Cancer Chemother Pharmacol (1999) 44: 267–274

ORIGINAL ARTICLE

Jill P. Smith · Sarathchandra Kanekal
Montesa B. Patawaran · Joy Y. Chen
Richard E. Jones · Elaine K. Orenberg · Ning Y. Yu

Drug retention and distribution after intratumoral chemotherapy with fluorouracil/epinephrine injectable gel in human pancreatic cancer xenografts

Received: 24 July 1998 / Accepted: 29 January 1999

Abstract *Purpose*: Pancreatic cancer is widespread, associated with high mortality, and rapidly fatal. Most cases are diagnosed too late for surgical treatment, and the disease responds poorly to systemic chemotherapy. Nevertheless, pancreatic cancer cells are sensitive to fluorouracil (5-FU) in a time- and dose-dependent manner, suggesting that improved retention of drug in the tumor may improve patient prognosis. In this study, we evaluated a novel drug delivery system, 5-FU/epinephrine injectable gel (5-FU/epi gel), designed to improve drug retention in tumors. *Methods*: We used a BxPC-3 human pancreatic cancer xenograft model in athymic mice to examine drug levels in tumor, liver, and kidney tissue following administration of: (a) 5-FU/epi gel (30 mg 5-FU/ml) intratumorally (i.t.); (b) 5-FU solution i.t.; and (c) 5-FU solution intraperitoneally (i.p.). [^{3}H]5-FU was added as a radiolabeled marker to all test formulations. Animals were sacrificed at designated times, and the tumor, liver, and one kidney from each animal were excised and processed for radioactivity analysis. Drug concentration was quantified by both storage-phosphor autoradiography (SPA) and liquid scintillation counting (LSC). *Results*: Higher and sustained i.t. drug levels were achieved following i.t. administration of 5-FU/epi gel (SPA AUC 18.4 mM · h, LSC AUC 13.0 mM · h) compared with 5-FU solution i.t. (SPA AUC 2.02 mM · h, LSC AUC 1.92 mM · h) or 5-FU solution i.p. (SPA AUC 0.07 mM · h, LSC AUC 0.04 mM · h). Use of the 5-FU/gel system was associated with lower drug levels in liver and kidney, indicating that it produces far less systemic exposure. *Conclusion*: In the human pancreatic cancer xenografts, i.t. administration of 5-FU/epi injectable gel provided significantly higher drug and/or metabolite concentrations for extended periods than was possible with either i.t. or i.p administration of drug solution. This i.t. drug delivery system could potentially be used to treat patients with pancreatic cancer to increase tumor exposure to drug and improve the therapeutic index in comparison to systemic drug administration.

Key words Sustained-retention drug delivery · 5-Fluorouracil/epinephrine injectable gel · Pancreatic cancer

This work was supported by a grant from Matrix Pharmaceutical, Inc., Fremont, Calif.

J.P. Smith (✉)
Department of Medicine, Division of Gastroenterology,
The Milton S. Hershey Medical Center,
The Pennsylvania State University,
P.O. Box 850, Hershey, PA 17033, USA
Tel. +1-717-531-6261; Fax +1-717-531-6770

S. Kanekal · M.B. Patawaran · J.Y. Chen · R.E. Jones
E.K. Orenberg · N.Y. Yu
Matrix Pharmaceutical Inc., Fremont, California 94555, USA

Introduction

Pancreatic cancer is the second most common gastrointestinal malignancy [25]. Recent reports indicate that the mortality associated with this disease represents 2.8% of all cancer deaths worldwide [33]. It is among the most rapidly fatal cancers with a 1-year survival rate of less than 20%. Because early detection of this disease is difficult, most patients (~75 to 85%) have advanced or nonresectable cancer at the time of diagnosis, which severely limits the potential for effective treatments such as surgery or radiation therapy [11]. Human pancreatic adenocarcinoma is also highly resistant to most chemotherapeutic drugs [32]. While systemic therapy is used to treat patients with widespread disease, the impact is insignificant.

Fluorouracil (5-FU) has been extensively studied as a single agent and has a response rate of about 20% in pancreatic cancer patients [3, 7, 12, 17]. Little improvement in response rates or survival has been achieved with other single agents in comparison with 5-FU alone, and most drug combination regimens only increase the toxicity [3, 9, 15, 27, 31].

Several factors may contribute to the unsatisfactory response of pancreatic cancers to chemotherapeutic

drugs: the histologic features of the tumor; the potential inherent chemoresistance of pancreatic cancer cells; and the inability to achieve adequate tumor drug concentrations. The avascular nature and local fibrosis of these tumors may partially contribute to poor drug exposure and suboptimal cytotoxic drug concentrations following systemic chemotherapy. In vitro studies in our laboratory [28] and others [20, 26] have shown that pancreatic cancer cell lines are sensitive to a variety of drugs in a time- and dose-dependent manner (e.g. 5-FU, cisplatin, doxorubicin, mitomycin C). Thus, chemotherapy could be used to improve the prognosis of patients with pancreatic cancer if a more effective drug delivery system was available.

Using pancreatic cancer xenografts as an in vivo model, we first explored the use of a therapeutic injectable gel system administered intratumorally (i.t.) to achieve higher tumoral drug levels than those from intravenous (i.v.) drug administration [28]. This site-specific, sustained-retention drug technology provides a new modality for local control of solid tumors. The injectable gel contains a cytotoxic drug (e.g. 5-FU, cisplatin), and epinephrine as a vasoconstrictor to enhance local drug retention. The drugs are incorporated into a biocompatible, biodegradable gel of purified bovine collagen. This gel formulation provides a homogeneous, reliable and safe delivery system. Use of this delivery format has been shown to enhance tumoral drug retention and increase antitumoral efficacy in a syngeneic mouse tumor model [34]. In our previous preclinical studies with athymic mice bearing BxPC-3 human pancreatic cancer xenografts, we have demonstrated that tumors treated with i.t. injectable gels (containing 5-FU, cisplatin, or doxorubicin) significantly decreased in size by 72–79% compared with vehicle-treated or untreated controls. Also, neither i.t. injection of drug solution or intraperitoneal (i.p.) injection of drug in the injectable gel delivery system produced tumor size reductions when compared with controls [28]. Several injectable gel systems have been used to treat a variety of spontaneous cutaneous and subcutaneous tumors in veterinary animals [16, 24]. In human clinical trials, direct i.t. administration of 5-FU/epinephrine gel or cisplatin/epinephrine gel has been used to treat patients with cutaneous epitheliomas [18, 21], and advanced solid tumors including cancers of the head and neck [4], esophagus [22], prostate [2] and liver [10]. Injection with endoscopic procedures for esophagus [6], and percutaneous techniques with ultrasound or computed tomography to guide the injections has been utilized for subcutaneous tumors or tumors in internal organs such as liver and prostate. For pancreatic cancer, treatment could be accomplished with a similar percutaneous route of administration of injectable gel under ultrasound guidance [8], or endoscopic delivery through the duodenum to the head of pancreas, or an initial injection provided intraoperatively for patients found to have non-resectable tumors.

Drug retention and distribution can vary depending on the morphological characteristics of different tumors. In this study, we examined the local distribution and retention of 5-FU in BxPC-3 human pancreatic cancer xenografts grown on the flanks of athymic mice, using [^{3}H]5-FU as a tracer. Distribution of label to distal organs was also monitored. The tumoral concentration and distribution of radiolabel was evaluated after i.t. administration of 5-FU/epinephrine injectable gel (5-FU/epi gel) or 5-FU solution. For comparison, tumors of mice treated i.t. or i.p. with 5-FU solution were also examined. Equivalent drug concentration was quantified using two analytical methods: storage-phosphor autoradiography (SPA) and liquid scintillation counting (LSC).

Materials and methods

Investigational agents

5-FU was administered either as an aqueous solution or as 5-FU/epi gel (Matrix Pharmaceutical, Fremont, Calif.). The 5-FU/epi injectable gel is an aqueous system consisting of 5-FU and epinephrine as the active agents, purified bovine collagen as the gellant, and various pharmaceutically acceptable salts as buffering and osmotic agents. Radiolabeled fluorouracil (6-^{3}H, 20 Ci/mmol) was obtained as a solution in 50% ethanol (American Radiolabeled Chemicals; St. Louis, Mo.). Fluorouracil Injection USP (50 mg/ml) was obtained as an injectable commercial product (SoloPak Laboratories, Elk Grove Village, Ill.), and epinephrine solution (1 mg/ml) was a custom preparation manufactured for Matrix Pharmaceutical by Chesapeake Biological Laboratories, Baltimore, Md.

Radiolabeled formulations

Tritium-labeled fluorouracil ([^{3}H]5-FU) was added as a radiolabeled marker to the test formulations, 5-FU solution and 5-FU/epi gel. The physical, chemical, and biochemical properties of [^{3}H]5-FU are the same as that of 5-FU. In preparing the [^{3}H]5-FU/epi gel, aliquots of the ethanolic [^{3}H]5-FU solution were evaporated overnight in a fume hood, reconstituted in epinephrine solution, and then mixed with 5-FU and collagen gel until homogeneous (~30 s) by means of syringe-to-syringe transfer back and forth through a Luer-lock mixing adapter. The final [^{3}H]5-FU/epi gel nominally contained 30 mg/ml fluorouracil, approximately 100 μCi/ml ^{3}H, 0.1 mg/ml epinephrine, and 20 mg/ml collagen. In preparing the [^{3}H]5-FU solution for i.t. administration, the evaporated [^{3}H]5-FU was reconstituted in 5-FU solution (50 mg/ml) and saline (0.9% Sodium Chloride Injection, USP). The resultant [^{3}H]5-FU solution nominally contained 30 mg/ml 5-FU and approximately 100 μCi/ml ^{3}H. The solution was further diluted fourfold with saline to yield the 7.5–mg/ml [^{3}H]5-FU solution for i.p. administration.

Animals

Male athymic NCr-nu mice (5 to 6 weeks old) were obtained from the National Cancer Institute. Average body weight was approximately 25 g. The animals were housed in sterile isolator cages made of flexible plastic film, with autoclaved bedding, food, and water. A diurnal cycle of 12 h light and 12 h dark was maintained. Food and water were available *ad libitum*.

Human pancreatic cancer xenografts

The human pancreatic cancer cell line BxPC-3, established from a well-differentiated human pancreatic adenocarcinoma [29] was

purchased from the American Type Culture Collection (Rockville, Md.). The BxPC-3 cells were maintained in culture in RPMI medium supplemented with 10% fetal bovine serum, L-glutamine (2 m*M*), penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells were maintained at 37 °C under a humidified atmosphere containing 5% CO_2 in an incubator. Single-cell suspensions were injected subcutaneously in a volume of 0.5 ml (1×10^6 cells) into the back of each athymic mouse. When tumors reached approximately 800 mm^3, mice were randomly assigned to treatment groups.

Experimental design

Local retention of ^3H and its distribution to distal tissues were assessed following administration of [^3H]5-FU solution i.p, [^3H]5-FU solution i.t., or [^3H]5-FU/epi gel i.t. The injection volume was 200 μl for i.p. administration and 50 μl for i.t. administration. The 5-FU dose for all groups was 1.5 mg/injection per animal, or approximately 60 mg/kg, and the ^3H dose was approximately 5 μCi per injection. At each designated time-point, one animal from each group was euthanized. The pancreatic cancer xenografts as well as the liver and one kidney from each animal were excised and immediately frozen and stored at −20 °C until sectioning. The tissues were then processed for analysis by both SPA and LSC.

Storage-phosphor autoradiography

Tumor imaging and quantitative autoradiography were performed as previously described by Kanekal et al. [14]. This method was used to compare the local retention and distribution of radiolabeled 5-FU delivered systemically (i.p.) as a drug solution or i.t. as either a drug solution or 5-FU/epi injectable gel. Frozen samples of tissue removed from mice were mounted onto cryomicrotome chucks with tissue-freezing medium and sectioned (using a cryomicrotome) at −25 °C. Tumors were sectioned serially along the longitudinal axis, and 10 to 18 sections (20–50 μm thick) traversing the entire thickness of the tumor were collected at approximately 400-μm intervals. For the liver and kidney, three to five longitudinal sections were removed at roughly the midpoint of the tissues. The tissue sections were placed on microscope slides and were air-dried at room temperature. The slides were placed in autoradiography cassettes and exposed, along with a ^3H standard slide (prepared using a [^3H]5-FU/epi gel formulation) to imaging plates for 15 h. The exposed plates were scanned, and radiation was quantified using a phosphor imaging analyzer (BAS 1000; Fuji Biomedicine Systems, Stamford, Ct.) equipped with the instrumental software (Fuji MacBas version 1.0).

Representative images of the sections were transferred to a computer graphics program (Canvas; Deneba Systems, Miami, Fl.) to construct time-course color plates. After imaging plates were scanned, the areas in which the radiolabel resided (as assessed by color) were delineated. Sections that produced autoradiograms with detectable radioactivity were used for quantification. The imaging software then integrated the total intensity (expressed as photostimulatable luminescence, PSL) over that area (square millimeters). Background (BG) radiation intensity was determined in a similar fashion from an identical area drawn on a blank imaging plate. This background intensity was subtracted from the total PSL over the area of interest. Average intensities are expressed in (PSL − BG)/mm^2 and converted to disintegrations per minute per milliliter or disintegrations per minute per cubic centimeter by comparison with standard curves. The disintegrations per cubic centimeter values were then converted to micromoles of 5-FU equivalent per cubic centimeter (or millimoles) based on the specific activity of the radiolabel in each formulation.

A graphics software package (Kaleidagraph; Abelbeck Software, Reading, Pa.) was used to plot concentration-versus-time profiles. The linear trapezoidal rule was used to calculate areas under the tumor concentration-versus-time curve ($AUC_{0\rightarrow \text{last time point}}$). The first-order rate constant was determined by monoexponential curve-fitting, performed by least-squares analysis of the semilog plots. The half-life ($t_{1/2}$) of ^3H in the tumor, defined as the time required for the concentration to decrease by 50%, was calculated from the rate constant obtained from the monoexponential curve fit. Tumoral clearance (cubic millimeters per hour) was calculated from the equation:

$$\text{Clearance} = \frac{\text{Dose}}{\text{AUC}}$$

Liquid scintillation counting

Retention of ^3H in tissue samples was determined by LSC. For this method, the 400-μm tissue chunks between the thin serial sections for SPA were collected and processed. Methanolic KOH was added to these samples, which were then heated at 37 °C and agitated intermittently until the tissue was fully solubilized (approximately 2 days). Aliquots (100 μl) of each sample were placed into scintillation vials and neutralized with 100 μl of 2 *N* HCl. After addition of 4 ml scintillation fluid (Cytoscint, ICN Biomedicals) to each sample, ^3H was counted (5-min count) in a liquid scintillation counter (Beckman LS3801). Data are presented as disintegrations per minute, percent of total administered ^3H dose, and 5-FU equivalent concentration (micrograms per gram and millimoles) in the specified tissue. The 5-FU equivalent concentration was calculated by converting the ^3H concentration (expressed as percent total administered dose) to 5-FU concentration, assuming an administered (100%) dose of 1.5 mg in an injection volume of either 200 or 50 μl, AUC, $t_{1/2}$, and clearance values were obtained as described above. All ^3H concentrations for SPA and LSC are expressed as 5-FU equivalents (millimoles). For both analytical methods, the average concentrations were calculated to include drug in the gel and drug in the tumor tissue.

Results

Storage-phosphor autoradiography

The qualitative time course of retention of ^3H (5-FU and/or its metabolites) in tumors is presented in Fig. 1. Retention and localization of ^3H were greater in the xenografts treated i.t. with [^3H]5-FU/epi gel than in those treated i.t. with [^3H]5-FU solution. Three-dimensional (3-D) images of the tumors were constructed based on the series of two-dimensional autoradiograms of tumor slices. These 3-D images showed that [^3H]5-FU was rapidly distributed throughout the tumor within 2 min of injection of either gel or solution. At 0.5 h after administration of the 5-FU solution, the radioactivity was substantially decreased. In contrast, substantial radioactivity was found even after 120 min in tumors treated with the 5-FU/epi gel and the distribution pattern was different.

The quantitative retention time course for the xenograft tumors is shown in Table 1 and Fig. 2. For up to 1 h after systemic (i.p.) administration of 5-FU solution, radiolabel in the tumor was barely discernible (less than 0.10 m*M*). Local (i.t.) administration of 5-FU solution resulted in tumor ^3H concentrations much higher than those observed after i.p. administration both immediately after injection and 0.5 h later. However, at 1 h postinjection, the tumor ^3H concentration had decreased to less than the concentration achieved after i.p. administration at the same time-point. In contrast, i.t.

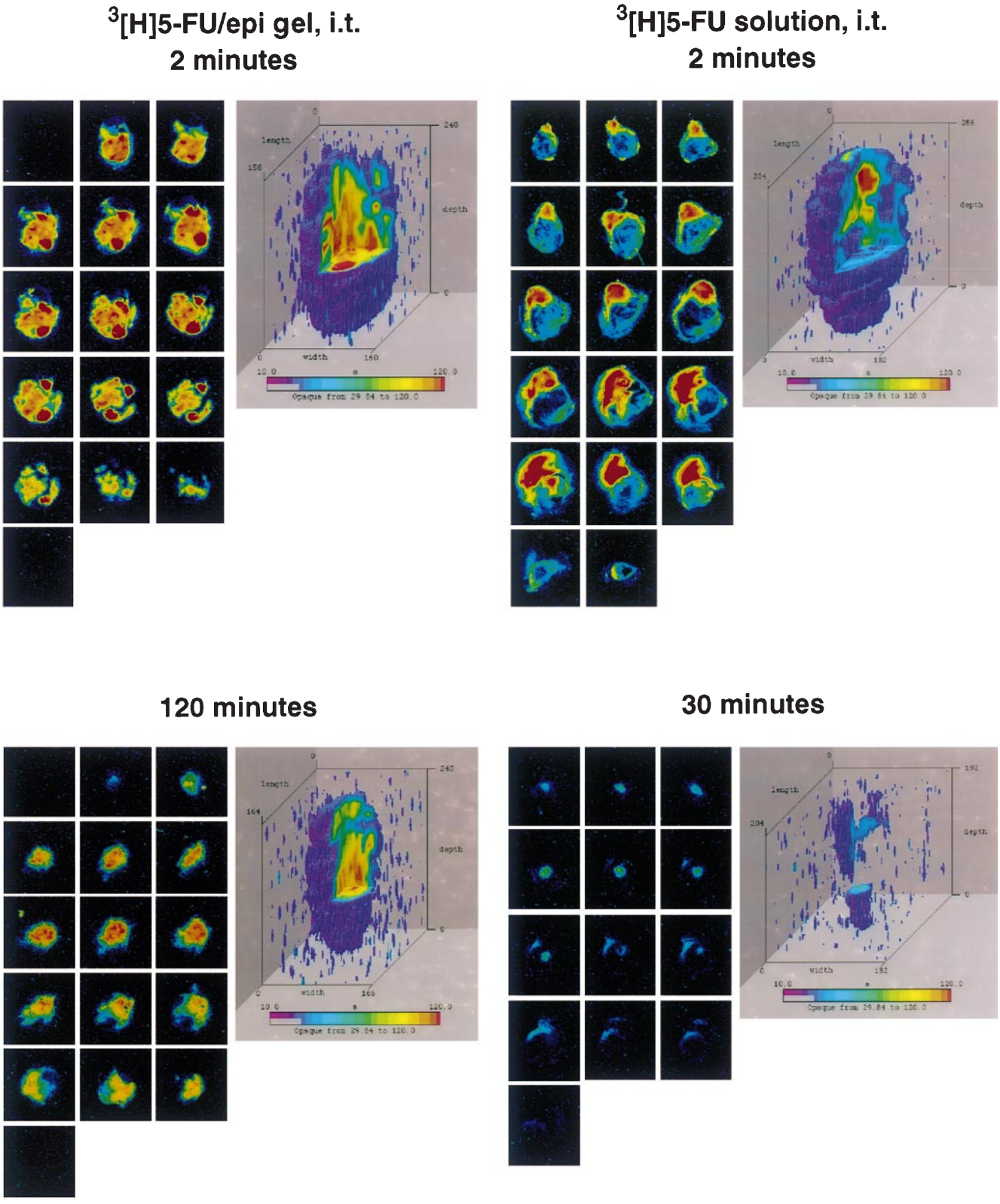

Fig. 1 Localization of ^{3}H in BxPC-3 human pancreatic cancer xenografts after intratumoral (i.t.) injection of [^{3}H]5-FU/epi gel or [^{3}H]5-FU solution. Two- dimensional autoradiograms (*left section of each panel*) of all cryosectioned tissue slices of each tumor were used to construct the three-dimensional images (*right of each panel*)

Table 1 ^{3}H retention in BxPC-3 human pancreatic cancer xenografts. The nominal 5-FU dose in all cases was 1.5 mg (11.5 μmol) per animal or ~60 mg/kg (*AUC* area under the curve, zero hour to last time-point)

Treatment	Time after injection (h)	Storage-phosphor autoradiography[a]		Liquid scintillation counting	
		% Total dose	5-FU equivalent (m*M*)	% Total dose	5-FU equivalent (m*M*)
5-FU solution, i.p. (7.5 mg/ml, 200 μl)	0	0.11	0.06	0.01	0.00
	0.5	0.15	0.09	0.23	0.06
	1	0.06	0.04	0.44	0.05
	AUC	0.12% · h	0.07 m*M* · h	0.23% · h	0.04 m*M* · h
5-FU solution, i.t. (30 mg/ml, 50 μl)	0	39.1	7.44	35.8	6.83
	0.5	0.90	0.26	0.90	0.26
	1	0.22	0.02	0.69	0.06
	2	0.12	0.04	0.30	0.07
	AUC	10.5% · h	2.02 m*M* · h	10.1% · h	1.92 m*M* · h
5-FU/epi gel, i.t.[b] (30 mg/ml, 50 μl)	0	81.6	10.6	78.9	10.2
	1	61.8	7.45	41.5	5.00
	2	14.6	3.40	6.46	1.51
	4	1.00	0.15	0.89	0.13
	8	0.20	0.05	0.45	0.11
	AUC	128% · h	18.4 m*M* · h	94.2% · h	13.0 m*M* · h

[a] Mean of 3–15 autoradiograms per time-point (obtained from tissue sections at equal intervals through the entire tumor)
[b] Drug retention represents overall retention and includes retention of drug in the gel as well as in the tumor

administration of the 5-FU/epi gel yielded tumor ^{3}H concentrations that were considerably higher than those observed after 5-FU solution administered either i.p. or i.t. at the same time-points; for example, at 1 h, the concentrations were nearly 200 and 400 times greater, respectively.

Tumor AUC values for 5-FU solution i.p., 5-FU solution i.t., and 5-FU/epi gel i.t. were 0.07, 2.02, and 18.4 m*M* · h, respectively. After i.t. administration of 5-FU as a simple solution, the concentration decreased rapidly, exhibiting a first-order rate constant of about 4.0 h^{-1}, corresponding to a $t_{1/2}$ of only about 10.4 min (Fig. 2). In contrast, the 5-FU/epi gel provided sustained i.t. availability of drug: the radiolabel was cleared much more slowly and exhibited a rate constant of only 0.70 h^{-1}, corresponding to a $t_{1/2}$ of about 59 min. The 5-FU solution i.p. was barely detectable at 30 min. Clearance of ^{3}H from the tumor was 9.58 times slower after i.t. administration of the 5-FU/epi gel than after administration of the aqueous 5-FU solution (Table 2).

Pharmacokinetics in the liver and kidney are presented in Table 3. In the liver, radiolabel concentrations after either systemic (i.p.) or local (i.t.) administration of 5-FU solution peaked earlier (at 0.5 h) and were twice those observed after i.t. administration of 5-FU/epi gel, which peaked at 2 h. Similarly, drug-derived radiolabel concentrations in kidney peaked 0.5 h after either i.p. or i.t. administration of 5-FU solution and were 35% higher than those after 5-FU/epi gel i.t., which peaked 2 h after administration. No radioactivity was detectable in liver or kidney 2 h after injection (i.t. or i.p.) of 5-FU solution.

Liquid scintillation counting

The drug-derived radiolabel concentrations in tumors treated i.t. with 5-FU/epi gel were initially twice those in tumors injected with the simple drug solution, and at all study time-points, they persisted at levels higher than those following administration of 5-FU solution either i.t. or i.p. (Table 1).

Tumor AUC values for 5-FU solution i.p., 5-FU solution i.t., and 5-FU/epi gel i.t. were 0.04, 1.92, and 13.0 m*M* · h, respectively (Table 1), indicating, as with the SPA method, far greater concentration of drug and/or metabolites over time with 5-FU/epi gel i.t. than with the other treatment schemes. The $t_{1/2}$ values for 5-FU solution i.t. and 5-FU/epi gel i.t. were 12 min and 55 min, respectively (Fig. 2). Clearance of ^{3}H from the tumor was 7.07 times slower after i.t. administration of the 5-FU/epi gel than after administration of the aqueous solution (Table 2).

Radiolabel concentrations in liver were substantially higher immediately after i.p. administration of 5-FU solution than after i.t. administration. Peak levels for both administration routes were reached 0.5 h after injection. In contrast, animals treated with 5-FU/epi gel did not manifest peak liver concentrations until 1 to 2 h after the injection (Table 3).

Compared with those in tumor and liver, radiolabel concentrations in kidney were considerably lower at all time-points, peaking 1 h after injection of 5-FU solution i.p., 30 min after injection of 5-FU solution i.t., and 2 h after injection of 5-FU/epi gel i.t. The kidney concentration produced by 5-FU/epi gel i.t. 2 h postinjection

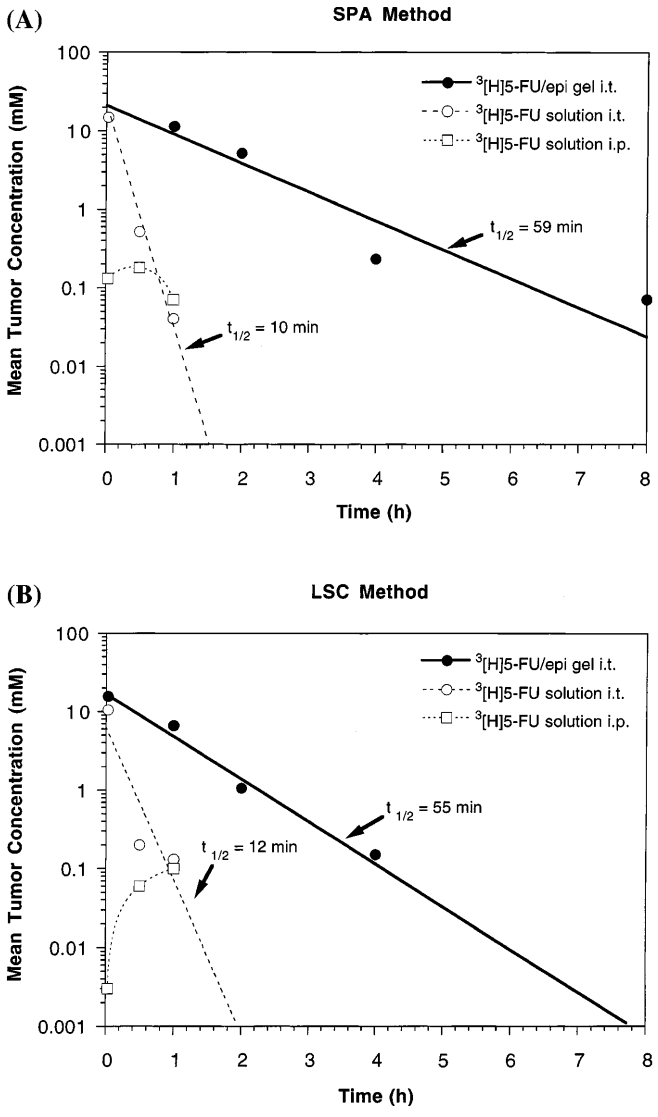

Fig. 2 Retention of ^{3}H in BxPC-3 human pancreatic cancer xenografts after various forms of drug delivery. Tumor concentrations over time were determined by storage-phosphor autoradiography (SPA, **A**) and liquid scintillation counting (LSC, **B**). Concentrations are expressed as 5-FU equivalents

Table 2 Clearance of ^{3}H in BxPC-3 human pancreatic cancer xenografts. The nominal 5-FU dose in all cases was 1.5 mg (11.5 μmol) per animal or ~60 mg/kg. (*SPA* storage-phosphor autoradiography, *LSC* liquid scintillation counting)

Detection method	Formulation	Clearance[a] (cm^3/h)	Clearance ratio (5-FU solution vs 5-FU/epi gel)
SPA[b]	5-FU/epi gel	0.635	9.58
	5-FU solution	6.085	–
LSC	5-FU/epi gel	0.904	7.07
	5-FU solution	6.389	–

[a] Clearance (dose/AUC) represents overall clearance (clearance from gel + clearance from tumor tissue)
[b] Mean of 3–15 autoradiograms per time point

was four times lower than that produced by 5-FU solution i.t. (Table 3).

Discussion

In the present experiments using pancreatic cancer xenografts, we studied a new drug delivery system designed for i.t. administration. This delivery system consists of an antineoplastic agent (5-FU) incorporated within an extended-retention gel. The gel consists of a biocompatible/biodegradable aqueous dispersion of a highly purified bovine dermal collagen, which physically enmeshes the drug and retards drug clearance after injection. The system also includes a small amount of epinephrine as an adjuvant, further inhibiting drug clearance into the circulation through local vasoconstriction. By measuring drug-derived radiolabel concentrations after injection, we demonstrated that the injectable gel not only provided higher drug and/or drug metabolite concentrations in the tumor than did drug administered as an aqueous solution i.t. or systemically, but also resulted in prolonged exposure of the tumor tissue to the drug and/or its metabolites. Concurrently, the gel was associated with lower levels of radioactivity in the liver and kidney, indicating that it produced far less systemic exposure.

The radiolabel technique used in these studies did not distinguish between 5-FU and its ^{3}H-containing metabolites. These metabolites can either be active (e.g. F-dUMP) or inactive (e.g. catabolism via dihydropyrimidine dehydrogenase, DPD). The latter enzyme is widespread in many tissues (particularly liver, kidney, lymphocytes, and the gastrointestinal mucosa) and is responsible for rapid metabolic inactivation of the drug following i.v. administration. With i.t. administration, such systemic inactivation pathways clearly affect the drug only after it leaves the local tumor environment. The levels of DPD in tumor tissue are reported to be variable, depending on tumor location and cell line [23]. DPD levels in the BxPC-3 line have not yet been characterized, although other pancreatic lines (e.g. PANC-3) are reported to have elevated DPD activity [1]. The long retention of radiolabel in the tumor following intratumoral administration of the 5-FU/epi gel could therefore, of course, simply have been due to the presence of inactive metabolite. However, there would appear to be little question that retention of parent drug and active metabolite would also be significantly enhanced with use of the gel delivery system as compared with the i.t. solution or, especially, the i.p. solution. This is supported by the enhancement of antitumor activity in vivo seen in earlier studies [28] and discussed briefly below.

In previous in vitro studies, we have demonstrated the sensitivity of BxPC-3 pancreatic cancer cells to 5-FU and other cytotoxic agents and the dependence on drug concentration and exposure time [28]. If the exposure time is increased, the required drug concentration can be reduced. For example, the concentration of 5-FU that

Table 3 ^{3}H levels in liver and kidney of mice bearing BxPC-3 xenografts. The nominal 5-FU dose in all cases was 1.5 mg (11.5 μmol) per animal or ~ 60 mg/kg (*SPA* storage-phosphor autoradiography, *LSC* liquid scintillation counting)

Treatment	Time (h)	Liver				Kidney			
		SPA[a]		LSC		SPA[a]		LSC	
		% Total dose	5-FU equivalent (m*M*)	% Total dose	5-FU equivalent (m*M*)	% Total dose	5-FU equivalent (m*M*)	% Total dose	5-FU equivalent (m*M*)
5-FU solution, i.p. (7.5 mg/ml, 200 μl)	0	1.18	0.10	2.21	0.19	0.22	0.13	0.23	0.14
	0.5	6.19	0.48	6.18	0.48	0.60	0.34	0.76	0.43
	1	3.75	0.28	5.19	0.39	1.30	0.62	1.20	0.57
5-FU solution, i.t. (30 mg/ml, 50 μl)	0	0.00	$<0.3 \times 10^{-3}$	0.01	0.00	0.00	$<0.3 \times 10^{-6}$	0.00	0.00
	0.5	6.23	0.45	13.2	0.95	0.59	0.34	1.50	0.76
	1	2.30	0.14	5.76	0.35	0.38	0.18	0.85	0.32
	2	0.62	0.05	1.55	0.12	0.20	0.09	0.20	0.11
5-FU/epi gel, i.t. (30 mg/ml, 50 μl)	0	0.00	$<0.3 \times 10^{-6}$	1.09	0.08	0.00	$<0.3 \times 10^{-6}$	0.59	0.32
	1	2.30	0.16	4.96	0.35	0.31	0.15	0.43	0.21
	2	2.83	0.22	5.52	0.43	0.46	0.25	0.78	0.42
	4	0.00	$<0.3 \times 10^{-6}$	2.00	0.13	0.00	$<0.3 \times 10^{-6}$	0.34	0.14
	8	0.00	$<0.3 \times 10^{-6}$	0.12	0.01	0.00	$<0.3 \times 10^{-6}$	0.11	0.06

[a] Mean of two to three sections per time-point (obtained approximately from the middle of the organ)

inhibits growth of 50–90% (IC_{50}, IC_{90}) of BxPC-3 cells is eight to ten times less with a 72-h drug exposure than with a 24-h exposure. If one uses an AUC to describe total exposure, these values at the IC_{90} for 24-h and 72-h exposures are 2.4 and 0.7 m$M \cdot$ h, respectively. Although such use of AUCs can be misleading, the apparent AUC for the 5-FU/epi gel was on the order of 13 to 18 m$M \cdot$ h after only 8 h (Table 1), and was nearly ten times the exposure after i.t. administration of 5-FU solution and roughly 300 times the exposure resulting from systemic (i.p.) administration of drug.

While 5-FU is the most clinically effective systemic drug for treatment of pancreatic cancer, it is far from successful. Dose intensification is required because 5-FU has a short plasma half-life and, because the major cytotoxic effect occurs during the S-phase of the cell cycle [5], is time-dependent [19]. To enhance antitumoral effects, protracted i.v. infusion of 5-FU has been one approach used concurrently with radiotherapy [13, 30]. Using the xenograft model, we have shown that delivery of drug i.t. in the 5-FU/epi gel formulation resulted in growth inhibition of the pancreatic cancer xenografts that could not be accomplished with either i.t. or i.p. 5-FU solution [28].

The BxPC-3 pancreatic cancer xenograft has histologic characteristics of a well-differentiated human pancreatic adenocarcinoma. The architecture of this experimental tumor consists of groups of closely packed cells arranged in a glandular pattern with large areas of interstitium. This ''porous'' morphology may permit rapid distribution of drug throughout the tumor within a short time after i.t. administration. This architecture and its associated pattern of drug distribution were easily visualized in the SPA images in the present study. Within 2 min of i.t. drug administration, either as 5-FU solution or 5-FU/epi gel, the radiolabel extended into the tumor margins despite the fact that the injection volume (50 μl) was only a small fraction of the tumor volume (~800 μl). The subsequent retention behavior, however, was quite different. Radioactivity was barely detectable 30 min after xenografts were injected i.t. with drug solution, whereas drug administered as 5-FU/epi gel resulted in prolonged concentrations of radiolabel equivalent to 5-FU concentrations in the tumor well above cytotoxic thresholds.

For the two methods of ^{3}H quantification (SPA and LSC), portions of the same tissue were used, providing the opportunity to compare and confirm drug retention profiles without the confounding influence of animal or tissue variability. SPA also revealed the spatial distribution of drug throughout the tumor. Quantification of ^{3}H concentrations and AUC values by SPA resulted in similar but slightly higher values than with LSC. These small differences are likely procedural because with SPA, only tissue sections containing detectable radioactivity were measured, and those sections without detectable radioactivity were not included in the determination of the mean tissue concentrations. With the LSC method, the entire tumor was sampled and weighed, resulting in a lower mean drug concentration. The detection limit for SPA was ~2 dpm/mm^2, or 1 fmol, compared with 20 dpm/mm^2, or 30 fmol for LSC.

In this pancreatic cancer xenograft model, we demonstrated that the therapeutic injectable gel, 5-FU/epi gel, provided high drug and/or metabolite concentrations in tumor for prolonged periods after i.t. administration. Similar concentrations were not obtainable by either i.t. or i.p. administration of drug solution. Drug delivery in the injectable gel formulation has previously been shown to enhance the antitumoral effect of 5-FU and produce significant growth inhibition of xenografts [28]. Because the injectable gel system may improve local tumor control and increase the therapeutic index, it may improve the prognosis or provide disease palliation in

patients with pancreatic cancer or other accessible solid tumors. This novel delivery system may also be applicable for other drugs used for the treatment of pancreatic cancer, such as gemcitabine.

Acknowledgement The authors thank Amar Sahai for his technical assistance, and Kim Hoag, Ph.D., Caren Rickhoff, Judith Simon, and Kimberly Dugoni for their assistance with preparation of the manuscript.

References

1. Beck A, Etienne MC, Cheradame S, Fischel JL, Formento P, Renee N, Milano G (1994) A role of dihydropyrimidine dehydrogenase and thymidylate synthase in tumor sensitivity to fluorouracil. Eur J Cancer 30A: 1517
2. Brawer MK, Ploch NR, Narayan P, Anderson RU, Albert E, Romas N, Lerner S, Korey A, Baughman J (1994) Intralesional administration of fluorouracil therapeutic implant (MPI 5011) in patients with prostatic cancer. Proc Am Soc Clin Oncol 13: 246
3. Brennan MF, Kinsella TJ, Casper ES (1993) Cancer of the pancreas. In: DeVita TJ Jr, Hellman S, Rosenberg S (eds) Principles and practices of oncology, 4th edn. JB Lippincott, Philadelphia, pp 800–835
4. Burris HA III, Vogel CL, Castro D, Mishra L, Schwarz M, Spencer S, Oakes DD, Korey A, Orenberg EK (1998) Intratumoral cisplatin/epinephrine-injectable gel as a palliative treatment for accessible solid tumors: a multicenter pilot study. Otolaryngol Head Neck Surg 118: 496
5. Calabro-Jones PM, Byfield JE, Ward JF, Sharp TR (1982) Time-dose response relationships for 5-fluorouracil cytotoxicity against human epithelial cancer cells *in vitro*. Cancer Res 42: 4413
6. Caletti G, Ferrari A (1996) Endoscopic ultrasonography. Endoscopy 28: 156
7. Carter SK, Comis RL (1975) The integration of chemotherapy into a combined modality approved for cancer treatment: VI. pancreatic adenocarcinoma. Cancer Treat Rev 2: 193
8. Chang KJ, Albers CG, Erickson RA, Butler JA, Wuerker RB, Lin F (1994) Endoscopic ultrasound-guided fine needle aspiration of pancreatic carcinoma. Am J Gastroenterol 89: 263
9. Cullinan S, Moertel CG, Wieand HS, Shutt AJ, Krook JE, Foley JF, Norris BD, Kardinal CG, Tschetter LK, Barlow JI (1990) A phase III trial on the therapy of advanced pancreatic carcinoma: evaluations of the Mallison regimen and 5-fluorouracil, doxorubicin, and cisplatin. Cancer 65: 2207
10. Curley SA, Carrasco CH, Charnsangavej C, Siddak Z, Korey A (1994) Phase I trial of intratumoral cisplatin therapeutic implant (MPI 5010) in patients with primary or metastatic cancer of the liver. Proc Am Soc Clin Oncol 13: 403
11. Gudjonsson B (1987) Cancer of the pancreas: 50 years of surgery. Cancer 60: 2284
12. Hansen R, Quebbeman E, Ritch P, Chitamar C, Anderson T (1988) Continuous 5-fluorouracil infusion in carcinoman of the pancreas. Am J Med Sci 295: 91
13. Ishii H, Okada S, Tokuuye K, Nose H, Okusaka T, Yoshimori M, Nagahama H, Sumi M, Kagami Y, Ikeda H (1997) Protracted 5-fluorouracil infusion with concurrent radiotherapy as a treatment for locally advanced pancreatic adenocarcinoma. Cancer 79: 1516
14. Kanekal S, Sahai A, Jones RE, Brown D (1995) Storage-phosphor autoradiography: a rapid and highly sensitive method for spatial imaging and quantitation of radioisotopes. J Pharmacol Toxicol Methods 33: 171
15. Kelsen D, Hudis C, Niedzwiecki D, Dougherty J, Casper E, Botet J, Vinciguerra V, Rosenbluth R (1991) A phase III comparison trial of streptozotocin, mitomycin, and 5-fluorouracil with cisplatin, cytosine arabinosine, and caffeine in patients with advanced pancreatic carcinoma. Cancer 68: 965
16. Kitchell BE, Brown DM, Luck EE, Woods L, Orenberg EK, Bloch DA (1994) Intralesional implant for treatment of primary oral malignant melanoma in dogs. J Am Vet Med Assoc 204: 229
17. Kovach JS, Moertel CG, Schutt AJ, Hahn RG, Reitemeier RJ (1974) A controlled study of combined 1,3-*bis*(2-chloroethyl)-1-nitrosourea and 5-fluorouracil therapy for advanced gastric and pancreatic cancer. Cancer 33: 563
18. Kraus S, Miller BH, Swinehart J, Shavin JS, Georgouras K, Jenner D, Griffin E, Korey A, Orenberg EK (1998) Intratumoral chemotherapy with fluorouracil/epinephrine injectable gel: A nonsurgical treatment of cutaneous squamous cell carcinoma. J Am Acad Dermatol 38: 438
19. MacMillan WE, Wolberg WH, Welling PG (1978) Pharmacokinetics of fluorouracil in humans. Cancer Res 38: 3479
20. Matsuno S, Hisano H, Kobari M, Akaishi S (1990) Growth-inhibitory effects of combination chemotherapy for human pancreatic cancer cell lines. Cancer 66: 2369
21. Miller BH, Shavin JS, Cognetta A, Taylor JR, Salasche S, Korey A, Orenberg EK (1997) Nonsurgical treatment approach for basal cell carcinomas with intralesional fluorouracil/epinephrine injectable gel. J Am Acad Dermatol 36: 72
22. Mishra L, Reilly J, Reilly H, Rofail M, Peters B, Kozam ML, Wadleigh R (1995) Treatment of esophageal cancer with endoscopic injection of methotrexate and cisplatin therapeutic gels. Gastroenterology 108: 168
23. Naguib FNM, el Kouni MH, Cha S (1985) Enzymes of uracil catabolism in normal and neoplastic human tissues. Cancer Res 45: 5405
24. Orenberg EK, Luck EE, Brown DM, Kitchell BE (1992) Implant delivery system. Intralesional delivery of chemotherapeutic agents for treatment of spontaneous skin tumors in veterinary patients. Clin Dermatol 9: 561
25. Parker SI, Tong T, Bolden S, Wingo PA (1996) Cancer statistics 1996. CA Cancer J Clin 46: 5
26. Sawabe Y, Yamagishi H, Yamaguchi N, Yamamura V, Oka T (1996) *In vitro* chemosensitivity of human pancreatic cancer cell lines. Int J Pancreat 20: 185
27. Smith FP, Hoth DV, Levin B, Karlin DA, MacDonald JS, Wooley PV III, Schein PS (1980) 5-fluorouracil, adriamycin and mitomycin C (FAM) chemotherapy for advanced adenocarcinoma of the pancreas. Cancer 46: 2014
28. Smith JP, Stock E, Orenberg EK, Yu NY, Kanekal S, Brown DM (1995) Intratumoral chemotherapy with a sustained-release drug delivery system inhibits growth of human pancreatic cancer xenografts. Anticancer Drugs 6: 717
29. Tan MH, Nowak NJ, Loor R, Ochi H, Sandberg AA, Lopez C, Pickren JW, Berjian R, Douglass HO Jr, Chu TM (1986) Characterization of a new primary human pancreatic tumor line. Cancer Invest 4: 15
30. Whittington R, Neuberg D, Tester WJ, Benson AB III, Haller HG (1995) Protracted intravenous fluorouracil infusion with radiation therapy in the management of localized pancreaticobiliary carcinoma: a phase I Eastern Cooperative Oncology Group trial. J Clin Oncol 13: 227
31. Wiggans RG, Woolley PV III, MacDonald JS, Smythe T, Ueno W, Schein PS (1978) Phase II trial of streptozocin, mitomycin C and 5-fluorouracil (SMF) in the treatment of advanced pancreatic cancer. Cancer 41: 387
32. Wils JA (1991) Chemotherapy in pancreatic cancer: a rational pursuit? Anticancer Drugs 2: 3
33. World Health Organization (1997) The World Health Report. Geneva
34. Yu N, Orenberg EK, Luck EE, Brown DM (1995) Antitumor effect of intratumoral administration of fluorouracil/epinephrine injectable gel in C3H mice. Cancer Chemother Pharmacol 36: 27