Intraarterial therapy using micellar nanoparticles incorporating SN-38 in a rat pancreatic tumor model

Abstract

Purpose

To evaluate advantages of micellar nanoparticles encapsulating SN-38, a biologically active metabolite of irinotecan, in intraarterial therapy for pancreatic cancer.

Materials and Methods

Rat pancreatic cancer cells (DSL-6A/C1) were implanted in Lewis rats under laparotomy. This study consists of two parts. Firstly, after confirming tumor formation by ultrasonography, celiac arteriography was performed, and tumor blood supply was visually evaluated by dye injection and CT during arteriography. Secondly, 18 rats were divided into two groups; the Micellar Nanoparticles group and the Irinotecan Infusion group. Micellar nanoparticles or irinotecan was injected via the celiac artery, and SN-38 and irinotecan concentrations in the tumor, duodenum and pancreatic parenchyma, were measured at 5 minutes, 6 hours and 24 hours.

Results

The maximum concentration (Cmax) of SN-38 were shown at 6 hours in the Micellar Nanoparticles group, while Cmax of irinotecan was shown at 5 minutes in the Irinotecan Infusion group. Tumor concentration in the Micellar Nanoparticles group maintained elevated for 24 hours without significant decrease (P=0.068). Conversely, a significant decrease was observed in the regular pancreas parenchyma (P=0.006) and duodenum (P=0.028). In the Irinotecan Infusion group, tumor irinotecan concentration significantly decreased at 24 hours (P=0.016).

Conclusion

Micellar nanoparticles may improve arterial infusion chemotherapy for pancreatic cancer. These nanoparticles have the potential to reduce SN-38 accumulation in duodenum while increasing it in the tumor.

Further research is warranted to validate and expand upon these findings.

Introduction

Pancreatic cancer is one of the most lethal and aggressive human malignancies, which is the fourth most common cause of cancer-related deaths [1]. Surgical treatment has the best long-term survival rate, but only 5-25% of patients can undergo radical surgery at diagnosis, and the overall 5-year survival rate for pancreatic cancer is around 10% [2]. Systemic chemotherapy such as FOLFIRINOX and gemcitabine plus nab-paclitaxel have been used for unresectable pancreatic cancer, but their clinical efficacy and results have not been satisfactory. The response rates of around 20-30 % were reported [3].

Arterial infusion chemotherapy has been reported as an effective treatment for unresectable pancreatic cancer with high response rates of around 70% that are not achievable with systemic chemotherapy [4-7]. However, achieving a high concentration of anticancer drugs in tumors presents a challenge due to the hypovascular nature of pancreatic cancer. Moreover, the inevitable distribution of drugs in the duodenum and adjacent normal pancreatic parenchyma raises concerns about side effects, such as duodenal ulcers and vomiting, along with the potential risk of pancreatitis [8].

Development of drug delivery system (DDS) formulations for solid tumors has been driven by the concept of enhanced permeability and retention (EPR). Polymeric materials encapsulating anticancer drugs can leak easily in tumor vessels with increased permeability and remain in tumor tissues with poor lymphatic collection system for a long time, thereby enhancing the antitumor effect. Also, DDS may reduce leakage in normal vessels, thereby preventing adverse events with minimalizing drug distribution in normal organs [9,10].

The biologically active metabolite of Irinotecan, SN-38, possesses potent antitumor activity against several types of cancer. However, it has not yet been used clinically due to its poor water solubility and high toxicity. Polymeric micelle encapsulating an irinotecan metabolite of SN-38 (NK012; Nippon Kayaku Corporation, Tokyo, Japan), developed as a novel DDS, have a particle size of 20-100 nm and show EPR effect with selectively accumulating in tumors [11,12,13]. In a previous study, our research group could

demonstrate advantages of the intraarterial micellar nanoparticles injection in a rat liver tumor model [11].

Based on these backgrounds, we hypothesized that micellar nanoparticles would be an effective DDS in intraarterial therapy for pancreatic cancer, which could decrease the distribution in the duodenum and normal pancreas and accumulate in tumors with sustaining release of active SN-38. Therefore, in this study, we conducted animal experiments using a rat pancreatic cancer model to evaluate drug concentrations in the tissues including tumor, duodenum and pancreas, by intraarterially injecting the micellar nanoparticles.

Materials & Methods

The State Committee on Animal Affairs in our institution approved this study, and all animals were treated under an approved protocol. In this study, we investigated the following two parts; firstly, arterial blood supply to the pancreatic tumor was investigated, and, secondly, the tumor and tissue SN-38 concentrations were evaluated after intraarterial injection of micellar nanoparticles encapsulating SN-38.

A flowchart of the experiment is shown in Fig1.

Preparation of Rat Pancreatic Cancer Models

The preparation of rat pancreatic cancer is described in the on-line supplement.

Interventional Techniques

Catheterization of rats was performed under fluoroscopy (Surginix: Cannon, Tokyo, Japan). The left common carotid artery was surgically exposed, and 4-0 silk threads were placed at the cephalic and caudal ends of the exposed vessel. A double ligation was performed distal to the common carotid artery to block retrograde blood flow. The common carotid artery was then retrogradely cannulated with a 20 G venous catheter (Angiocath-IV catheter, 20Gx1.16) using a dissecting microscope. A 40 cm long custom-made microcatheter (Tokai Medical, Aichi, Japan) 1.7 Fr, was inserted over a 0.014inch guidewire (Transend;

Boston scientific, Marlborough, USA). The microcatheter was subsequently inserted into the celiac artery, and digital subtraction angiography was obtained (Figure2a).

Evaluation of Tumor Perfusion and Blood Supply into the Tissues

Prior to transcatheter therapy, blood supply into the tissues in the rat pancreas cancer model was confirmed by dye injection and also CT arteriography (CTA).

Indocyanine Green (Diagnogreen®; Daiichi Sankyo Co., Tokyo, Japan) at a dose of 25 mg was dissolved in 5 ml of water. In 3 rats, the pancreas and tumor exposed under laparotomy, 1 mL of the indocyanine green solution was injected through the celiac artery and the staining of the pancreas parenchyma, duodenum and tumor was confirmed.

In addition, in two other rats, without laparotomy, contrast medium (Visipaque™ 270mgI/mL; GE Healthcare, Chicago, USA) at a dose of 1 mL was injected via the celiac artery and CT (LCT200; Hitachi,Tokyo, Japan) was obtained. Then, the enhanced area was evaluated on the CT images.

Preparation of Micellar Nanoparticles

A micelle-forming macromolecule was prepared by connecting SN-38 with the polyglutamate copolymer via an ester bond (NK012). The hydrophilic polyethylene glycol forms the outer shell of the micelle, and the hydrophobic SN-38–bound polyglutamate forms the inner core of the micelle [13]. The particle size has a range of from 20 to 100 nm in diameter. These materials were supplied by Nippon Kayaku Co. Ltd. The nanoparticles were stored in the freeze-dried state and dissolved in distilled water at a dose equivalent to 5 mg/mL SN-38 immediately before administration.

Drug Distribution and Concentration

The experimental design involved the division of animals into two distinct groups: The Micellar

Nanoparticles group (N=9), serving as the test group, and the Irinotecan Infusion group (N=9), functioning as the control group. A microcatheter was inserted into the celiac artery via the left carotid artery. The Micellar Nanoparticles group received NK012 (SN-38 30mg/kg) through the celiac artery under fluoroscopy, and the Irinotecan Infusion group received irinotecan (Nippon Kayaku) at a dose of 40mg/kg through the celiac artery as well. Animals of each group was euthanized at 5 minutes (N=3), 6 hours (N=3), and 24 hours (N=3) post-injection. Subsequently, tumors, duodenum, and pancreas were excised, and these tissue samples, after measuring their weight, were promptly frozen in liquid nitrogen and stored at -80°C. SN-38 concentrations were measured in the Micellar Nanoparticles group, and irinotecan concentrations were measured in the Irinotecan Infusion group, respectively. The concentration of SN-38 or Irinotecan in the tumor, pancreas, and duodenum tissues were analyzed using high-performance liquid chromatography system (Alliance 2795; Waters, Milford, MA).

Statistical Analysis

The analysis focused on assessing the timing of the maximum concentration (Cmax) and the duration of elevated concentrations over a 24-hour period. To compare the Cmax with that at 24 hours, Student's t-test was employed. Statistical analysis was conducted using SPSS software version 22.0 (SPSS, Inc, Chicago, Illinois). $P < 0.05$ was considered statistically significant.

RESULTS

Tumor Perfusion and Blood Supply into the Tissues

In all three rats in which dye was injected, the staining of the pancreatic parenchyma, tumor and duodenum was visually confirmed (figure3a). In both two rats in which CTA was performed, there were enhancement effects on the pancreatic parenchyma and peripheral sites of the tumor which covered the whole tumor and duodenum (figure3b).

SN-38 and Irinotecan Tissue Concentrations

The mean body weight of 18 rats received intraarterial treatment was 321 g (range 281-351 g). The mean administered dose of SN-38 was 9.77 mg (9.1-11 mg) and the mean dose of irinotecan was 12.7 mg (11.2-14 mg). All intraarterial infusions were completed without procedural problems. The tissue concentrations of SN-38 in the Micellar Nanoparticles groups are shown in Table1 and Figure4a.In all tissues, Cmax were shown at 6 hours. The study examined alterations in SN-38 concentrations during the 6-hour (Cmax) to 24-hour periods. Notably, the concentration in the tumor did not exhibit a significant decrease, maintaining elevated values (P=0.068). In contrast, a statistically significant decrease was observed in both the pancreas (P=0.006) and the duodenum (P=0.028). The tissue concentrations of irinotecan in the Irinotecan Infusion group are shown in Table2 and figure4b. In all tissues, Cmax were observed at 5 minutes post-infusion, followed by a rapid decrease at 6 hours. Changes in tissue concentrations between the 5-minute (Cmax) and 24-hour periods were examined. Notably, a significant decrease in the concentration of irinotecan in the tumor was observed (P=0.016).

Discussion

Arterial infusion chemotherapy is a treatment modality that can achieve high drug concentrations at the target site while keeping systemic drug concentrations low. A meta-analysis of local intraarterial chemotherapy for pancreatic cancer reported an improvement in median survival [14]. However, due to the location of pancreatic cancer adjacent to the normal pancreatic parenchyma and duodenum, it is anatomically impossible to inject chemo-agents selectively into the pancreatic cancer, and the inevitable occurrence of high gastrointestinal complications such as nausea and vomiting after injection and also risk of pancreatitis may be a problem [8].

Current DDS technology could overcome the above mentioned problem, which can increase drug

concentration in the tumor with decreasing concentrations normal tissues. The EPR effect is based on the tendency of macromolecules and microparticles to extravasate from blood vessels in solid tumors due to hypervascularization, incomplete vascular architecture, and increased vascular permeability. It is also based on retention effects due to lack of macromolecular efflux caused by lymphatic defects. Micellar nanoparticles incorporating SN-38 have two major advantages. First, macromolecular polymeric micelles (MPMs) can directly deliver more SN-38, the biologically active metabolite of irinotecan, to tumors. Second, MPMs continuously exert time-dependent cytotoxic effects after accumulation in tumor tissue.

In a previous study our research team demonstrated pharmacokinetic advantages of the intraarterial micellar nanoparticles injection in a rat liver tumor model [11]. The study compared intraarterial and intravenous administration SN-38. In both groups, the 2-hour period exhibited higher concentrations compared to the 3-minute period. Notably, sustained high values were maintained for 24 hours in both groups, emphasizing the achievement of prolonged and elevated drug concentrations. Furthermore, the intraarterial group consistently demonstrated higher values than the intravenous group, underscoring the sustained efficacy of intraarterial delivery. In this study cancer model, while we did not evaluate an intravenous group, similar trend in tumor concentration of SN-38were observed. After 6 hours the concentration was significantly higher compared to the 5-minute period, a pattern that differed significantly from the irinotecan infusion in the non-DDS control group. This suggests consistency in the sustained and enhanced drug delivery achieved with intraarterial micellar nanoparticles,.

In alignment with these findings, our pancreatic cancer model exhibited a decrease in SN-38 concentration in both the duodenum and pancreas parenchyma. This observation suggests a favorable EPR effect in our pancreatic cancer model, akin to the previously studied hepatocellular carcinoma model. These results imply that the utilization of micellar nanoparticles could potentially enhance the antitumor effect while concurrently reducing complications, such as gastrointestinal symptoms, through minimized drug washout in adjacent organs. The observed lower values of SN-38 and irinotecan in tumors compared to

duodenum and pancreas tissues can be attributed to the unique hypovascularity of the pancreatic cancer model. This characteristic contrasts with the hypervascular nature of the hepatocellular carcinoma model previously studied.

Irinotecan is one of the key drugs of pancreatic cancer. FOLFIRINOX regimen is recommended as a first-line treatment for metastatic pancreatic cancer. There is also a clinically used irinotecan-loadable DC-Bead^{TM} as a particle material, but it is not generally used for pancreatic arterial infusion due to its large particle size and the complications associated with embolization. Irinotecan is primarily metabolized in the liver, it is also known to undergo some metabolism in tumors and other organs [15]. However, to the best of our knowledge, no data exist on the extent to which irinotecan is metabolized to SN-38. Therefore, irinotecan was administered at 40 mg/kg BW, and the concentrations of irinotecan and SN-38 in each tissue were compared separately. Although the dosages of SN-38 and irinotecan used in this study were different, a direct comparison between the SN-38 group and the irinotecan group was not conducted.

The rat-based research model is playing an increasingly important role in interventional oncology research. The creation of rat pancreatic cancer model was based on the report of Zhibin [16]. The rat pancreatic cancer cells, DSL-6A/C1, exhibits pathologically similar characteristics to human pancreatic cancer, such as intermediate to highly differentiated ductal adenocarcinoma, a tendency to invade adjacent organs, intraperitoneal dissemination, and a tumor with poor blood flow.

Several limitations were identified in our study. These include: (1) The study was constrained by a relatively small sample size, potentially limiting the generalizability of the findings. (2) The duration of observation was relatively short, which may restrict our ability to capture long-term effects or variations in drug concentrations over an extended period. (3) This study did not include pathological examinations, and evaluations of tumor specific effects and necrosis rates were not conducted. (4) Adverse events related to the treatment were not systematically examined. (5) The blood supply to the pancreatic tumor were assessed using dye injection and CTA. However, the density of the nanoparticle solution differs from those of the

contrast agent and the dye, which might influence its distribution. (6) This experiment was conducted on rats, and it remains unclear whether these results can be replicated in humans. Acknowledging these limitations is crucial for interpreting the study's outcomes and guiding future research endeavors to address these constraints for a more comprehensive understanding of the therapeutic approach.

In conclusion, micellar nanoparticles could be an effective DDS of intraarterial therapy for pancreatic cancer to reduce drug concentration in adjacent normal organs with increasing tumor drug concentration.

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Abbreviations

DDS = drug delivery system, EPR = enhanced permeability and retention, Cmax=maximum concentration

MPMs = macromolecular polymeric micelles

Figure1 A Flow Chart of the Experiment

Figure2

Figure2a In digital subtraction angiography (DSA) using x-rays, iodine is injected through a catheter placed in the celiac artery, and a dark stain of the pancreatic parenchyma along the splenic artery is observed.(arrows) Tumor was difficult to identify because of poor intra-tumoral blood flow.

Figure2b Angiography in rats via carotid artery.

Figure3

Figure3a Dye injection from the celiac artery was performed in pancreatic cancer model rats. Tumor staining from the margins is observed under direct vision.(arrows)

Figure3b CTA of pancreatic cancer model rats via the celiac artery. Contrast enhanced effect is observed at the tumor margins.(arrow heads)

Table 1. The concentrations of SN-38 in the tumor, pancreas, and duodenum at 5 minutes, 6 hours, and 24 hours after infusion. The P value compares Cmax (6 hours) with 24 hour.

Table2. The concentrations of Irinotecan in the tumor, pancreas, and duodenum at 5 minutes, 6 hours, and 24 hours after infusion. The P value compares Cmax (5min) with 24 hour.

Figure4a Graphs showing the concentrations of SN-38 in the tumor, pancreas, and duodenum at 5 minutes, 6 hours, and 24 hours after infusion NK012.

Figure4b

Graphs showing the concentrations of Irinotecan in the tumor, pancreas, and duodenum at 5 minutes, 6 hours, and 24 hours after infusion Irinotecan.

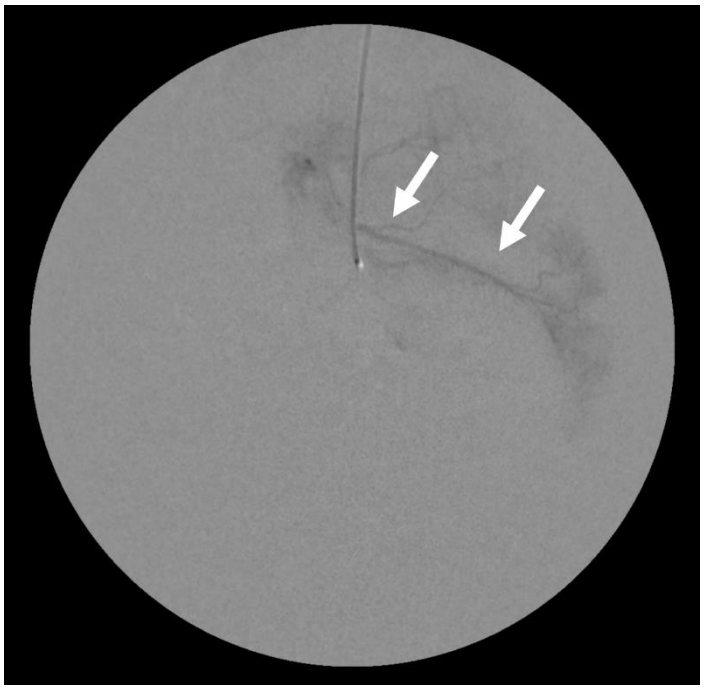

Figure 2a

Figure 2b

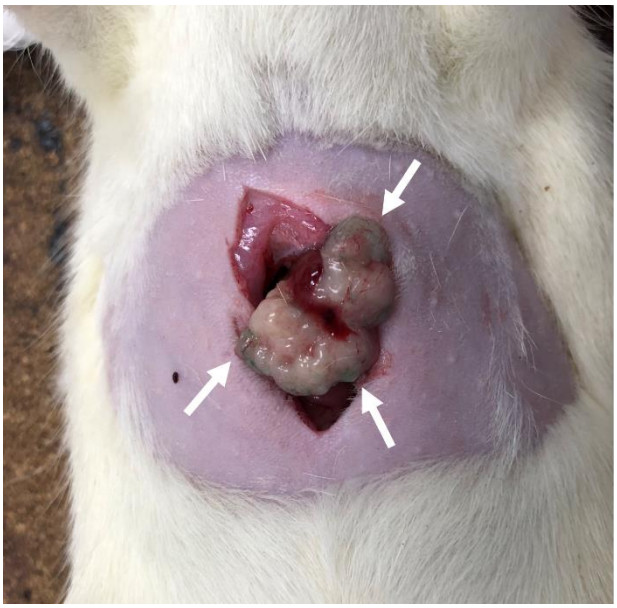

Figure 3a

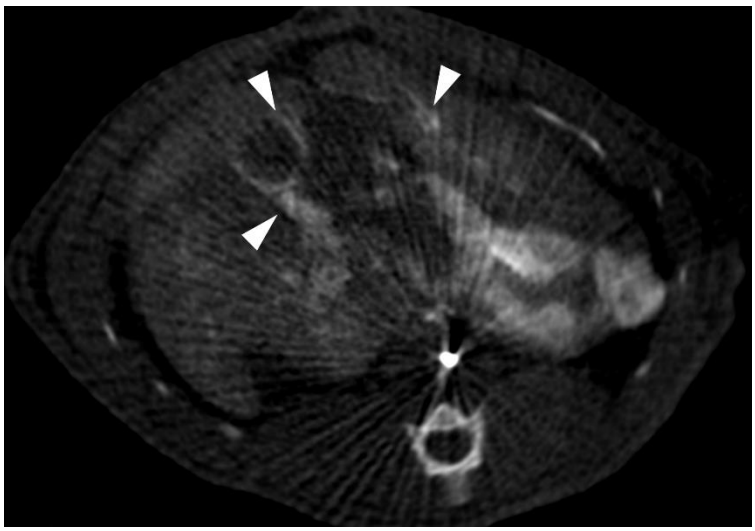

Figure 3b

Table 1. SN-38 concentration in Tumor ,Pancreas , and Duodenum

	SN-38 concentration(μg/g)			
	5min	**6h**	**24h**	**P Value ***
Tumor	0.181 ± 0.047	0.45 ± 0.089	0.436 ± 0.081	0.068
Pancreas	0.825 ± 0.009	1.25 ± 0.22	0.213 ± 0.024	0.006
Duodenum	0.251 ± 0.043	3.15 ± 0.77	1.19 ± 0.27	0.028

* The P value compares C max (6 hours) with 24 hour

Table 2. Irinotecan concentration in Tumor ,Pancreas , and Duodenum

	Irinotecan concentration(μg/g)			
	5min	**6h**	**24h**	**P Value ***
Tumor	554 ± 200	88.9 ± 20.7	61.6 ± 39.8	0.016
Pancreas	647 ± 446	165 ± 37.7	39.7 ± 47.6	0.104
Duodenum	1027 ± 517	102 ± 26.2	2.76 ± 2.06	0.034

* The P value compares C max (5min) with 24 hour

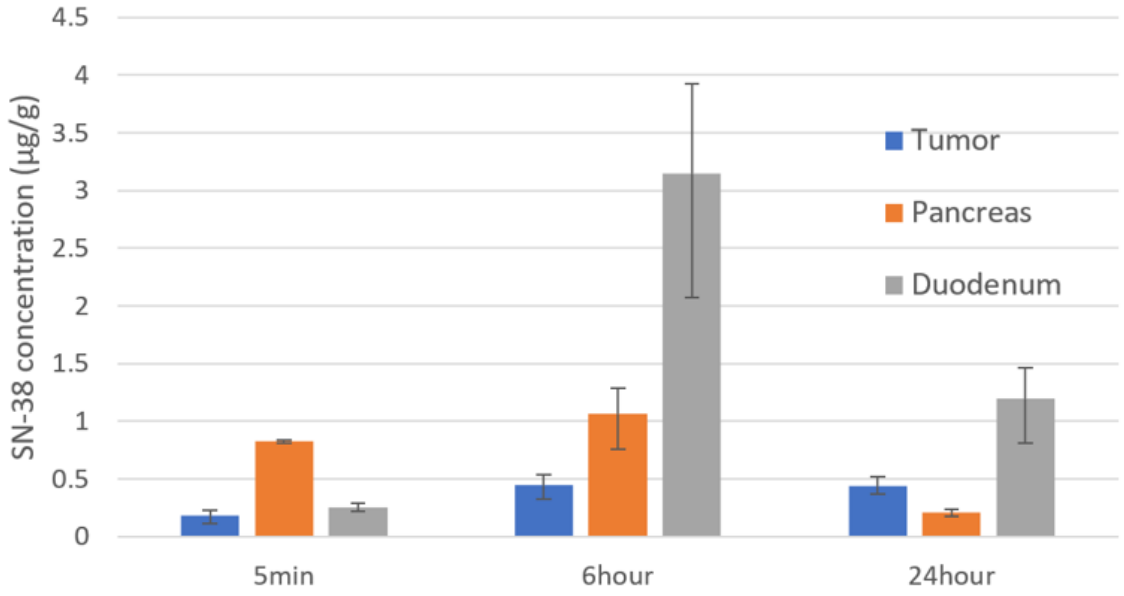

Figure 4a

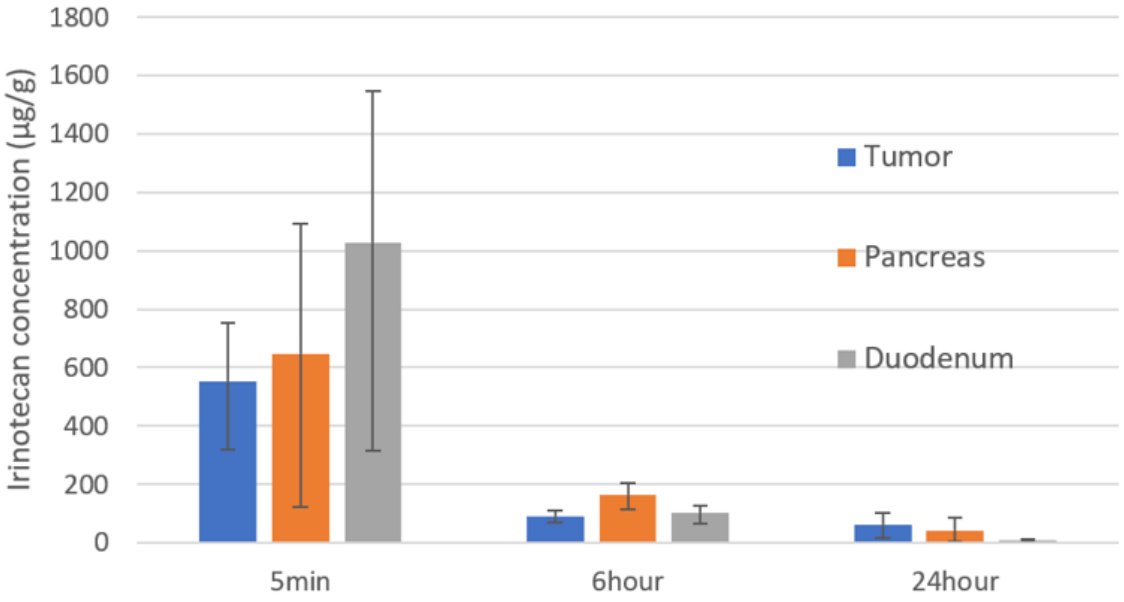

Figure 4b