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Original Paper

Evaluation of Antitumour Effects of Docetaxel (Taxotere®) on Human Gastric Cancers *In Vitro* and *In Vivo*

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***In vitro* antitumour effects of docetaxel (Taxotere®) were examined in nine cultured human gastric cancer cell lines and 18 clinical gastric cancer specimens. *In vivo* antitumour effects were examined in human gastric cancer xenografts in nude mice. The activity was compared with paclitaxel (Taxol®). Docetaxel was more effective than paclitaxel in six of the nine cell lines and the effectiveness rates of docetaxel and paclitaxel were 56% (10/18) and 6% (1/17), respectively, in the clinical gastric cancer specimens. *In vivo* docetaxel showed superior antitumour effect on well differentiated (MKN-28), poorly differentiated (MKN-45) and undifferentiated (KKLS) gastric cancer xenografts. We conclude that docetaxel promises to be clinically active against gastric carcinomas.**

Key words: antitumour effect, docetaxel, gastric cancer
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INTRODUCTION

DOCETAXEL IS a semisynthetic diterpene extracted from leaves of the European yew, *Taxus baccata* [1]. The structure of docetaxel resembles that of another antitumour diterpene, paclitaxel, which is extracted from the Pacific yew *Taxus brevifolia*. These compounds are mitotic spindle poisons that promote tubulin polymerisation and inhibit depolymerisation of microtubules [2, 3]. Both are active in preclinical animal screening systems for anticancer drugs [4] and are also active against tumour xenografts such as LX-1 lung, CX-1 colon, and MX-1 breast tumours [5]. Both underwent clinical trials in U.S.A., Europe and Japan, and showed clinical activity against refractory breast, lung and ovarian cancers [6–8]. *In vitro*, docetaxel has been reported to be 2.5-fold more active than paclitaxel against murine and human tumour cell lines and clinically obtained human cancer specimens [9–11].

A number of chemosensitivity tests have been reported for their ability to predict the efficacy of chemotherapeutic agents. Among them, a tetrazolium based colorimetric assay (the MTT assay) and a collagen gel supported three dimensional culture (the CGM assay) have been reported to be the most promising methods [12–14]. Not only these chemosensitivity tests but also human tumour xenografts might be useful as the screening systems of novel anticancer drugs. We report here the systematic evaluations of docetaxel with these screening methods using cultured human gastric cancer cells and clinical specimens from patients.

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MATERIALS AND METHODS

Drugs

Docetaxel was kindly provided by Rhône-Poulenc Rorer, Japan, Tokyo. Paclitaxel was purchased from Sigma (St. Louis, Missouri, U.S.A.). Both drugs were dissolved in 100% ethanol and diluted with distilled water just before use. The maximum final concentration of ethanol was less than 0.1% in the assays determining cytotoxicity. This ethanol concentration had no significant toxic effect on the cells used here.

Cisplatin, doxorubicin and pirarubicin, which are drugs used in chemotherapy of gastric cancer, were purchased from Nippon Kayaku (Tokyo), Pharmacia (Tokyo) and Meiji Seika Kaisya (Tokyo), respectively. They were diluted or dissolved in physiological saline solution just before use.

Tumours

Nine human gastric cancer cell lines were used in this study. MKN-7, −28, −45, NUGC-4 and KATO-III were supplied by the Japanese Cancer Research Resources Bank (Tokyo). ST-SA-1, NAKAJIMA and ST-KM, were kindly donated by Dr Yanoma (Kanagawa Cancer Centre, Yokohama). The KKLS was obtained from the Department of Surgery in our Institute. Cells were propagated in Dulbecco's minimum essential medium (Nissui Pharmaceutical Co., Osaka) supplemented with 10% heat-inactivated fetal bovine serum

(Gibco Lab., Grand Island, New York, U.S.A.), penicillin (100 U/ml) and streptomycin (100 μg/ml). Single-cell suspension was obtained by trypsinisation of monolayer cultures, and viable cells were counted using the Trypan Blue dye exclusion test.

Tumour specimens from surgery and biopsy were obtained by standard sterile techniques as part of routine clinical procedures. The specimens were stored in a refrigerator and used within 24 h after collection.

MTT assay

The tetrazolium-based semi-automated colorimetric assay (MTT assay) developed by Carmichael and colleagues [12] was modified and used to determine the cytotoxicity of docetaxel and paclitaxel. Cultured human cells (2000 cells in 180 μl of medium) were seeded in a 96-well flat-bottom micro test plate (InterMed, Roskilde, Denmark), and 20 μl of drug solutions with graded concentrations were simultaneously added in triplicate to each well. The plate was incubated for 3 days at 37°C in a humidified atmosphere of 5% CO_2. MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, Missouri, U.S.A.), was prepared at a concentration of 2 mg/ml in Dulbecco's phosphate buffered saline (PBS) without calcium and magnesium. On day 3, 25 μl of the MTT reagent were added to each well. After another 4 h of incubation, the medium was removed by aspiration. To dissolve the resulting MTT-formazan crystals, 0.2 ml of dimethylsulphoxide (DMSO) were added to each well and thoroughly mixed by using a mechanical plate mixer. Absorbance at 540 nm (OD_{540}) was measured with an Immuno Reader NJ-2000 (InterMed Japan, Tokyo). The percentage of cell growth inhibition was calculated by the following formula: per cent of cell growth inhibition = $(1 - T/C) \times 100$, where C is the mean OD_{540} of the control group and T is that of the treated group. The 50% inhibitory drug concentration (IC_{50} value) was determined graphically from the dose–response curve with at least three drug concentration points.

CGM assay

A 1 cm cube of specialised collagen gel matrix derived from pig skin (Spongostan®, Health Design Industries, Rochester, New York, U.S.A.) was placed in each well of a 24-well culture plate. The matrix was hydrated adequately with RPMI-1640 medium (Nissui Pharmaceutical Co. Ltd, Tokyo) supplemented with 10% heat inactivated fetal bovine serum and antibiotics. The tumours obtained from surgery were cut into thin fragments (approximately 10 mg wet weight). For uniform incorporation of MTT reagent to the fragments, they had to be less than 1 mm thick. They were weighed, placed on the matrix and incubated at 37°C in a humidified atmosphere of 5% CO_2. At that time, the top of the matrix and the tumour fragments were not covered with medium. Four days later, the medium was replaced with medium containing the drugs. The plate was incubated for 3 more days. To evaluate the viability of the tumour fragments, 50 μl of the MTT reagent described above and 50 μl of 0.5% collagenase (Worthington Biochemical Co., St. Louis, Missouri, U.S.A.) in PBS to digest the matrix were added to each well. After incubation for 4 h, the plate was centrifuged at 1500 rpm for 10 min to precipitate the resulting MTT-formazan and the supernatant was then discarded. Three hundred microlitres of DMSO were added to each well, and the plate was allowed to stand overnight in a dark place at room temperature to dissolve the MTT-formazan. Two hundred microlitres of the MTT-formazan solution were pip-

Table 1. Cytotoxic effects of docetaxel, paclitaxel, cisplatin, doxorubicin and pirarubicin on cultured human gastric cancers

Cell line	IC_{50} (μM) Docetaxel	Paclitaxel	Cisplatin	Doxorubicin	Pirarubicin
MKN-7	>1.2	>1.2	4.3	0.59	0.17
MKN-28	0.00064	0.0014	5.0	0.11	0.057
MKN-45	<0.00012	<0.00012	21.3	4.8	0.31
NUGC-4	0.0035	0.059	4.0	0.19	0.062
KATO-III	0.004	0.019	2.9	0.11	0.027
KKLS	0.00094	0.0028	3.7	1.2	0.24
ST-SA-1	0.099	0.094	4.3	0.21	0.09
NAKAJIMA	0.00039	0.0032	3.3	0.12	0.027
ST-KM	0.0005	0.0014	0.97	0.62	0.071

Table 2. Antitumour effects of docetaxel, paclitaxel, cisplatin, doxorubicin and pirarubicin on human gastric cancers obtained by surgery or biopsy using the collagen gel matrix assay

Drugs	10-fold equivalent therapeutic peak plasma concentration (μg/ml)	Clinical dosage (mg/m^2)	Number of sensitive specimens/number tested (effectiveness rate)
Docetaxel	15.6	50	10/18 (56%)
Paclitaxel	5.4	150	1/17 (6%)
Cisplatin	25.0	100	9/18 (50%)
Doxorubicin	6.0	60	4/16 (25%)
Pirarubicin	10.0	40	4/6 (67%)

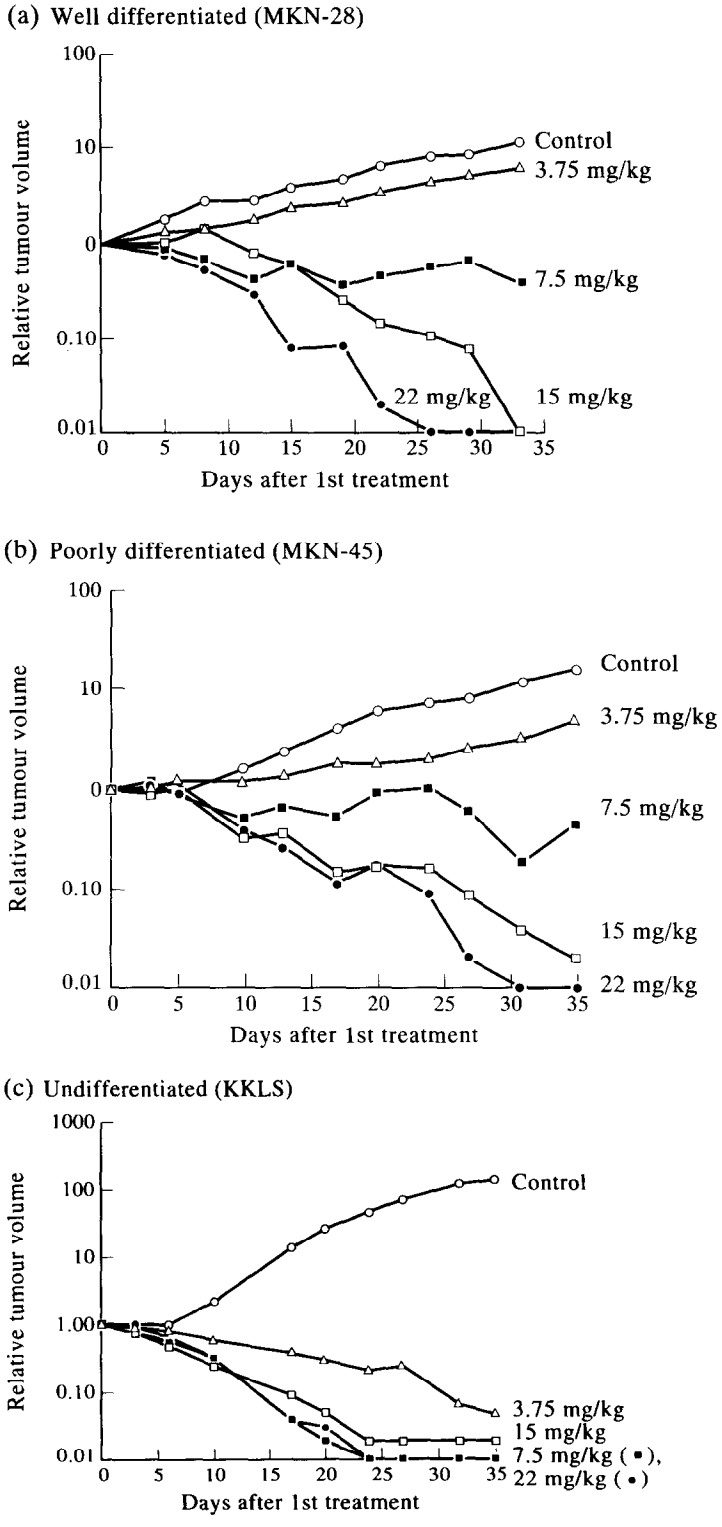

Figure 1. Antitumour effect of docetaxel on human gastric cancer xenografts. Docetaxel was intravenously injected three times at indicated doses with 4-day intervals from the day when the tumour size reached 5–8 mm.

etted to the 96-well flat-bottom micro plate, and OD_{540} was measured. The OD_{540} of untreated cancer cells decreased after 2 days' incubation, but thereafter it increased [14, 15]. The response rate was calculated from the OD_{540} adjusted for tumour weight, as described for the MTT assay. The 10-fold equivalent therapeutic peak plasma concentrations, as estimated by the clinical Phase I study for docetaxel [16] and paclitaxel [17] in Japan and by Scheithauer [18], were used for the present assays. Since the adverse side-effects in Japanese patients were more severe than those in Western patients, the recommended clinical dosages of docetaxel and paclitaxel in Japan were lower relatively than those in Europe and in the United States [16, 17]. The growth inhibition rate was calculated as for the cultured cells. Effectiveness was con-

sidered significant when the cell growth inhibition rate was 30% or more.

Antitumour activity of docetaxel on human tumour xenograft

Five-week-old athymic nude mice (BALB/c nu/nu) weighing approximately 20 g were purchased from Charles River Japan Inc., Atsugi, Japan. They were maintained on a standard diet and water throughout the experiments under the specific pathogen-free conditions. Six mice for each group were used in these experiments. Three human gastric cancer cell lines of different histological types were used for this assay: well differentiated gastric cancer (MKN-28), poorly differentiated gastric cancer (MKN-45) and undifferentiated gastric cancer (KKLS).

Cultured cells (5×10^6 cells) were transplanted into the right groin of mice. Docetaxel at doses of 22, 15, 7.5 or 3.75 mg/kg was intravenously injected three times with 4-day intervals from the day that the tumour size reached 5–8 mm. The tumour volume (V) was calculated for an ellipsoid with the formula $V = (a \times b^2)/2$, where a and b are the measurements (in mm) of length and width, respectively. According to the Battelle Columbus Laboratories Protocol [19], each tumour volume was expressed as the relative tumour volume (RV) calculated by the formula $RV = V_n/V_0$, where V_n is the mean tumour volume at day n and V_0 is the mean initial tumour volume at the start of treatment (day 0). The calculated tumour volume and RV value were graphically plotted and the effectiveness of each drug was statistically evaluated by the Mann–Whitney U-test.

RESULTS

Table 1 shows the IC_{50} value (μM) of docetaxel, paclitaxel, cisplatin, doxorubicin and pirarubicin on nine cultured human gastric cancer cells using the MTT assay. The cytotoxic effect of docetaxel was relatively greater than that of paclitaxel in six of the nine cells. The effect of docetaxel and paclitaxel on MKN-7 and ST-SA-1 cells was less than on the other seven cultured cells. However, the cytotoxic effect of docetaxel and paclitaxel were much higher than that of cisplatin, doxorubicin and pirarubicin.

The antitumour effect of docetaxel on 18 clinical specimens obtained by surgery or biopsy was evaluated by use of the CGM assay (Table 2). The activity was compared with paclitaxel and clinically used drugs. According to our criteria for evaluating effectiveness, docetaxel was effective for 10 of the 18 specimens (56%) while paclitaxel, doxorubicin, pirarubicin and cisplatin were effective in 6% (1/17), 25% (4/16), 67% (4/6) and 50% (9/18), respectively. Docetaxel showed a potent antitumour effect on these gastric cancers. Interestingly, paclitaxel was no more active than conventional anticancer drugs for gastric cancers.

The antitumour effect of docetaxel on human gastric cancers xenografts (MKN-28, MKN-45 and KKLS) was also measured. Docetaxel inhibited tumour growth dose-dependently, particularly at doses of 22 and 15 mg/kg when complete tumour regression was observed in all mice (Figure 1). Tumour regression rates, calculated by the Battelle Columbus Laboratories Protocol, was 98% at 7.5 mg/kg ($P < 0.01$ compared with control) and 47% at 3.75 mg/kg ($P < 0.05$ compared with control) for MKN-28 xenograft. As a side-effect of docetaxel, body weight was decreased by 18% in the treatment period at a dose of 22 mg/kg, but no mice were sacrificed due to treatment-related toxicity.

DISCUSSION

Gastric cancer continues to be a leading cause of cancer mortality in Japan. Despite the progress of surgical approaches, prognosis of gastric cancer patients remains very poor. Chemotherapy has greatly contributed to the improvement of the 5-year survival rates of patients with advanced gastric cancer. However, although cisplatin, 5-fluorouracil, mitomycin C and doxorubicin are usually used for the chemotherapy of gastric cancer, the effectiveness of chemotherapy has been unsatisfactory. Therefore, a novel anticancer drug for gastric cancer is needed.

Microtubules play an important role in mitosis, cell movement, cell adhesion, and intracellular transportation. Microtubules are unstable and maintain a balance between polymerisation and depolymerisation of tubulin. Microtubules are the most strategic subcellular targets for anticancer agents [20]. Recently, such agents have been introduced for clinical use in cancer chemotherapy. Vinca alkaloids inhibit the polymerisation of tubulin [21] and show a strong antitumour effect on malignant lymphoma, lung carcinoma and leukaemia. Docetaxel inhibits microtubule depolymerisation [22].

In this report, the antitumour effect of docetaxel on human gastric cancers was systematically evaluated both by *in vitro* and *in vivo* screening systems. An *in vitro* MTT assay revealed that docetaxel was more active than paclitaxel in six of the nine cultured human gastric cancer cell lines and its cytotoxicity was 2–80 times greater than that of paclitaxel. Docetaxel showed relatively broad cytotoxic effects on a panel of 30 tumour cell lines (data not shown). To confirm the results of the MTT assay on cultured human gastric cancers, the chemosensitivity of tumour specimens from different patients was tested using the CGM assay. Docetaxel showed a cytotoxic effect in 10 of the 18 clinical specimens (56%). This effectiveness rate was very similar to that of cisplatin (50%) on the same specimens. An *in vivo* nude mouse assay also revealed that docetaxel showed high antitumour effect on human gastric cancer xenografts, and the effect was seen irrespective of histological type.

The assay systems used here have been tested for correlations between assay and clinical responses in many institutions [23], and have been reported to have excellent clinical predictability in spite of some drawbacks [24]. An early Phase II study of docetaxel for gastric cancer is underway in Japan, and the response rate of docetaxel is reported to be 20% [24]. This result shows that the systematic screening systems may predict the clinical response of docetaxel. Accordingly, docetaxel is anticipated to be a novel, clinically useful anticancer drug for gastric cancer with a unique mechanism of action and a broad spectrum of antitumour activity.

1. Ringel I, Horwitz SB. Studies with RP 56976 (docetaxel): a semisynthetic analogue of taxol. *J Natl Cancer Inst* 1991, **83**, 288–291.
2. Diaz JF, Andreu JM. Assembly of purified GDP-tubulin into microtubules induced by taxol and taxotere: reversibility, ligand stoichometry, and competition. *Biochemistry* 1993, **32**, 2747–2755.
3. Schiff PB, Horwitz SB. Taxol stabilized microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA* 1980, 77, 1561–1565.
4. Bissery MC, Guenard D, Gueritte-Voegelein F, Lavelle F. Experimental antitumor activity of taxotere (RP 56976, NSC 628503), a taxol analogue. *Cancer Res* 1991, **51**, 4845–4852.
5. Harrison SD, Dykes DJ, Bissery MC, Griswold DP. Response of four human tumor xenografts in nude mice to taxotere. 7th NCI-

EORTC Symposium on New Drugs in Cancer Therapy 1992, Abstract no. 251, 121.
6. Grem JL, Tutsch KD, Simson KJ, *et al.* Phase I study of taxol administered as a short iv infusion daily for 5 days. *Cancer Treat Rep* 1987, **71**, 1179–1184.
7. Pazdur R, Newman RA, Newman BM, *et al.* Phase I trial of taxotere: five-day schedule. *J Natl Cancer Inst* 1992, **84**, 1781–1788.
8. Pazdur R, Kudelka AP, Kavanagh JJ, Cohen PR, Raber MN. The taxoids: paclitaxel (Taxol®) and docetaxel (Taxotere®). *Cancer Treat Rev* 1993, **19**, 351–386.
9. Hanauske AR, Degen D, Hilsenbeck SG, Bissery MC, Von Hoff DD. Effect of taxotere and taxol on in vitro colony formation of freshly explanted human tumor cells. *Anticancer Drugs* 1992, **3**, 121–124.
10. Riou JF, Naudin A, Lavelle F. Effect of taxotere on murine and human tumor cell lines. *Biochem Biophys Res Commun* 1992, **187**, 164–170.
11. Vogel M, Hilsenbeck SG, Depenbrock H, *et al.* Preclinical activity of taxotere (RP56976, NSC 628503) against freshly explanted clonogenic human tumour cells: comparison with taxol and conventional antineoplastic agents. *Eur J Cancer* 1993, **29**, 2009–2014.
12. Carmichael J, DeGraff WG, Gazdar AF, Minna JD, Mitchell JB. Evaluation of a tetrazolium-based semiautomated colorimetric assay: assessment of chemosensitivity testing. *Cancer Res* 1987, **47**, 936–942.
13. Furukawa T, Kubota T, Watanabe M, *et al.* High *in vitro–in vivo* correlation of drug response using sponge-gel-supported three-dimensional histoculture and the MTT end point. *Int Natl J Cancer* 1992, **51**, 489–498.
14. Ohyama S, Tanaka M, Yonemura Y, Kinoshita K, Miyazaki I, Sasaki T. *In vitro* chemosensitivity test of gastric carcinomas using collagen gel matrix. *Jpn J Cancer Res* 1991, **82**, 607–612.
15. Egawa M, Hisazumi H, Uchibayashi T, Tanaka M, Sasaki, T. Comparative study of 3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyl-tetrazolium bromide and tritiated thymidine in a chemosensitivity test using collagen gel matrix. *Urological Res* 1993, **21**, 83–88.
16. Taguchi T, Furue H, Niitani H, *et al.* Phase 1 clinical trial of RP 56976 (docetaxel) a new anticancer drug. *Jpn J Cancer Chemother* 1994, **21**, 1997–2005.
17. Horikoshi N, Inoue K, Aiba K, *et al.* Phase I study of paclitaxel. *Jpn J Cancer Chemother* 1994, **21**, 2407–2414.
18. Scheithauer W, Clark GM, Salmon SE, Dorda W, Shoemaker RH, Von Hoff DD. Model for estimation of clinically achievable plasma concentrations for investigational anticancer drugs in man. *Cancer Treat Rep* 1986, **70**, 1379–1382.
19. Ovejera AA, Houchens DP, Baker AD. Chemotherapy of human tumor xenografts in genetically athymic nude mice. *Ann Clin Lab Sci* 1978, **8**, 50–56.
20. Rowinsky EK, Cazenave LA, Donehover RC. A novel investigational antimicrotubule agent. *J Natl Cancer Inst* 1990, **82**, 1247–1259.
21. Csuka O, Sugar J, Palyi I, Somfai-Relle S. The mode of action of vinca alkaloids. *Oncology* 1980, **37** (Suppl. 1), 83–87.
22. Gueritte-Voegelein F, Guenard D, Lavelle F, Le Goff MT, Mangatal L, Potier P. Relationship between the structure of taxol analogues and their antimitotic activity. *J Med Chem* 1991, **34**, 992–998.
23. Phillips RM, Bibby MC, Double JA. A critical appraisal of the predictive value of *in vitro* chemosensitivity assays. *J Natl Cancer Inst* 1990, **82**, 1457–1468.
24. Kubota T, Furukawa T, Kitajima M. High clinical predictability of histoculture drug response assay for drug-sensitivity of cancer of the digestive organs. *Jpn J Cancer Chemother* 1993, **20**, 461–466.
25. Taguchi T. An early phase II clinical study of RP 56976 (docetaxel) in patients with cancer of the gastrointestinal tract. *Jpn J Cancer Chemother* 1994, **21**, 2431–2437.

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