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ORIGINAL ARTICLE

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Comparison of intraperitoneal continuous infusion of floxuridine and bolus administration in a peritoneal gastric cancer xenograft model

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Abstract *Purpose*: To identify the optimal schedule for intraperitoneal (i.p.) infusion of floxuridine (FUDR) against peritoneal micrometastases from gastric cancer. *Methods*: The efficacy of continuous i.p. infusion of FUDR was compared with that of bolus i.p. administration in peritoneal gastric cancer (MKN45) xenografts. The FUDR continuous delivery system in this study was in the form of injectable poly(lactic-coglycolic) acid (PLGA) microspheres intended for i.p. injection. Animals were treated by continuous i.p. infusion using FUDR-loaded microspheres or bolus i.p. administration of FUDR. *Results*: In vitro testing demonstrated that FUDR was released slowly from the microspheres at a rate of approximately 5% of the total encapsulated drug per day. In in vivo studies, the peritoneal level was found to persist and was approximately 5- to 50-fold higher than that of plasma for more than 2 weeks following a single injection of the microspheres. An in vitro MTT assay showed that exposure time clearly influenced the cytotoxic potency of FUDR. In vivo, continuous infusion was more effective against peritoneal tumor than bolus administration at equivalent doses. However, compared with bolus administration, toxicity was increased, resulting in a reduced maximum tolerated dose (MTD) with continuous infusion. When the treatment was carried out at each MTD (continuous 1 mg/kg, bolus 600 mg/kg), continuous infusion had no advantage in inhibiting tumor growth. *Conclusions*: Owing to the higher toxicity and the equal efficacy of continuous infusion compared with bolus administration, continuous infusion is not recommended in i.p. FUDR treatment.

Keywords FUDR · Adjuvant chemotherapy · PLGA · Microsphere · Drug delivery system

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Introduction

Until recently, gastric cancer was the second most common cancer worldwide. Now, however, an estimated 974,000 new cases in 2000 put it in third place behind breast cancer [1]. Despite its continued decline in incidence, it remains the second most common cause of cancer death, with 734,000 deaths annually.

Complete surgical resection is the only therapy that is potentially curative. However, even after a complete resection with negative margins (an R0 resection), many patients, particularly those with more locally advanced tumors, will experience recurrence. In the case of recurrence, in general only palliative therapy is possible. There have been many attempts over the past two decades to improve cure rates by using postoperative systemic chemotherapy (adjuvant chemotherapy) [2]. No single trial has been sufficiently powerful to discern a significant survival advantage for adjuvant chemotherapy versus surgery alone. Several recent meta-analyses have indicated that adjuvant chemotherapy may produce a small survival benefit of borderline significance in patients who undergo R0 resection; however, any impact on outcome is modest [3, 4, 5, 6]. As a result, for the past decade, the standard therapy for the treatment of resectable gastric cancer has been surgery alone.

Clearly, because the most common type of recurrence is peritoneal metastases, it is important to decrease the

risk of peritoneal failure [7, 8]. One hypothesis is that microscopic residual cancer occurs in the operative site and on the peritoneal surface and causes local and regional peritoneal recurrence. Intraperitoneal (i.p.) chemotherapy is currently being studied to address this point. For peritoneal disease, i.p. delivery of chemotherapy offers a potential therapeutic advantage over systemic chemotherapy by producing high regional concentrations of drug while simultaneously minimizing systemic toxicities [9]. Drugs that achieve the best peritoneal/plasma concentration ratio are those that have a high molecular weight, extensive first-pass hepatic inactivation, and rapid plasma clearance.

Floxuridine (FUDR) is a deoxynucleoside metabolite of fluorouracil (5-FU) that inhibits thymidylate synthase resulting in blockage of DNA synthesis. Chemically, FUDR is 2′-deoxy-5-fluorouridine with an empirical formula of $C_9H_{11}FN_2O_5$. Bolus i.p. administration of FUDR has been investigated in an adjuvant setting in gastric cancer as well as colon cancer and epithelial ovarian cancer patients since 1990 [10, 11, 12, 13, 14, 15]. When given by the i.p. route, FUDR shows an appreciable catabolic clearance mainly by the liver and i.p. drug levels two to three logs higher than plasma levels. Moreover, i.p. FUDR is well tolerated and not associated with the high risk of chemical peritonitis observed with i.p. 5-FU. A bolus 3000-mg dose given for three consecutive days has been shown to have antitumor activity and to produce no systemic toxicity except nausea and vomiting. However, the activity of FUDR is relatively S-phase-dependent and its half-life in the peritoneal cavity is short. Continuous i.p. infusion may expose a relatively larger proportion of peritoneal tumor cells to FUDR and tumor cells are more likely to be killed.

The ability to deliver drugs into the abdominal cavity on a continuous basis has frequently been frustrated by the non-availability of a safe delivery system. Injectable microspheres prepared using biodegradable polymers, polylactic acid (PLA) and poly(lactic-coglycolic) acid (PLGA) have been widely used for controlled release of many drugs including peptides and proteins [16]. We consider that PLGA microspheres are one of the most desirable dosage forms for the drug since they are able to maintain therapeutic levels for more than 2 weeks following a single injection.

The purpose of this study was to develop FUDR-containing PLGA microspheres and to test the efficacy of continuous infusion compared with bolus administration of i.p. FUDR therapy.

Materials and methods

Preparation of FUDR-loaded PLGA microspheres

FUDR-loaded microspheres were prepared using a water-in-oil-in-water (w/o/w) emulsion solvent extraction method. A weighed amount of FUDR (5-fluoro-2′-deoxyuridine; Sigma Chemical Company, St. Louis, Mo.) was dissolved in 0.1 ml distilled water. A weighed amount of a 50:50 lactide/glycolide PLGA (Sigma) was dissolved in 2 ml dichloromethane, and then a similar amount of a 75:25 lactide/glycolide PLGA (Wako Pure Chemical Industries, Osaka, Japan) was added to the organic solution (the total mass of PLGA and FUDR was held constant at 1000 mg). Both solutions were mixed and vigorously homogenized in a model G-560 Vortex Genie 2 (Scientific Industries, Bohemia, N.Y.) for 1 min. The w/o emulsion was passed through a thin nozzle into 200 ml of a 2.0% polyvinyl alcohol solution and the resulting emulsion was stirred at 1200 rpm with a magnetic stir bar for 3 h under reduced pressure at room temperature until the organic solvent had evaporated. The 20–800 μm microspheres were collected by filtration, rinsed with 500 ml water, and then dried.

Morphology and drug content of microspheres, and drug release

For morphological examination, the microspheres were mounted on double-sided adhesive tape, sputtered with platinum and viewed in a Hitachi S-4700 scanning electron microscope (SEM). The particle size distribution of the microspheres was determined by optical microscopy using a stage micrometer. In the field of view of the microscope 100 microspheres were examined, and at least three fields of view were examined for each sample. To determine the FUDR content of the PLGA microspheres, 50 mg of microspheres was dissolved in 5 ml dichloromethane and FUDR was extracted into 5 ml water. FUDR in the aqueous layer was determined by high-performance liquid chromatography (Shimadzu, Kyoto, Japan) using a mobile phase of 25 mmol/l ammonium phosphate with 3% (v/v) acetonitrile. Analyses were performed on a Zorbax RX column, 5 μm particle size (25 cm × 4.6 mm ID), plus a 12.5×4.6 mm ID guard cartridge of the same material and from the same manufacturer. Injection volumes were 20 μl with a flow-rate of 1 ml/min and column eluates was monitored at 270 nm. To examine FUDR release from the PLGA microspheres, 500 mg of drug-loaded microspheres were weighed into 15-ml glass test tubes (Asahi Techno Glass Company, Tokyo, Japan). Phosphate-buffered saline (10 ml, pH 7.4) was added to the tubes. The vials were then capped and stirred continuously at 37°C with a gyrating shaker (Taitec, Saitama, Japan). Samples were removed every day, and the FUDR concentration in the samples was determined by the HPLC method described above.

Animal studies

Pharmacokinetic studies were performed in healthy male F344 JCL rats (Cler Japan, Tokyo, Japan) at 5–6 weeks of age. Athymic male BALB/$c^{nu/nu}$ mice (Cler Japan) at 5 weeks of age were used for therapeutic efficacy studies. The animals were acclimated for 1 week while caged in groups of three. All animal studies were carried out in accordance with the "Guidelines for Animal Experimentation" under a protocol approved by the Animal Experimentation Committee of Kansai Medical University.

Cell culture

The MKN45 human gastric cancer cells were obtained from the Riken Cell Bank (Tsukuba, Japan). These cells were cultured in RPMI 1640 (Nissui Pharmaceutical Company, Tokyo, Japan) supplemented with 10% fetal bovine serum (Sigma F9423, Tokyo, Japan) and maintained in an atmosphere containing 5% CO_2 at 37°C in a humidified incubator. Cells in exponential growth were harvested by brief treatment with a 0.25% trypsin/1 m*M* EDTA solution (Gibco, Gaithersburg, Md.).

Pharmacokinetic studies

Healthy rats weighting 100–120 g received bolus or continuous i.p. infusions of FUDR. The dose of FUDR used in this pharmacokinetic study was chosen to approximate the i.p. dosage

recommended for use in humans (3000 mg/1–2 l per patient), and to be above the analytical detection limit in fluid samples [10, 11, 12, 13]. For the bolus i.p. pharmacokinetic study, FUDR at a dose of 4 mg was administered to a group of six animals. The drug was prepared in 5 ml normal saline and warmed to 37°C. The FUDR solution was infused rapidly into the peritoneal cavity through a 27-gauge needle. For the continuous i.p. pharmacokinetic study, a total dose of 1000 mg 0.4% FUDR-loaded microspheres was administered i.p. to a group of 12 animals. The microspheres were prepared in 5 ml normal saline and warmed to 37°C. The microspheres were injected rapidly into the peritoneal cavity through a small incision. Blood was obtained by cardiac puncture under inhalation of diethyl ether at various times and placed in heparinized microtubes. Plasma was separated immediately from the blood by microcentrifugation (2100 *g* for 5 min) and stored at −20°C until assayed. At each time point, the peritoneal fluid was collected from the animals and stored at −20°C until assayed.

The FUDR concentration in the samples was determined by HPLC based on the method of Smith-Rogers et al. [17] with the following modifications. Plasma samples (100 µl) were pipetted into Eppendorf tubes. Bromouridine as internal standard was added at 10 µg/ml to all samples. After addition of 80 µl 12.5% perchloric acid, all samples were thoroughly mixed with a model G-560 Vortex Genie 2 (Scientific Industries, Bohemia, N.Y.) and allowed to remain for at least 12 h at 4°C to allow maximum protein precipitation of the plasma. The samples were then centrifuged (high-speed refrigerated microcentrifuges; Tomy, Tokyo, Japan) for 10 min at 2100 *g* at 4°C. The supernatant, which contained the drug was saved for future analysis and the precipitate was discarded. Peritoneal samples were diluted 1:3 with water and 160 µl was pipetted into Eppendorf tubes. After addition of 20 µl bromouridine (100 µg/ml) and 20 µl 50.0% perchloric acid, all samples were prepared in the same way as the plasma.

The assay utilized a gradient mobile phase system. Mobile phase A consisted of 25 mmol/l ammonium phosphate, and mobile phase B of consisted 25 mmol/l ammonium phosphate with 10% (v/v) acetonitrile. HPLC was performed at 15°C with a flow-rate of 1 ml/min. The gradient was run with 100% mobile phase A for the first 3 min, then a linear gradient to 100% mobile phase B over the next 3 min. The system was then held for 5 min with 100% mobile phase B and finally returned to 100% mobile phase A over 1 min. The HPLC apparatus was then allowed to equilibrate for 20 min in mobile phase A before making the next injection. Injection volumes were 20 µl with a flow rate of 1 ml/min and column eluates was monitored at 270 nm. The retention time for FUDR was 6–7 min and the retention time for bromouridine, the internal standard, was 10 min. Pharmacokinetic parameters were calculated by moment analysis with the computer program MOMENT (EXECL) using Microsoft Excel.

MTT assay

To examine the time and dose effect of FUDR on MKN45 cells, a modified tetrazolium salt (MTT) assay was performed. Cells were seeded into 96-well plates at a density designed to reach 70–80% confluency at the time of assay. At 24 h after seeding, cells were treated with various concentrations of FUDR. After 0.5, 1, 2, 3, 4, 24, 48, 72, 120, and 168 h of treatment, the tetrazolium salt was added at a concentration of 0.3 mg/ml to each well, and the plate was incubated for 4 h at 37°C. The medium was removed, and the MTT crystals were solubilized in dimethyl sulfoxide (Nacalai Tesque, Kyoto, Japan). The absorbance of each sample was measured spectrophotometrically at 540 nm using a Spectra microplate reader (Multiskan Labsystems, Helsinki, Finland). The untreated controls were assigned a value of 100%.

Treatments

On the basis of phase I/II FUDR i.p. trials, bolus i.p. treatments were given once daily for three consecutive days [10, 11,12, 13]. Through a small midline abdominal incision, each group received a bolus i.p. infusion with a weighed amount of FUDR in 1 ml of saline solution. For the next 2 days, the drugs were infused through a 27-gauge needle. For continuous i.p. treatment, a total of 20 g/kg of several percent FUDR-loaded microspheres in 1 ml saline solution was injected through a small midline abdominal incision. No further treatment was carried out.

Maximally tolerated doses

To determine the respective maximally tolerated doses (MTDs) of bolus i.p. infusion and continuous i.p. infusion, dose escalation studies were completed with three nontumor-bearing nude mice entered at each dose level. Toxicity was monitored by daily weighing over 28 days for a total of seven doses. Dose-limiting toxicity was determined as a weight loss of greater than 20% compared with before treatment. Where this was observed, the next lower dose levels were defined as the MTDs for treatment of tumor-bearing animals.

Tumor xenograft model

Gastric cancer cells (MKN45) were grown in cell culture as described above. The tumors were harvested by brief exposure to 0.25% trypsin/1 m*M* EDTA solution. A suspension of 2×10^6 tumor cells in 1.0 ml RPMI 1640 was injected into the peritoneal cavity of nude mice through a 27-gauge needle.

Effects in xenograft model

To evaluate whether continuous i.p. FUDR treatment is a clinically promising therapeutic approach compared with bolus i.p. treatment, we compared the efficacy of these treatments in tumor-bearing nude mice at each MTD. A comparison of the efficacies of continuous i.p. treatment and bolus i.p. treatment against xenografts was also performed at an equivalent dose. One week after injection of tumor cells, nude mice were randomly divided into the following five i.p. treatment groups: (1) control group treated with 1.0 ml saline once daily for three consecutive days; (2) group treated with FUDR-unloaded PLGA microspheres; (3) continuous treatment group treated with FUDR-loaded PLGA microspheres at the MTD; (4) bolus group treated with FUDR solution at a dose equivalent to that used in group 3 (divided into daily doses) once daily for three consecutive days; and (5) bolus group treated with FUDR solution at the MTD once daily for three consecutive days. For each experiment there were five mice per group. Peritoneal tumors were determined at the end of the experiments (28 days after tumor cell inoculation). In vivo efficacy was assessed by comparing the weight of peritoneal tumors between the treatment and control groups.

Statistical analysis

One-way factorial ANOVA and multiple comparison tests (Scheffe and Bonferroni/Dunn) on StatView for Macintosh version 5.0 were used to compare the different treatment groups of tumor-bearing mice.

Results

Morphology and drug content of FUDR-loaded microspheres, and drug release

SEM showed that the microspheres were spherical and showed no aggregation (a representative micrograph is

shown in Fig 1). The mean size of the microspheres was 218 μm (range 50–750 μm). An encapsulation efficiency of 50–80% was achieved in all the preparations (Table 1). In vitro studies showed that FUDR was released slowly from the microspheres (Fig 2). After an initial burst of FUDR release over the first day (approximately 20% of the encapsulated drug), the release rate decreased to 5% per day for the next 2 weeks. On increasing the theoretical drug loading from 0.001% to 1.0%, no significant differences in morphology, encapsulation efficiency, or drug release were detectable.

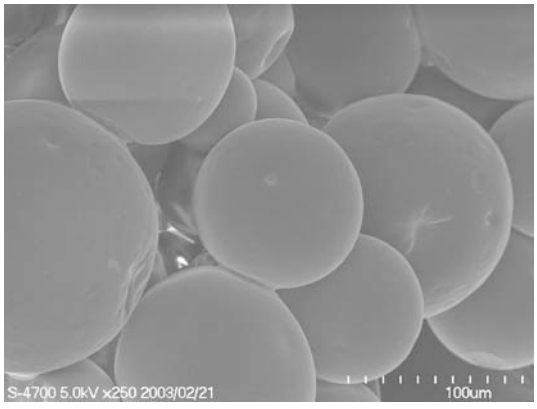

Fig. 1 Representative scanning electron microscope (SEM) micrograph of the FUDR-loaded microspheres

Table 1 Characteristics of FUDR-loaded microspheres

Theoretical drug content (%)	Particle size (μm)		Recovery rate (%)		Encapsulation efficiency (%)	
	Mean	SD	Mean	SD	Mean	SD
0.001	219.4	135.5	67.0	4.8	65.7	8.1
0.01	221.8	134.8	65.0	3.8	64.8	6.5
0.1	218.5	140.3	64.9	3.9	64.9	10.1
1.0	213.0	138.5	68.1	5.2	66.6	5.1

Pharmacokinetic studies

Peritoneal and plasma concentrations of FUDR from 0.5 h to 4 h after bolus i.p. administration (4 mg/5 ml per rat) are shown in Fig 3A. Peritoneal concentrations of FUDR declined rapidly. The terminal half-life in peritoneal fluid was 45 min. Peritoneal concentrations were near the limit of detection (< 1 μg/ml) at 4 h after administration. The $AUC_{0-4\ h}$ values in peritoneal fluid and plasma were 734.0 and 20.2 μ g/ml·h, respectively. Peritoneal and plasma concentrations of FUDR from 1 to 18 days after FUDR PLGA microsphere administration (4 mg/5 ml per rat) are shown in Fig 3B. The peritoneal level was found to be persistent at approximately 5- to 50-fold higher than that in plasma throughout the time-course. Thus, the decay half-life was not measurable during the experimental period. The $AUC_{0-18\ day}$ values in peritoneal fluid and plasma were 4652.4 and 597.6 μg/ml·h, respectively.

MTT assay

We analyzed the effects of FUDR on gastric cancer cells in vitro. The concentration-response curves of FUDR with MKN45 at various exposure times are shown in Fig 4. The cytotoxicity of FUDR against MKN45 cells was concentration-dependent. However, when comparing the curves at various times there was an obvious downward shift, indicating that exposure time had a clear influence on the cytotoxicity of FUDR.

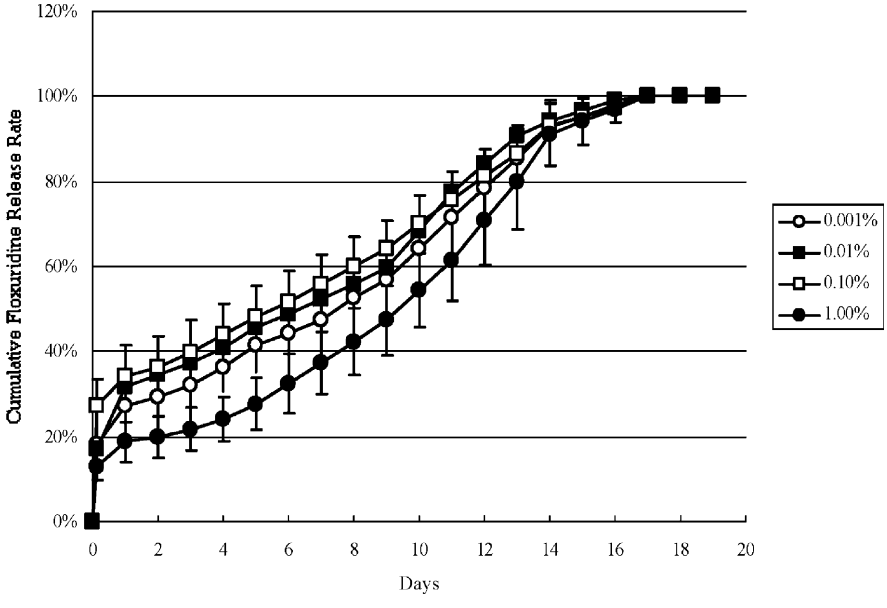

Fig. 2 Release profiles of FUDR from PLGA microspheres. The values presented are means ± SD, $n = 5$

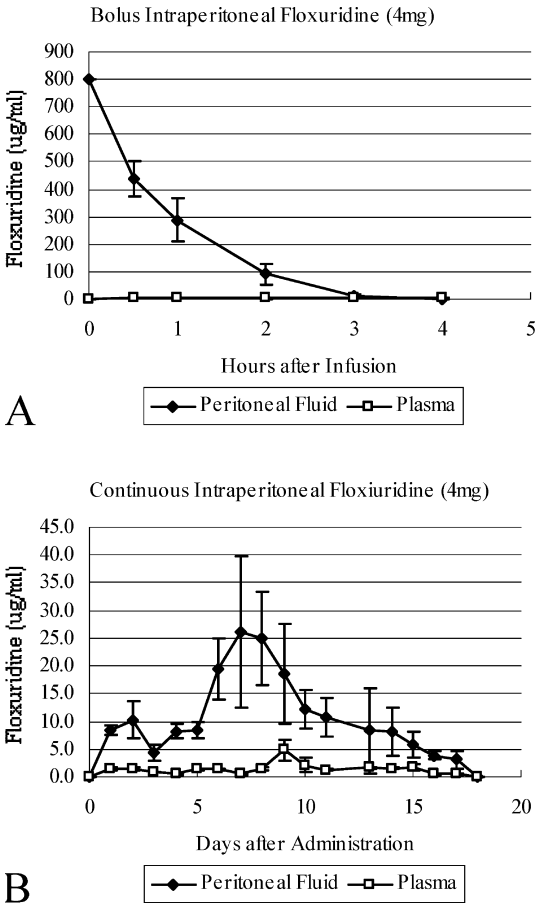

Fig. 3A, B Peritoneal fluid and plasma FUDR concentrations (means ± SD) in healthy male F344 JCL rats receiving (**A**) bolus i.p. FUDR 4 mg or (**B**) a total dose of 1000 mg 0.4% FUDR-loaded microspheres

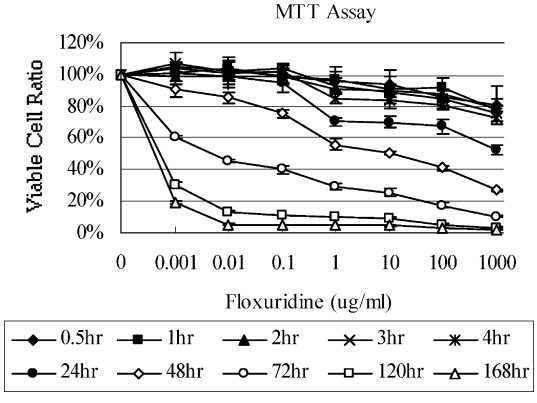

Fig. 4 Time and dose effect of FUDR on MKN45 cells. Cell viability was determined by MTT assay. Results are presented as cell viability (means ± SD, $n = 6$) in relation to that of untreated control cells taken as 100%

Maximally tolerated dose

Mice in each group were weighed throughout the study period, and the incidences of weight loss greater than 20% compared with before treatment are shown in Table 2. There was no weight loss greater than 20% at 600 mg/kg (200 mg/kg daily) in the bolus FUDR treatment group. However, with continuous FUDR treatment using FUDR-loaded PLGA microspheres, weight loss of greater than 20% was observed at 1.5 mg/kg, indicating that continuous FUDR treatment was extremely toxic. Reduced food consumption and diarrhea occurred in mice that had lost more than 20% of their pretrial weight in both treatment groups. The dose-limiting toxic effect of i.p. FUDR was therefore primarily gastrointestinal toxicity. From the data shown, we determined that the MTDs of the i.p. treatments for tumor-bearing mice were 600 mg/kg (200 mg/kg daily) and 1.0 mg/kg, respectively.

Effects in xenograft model

Animals bearing MKN45 tumors were randomly divided into five i.p. treatment groups: (1) control group treated with 1.0 ml saline once daily for three consecutive days; (2) group treated with FUDR-unloaded PLGA microspheres (16.1 g/kg); (3) group treated with continuous FUDR (1.0 mg/kg) with 0.0062% FUDR-loaded PLGA microspheres (16.1 g/kg); (4) bolus group treated with FUDR (1.0 mg/kg) solution divided into daily doses once daily for three consecutive days; and (5) bolus group treated with FUDR (200 mg/kg) solution once daily for three consecutive days. These doses were chosen on the basis of the MTD studies described above. These doses caused no signs of toxicity in the tumor xenograft models.

The effects of treatment schedule on the antitumor activity of FUDR in MKN45 tumor-bearing mice are shown in Fig 5. There was no difference in tumor weight between the group treated with FUDR-unloaded PLGA microspheres and the control group, indicating that PLGA microspheres themselves have no impact on tumor growth. Bolus FUDR treatment at 1 mg/kg did not reduce tumor growth, but continuous treatment at an equivalent dose using FUDR-loaded PLGA microspheres did reduce tumor growth. This difference was significant. At equivalent doses, continuous i.p. FUDR therapy using PLGA microspheres was therefore more effective against the MKN45 peritoneal metastases model than conventional bolus i.p. treatment. However, there was no significant difference in tumor weight between continuous i.p. treatment and bolus i.p. treat-

Table 2 Incidences of a weight loss of greater than 20% compared with before treatment

Bolus	Dose (mg/kg)	0	150	300	600	900	1200	1500
	Incidence	0 (3)	0 (3)	0 (3)	0 (3)	1 (3)	3 (3)	3 (3)
Continuous	Dose (mg/kg)	0	0.1	0.25	0.5	1	1.5	3
	Incidence	0 (3)	0 (3)	0 (3)	0 (3)	0 (3)	2 (3)	3 (3)

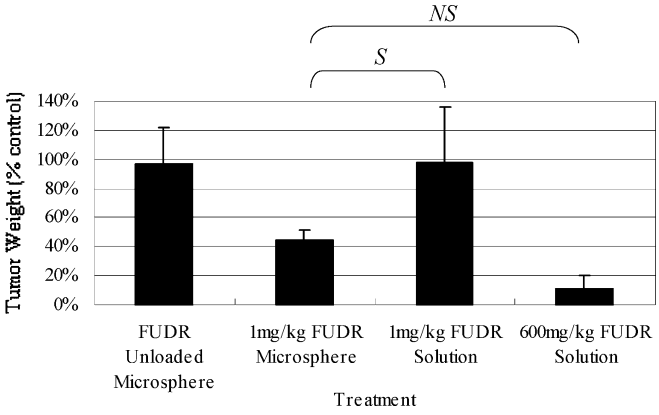

Fig. 5 Effects of treatment schedule on the antitumor activity of FUDR on growth of human gastric cancer xenografts in athymic mice. Each column represents tumor weight (mean ± SD, $n = 6$) in relation to that of control tumors taken as 100%. One-way factorial ANOVA and multiple comparison tests (Scheffe and Bonferroni/Dunn) were used to compare the different treatment groups of tumor-bearing mice (*S* significant, *NS* not significant)

ment at their MTDs of 1.0 mg/kg and 600 mg/kg, respectively. Both treatments at their MTDs were very active in inhibiting peritoneal tumor growth.

Discussion

In the studies reported here, continuous i.p. infusion of FUDR was compared with bolus i.p. administration. Continuous infusion of chemotherapy is a promising development in the attempt to improve the treatment of metastatic cancer [18]. There is a sound theoretical rationale for delivering cytotoxic drugs as a continuous infusion. Drugs especially suitable for continuous infusion are those that have a short half-life and a specific cell cycle effect. By continuously exposing the tumor to these drugs, tumor cells are more likely to be killed. Another advantage of a continuous infusion schedule is that a steady-state plasma level can be reached more easily, which reduces the risk of toxicity related to peak plasma levels of cytostatic drugs. One of the major breakthroughs that has encouraged the increased interest in continuous infusion schedules is the development of implanted subcutaneous port catheters and portable infusion pumps. Several authors have reported their experience with the use of implanted subcutaneous port catheters and the administration of i.p. chemotherapy [19]. However, the ability to deliver drugs into the abdominal cavity on a continuous basis has frequently been frustrated by the inadequacy of drug distribution with the use of portable infusion pumps. As any advantage of i.p. administration over systemic delivery of the agent relies on free-surface diffusion of the drug into tumor cells, it is critical that the drug actually reaches the tumor site within the peritoneal cavity. In most patients a treatment volume of two or more liters should be employed [20].

The FUDR delivery system in this study was in the form of injectable microspheres intended for i.p. injection. This forms a controlled-release implant that releases the drug locally at the site of action. In vitro studies showed that this formulation released FUDR at a rate of approximately 5% of the total encapsulated drug per day (Fig 2). In in vivo studies, the peritoneal level was found to persist and was approximately 5- to 50-fold higher than that of plasma for more than 2 weeks following a single injection (Fig 3B). The therapeutic efficacy of continuous FUDR in MKN45 tumors was documented both in vitro and in vivo. Our MTT assay showed that exposure time had a clear influence on the cytotoxic potency of FUDR. This finding is consistent with a previous report that the sustained exposure of tumor cells to low concentrations of FUDR may be more efficacious than shorter exposure to higher concentrations [21]. The experiment compared a single i.p. administration of FUDR-loaded microspheres to equivalent dosing with conventional bolus i.p. FUDR in terms of their ability to inhibit the development of peritoneal metastases in cells implanted in the peritoneal cavity of nude mice. The FUDR-loaded microspheres caused a sustained, significantly greater reduction in tumor growth than the conventional bolus therapy. However, the goal of the studies was to determine whether continuous i.p. infusion is superior to bolus i.p. administration in terms of objective therapeutic results. Compared with bolus administration, toxicity was increased, resulting in a reduced MTD with the continuous schedule. The dose-limiting toxicity of i.p. FUDR was gastrointestinal. When the treatment was carried out at each MTD, the continuous schedule had no advantage in inhibiting tumor growth.

To our knowledge, no clinical or preclinical studies have been performed on continuous i.p. infusion of FUDR compared with bolus i.p. administration. Clinical studies of intravenous (i.v.) FUDR treatment have indicated that continuous infusion of FUDR is significantly inferior to bolus administration. Several phase I studies have indicated the necessity to reduce the dose of FUDR when the infusion period is increased. Sullivan and Miller have reported that a 24-h continuous infusion schedule with a dose of 3 mg/kg daily for 5 days produces the same toxicity as bolus administration of 30 mg/kg daily for 5 days [22]. Lokich et al. have reported that the recommended daily dose rate for continuous-infusion schedules of 14 days is 0.15 mg/kg daily [23]. Dose-limiting toxicity consisted mainly of diarrhea and stomatitis. A randomized controlled trial has shown that 24-h continuous i.v. infusion is significantly inferior to bolus injection with response rates of 6% and 17%, respectively [24]. This study also showed that each schedule of administration produced its own distinctive toxicity pattern. The predominant toxicity was stomatitis for continuous infusion and leucopenia for bolus administration. What is remarkable is that if the treatment period was increased, the FUDR dose needed to be reduced due to toxicity. This is in contrast

to continuous i.v. infusion of 5-FU, which is closely related to FUDR. It is well known that continuous infusion allows higher doses of 5-FU, produces higher tumor response and achieves a slight increase in overall survival compared to rapid bolus administration [25].

Due to the higher toxicity, the higher costs and the disappointing results with continuous i.v. infusion of FUDR compared to 5-FU, this drug has not been used extensively. However, because of superior properties for hepatic arterial (HA) infusion, FUDR has been investigated in patients with unresectable liver metastases from colorectal cancer. Ensminger et al. have reported that FUDR has a 94% to 99% extraction rate by the liver during the first pass, making it an ideal agent for HA directed therapy [26]. A meta-analysis combining the results of seven randomized controlled trials supports the use of continuous HA infusion of FUDR in the treatment of unresectable liver metastases from colorectal cancer [27]. A significantly better local response rate of 41% was achieved with HA infusion of FUDR compared with a 14% response rate for systemic chemotherapy. The therapeutic dosage schedules of HA infusion of FUDR were continuous administration at 0.1 to 0.3 mg/kg daily for 14 days every 28 days.

In two recently published randomized controlled trials, the potential benefit of continuous HA infusion of FUDR (0.05 to 0.25 mg/kg daily for 14 days every 28 days, four to six cycles) combined with systemic chemotherapy as adjuvant treatment after resection of colorectal liver metastases has been evaluated [28, 29]. These studies demonstrated that continuous HA infusion of FUDR associated with systemic chemotherapy can reduce the risk of recurrence after surgery for liver metastases. To our knowledge, no clinical or preclinical studies have been performed on continuous HA infusion of FUDR compared with bolus HA administration. However, because of good results in patients with hepatic metastases from colorectal cancer of continuous HA infusion of FUDR, we undertook this study to test the efficacy of continuous i.p. infusion of FUDR compared with bolus i.p. administration against peritoneal micrometastases from gastric cancer.

Our study demonstrated that the continuous schedule was more effective for peritoneal tumor than bolus i.p. treatment at an equivalent dose. From the standpoint of objective therapeutic results, however, continuous i.p. therapy had no advantages over bolus injection. Owing to the higher toxicity and the equal efficacy of continuous infusion compared with bolus administration, this schedule is not recommended for i.p. FUDR treatment.

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