Combination Therapy of S-1 with Selective Cyclooxygenase-2 Inhibitor for Liver Metastasis of Colorectal Carcinoma

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Abstract. *Background: Hepatic resection, the only curative treatment for liver metastasis of colorectal cancer has become standard treatment, but most cases of liver metastases are inoperable, and of the patients treated with hepatectomy about 50% have a recurrence in the liver. The aim of this study was to establish preventive therapy for the liver metastasis of colorectal cancer. Materials and Methods: In this study, a combined treatment with S-1 and a selective COX-2 inhibitor for liver metastasis of colorectal cancer was developed. The effect of these agents on the proliferation and invasion of a highly metastatic human colon cancer cell line, LM-H3, was examined. Results: 5-Fluorouracil (5-FU) had an inhibitory effect on the proliferation of LM-H3 cells, but no inhibitory effect on the invasion of LM-H3 cells in in vitro experiments. 5-Chloro-2,4-dihydroxypyridine (CDHP) had no antitumor activity itself, but the inhibitory effect of 5-FU on the proliferation was enhanced by adding CDHP. COX-2 inhibitors, etodolac and rofecoxib, did not have an inhibitory effect on the proliferation of LM-H3 cells at low concentrations, but had significant inhibitory effect on the invasion of LM-H3 cells in in vitro experiments. In a nude mouse liver metastasis model, combined treatment with S-1 and a COX-2 inhibitor more effectively restrained liver metastasis of LM-H3 cells than either alone. This outcome was most likely due to S-1 inhibiting proliferation of and the COX-2 inhibitor inhibiting invasion of LM-H3 cells. Conclusion: Combined therapy with S-1 and a COX-2 inhibitor might hold promise for prophylaxis of liver metastasis of colorectal cancer.*

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Liver metastasis is an important negative prognostic factor in colorectal carcinoma. Fifteen to 25% of patients have metastatic liver disease when the primary tumor is diagnosed and an additional 35% to 45% of patients will develop hepatic metastases during the course of the disease (1). Hepatic resection is the only curative treatment and has become the standard treatment for liver metastasis of colorectal cancer. The 5-year survival rate of patients treated with hepatectomy ranges between 20%-50% (2-5). Unfortunately, most liver metastases are inoperable because of tumor number, size, or location, and about 50% of patients treated with hepatectomy have a recurrence in the liver. Adjuvant therapy is necessary to reduce recurrence in the liver after resection of the primary tumor and resection of liver metastases.

Recently, oral anticancer drug therapy for colorectal cancer has attracted increasing attention, especially the oral fluoropyryzine drugs, UFT, S-1 and capecitabine (6, 7). Oral administration of UFT plus leucovorin (LV) achieved similar overall survival to intravenous administration of 5-fluorouracil (5-FU) plus LV, with no difference in toxicity, and obtained good compliance. As oral administration is more convenient for patients, we believe that oral administration may become a standard in the chemotherapy for colorectal cancer.

S-1 consists of tegafur (a prodrug of 5-FU) and two modulators, 5-chloro-2,4-dihydroxypyridine (CDHP) and potassium oxonate, at a molar ratio of 1:0.4:1 (8). CDHP is a reversible competitive inhibitor of dihydroxypyridine dehydrogenase, which is an enzyme for 5-FU degradation. Therefore, CDHP with tegafur is expected to yield prolonged and high serum and tumor tissue 5-FU concentrations. Oxonate is a reversible competitive inhibitor of orotate phosphoribosyltransferase, which is an enzyme for 5-FU phosphoribosylation in the gastrointestinal mucosa. It is reported that oxonate concentrates selectively in gastrointestinal tissues after oral administration and suppresses gastrointestinal toxicity caused by phosphoribosylation of 5-FU in the gastrointestinal tract without decreasing the antitumor activity (9).

On the other hand, epidemiological studies have suggested that non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of colorectal cancer (10-14) and reduce, in number and size, polyps in patients with familial adenomatous polyposis (FAP) (15-17). These studies implied that NSAIDs could modulate carcinogenesis and the development of colorectal carcinoma. The mechanisms of this effect are presently under investigation. NSAIDs are known to inhibit cyclooxygenase (COX), which is the key enzyme in conversion of arachidonic acid to prostaglandins. Two isoforms of COX, COX-1 and COX-2, are recognized (18). COX-1 is constitutively expressed in many normal tissues. In contrast, COX-2 is induced by several inflammatory stimuli, such as cytokines, growth factors, and tumor promoters (19), and is expressed in colorectal cancer (20, 21). COX-2 is thought to influence carcinogenesis and the development of colorectal carcinoma. Several reports have suggested that COX-2 inhibitors can reduce progression (22-27) and invasion (22, 28-30) of colon cancer, and reduce angiogenesis (31-33). We previously reported that a COX-2 inhibitor suppressed liver metastasis of colorectal carcinoma cells in a nude mouse model (34), indicating that COX-2 inhibitors might have clinical potential for preventing liver metastasis and that the clinical application of COX-2 inhibitors might become routine.

The aim of the present study was to examine the effects of these agents on the proliferation and invasion of colorectal carcinoma and to investigate the preventative effects of a combined treatment of S-1 and a selective COX-2 inhibitor on liver metastasis of colorectal cancer in preclinical models.

Materials and Methods

Drugs. 5-FU was kindly provided by Wako Pure Chemical Industries (Osaka, Japan). CDHP and S-1 were kindly provided by Taiho Pharmaceutical Co. (Tokyo, Japan). Etodolac, a selective COX-2 inhibitor, was kindly provided by Nippon Shinyaku Co., Ltd. (Kyoto, Japan). Rofecoxib, a selective COX-2 inhibitor, was kindly provided by Merck & Co., Inc., (NJ, USA).

Animals. Specific, pathogen-free, athymic BALB/c-*nu*/*nu* nude mice were purchased from Oriental Kobo (Tokyo, Japan). The mice were maintained under specific pathogen-free conditions and given sterile food and water *ad libitum*. Four-week-old female mice were used for the experiments. Studies were performed according to Osaka City University Medical School's standard guidelines for animal experiments.

Cell line. A highly metastasizing colon carcinoma cell line, LM-H3, which was previously established in our laboratory was used (35). LM-H3 cells were cultured *in vitro* at 37˚C in Dulbecco's modified Eagle's medium (DMEM; Whittaker Bioproducts, Walkersville, MD, USA), with 10% fetal bovine serum (FBS; GIBCO, Grand Island, NY, USA), 100 μg/ml of streptomycin penicillin (ICN Biomedicals, Costa Mesa, CA, USA) and 0.5 mM sodium pyruvate (Whittaker Bioproducts).

Proliferation assay. LM-H3 cells ($1x10^3$ cells/100 μl) were seeded into 96-well microplates, and incubated for 24 h at 37˚C in a 5% CO_2 atmosphere. After centrifuging, the supernatant was aspirated completely. Cells were then treated with 150 μl of 5-FU (0, 0.01, 0.1, 1, 10, 100 μg/ml) alone or in combination with CDHP at a concentration ratio of 1:0.4. Cells were also treated with 150 μl of CDHP (0, 0.004, 0.04, 0.4, 4, 40 μg/ml) or COX-2 inhibitors (rofecoxib or etodolac) (0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M).

After 72 h treatment, the number of cells was quantified by 3-(4,5-dimethyl-2-thiazol)-2H tetrazolium bromide (MTT; Wako Pure Chemical Industries) colorimetric assay. The assay was designed to measure the formazan product of MTT, using an MTP-120 microplate reader (Corona Electric, Ibaragi, Japan) to measure optical density (OD) at 550 nm.

Invasion assay. Transwell double chambers with a 12 mm pore size (Millipore, Bedford, MA, USA) were used for the invasion assay. Matrigel was diluted to 0.1 mg/ml with conditioned medium and 100 μl of diluted matrigel was applied to the filter of the upper chamber. The filter was then air-dried for 2 h at room temperature. LM-H3 cells ($2x10^5$ cells/100 μl) were seeded in the upper chamber, 700 μl of conditioned medium were added to the lower chamber, and 100 μl of 5-FU (0, 0.01, 0.1, 1, 10, 100 μg/ml) or of rofecoxib or etodolac (0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4} M), or CDHP (0, 0.004, 0.04, 0.4, 4, 40 μg/ml) were added to the upper chamber. After incubation for 48 h at 37˚C in a 5 % CO_2 atmosphere, the matrigel and the cells on the upper surface of the filter were completely removed by wiping with a cotton swab. Cells that had invaded the matrigel and had migrated through the filter and adhered to its lower surface were stained with Mayer's hematoxylin. The number of cells was counted under a light microscope at x400 magnification.

Formation of liver metastases. BALB/c nude mice were used. Under anesthesia, the abdomen was opened. A total of $5x10^5$ cells/0.1 ml LM-H3 cells suspended in phosphate-buffered saline (PBS) were injected into the lower lobe of the spleen. Splenectomy was conducted 2 to 3 min after injection. Mice were randomly divided into 6 groups: controls, etodolac (10 mg/kg), rofecoxib (10 mg/kg), S-1 (10 mg/kg) and two combined groups (S-1 + etodolac group, and S-1 + rofecoxib group). Each drug was administered orally 5 days per week for 4 weeks from the day after injection of LM-H3 cells. After 4 weeks, mice were sacrificed, and the livers were removed and weighed. The number of metastatic nodules on the liver surface were counted.

Statistical analysis. Data are expressed as means±SD and taken from at least 4 independent determinations. Significant differences were analyzed using the unpaired Student's *t*-test. A *p*-value less than 0.05 was considered to indicate a statistically significant difference.

Results

Effect of 5-FU with or without CDHP on cell proliferation. The effect of 5-FU with or without CDHP on cell proliferation is shown in Figure 1. 5-FU significantly inhibited proliferation of LM-H3 cells in a dose-dependent manner. CDHP enhanced the inhibitory effect of 5-FU on cell proliferation at the dose used. CDHP had no inhibitory effect on the proliferation of LM-H3 cells itself (Figure 2).

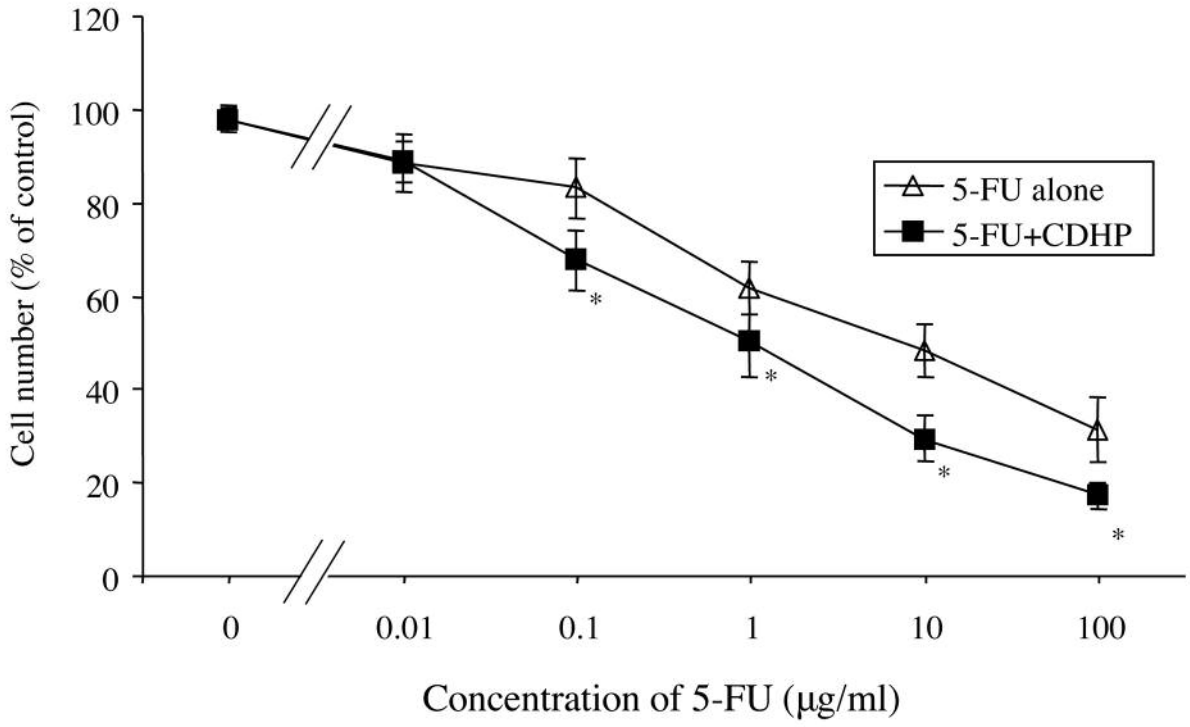

Figure 1. *Inhibitory effect of 5-FU with or without CDHP on proliferation of LM-H3 cells. 5-FU significantly inhibited proliferation of LM-H3 cells in a dose-dependent manner. CDHP enhanced the inhibitory effect of 5-FU on cell proliferation at the dose used here. Statistically significant differences from 5-FU alone values: *p<0.01.*

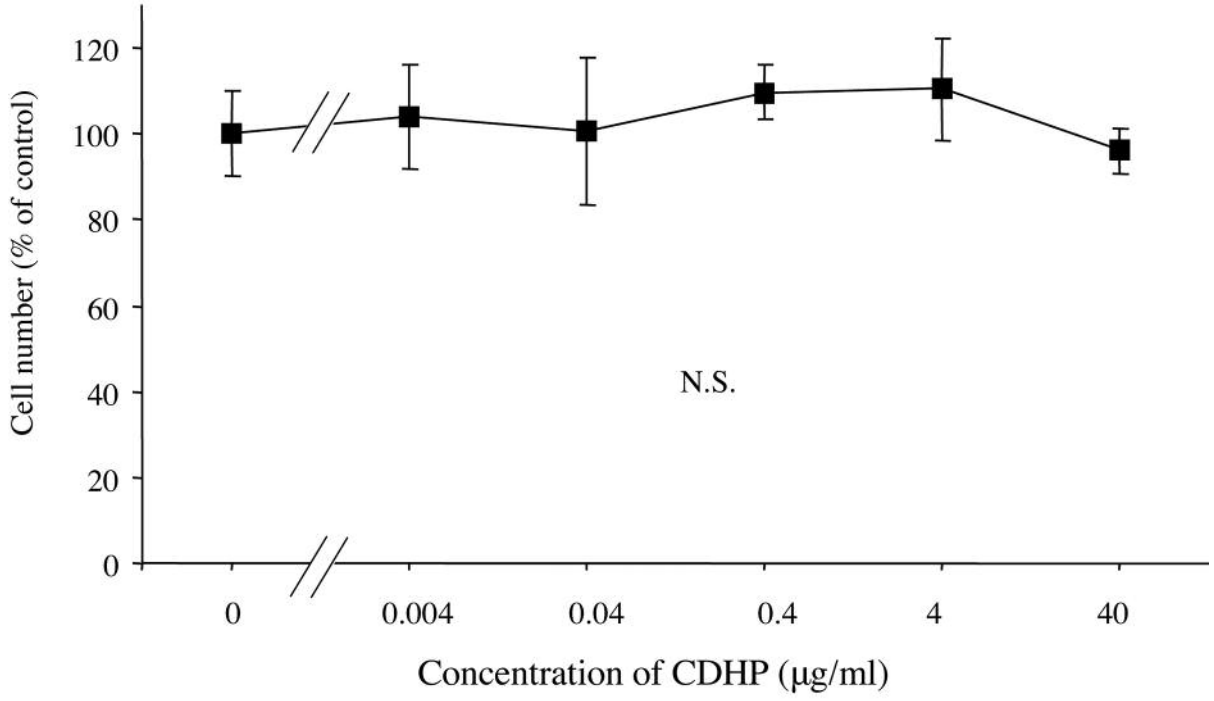

Figure 2. *The effect of CDHP on proliferation of LM-H3 cells. CDHP did not have an inhibitory effect on proliferation of LM-H3 cells. N.S.: Not significant.*

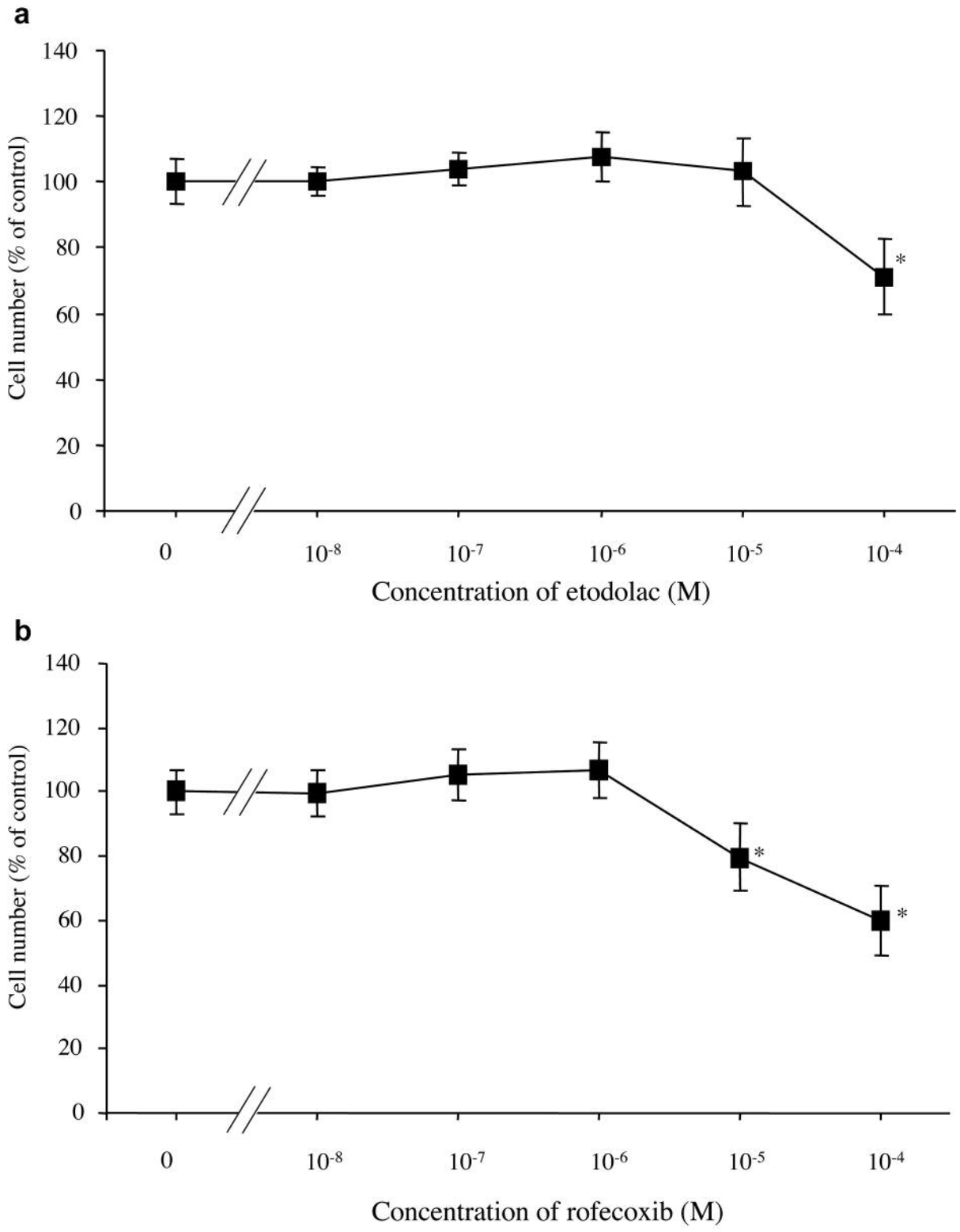

Figure 3. *The effect of COX-2 inhibitors on proliferation of LM-H3 cells. Etodolac had an inhibitory effect on the proliferation of LM-H3 cells only at the high concentration of 10^{-4}M (a). Rofecoxib also had an inhibitory effect on the proliferation of LM-H3 cells, however only at the high concentration of 10^{-5}M and 10^{-4}M (b). Etodolac and rofecoxib did not have an inhibitory effect at the concentration ≤10^{-6}M. Statistically significant differences from control values: *$p<0.01$.*

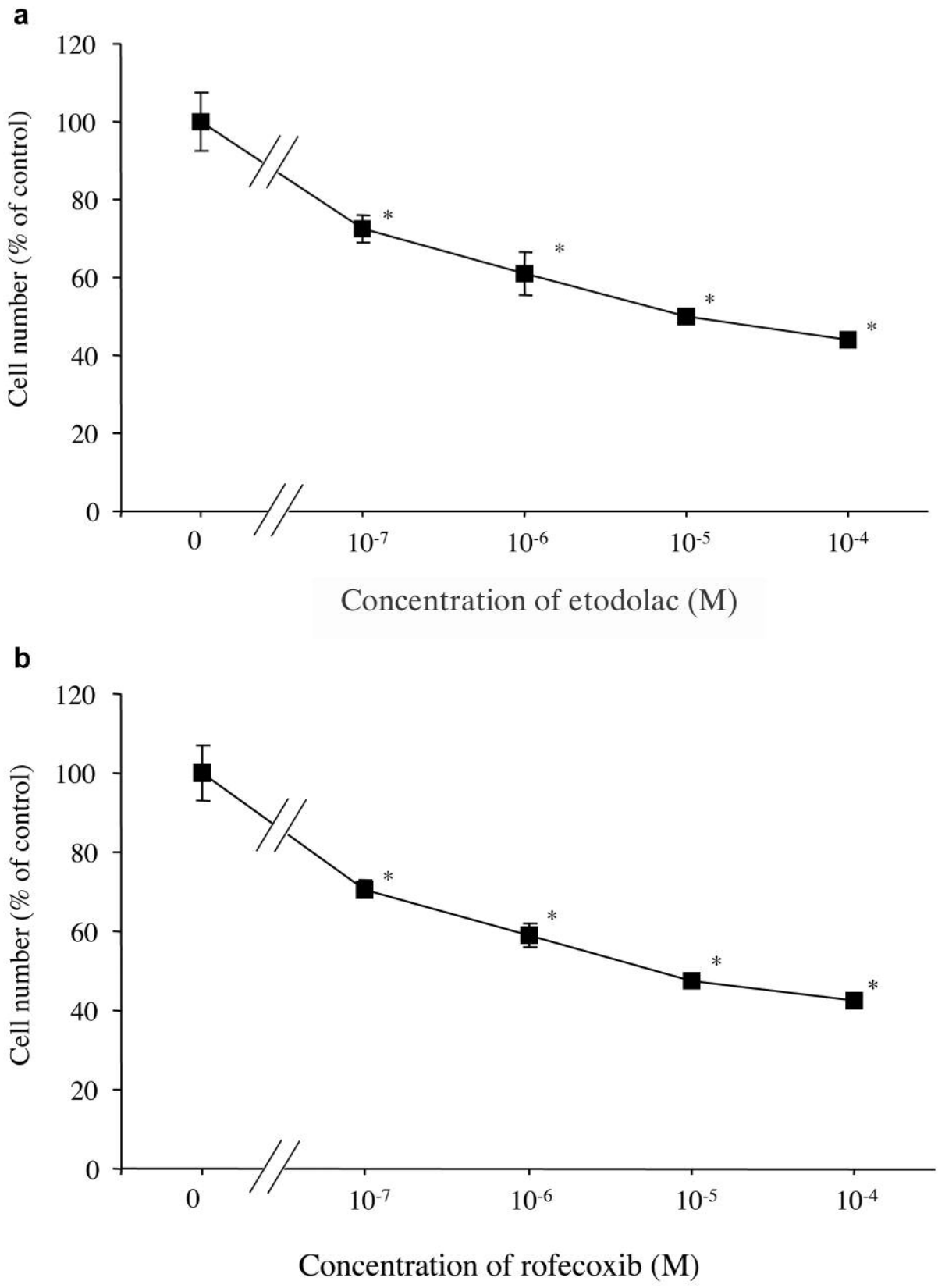

Figure 4. *Effect of COX-2 inhibitors on cell invasion. Etodolac inhibited cell invasiveness in a dose-dependent manner (a). Rofecoxib also inhibited cell invasiveness in a dose-dependent manner (b). Statistically significant differences from control values: *p<0.01.*

Effect of etodolac and rofecoxib on cell proliferation. Etodolac had an inhibitory effect on the proliferation of LM-H3 cells only at the high concentration of 10^{-4}M (Figure 3a). Rofecoxib had inhibitory effect on the proliferation of LM-H3 cells only at the high concentration of 10^{-5}M and 10^{-4}M. (Figure 3b). Neither etodolac nor rofecoxib had any have inhibitory effect at concentrations ≤10^{-6}M, nor did they enhance the inhibitory effect of 5-FU on cell proliferation (data not shown).

Effect of etodolac, rofecoxib, 5-FU and CDHP on cell invasion. Both etodolac and rofecoxib significantly inhibited cell invasiveness in a dose-dependent manner (Figure 4a, b). There was no difference between etodolac and rofecoxib in the inhibitory effect on the invasion of LM-H3 cells. 5-FU and CDHP had no inhibitory effect on the invasion of LM-H3 cells (Figures 5 and 6).

Inhibitory effect of S-1 and a COX-2 inhibitor on liver metastasis. The inhibitory effect of S-1 and etodolac or rofecoxib on liver metastasis is summarized in Table I. The macroscopic appearance of the liver metastatic foci is shown in Figure 7. Compared with the control group, the etodolac, rofecoxib and S-1 groups showed significant inhibition of liver metastasis ($p<0.05$). The S-1 group showed more significant inhibition of liver metastasis than the COX-2 inhibitor groups ($p<0.01$). The combined groups had the most markedly reduced amount of liver metastasis.

Table I. *The inhibitory effect of S-1 with etodolac or rofecoxib on liver metastasis of colon cancer.*

	Number of metastatic nodules of liver	Weight of liver (mg)
Control	243±40	3732±159
S-1	51±42**	1540±416**
Etodolac	174±8*	3104±480*
Rofecoxib	167±22*	2965±452*
S-1+ Etodolac	34±12**	1314±79**
S-1+ Rofecoxib	22±7.1**	1260±81**

Statistically significant differences from control values: *$p<0.05$,**$p<0.001$.

Discussion

We set out to establish an experimental basis for the development of treatment to prevent liver metastasis of colorectal cancer. 5-FU had an inhibitory effect on the proliferation of LM-H3 cells, but no inhibitory effect on the invasion of LM-H3 cells in *in vitro* experiments. CDHP had no antitumor activity itself, but the inhibitory effect on the proliferation was enhanced by adding CDHP to 5-FU. 5-FU has been widely used as a key drug in the treatment of colorectal cancer. It has been reported that the tumoral expression level of dihydropyrimidine dehydrogenase (DPD) is associated with the chemoresistance to 5-FU in colorectal cancer because DPD is a rate-limiting enzyme in the catabolic processing of 5-FU.

In order to maintain stable plasma 5-FU levels and superior antitumor activity, several oral fluoropyrimidine derivatives with DPD inhibitory activity have been developed. UFT is a combination drug consisting of 1 M tegafur, a prodrug of 5-FU and 4 M uracil. Uracil selectively inhibits the degradation by DPD of the 5-FU formed by conversion from tegafur (36).

S-1 consists of tegafur, CDHP and oxonate. CDHP is 200-fold more potent than uracil in inhibiting DPD, and 5-FU peak levels and the area under the curve are significantly higher for tegafur/CDHP than for tegafur/uracil. CDHP competitively inhibits DPD, resulting in prolonged maintenance of 5-FU concentration. It has been reported that the serum concentration of 5-FU is equal to that achieved by continuous 5-FU infusion when S-1 is orally administered. S-1 might be effective for the suppression of proliferation, especially in colorectal cancer that has a high DPD activity.

COX-2 inhibitors did not have an inhibitory effect on the proliferation of LM-H3 cells at a low concentration, but had significant inhibitory effect on the invasion of LM-H3 cells in a dose-dependent manner. There is currently no consensus on the suppressive effects of COX-2 inhibitors on cell proliferation, although several studies investigated the induction of apoptosis (23-27) and suppression of growth factor production (34, 37). Chen *et al.* reported cytotoxicity of etodolac on several colorectal carcinoma cell lines. The sensitivity to etodolac is different between cell lines (28). Yamazaki *et al.* compared different COX-2 inhibitors on proliferation. They reported that celecoxib, another selective COX-2 inhibitor, induced apoptosis and inhibited proliferation of cancer cells expressing COX-2, whereas etodolac failed to do so (38). There might be differences in the inhibitory effects on proliferation among different cell lines or COX-2 inhibitors.

We used two kinds of COX-2 inhibitors, etodolac and rofecoxib, in this study. Rofecoxib is a more selective cyclooxygenase-2 inhibitor than etodolac, but there was no difference between etodolac and rofecoxib in the inhibitory effect on the proliferation of LM-H3 cells. On the other hand, many reports indicated that COX-2 inhibitors had an inhibitory effect on the invasion of cancer cells, and both etodolac and rofecoxib inhibited the invasion of LM-H3 cells in our study. Tsujii *et al.* showed COX-2 expression led to activation of metalloproteinase (MMP)-2 and increased cell invasiveness in colon cancer and that sulindac sulfide, a known COX inhibitor, decreased the invasiveness (29). Abiru *et al.* observed that aspirin and NS-398, a selective COX-2 inhibitor, decreased the hepatocyte growth factor (HGF) -induced invasiveness of hepatocellular carcinoma (39). According to our previous report, JTE-522, a selective COX-2 inhibitor,

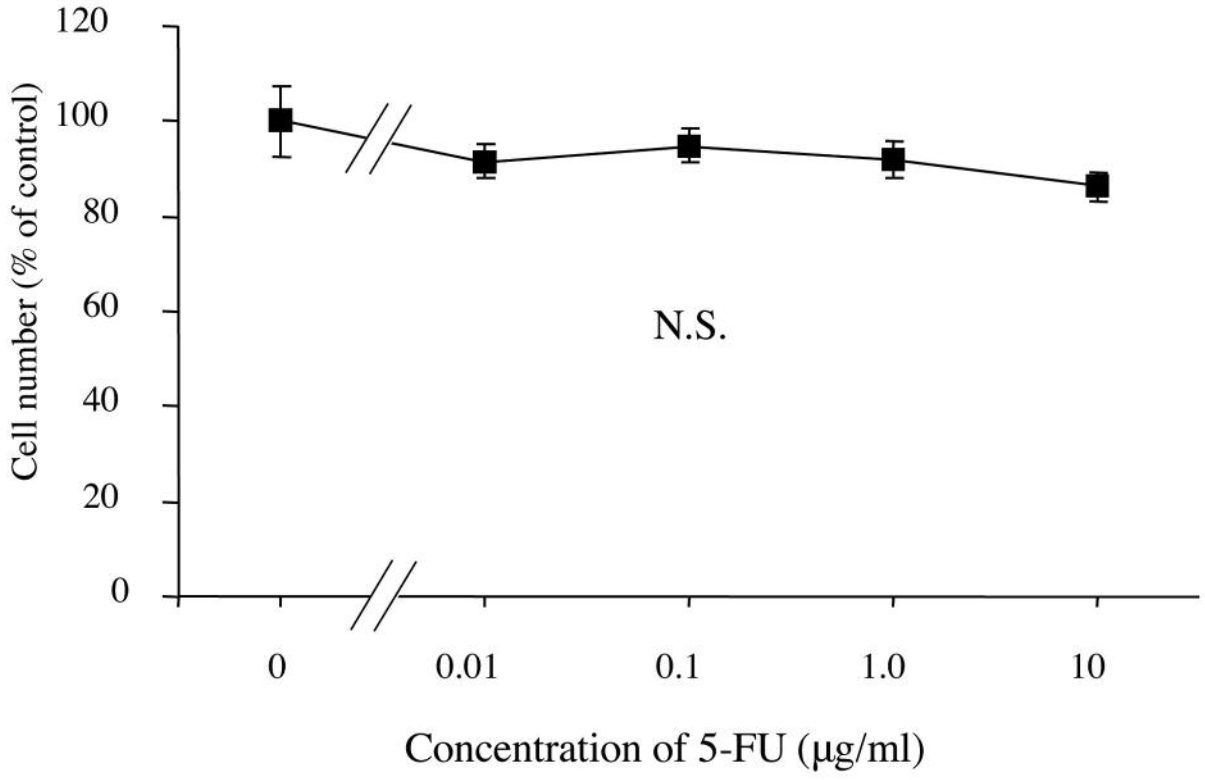

Figure 5. *Effect of 5-FU on cell invasion. 5-FU had no inhibitory effect on the invasion of LM-H3 cells. N.S.: Not significant.*

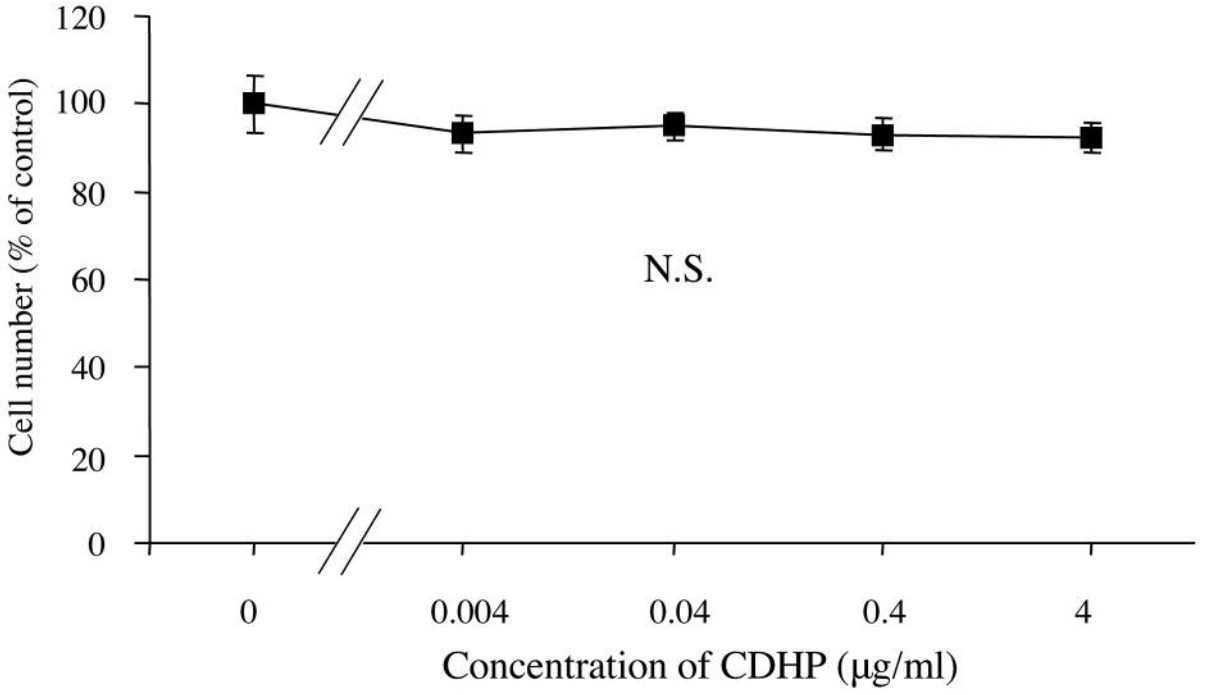

Figure 6. *Effect of CDHP on cell invasion. CDHP had no inhibitory effect on the invasion of LM-H3 cells. N.S.: Not significant.*

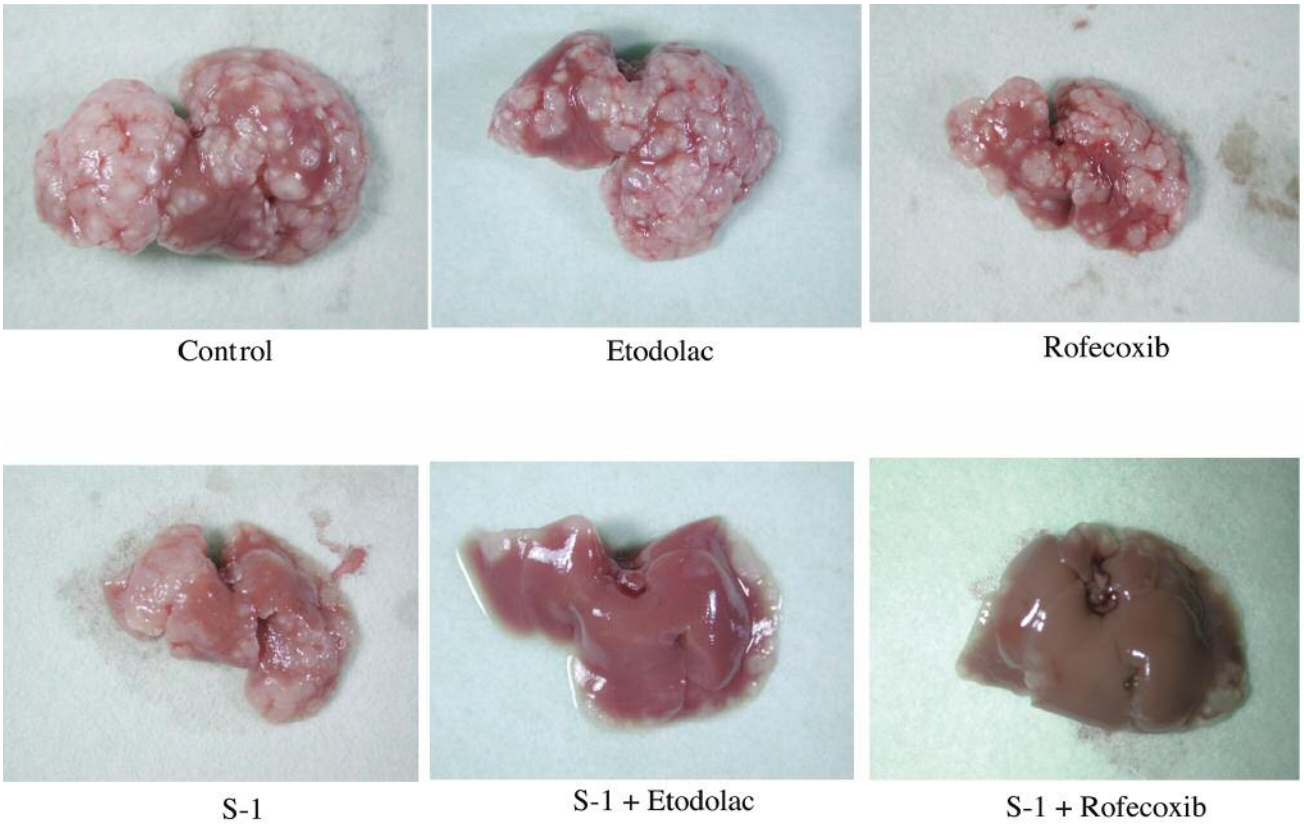

Figure 7. *Macroscopic appearance of the liver metastatses in nude mice.*

inhibited the invasion of LM-H3 cells and the inhibitory effect on the invasion of LM-H3 cells is probably due to suppression of platelet-derived growth factor (PDGF) and MMP-2 production (34). We supposed that COX-2 expression might be associated with the invasion potency of colorectal cancer and COX-2 inhibitors might be an effective drug for the suppression of the invasion of colorectal cancer.

In this nude mouse liver metastasis model, combined treatment with S-1 and a COX-2 inhibitor more effectively restrained liver metastasis of LM-H3 cells than either drug alone. This outcome was most likely due to S-1 inhibiting their proliferation and the COX-2 inhibitor inhibiting invasion of LM-H3 cells. DPD activity is very high in the liver and the degradation of 5-FU is more rapid in the liver than in other tissues. Since S-1 administration might maintain a high concentration of 5-FU in the liver, it could yield a prolonged high concentration of 5-FU, providing an inhibitory effect on liver metastasis. It has been reported that S-1 shows more inhibitory effect on liver metastasis in nude mice than does UFT, and both tumor and plasma 5-FU levels are significantly higher with S-1 administration than UFT administration (40).

To the best of our knowledge, there is no report to demonstrate the inhibitory effect of combined treatment with S-1 and a COX-2 inhibitor on liver metastasis of colorectal cancer. In this study, each drug was administered orally 5 days per week for 4 weeks from the day after injection of LM-H3 cells. The high rate of recurrence in the liver after resection of the primary tumor or liver metastases is due to the presence of micrometastases in the liver. In our liver metastasis model, each drug was administered under conditions of micrometastases in the liver. Therefore, this combined therapy with S-1 and a COX-2 inhibitor may be a useful chemopreventive therapy for liver metastasis of colorectal cancer after resection of the primary tumor or liver metastases.

The aim of the present study was to establish a preventative therapy for the liver metastasis of colorectal cancer. In consideration of clinical applications, we designed an oral medication regimen, thought to be the most useful procedure. Combined therapy with S-1 and a COX-2 inhibitor hold promise as a preventative therapy for liver metastasis of colorectal cancer.

References

1 August DA, Sugarbaker PH, Ottow RT, Gianola FJ and Schneider PD: Hepatic resection of colorectal metastases. Influence of clinical factors and adjuvant intraperitoneal 5-fluorouracil *via* Tenckhoff catheter on survival. Ann Surg *201*: 210-218, 1985.

2 Rodgers MS and McCall JL: Surgery for colorectal liver metastases with hepatic lymph node involvement: a systematic review. Br J Surg *87*: 1142-1155, 2000.

3 Martin LW and Warren RS: Current management of colorectal liver metastases. Surg Oncol Clin N Am *9*: 853-876, 2000.

4 Penna C and Nordlinger B: Colorectal metastasis (liver and lung). Surg Clin North Am *82*: 1075-1090, 2002.

5 Kato T, Yasui K, Hirai T, Kanemitsu Y, Mori T, Sugihara K, Mochizuki H and Yamamoto J: Therapeutic results for hepatic metastasis of colorectal cancer with special reference to effectiveness of hepatectomy: analysis of prognostic factors for 763 cases recorded at 18 institutions. Dis Colon Rectum *46*: 22-31, 2003.

6 Malet-Martino M and Martino R: Clinical studies of three oral prodrugs of 5-fluorouracil (capecitabine, UFT, S-1): a review. Oncologist *7*: 288-323, 2002.

7 Johnston PG and Kaye S: Capecitabine: a novel agent for the treatment of solid tumors. Anticancer Drugs *12*: 639-646, 2001.

8 Shirasaka T, Shimamato Y, Ohshimo H, Yamaguchi M, Kato T, Yonekura K and Fukushima M: 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, 1996.

9 Shirasaka T, Shimamoto Y and Fukushima M: Inhibition by oxonic acid of gastrointestinal toxicity of 5-fluorouracil without loss of its antitumor activity in rats. Cancer Res *53*: 4004-4009, 1993.

10 Vinogradova Y, Hippisley-Cox J, Coupland C and Logan RF: Risk of colorectal cancer in patients prescribed statins, nonsteroidal anti-inflammatory drugs, and cyclooxygenase-2 inhibitors: nested case-contorol study. Gastroebterology *133*: 393-402, 2007.

11 Thun MJ, Namboodiri MM and Heath CW Jr: Aspirin use and reduced risk of fatal colon cancer. N Engl J Med *325*: 1593-1596, 1991.

12 Giovannucci E, Egan KM, Hunter DJ, Stampfer MJ, Colditz GA, Willett WC and Speizer FE: Aspirin and the risk of colorectal cancer in women. N Engl J Med *333*: 609-614, 1995.

13 Suh O, Mettlin C and Petrelli NJ: Aspirin use, cancer, and polyps of the large bowel. Cancer *72*: 1171-1177, 1993.

14 Peleg, II, Maibach HT, Brown SH and Wilcox CM: Aspirin and nonsteroidal anti-inflammatory drug use and the risk of subsequent colorectal cancer. Arch Intern Med *154*: 394-399, 1994.

15 Hisamuddin IM, Wehbi MA, Chao A, Wyre HW, Hylind LM, Giardiello FM and Yang VW: Genetic polymorphisms of human flavin monooxygenase 3 in sulindac-mediated primary chemoprevention of familial adenomatous polyposis. Clin Cancer Res *10*: 8357-8362, 2004.

16 Giardiello FM, Hamilton SR, Krush AJ, Piantadosi S, Hylind LM, Celano P, Booker SV, Robinson CR and Offerhaus GJ: Treatment of colonic and rectal adenomas with sulindac in familial adenomatous polyposis. N Engl J Med *328*: 1313-1316, 1993.

17 Steinbach G, Lynch PM, Phillips RK, Wallace MH, Hawk E, Gordon GB, Wakabayashi N, Saunders B, Shen Y, Fujimura T, Su LK and Levin B: The effect of celecoxib, a cyclooxygenase-2 inhibitor, in familial adenomatous polyposis. N Engl J Med *342*: 1946-1952, 2000.

18 Smith WL, DeWitt DL and Garavito RM: Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem *69*: 145-182, 2000.

19 Williams CS, Mann M and DuBois RN: The role of cyclooxygenases in inflammation, cancer, and development. Oncogene *18*: 7908-7916, 1999.

20 Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S and DuBois RN: Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. Gastroenterology *107*: 1183-1188, 1994.

21 Sano H, Kawahito Y, Wilder RL, Hashiramoto A, Mukai S, Asai K, Kimura S, Kato H, Kondo M and Hla T: Expression of cyclooxygenase-1 and -2 in human colorectal cancer. Cancer Res *55*: 3785-3789, 1995.

22 Yao M, Lam EC, Kelly CR, Zhou W and Wolfe MM: Cyclooxygenase-2 selective inhibition with NS-398 suppresses proliferation and invasiveness and delays liver metastasis in colorectal cancer. Br J Cancer *90*: 712-719, 2004.

23 Erickson BA, Longo WE, Panesar N, Mazuski JE and Kaminski DL: The effect of selective cyclooxygenase inhibitors on intestinal epithelial cell mitogenesis. J Surg Res *81*: 101-107, 1999.

24 Li M, Wu X and Xu XC: Induction of apoptosis in colon cancer cells by cyclooxygenase-2 inhibitor NS398 through a cytochrome c-dependent pathway. Clin Cancer Res *7*: 1010-1016, 2001.

25 Hanif R, Pittas A, Feng Y, Koutsos MI, Qiao L, Staiano-Coico L, Shiff SI and Rigas B: Effects of nonsteroidal anti-inflammatory drugs on proliferation and on induction of apoptosis in colon cancer cells by a prostaglandin-independent pathway. Biochem Pharmacol *52*: 237-245, 1996.

26 Williams CS, Watson AJ, Sheng H, Helou R, Shao J and DuBois RN: Celecoxib prevents tumor growth *in vivo* without toxicity to normal gut: lack of correlation between *in vitro* and *in vivo* models. Cancer Res 60: 6045-6051, 2000.

27 Chan TA, Morin PJ, Vogelstein B and Kinzler KW: Mechanisms underlying nonsteroidal antiinflammatory drug-mediated apoptosis. Proc Natl Acad Sci USA *95*: 681-686, 1998.

28 Chen WS, Wei SJ, Liu JM, Hsiao M, Kou-Lin J and Yang WK: Tumor invasiveness and liver metastasis of colon cancer cells correlated with cyclooxygenase-2 (COX-2) expression and inhibited by a COX-2-selective inhibitor, etodolac. Int J Cancer *91*: 894-899, 2001.

29 Tsujii M, Kawano S and DuBois RN: Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci USA *94*: 3336-3340, 1997.

30 Attiga FA, Fernandez PM, Weeraratna AT, Manyak MJ and Patierno SR: Inhibitors of prostaglandin synthesis inhibit human prostate tumor cell invasiveness and reduce the release of matrix metalloproteinases. Cancer Res *60*: 4629-4637, 2000.

31 Tsujii M, Kawano S, Tsuji S, Sawaoka H, Hori M and DuBois RN: Cyclooxygenase regulates angiogenesis induced by colon cancer cells. Cell *93*: 705-716, 1998.

32 Jones MK, Wang H, Peskar BM, Levin E, Itani RM, Sarfeh IJ and Tarnawski AS: Inhibition of angiogenesis by nonsteroidal anti-inflammatory drugs: insight into mechanisms and implications for cancer growth and ulcer healing. Nat Med *5*: 1418-1423, 1999.

33 Kobayashi H, Gonda T, Uetake H, Higuchi T, Enomoto M and Sugihara K: JTE-522, a selective COX-2 inhibitor, interferes with the growth of lung metastases from colorectal cancer in rats due to inhibition of neovascularization: a vascular cast model study. Int J Cancer *112*: 920-926, 2004.

34 Nagatsuka I, Yamada N, Shimizu S, Ohira M, Nishino H, Seki S and Hirakawa K: Inhibitory effect of a selective cyclooxygenase-2 inhibitor on liver metastasis of colon cancer. Int J Cancer *100*: 515-519, 2002.

35 Yamada N, Chung YS, Takatsuka S, Arimoto Y, Sawada T, Dohi T and Sowa M: Increased sialyl Lewis A expression and fucosyltransferase activity with acquisition of a high metastatic capacity in a colon cancer cell line. Br J Cancer *76*: 582-587, 1997.

36 Taguchi T: Clinical application of biochemical modulation in cancer chemotherapy: biochemical modulation for 5-FU. Oncology *54(Suppl 1)*: 12-18, 1997.

37 Nishimura G, Yanoma S, Mizuno H, Kawakami K and Tsukuda M: A selective cyclooxygenase-2 inhibitor suppresses tumor growth in nude mouse xenografted with human head and neck squamous carcinoma cells. Jpn J Cancer Res *90*: 1152-1162, 1999.

38 Yamazaki R, Kusunoki N, Matsuzaki T, Hashimoto S and Kawai S: Selective cyclooxygenase-2 inhibitors show a differential ability to inhibit proliferation and induce apoptosis of colon adenocarcinoma cells. FEBS Lett *531*: 278-284, 2002.

39 Abiru S, Nakao K, Ichikawa T, Migita K, Shigeno M, Sakamoto M, Ishikawa H, Hamasaki K, Nakata K and Eguchi K: Aspirin and NS-398 inhibit hepatocyte growth factor-induced invasiveness of human hepatoma cells. Hepatology *35*: 1117-1124, 2002.

40 Konno H, Tanaka T, Baba M, Kanai T, Matsumoto K, Kamiya K and Nakamura S: Therapeutic effect of 1 M tegafur -0.4 M 5-chloro-2,4-dihydroxypyridine -1 M potassium oxonate (S-1) on liver metastasis of xenotransplanted human colon carcinoma. Jpn J Cancer Res *90*: 448-453, 1999.

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