Pharmacokinetics of a retrograde infusion of Paclitaxel into the thoracic duct in a porcine model

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Research Article

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Abstract

Gastrointestinal cancer with massive nodal metastases is a lethal disease. In this study, using a porcine model, we attempted to infuse the anti-cancer drug, Paclitaxel (PTX), into the thoracic duct to examine the efficiency of drug delivery to intra-abdominal lymph nodes. We established a technical method to catheterize the thoracic duct in the necks of pigs. Serum, liver, and spleen concentrations of PTX were significantly lower after thoracic duct (IT) infusion than after intravenous (IV) administration. Approximately 12–24 h after infusion, PTX concentrations in abdominal lymph nodes tended to be higher with IT than with IV infusion; however, increased levels of PTX were much lower than expected. Unexpectedly, concentrations of PTX in urine were much higher after IT administration than after IV administration, demonstrating that most PTX administered via the thoracic duct was promptly excreted from the kidneys. These findings suggest that infusion of anti-cancer drugs into the thoracic duct will not produce clinical benefits for patients with extensive lymphatic metastases in abdominal malignancies.

Introduction

Lymphatic metastasis usually occurs in the direction of lymph flow. In abdominal malignancies, such as gastrointestinal or ovarian cancer, metastatic tumor cells in regional lymph nodes move to the retroperitoneal para-aortic nodes and then spread to the left neck lymph nodes (Virchow's nodes) through the thoracic duct. Extensive lymph node metastases (ELM), including para-aortic lymph node metastasis, are commonly regarded as unresectable. These malignancies are treated with a combination of surgery, radiotherapy, and systemic chemotherapy. However, outcomes of patients with ELM are still very poor in gastric [1–3], pancreatic [4], and colorectal [5,6] cancer. In comparison, prognoses of patients with ELM from ovarian cancers tend to be better, which is likely due to their greater chemosensitivity [7,8]. It is possible that exposure of metastatic lymph nodes to higher concentrations of anti-cancer drugs could improve outcomes of patients with ELM, even for gastrointestinal cancers.

The thoracic duct, the body's central lymphatic vessel, originates in the cisterna chyli in the retroperitoneum, ascends between the esophagus and the descending aorta in the mediastinum, and flows into the left venous angle in humans. We hypothesized that high doses of anti-cancer drugs might be administered with low systemic toxicity if given via the thoracic duct in retrograde fashion to selectively infuse metastatic lymph nodes in the retroperitoneum. Based on this hypothesis, we catheterized the thoracic duct of pig necks and infused Paclitaxel (PTX) via catheters. We then compared pharmacokinetics of PTX administered intrathoracically with those of systemic (intravenous) infusion.

Results

Concentrations of PTX in serum and urine

When PTX was first administrated through the intrathoracic duct (IT), considerable amounts of PTX were detected in sera. However, concentrations of PTX 1 to 12 h after administration were significantly lower

than those after intravenous administration (IV). The PTX concentration decreased with time, ultimately disappearing from the serum after 24 h with both IT and IV administration (Fig. 2a). In contrast, concentrations of PTX in urine samples were significantly higher with IT administration than with IV administration at all times (Fig. 2b)

PTX concentrations in various organs

In the first set of experiments, PTX accumulation in various organs was examined at 1 and 3 h post-infusion. In the lungs, liver, and spleen, PTX concentrations after IV administration were more than twice as high as after IT administration. However, there were no significant differences between the two infusion methods in terms of PTX concentrations in mesenteric lymph nodes (Fig. 3).

In the next set of experiments, we compared PTX concentrations 8, 12, and 24 h after IV and IT infusion. Although PTX concentrations were still high in the liver and spleen at 8 h, there were no differences in PTX levels in any organs between IV and IT administration after 12 h. However, 24 h after IT administration, relatively high PTX concentrations were still present in mesenteric and para-aortic lymph nodes compared to those following IV administration (Fig 4).

Discussion

The thoracic duct is the main collecting vessel of the lymphatic system, and it drains lymph from the abdomen and lower extremities into the venous blood stream [9]. Thus, the thoracic duct is a direct pathway to the retroperitoneal lymph nodes through the cisterna chyli. In fact, clinical studies have documented retrograde spreading of esophageal and lung cancers to abdominal lymph nodes through the thoracic duct [10,11]. These findings inspired us to examine the possibility of retrograde intrathoracic duct chemotherapy for gastrointestinal cancers with extensive metastases to the retroperitoneal lymph nodes.

We succeeded in cannulating the terminal thoracic duct in pig necks, and attempted to achieve selective retrograde administration of PTX to the lymphatic system. We selected PTX because the concentration of PTX in lymph in the thoracic duct is maintained at high levels after intraperitoneal infusion, probably due to its high molecular weight and hydrophobic properties [12]. Using this non-invasive model, we were able to compare pharmacokinetics in various organs between intrathoracic (IT) and intravenous (IV) infusions of drugs up to 24 h after infusion. Our hypothesis was that IT administration of PTX would result in much higher PTX accumulation in abdominal lymph nodes than IV administration, with less loss to circulating blood. In fact, within 12 h of infusion, PTX concentrations in the serum, liver, and spleen were significantly lower with IT than with IV administration; however, this difference was smaller than expected. Comparing the two routes, no significant differences were detected in PTX concentrations in the abdominal lymph nodes, the stomach, intestines, or omentum. Moreover, concentrations of PTX in mesenteric or para-aortic lymph nodes tended to be higher after IT than IV administration only 24 h after infusion.

On the other hand, PTX concentrations in urine were considerably higher with IT than IV administration, especially at early time points. This suggests that most IT-infused PTX was retrogradely transported to the renal cortex and excreted from the kidneys, since renal lymphatic outflow is connected to the para-aortic lymph nodes through the thoracic duct [13].

In summary, we examined drug delivery efficiency of IT infusion for extensive metastatic lymph nodes in the abdomen. After IT infusion, the same concentrations of PTX were obtained in abdominal lymph nodes, but with lower serum concentrations than after systemic infusion. Thus, it may be possible to achieve higher PTX doses with IT than with IV administration. However, the amount of urinary excretion may present a high risk of renal toxicity. In conclusion, IT administration of anti-cancer drugs does not offer a significant pharmacokinetic advantage and has little clinical merit.

Materials And Methods

Drugs and animals

Paclitaxel (Taxol) was purchased from Bristol-Myers Squibb Japan (Tokyo, Japan). Porcine models, comprising 4 female pigs with an average body weight of 29.8 kg (range: 20.8–21.9 kg), were purchased from Sanesu Breeding Co, Ltd. (Funabashi, Japan) and were housed individually at the Center for Development of Advanced Medical Technology (CDAMTech), Jichi Medical University. All animal handling procedures in this study complied with the Jichi Medical University Guide for Laboratory Animals, the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication, eighth edition, 2011), and the ARRIVE guidelines. [14] The Institutional Animal Care and Concern Committee at Jichi Medical University approved all experimental protocols (Approval number: 20139-01).

Porcine thoracic duct cannulation model

After the pigs were placed in a supine position under inhalation anesthesia with sevoflurane, a 15-cm longitudinal incision was made 1 cm to the left of the midline at the 1/3 level on the cranial crest, caudal side. The left anterior cervical muscle was dissected, the left external jugular vein and the left subclavian vein were ligated and dissected, and then the first rib was dissected and removed 2 cm from the sternum attachment site. We then performed a laparotomy, and 2 mL of Patent Blue (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) were injected into the mesenteric lymph nodes of the small intestine using a fine needle. Cervical lymphatic vessels could be identified in the remaining left internal carotid artery and left subclavian artery bifurcation a few minutes after the dye injection. Discharge of lymph was confirmed after cannulation with a 24G Surflo™ needle was performed (Fig. 1a, b). The thoracic duct was identified by the C-arm due to injection of the radiocontrast Omnipark® (Fig. 1c).

Measurement of PTX concentrations in various organs with high-performance liquid chromatography-mass spectrometry (LC-MS/MS).

After intravenous administration of 100 mg of hydrocorton (Nichiiko, Tokyo, Japan), 30 mg of PTX (MOCHIDA, Tokyo, Japan) were administrated from the cannulated thoracic duct, as described in the Materials and Methods. Pigs were systematically cannulated in the left internal jugular vein with a 24G Surflo^{TM} needle, where PTX was then intravenously infused. At 1, 3, 8, 12, and 24 h post-infusion, blood and urine samples were collected and centrifuged at 2000 rpm for 10 min at 4°C. Supernatants were then collected, placed in cryotubes for freezing, and stored at -80°C. After thawing the samples, PTX concentrations were measured with high-performance liquid chromatography-mass spectrometry (LC-MS/MS) (Shimadzu, Kyoto, Japan).

Fifty µL of each liquid sample (serum, lymph, ascites, urine), to which 200 µL of methanol and 20 µL of internal standard reagent IS (Paclitaxel-d5, 1 µg/mL-MeOH) had been added, were vortexed for 30 s and then centrifuged (12,000 rpm for 10 min at 4°C). The resultant supernatants were then subjected to spin filtering. Filtrates were analyzed by LC-MS/MS (LCMS-8050 System, Shimadzu, Kyoto, Japan). Tissue samples (10 mg), to which 20 µL of internal standard reagent (Paclitaxel-d5, 1 µg/mL-MeOH) and 1 mL of 0.1% formic acid-MeOH had been added, were homogenized and centrifuged (12,000 rpm for 10 min at 4°C). The resultant supernatants (500 µL) were transferred to other tubes, to which 200 µL of water had been added. Samples were then vortexed and centrifuged (12,000 rpm for 10 min at 4°C), and the resulting supernatants were analysed.

For LC analyses, YMC-Triart C_{18} (50 × 2 mm 1.9 µm) was used as an analytical column, and the column oven and autosampler were set to 40°C and 4°C, respectively. Mobile phases A was 0.1% formic acid-water and mobile phase B was acetonitrile. The flow rate was set to 0.5 mL/min and the injection volume was 3 µL. The gradient was 40% B for 2.7 min, ramping up to 100% by 4 min, remaining at 100% until 6 min, then decreased over 0.5 min, followed by an equilibration step at 40% B for 1.5 min.

Paclitaxel and Paclitaxel-d5 were detected in ESI positive mode. LC/MS-MS conditions were as follows: Nebulizer gas flow (3L/min), heating gas flow (10L/min), interface temperature (300°C), desolvation temperature (526°C), heat block temperature (400°C), and drying gas flow (10L/min). Collision energies were 24 V, 22 V, and 21 V for Paclitaxel, and 24 V, 25 V, and 21 V for Paclitaxel-d5. Paclitaxel, and Paclitaxel-d5 were observed at m/z 854 > 286 and m/z 859 > 291, respectively. Paclitaxel concentrations of biological samples were quantified by the area ratio with the internal standard reagent (Paclitaxel-d5) added to the samples.

Paclitaxel was added to the serum at a concentration of 0.005-5 µg/mL to prepare an 8-point calibration curve. Accuracy and linearity of the calibration curve, carryover, diurnal variation, inter-day variation, and sample stability were confirmed. Recovery tests of Paclitaxel added to porcine serum (4 animals) and porcine ascites (2 animals) were performed, and accuracies were 110% and 95% for porcine serum and ascites, respectively. For urine analyses, Paclitaxel was added to pig urine, derived from pigs to which Paclitaxel had not been administered, at a concentration of 0.125-1 µg/mL to prepare an 8-point calibration curve.

Concentrations in various organs were measured in the early and late phases, since frequent tissue collection with such an invasive maneuver may seriously affect pharmacokinetics. In the early phase analysis, lung, stomach, liver, spleen, small intestine, omentum, and mesenteric lymph nodes were taken 1 and 3 h after PTX infusion. In the late phase analysis, some organs were taken at 8, 12, and 24 h after PTX infusion. Para-aortic lymph nodes were additionally taken only at the end of the experiment. Immediately after collection, each organ was stored in its collected state in a cryotube at -80°C. Later, 10-mg samples of these organs were homogenized and PTX concentrations were measured, as described above.

Statistical Analysis

Results were analyzed using Student's t-tests, and p values < 0.05 were considered statistically significant.

Declarations

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Author contributions

SA, JK and KA designed and conducted the experiments, analyzed the data, and prepared the figures and manuscript. KY performed experiments and provided support for the experimental design. NK and KA developed the analytical settings for mass spectrometry and data acquisition. OH, MH, YH, KLA, RN and SA supervised and edited the manuscript. All aspects of the work in the final version of the manuscript were approved and agreed on by all authors, which guarantees that the accuracy and integrity of any part of the work is suitably examined and resolved.

Additional information

Competing interests

The authors declare no competing interests.

References

1. Fujiwara, Y. *et al.* A Multidisciplinary Approach for Advanced Gastric Cancer with Paraaortic Lymph Node Metastasis. *Anticancer Res*, **35**, 6739–6745 (2015).

2. Kim, J. H. *et al.* Phase II Study of Induction Chemotherapy with Docetaxel, Capecitabine, and Cisplatin Plus Bevacizumab for Initially Unresectable Gastric Cancer with Invasion of Adjacent Organs or Paraaortic Lymph Node Metastasis. *Cancer Res Treat*, **50**, 518–529 https://doi.org/10.4143/crt.2017.005 (2018).
3. Morita, S., Fukagawa, T., Fujiwara, H. & Katai, H. The clinical significance of para-aortic nodal dissection for advanced gastric cancer. *Eur J Surg Oncol*, **42**, 1448–1454 https://doi.org/10.1016/j.ejso.2016.01.002 (2016).
4. Yamada, S. *et al.* Pancreatic cancer with paraaortic lymph node metastasis: a contraindication for radical surgery?, **38**, e13–17 https://doi.org/10.1097/MPA.0b013e3181889e2d (2009).
5. Bae, S. U. *et al.* Oncologic Outcomes of Colon Cancer Patients with Extraregional Lymph Node Metastasis: Comparison of Isolated Paraaortic Lymph Node Metastasis with Resectable Liver Metastasis. *Ann Surg Oncol*, **23**, 1562–1568 https://doi.org/10.1245/s10434-015-5027-9 (2016).
6. Wong, J. S., Tan, G. H. & Teo, M. C. Management of para-aortic lymph node metastasis in colorectal patients: A systemic review. *Surg Oncol*, **25**, 411–418 https://doi.org/10.1016/j.suronc.2016.09.008 (2016).
7. Martinez, A. *et al.* Prognostic relevance of celiac lymph node involvement in ovarian cancer. *Int J Gynecol Cancer*, **24**, 48–53 https://doi.org/10.1097/IGC.0000000000000041 (2014).
8. Harter, P. *et al.* A Randomized Trial of Lymphadenectomy in Patients with Advanced Ovarian Neoplasms. *N Engl J Med*, **380**, 822–832 https://doi.org/10.1056/NEJMoa1808424 (2019).
9. el-Gendi, M. A. & Zaky, H. A. Thoracic duct lymph flow: a theoretical concept. *Surgery*, **68**, 786–790 (1970).
10. Oshiro, H. *et al.* Retrograde Lymphatic Spread of Esophageal Cancer: A Case Report. *Med. (Baltim)*, **94**, e1139 https://doi.org/10.1097/MD.0000000000001139 (2015).
11. Ryu, W. *et al.* Abdominal lymph node metastasis by lymphatic spread through the thoracic duct in patients with non-small-cell lung cancer. *Thorac Cancer*, https://doi.org/10.1111/1759-7714.14014 (2021).
12. Soma, D., Kitayama, J., Ishigami, H., Kaisaki, S. & Nagawa, H. Different tissue distribution of paclitaxel with intravenous and intraperitoneal administration. *J Surg Res*, **155**, 142–146 https://doi.org/10.1016/j.jss.2008.06.049 (2009).
13. Russell, P. S., Hong, J., Windsor, J. A., Itkin, M. & Phillips, A. R. J. Renal Lymphatics: Anatomy, Physiology, and Clinical Implications. *Front Physiol*, **10**, 251 https://doi.org/10.3389/fphys.2019.00251 (2019).
14. Kilkenny, C. *et al.* Animal research: reporting in vivo experiments–the ARRIVE guidelines. *J Cereb Blood Flow Metab*, **31**, 991–993 https://doi.org/10.1038/jcbfm.2010.220 (2011).

Figures

Figure 1

Thoracic duct cannulation. The lymphatic vessel was identified between the left internal carotid artery and left subclavian artery at their bifurcation (a) and the terminal thoracic duct was cannulated with a 24G needle (b). The whole length of the thoracic duct was visualized by injecting the radiocontrast, Omnipark® (c).

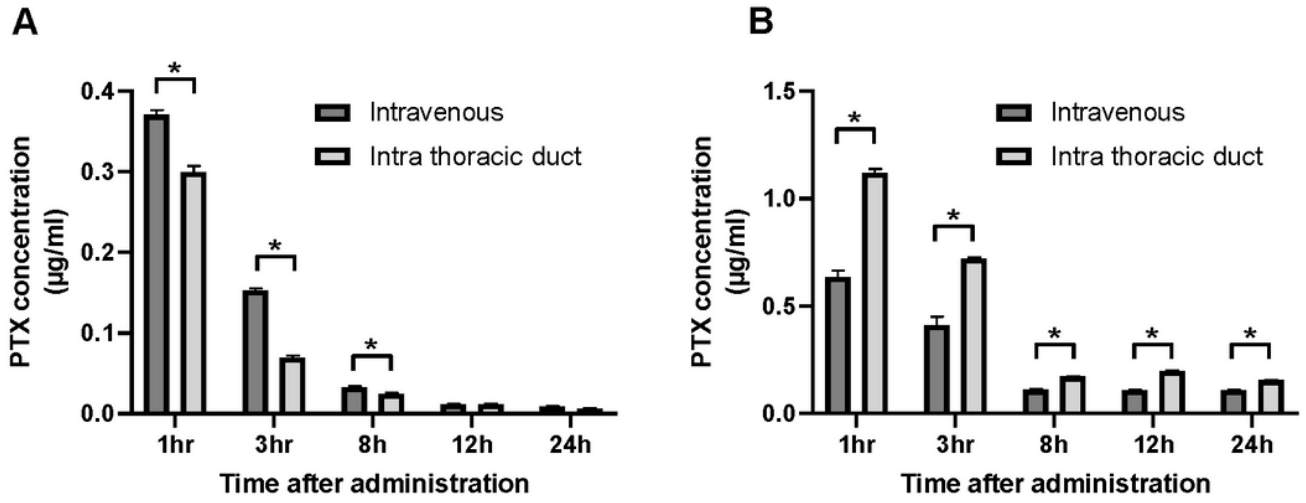

Figure 2

PTX concentrations in serum (a) and urine (b) after IT or IV injection. Serum and urine samples were obtained 1 to 24 h after infusion. PTX concentrations were measured with a mass spectrometer (LC-MS/MS). Data show the mean±SD of 3 experiments. *: $p < 0.01$

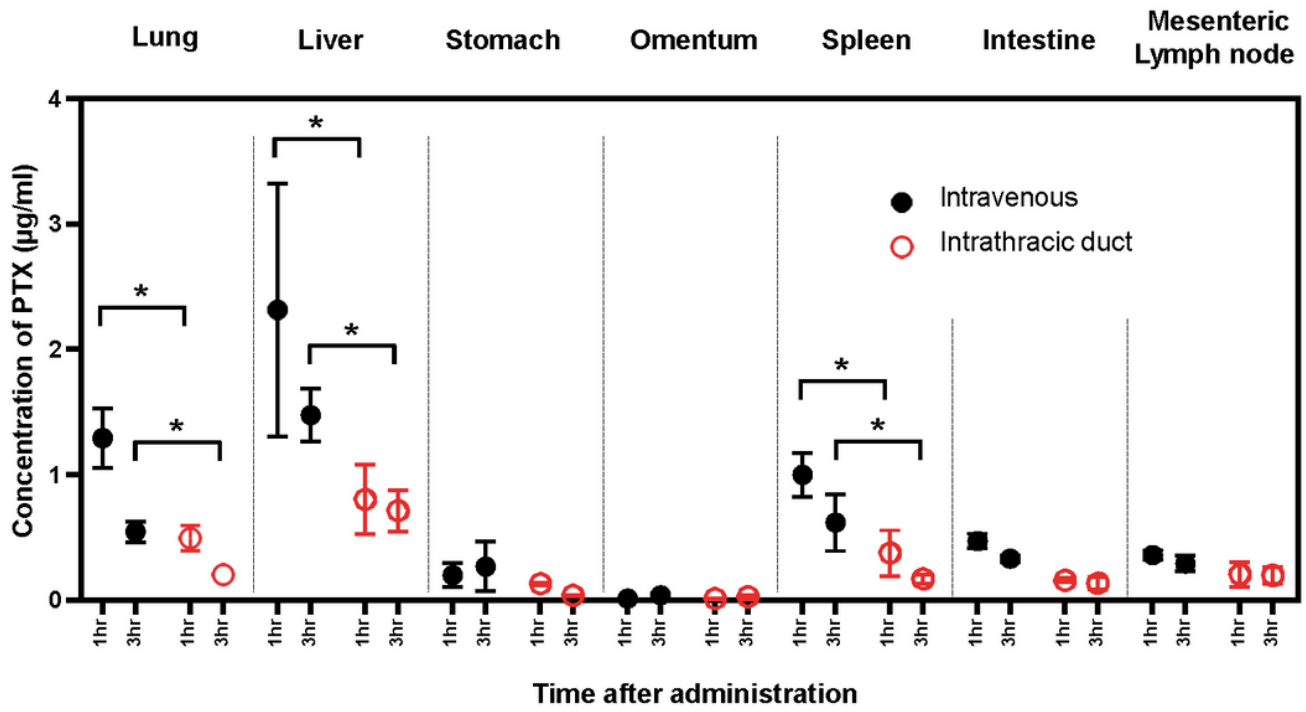

Figure 3

Concentrations of PTX in various organs soon after IT or IV injection. Three samples were obtained from each organ 1 and 3 h after infusion, and PTX concentrations were measured with a mass spectrometer (LC-MS/MS). Data show the mean±SD of 3 samples. *: $p < 0.01$

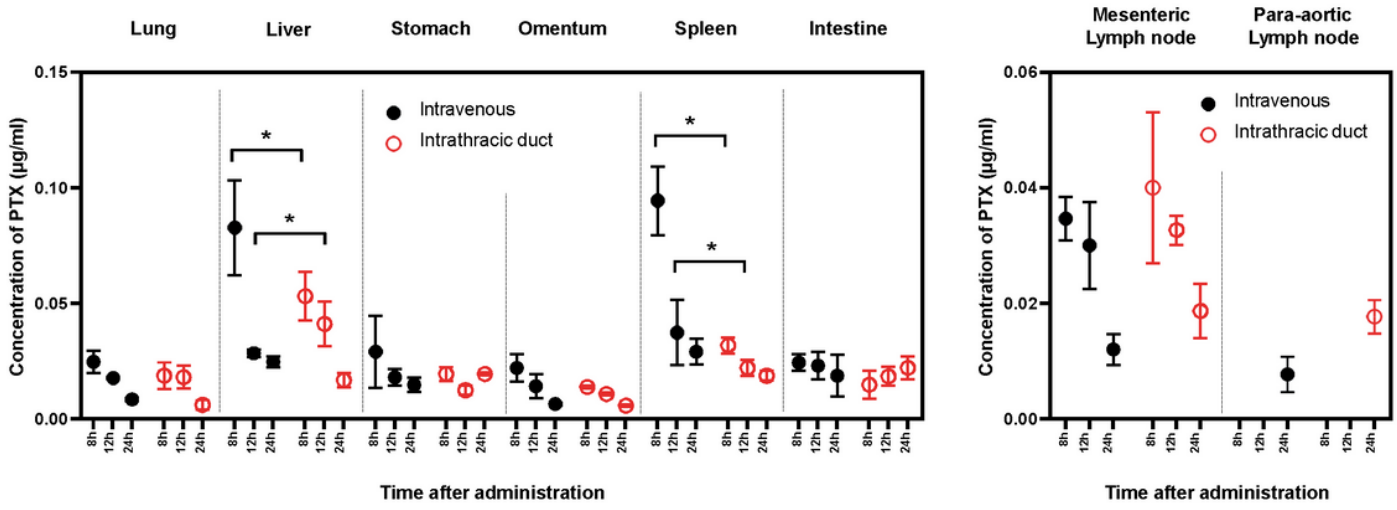

Figure 4

PTX concentrations in various organs at later time points after IT or IV injection. Three samples were obtained from each organ 8, 12, and 24 h after infusion. PTX concentrations were measured with a mass spectrometer (LC-MS/MS). Data show the mean±SD of 3 samples. *: $p < 0.01$