Cancer Chemother Pharmacol (1995) 36: 27–34

ORIGINAL ARTICLE

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Antitumor effect of intratumoral administration of fluorouracil/epinephrine injectable gel in C3H mice

Received: 19 May 1994/Accepted: 22 September 1994

Abstract Fluorouracil/epinephrine injectable gel (5-FU/epi gel) was evaluated in vitro for its drug-release profile characteristics and in a mouse tumor model for its antitumor effectiveness. In vitro chemosensitivity studies with 5-FU in RIF-1 fibrosarcoma cells showed less than 1 log of cell kill at 1 m*M* after 2 h of exposure. Increasing the exposure time to 24 h resulted in greater cell killing (~ 2.5 log cell kill at 0.5 m*M*), suggesting that sustained drug levels in tumors would result in an increased efficacy outcome in vivo. A 5-FU/epi injectable gel was designed, providing drug release in vitro of 50% by ~ 4 h and of 80% by 24 h. The retention of 5-FU in RIF-1 mouse tumors was determined after intratumoral administration of 5-FU/epi gel or various combinations of the formulation components. Area-under-the-curve ($AUC_{0-24\ h}$) calculations resulted in an AUC value of 146.4 % h for the 5-FU/epi gel formulation as compared with 45.7 % h for 5-FU solution. Tumor growth was significantly delayed ($P < 0.05$) with the 5-FU/epi gel (60 mg/kg) as compared with 5-FU solution given intratumorally or systemically. A fluorouracil dose of 150 mg/kg in the 5-FU/epi gel given weekly for 13 weeks was not lethally toxic, whereas the same dose given as drug solution was 100% lethal, suggesting that the therapeutic index for 5-FU in the gel formulation may be much greater than that for aqueous drug solution delivered intratumorally.

Key words Fluorouracil · Intratumoral injection · In vivo efficacy

Introduction

The narrow therapeutic index of most cancer chemotherapeutic agents used systemically has limited their potential clinical application despite the benefits that have been identified for these agents in preclinical laboratory research. In the clinic, the ability to achieve an antitumor response is compromised by acute toxicity of anticancer agents to other rapidly dividing tissues, such as bone marrow and gastrointestinal mucosa. Dose-limiting normal-tissue toxicities often subvert the achievement of maximal drug concentrations in the tumor cells. This may be especially true for solid tumors, where the blood flow and diffusion of drug affect tumor drug concentrations [4, 25]. Furthermore, for many therapeutic agents, maintenance of cellular drug exposure is important to maximize the cell-killing potential either for cell-cycle-specific drugs or for those agents for which accumulation of damage to cellular targets is important. In an effort to increase and maintain chemotherapeutic drugs in tumors and to spare exposure to sensitive normal tissues, researchers have developed a variety of drug-delivery systems designed to improve the therapeutic index. Such delivery systems include liposomes [6, 20, 36], drug-containing microparticles [41], chemoembolic materials used intra-arterially [24, 26], monoclonal antibodies or antibody fragments coupled with therapeutic agents [1, 10, 13, 21], and implantable synthetic polymers [14, 40]. Most of these strategies have focused on systemic or regional delivery of the therapeutic agent in an effort to control both local and metastatic disease.

Local control of tumors is a primary clinical objective and is usually achieved by surgery or radiation. However, local control is currently limited for some types of cancer, including head and neck, liver, prostate, and brain tumors, because of the inability to resect the tumor completely or because the normal tissue is sensitive to radiation. Since these tumors can be accessed directly or with the assistance of ultrasound or computerized tomography, the potential exists for direct administration of drug to the tumor. Local drug intervention could be used either in combination with

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radiation or hyperthermia preoperatively to shrink the tumor mass before resection, as a stand-alone therapy for nonresectable and radio-resistant lesions, or in combination with systemic chemotherapy protocols to attack a nonresponsive lesion selectively. Administration of various drug solutions directly into superficial tumors has met with some limited success in the treatment of cutaneous lesions, including keratoacanthoma [16], basal-cell carcinoma [18, 30], verruca plantaris [22, 28], condyloma acuminatum [12, 15, 43], and Kaposi's sarcoma [5]. These investigations have typically focused on injection of aqueous drug solutions into the lesion site; it is likely that the potential for this delivery methodology has been limited by rapid diffusion of drug out of the diseased tissue following administration.

To improve the efficacy of local chemotherapy, we developed a sustained-release drug-delivery system for direct intratumoral administration. Bovine collagen is used as a gellant to retard the rate of drug clearance from the site of injection in the diseased tissue and, thereby, to maintain longer drug exposure to tumor cells. Collagen is biocompatible and biodegradable and has previously been used in several forms in a variety of medical devices [8, 9, 19, 32, 38]. A vasoactive agent, epinephrine (epi), is also added in the gel formulation to enhance further the therapeutic effect by delaying drug clearance via the local vasculature. Preclinical and clinical studies are currently under way to deliver fluorouracil (5-FU) [23, 33, 34], cisplatin [2, 23, 27, 29, 44], methotrexate [33], and vinblastine [39] in this gel system. In the studies described herein, we evaluated the ability of 5-FU/epi gel to enhance drug retention and antitumor efficacy in an in vivo tumor model in comparison with aqueous drug solution. We decided to use 5-FU because it is a cell-cycle-specific drug with a short half-life in plasma [31, 35, 37], has an in vitro chemosensitivity concentration requirement of $> 100\ \mu M$ [11], and is a common drug in the management of many cancers.

Materials and methods

5-FU/epi injectable gel

The 5-FU/epi gel is a viscous, injectable gel containing 5-FU and epi as active agents, with a purified bovine collagen serving as the gellant and various pharmaceutically acceptable salts acting as buffering and osmotic agents. The components of this 5-FU/epi gel were obtained from the following sources: 5-FU solution (50 mg/ml), Quad Pharmaceuticals, Inc. (Indianapolis, Ind.), epi solution (1:1,000, v/v) Parke-Davis (Morris Plains, N.J.); collagen gel (6.5%), Koken Co., Ltd. (Tokyo, Japan); and 0.9% NaCl solution, Abbott Laboratories (North Chicago, Ill.). In the studies in which tritium-labeled 5-FU was used as a tracer, the 5-FU stock solution consisted of both tritium-labeled 5-FU (5-[6-^{3}H]-fluorouracil, 19.2 Ci/mmol; Du Pont, NEN Research Products, Boston, Mass.) and unlabeled 5-FU solution.

Formulation

The 5-FU/epi gel mixture was prepared immediately before use to yield the following component concentrations: 5-FU, 30 mg/ml; epi, 0.1 mg/ml; and collagen, 20 mg/ml. Briefly, 0.6 ml of the 5-FU solution was combined with 0.1 ml of the epi solution in a 1-ml Luer-lock syringe, then the syringe was attached with a connector unit to a second 1-ml syringe containing 0.3 ml of collagen gel. The materials in both syringes were transferred back and forth until they became homogeneous (~ 30 s). Other formulations (5-FU gel, 5-FU/epi solution, 5-FU solution) were also prepared similarly by substituting 0.9% NaCl solution for the missing component(s).

In vitro release profile

Triplicate 100-µl aliquots of the tritiated gel formulation were placed into the bottom of 1.5-ml microcentrifuge tubes. At time zero, 0.9 ml of 0.9% NaCl solution was carefully layered over each aliquot. The centrifuge tube was capped and incubated at 37 °C. At designated time points (0, 0.5, 1, 2, 4, 6, and 24 h), a 20-µl aliquot of the supernatant (which was gently mixed before sampling) was removed for liquid-scintillation-counter analysis, and 20 µl of fresh 0.9% NaCl solution at 37 °C was added back to the test tube, which was returned to the incubator until the next sampling time. The 100-µl gel formulation remained as a pellet in the bottom of the test tube throughout the study. On addition of the scintillation fluid, the radioactivity of each sample was counted for 5 min (Beckman liquid scintillation counter LS 3801). The data, representing percentages of release of the initial dose, are expressed as mean values ± standard deviation (SD).

In vitro chemosensitivity as determined by clonogenic assay

RIF-1 fibrosarcoma cells [42] maintained in vitro (Waymouth medium with 20% fetal bovine serum) at 37 °C in a humidified incubator containing 5% CO_2 were harvested and plated overnight in triplicate 100- × 20-mm petri dishes for cell attachment. Appropriate amounts of 5-FU were added to the dishes, resulting in a range of concentrations of up to 1 m*M*. After a 2- or 24-h incubation in the presence of drug, dishes were washed twice with Hanks' balanced salt solutions and fresh medium was added. After 12 days of incubation, all dishes were fixed and stained with crystal violet, and macrocolonies (> 50 cells) were counted.

Mice

Normal C3H/Sed inbred female mice (Massachusetts General Hospital Cancer Center, Boston, Mass.) aged 3–4 months were used. The average body weight was approximately 25 g. Animals were maintained in isolator cages with a diurnal 12-h/12-h light/dark cycle. Food and acidified water (pH 2.6) were available ad libitum.

Tumor implantation

Log-phase RIF-1 cells were treated with trypsin and harvested from cell-culture flasks to yield a concentration of 4×10^6 cells/ml. Tumor cells were injected intradermally in a volume of 50 µl on shaved flank(s) of mice. Approximately 2–3 weeks later, when tumors reached the experimental volume called for in the study protocol, mice were randomized into different treatment groups.

Tumor drug-retention study.

Tumor-bearing mice (with ~ 400-mm^3 tumor volume) were used for this study, and three mice per time point were included in each treatment group. A 1.5- × 1.5-cm template was placed around the tumor, and the area was marked with a permanent ink pen. Tritiated 5-FU/epi gel, 5-FU gel, 5-FU/epi solution, or 5-FU solution (each in 50 µl) was injected into the center of the tumor. At designated time points after the injections (0.33, 0.67, 1, 2, 4, 6, 8, 18, and 24 h), mice were anesthetized and exsanguinated via cardiac puncture. The 1.5- × 1.5-cm designated area of skin including the tumor was excised. The excised skin/tumor samples were solubilized in 2 *N* KOH in MeOH at 37 °C. Blood samples were decolorized by 30% H_2O_2. The blood volume of each animal was calculated as 8% of the body weight at the time of drug administration. The amount of tritium was determined by liquid scintillation counting. Data are expressed as percentages of the initial total dose. The area under the curve ($AUC_{0-24\,h}$) was calculated as the sum of trapezoid areas for each set of data points and was expressed in percent times hours (% h). At time 0, 100% was assumed for the tritium content in tumors and 0 was assumed in blood, and these values were used in the AUC calculations.

Efficacy study

The effect of a single intratumoral administration of 5-FU/epi gel was compared with that of 5-FU gel, 5-FU/epi solution, or 5-FU solution. Each animal was implanted with two tumors, one on each flank. Eight animals were used per group. The tumor volume at the time of treatment was ~ 100 mm^3. One of the two tumors on each mouse was injected intratumorally (i.t.) using a fanning technique, which created several tracks within the tumor tissue to ensure a better drug distribution. The other contralateral tumor served as an untreated control. Another group of animals received intraperitoneal (i.p.) injections of 5-FU solution as a positive control, and both tumors were considered as treated. Also included as a negative control was a group of animals that received no treatment. The administration volume was 50 µl for i.t. injection and 100 µl for i.p. injection. The final 5-FU dose was 1.5 mg/injection, or ~ 60 mg/kg. Tumors were measured three times per week with vernier calipers for length, width, and height. The tumor volume (V, in cubic millimeters) was calculated according to the formula:

$$V = \frac{\pi}{6} \times D_1 \times D_2 \times D_3,$$

where D_1, D_2, and D_3 are three perpendicular diameters measured in millimeters.

The time (in days) required for treated and untreated control tumors to grow to 4 times (4 ×) their initial experimental volume was used as the study endpoint. Data are expressed as mean values ± standard error (SE), and Student's *t*-test was used for statistical analysis.

Toxicity study

Five normal C3H female mice were used in each treatment group. This non-GLP study compared the toxicity of 5-FU/epi gel versus 5-FU solution given once a week for 13 weeks. Doses of 5-FU ranging from 60 to 150 mg/kg per week were evaluated. An intradermal injection was used with a 0.1-ml volume for all doses except the highest dose (150 mg/kg), for which the injection volume was 0.15 ml. Body-weight changes were evaluated and local skin reactions were described as follows: normal, erythema, callous, scab formation, and necrosis. The study duration was approximately 6 months after the last treatment.

Results

In vitro release profile

Figure 1 shows the percentage of radioactive tracer released from collagen gel as a function of time. The release profile determined over 24 h demonstrated that ~ 50% of the 5-FU was slowly released from the gel by 3–4 h and ~ 80% was released by 24 h. The collagen gel remained as a pellet in the test tube throughout the study period. High-performance liquid chromatography (HPLC) analyses [7] for 5-FU produced a similar release profile, indicating that tritium label was associated with intact drug under these in vitro conditions (data not shown). Other drug-release assays that involved slow agitation and sink conditions produced similar release profiles, except that they were obtained over a shorter period. Complete recovery of 5-FU was achieved on repeated washing or complete dissolution of the remaining gel. The lack of residual binding of the drug to the gel demonstrated the compatibility between 5-FU and collagen gel and the complete bioavailability of 5-FU.

In vitro chemosensitivity as determined by clonogenic assay

The clonogenicity of RIF-1 cells after exposure to varying concentrations of 5-FU was measured in vitro (Fig. 2). Less than 1 log (0.4, surviving fraction at 0.25 m*M*) of cell kill was observed after a 2-h drug exposure. Increasing the drug concentration to 1 m*M* did not result in increased cell killing. However, after the 24-h exposure, a similar surviving fraction was obtained with a much lower drug concentration (e.g., 0.1 m*M*), and ~ 2.5 log of cell kill was obtained at

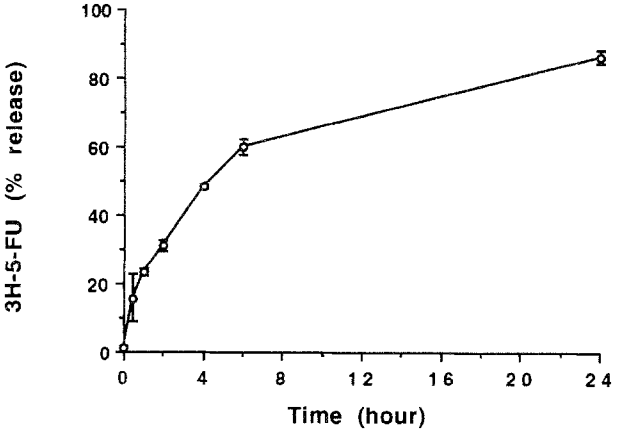

Fig. 1 Release kinetics of [^{3}H]-5-FU from a collagen gel as determined in vitro. Each data point represents the mean value ± SD for triplicate samples. A drug release of 50% was observed by 4 h and ~ 80% by 24 h

0.5 m*M* 5-FU. These results suggested that sustained exposure of tumor cells to drug would enhance the antitumor response in vivo. Our in vitro data correlate well with cell-sensitivity studies for 5-FU with human cell lines [11].

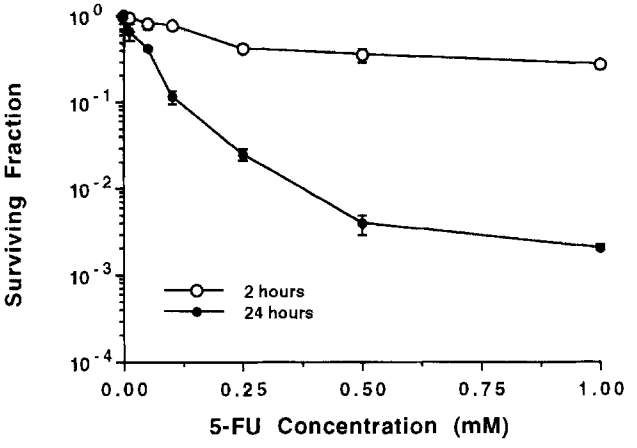

Fig. 2 In vitro RIF-1 cell surviving fraction after 2- and 24-h periods of exposure to 5-FU. Each data point represents the mean value ± SD for triplicate petri dishes. When the drug-exposure time was increased from 2 to 24 h, a 2.5-log (~99%) cell kill was obtained at a 0.5-m*M* 5-FU concentration

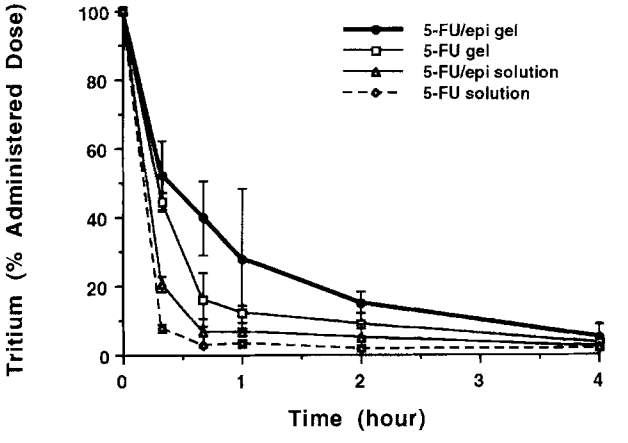

Fig. 3 Tritium retention in RIF-1 tumors after intratumoral injection of [^{3}H]-5-FU in various formulations into C3H mice. Each data point represents the mean value ± SD for three animals

Tumor drug-retention study

After injecting the mouse tumors with 5-FU/epi gel or other formulations with various components, we determined the amount of 5-FU retained locally by measuring the radioactivity remaining at the injection site and surrounding tissue (Fig. 3). Total drug retention in the tumor tissue was lowest for animals treated with 5-FU solution and progressively higher for animals treated with 5-FU/epi solution and 5-FU gel. The 5-FU/epi gel produced the highest levels of tumor drug retention at all time points. The area-under-the-curve ($AUC_{0-24\ h}$) analyses showed a similar ranking, with 5-FU/epi gel providing the greatest AUC value of 146.4 % h, followed by 5-FU gel (96.7 % h), 5-FU/epi solution (68.8 % h), and 5-FU solution (45.7 % h). The contribution of adding epi to 5-FU, expressed as the AUC ratio to 5-FU solution, was 1.51, that of adding collagen gel to the drug was 2.12, and that of adding both collagen gel and epi to the drug was 3.20 (Table 1).

Blood levels of radioactive 5-FU detected after i.t. injection of 5-FU/epi gel or other formulations are shown in Fig. 4. Peak blood levels of 5-FU occurred at 40 min with 5-FU/epi gel (1.5%) and 5-FU gel formulation (2.0%), whereas peak blood levels for 5-FU solution (3.9%) and 5-FU/epi solution (3.4%) occurred at 20 min or earlier. The slightly delayed and slightly lower peaks associated with both gel formulations were likely due to the enhanced local retention of drug by the gel at the administration site in comparison with drug solutions. However, the overall drug exposure in blood, or cumulative AUC values, were similar for all four formulations (Table 1).

In a separate study (data not shown), 5-FU retention in tissue samples after i.t. injection of 5-FU/epi gel was monitored using HPLC. The HPLC results indicate a similar pharmacokinetic profile in tumor drug retention until approximately 4 h. After this time point, the residual tritium levels, including both the parent compound and its metabolites, were higher and persisted longer than the 5-FU levels measured by HPLC. By 24 h, approximately 1%–2% of the drug remained in the tumor as determined in the HPLC assay. Since 5-FU converts to 5-fluorodeoxyuridine monophosphate

Table 1 Tumor and blood content of tritium (AUC) after intratumoral injection of [^{3}H]-5-FU in mice with RIF-1 tumors[a] (*sol'n* – solution)

$AUC_{(0-24\ h)}$	5-FU/epi gel	5-FU gel	5-FU/epi sol'n	5-FU sol'n
Tumor AUC (% h)	146.4	96.7	68.8	45.7
Ratio to 5-FU sol'n	3.20	2.12	1.51	(1.00)
Blood AUC (% h)	23.2	27.7	28.9	30.1
Ratio to 5-FU sol'n	0.77	0.92	0.96	(1.00)

[a] Tritiated 5-FU formulations (in 50 µl) were injected into the center of the tumor (~ 400 mm^3). Three animals were used for each time point, for a total of nine time points (20 and 40 min and 1, 2, 4, 6, 8, 18, and 24 h). The amount of tritium radioactivity was determined by liquid scintillation counting. Data are expressed as percentages of the initial total dose. The AUC value (concentration × time) was calculated as the sum of trapezoid areas for each set of data points and expressed in % h

(FUdMP) in the metabolic pathway so as to inhibit thymidylate synthetase for cell killing, the parent 5-FU concentration measured by HPLC was probably an underestimate.

Efficacy study

In the efficacy study we used mice bearing two tumors, one on each flank, and measured the tumor growth after one of the two tumors received a single i.t. injection of 5-FU/epi gel or formulation components. Intratumoral injection of placebos, e.g., saline, collagen gel alone, or collagen gel containing epi, produced no apparent delay in tumor growth (data not shown). For comparison, we also measured the tumor growth rate after a single i.p. injection of 5-FU solution. The results obtained using the various formulation components are shown in Table 2. Overall, the tumors treated i.t. with the test formulations showed statistically significant ($P < 0.05$) delays in 4× tumor growth as compared with the untreated contralateral tumors.

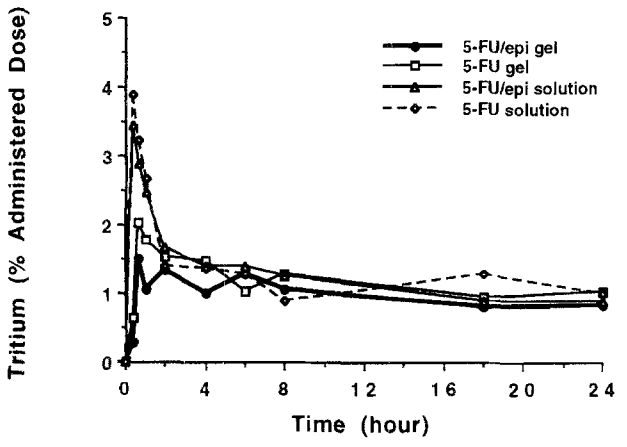

Fig. 4 Tritium content in blood after intratumoral injection of [^{3}H]-5-FU in various formulations into C3H mice. Each data point represents the mean value ± SD for three animals

Treated tumors.

All tumors treated i.t. with the various test formulations showed statistically significant ($P < 0.05$) delays in 4× tumor growth as compared with tumors in the untreated control mice. Tumors treated with formulations that did *not* contain epi [5-FU solution (i.p. or i.t.), 5-FU gel (i.t.)] exhibited similar delays in tumor growth, with the 4× endpoint being reached at an average of 13.5, 13.3, and 13.8 days, respectively. In contrast, tumors treated with formulations that *did* contain epi (5-FU/epi solution, 5-FU/epi gel) displayed further delays in tumor growth, extending the 4× endpoints to 15.3 and 16.8 days, respectively, which were significantly longer ($P < 0.05$) than those obtained with formulations that did not contain epinephrine. Thus, including epi in the formulation improved the therapeutic efficacy as compared with the treatments without epi.

Untreated contralateral tumors.

Untreated contralateral tumor sites for all i.t. treatments had statistically significant ($P < 0.05$) delays in tumor growth as compared with untreated tumors in the control mice. This finding suggests that after one of the two tumors on each mouse had been injected with the drug formulation, the untreated contralateral tumor was exposed to drug through the systemic circulation and, thus, contralateral tumor growth was delayed. However, these delays were significantly shorter than those achieved in the treated tumors. Moreover, the rates of contralateral tumor growth observed in mice treated i.t. with gel formulations (5-FU gel, 5-FU/epi gel) were faster than the corresponding rates attained using formulations without gel (5-FU solution, 5-FU/epi solution), presumably because the gel enhanced the localization of the drug at the administration site and reduced systemic exposure.

Table 2 Effect of 5-FU/epi injectable gel on RIF-1 tumor growth in C3H mice: evaluation of formulation components[a]

Group	Treatment	Number of animals	4× Tumor growth (days ± SE)	
			Treated side	Untreated side
1	Untreated controls	8		5.4 ± 0.2
2	5-FU solution, i.p.	8		13.5 ± 0.4
3	5-FU solution, i.t.	8	13.3 ± 0.6	11.8 ± 0.5
4	5-FU gel, i.t.	8	13.8 ± 0.8	7.6 ± 0.5
5	5-FU/epi gel, i.t.	8	16.8 ± 1.0	8.7 ± 1.1
6	5-FU/epi solution, i.t.	8	15.3 ± 0.5	11.7 ± 0.6

[a] Single doses of 5-FU formulations were injected intraperitoneally (i.p.) or intratumorally (i.t.) into one of the two tumors (located on each flank, approximately 100 mm^3 in size) in C3H female mice. The other contralateral tumor served as an untreated control. The 5-FU dose was 1.5 mg per injection/animal, or approximately 60 mg/kg. The time required for tumors to grow to 4 times (4×) their initial experimental size was used as the study endpoint

Table 3 Toxicities determined at 13 weeks for 5-FU/epi gel and 5-FU solution injected intradermally once a week into normal mouse skin[a]

Group	Treatment	Weekly 5-FU dose (mg/kg)	Cumulative 5-FU dose (mg/kg)	Mortality (%)
1	5-FU solution	60	780	0
2	5-FU solution	80	1040	0
3	5-FU solution	100	1300	0
4	5-FU solution	120	1560	40
5	5-FU solution	150	1950	100
6	5-FU/epi gel	60	780	0
7	5-FU/epi gel	80	1040	0
8	5-FU/epi gel	100	1300	0
9	5-FU/epi gel	120	1560	0
10	5-FU/epi gel	150	1950	0

[a]A total of 13 weekly doses of 5-FU solution or 5-FU/epi gel (100–150 µl/injection) was injected intradermally into normal C3H mouse skin. Five animals were used in each treatment group. The study was terminated approximately 6 months after the last treatment

These results indicate that both epi and collagen gel contribute to the efficacy of the 5-FU/epi gel formulation in producing (a) the greatest growth delay on the treated-site tumors (epinephrine effect) and (b) the least systemic drug availability (gel effect) as demonstrated by the faster growth of contralateral tumors in mice treated with gel formulations.

Toxicity study

Table 3 shows the results of a non-GLP, 13-weekly-injection lethal dose (LD) study in which the toxicity of 5-FU/epi gel was compared with that of 5-FU solution given intradermally to normal mice. All mice treated with 5-FU/epi gel (60–150 mg/kg) survived for > 6 months after the last treatment, at which time the experiment was terminated. For the 5-FU solution group, an LD_{100} was obtained at 150 mg/kg and an LD_{40}, at 120 mg/kg. At lower doses ($\leq$ 100 mg/kg) all animals survived. All mice receiving 150 mg/kg of the 5-FU solution had significant body-weight loss (> 10%) and died before the fifth weekly injection. In the mice treated with 120 mg/kg of the 5-FU solution, no significant body-weight loss was observed, and death occurred near the end of the treatment regimen.

Local cutaneous toxicity was evaluated and scored throughout the study. No skin reaction occurred in mice treated with free 5-FU solution at any of the doses tested. Injection sites of animals treated with 5-FU/epi gel showed dose-related skin reactions. Scabbing confined to the injection site developed during the treatment period (day 0 to day 84) but healed progressively during the recovery period until the study ended at 6 months. No necrosis was observed.

Discussion

The potential of using collagen gel as a biologically compatible drug carrier for the i.t. administration of cancer chemotherapeutics, such as fluorouracil, cisplatin, and vinblastine, has been established in animal models [27, 32, 39, 44] and human clinical trials [34]. Drug delivered in collagen gel enhanced the antitumor response in subcutaneously (s.c.) grown syngeneic experimental fibrosarcomas as compared with i.p. or i.t. administration of aqueous drug solution. Furthermore, the normal-tissue toxicities encountered with this drug-delivery system were much less frequent than those encountered with drug solutions given i.t., i.p., or intracranially. From these reports, it has been shown that the gel formulation retains the drug within the tumor tissue for several hours, resulting in an improved antitumor response, less systemic drug exposure, and a reduction of normal-tissue toxicities.

The efficiency of delivering drug to tumors through systemic administration depends on many factors. For example, drug molecules must first cross the blood vessel to enter the interstitial space in the tumor, where the fluid pressure is probably higher; the vasculature in the tumor tissue usually presents a heterogeneous blood supply, resulting in variable drug distribution; the distance that the drug molecules travel once they are in the interstitial space is often limited by their size; and, finally, the drug molecules have to enter the tumor cells and reach the cellular targets to act. The concentration of drug at the cellular level is the determining factor for cell killing. Another disadvantage of systemic chemotherapy for solid tumors is the typically rapid clearance of drug from the vascular circulation, which reduces the drug availability to the tumor. Furthermore, normal-tissue sensitivity or toxicity is a major limiting factor in achieving adequate drug dosing and delivery.

Clinically, it has not been feasible to achieve and maintain the 5-FU concentration at the therapeutic dose (e.g., > 100 µ*M* as indicated in vitro [11]) in plasma or tumor for any extended period. Because tumor drug concentrations are not readily measurable in patients, comparisons must be based on the plasma concentrations. Single i.v. bolus injection to humans with 8.5–12 mg/kg of 5-FU ($\sim$ 700- to 800-mg total dose) resulted in a mean peak plasma concentration of 61 µg/ml, or close to 0.5 m*M*, at 5 min after the injection [31]. However, the mean half-life was 11.4 min, and the drug was essentially cleared from the plasma in 1 h. By i.v. continuous infusion (400–500 mg/m^2 per day), it was possible to maintain 5-FU levels at 1–1.5 ng/ml or 7.7–11.5 n*M*, in plasma for up to 30 days [35]. However, these levels were unlikely to produce a significant antitumor response. For example, regional therapy by subconjunctival administration (intended to increase the exposure of tumors to drug)

has shown only a moderate improvement in the antitumor response [35].

In the mouse tumor model, the tumor drug-retention study (Table 1, Fig. 3) using the i.t. 5-FU/epi gel demonstrated the feasibility and potential of this drug-delivery system in solid tumor management. When the 5-FU/epi gel is used to deliver 5-FU directly into the tumor, 5-FU diffuses from the gel matrix over several hours and enters the surrounding tumor tissue, i.e., the interstitial space. This step bypasses some of the above-described issues related to systemic chemotherapy. Although the drug concentration at the cellular level was not measured in this study and the majority of the drug was not measurable in the tumor tissue by 4 h, the data showed that the concentration/residence time of the drug in the remaining gel and interstitial space in the tumor was highest for the 5-FU/epi gel formulation than for other formulations given i.t. at an equivalent drug dose. Intraperitoneal administration of 5-FU solution produced the least drug retention in the tumors (data not shown) as compared with i.t. administration of 5-FU solution.

Although the measurement of tritium label in vivo does not necessarily represent the exact amount of intact drug remaining and the concentrations measured do not necessarily represent *cellular* drug levels, the 5-FU/epi gel clearly retained the drug at the site of the injection and provided protracted exposure of the tumor tissue to previously unachievable drug levels in vivo.

In terms of the antitumor response obtained using the growth-delay efficacy model, the results were less pronounced. This could be due to the nature of the murine tumor system, and fibrosarcoma may inherently be more resistant to drug than other tumor types. RIF-1 is a fast-growing tumor with a volume-doubling time of ~ 3 days. Thus, it was more difficult to differentiate quantitatively the component contribution effects that lasted < 24 h in an equidose evaluation model with a late-stage study endpoint of tumor growth delay. Nevertheless, the antitumor effect was most pronounced in the tumors treated with 5-FU/epi-gel, which also resulted in the most localized effect. Faster tumor growth to $4\times$ the initial tumor volume on the untreated side is demonstrated when less drug is available in the systemic circulation.

Because the drug was injected directly into the lesion and because the drug was retained much longer at the injection site with the gel system, the general normal-tissue toxicity caused by this treatment was minimal. When the acute systemic toxicity was evaluated at an equidose level after a total of 13 weekly administrations of 5-FU, the 5-FU/epi gel was much less toxic than drug solution (Table 3). As expected, the local effects of the 5-FU in the gel system were somewhat greater than those of the simple solution; local skin reactions were observed with the 5-FU/epi gel treatments, for example, erythema and scabbing, that were related to the drug dose (data not shown). However, on termination of the treatment at the end of week 13, healing of the skin reactions was noticeable and complete within a few weeks. Again, skin reactions were expected because most of the drug was retained at the injection site for at least a short time. Local cutaneous reactions after treatment with 5-FU have been observed in our work [33, 34] and that of other investigators [3, 17].

The feasibility of the intralesional drug-delivery technology has been examined in 173 veterinary patients (cats, dogs, and horses) with 434 spontaneous and accessible skin and s.c. tumors [27, 33]. The most frequent tumor types were squamous-cell carcinoma, oral melanoma; sarcoid, eosinophilic granuloma and fibrosarcoma. Animals had recurrent disease or disease with a poor prognosis, and many had undergone unsuccessful ablative procedures or radiation therapy. Either single- or multiple-drug regimens were used. Some patients were treated adjunctively with surgery, radiation, or hyperthermia. These studies demonstrated the clinical utility of seven different chemotherapeutic drugs: 5-FU, methotrexate, cisplatin, vinblastine, carmustine, bleomycin, and triamcinolone. Positive clinical responses ($\geq 50\%$ tumor-volume decrease) occurred in 79 % (342/434) of tumors, and 51 % (217/434) had a complete clinical resolution. A total of 2021 treatments were given to these 434 tumors and all treatments were well tolerated. Acute local reactions were observed in $< 2\%$ (33/2021) of the treatments. Systemic reactions, potentially related to treatment, were noted in six animals. As a result of these promising preclinical and veterinary studies, the 5-FU/epi gel is under evaluation in humans for treatment of cutaneous diseases such as basal-cell carcinoma [34] and condylomata acuminata and of deeper tumors, including prostate and liver tumors.

In conclusion, using collagen as a drug carrier for intralesional chemotherapy is feasible. It can retain the therapeutic dose at the lesion site for sustained periods and, thus, improves response rates while sparing normal-tissue exposure. The injectable gel is both biocompatible and biodegradable. This drug-delivery system permits repeated administration to any lesion that is accessible by needle. Furthermore, a broad range of chemotherapeutic agents have been shown to be compatible, suggesting that this gel system can be used as a single agent alone or that it can be combined with other drugs or other forms of therapy to enhance local tumor control.

Acknowledgements. We thank Joe Palecek and Anny Wong for their excellent technical assistance and Luanne Porter and Caren Rickhoff for their help in preparing the manuscript.

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