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Impact of formulation on the iontophoretic delivery of the FOLFIRINOX regimen for the treatment of pancreatic cancer

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Abstract

Purpose—Effective treatment of patients with locally advanced pancreatic cancer is a significant unmet clinical need. One major hurdle that exists is inadequate drug delivery due to the desmoplastic stroma and poor vascularization that is characteristic of pancreatic cancer. The local iontophoretic delivery of chemotherapies provides a novel way of improving treatment. With the growing practice of highly toxic combination therapies in the treatment of pancreatic cancer, the use of iontophoresis for local delivery can potentiate the anti-cancer effects of these therapies while sparing unwanted toxicity. The objective of this study was to investigate the impact of

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Conflicts of Interest

J.D.B., J.M.D., and J.J.Y. hold equity in the start-up company, Advanced Chemotherapy Technologies L.L.C.

Associated Content

Supporting Information

Iontophoretic transport of the FOLFIRINOX formulations characterized according to liver and kidney exposure after a single device treatment.

formulation on the electrotransport of the FOLFIRINOX regimen for the development of a new treatment for pancreatic cancer.

Methods—Three formulations of the FOLFIRINOX regimen (5-fluorouracil, leucovorin, irinotecan, and oxaliplatin) were generated at a fixed pH of 6.0 and were referred to as formulation A (single drug solution with all four drugs combined), formulation B (two drug solutions with two drugs per solution), and formulation C (four individual drug solutions). Anodic iontophoresis of the three different formulations was evaluated in orthotopic patient-derived xenografts of pancreatic cancer.

Results—Iontophoretic transport of the FOLFIRINOX drugs was characterized according to organ exposure after a single device treatment *in vivo*. We report that the co-iontophoresis of two drug solutions, leucovorin + oxaliplatin and 5-fluorouracil + irinotecan, resulted in the highest levels of cytotoxic drugs in the tumor compared to drugs delivered individually or combined into one solution. There was no significant difference in plasma, pancreas, kidney, and liver exposure to the cytotoxic drugs delivered by the three different formulations. In addition, we found that reducing the duration of iontophoretic treatment from 10 to 5 minutes per solution resulted in a significant decrease in drug concentrations.

Conclusions—Underlying the difference in drug transport of the formulations were electrolyte concentrations, which includes both active and inactive components. Electrolyte concentrations can hinder or improve drug electrotransport. Overall, balancing electrolyte concentration is needed for optimal electro-transport.

Keywords

iontophoresis; FOLFIRINOX; pancreatic cancer; device delivery; chemotherapy

Introduction

Pancreatic cancer is among the most devastating cancers because of the very low survival rate and poor response to therapy [1]. Current treatment options for patients with pancreatic cancer include surgical resection, systemic chemotherapy, and radiation. Surgical resection offers the best chance of cure, but only 15% of patients have resectable disease at the time of diagnosis. Approximately 40% of patients are diagnosed with locally advanced, unresectable disease due to the anatomical involvement of the tumor [2]. The 5-year overall survival rate in all patients with pancreatic cancer is less than 5% [1].

The microenvironment of pancreatic tumors limits the delivery of anticancer drugs, especially to cells that are situated distal to functioning blood vessels [3–4]. Stroma acts as a major resistance mechanism to systemically administered cytotoxic treatments [4]. To circumvent the problems associated with limited drug transport into pancreatic tumors, we have designed an implantable iontophoretic device that can be adapted to deliver cytotoxic chemotherapies directly to pancreatic tumors [5–6]. The application of an electrical potential can drive drug molecules into poorly vascularized tissues, including tumors, at higher concentrations than achievable by systemic administration [7–8].

One tremendous advantage of the iontophoretic device is the delivery of agents that are limited by systemic toxicity. A recently identified first-line treatment for pancreatic cancer known as FOLFIRINOX is a promising mixture of cytotoxic agents, including folinic acid (leucovorin), 5-fluorouracil, irinotecan, and oxaliplatin; however, it is not suitable for all patients due to its degree of toxicity when delivered intravenously [9]. Therefore, iontophoretic device delivery of FOLFIRINOX would have the potential to increase the proportion of patients able to receive this therapy and ultimately may provide an adjunct for better local control in the percentage of patients undergoing surgical resection. In addition, for patients with metastatic disease and debilitating local symptoms, this device treatment can provide a palliative modality to improve symptom control.

The use of combination chemotherapies has yet to be fully explored for iontophoretic treatments. The aim of our study was to elucidate the effect of formulation on the electrotransport of the drugs that make up FOLFIRINOX, so as to optimize delivery efficiency. We evaluated the transport of these formulations in pancreatic cancer patient-derived xenografts (PDX), which maintain the same tumor heterogeneity and stromal network as human tumors [1, 10]. We report that the iontophoretic delivery of the combination of drugs divided into two solutions results in higher levels of cytotoxic drugs in the tumor compared to drugs delivered individually or combined in one solution. The co-iontophoresis of FOLFIRINOX represents a promising strategy for the local delivery of this highly toxic chemotherapy regimen.

Materials and Methods

Materials

Platinum foil (0.025 mm thickness) was acquired from Strem Chemicals. Four-lumen medical-grade polyurethane tubing was custom-made by A.P. Extrusion. Steryalloy 2056 polyurethane for the device reservoir was obtained from Hapco Inc. All other materials, including 32 gauge stainless steel wire, 14,000 Dalton (kDa) cellulose membrane, and chemicals for analysis, were obtained from Fisher Scientific. All drugs used for the formulations, including leucovorin (Sagent Pharmaceuticals), 5-fluorouracil (APP Pharmaceuticals), irinotecan (Sun Pharmaceuticals), and oxaliplatin (Hospira), were obtained directly from the University of North Carolina (UNC) hospital pharmacy.

Device Fabrication

The fabrication and testing of the device have been previously described [5–6]. Briefly, 5 millimeter (mm) disks were cut from 0.1 mm thick platinum foil using a 5 mm hole punch. The platinum disk was soldered to a stainless steel cable wire and embedded in a polyurethane reservoir generated from a custom-made mold. The steel wire was then threaded through a multi-luminal polyurethane tubing, and a semipermeable 14 kDa cellulose membrane was adhered for enclosure of the reservoir. All connections were reinforced with polyurethane and cured at 60 °C for at least 4 hours. The device was designed for intra-abdominal implantation with external access to power and drug supply through the nape of the animal (Figure 1). The reservoir-based system allowed for the

delivery of multiple drug solutions sequentially without risk of cross-contamination. This ability to administer a variety of drug solutions enabled the study of different formulations.

Drug Formulation

The formulations were generated using commercially available and approved drugs from the UNC pharmacy. All formulations were fixed at a pH of 6.0, and the drug concentration in each formulation was based upon the maximum concentration that each drug was stable and active at pH 6.0. Three formulations were generated and tested: formulation A was a single drug solution with all four drugs combined, formulation B was two drug solutions with two drugs per solution, and formulation C was four individual drug solutions. Formulation A was generated as a single solution: leucovorin was dissolved using oxaliplatin solution, and irinotecan and 5-fluorouracil solutions were subsequently added. Hydrochloric acid was used to lower the pH of the solution to 6.0 to limit conversion of irinotecan to its inactive form. Formulation B was generated as two solutions: (1) leucovorin was dissolved using an oxaliplatin solution; (2) irinotecan solution was added to a 5-fluorouracil solution, with hydrochloric acid being used to lower the pH to 6.0. Formulation C involved (1) dissolution of 50 milligrams (mg) leucovorin in 10 milliliters (ml) of sterile water; (2) oxaliplatin was used directly from the clinical formulation; (3) 5-fluorouracil was diluted with sterile water and the pH reduced to 6.0 using hydrochloric acid, and (4) irinotecan was diluted with sterile water and the pH brought to 6.0 using sodium hydroxide. The final concentrations of the drugs in the formulations can be found in Table 1. The mass of the excipients can be found in Table 2. Formulation parameters including drug concentration and pH were held constant, except for formulation A, which had lower irinotecan and oxaliplatin concentrations due to the use of clinical drugs for generating the formulation. The pH of the drug solutions was chosen to maximize electroosmotic drug transport. Lastly, leucovorin was not quantified due to being a non-cytotoxic component of FOLFIRINOX. Intravenous (IV) FOLFIRINOX was included for comparison as the standard method of delivery; IV dosing was adjusted from clinical dosage in accordance with prior maximum tolerated dose studies [11–13]. The drugs were delivered via tail vein injection and dosed at 100 mg/kilogram (kg) leucovorin, 5 mg/kg oxaliplatin, 50 mg/kg 5-fluorouracil, and 50 mg/kg irinotecan. These drug concentrations were lower than the concentrations used in clinical practice [9].

Animal Studies

All studies involving animals were approved by the appropriate Institutional Animal Care and Use Committee before initiation. Devices were surgically implanted onto the orthotopic pancreatic tumors when they reached a median volume of 280 mm³ (range 180–392 mm³) as previously described [6]. Treatment was started 1 week post-implantation. Figure 1 shows the device setup used for the *in vivo* studies. The positive lead was connected to the device, and the negative lead was connected to a silver chloride electrode placed on the skin. Drug solutions were administered into the device at a flow rate of 50 µl/min; the volume of the drug reservoir was 7 mm³. Drug solution circulated into and out of the device reservoir, which allowed for continuous replenishment of the drug at the electrode surface. The pH of the drug solution was not monitored on flow out of the system. When the constant current was applied using a direct current power system, drug was driven from the device reservoir into the tumor tissue. To compare the formulations, 2 milliAmps (mA) of current was

applied for 10 minutes per drug solution with a 5 minute washout period between drug solutions. The time of current application was also evaluated for formulation B, where 2 mA of current was applied for 5 or 10 minutes per drug solution with a 5 minute washout period between drugs solutions. Data generated for formulation B has been previously reported and has been incorporated for comparison [6].

Drug Quantification

For quantitation of drug plasma and organ concentrations, blood samples were collected into K3-EDTA tubes and plasma was generated and frozen with liquid nitrogen; tissue and device were extracted and snap frozen with liquid nitrogen and stored at -80°C . Intact tissues and sectioned tumors were diluted in a 1:3 ratio with double distilled (dd)H₂O and homogenized using zirconium oxide beads (Omni International, Inc, Kennesaw GA) in 2 ml polypropylene tubes using a Precellys 24 homogenizer (Omni international, Inc).

Irinotecan—samples were processed by protein precipitation using acetonitrile/0.1% formic acid containing 300 ng/ml CPT internal standard [14]. The supernatants were dried under nitrogen and reconstituted in 50% acetonitrile/0.1% formic acid for analysis by liquid chromatography-mass spectrometry (LC-MS). Irinotecan and SN-38 were spiked together into blank mouse plasma (CD-1, K3-EDTA, Bioreclamation IVT, Hicksville, NY) to generate the standard curves (1–5000 nanogram (ng)/ml) and QCs (4, 40, 400, and 4000 ng/ml), and processed as described above. Additionally, quality controls (QCs) were prepared (400 ng/ml) in blank tissue homogenates for each matrix evaluated, and a correction factor applied to adjust final concentrations for matrix effects when necessary. Compounds were separated on an XBridge C18 column (2.5 micrometer (μm), 2.1×50 mm; Waters, Milford, MA) fitted with an XBridge C18 guard column (2.5 μm , 2.1×10 mm; Waters) using 0.1% formic acid (mobile A) and a gradient of 10% to 45% acetonitrile/0.1% formic acid (mobile phase B) over 2 min on an LC-20AD liquid chromatograph (Shimadzu Corporation, Columbia, MD). Analytes were detected using either an Exactive or LTQ-Orbitrap Discovery mass spectrometer (Thermo Fisher Scientific, Waltham, MA) in the positive-ion, full-scan mode. The peak areas for each analyte and internal standard were extracted from the ion chromatogram and standard curves generated by linear regression from the subsequent response ratios with a 1/X² weighting using Xcalibur software (Thermo Fisher Scientific).

5-fluorouracil—Samples were spiked with 5 $\mu\text{g}/\text{ml}$ 5-chlorouracil internal standard and processed by an ammonium sulfate precipitation (4.1 Molar (M)) followed by extraction with ethyl acetate: isopropanol (10:1) [15]. The supernatants were dried under nitrogen, reconstituted in 100 μl of 1 mM ammonium acetate, pH 5.0, and passed through a 0.2 mm GHP filter (Pall Corporation, Ann Arbor, MI) for analysis by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). 5-fluorouracil was spiked into blank mouse plasma (CD-1, K3-EDTA)(Bioreclamation IVT) to generate the standard curves (1–1000 ng/ml) and QCs (25, 75, 750 ng/ml), and processed as described above. Compounds were separated using an Atlantis T3 column (3 μm , 2.1×50 mm; Waters) fitted with an Atlantis T3 guard column (Waters) using 1 mM ammonium acetate, pH 5.0 (mobile A) and a gradient of 2–75% MeOH: ACN (1:1) (mobile phase B) on an LC-20AD liquid

chromatograph (Shimadzu). Analytes were detected using a TSQ Ultra triple quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with a heated ESI source in the positive-ion mode. 5-fluorouracil and 5-chlorouracil were measured by selected-ion monitoring using the transitions 129->42 and 145->42, respectively, at a collision energy of 17 Volts. Calibration curves were generated in XCalibur (Thermo Fisher Scientific) using linear regression and a 1/X² weighting.

Oxaliplatin—samples were processed by direct acid digestion using 200 µl of 70% HNO₃ containing 50 ng/mL of Iridium (Inorganic Ventures, Christiansburg, VA) external standard added to 75 microliter (µl) of plasma or tissue homogenate [16]. Samples were heated at 90 °C for 1.5 hours and then brought to final volume (HNO₃ concentration of 3.5%) with deionized water and stored at 4 °C until analysis. Inductively-coupled plasma mass spectroscopy (ICP-MS) elemental analysis was performed on an Agilent 7500cx (Agilent Technologies, Tokyo, Japan). Instrumental parameters were optimized daily to obtain best sensitivity before analysis was performed. The lower limit of detection for platinum and iridium was 1.0 ng/ml. Indium was used for the external instrument standard.

Statistical Analysis

Unpaired t tests were used to make comparisons of continuous values between groups. Unadjusted p values were reported for pairwise comparisons when an overall difference was detected.

Results

Iontophoretic transport of the FOLFIRINOX drugs was characterized according to organ exposure after a single device treatment *in vivo*. Drug delivery into the tumor using formulation B was superior to formulation A and C for all of the cytotoxic drugs (211.14 vs. 47.17 and 105.46 µg/g for 5-fluorouracil, 21.68 vs. 9.22 and 1.73 µg/g for irinotecan, and 16.29 vs. 9.99 and 5.22 µg/g for oxaliplatin), as seen in Figure 2. The average tumor penetration distances for FOLFIRINOX were not able to be quantified due to the amount of tissue required for measurement of the 3 cytotoxic drugs. Plasma levels of the 3 cytotoxic drugs delivered by formulations A, B, and C were remarkably similar (0.09, 0.17, and 0.39 µg/ml for 5-fluorouracil, 0.0, 0.0, and 0.30 µg/ml for irinotecan, and 0.01, 0.0, and 0.02 µg/ml for oxaliplatin, respectively). There was no significant difference in pancreas, kidney, and liver exposure to the cytotoxic drugs between formulations A, B, and C, as shown in Figure 2 and Supplemental Figure 1. In addition, there was no toxicity noted as a result of the treatment.

The duration of current application was evaluated for the *in vivo* transport of formulation B. We found that reducing the duration of iontophoretic treatment from 10 to 5 minutes per solution resulted in a significant decrease in drug concentrations (211.14 vs. 102.56 µg/g for 5-fluorouracil, 21.68 vs. 9.23 µg/g for irinotecan, and 16.29 vs. 7.19 µg/g for oxaliplatin) shown in Figure 3.

Discussion

Over the last 40 years, systemic chemotherapies have been shown to be largely ineffective in the treatment of pancreatic cancer [1]. Surgery remains the only potentially curative treatment for pancreatic cancer. However, the occurrence of relapse is common. The role of chemoradiotherapy is also increasingly being questioned for use in locally advanced pancreatic cancer [17–18]. There is a need for new treatments, such as iontophoretic devices and local drug eluting matrices, to improve resection rates and reduce recurrence [5–6, 19–20]. Through better delivery of cytotoxic agents, we may be able to increase the number of patients that could undergo tumor resection and thus improve long-term patient outcomes.

The aim of this study was to investigate the impact of formulation on the electrotransport of the FOLFIRINOX regimen for the development of a new treatment for pancreatic cancer. Given that the co-administration of multiple systemic chemotherapies is routinely performed, it is important to understand the optimal electrotransport conditions of multiple drugs for the delivery of clinically relevant drug regimens. Here, we tested three different formulations, including a single drug solution with all four drugs, two solutions with two drugs each, and four individual drug solutions. We showed that the co-iontophoresis of FOLFIRINOX in two solutions increased intratumoral drug concentrations compared to iontophoresis of each individual cytotoxic agent or all agents together in one solution. Systemic exposure was found to be minimal for all three formulations. The time of current application was also evaluated for one of the formulations and was found to be directly proportional to the amount of drug delivered. It was shown that both drug formulation and time of current application play important roles in the electrotransport of chemotherapies.

The FOLFIRINOX regimen offered a unique formulation challenge due to the number of drugs, drug stability in combination, and balance between drug electroosmosis and electromigration under an applied electric current. Given the clinical translatability of the treatment, the primary focus was on using clinically available formulations with very little manipulation of the drug regimens. At the pH used in these studies, the degree of ionization of each drug largely favored electroosmotic transport using anodal iontophoresis. Irinotecan was the only drug in this regimen that was positively charged (pKa of 8.79) [21].

Underlying the difference in drug transport of the formulations were electrolyte concentrations, which includes both active and inactive components. Electrolyte concentrations can hinder or improve drug electrotransport [22]. The inactive ingredients in the commercially available formulations making up the FOLFIRINOX regimen largely consisted of sodium chloride and sodium hydroxide [23–26]. Electroosmotic flux has been found to decrease sharply as the electrolyte concentration increases, which explains the improved transport in the two drug solutions of Formulation B compared to the one drug solution of Formulation A [22]. However, there must be enough electrolytes in solution to allow for electroosmotic transport, which is likely the reason for the inferiority of Formulation C. Overall, balancing electrolyte concentration is needed for optimal electrotransport. The intra-tumoral drug concentrations achieved for all three formulations were above the published half maximal inhibitory concentrations (IC₅₀) values for most pancreatic cancer cell lines [27–29].

Total drug transport of each formulation could be further improved by altering drug counterions or excipients, increasing drug concentration in the solutions, and extending the time of current application. Although our preliminary investigation into a single drug solution (formulation A) showed promise, the irinotecan and oxaliplatin concentrations in the pre-experiment solution were less than the drug concentrations used in formulation B and C, which warrants additional testing. As determined in this study, the time of current application directly impacts the amount of drug delivered, and by extending the time of current application, the effectiveness of the treatment may be improved by delivering more drug.

The local iontophoretic delivery of chemotherapies may be applied to a variety of other cancers, including sarcomas, gastroesophageal, head and neck, and recurrent rectal cancers. By reducing systemic chemotherapy exposure, iontophoresis may allow more patients to tolerate therapy; in addition, new systemic regimens can be evaluated in combination with iontophoresis for further additive effect. This device treatment has tremendous potential to improve overall outcomes in pancreatic cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

FOLFIRINOX	<u>F</u> olinic acid, 5- <u>F</u> luorouracil, <u>I</u> rinotecan, and <u>O</u> xaliplatin
UNC	University of North Carolina
LC-MS	liquid chromatography-mass spectrometry
QC	quality controls
ICP-MS	inductively-coupled plasma mass spectroscopy

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Figure 1.

Iontophoretic device used for the local delivery of the FOLFIRINOX formulations. (A) Front and side images of the device. (B) Device treatment setup where the drug solution is supplied to the device using a syringe pump and electrical current via a direct current (DC) power supply. Abbreviations: LV – leucovorin, FU – 5-fluorouracil, OX – oxaliplatin, and IR – irinotecan.

Figure 2.

Iontophoretic transport of the FOLFIRINOX formulations was characterized according to organ exposure after a single device treatment. Organs were collected from each animal at 10 minutes after the last treatment, and total drug concentrations were analyzed. *P* values were determined by unpaired *t* test. Data are means $\pm$ SD ($n = 4 - 5$ animals per group). Limit of quantitation for oxaliplatin was 1 ng/ml; limits of quantitation for irinotecan and 5-fluorouracil were 30 ng/ml.

Figure 3.

Iontophoretic transport of formulation B as a function of time was characterized according to tumor exposure after a single device treatment. *P* values were determined by unpaired *t* test. Data are means $\pm$ SD (*n* = 4 – 5 animals per group).

Table 1.

Formulations of the FOLFIRINOX regimen.

Formulation	Drug	Drug Concentration	Number of Drug Solutions
A	Leucovorin	10 mg/ml	1
	Oxaliplatin	1.8 mg/ml	
	5-fluorouracil	14 mg/ml	
	Irinotecan	7.2 mg/ml	
B	Leucovorin	10 mg/ml	2
	Oxaliplatin	5 mg/ml	
	5-fluorouracil	14 mg/ml	
	Irinotecan	14.4 mg/ml	
C	Leucovorin	10 mg/ml	4
	Oxaliplatin	5 mg/ml	
	5-fluorouracil	14 mg/ml	
	Irinotecan	14.4 mg/ml	

Table 2.

Excipients in each clinically available drug of the FOLFIRINOX regimen, as found in the package inserts.

Drug	Excipient	Excipient Mass
Leucovorin	Calcium	3.92 mg
	Sodium chloride	*
	Hydrochloric acid or sodium hydroxide	*
Oxaliplatin	Tartaric acid	*
	Sodium hydroxide	*
5-fluorouracil	Sodium hydroxide	*
Irinotecan	Chloride	1.04 mg
	Sorbitol	45 mg
	Lactic acid	0.9 mg
	Sodium hydroxide	*
	Hydrochloric acid	*

* Titrated according to pH and amounts not directly stated.