawa, Mitsuo Shimada, Masanori Nishioka, Nobuhiro Kurita, Takashi Iwata, Jun

Higashijima, Tomohiko Miyatani, Motoya Chikakiyo, Toshihiro Nakao, and Masato Komatsu have no conflicts of interest or financial ties to disclose.

References

1. Ekelund M, Ekelund M, Qader SS, Hallen MH, Ekblad E. Effects of total parenteral nutrition on rat enteric nervous system, intestinal morphology, and motility. *J Surg Res*. 2005;124:187–93.
2. MacFie J, Reddy BS, Gatt M, Jain PK, Sowdi R, Mitchell CJ. Bacterial translocation studied in 927 patients over 13 years. *Brit J Surg*. 2006;93:87–93.
3. Anderson ADG, McNaught CE, Jain PK, MacFie J. Randomised clinical trial of symbiotic therapy in elective surgical patients. *Gut*. 2004;53:241–5.
4. Sato Y, Inoue S, Katagiri F, Itoh H, Takeyama M. Effects of Pirenzepine on Daikenchuto-induced elevation of the plasma neuropeptide levels in humans. *Biol Pharm Bull*. 2006;29:166–71.
5. Satoh K, Kase Y, Yuzurihara M, Mizoguchi K, Kurauchi K, Ishige A. Effect of Daikenchuto (Da-Jian-Zhong-Tang) on the delayed intestinal propulsion induced by chlorpromazine in mice. *J Ethnopharmacol*. 2003;86:37–44.
6. Hashimoto K, Satoh K, Murata P, Makino B, Sakakibara I, Kase Y, et al. Components of Panax ginseng that improve accelerated small intestinal transit. *J Ethnopharmacol*. 2003;84:115–9.
7. Nakamura T, Sakai A, Isogami I, Noda K, Ueno K, Yano S. Abatement of morphine-induced slowing in gastrointestinal transit by Daikenchuto, a traditional Japanese herbal medicine. *Jpn J Pharmacol*. 2002;88:217–21.
8. Satoh K, Hashimoto K, Hayakawa T, Ishige A, Kaneko M, Ogihara S, et al. Mechanism of atropine-resistant contraction induced by Daikenchuto in guinea pig ileum. *Jpn J Pharmacol*. 2001;86:32–7.
9. Suehiro T, Matsumata G, Shikada Y, Sugimachi K. The effect of the herbal medicines Daikenchuto and Keishi-bukuryo-gan on bowel movement after colorectal surgery. *Hepatogastroenterology*. 2005;52:97–100.
10. Itoh T, Yamakawa J, Mai M, Yamaguchi N, Kanda T. The effect of the herbal medicine Daikenchuto on post-operative ileus. *J Int Med Res*. 2002;30:428–32.
11. Hayakawa T, Kase Y, Saito K, Hashimoto K, Ishige A, Komatsu Y, et al. Pharmacological studies of the effect of Daikenchuto on spontaneous contraction of isolated rabbit jejunum. *J Smooth Muscle Res*. 1999;35:55–62.
12. Murata P, Kase Y, Ishige A, Sasaki H, Kurosawa S, Nakamura T. The herbal medicine Daikenchuto and one of its active components 6-shogaol increase intestinal blood flow in rats. *Life Sci*. 2002;70:2061–70.
13. Kaiho T, Tanaka T, Tsuchiya S, Yanagisawa S, Takeuchi O, Miura M, et al. Effect of the herbal medicine Daikenchuto for serum ammonia in hepatectomized patient. *Hepatogastroenterology*. 2004;52:161–5.
14. Hayakawa T, Kase Y, Saito K, Hashimoto K, Ishige A, Komatsu Y, et al. Effect of Daikenchuto on intestinal obstruction following laparotomy. *J Smooth Muscle Res*. 1999;35:47–54.
15. Yoshiakwa K, Kurita N, Higashijima J, Miyamoto T, Miyamoto H, Nishioka M, et al. Kampo medicine “Daikenchuto” prevents bacterial translocation in rats. *Dig Dis Sci*. 2008;53:1824–31.
16. Ohya T, Usui Y, Arai S, Iwai T. Effect of Daikenchuto on obstructive bowel disease in children. *Am J Chin Med*. 2003;31:129–35.
17. Jin X, Shibata C, Naito H, Ueno T, Funayama Y, Fukushima K, et al. Intraduodenal and intrajejunal administration of the herbal medicine, Daikenchuto, stimulates small intestinal motility via