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Intratumoral chemotherapy with a sustained-release drug delivery system inhibits growth of human pancreatic cancer xenografts

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This study provides the first evidence that treatment of human pancreatic adenocarcinoma is markedly improved by the intratumoral administration of chemotherapeutic agents in a novel drug delivery system. The effect of chemotherapeutic agents delivered in a sustained-release, protein-based, injectable gel was evaluated on the growth of human pancreatic adenocarcinoma cell line, BxPC-3. *In vitro* chemosensitivity of BxPC-3 cells exposed for 24 or 72 h to fluorouracil (0.01–5 mM), cisplatin or doxorubicin (0.1–50 μM) and floxuridine, vinblastine, mitomycin or paclitaxel (1.0–100 μM) was compared with that of untreated cells. *In vitro* chemosensitivity was also studied with fluorouracil and mitomycin in the poorly differentiated PANC-1, human pancreatic cancer cell line. Survival was determined after 7–10 days. All drugs decreased cell growth in a dose-dependent fashion. The efficacy of fluorouracil, cisplatin and doxorubicin increased with prolonged exposure, rendering these drugs most appropriate for a sustained-release preparation. For *in vivo* studies, athymic nude mice bearing BxPC-3 xenografts were treated either with fluorouracil, cisplatin or doxorubicin in the therapeutic injectable gel containing epinephrine or with vehicle alone administered intratumorally on days 1 and 4. After 28 days, the mice were sacrificed and tumors dissected and weighed. Tumors in mice treated with the injectable gel decreased in size by 72–79% compared with tumors in untreated controls and tumors treated with vehicle alone. Intratumoral injection of drug solution and intraperitoneal injection of drug in the injectable gel did not change tumor size compared with controls. In a drug-retention study, mice were injected intratumorally with [^{3}H]fluorouracil either in the injectable gel or in solution. Sustained radioactivity was observed in tumors injected with the gel, and, conversely, greater radioactivity was detected in the liver and kidneys in mice receiving the radiolabeled solution. hese results suggest that the therapeutic injectable gel chemotherapy, when given intratumorally, may improve tumor response with less systemic toxicity in comparison with conventional systemic chemotherapy.

Key words: Intratumoral drug delivery, pancreatic cancer.

Introduction

Pancreatic cancer is a lethal malignancy occurring in more than 28 000 Americans yearly and ranks as the fifth most common cause of cancer-related mortality in the US.[1] Unfortunately, the 5-year survival rate of individuals with pancreatic cancer has remained less than 1% with most patients surviving for only 3–6 months.[2] Surgical resection of early-stage tumors may improve survival to 17–20%;[3,4] however, most patients have advanced disease at the time of diagnosis.[5] Treatment of pancreatic cancer has been overwhelmingly unsuccessful,[6] and many oncologists and gastroenterologists tend to take a nihilistic or defeatist attitude toward this disease.[7,8] Human pancreatic adenocarcinoma is known to be resistant to most clinically active antitumor agents.[6] Of all the chemotherapeutic agents tested over the past three decades, only 5-fluorouracil (5-FU) routinely resulted in a 20% response rate with 95% confidence intervals.[9–11] Slightly improved response rates have been reported with drug combinations such as FAM (5-FU, doxorubicin [Adriamycin] and mitomycin)[12] or SMF (streptozoticin, mitomycin and 5-FU).[13] However, survival rates have not improved considerably over those achieved with single-agent fluorouracil therapy.

Histologically, pancreatic tumors are quite avascular and tend to induce an intense localized fibrosis.[7] These histologic features may be partially responsible for the poor penetration of systemic chemotherapeutic agents in the tumors. It remains unclear whether failure to adequately control local

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pancreatic disease is due to the ineffectiveness of combination chemotherapy, the inability to achieve adequate drug concentrations in the tumor or in the inherent chemoresistance of pancreatic cancer cells.

Most patients with pancreatic cancer die as a result of direct tumor extension of the tumor into vital organs and vessels. Although distant metastases are occasionally seen, they are rarely relevant to a patient's clinical course.[9] New approaches to drug delivery might provide the opportunity to improve drug dosing intensity and thereby improve clinical efficacy. For these reasons, intra-arterial injection of chemotherapeutic drugs has been tried in an attempt to increase tumoral drug concentrations.[14] However, this approach is unsuccessful because the gastroduodenal and splenic arteries that feed the pancreatic cancers supply more blood to the stomach, duodenum and spleen.

Another approach to achieving higher tumor-drug levels than those obtained from systemic administration is by intralesional injection. Intratumoral administration of chemotherapy into cutaneous tumors improves response rate and appears to decrease toxicity[15] when compared with intraperitoneal therapy in mice. Administration of chemotherapeutic agent in a slow-release device[16] or conjugated to monoclonal antibodies[17,18] enhanced response rate and reduced toxicity. Intratumoral chemotherapy with mitomycin C absorbed to activated carbon particles has been performed safely by ultrasound guidance in patients with pancreatic cancer.[19] When a conventional chemotherapeutic, such as 5-FU, cisplatin, methotrexate or vinblastine, is administered intralesionally in animals[20–22] in a sustained-release gel delivery system, tumoral drug retention is enhanced and antitumor efficacy increased. In human patients, intratumoral chemotherapeutic gel has been used to treat accessible solid tumors,[23,24] including head and neck squamous cell carcinoma, esophageal cancer,[25] liver cancer,[26] and prostate cancer.[27] Because of the local aggressiveness of pancreatic tumors, it was of interest to see whether the chemotherapeutic response rate of this malignancy could possibly be enhanced by intratumoral medication. In this study, we examined and evaluated the chemosensitivity of human pancreatic cancer cells *in vitro*, the tumoral concentration of drug administered locally via a therapeutic injectable gel and its effectiveness on the growth of human pancreatic cancer xenografts *in vivo*.

Materials and methods

Investigational agents

The *in vitro* chemosensitivity tests were conducted using seven different chemotherapeutic drugs in solution, whereas the *in vivo* tests were performed using three chemotherapeutic drugs each formulated in the therapeutic injectable gel. The injectable gel formulation consists of a chemo-therapeutic drug and epinephrine as the active agents, purified bovine collagen (gel) as the gellant, and various pharmaceutically acceptable salts as buffering and osmotic agents. Epinephrine solution (1 : 1000) was obtained as a commercial product from Parke-Davis (Morris Plains, NJ) and collagen (6.5% w/w) was obtained from Koken (Tokyo, Japan). Chemotherapeutic agents used in this study included 5-FU and vinblastine purchased from LyphoMed (Rosemont, IL); 5-FU from Hoffmann-La Roche (Nutley, NJ); cisplatin and mitomycin from Bristol (Evansville, IN); doxorubicin (DOX) from Cetus (Emeryville, CA); and paclitaxel from Sigma (St Louis, MO). Radiolabeled fluorouracil (6-^{3}H, 87–100 μCi/mmol) was obtained from American Radiolabeled Chemicals (St Louis, MO).

Injectable gel formulations

The injectable gels (IntraDose™ gel; Matrix Pharmaceutical, Menlo Park, CA) contained either 5-FU, cisplatin or DOX along with epinephrine (epi) and collagen (gel), and were designated as 5-FU/epi gel, cisplatin/epi gel and DOX/epi gel, respectively. All formulations were prepared by combining each chemotherapeutic agent with epinephrine solution in a 1 ml Luer-lock syringe. The syringe was then attached with a connector unit to a second 1 ml syringe containing collagen gel. The materials in both syringes were transferred back and forth until homogeneous ($\sim$30 s). The injectable gels were prepared to contain 0.1 mg/ml of epinephrine, 20 mg/ml of collagen and the following concentrations of drugs: the 5-FU/epi gel contained 15 or 30 mg/ml of 5-FU, the cisplatin/epi gel contained 1.5 or 2.0 mg/ml of cisplatin and the DOX/epi gel contained 1.2 or 2.0 mg/ml of DOX.

Human pancreatic carcinoma cell lines

The human pancreatic cancer cell lines BxPC-3 and PANC-1 were purchased from the American Type Culture Collection (Rockville, MD). BxPC-3 cell

line[28] was established from a well-differentiated pancreatic adenocarcinoma, whereas PANC-1 was established from a poorly differentiated primary pancreatic ductal adenocarcinoma and characterized by Lieber *et al.*[29]

In vitro chemosensitivity tests

The chemosensitivity of human pancreatic cancer cells to several chemotherapeutic agents was tested *in vitro*. BxPC-3 and PANC-1 cells were maintained in culture in RPMI media and Dulbecco's MEM media, respectively, and supplemented with 10% fetal bovine serum, L-glutamine (2 mM), penicillin (100 units/ml) and streptomycin (100 μg/ml). Cells were maintained at 37°C in a humidified 5% CO_2 incubator. Two hundred thousand cells were harvested and plated overnight into each 4 cm^2 well of 12-well plates for cell attachment. Appropriate amounts of drugs were added to the wells to create final concentrations of 0.01, 0.1, 1.0 and 5 mM 5-FU; 1, 10, 50 and 100 μM mitomycin, vinblastine, floxuridine or paclitaxel; and 0.1, 1, 10 and 50 μM DOX or cisplatin. PANC-1 cells were treated with 5-FU and mitomycin at the same concentrations described above. Each drug concentration was evaluated in four replicates. After a 24 or 72 h incubation in the presence of drug, the plates were washed twice with Hanks' balanced salt solution (pH 7.4) and fresh medium was added. BxPC-3 cells were allowed to grow for 10 days and PANC-1 cells for 7 days after the initial plating, such that the untreated control cells passed through 1.5–3 doubling times. Cells were then harvested with a solution containing trypsin (0.25%) and EDTA (0.05%), and were counted manually by the Trypan blue exclusion technique.

In vivo tests

Mice. Male 5–6 week old, athymic (Ncr-*nu*) mice were obtained from the National Cancer Institute. The average body weight was approximately 25–28 g. Animals were housed in sterile, plastic flexible film isolator cages 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. Single-cell suspensions were made from BxPC-3 cancer cells grown in culture. Each animal was inoculated by a subcutaneous injection of 10×10^6 BxPC-3 cells in a volume of 0.5 ml in the flank. By 1 week, 100% of the mice had palpable tumors of the designated experimental size ($\sim$100–125 mm^3) and were randomized into different treatment groups.

Table 1. Study design for treatment of human pancreatic cancer xenografts

Treatment	Drug concentration (mg/kg)	Injection route	Injection schedule (days)	No. of mice/group
Study 1				
untreated control	—	—	—	5
placebo gel control	—	i.t.	1, 4	5
epi gel control	—	i.t.	1, 4	5
DOX/epi gel	5, 8	i.t.	1, 4	8
cisplatin/epi gel	6, 8	i.t.	1, 4	8
5-FU/epi gel	60, 120	i.t.	1, 4	8
5-FU/epi gel	60	i.t.	1, 4, 8, 12	8
Study 2				
untreated control	—	—	—	5
5-FU/epi gel	60	i.p.	1, 4	8
5-FU/epi gel	60	i.t.	1, 4	8
cisplatin/epi gel	8	i.p.	1, 4	8
5-FU/epi gel	60	i.t.	1, 4 &	8
+ cisplatin/epi gel	6	i.t.	8, 12	
Study 3				
untreated control	—	—	—	5
5-FU solution	60	i.t.	1, 4	8

Effect of chemotherapeutic agents on growth of pancreatic xenografts. The effects of three chemotherapeutic agents in the injectable gel formulations were evaluated: 5-FU/epi gel, cisplatin/epi gel and DOX/epi gel. Eight animals were used for each drug-treated group and five animals were used for each control group. A series of three separate studies were performed. The study design for treatment is summarized in Table 1. The intratumoral administration of both controls and drugs was given in a volume of 100 µl. The final drug doses per injection per animal were as follows: 5-FU, 1.5 or 3.0 mg (~60 or 120 mg/kg); cisplatin, 150 or 200 µg (~6 or 8 mg/kg); and DOX, 125 or 200 µg (~5 or 8 mg/kg). Control groups consisted of untreated animals or animals receiving an intratumoral injection of either placebo gel or epi gel. In study 1, we evaluated the effects of 5-FU/epi gel, cisplatin/epi gel or DOX/epi gel on BxPC-3 tumor growth. All injections were administered intratumorally on days 1 and 4 of week 1, except for one group of animals that received the 5-FU/epi gel for two consecutive weeks on days 1, 4, 8 and 12. In study 2, we evaluated the antitumor efficacy of 5-FU/epi gel and cisplatin/epi gel when administered intraperitoneally and compared with those of 5-FU/epi gel administered intratumorally on days 1 and 4 for 1 week, and untreated controls. An additional group of animals in study 2 was treated sequentially with 5-FU/epi gel and then with cisplatin/epi gel. In study 3 we compared the growth inhibition of pancreatic xenografts injected intratumorally with 5-FU solution with untreated controls.

Tumors were measured weekly with Vernier calipers for length (L) and width (W). Tumor volume (V in mm^3) was calculated according to the formula: $V = L \times W^2 \times 0.5$, where L and W were the length and width measured in millimeters.

Twenty-eight days after the first treatment, the animals were sacrificed, and the tumors were dissected and weighed. Data are expressed as mean tumor weight or tumor volume ± SEM. Statistical analysis was performed with ANOVA and the nonparametric Mann–Whitney analysis.

Retention and distribution of radiolabeled 5-FU in tumor xenografts. A quantitative autoradiographic study was conducted to compare the retention and local distribution of radiolabeled 5-FU delivered intratumorally either as a simple drug solution or in the 5-FU/epi gel formulation as previously described by Kanekal *et al.*[30] The [^{3}H]5-FU was added as a tracer to the 5-FU solution or 5-FU/epi gel. A 50 µl (1 µCi) volume of either formulation was injected into the center of each pancreatic cancer xenograft (800–1000 mm^3). The mice were sacrificed 30 min after the injection. The tumor xenografts, as well as the liver and kidneys, were excised and immediately frozen in liquid nitrogen, then stored at −20°C until sectioning. Each tissue was cut entirely into serial sections (20 µm thick) using a Microm cryostat (HM505E) at −20°C. Tissue sections from various tumor depths were placed on glass slides, allowed to dehydrate in the cryostat chamber, then exposed along with ^{3}H standards to imaging plates for 72 h. The exposed plates were scanned and the radioactivity was quantified using a Fuji BAS 1000 phosphor imaging analyzer (Fuji Biomedicine Systems, Stamford, CT). The image analysis was performed on the entire area of tissue sections on the autoradiograms. The instrument response is expressed as photostimulated luminescence (*PSL*, corrected for background, *BG*), as a function of surface area (mm^2) of the tissue. The detection limit for tritium is approximately 2 d.p.m./mm^2 and measurements are linear up to 8000 d.p.m./mm^2. The total radioactivity in a tissue section is calculated using the formula: $(PSL-BG)/mm^2 = 0.052\ X^{0.903}$, where X is d.p.m./mm^2.

Results

In vitro chemosensitivity tests

The chemosensitivity of both BxPC-3 and PANC-1 pancreatic cancer cells after exposure to various concentrations of therapeutic agents was measured *in vitro*. The BxPC-3 cells were sensitive to all the drugs tested. Drug concentrations (µM) that inhibited growth of either 50% (IC_{50} (or 90%) IC_{90}) of the BxPC-3 cells after a 24 or 72 h exposure are shown in Table 2. The drugs are listed in order of potency at the 24 h exposure time. With the longer exposure time (72 h), an equal or higher concentration of the drug is necessary to kill 50 or 90% of the BxPC-3 cells with all the agents tested except for 5-FU, cisplatin and DOX. The concentration of 5-FU that inhibited growth of 50–90% of the cells after 72 h was approximately eight to 10 times less than the concentration required to inhibit growth of the same percentage of the cells at the shorter exposure time (24 h). Likewise, for DOX and cisplatin, lower drug concentrations were needed to inhibit growth of a comparable number of BxPC-3 cells longer exposure time: 15 to 20 times less for DOX and 1.2 to 1.5 less for cisplatin. Because these three drugs increased the potency with longer exposure time,

Table 2. *In vitro* chemosensitivity of human pancreatic cells[a]

Treatment	24 h exposure (μM)		72 h exposure (μM)	
Groups	IC_{50}	IC_{90}	IC_{50}	IC_{90}
BxPC-3				
floxuridine	0.40	0.80	2.50	>100.00
paclitaxel	0.50	0.88	0.52	0.93
vinblastine	0.52	0.90	0.60	9.20
mitomycin	0.50	1.00	0.60	1.00
DOX	0.75	7.70	0.05	0.34
cisplatin	5.00	9.20	2.00	8.25
5-FU	42.00	99.00	5.10	9.10
PANC-1				
mitomycin	1.25	21.50	1.25	25.50
5-FU	65.00	600.00	9.90	500.00

[a] 2×10^5 cells were placed into each 4 cm^2 well of 12-well plates. Drug exposure to attached cells was for 24 or 72 h.

it was thought that these agents would be particularly effective in the *in vivo* xenograft model when administered intratumorally in a sustained drug-release system.

Results of the *in vitro* chemosensitivity studies with PANC-1 cells to mitomycin and 5-FU are also shown in Table 2. No differences were observed in PANC-1 chemosensitivity to mitomycin by increasing the exposure time. In contrast, prolonged exposure to 5-FU increased chemosensitivity of PANC-1 cells. PANC-1, the poorly differentiated tumor, required a 26- and 55-fold higher concentration of mitomycin and 5-FU, respectively, to inhibit growth as compared to BxPC-3 cells.

In vivo antitumor efficacy

Growth studies on pancreatic cancer xenografts. The results on antitumor efficacy following the drug treatments are shown in Figures 1–3. Throughout these experiments, there were no differences in body weight changes among all the groups. Intratumoral injections of either placebo gel or epi gel produced no antitumor effect and the average tumor weight of mice in these control treatment groups was similar to that of the untreated

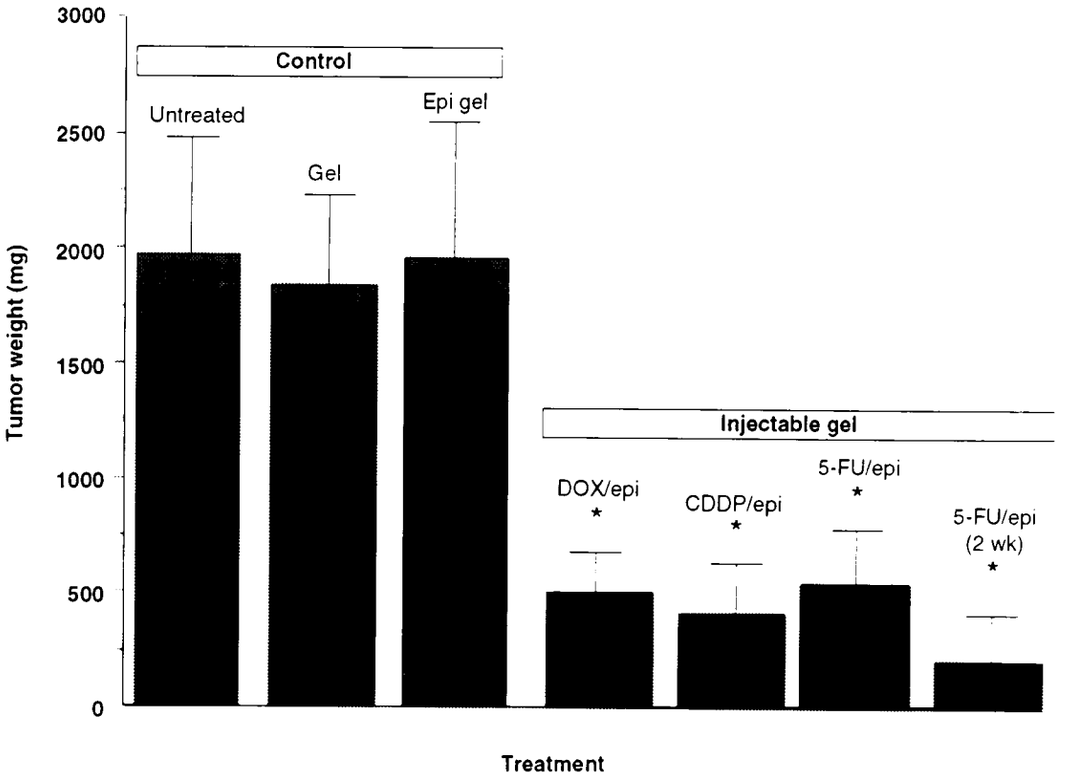

Figure 1. Effects of intratumoral chemotherapy administered in injectable gels on BxPC-3 human pancreatic cancer xenografts in athymic mice. Effects of DOX (8 mg/kg), cisplatin (CDDP, 8 mg/kg) and 5-FU (60 mg/kg) are compared with controls in tumors removed and weighed at day 28 after the first treatment. Values shown are mean ± SEM of tumor weights of five to eight mice. Significant decreases in tumor weight compared with controls are represented ($^*p < 0.05$).

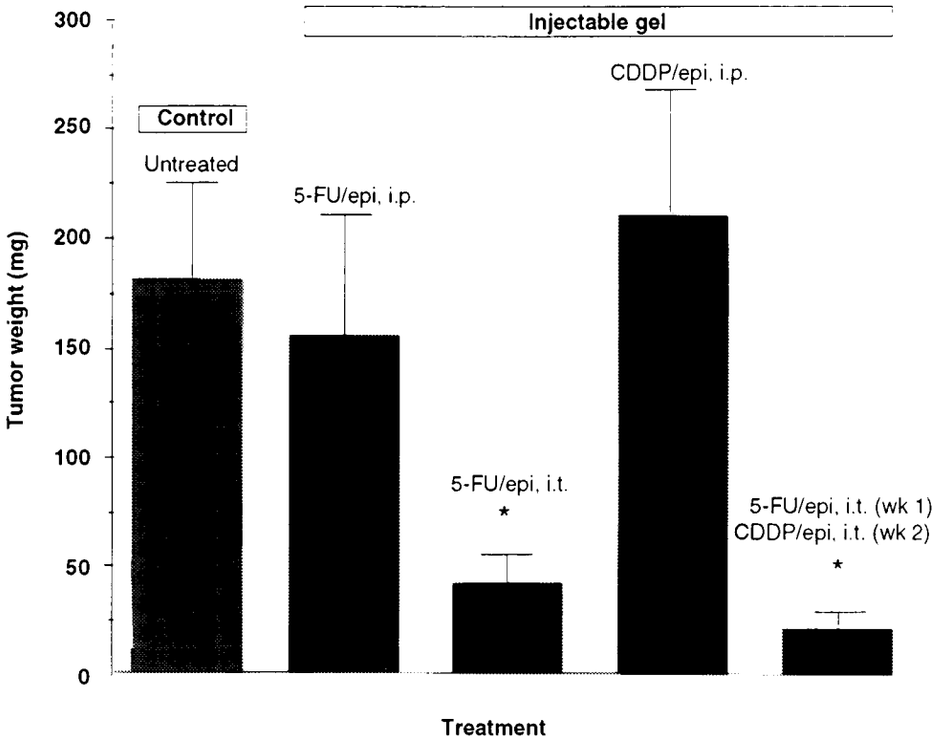

Figure 2. Comparison of the intratumoral and intraperitoneal chemotherapy administered in 5-FU/epi gel (60 mg/kg) and cisplatin/epi gel (8 mg/kg). Intratumoral 5-FU/epi gel produces a significant reduction of tumor growth ($^*p < 0.05$) compared with untreated control or intraperitoneal administration. Although sequential intratumoral administration of both drugs reduced tumor weight compared to controls ($^*p < 0.05$), the reduction was not significantly different than that achieved using the 5-FU/epi gel intratumorally alone.

control mice at the end of the study period (Figure 1). The mean tumor weights in animals treated with cisplatin/epi gel (6 mg/kg) and DOX/epi gel (5 mg/kg) were also similar to those of control animals (data not shown). Although 5-FU/epi gel (120 mg/kg) significantly reduced tumor volume ($p = 0.03$, data not shown), four of the eight animals in this treatment group died before day 28. Autopsies failed to reveal metastatic disease, hence suggesting 5-FU toxicity.

Tumors in animals treated with intratumoral injections of 5-FU/epi gel (5-FU: 60 mg/kg), cisplatin/epi gel (cisplatin: 8 mg/kg) and DOX/epi gel (DOX: 8 mg/kg) on days 1 and 4 during week 1 decreased in size by 72–79% (Figure 1). This decrease was significantly different ($p < 0.05$) from the tumors in the untreated control, placebo gel control or epi gel control animals. Animals treated intratumorally with the 5-FU/epi gel for two consecutive weeks showed an additional 62% reduction in tumor weight as compared to the animals that received 5-FU/epi gel (60 mg/kg) for only 1 week. However, three of the eight animals that received the 5-FU/epi gel for 2 weeks died on day 26 possibly due to 5-FU toxicity.

When 5-FU/epi gel (5-FU: 60 mg/kg) and cisplatin/epi gel (cisplatin: 8 mg/kg) were administered intraperitoneally, there were no differences in tumor weights compared with untreated controls at the end of the study (Figure 2). However, intratumoral drug delivery of the 5-FU/epi gel alone or in combination with cisplatin/epi gel produced significant tumor weight reductions in comparison to 5-FU/epi gel ($p < 0.001$) or cisplatin/epi gel ($p < 0.001$) administered intraperitoneally. Intratumor 5-FU/epi gel ($p < 0.001$) or 5-FU/epi gel followed by cisplatin/epi gel ($p < 0.001$) produced significant reductions in tumor weight when compared with untreated controls. The sequential therapeutic treatment also produced an approximate 52% tumor weight reduction as compared to 5-FU/epi gel alone, suggesting a therapeutic advantage, but this difference was not statistically significant ($p = 0.07$).

Tumor volumes measured over 28 days after the intratumoral injection of 5-FU solution are shown in Figure 3. This treatment produced no antitumor ac-

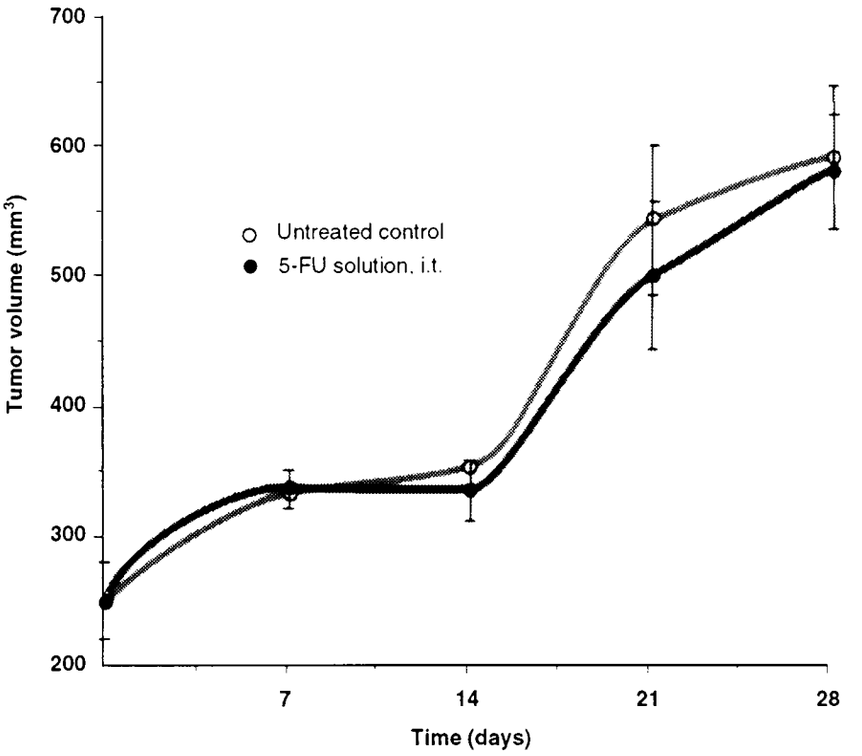

Figure 3. Effects of intratumoral injection of free drug on BxPC-3 human xenografts. Intratumoral administration of free 5-FU solution shows no antitumor activity (mean ± SEM for tumor volume) over 28 days after treatment after two doses (60 mg 5-FU/kg) compared with untreated controls.

tivity and the tumor growth rate was comparable to that of the untreated control tumors.

Visualization and quantification of retention of radiolabeled 5-FU after intratumoral injection in tumor xenografts. Representative autoradiograms of tumors treated with [^{3}H]5-FU/epi gel and [^{3}H]5-FU solution showed localization of radiolabeled drug at the site of injection, i.e. center of the tumor (Figure 4). Total radioactivity (d.p.m.) in each tissue section was calculated from the quantitative profile analysis. At 30 min after drug injection, the radioactivity in tissue sections of the tumor treated with [^{3}H]5-FU/epi gel was 11 108 d.p.m. compared with 1327 d.p.m. per tissue section from the tumor treated with drug solution. Thus, delivery of [^{3}H]5-FU in the injectable gel formulation resulted in 8.4-fold greater radioactivity, reflecting increased drug retention and localization in tumor.

When the kidney and liver of mice with treated xenografts were examined, the autoradiograms revealed substantial amounts of [^{3}H]5-FU at 30 min post-treatment in the organs of mice treated with 5-FU solution; radioactivity levels in mice treated with 5-FU/epi gel were close to background levels.

Discussion

The present work demonstrates the first evidence that human pancreatic cancer is sensitive to chemotherapeutic agents when administered intratumorally in a novel drug delivery system (therapeutic injectable gel). The therapeutic injectable gel provided high tumoral drug concentrations in the xenografts not obtainable by either intratumoral administration of drug solution or intraperitoneal administration of gel. Because patients with pancreatic cancer usually succumb to the local invasiveness of this tumor into surrounding vital organs and vessels rather than distant metastases, intratumoral drug delivery via the injectable gel represents a potential therapeutic approach to achieve local tumor control or tumor debulking. Percutaneous administration of therapeutic gel under ultrasound guidance

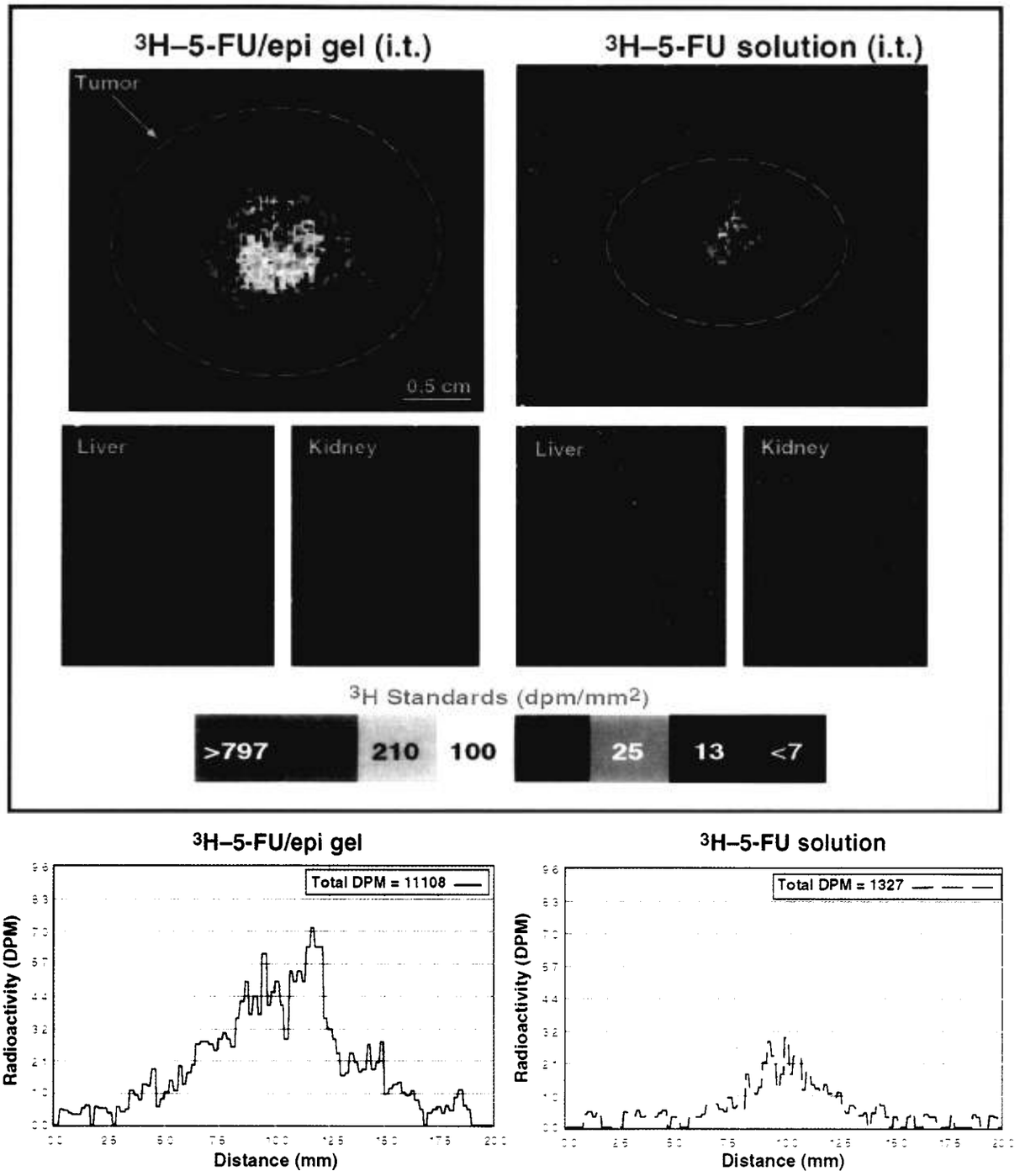

Figure 4. Comparison of [^{3}H]5-FU retention in human pancreatic cancer xenografts at 30 min after intratumoral administration either of radiolabeled 5-FU/epi gel or 5-FU solution. A dose of 50 μl of drug was injected into each tumor. These representative autoradiograms of frozen tissue sections (20 μm thick) from treated tumor, liver and kidney of each animal were obtained by scanning the radioactivity on a phosphor imaging bioanalyzer. The highest level of radioactivity is shown as red, the lowest levels as blue or black. The graphs show d.p.m./mm^2. Administration of 5-FU in the sustained-release gel formulation results in greater drug localization and retention.

has been demonstrated to be feasible in patients with liver cancer.[26] For pancreatic cancer, a similar percutaneous administration technique under ultrasound could be used, as is also done for percutaneous aspiration cytology, or the gel could be initially injected intraoperatively, especially in patients found to have non-resectable cancer.

Several chemotherapeutic agents were first exposed to cells *in vitro* at both 24 and 72 h. Although BxPC-3 cells appeared to be chemosensitive to floxuridine, paclitaxel, vinblastine and mitomycin after 24 h of exposure, higher concentrations were needed to kill the same percentage of cells after 72 h of exposure. One possible explanation may be the rapid development of drug resistance in BxPC-3 cells with the longer exposure time. The three drugs that increased in potency with longer exposure times (doxorubicin, cisplatin and 5-FU) were the drugs selected to test *in vivo* because the intratumoral administration of therapeutic injectable gel simulates the *in vitro* test conditions. Chemosensitivity was not unique to BxPC-3 pancreatic cancer cells, but was also observed in the poorly differentiated PANC-1 human pancreatic cancer cells where higher drug concentrations were needed to kill the same percentage of cancer cells. Hence, a more histologically aggressive tumor requires a greater amount of drug to kill the same number of cells.

In the *in vivo* studies we used a new drug delivery system in which chemotherapeutic agents are administered intratumorally in a sustained-release, therapeutic injectable gel. The therapeutic gels are intended to improve local tumor control by enhancing the concentration of active drug in the tumor, compared with that of systemic chemotherapy. The therapeutic gel is a viscous gel composed of a chemotherapeutic drug and epinephrine (epi) as an adjuvant that are formulated with a biodegradable protein carrier matrix of purified bovine collagen. Collagen acts as a gellant that incorporates the drug within the spaces of the triple-helix molecular structure, retards the rate of drug diffusion and maintains a high drug dose exposure to tumor cells.

Although the carrier matrix gel (collagen) and vasoconstrictor epinephrine when formulated with drug contribute to make chemotherapeutic agents efficacious in the tumor models, these agents alone had no effect on tumor growth. Free drug (5-FU) alone given to BxPC-3 tumors also had no effect on tumor size. However, the combination of the chemotherapeutic agent with the gel and epinephrine is cytotoxic. This gel formulation allows for the maintenance of higher intratumoral concentrations of drug as we have previously shown[19] without the toxicity because peripheral blood levels are lower. High drug concentrations are thought to be achieved secondary to the local vasoconstriction from epinephrine and the slow drug release due to the collagen base. Therapeutic gels have shown efficacy in animal[21,22] and human cancers[23–27] in which drug is administered intratumorally by direct needle injection, endoscopically in a manner similar to biopsy procedure, or with assistance of computerized tomography or ultrasound visualization.

Antitumor response in the *in vivo* model was also dose dependent. The higher fluorouracil dose (120 mg/kg) was toxic and resulted in 50% mortality. The lower doses of doxorubicin and cisplatin in the injectable gel (5 and 6 mg/kg, respectively) did not significantly inhibit the growth of BxPC-3 xenografts, whereas higher doses were effective. Hence, a therapeutic index exists where the proper concentration of drug is necessary to exert a therapeutic effect, but excessive drug may be toxic.

Conclusions

In this study, we demonstrated that the retention of tritium label in the [^{3}H]5-FU/epi gel-injected tumors was significantly greater and longer than in those treated with [^{3}H]5-FU solution. The amount of radioactivity detected in the corresponding liver and kidney of these gel-treated animals thus was significantly lower, indicating a lesser drug exposure or systemic toxicity. These resultant higher sustained tumor drug concentrations, as expected, enhanced the antitumor activity in this xenograft model. In contrast, intratumoral or intraperitoneal injection of similar doses of free drugs had no effect. This suggests that greater efficacy may be achieved using local therapy with therapeutic gels than using a systemic approach. Although drug synergism was not fully explored in the xenograft model, it is interesting to note that the combination sequential therapy with 5-FU/epi gel and cisplatin/epi gel did *not* produce a significantly greater growth inhibition than 5-FU/epi gel alone at the doses and schedules used here. In the human pancreatic cancer clinical experience with systemic treatment, only a slight improvement in response rate was reported with combination chemotherapy. Thus, the pancreatic cancer xenograft model and the human pancreatic cancer clinical experience may be similar with regard to combination chemotherapy.

Pancreatic cancer carries the worse prognosis of all gastrointestinal cancers with a marked clinical resistance to systemic chemotherapy. Because pan-

creatic cancer is locally invasive, a new local drug delivery system such as therapeutic injectable gel may prove to be useful in the treatment of human subjects with pancreatic cancer.

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