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ORIGINAL ARTICLE

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Docetaxel: pharmacokinetics and tissue levels after intraperitoneal and intravenous administration in a rat model

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Abstract *Purpose:* Docetaxel (Taxotere) has been shown to possess a broad spectrum of antitumor activity against various malignancies such as breast and lung cancers, but also against intraabdominal malignancies such as mesothelioma and ovarian cancer. For cancers occurring within the abdominal cavity, the advantage of intraperitoneal chemotherapy is the prolonged high drug concentration that can be achieved locally with low systemic toxicity. Using a rat model, this study was designed to compare the pharmacokinetics and tissue distribution of intraperitoneal versus intravenous docetaxel. *Methods:* The study animals were comprised of 15 Sprague Dawley rats. They were randomized into three groups according to dose and route of administration (15 mg/kg intravenously, 15 mg/kg intraperitoneally, or 150 mg/kg intraperitoneally) and then given a single dose of docetaxel. Blood and peritoneal fluid were sampled using a standardized protocol for 90 min. At the end of the procedure the rats were killed and docetaxel concentrations in peritoneal fluid, plasma and selected tissue samples were determined by high-performance liquid chromatography (HPLC). *Results:* When docetaxel was delivered at 15 mg/kg the area under the curve (AUC) of the peritoneal fluid was significantly higher with intraperitoneal administration (110.6 μg/ml·min) as compared to intravenous administration (0.043 μg/ml·min; $P = 0.0079$). This represents more than a 2500-fold increase in exposure for tissues at peritoneal surfaces after intraperitoneal administration. Conversely, at the same dose the AUC of the plasma was significantly lower with intraperitoneal administration (0.11 μg/ml·min) as compared to intravenous administration (4.25 μg/ml·min; $P = 0.0079$). The AUC ratio (AUC peritoneal fluid/AUC plasma) was 976 for intraperitoneal administration as opposed to 0.01 for intravenous delivery. The AUC ratio for intraperitoneal docetaxel at 150 mg/kg was 3004. There were significantly different concentrations in the heart and the abdominal wall ($P = 0.0079$) and in the stomach and colon ($P = 0.0159$) when intraperitoneal versus intravenous docetaxel were compared. *Conclusions:* The exposure of the peritoneal surface to docetaxel is significantly increased and the systemic exposure decreased with intraperitoneal docetaxel administration. Also, high concentrations of drug were observed in the abdominal wall and in the colon after intraperitoneal delivery. This experiment suggests the need for clinical studies to evaluate intraperitoneal administration of docetaxel in humans.

Keywords Docetaxel · Intraperitoneal chemotherapy · Pharmacokinetics · Tissue distribution

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Introduction

Docetaxel (Taxotere) is the second member of the taxane class of cytotoxic agents to reach clinical use. The drug induces polymerization of tubulin monomer and inhibits depolymerization leading to mitotic arrest in the G_2M phase of the cell cycle [4]. Docetaxel also induces apoptotic cell death by stimulating the phosphorylation of bcl-2, a protein presenting antiapoptotic effects [15]. Other mechanisms are likely to be involved in the apoptotic effects of taxanes [16]. Preclinical cytotoxic activity was evident in mice bearing human cancer xenografts subcutaneously implanted and treated with intravenous docetaxel (colon, lung and mammary carcinomas) and in mice bearing ovarian carcinoma xenografts implanted intraperitoneally and treated with intraperitoneal docetaxel [5]. The growth of mesothelioma cells has been shown to be inhibited by docetaxel in vitro [11]. In humans, therapeutic activity with systemic drug administration has been demonstrated in a wide

range of tumors which include breast, lung, prostate, ovarian, head and neck, gastric, pancreatic and bladder cancers [6].

Following intravenous administration, cytotoxic agents such as docetaxel may not penetrate into cancerous tissues at high enough concentrations to effectively eradicate the cancer. For cancer that may disseminate to peritoneal surfaces, the therapeutic index of docetaxel may be improved by changing the route of administration. The major advantage of intraperitoneal chemotherapy is the high drug concentration that can be achieved locally with low systemic exposure. After surgical removal of an ovarian cancer with peritoneal seeding or of a peritoneal mesothelioma, intraperitoneal docetaxel may be able to eliminate microscopic residual disease or small foci of persistent cancer.

In order to establish the therapeutic potential for docetaxel for malignancies that disseminate to peritoneal surfaces, greater knowledge of the intraperitoneal pharmacokinetics of the drug is necessary. Using a rodent model this study was conducted to determine and compare the pharmacokinetics and the tissue distribution of intraperitoneal versus intravenous docetaxel.

Materials and methods

Rats

The study animals were comprised of 15 male Sprague Dawley rats weighing between 350 and 450 g obtained from a single breeding colony (Harlan Sprague Dawley, Indianapolis, Ind.). Animals were individually housed and were allowed free access to food and water. These experiments were conducted after approval by the Animal Care and Use Committee. After completion of the experiment the rats were killed by lethal injection.

Surgical procedure

All rats were anesthetized with an intraperitoneal injection of sodium phenobarbital (50 mg/kg) and underwent laparotomy. A multiperforated catheter (Silicone tubing, 3.2 mm ID, 6.4 mm OD; Fisher Scientific, Norcross, Ga.) was placed in the pelvis through the abdominal incision. The catheter was coated with gauze in a standardized fashion to prevent occlusion by omentum or small bowel loops [7]. The intraperitoneal catheter was used for both dispensing intraperitoneal fluids and for peritoneal fluid sampling and was secured within the abdominal incision with a running suture used to close the laparotomy. A catheter (Polypropylene tubing, 0.58 mm ID, 0.965 mm OD; Becton Dickinson, Parsippany, N.J.) was inserted in the left femoral vein. The intravenous catheter was used for drug delivery and blood sampling.

Experimental design

The 15 rats were randomized into three groups according to the route of administration of the chemotherapy and the drug dose. The first group received intravenous chemotherapy consisting of a single bolus dose of docetaxel (15 mg/kg) through the venous catheter, and a bolus administration of 0.1 ml/g body weight of 5% dextrose solution through the abdominal catheter. The docetaxel used in these 15 experiments was supplied as a concentrate in Polysorbate 80. Rats in the second group received intraperitoneal chemotherapy with a single bolus administration of docetaxel 15 mg/kg in 0.1 mg/g body weight of 5% dextrose solution though the abdominal catheter. To determine if the pharmacokinetics of intraperitoneal docetaxel were consistent over a wide range of doses, the third group underwent the same procedure as group 2 but with 150 mg/kg of docetaxel. Prior phase 1 studies had established that no precipitation occurs with docetaxel up to an approximate concentration of 2.5 mg/ml [2].

All rats were studied for 90 min. For each group, 1 ml peritoneal fluid and 1 ml blood were collected at 5, 15, 30, 60 and90 min after initiation of the chemotherapy. A new syringe was used to obtain each intraoperative sample and the catheter was lavaged three times to thoroughly mix the syringe contents with the intraperitoneal fluid. The venous catheter was flushed with 0.8 ml heparinized saline after intravenous drug administration and with 0.2 ml after each blood sampling. In vitro and in vivo studies had shown that there is less than 0.05% residual docetaxel in the catheter when this flushing technique is used. At the end of the procedure (90 min) the rats were killed and tissue samples were taken from the heart, liver, stomach, colon and abdominal wall. Docetaxel concentrations in peritoneal fluid, plasma and tissue samples were determined by high-performance liquid chromatography (HPLC) immediately at the end of the experiment.

HPLC analysis

Docetaxel levels were determined in plasma, peritoneal fluid and tissue samples, using a modification of the HPLC procedures described by Lee et al. [10]. The HPLC system consisted of a Shimadzu LC7A instrument equipped with an SPD-6AV (UV-VIS) detector set at 227 nm-UV, along with a C-R6A Chromopac data processor. A 250×4.6-mm reversed-phase column of Dynamax 300A 5 μm silica was used, coupled to a guard column of the same chemical consistency (Varian Associates, Walnut Creek, Calif.). The mobile phase consisted of an isocratic mixture of acetonitrile and 0.1% phosphoric acid in deionized water (50:50 v/v) run at a flow rate of 1.1 ml/min. Sample injections were 50 μl. All solvents used were HPLC grade (Fisher Scientific, Norcross, Ga.).

Sample preparation and analysis

Blood samples were centrifuged and the plasma was separated from the cells. Using a 15-ml polypropylene conical tube, a 300-μl sample of plasma was treated with 6 ml acetonitrile and mixed thoroughly on a vortex mixer. After centrifugation the acetonitrile extract was transferred to another polypropylene tube and evaporated at approximately 45°C under a gentle stream of nitrogen. The residue was resuspended in 150 μl mobile phase and filtered through a 0.45-μm syringe filter for injection into the HPLC system. Peritoneal fluid samples were diluted with mobile phase as required and filtered through 0.45 μm nylon syringe filters for injection into the HPLC system. Tissue samples were dried of surface moisture, accurately weighed (250 mg), and homogenized in 5 ml acetonitrile. The homogenate was centrifuged and the acetonitrile extract was removed and evaporated as with the plasma samples. The residue was redissolved in 1 ml mobile phase and filtered for injection into the HPLC system.

Statistical procedures

The AUC of peritoneal fluid vs time, and plasma vs time were obtained using Prism for Windows, version 3.0 (GraphPad Software, San Diego, Calif.). All pharmacokinetic data and tissue concentrations were compared between groups using the Mann-Whitney test (two-tailed) also using Prism for Windows, version 3.0. For all statistical procedures, P values < 0.05 were taken as significant.

Results

Intravenous docetaxel

After the bolus intravenous injection of 15 mg/kg of docetaxel in 1 ml normal saline solution, a rapid clearance of the drug from the systemic circulation was observed during the first 15 min (Fig. 1). This was followed by a slower more steady decrease in plasma docetaxel concentration over the next 75 min. Peritoneal fluid concentrations showed a gradual increase during the first 60 min and a slow decrease during the last 30 min. A concentration equilibrium was not observed at 90 min. At that time the mean plasma and peritoneal fluid levels were respectively 1.18 ± 0.86 and 0.046 ± 0.005 μg/ml. The mean AUC ratio of peritoneal fluid to plasma docetaxel over the 90-min period was 0.01.

Intraperitoneal docetaxel at 15 mg/kg

When docetaxel was delivered intraperitoneally there was a delayed clearance of the drug from the peritoneal fluid (Fig. 2). The mean half-life of docetaxel in the peritoneal fluid was more than 90 min with approximately 40% of the drug absorbed at the end of the experiment. There was a gradual slow increase in plasma concentration over the 90-min period with a mean plasma level of 0.19 ± 0.1 μg/ml at the end of the experiment. The AUC ratio of peritoneal fluid to plasma was 976.

Intraperitoneal docetaxel at 150 mg/kg

With a high dose of intraperitoneal docetaxel there was a delayed clearance of drug from the peritoneal cavity similar to that seen with 15 mg/kg (Fig. 3). The mean half-life of docetaxel in the peritoneal fluid was more than 90 min with approximately 44% of the drug absorbed at the end of the experiment. There was a gradual increase in plasma concentration over the 90-min period with a mean plasma level of 0.68 ± 0.2 μg/ml at the end of the experiment. The AUC ratio of peritoneal fluid to plasma was 3004.

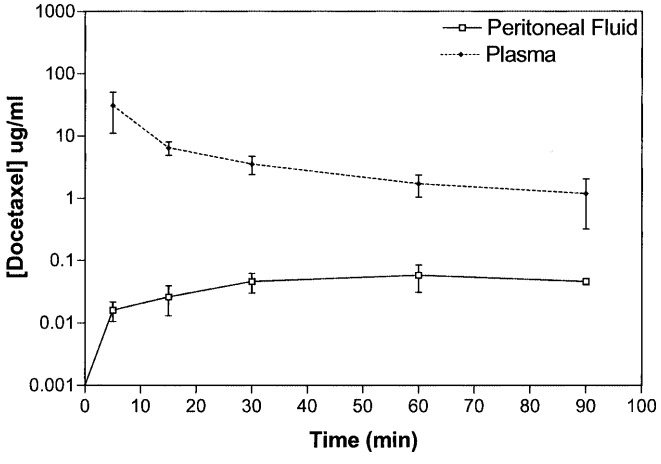

Fig. 1. Plasma and peritoneal fluid concentration vs time after intravenous administration of 15 mg/kg docetaxel

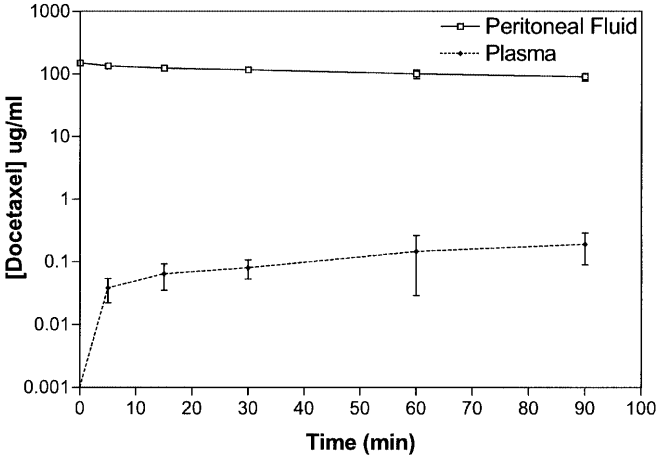

Fig. 2. Plasma and peritoneal fluid concentration vs time after intraperitoneal administration of 15 mg/kg docetaxel

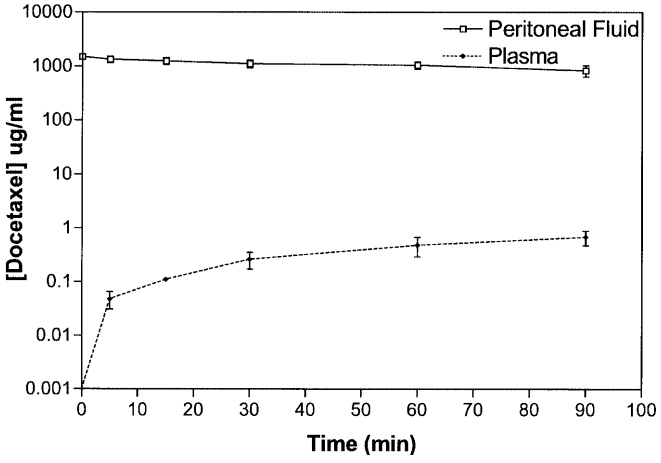

Fig. 3. Plasma and peritoneal fluid concentration vs time after intraperitoneal administration of 150 mg/kg docetaxel

Analysis of drug concentrations by route of docetaxel administration

A comparison of the AUC for plasma concentrations after intravenous and intraperitoneal administration of 15 mg/kg docetaxel revealed a significant decrease in systemic exposure when docetaxel was administered intraperitoneally. The AUC for plasma docetaxel with intravenous delivery was 4.25 μg/ml·min as opposed to 0.11 μg/ml·min with intraperitoneal delivery ($P = 0.0079$). Conversely, there was a significant increase ($P = 0.0079$)

in the AUC for peritoneal fluid concentrations after intraperitoneal administration. The AUC for peritoneal fluid docetaxel with intraperitoneal delivery was 110.6 μg/ml·min as opposed to 0.043 μg/ml·min after intravenous administration. The data apply only to the 90 min over which these experiments were conducted and should not be extrapolated to longer time periods.

Tissue absorption

The highest mean tissue concentrations were observed in the heart and the liver (26.1 ± 7.8 and 25.3 ± 26.1, respectively) after intravenous docetaxel administration and in the abdominal wall and the colon (23.6 ± 15.1 and 21.7 ± 5.7, respectively) after intraperitoneal administration. The differences in the tissue concentrations observed after intraperitoneal delivery as opposed to intravenous delivery (Fig. 4) were statistically significant in the heart ($P = 0.0079$), stomach ($P = 0.0159$), colon ($P = 0.0159$) and abdominal wall ($P = 0.0079$).

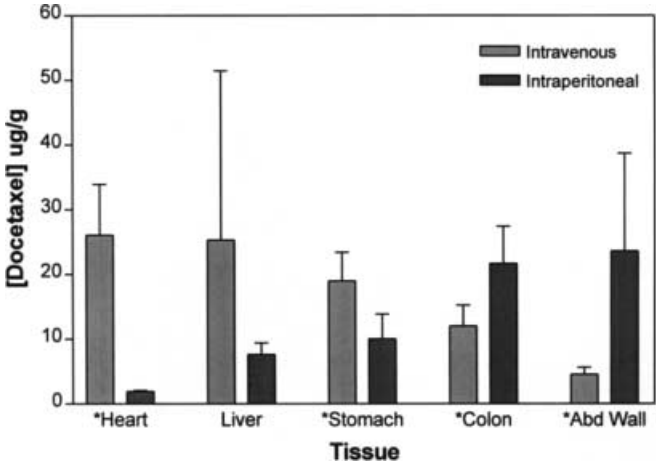

Fig. 4. Comparison of docetaxel in tissues after 90 min of intravenous and intraperitoneal docetaxel at 15 mg/kg. *Asterisks* statistical significance

Discussion

High molecular weight drugs are retained in high concentrations and for prolonged periods in the peritoneal cavity after intraperitoneal administration [3, 9], and docetaxel, with a molecular weight of 861.9, is no exception. The ratio of the AUC of docetaxel in peritoneal fluid following intraperitoneal administration to that following intravenous administration was 2500:1. This means a 2500-fold increase in exposure for peritoneal surfaces with intraperitoneal administration. The AUC ratio of peritoneal fluid to plasma docetaxel was 976 with intraperitoneal administration at 15 mg/kg and 3004 at 150 mg/kg as opposed to 0.01 with intravenous administration. The marked difference between peritoneal fluid and plasma docetaxel concentrations was maintained over 90 min and across great differences in docetaxel concentration. Apparently there is a more systemic clearance as compared to peritoneal absorption over a wide dose range. A summary of the peritoneal fluid to plasma AUC ratios of different drugs previously studied in our laboratory and delivered intraperitoneally is shown in Table 1.

Failure analysis of the surgical treatment of peritoneal surface malignancy shows that local-regional cancer recurrence is seen in a large proportion of patients even though all visible evidence of disease is removed surgically. One hypothesis is that microscopic residual disease persists on peritoneal surfaces and results in cancer recurrence [18]. Recently, methodologies to add intraperitoneal chemotherapy to the surgical removal of the cancer have been described [17]. In this combined approach the cancer within the abdomen or pelvis is removed by cytoreductive surgery and then chemotherapy is administered intraperitoneally. Intraperitoneal chemotherapy can be considered in the treatment of several intraabdominal malignancies that disseminate to peritoneal surfaces such as malignant peritoneal mesothelioma, and gastric and ovarian cancer [1, 19, 20]. Data suggest that intraperitoneal chemotherapy can significantly improve the survival of patients with gastric cancer and is more effective than systemic treatment for malignant peritoneal mesothelioma [19, 20]. Because of

Table 1. Peritoneal fluid to plasma AUC ratios of different drugs previously studied in our laboratory and delivered intraperitoneally

Drug	Reference	Molecular weight	Experiment duration (min)	Drug dose (mg/kg)	AUC ratio
Docetaxel		861.9	90	15	976
Paclitaxel	Marchettini et al. (2001) Unpublished data	853.9	90	15	2301
Liposome-entrapped doxorubicin	Pestieau et al. (1999) Unpublished data		90	6	670
Doxorubicin	7	579.9	60	4	74
Multitargeted antifolate	13	471.3	90	10	50
Oxaliplatin	14	397.6	90	5	16
Gemcitabine	12	263.2	90	12.5	16

the high incidence of intraabdominal tumor recurrences after resection of abdominal and pelvic malignancies, intraperitoneal docetaxel may diminish the incidence of local and regional disease recurrence.

In this study docetaxel was shown to be a pharmacokinetically advantageous drug for intraperitoneal administration. High intraperitoneal docetaxel concentrations were achieved over the 90-min experiment with very low plasma levels. This marked difference in drug concentrations with paclitaxel continues for at least 24 h (Mohamed et al., work in progress). The high AUC ratio demonstrates excellent regional exposure of the drug and a potential for a favorable antitumor effect on small peritoneal surface tumor deposits or microscopic residual disease. Intraperitoneal delivery resulted in statistically significantly increased docetaxel concentration in the abdominal wall and in the colon, suggesting high direct penetration of docetaxel into the tissues; in contrast there was a statistically significantly reduced drug concentration in the heart. High and variable docetaxel concentrations were observed in the liver after intravenous delivery while they were low and stable after intraperitoneal administration. The lower docetaxel concentrations observed in the stomach when the drug was administered intraperitoneally as compared to intravenously could have been due to the thickness of the stomach wall. The direct penetration of drug into tissue may result in a low mean concentration when determined on a tissue as thick as the stomach wall. Johansen documented a significant difference in uptake of other chemotherapy agents in tissues following intraperitoneal vs intravenous administration [8].

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