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Pharmacokinetic advantage of intraperitoneal injection of docetaxel in the treatment for peritoneal dissemination of cancer in mice

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Abstract

Intraperitoneal administration of docetaxel has been used to treat peritoneal dissemination of cancer, but its safety has not yet been confirmed. We have compared the pharmacokinetic behaviour of docetaxel after intravenous and intraperitoneal administration in CD-1-*nu/nu* mice bearing MKN-45P, a gastric cancer variant line producing peritoneal dissemination. Docetaxel (8 mg kg^{-1}) was intravenously or intraperitoneally injected into the mice and at designated times the drug concentration was measured in plasma, ascites fluid, and abdominal tissues (liver, kidney, intestine and spleen, solid cancer, and suspended free cancer). The pharmacokinetic behaviour of docetaxel was similar in control mice and cancer-bearing mice after administration via either route, except that the transfer of docetaxel from the abdominal cavity to systemic blood (plasma) was slower in cancer-bearing mice than in control mice. As expected, the intraperitoneal drug concentration was much higher (approximately 100-fold) and was maintained for a longer time in the intraperitoneal injection group than in the intravenous injection group. The drug concentrations in peritoneal solid cancer tissue and suspended free cancer cells were also significantly higher for a longer time in the intraperitoneal injection group than in the intravenous injection group. The values of the plasma area under concentration–time curves (AUC) were similar for both administration routes. The ratio of AUC ascite/AUC plasma after intraperitoneal administration was higher than after intravenous administration. The drug concentration in abdominal organs after intraperitoneal injection was lower during the first 2 h, then became similar to those after intravenous injection. These results indicated that the intraperitoneal administration of docetaxel for peritoneal dissemination was likely to be an effective treatment method, without causing any increase in systemic toxicity.

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Introduction

Chemotherapy for patients with peritoneal dissemination has generally been unsatisfactory. In most cases, drugs have been given by intravenous (i.v.) injection, and the insufficient effect might have been due to failure of the drugs to reach abdominal cancerous tissues at sufficient concentration to eradicate the cancer. Intraperitoneal (i.p.) injection, on the other hand, would be expected to produce a higher drug concentration in the abdominal cavity and a lower systemic toxicity compared with intravenous administration. In Kanazawa University Hospital, intraperitoneal infusion chemotherapy using CMV therapy (cisplatin, mitomycin and etoposide) or taxane has recently been tried in patients with peritoneal dissemination of gastric cancer, with monitoring of the drug concentration in plasma and peritoneal fluid. It provided significant effectiveness with a low level of side effects (Fushida et al 2002a, b; Furui et al 2003). There have been some basic pharmacological and pharmacokinetic studies on the intraperitoneal injection of anticancer drugs and the safety of the chemotherapy in experimental animals and man (Alberts et al 1996; Marchettini et al 2002; de Bree et al 2003; Maruyama et al 2003; Morgan et al 2003). It is important for the prediction of safety and effectiveness to know the behaviour of anticancer drugs in the systemic blood flow and in organs and cancer tissues in the peritoneal cavity.

Docetaxel, a member of the taxane class of cytotoxic agents, induces polymerization of tubulin monomer and causes mitotic arrest in the G_2M phase of the cell cycle (Diaz & Andreu 1993). It is used to treat a wide range of tumours by intravenous or intraperitoneal injection, including breast, lung, prostate, ovarian, head and neck, gastric, pancreatic and bladder cancer (van Oosterom 1999). We have compared the pharmacokinetics after intraperitoneal injection and intravenous injection of docetaxel in CD-1-*nu*/*nu* mice that had been intraperitoneally inoculated with MKN-45P human gastric cancer.

Materials and Methods

Materials

Docetaxel (Taxotere) (10 mg mL^{-1}) was kindly provided by Aventis Pharma Ltd (Tokyo, Japan). This preparation was diluted with physiological saline to 4 mg mL^{-1} docetaxel for intravenous injection and to 0.25 mg mL^{-1} docetaxel for intraperitoneal injection.

Animals and cell line

Female CD-1 (ICR)-*nu*/*nu* mice were obtained from Charles River Japan, Inc. (Yokohama, Japan), and treated in accordance with the guidelines of the Institutional Animal Care and Use Committee of Kanazawa University. A human gastric cancer cell line MKN-45P (10^7 cells) was inoculated intraperitoneally into 6-week-old CD-1-*nu*/*nu* mice. After three weeks, docetaxel (8 mg kg^{-1}) was administered intravenously or intraperitoneally into control and cancer-bearing mice. The injection volume was 2 μL g^{-1} body weight for intravenous administration and 30 μL g^{-1} body weight for intraperitoneal administration. Blood, ascites fluid and major peritoneal organs (liver, spleen, kidney, intestine), solid cancer, and suspended free cells were collected at designated times (0.5, 1, 2, 4 and 8 h). Tissue samples were homogenized using a Teflon homogenizer. Plasma was separated by centrifugation at 3000 *g* for 10 min. Each sample was stored at −20°C until use.

Measurement of docetaxel

The assay for docetaxel was performed according to Loos et al (1997). Briefly, a 0.5–1.0 mL sample, 3 mL acetonitrile: *n*-butyl chloride (1:4, v/v) and 100 μL paclitaxel (6 μg mL^{-1}) as an internal standard were added to a glass tube. The sample was mixed vigorously for 30 s, followed by centrifugation for 10 min at 3000 *g*. The organic layer was collected in another glass tube and evaporated for 40 min at 55°C. A volume of 250 μL methanol:water (1:1, v/v) was added to the residue and after vortex-mixing, 150 μL of the supernatant was injected into the HPLC system. All samples were analysed on an HPLC system equipped with a Shim-pack CLC-ODS column (150 × 6.0 mm i.d., Shimadzu). The absorbance was detected at a wavelength of 232 nm. The mobile phase consisted of methanol:0.3% phosphoric acid (2:1, v/v) and was pumped at a rate of 1 mL min^{-1}. The detection limit was approximately 0.05 μg mL^{-1} and the linear regression coefficients were 0.985–0.998. The coefficients of variation for the within-run and between-run precisions were below 7%.

Data analysis

The statistical significance was calculated using the Mann-Whitney U test. The criterion of a significant difference between sets of data was taken to be $P < 0.05$. The experiments were performed in triplicate.

Results

The plasma concentration–time courses of docetaxel after intravenous or intraperitoneal administration (8 mg kg^{-1}) in control and cancer-bearing mice are shown in Figure 1. In normal control mice, the drug appeared in the systemic blood and reached the maximum plasma concentration at 1 h after intraperitoneal injection, while in cancer-bearing

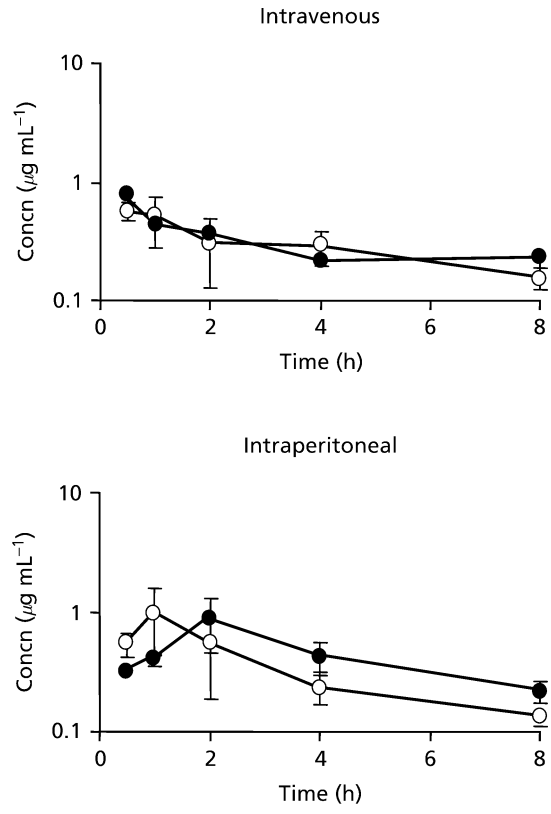

Figure 1 Time course of plasma concentration of docetaxel after an intravenous or intraperitoneal injection of docetaxel in normal control and MKN-45P-bearing mice. Docetaxel (8 mg kg^{-1}) was injected (i.v. or i.p.) into normal control mice (open symbols) and cancer-bearing mice (closed symbols) on day 21 after intraperitoneal inoculation of 10^7 MKN-45P gastric cancer cells. Each point represents the mean ± s.d. of three mice.

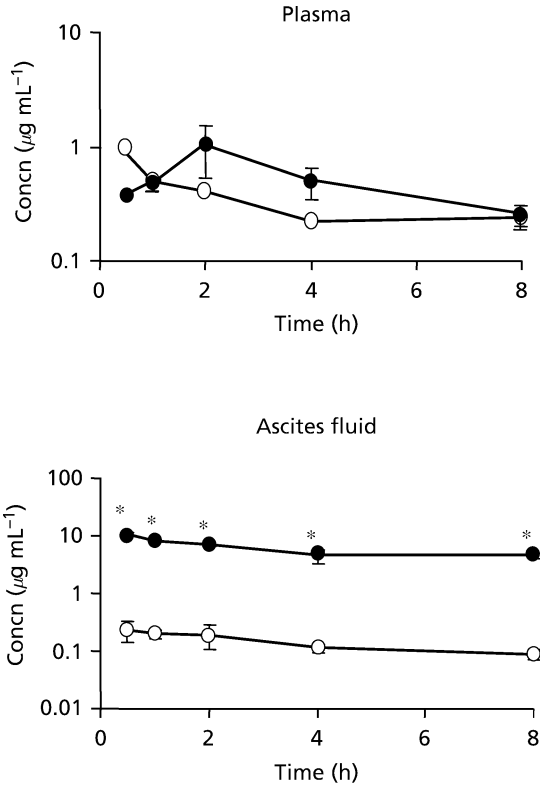

Figure 2 Time course of the concentration of docetaxel in plasma and ascites fluid after an intravenous or intraperitoneal injection of docetaxel in MKN-45P-bearing mice. Docetaxel ($8\,mg\,kg^{-1}$) was intravenously (open symbols) or intraperitoneally (closed symbols) injected into cancer-bearing mice on day 21 after intraperitoneal inoculation of 10^7 MKN-45P gastric cancer cells. Each point represents the mean ± s.d. of three mice. $*P < 0.05$ compared with intravenous injection.

mice the maximum plasma concentration was similar to that in normal mice, but the peak time was delayed. After intravenous administration, the plasma concentrations showed the same profiles in control and cancer-bearing mice. The cancer-bearing mice had approximately 10–20 mL ascites fluid, and so the peritoneal drug concentration could be measured after drug administration, but this was not possible in normal control mice, which had no ascites fluid. Figure 2 shows the time courses of docetaxel concentration in plasma and ascites fluid in cancer-bearing mice after an intravenous or an intraperitoneal injection of docetaxel ($8\,mg\,kg^{-1}$). The drug concentration in the peritoneal cavity was approximately 100-fold higher after intraperitoneal injection than after intravenous injection, while the plasma concentrations were similar. Table 1 gives the values of the area under the concentration–time curves (AUC) in plasma and ascites fluid for the 8 h after injection. From these data, after intraperitoneal injection, docetaxel was not readily transferred into the systemic blood flow, at 8 h 50% remained in the peritoneal cavity, whereas after intravenous injection, the drug seemed to pass comparatively easily into the peritoneal cavity from the blood flow, although the intraperitoneal concentration was low.

Table 1 The values of AUC of docetaxel in plasma and ascites fluid after an intravenous or an intraperitoneal injection

Injection route	AUC_p ($mg\,h\,mL^{-1}$)	AUC_a ($mg\,h\,mL^{-1}$)	Ratio of AUC_a/AUC_p
Intravenous	4.85 ± 0.59	1.13 ± 0.13	0.233
Intraperitoneal	3.37 ± 0.89	47.3 ± 5.2	14.0

AUC_p and AUC_a indicate the AUC values in plasma and ascites fluid, respectively, from 0 to 8 h after an intravenous or an intraperitoneal injection of $8\,mg\,kg^{-1}$ docetaxel in MKN-45P-bearing mice. Data are mean ± s.d.

Figure 3 shows the time course of the concentration of docetaxel in peritoneal cancer after an intravenous or intraperitoneal injection of docetaxel ($8\,mg\,kg^{-1}$). In suspended free cells, the drug concentration was much higher in the intraperitoneal group than in the intravenous group, paralleling the concentrations in ascites after drug injection via the respective routes. In solid cancer tissue, the drug concentration gradually decreased after intravenous injection,

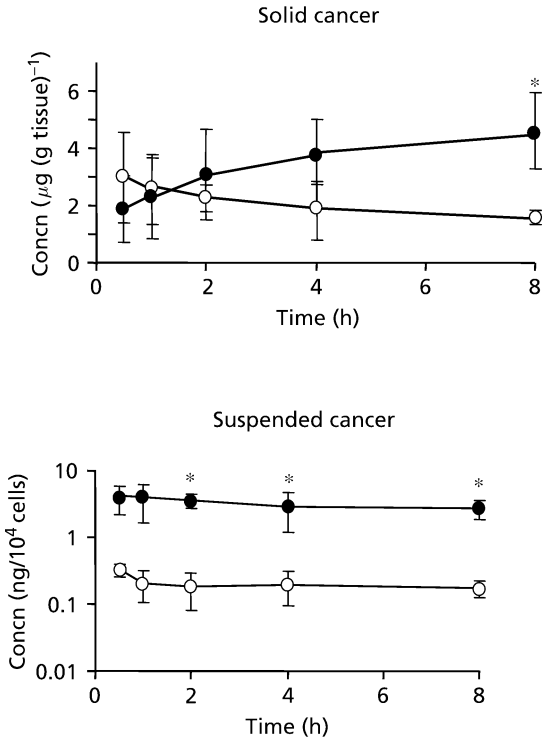

Figure 3 Time course of the concentration of docetaxel in solid cancer and suspended free cancer cells after an intravenous or intraperitoneal injection of docetaxel in MKN-45P-bearing mice. Docetaxel ($8\,mg\,kg^{-1}$) was intravenously (open symbols) or intraperitoneally (closed symbols) injected into cancer-bearing mice on day 21 after intraperitoneal inoculation of 10^7 MKN-45P gastric cancer cells. Each point represents the mean ± s.d. of three mice. $*P < 0.05$ compared with intravenous injection.

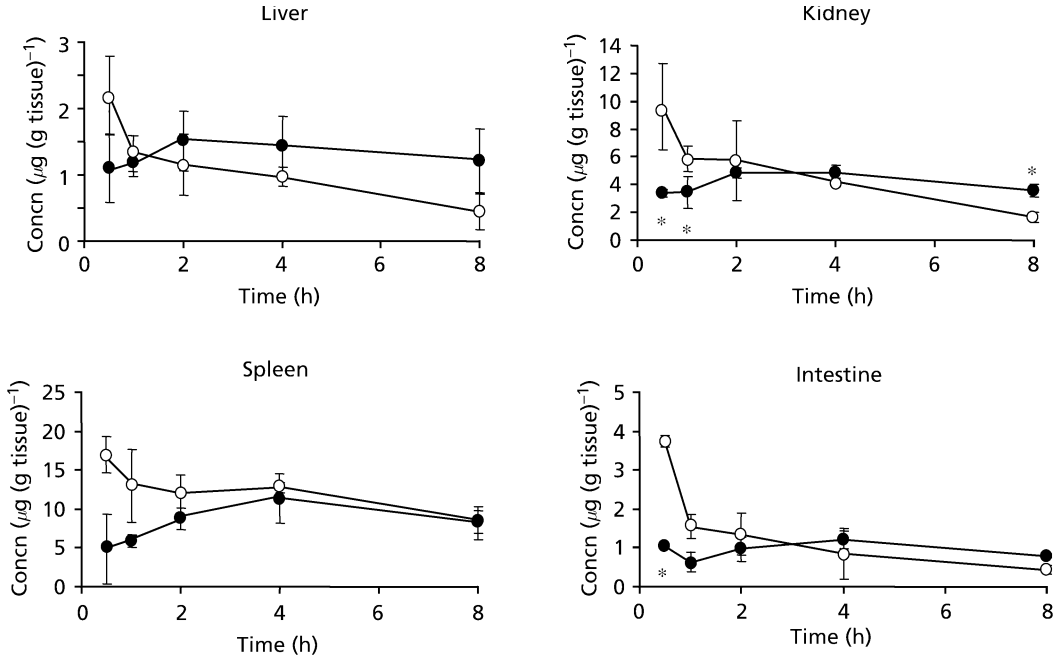

Figure 4 The tissue concentration–time course of docetaxel after an intravenous or intraperitoneal injection of docetaxel in MKN-45P bearing mice. Docetaxel (8 mg kg^{-1}) was intravenously (open symbols) or intraperitoneally (closed symbols) injected into cancer-bearing mice on day 21 after intraperitoneal inoculation of 10^7 MKN-45P gastric cancer cells. Each point represents the mean ± s.d. of three mice. *$P < 0.05$ compared with intravenous injection.

following the change in the plasma concentration. After intraperitoneal injection the concentration increased up to 8 h after injection, so that the docetaxel concentration in solid cancer was maintained at a higher level from 2 h after intraperitoneal injection as compared with that after intravenous injection. On the other hand, docetaxel appeared to be distributed into the liver and intestine to a similar extent to that in solid cancer tissue after intravenous injection, but was concentrated much more in the kidney and spleen (Figure 4). The docetaxel concentrations in these tissues rapidly decreased up to 1 h and then gradually decreased in the intravenous group, while in the intraperitoneal group the concentrations increased up to 2 or 4 h after injection, then slowly decreased, although the concentration in solid cancer continued to increase for at least 8 h after intraperitoneal injection.

Discussion

The results provided evidence to support treatment of peritoneal carcinomatosis by intraperitoneal administration of docetaxel rather than by intravenous administration. When docetaxel was injected via the intravenous and intraperitoneal routes at the same dosage (8 mg kg^{-1}) into gastric cancer MKN-45P-bearing mice, the drug concentration in ascites fluid was much higher (approximately 100-fold) and was maintained for a longer time in the intraperitoneal group than in the intravenous group. Also, the drug concentrations in peritoneal solid cancer tissue and suspended free cancer cells were significantly higher for a longer time in the intraperitoneal group than in the intravenous group. The AUC values in plasma were similar for both administration routes. The ratio of AUC ascite/AUC plasma after intraperitoneal administration was higher than after intravenous administration. These results suggested that the intraperitoneal administration of docetaxel was more effective for treatment of peritoneal dissemination than intravenous administration. It has been reported that anticancer drugs administered intravenously might not pass readily into the peritoneal cavity due to the plasma–peritoneal barrier, consisting of the endothelium, mesothelium and intervening interstitium (Jacquet & Sugarbaker 1996). However, there is little information regarding drug transfer from the peritoneal cavity to the blood. We have indicated that docetaxel injected into the peritoneal cavity was transferred rather slowly to the peripheral blood flow, especially in cancer-bearing mice (Figure 1). This suggested that intraperitoneal administration might have caused less severe systemic adverse effects than intravenous administration, even at the same dosage. The reason for this might be as follows: docetaxel was used as a micellar preparation with Polysorbate 80, which was hydrolysed by esterases in plasma, leading to release of the drug (van Tellingen et al 1999); however, esterase activity was low in ascites fluid, so that the free drug concentration was low, and therefore the drug penetration through the abdominal wall into blood was very slow. Also, the mechanism underlying our findings that the transfer rate in cancer-bearing mice was slower compared with normal mice was unclear. Oku et al (1988)

reported that it became hard for a drug to penetrate from the peritoneal to systemic blood flow when the disseminated peritoneum adhered and became hard. This suggested that an anticancer drug administrated intraperitoneally was more effective when the peritoneal dissemination of the cancer became worse. On the other hand, Taxol is a paclitaxel preparation dissolved in Cremophol EL. Cremophol EL is less hydrolysable than polysorbate 80, and so when injected into the peritoneal cavity, the free concentration of paclitaxel in ascites fluid was very low and its penetration into the systemic blood flow and tissues was lower than docetaxel (Fushida et al 2002b; Furui et al 2003).

The drug concentration in suspended free cancer cells was much higher after intraperitoneal injection than after intravenous injection, presumably because the drug concentration in the peritoneal cavity was approximately 100-fold after intraperitoneal injection compared with after intravenous injection (Figure 2). The drug behaviour in tissues of the abdominal organs was similar to the course of plasma concentration irrespective of the injection route, although the drug concentrations were different in each tissue (Figure 4). This suggested that the translocation to tissue, except for solid cancer, was dependent on the blood concentration. However, the intraperitoneally injected drugs might have been distributed into cancer tissue in the peritoneal cavity both via the blood flow and by direct transfer from ascites fluid. Indeed, the drug concentration in solid cancer tissue increased for some time after intraperitoneal injection, while after intravenous injection the drug concentration decreased in parallel with the plasma concentration (Figure 3). Previously, we reported that docetaxel ($8\,mg\,kg^{-1}$) was significantly effective in a dose-dependent manner against peritoneal dissemination in MKN-45P-bearing mice when given by intraperitoneal administration (Yonemura et al 2004). Dykes et al (1995) reported that the intraperitoneal administration of docetaxel ($15\,mg\,kg^{-1}$) resulted in an increase in the life span of mice bearing peritoneal dissemination of ovarian cancer, melanoma, and mammary carcinoma, while the effect of intravenous administration of the same dose of docetaxel was significantly less. Recently, there have been a few papers regarding intraperitoneal docetaxel pharmacokinetic studies (Fushida et al 2002a, b; Morgan et al 2003). The significant advantage of intraperitoneal chemotherapy for peritoneal cancers was supported by a study by Alberts et al (1996) using cisplatin.

In summary, the intraperitoneal injection of docetaxel was considered to be advantageous as a treatment method for peritoneal dissemination of cancers, offering higher local drug concentration and lower systemic toxicity compared with intravenous injection.

References

Alberts, D. S., Liu, P. Y., Hannigan, E. V., O'Toole, R., Williams, S. D., Young, J. A., Franklin, E. W., Clarke-Pearson, D. L., Malviya, V. K., DuBeshter, B. (1996) Intraperitoneal cisplatin plus intravenous cyclophosphamide versus intravenous cisplatin plus intravenous cyclophosphamide for stage III ovarian cancer. *N. Engl. J. Med.* **335**: 1950–1955

Diaz, J. F., Andreu, J. M. (1993) Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichiometry, and competition. *Biochemistry* **32**: 2747–2755

Dykes, D. J., Bissery, M. C., Harrison, S. D., Waud, W. R. (1995) Response of human tumor xenografts in athymic nude mice to docetaxel (RP 56976, Taxotere). *Invest. New Drugs* **13**: 1–11

de Bree, E., Romanos, J., Michalakis, J., Relakis, K., Georgoulias, V., Melissas, J., Tsiftsis, D. D. (2003) Intraoperative hyperthermic intraperitoneal chemotherapy with docetaxel as second-line treatment for peritoneal carcinomatosis of gynaecological origin. *Anticancer Res.* **23**: 3019–3027

Furui, N., Yamazaki, T., Yokogawa, K., Fushida, Y., Miwa, K., Miyamoto, K. (2003) Ascites and the plasma concentration-time courses of the taxanes after an intraperitoneal administration in patients with peritoneal tumors. *Jpn. J. Health Care Sci.* **29**: 263–269

Fushida, S., Nao, F., Kinami, S., Ninomiya, I., Fujimura, T., Nishimura, G., Ohta, T., Yokogawa, K., Miyamoto, K., Miwa, K. (2002a) Pharmacologic study of intraperitoneal docetaxel in gastric cancer patients with peritoneal dissemination. *Jpn. J. Cancer Chemother.* **29**: 1759–1763

Fushida, S., Nao, F., Kinami, S., Ninomiya, I., Fujimura, T., Nishimura, G., Ohta, T., Yokogawa, K., Miyamoto, K., Miwa, K. (2002b) Pharmacologic study of intraperitoneal paclitaxel in gastric cancer patients with peritoneal dissemination. *Jpn. J. Cancer Chemother.* **29**: 2164–2167

Jacquet, P., Sugarbaker, P. H. (1996) Peritoneal-plasma barrier. *Cancer Treat. Res.* **82**: 53–63

Loos, W. J., Verweij, J., Nooter, K., Stoter, G., Sparreboom, A. (1997) Sensitive determination of docetaxel in human plasma by liquid-liquid extraction and reversed-phase high-performance liquid chromatography. *J. Chromatogr. B: Biomed. Sci. Appl.* **693**: 437–441

Marchettini, P., Stuart, O. A., Mohamed, F., Yoo, D., Sugarbaker, P. H. (2002) Docetaxel: pharmacokinetics and tissue levels after intraperitoneal and intravenous administration in a rat model. *Cancer Chemother. Pharmacol.* **49**: 499–503

Maruyama, M., Ebuchi, M., Nagahama, T., Takashima, I., Hasegawa, K. (2003) Experimental study on intraperitoneal versus intravenous paclitaxel for peritoneal seeding and liver metastasis. *Jpn. J. Cancer Chemother.* **30**: 1602–1604

Morgan, R. J., Doroshow, J. H., Synold, T., Lim, D., Shibata, S., Margolin, K., Schwarz, R., Leong, L., Somlo, G., Twardowski, P., Yen, Y., Chow, W., Lin, P., Paz, B., Chu, D., Frankel, P., Stalter, S. (2003) Phase I trial of intraperitoneal docetaxel in the treatment of advanced malignancies primarily confined to the peritoneal cavity: dose-limiting toxicity and pharmacokinetics. *Clin. Cancer Res.* **9**: 5896–5901

Oku, M., Noda, T., Kiyozuka, Y., Ninomiya, Y., Hino, K., Okamura, Y., Maruyama, M., Ichijo, M. (1988) Pharmacokinetic analysis of intraperitoneal cisplatinum administration. *Nippon Gan Chiryo Gakkai Shi.* **23**: 657–664

van Oosterom, A. T. (1999) Docetaxel (Taxotere) and gemcitabine phase I and early phase II studies: results to date and an overview of ongoing studies. *Semin. Oncol.* **26**: 8–11

van Tellingen, O., Beijnen, J. H., Verweij, J., Scherrenburg, E. J., Nooijen, W. J., Sparreboom, A. (1999) Rapid esterase-sensitive breakdown of Polysorbate 80 and its impact on the plasma pharmacokinetics of docetaxel and metabolites in mice. *Clin. Cancer Res.* **5**: 2918–2924

Yonemura, Y., Endou, Y., Bando, E., Kuno, K., Kawamura, T., Kimura, M., Shimada, T., Miyamoto, K., Sasaki, T., Sugarbaker, P. H. (2004) Effect of intraperitoneal administration of docetaxel on peritoneal dissemination of gastric cancer. *Cancer Lett.* **210**: 189–196