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A Comparative Pharmacology Study Between the Intracolonic and Oral Routes of 5-FU Administration in a Colon Cancer-Bearing Yoshida Sarcoma Rat Model

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Abstract. We prepared a colon cancer-bearing Yoshida sarcoma rat model to examine the dose-response relationship of antitumor activity of intracolonically or orally administered 5-fluorouracil (5-FU; 45, 30, 20, 13, and 8 mg/kg). At doses of ≥20 mg/kg and ≥30 mg/kg, the 5-FU intracolonic and oral administration groups each showed a statistically significant difference in antitumor activity against the control group ($P<0.05$, Williams' test). A statistically significant dose-response relationship was noted in the two routes of administration, with an ED_{50} value of 29 mg/kg. White blood cell count tended to decrease at high doses when 5-FU was administered intracolonically and showed a statistically significant decrease at doses of ≥30 mg/kg when 5-FU was administered orally. Regarding the time-course of body weight, even the 5-FU highest dose (45 mg/kg) intracolonic administration group showed no inhibited body weight increase compared to the control group. However, the 5-FU (≥20 mg/kg) oral administration groups showed a statistically significant difference in body weight increase against the control group. These facts suggested that the intracolonic administration of 5-FU, while exhibiting more potent antitumor activity than that observed in oral administration, allows an extensive reduction in its toxicities compared to oral administration.

Keywords: 5-FU, pharmacological evaluation, intracolonic administration, colon cancer, oral administration

Introduction

Pre- and postoperative chemotherapies are considered indispensable, although surgical therapy constitutes the core for the treatment of colon cancer (1 – 3). However, cancer chemotherapy as applied currently is mostly mediated by the systemic circulation, which generates the current situation where dose reduction or drug withdrawal is necessarily required due to the development of serious adverse reactions, for example, gastrointestinal disorders and myelosuppression (4, 5). 5-Fluorouracil (5-FU) and its derivatives, which represent first-line drugs for the treatment of gastrointestinal cancers in Japan, are commercially provided as injectable and oral preparations. Therefore, the drugs involve the above-mentioned issues. Gastrointestinal disorders, represented by diarrhea and other adverse reactions, develop because the small intestinal epithelium is damaged at the time of drug absorption, and intestinal villi atrophy and lose (6, 7). Therefore, diarrhea is considered to be an adverse reaction that is inevitable by a method to inhibit 5-FU activation at the time of absorption (8) or by a method to administer 5-FU through a mechanism other than small intestinal absorption. Furthermore, myelosuppression-induced leukopenia and neutropenia — factors which determine the maximal tolerated dose (MTD) (5, 9, 10) — are considered eludible by maintaining systemic blood drug concentrations low. In the intracolonic administration of 5-FU, the drug does not come in direct contract with the small intestinal epithelium which presents increased turnover due to active transport. Epithelial toxicity varies due to a difference in cellular uptake, while

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systemic toxicity varies due to a difference in absorption rate. Therefore, this approach allows a reduction in gastrointestinal disorders. Unlike the small intestine, the absorption of 5-FU from the colon does not depend on active transport (11, 12), thus inhibiting the excessive absorption of 5-FU into the systemic circulation and reducing myelosuppression. Moreover, a devise to make 5-FU be absorbed from colon cancer tissue and from the vicinity thereof maintains high concentrations of 5-FU in colon cancer tissue for a long time and permits the effective supply of the drug into the portal vein and mesenteric lymph nodes that are considered to constitute the pathway for metastasis. Therefore, the intracolonic administration of 5-FU is expected to advantageously promote long-term adjuvant chemotherapy for colon cancer compared to the conventional oral administration of the drug.

To elucidate the usefulness of intracolonic administration of 5-FU, we conducted a pharmacological study using a colon cancer-bearing Yoshida sarcoma rat model in which an innovative method of utilizing the ascending colon as the route of administration was used; intracolonic administration was compared with oral administration at the same doses of the drug.

Materials and Methods

Test substances

The test substance was 5-FU [5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione; molecular formula: $C_4H_3NF_2O_2$; molecular weight: 130.08; purity: 99.6%; Fuji Chemical Industry Co., Ltd., Toyama]. It occurs as a white crystal or crystalline powder and is odorless. It is freely soluble in dimethylformaldehyde, is sparingly soluble in water, is slightly soluble in ethanol, and is practically insoluble in ether. The test substance, scaled according to the concentration of the dosing solution for each dose, was suspended in the 0.5% solution of carboxymethylcellulose (Wako Pure Chemical Industries, Ltd., Tokyo) before use. The 0.5% solution of carboxymethylcellulose only was administered to animals in the control group.

Animals

Male Donryu rats, 7 weeks of age, were purchased from Charles River Japan, Inc., Tokyo; 63 animals were used for the intracolonic administration study and 62 animals, for the oral administration study. Animals were used after 7-day quarantine and acclimatization and were housed in an animal room whose environmental conditions were maintained under the following conditions: room temperature of 22 – 26°C, relative humidity of 30 – 60%, ventilation frequency of ventilations/h, and 12:12-h light-dark cycle (06:00 – 18:00) for the lightening time. Animals, housed in the cage (5 animals per cage), were allowed to take solid pellet CE-2 (Clea Japan, Inc., Tokyo) and tap water from the automatic water supplier ad libitum. Body weights at 8 weeks (age in weeks at the onset of study) were 244.4 – 291.9 g and 252.8 – 302.8 g for rats that were used in the intracolonic administration study and the oral administration study, respectively.

All animals, whose acclimatization period was completed, were subjected to cannulation surgery; of these animals, 54 were allotted into 6 groups of 9 animals to avoid bias. After surgery, animals were housed (1 animal per cage) and then allowed to take CE-2 and tap water ad libitum.

The present study was conducted in compliance with Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society and with the Jichi Medical School Guide for Use of Laboratory Animals.

Method to prepare the intracolonic administration model and method to xenograft cancer cells

Animals were anesthetized with the stock solution of the Nembutal™ (Dainippon Pharmaceutical Co., Ltd., Osaka) injection solution (pentobarbital sodium, 50 mg/mL) at a dose of 1 mL/kg. After shaving the cervico-dorsal and abdominal regions, the shaved regions were disinfected with ethanol; subsequently, a small incision was made in the abdominal (median area) and cervico-dorsal regions. A catheter (Atom nutrition catheter 4 Fr; Atom Medical Corporation, Tokyo) with saline (Otsuka Pharmaceutical Co., Ltd., Tokyo) was inserted from the cervico-dorsal region into the abdominal region of the animal, and the plug was sutured for its fixation to the cervico-dorsal region. A small incision was made in abdominal muscles (median area) to insert a catheter by 5 cm (1 to 2 cm within the colon) through the lower portion of the ileocecum. 6-0 DEXON™ 2 (synthetic and absorptive suture thread; Lederle Japan, Inc., Tokyo) was used to fix the catheter by purse-string suture and the Witzel technique. The sigmoid colon was exposed, and an injection needle (27 G; Terumo Corporation, Tokyo) and a 0.25-mL glass syringe (Terumo Corporation) were used to subserosally inject the Yoshida sarcoma cell suspension (Kyowa Hakko Kogyo Co., Ltd., Tokyo; 5×10^7/mL) at a volume of 30 μL/animal (1.5×10^6/animal). The peritoneal cavity was washed with about 10 mL of saline, followed by the suture of abdominal muscles and skin. Saline (approximately 0.5 mL) was injected into a catheter, and the sutured area was disinfected with acrinol (Kenei Pharmaceutical Co., Ltd., Osaka).

Subsequently, animals were warmed before replacement into the cage.

Study design

For each of the oral and intracolonic routes of administration, the following six groups were established in the present study: five 5-FU (45, 30, 20, 13, and 8 mg/kg, 2 mL/kg) administration groups and one control (saline) group. Subsequent to the next day of surgery, 5-FU was administered intracolonically or orally by gavage using a gastric tube. The study drug was administered once daily for 5 days. On the sixth day of study, animals were sacrificed. Animals were weighed prior to drug administration every day, and body weight was based to determine the dosing volume for the day. At the end of the study, day 6, blood was collected from the postcava under ether anesthesia. Subsequently, animals were subjected to fatal exsanguination and were then necropsied. After isolation of the sigmoid colon, red sarcoma only was removed to measure its diameter and weight. Furthermore, blood chemistry, hematology, necropsy, and clinical sign observation were conducted.

Method to measure hematology and blood chemistry values

On the day of necropsy, approximately 5 mL of blood was collected from the postcava of rats under ether anesthesia and then sampled into a 10-mL tube containing 30 μL of heparin at 1000 U/mL. Of the blood sampled, 1 mL of whole blood was transferred into the 1.5-mL plastic tube and was used as the sample for blood cell counting. The remaining blood was centrifuged (3000 rpm, 10 min), and the supernatant obtained was used as the sample for blood chemistry. A multichannel automated blood cell counter (K-2000; Sysmex Corporation, Kobe) was used to conduct blood cell counting. AU-510 (Olympus Corporation, Tokyo) was used to determine blood chemistry values, that is, total cholesterol (enzymatic method), triglyceride (enzymatic method), GOT (Henry method), GPT (Henry modified method), and LDH (G.S.C.C. equivalent method).

Statistical procedures

All data were expressed as means ± S.E.M. The SAS system (SAS Institute Japan, Tokyo) and EXSAS (version 3.00, Arm Ltd., Osaka) were used to test the level of significance. The Tukey type multiple comparison test was made for intergroup comparisons. The Williams type multiple comparison test was made for the dose-response relationship of tumor weight.

Results

Time-course of body weights

During the intracolonic administration study period, animals in the control group showed body weight decrease on the next day of surgery (day 1). However, no body weight decrease occurred subsequent to day 2, and body weights followed a nearly flat time-course up to day 6 of administration when the study was completed. Animals in the 5-FU intracolonic administration groups also showed body weight decrease on the next day of surgery. However, body weights followed a nearly flat time-course up to day 6 of administration similarly to the control group. Body weights followed a time-course at slightly lower levels in the 5-FU maximal dose (45 mg/kg) intracolonic administration group than in the control group, with no statistically significant difference (Tukey's test) (Fig. 1).

During the oral administration study period, animals in the control group showed body weight decrease on the day after surgery (day 1). However, no body weight decrease occurred subsequent to day 2 of administration, and body weights followed a nearly flat time-course up to day 6 of administration when the study was completed. Animals in the 5-FU (≤13 mg/kg) oral administration groups showed a time-course of body weight that was nearly comparable to that in the control group. However, animals in the 5-FU (45, 30, and 20 mg/kg) oral administration groups showed statistically significant decreases in body weight compared to the control group subsequent to day 4 of administration, subsequent to day 5 of administration, and on day 6

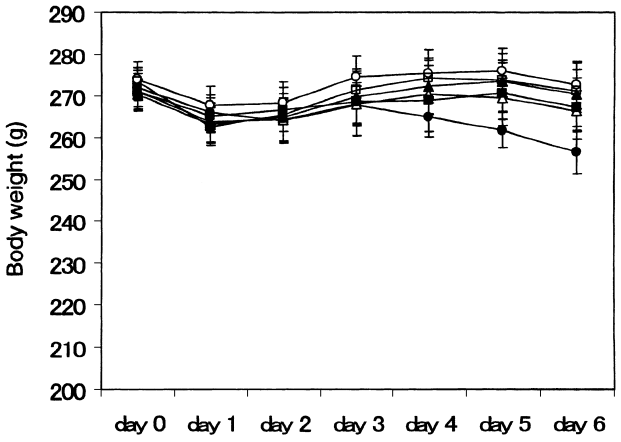

Fig. 1. Time-courses of body weight in colon-cancer-bearing Yoshida sarcoma rats after intracolonic administration of 5-FU. No significant difference was found. Mean ± S.E.M. Open circle: control (n = 9), closed circle: 45 mg/kg (n = 9), open triangle: 30 mg/kg (n = 9), closed triangle: 20 mg/kg (n = 6), open square: 13 mg/kg (n = 8), closed square: 8 mg/kg (n = 8).

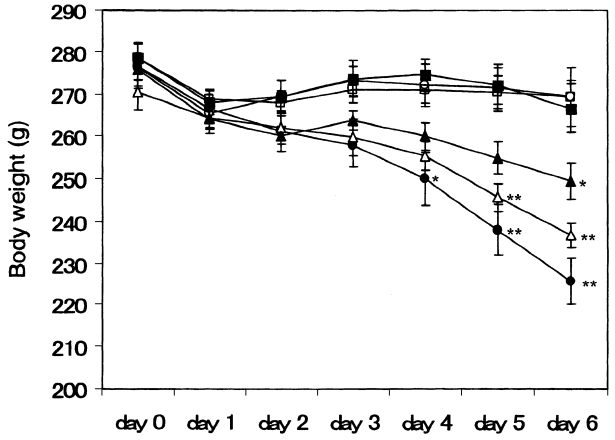

Fig. 2. Time-courses of body weight in colon-cancer-bearing Yoshida sarcoma rats after oral administration of 5-FU. **P*<0.05, ***P*<0.01 (*vs* control group, Tukey's test). Mean ± S.E.M. Open circle: control (n = 8), closed circle: 45 mg/kg (n = 9), open triangle: 30 mg/kg (n = 9), closed triangle: 20 mg/kg (n = 9), open square: 13 mg/kg (n = 8), closed square: 8 mg/kg (n = 8).

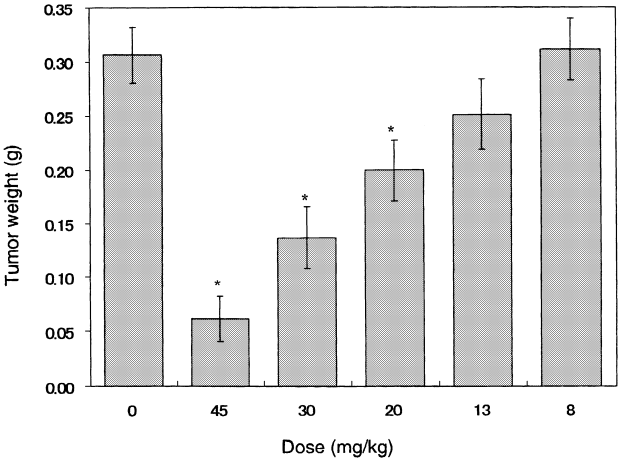

Fig. 3. Dose-response relationship of tumor weight in colon-cancer-bearing Yoshida sarcoma rats after intracolonic administration of 5-FU. **P*<0.05 (*vs* control group, Williams' test). Mean ± S.E.M. ED_{50} value: 29.2882 mg/kg.

of administration, respectively (*P*<0.05, Tukey's test) (Fig. 2).

Tumor weights

The mean weights of the tumor in the sigmoid colon at the end of the intracolonic administration study were as follows: 306.1 ± 25.5 mg in the control group; 61.6 ± 20.9 mg in the 5-FU (45 mg/kg) intracolonic administration group, 137.1 ± 28.9 mg in the 5-FU (30 mg/kg) intracolonic administration group, 199.5 ± 27.9 mg in the 5-FU (20 mg/kg) intracolonic administration group, 250.6 ± 32.5 mg in the 5-FU (13 mg/kg) intracolonic administration group, and 310.9 ± 29.3 mg in the 5-FU (8 mg/kg) intracolonic administration group. A comparison of each 5-FU intracolonic administration group with the control group revealed that the 5-FU (≥20 mg/kg) intracolonic administration groups showed a statistically significant difference in antitumor activity (*P*<0.05, Williams' test). Furthermore, a statistically significant dose-response relationship was also noted, with an ED_{50} value of 29.29 mg/kg (Fig. 3).

The mean weights of the tumor at the end of the oral administration study were as follows: 354.7 ± 34.6 mg in the control group, 13.3 ± 9.3 mg in the 5-FU (45 mg/kg) oral administration group, 154.1 ± 31.5 mg in the 5-FU (30 mg/kg) oral administration group, 333.8 ± 39.0 g in the 5-FU (20 mg/kg) oral administration group, 346.0 ± 27.6 mg in the 5-FU (13 mg/kg) oral administration group, and 330.4 ± 32.4 mg in the 5-FU (8 mg/kg) oral administration group. A comparison of each 5-FU oral administration group with the control group revealed that the 5-FU (≥30 mg/kg) oral

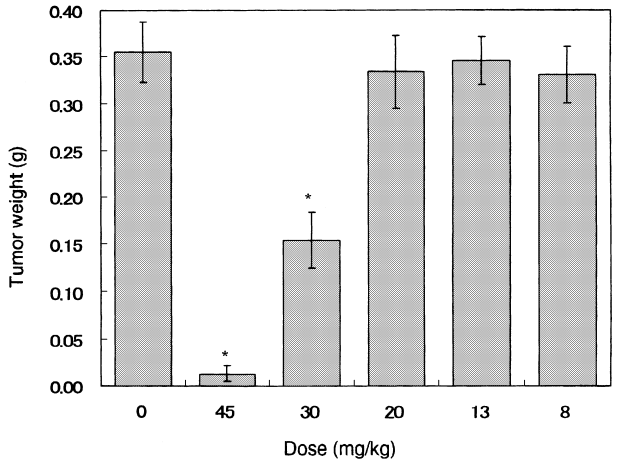

Fig. 4. Dose-response relationship of tumor weight in colon-cancer-bearing Yoshida sarcoma rats after oral administration of 5-FU. **P*<0.05 (*vs* control group, Williams' test). Mean ± S.E.M. ED_{50} value: 29.2908 mg/kg.

administration groups showed a statistically significant difference in antitumor activity (*P*<0.05, Williams' test). Furthermore, the statistically significant dose-response relationship was also noted, with an ED_{50} value of 29.29 mg/kg (Fig. 4).

Hematology

Hematological values of the blood collected at the completion of the intracolonic administration study are shown in Fig. 5. No statistically significant difference was found, although a comparison of mean white blood cell counts revealed slightly lower values in the

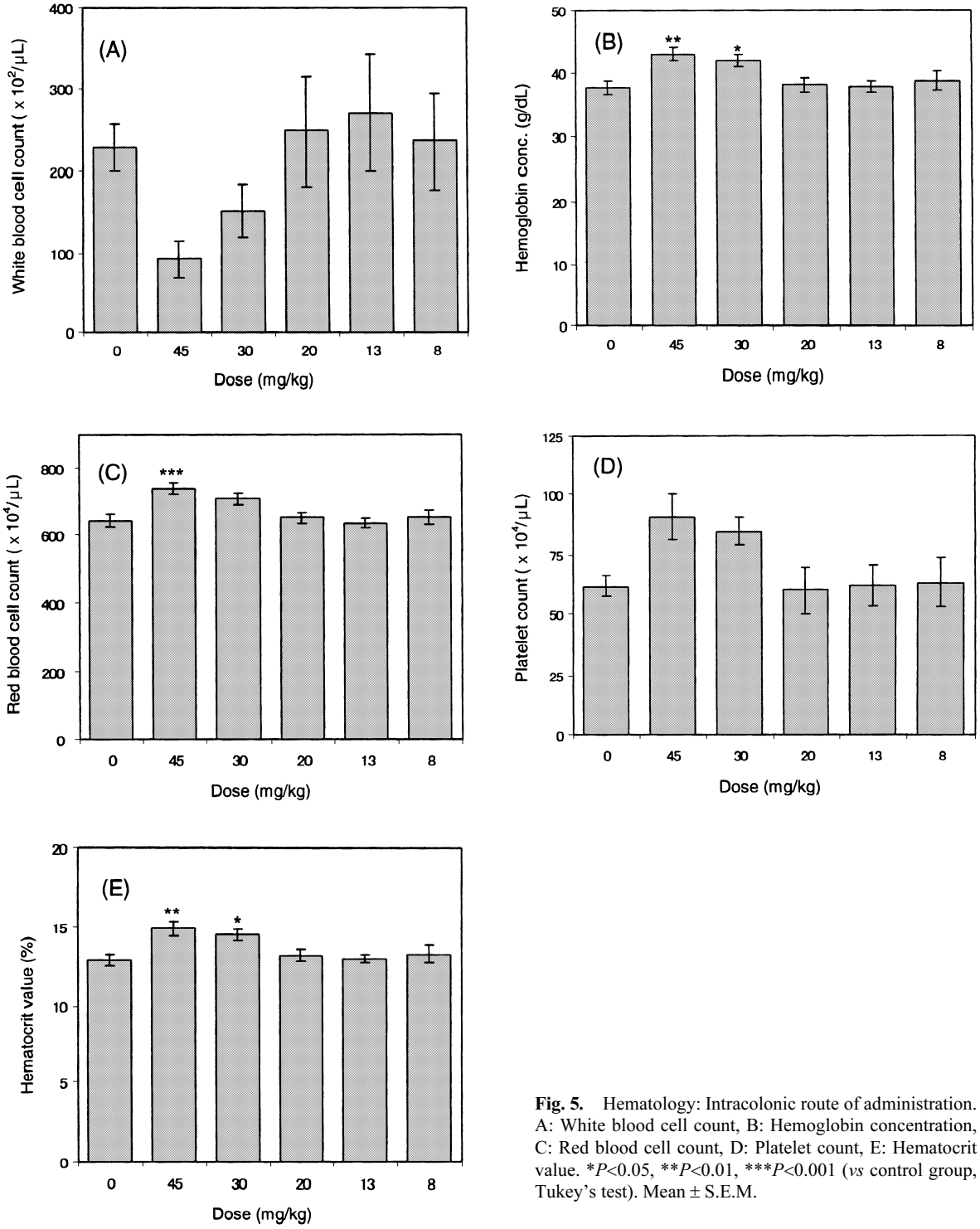

Fig. 5. Hematology: Intracolonic route of administration. A: White blood cell count, B: Hemoglobin concentration, C: Red blood cell count, D: Platelet count, E: Hematocrit value. **P*<0.05, ***P*<0.01, ****P*<0.001 (*vs* control group, Tukey's test). Mean ± S.E.M.

5-FU (≥20 mg/kg) intracolonic administration groups than in the control group. The 5-FU (45 mg/kg, ≥30 mg/kg, and ≥30 mg/kg) administration groups showed statistically significantly higher values in red blood cell count, hemoglobin concentration, and hematocrit value, respectively, compared to the control group (P<0.05, Tukey's test).

Among other laboratory test values, platelet count revealed no marked change which was considered attributable to the effects of 5-FU.

Hematological values of the blood collected at the completion of the oral administration study are shown in

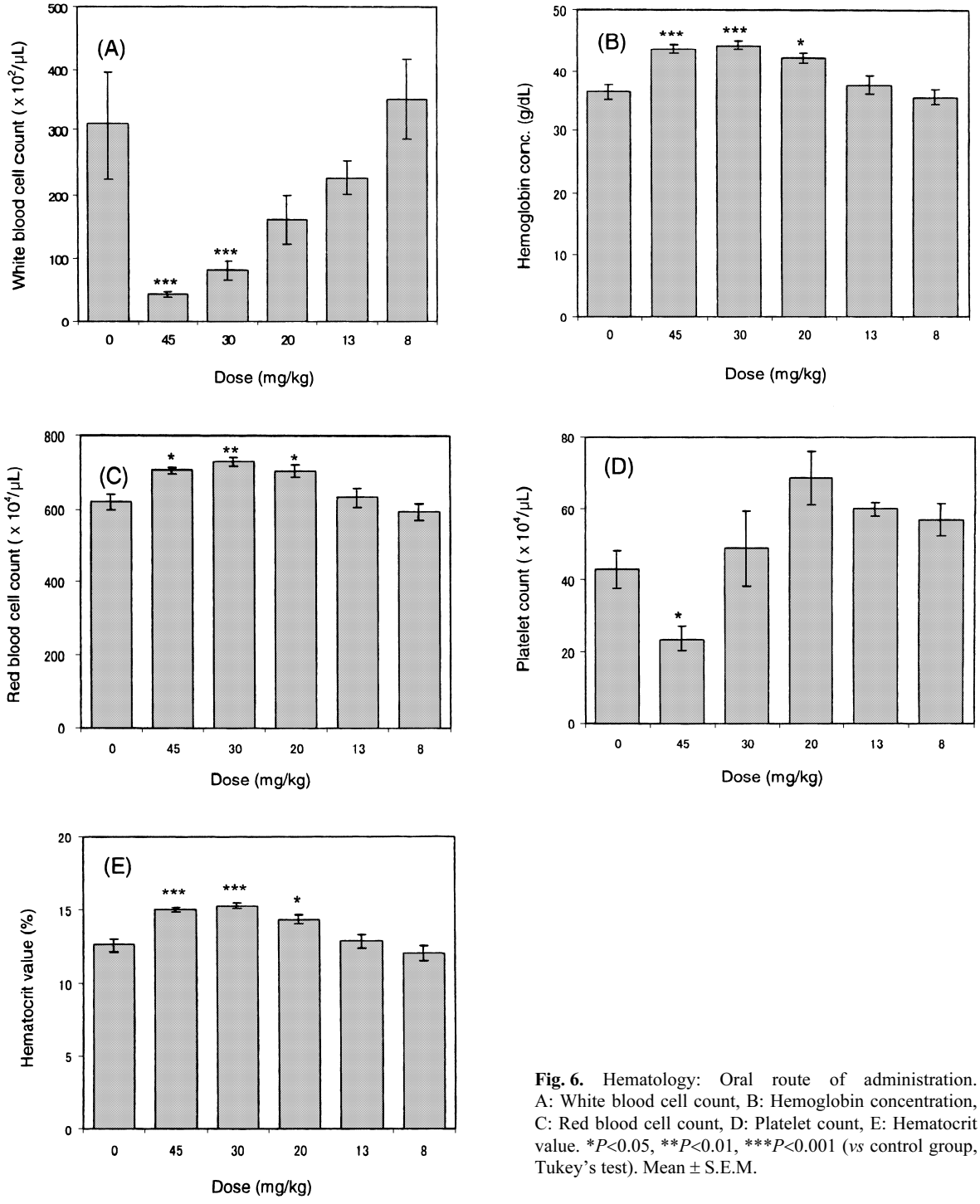

Fig. 6. Hematology: Oral route of administration. A: White blood cell count, B: Hemoglobin concentration, C: Red blood cell count, D: Platelet count, E: Hematocrit value. **P*<0.05, ***P*<0.01, ****P*<0.001 (*vs* control group, Tukey's test). Mean ± S.E.M.

Fig. 6. White blood cell count and platelet count tended to decrease dose-dependently. The 5-FU (≥30 mg/kg) and (45 mg/kg) oral administration groups showed statistically significantly low while blood cell count and platelet count, respectively, compared to the control group. Furthermore, red blood cell count, hemoglobin concentration, and hematocrit value tended to increase dose-dependently. The 5-FU (≥20 mg/kg) oral administration groups showed statistically significantly high levels of these variables compared to the control group (P<0.05, Tukey's test).

Blood chemistry values

Blood chemistry values of the plasma which was

collected at the completion of the 5-FU intracolonic administration study are shown in Fig. 7. The 5-FU (≥20 mg/kg) intracolonic administration groups showed statistically significantly low plasma triglyceride levels against the control group (P<0.05, Tukey's test). Furthermore, the 5-FU highest dose (45 mg/kg) intracolonic administration group inversely showed a statistically significantly high plasma total cholesterol level against the control group (P<0.05, Tukey's test). On the other hand, the 5-FU (≥30 mg/kg) intracolonic administration groups showed slightly low plasma levels of LDH, GOT, and GPT compared to the control group, with no statistically significant difference.

Blood chemistry values of the plasma which was collected at the completion of the 5-FU oral administration study are shown in Fig. 8. Plasma levels of LDH, GPT, γ-GTP, and GOT decreased dose-dependently. The 5-FU (≥30 mg/kg) oral administration groups showed statistically significantly low levels of these variables compared to the control group. The 5-FU (up to 30 mg/kg) oral administration groups similarly showed a dose-dependent decrease in plasma triglyceride level, and the 5-FU (20 and 30 mg/kg) oral administration groups showed statistically significantly low plasma triglyceride levels against the control group. However, the 5-FU highest dose (45 mg/kg) oral administration group showed no statistically significant difference. On the other hand, plasma total cholesterol level inversely tended to increase dose-dependently; the 5-FU (≥30 mg/kg) oral administration groups showed statistically significantly higher plasma total cholesterol levels against the control group (P<0.05, Tukey's test).

Necropsial findings and clinical signs

Among necropsial findings at the completion of the 5-FU administration studies, an assessment was made with respect to the metastasis of sarcoma to the peritoneum, greater omentum, mesenteric lymph nodes, intraperitoneal organ surface, thymus, and lung. None of the animals in all the 5-FU intracolonic administration groups was completely free of metastasis. However, the metastasis to the peritoneum, greater omentum, and lung tended to lessen dose-dependently. As another organ/tissue finding worthy of particular mention, furthermore, tumor tissue of Yoshida sarcoma tended to whiten in the 5-FU high dose intracolonic administration groups. In addition, thymic atrophy — considered to be one of toxicity findings — was not observed in all animals (Tukey's test). On the other hand, only 2 animals in the 5-FU (45 mg/kg) oral administration group were completely free of macroscopic metastasis. Metastases to the peritoneum, greater omentum, and lung tended to lessen dose-dependently. Furthermore, thymic atrophy — considered to be one of toxicity findings — was observed in the 5-FU (≥30 mg/kg) oral administration groups.

Discussion

Tumor weight as a variable for the pharmacological actions of 5-FU was compared by route of administration and by dose. Tumor weight decreased dose-dependently in both intracolonic and oral routes. The statistically significant dose-response relationship was also observed. The ED_{50} values were nearly equivalent. The 5-FU (≥20 mg/kg) intracolonic administration groups showed a statistically significant difference in tumor weight against the control group, while the 5-FU (≥30 mg/kg) oral administration groups showed a statistically significant difference (P<0.05, Williams' test). Therefore, the 5-FU low dose intracolonic administration groups showed obviously superior antitumor activity.

Another pharmacokinetics study analyzed, after the intracolonic and oral administration of ^{14}C-5-FU, plasma from the portal vein, plasma from the postcava, liver, mesenteric lymph nodes, small intestinal wall, walls of descending colon, small intestinal content, colonic content, urinary metabolites, and biliary metabolites. Consequently, a high tissue concentration of 5-fluorouridine (FUR), a major active metabolite of 5-FU, was observed in walls of the descending colon — the site of direct action of intracolonically administered 5-FU, and both 5-FU and FUR were present up to 24 h after administration. On the other hand, neither 5-FU nor FUR was detected in the lower portion of colonic walls when ^{14}C-5-FU was administered orally. Furthermore, higher concentrations of 5-FU and FUR in the portal vein and mesenteric lymph nodes, pathways for colon cancer metastasis, persisted in the 5-FU intracolonic administration groups than in the oral administration group. Therefore, 5-FU in the caval plasma was also found to persist in the 5-FU intracolonic administration groups for a longer time than in the 5-FU oral administration groups. Concentrations of unchanged 5-FU and its active metabolites in colonic tissue were found to persist for a long time in intracolonic administration compared to oral administration (data not shown).

We did not measure xenograft and metastatic tumor concentrations of 5-FU in the present study because we do not consider that 5-FU is delivered into tumor tissue by active transport in the colon. In a pharmacokinetics study using ^{14}C-5-FU in normal rats, however, intracolonic and oral administration of 5-FU 2 mg/kg yielded the maximum plasma concentrations of 0.534 ± 0.159 μg·eq/mL (1-h value) and 0.919 ± 0.232 μg·eq

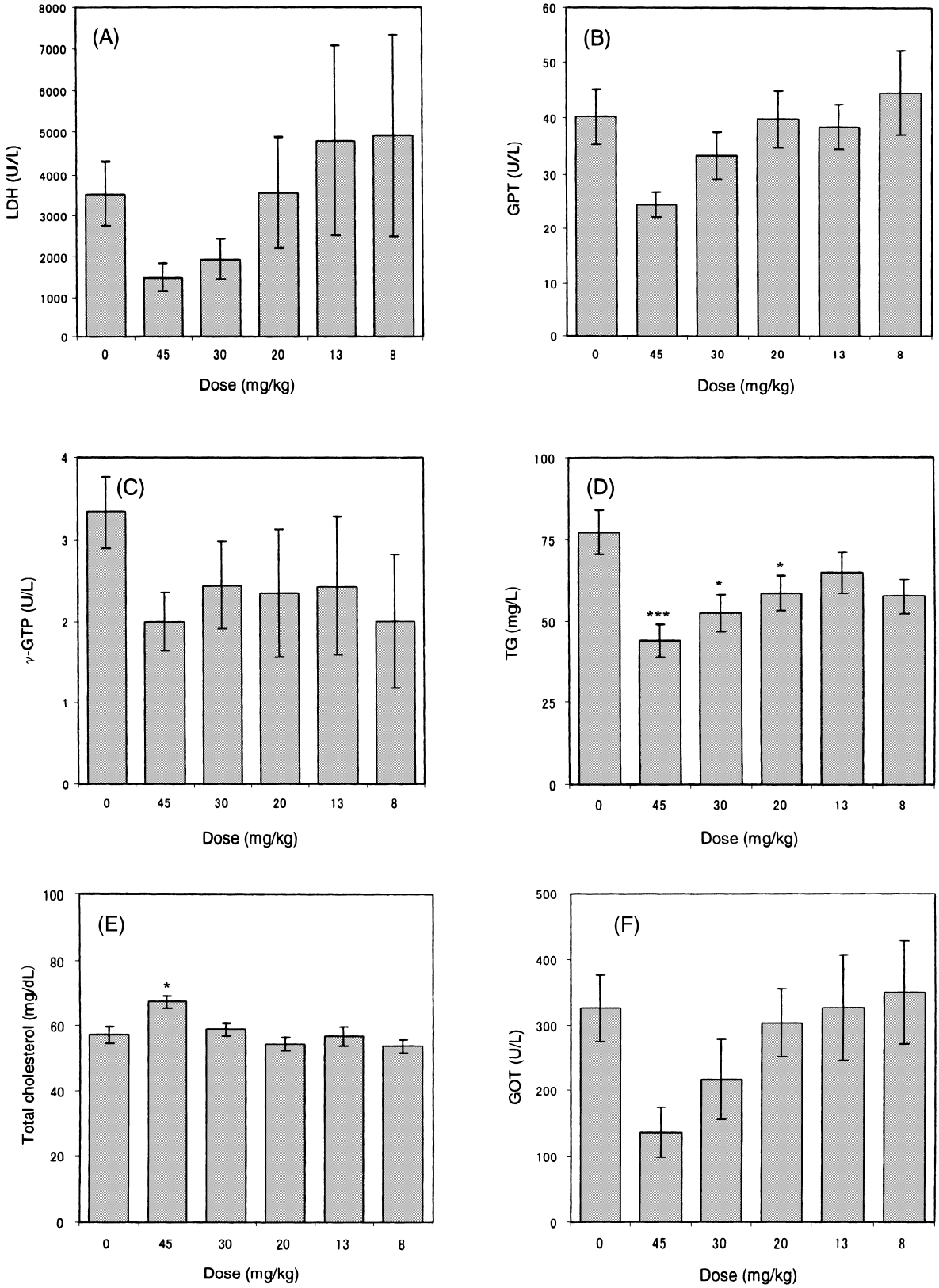

Fig. 7. Blood chemistry: Intracolonic route of administration. A: LDH, B: GPT, C: γ-GTP, D: Triglyceride, E: Total cholesterol, F: GOT. *P<0.05, ***P<0.001 (*vs* control group, Tukey's test). Mean ± S.E.M.

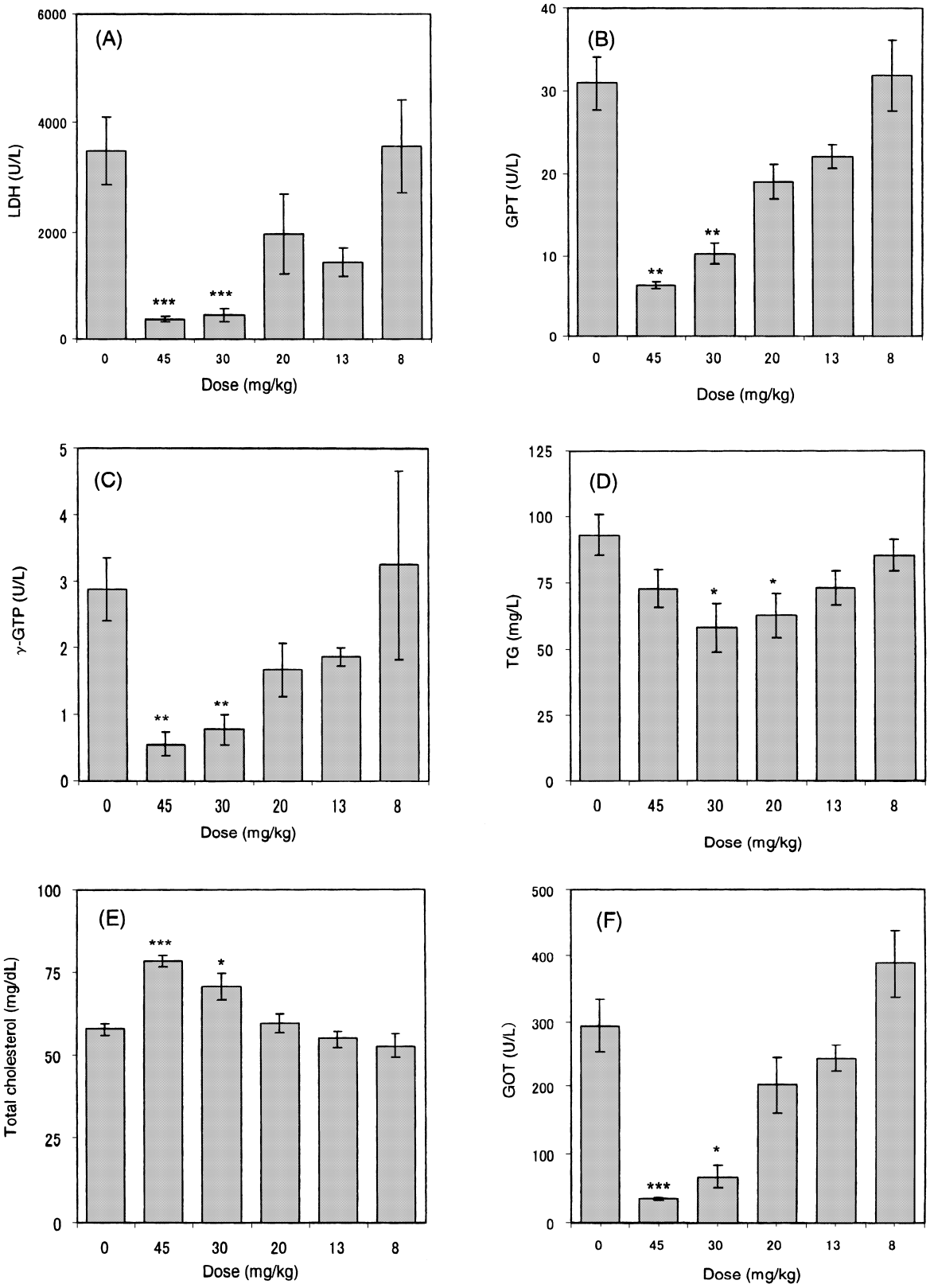

Fig. 8. Blood chemistry: Oral route of administration. A: LDH, B: GPT, C: γ-GTP, D: Triglyceride, E: Total cholesterol, F: GOT. *P<0.05, **P<0.01, ***P<0.001 (*vs* control group, Tukey's test). Mean ± S.E.M.

/mL, respectively, and the maximum colonic concentrations of 156.491 ± 21.236 and $<1\ \mu g \cdot eq/mL$ (1-h value), respectively. These data support the superior pharmacological effects of 5-FU when given by intracolonic administration, compared to those by oral administration, in the present study. In a toxicokinetics study of 5-FU at 30 mg/kg to examine its plasma concentrations, on the other hand, ≥5-fold differences were noted in plasma 5-FU concentrations between intracolonic (100 ng/mL) and oral (≥1,000 ng/mL) administration; differences in plasma concentrations were manifested as statistically significant differences in the incidences of systemic adverse reactions.

The pharmacological actions of 5-FU as observed in the present study reflect the results of the relevant pharmacokinetics study. However, the difference in pharmacological actions was not so much as the difference in drug concentrations; this fact leads us to propose the hypothesis that there is a difference between tissue drug concentration in the entire colon tissue and tissue drug concentration at the tumor site. Another permissible hypothesis is that a devise of making 5-FU be absorbed from colon cancer tissue and from the vicinity thereof maintains 5-FU concentrations in colon cancer tissue high for a long time, thus allowing the efficacious supply of 5-FU into the portal vein and mesenteric lymph nodes which are considered to constitute the pathway for metastasis. However, necropsial findings in the present study failed to find merits of intracolonic administration with respect to tumor tissue, which had been observed in other organs. In tumor tissue observed in other organs, 5-FU showed no statistically significant difference in anticancer activity between oral and intracolonic routes of administration (data not shown). We interpret these results to mean that cancer cell retention could not be confined to the site of colon xenograft when xenografting cultured cancer cells, thus causing dispersion thereof at the time of administration. Therefore, we considered that the present model is not in the condition to reflect tumor metastasis.

Time-courses of blood chemistry, hematology, and body weight by route of administration and by dose were compared as variables for toxicity evaluation.

Regarding hematology, the 5-FU intracolonic administration groups tended to show a dose-dependent decrease in white blood cell count, although no statistically significant difference was noted. The 5-FU oral administration groups tended to show a dose-dependent decrease in white blood cell count, with a statistically significant decrease at doses of 30 mg/kg. These changes represent the development of 5-FU's toxicities to the hematopoietic system and suggest the advantage of intracolonic administration over oral administration, even though it only slightly alleviates myelosuppression, one of the serious systemic adverse reactions of the drug. Furthermore, the 5-FU maximal dose (45 mg/kg) intracolonic administration group showed statistically significant increases in red blood cell count, hemoglobin concentration, and hematocrit value. In the 5-FU (≥20 mg/kg) oral administration groups, there were also statistically significant increases. These changes were considered attributable to hemoconcentration that was associated with deterioration of clinical signs.

Regarding blood chemistry, the 5-FU intracolonic administration groups tended to show dose-dependent decreases in LDH, GPT, and GOT activity. The 5-FU oral administration groups tended to show dose-dependent decreases in LDH, GPT, γ-GTP, and GOT activity, with a statistically significant difference at doses of ≥30 mg/kg. The normal levels of GOT and LDH in rats are approximately 100 U/L and approximately 40 U/L, respectively. In the present study, the GOT and LDH levels in animals in the control group were approximately 300 U/L and approximately 3500 U/L, respectively. These facts indicate that sarcoma-bearing rats in the present study, for which catheterization surgery had been conducted, were in the condition where extensive inflammation was induced at the onset of the study. Therefore, a dose-dependent tendency for decrease in these values was considered to be based on a reduction in inflammation due to the immunosuppressive activity of 5-FU. Furthermore, the 5-FU highest dose (45 mg/kg) intracolonic administration group and the 5-FU high dose (≥30 mg/kg) oral administration groups showed statistically significant increases in plasma total cholesterol compared to the control group. However, the cause for the increases was not elucidated.

Even the 5-FU highest dose (45 mg/kg) intracolonic administration group showed no statistically significant difference in body weight against the control group, although it followed a time-course at slightly low levels. The 5-FU (≥20 mg/kg) oral administration groups showed statistically significant decreases in body weight compared to the control group (Fig. 1). The fact that the 5-FU highest dose (45 mg/kg) intracolonic administration group showed no decrease in rat body weight while exhibiting an approximately 80% tumor reduction rate (Fig. 2) strongly suggests that the intracolonic administration of 5-FU provides a very promising route of administration that successfully reduces its toxicities while conserving its efficacies.

Furthermore, the 5-FU (20 mg/kg) intracolonic administration group showed marked pharmacological actions without exhibiting any signs of toxicities. The

fact that the 5-FU (20 mg/kg) oral administration group only showed toxicities without exhibiting pharmacological actions further strongly supports the advantage of the abovementioned intracolonic administration of 5-FU, that is, marked efficacies with less toxicities. The above facts revealed that the intracolonic administration of 5-FU is a highly useful route of administration that allows an expectation for increased efficacies of the drug while extensively reducing its systemic toxicities. We have developed a prototype, colon-targeted, two-layer coated preparation as a multipurpose drug delivery system. This preparation, when administered orally, passes intact through the stomach, its outer layer dissolves in the small intestine, and its inner layer swells and dissolves to release a drug into the colon. We have already verified the integrity of the system in humans (13). We expect the outcomes of the present study and the relevant system to be useful for the development of a new colon-targeted drug delivery system containing 5-FU in the future.

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References

1 Hoff PM, Janjan N, Saad ED, et al. Phase I study of preoperative oral uracil and tegafur plus leucovorin and radiation therapy in rectal cancer. J Clin Oncol. 2000;18:3529–3534.

2 Sakamoto J, Hamada C, Kodaira S, Nakazato H, Ohashi Y. Adjuvant therapy with oral fluoropyrimidines as main chemotherapeutic agents after curative resection for colorectal cancer: individual patient data meta-analysis of randomized trials. Jpn J Clin Oncol. 1999;29:78–86.

3 Taylor I, Mchini D, Mullee M, et al. A randomized controlled trial of adjuvant portal vein cytotoxic perfusion in colorectal cancer. Br J Surg. 1985;72:359–363.

4 Hines JD, Zakem MH, Adelstein DJ, Rustum YM. Treatment of advanced-stage colorectal adenocarcinoma with fluorouracil and high-dose leucovorin calcium: a pilot study. J Clin Oncol. 1988;6:142–146.

5 Laufman LR, Krzeczowski KA, Roach R, Segal M. Leucovorin plus 5-fluorouracil: an effective treatment for metastatic colon cancer. J Clin Oncol. 1987;5:1394–1400.

6 Tamaki T, Naomoto Y, Kimura S, et al. Apoptosis in normal tissues induced by anti-cancer drugs. J Int Med Res. 2003;31:6–16.

7 Vidon N, Perchellet E, Huchet B, Bernier JJ. Hydroelectrolytic movements in rat jejunum during the alterations of the mucosa induced by a single injection of 5-fluorouracil. Digestion. 1979;19:370–374.

8 Taguchi T, Inuyama Y, Kanamaru R, Hasegawa K. Phase I study of S-1. S-1 Study Group. Jpn J Cancer Chemother. 1997; 24:2253–2264. (text in Japanese with English abstract)

9 Nakatsu T, Yokoyama, Tsuyuki K, et al. Clinical reevaluation of continuous intravenous infusion of 5-fluorouracil — plasma concentrations and clinical dose by continuous intravenous and 60-min infusions. Jpn J Cancer Chemother. 1990;17:253–258. (text in Japanese with English abstract)

10 Akimoto M, Ueki H, Nakajima Y, et al. Prevention of 5-FU induced toxicity in C3 H/HE mice with interferon or with interferon inducers (poly I:C, OK-432, Lentinan). Jpn J Cancer Chemother. 1984;11:1462–1467. (text in Japanese with English abstract)

11 Tomei S, Hayashi Y, Inoue K, et al. Search for carrier-mediated transport systems in the rat colon. Biol Pharm Bull. 2003; 26:274–277.

12 Yuasa H, Matsuhisa E, Watanabe J. Intestinal brush border transport mechanism of 5-fluorouracil in rats. Biol Pharm Bull. 1996;19:94–99.

13 Goto T, Tanida N, Yoshinaga T, et al. Pharmaceutical design of a novel colon-targeted delivery system using two-layer coated tablets of three different pharmaceutical formulations, supported by clinical evidence in humans. J Control Release. 2004 (in press)