Arch Pharm Res Vol 25, No 4, 550-555, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Which Dosing Scheme is Suitable for the Taxanes? An *in Vitro* Model

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(Received January 18, 2002)

The discovery and development of the taxane class of antitumor compounds represent significant advances in the treatment of patients with a variety of malignancies. These drugs are effectively used in the treatment of breast cancer. In this study we evaluated the efficacy of fractionated usage of both paclitaxel and docetaxel as a single agent in the breast cancer cell line MCF-7. It has been shown that the cytotoxic effect of paclitaxel was increased when the divided IC_{50} concentrations were used sequentially and in contrast to paclitaxel, cytotoxic effect of docetaxel was decreased with the same schema and the single dose of IC_{50} concentration was optimal. The cause of the difference between the cytotoxic effects of two agents with this schedule is obscure. Demonstrating mechanisms, which are responsible for these differences, will be important for more rational use of taxoids and to provide basis for the following clinical trials.

Key words: Breast cancer, Docetaxel, Mcf-7, Paclitaxel, Cytotoxicity

INTRODUCTION

Long term disease free survival is offered to very few patients with metastatic breast cancer (MBC) by current treatment modalities (Perez, 1999; Esteva *et al.*, 2001). As an initial diagnosis, MBC accounts for low percentage of breast cancer patients, however at least 20 to 30 percent of patients initially diagnosed with early stage disease will eventually develop MBC (Perez, 1999). Although several cytotoxic agents are available for the treatment of MBC but, quick establishment of resistance or low tolerability of patients to these drugs leads to lack of success (Greenberg *et al.*, 1996; Esteva *et al.*, 2001). Therefore newer treatment modalities are definitely needed for women with MBC.

Taxanes, both Paclitaxel and Docetaxel are very effective agents in the treatment of MBC and have changed the treatment of patients with MBC. They are the first drugs to show a high level of efficacy in anthracycline resistant tumors (Cortes *et al.*, 1995; Valero, 1997; Paridaens et al., 2000). The combination of taxane with anthracycline has produced high response rate and better survival in patients with MBC (Carmichael *et al.*, 1997; Nabholtz, 1999; Lembersky *et al.*, 2000; Jassem J *et al.*, 2001). Therefore, taxanes are now standard part of the therapy of MBC. Evaluation of optimal dosing and scheduling of paclitaxel are continuing with discussions on the three or 24 hour infusion once every 3 weeks, however schema for docetaxel is now standard as a bolus dose once every 3 weeks (Rowinsky, 1997; Nabholtz *et al.*, 2000).

Tolerability of treatment is also with great consideration for the patients with MBC, because of most of patients will have previously received chemotherapy either as adjuvant or for advanced disease (Carlson, 1998). Taxanes, either paclitaxel or docetaxel as single agents or in combination have showed moderate to serious toxicity in patients (Pazdur *et al.*, 1993). These toxicities are mostly unacceptable for patients especially previously heavily treated for MBC. Clinical research has done to reduce toxicity without decreasing the effectivity of these two drugs.

Although Paclitaxel and Docetaxel belong to same class of cytotoxic agents, each of them has different pharmacokinetic and pharmacodynamic properties. Paclitaxel may have different effects rather than its unique antitubulin effect, such as inducing some genes

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e.g TNF and IL-8 which may lead to other mechanism of action (Burkhart *et al.*, 1994; Mullins *et al.*, 1997; White *et al.*, 1998). On the other hand, the dose-response relationship is better understood for docetaxel compared to paclitaxel, since it is believed that docetaxel has only one mechanism of action (Von Hoff, 1997). Therefore, any clinical results come from one agent cannot be generated for the other one.

After reports of the activity and tolerability of weekly administration paclitaxel (Chang *et al.*, 1997; Seidman *et al.*, 1998), a strong clinical interest has been generated to this scheduling of both two taxanes and several clinical trials has been conducted (Burstein *et al.*, 2000; Stemmler *et al.*, 2001). It is really difficult to argue on weekly schedule is more or less active than standard regimen, even equivalent, since none of these trials are randomized for the dosing and scheduling. All these clinical studies are lack of in vitro background and justification. They are empirically designed to attempt to reduce toxicity. This approach would lead to underestimation of the effectivity of taxanes.

In this study, the differences between two taxanes, Paclitaxel and Docetaxel were evaluated, using MCF-7 Breast carcinoma cell line and different schedules mimicking the bolus once every 3-week and weekly clinical usage of both two drugs. We believe that, these results will strengthen the weekly usage of Paclitaxel, but not the Docetaxel and allow the clinicians to use taxanes more effectively and promptly.

MATERIALS AND METHODS

Tumor cells

The human breast cancer cell line MCF-7 was kindly provided by Dr J.R. Bertino from Memorial Sloan-Kettering Cancer Center.

This tumor cell line was maintained in culture as adherent cells and cultured in RPMI 1640 (Sigma Chemical Co., St. Louis, Missouri) plus 10% heat inactivated fetal calf serum (FCS) (Sigma Chemical Co., St. Louis, Missouri) added to 1% L-glutamine (Sigma Chemical Co., St. Louis, Missouri), 1% non-essential amino acids (Sigma Chemical Co., St. Louis, Missouri), 10.000 units/ml penicillin (Sigma Chemical Co. St. Louis, Missouri), and 10 mg/ml streptomycin (Sigma Chemical Co., St. Louis, Missouri). Cell line was grown in a humidified atmosphere at 37°C in 5% CO_2. When the tumor cell line was used as target cells, they were treated with tyrpsin-EDTA (Sigma Chemical Co., St. Louis, Missouri), washed, and resuspended in complete medium.

Reagents

Paclitaxel, MTT, DMSO, and PBS were purchased from Sigma Chemical Co. St. Louis, Missouri. Docetaxel was kindly provided by W. Tong from MSKCC of HPLC quality. Stock solutions of paclitaxel, docetaxel were prepared in DMSO (Sigma Chemical Co., St. Louis, Missouri) and the DMSO concentration in the assay did not exceed 0.1% and was not cytotoxic to MCF-/ cells in comparison to media control.

The determination of doubling time of MCF-7 cells

The trypan blue dye exclusion assay was used to determine doubling time of MCF-7 cells as described previously (Warburton *et al.*, 1994). Each well of the six well plates included 5×10^5 cells in duplicated with control and total volume was made to 2 ml. with medium. The plates were incubated in a humidified 5% CO_2 atmosphere. Cell viability was determined at 24, 48, 72, and 96 hours.

Cytotoxicity assay

The MTT assay (Hansen *et al.*, 1989) and trypan blue dye exclusion test (Warburton *et al.*, 1994) were used to determine drug-mediated cytotoxicity as described previously.

MTT assay, briefly, target tumor cells were resuspended in medium at 1×10^5 cells/ml after verifying cell viability by trypan blue dye (Sigma Chemical Co., St. Louis, Missouri) exclusion assay. One hundred mL of cell suspension were distributed into each well of 96-well flat-bottomed microtiter plate (Greiner Labortechnik, Frickenhausen, Germany), and each plate was incubated for 24 h at 37°C and 5% CO_2 atmosphere. Following the incubations, 100 ml reagent solutions or media at the desired concentrations were distributed into each well. Treatments were performed in triplicate. Two hundred μl of the medium alone without cells and reagent were used as a control. The microtiter plate was incubated for desired period of time. Thereafter, 20 μl of the MTT dye (5 mg/ml) was added into each well. The unreactive supernatants in the well were carefully aspirated and replaced with 100 μl of isopropanol (Sigma Chemical Co., St. Louis, Missouri) supplemented with 0.05 N HCl to solubilize the reactive dye. The absorbance (A) values of each well at 540 nm were read using an automatic multiwell spectrophotometer (Biorad-Coda, Richmond, CA). The control well was used for zeroing absorbance. The percentage of cytotoxicity was calculated using the background-corrected absorbance as follows:

% Cytotoxicity = [1 (A of experimental well/A of control well)]×100

Experiments were performed at least three times with

representative data presented.

The determination of cytotoxicity with PAC and DOC in the MCF-7 breast cancer cell line

The stock solutions of PAC and DOC were prepared as 40 ng/ml and final concentrations were planned to be 2,5, 5, 10, 15, 20 ng/ml. For each concentration, cell viability was determined after 72 h incubation.

Preparation of fractional concentrations and treatment

Paclitaxel

100% of the IC_{50} of PAC (10 ng/ml) was placed to the first well, and then 75% of IC_{50} (7,5 ng/ml) to the second well, 25% of IC_{50} (2,5 ng/ml) and 50% of IC_{50} (5 ng/ml) to the third and fourth wells respectively. They were incubated for 24 hours. After this first incubation period all of the supernatants containing the drug were aspirated and replaced by normal medium, they were incubated for another 24 hours. After this second incubation period, drug free medium was placed to the first well, and then 25% of IC_{50} (2,5 ng/ml) to the second well, 75% of IC_{50} (7,5 ng/ml) and 50% of IC_{50} (5 ng/ml) to the third and fourth wells respectively. They were incubated for 24 hours more. The cytotoxicity was determined after this last period. By this process 100% of IC_{50} (control) and 75%-25%, 25%-75%, 50%-50% fractionated dosages were applied sequentially (Fig. 3).

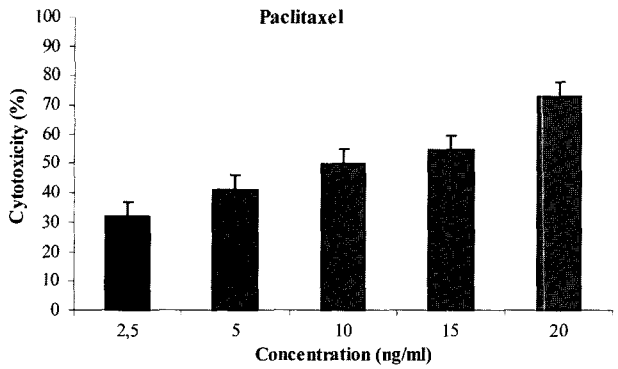

Fig. 1. The cytotoxic effect of paclitaxel on MCF-7 cell line (% cytotoxicity-IC_{50}=10 ng/ml). Cytotoxicity was assessed by trypan blue dye exclusion test and MTT assay following 72 hr culture. The data represent the mean of three different experiments with a SD not exceeding 5%.

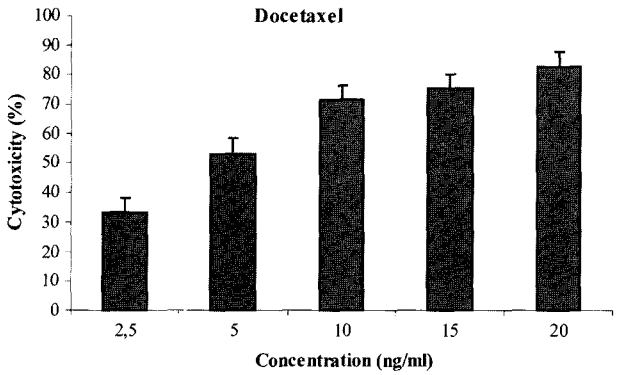

Fig. 2. The cytotoxic effect of docetaxel on MCF-7 cell line (% cytotoxicity-IC_{50}=5 ng/ml). Cytotoxicity was assessed by trypan blue dye exclusion test and MTT assay following 72 hr culture. The data represent the mean of three different experiments with a SD not exceeding 5%.

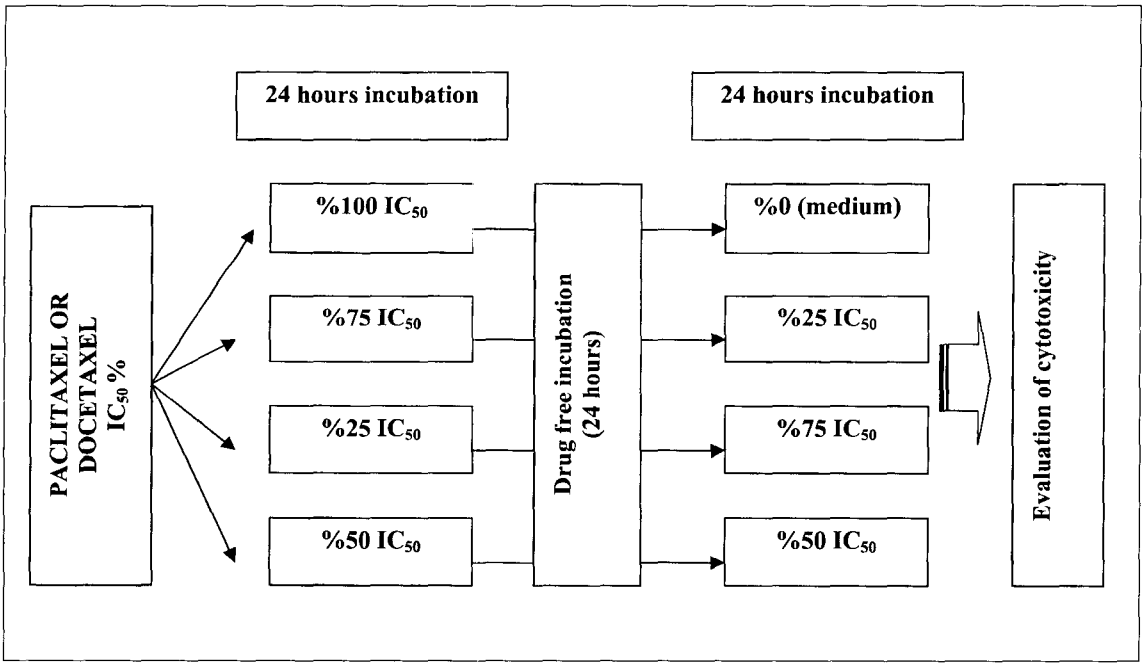

Fig. 3. Preparation of fractional concentrations and sequential treatment of paclitaxel and docetaxel

Docetaxel

The same procedure was applied for DOC, too. 100% of the IC_{50} of DOC (5 ng/ml) was placed to the first well, and then sequentially 75% (3,75 ng/ml) to the second well, 25% (1,25 ng/ml) and 50 % (2,5 ng/ml) to the third and fourth wells respectively. They were incubated for 24 hours After this second incubation period, drug free medium was placed to the first well, and then 25% of IC_{50} (1,25 ng/ml) to the second well, 75% of IC_{50} (3,75 ng/ml) and 50% of IC_{50} (2,5 ng/ml) to the third and fourth wells respectively. They were incubated for 24 hours more. The cytotoxicity was determined after this last period. By this process 100% of IC_{50} (control) and 75%-25%, 25%-75%, 50%-50% frac-tionated dosages were applied sequentially (Fig. 3).

RESULTS

Doubling time of MCF-7 cells

The doubling time of MCF 7 cells was found to be 72 h.

IC_{50} concentrations of PAC and DOC

IC_{50} value for PAC was 10 ng/ml. and for DOC 5 ng/ml. (Fig 1-2).

The sequential treatment with the fractionation of total IC_{50} dosage

Paclitaxel

Fractionation of total IC_{50} dose increases the cytotoxicity. This increase was best observed when IC_{50} dose is divided to half and half (Fig. 4).

Docetaxel

For docetaxel, single dose is found to be most cytotoxic when compare to divided dosage. Therefore, fractionation decreases the antitumor effect (Fig. 5).

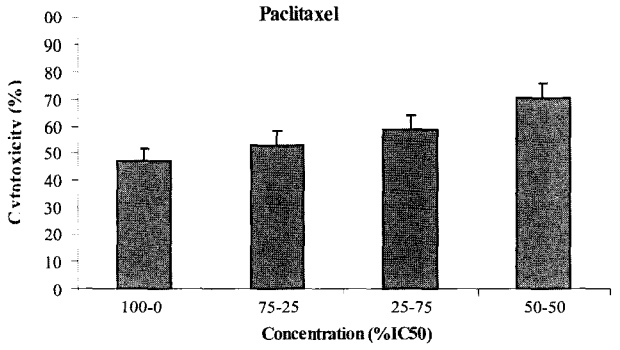

Fig. 4. The cytotoxicity for paclitaxel with the fractionation of total IC_{50}. Fractionation of total cytotoxic dose and usage of sequentially of paclitaxel augments the cytotoxicity.

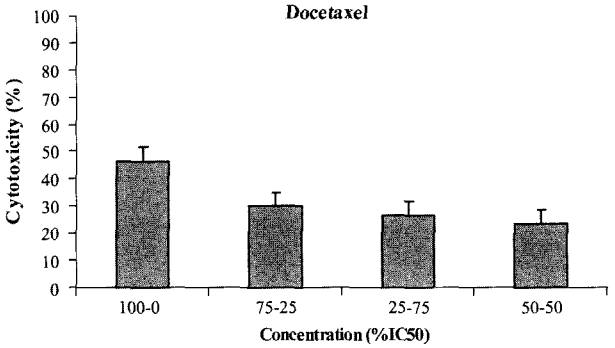

Fig. 5. The cytotoxicity for docetaxel with the fractionation of total IC_{50}. The cytotoxicity with the fractionation was less than non-fractionated total IC_{50} dose for docetaxel.

DISCUSSION

It is widely accepted that both 3-weekly and weekly low-dose schedules of taxanes are effective for the treatment of MBC, albeit this was shown in only nonrandomized Phase 2 studies (Klaassen *et al.*, 1996; Hainsworth *et al.*, 1998; Hainsworth *et al.*, 1999). These clinical studies are designed to show that dividing to effective doses of drugs would reduce the toxicity while effectivity stands equivalent. This approach would allow the increase the usage of taxanes in more patients, however, does not provide an attractive additional option for the treatment of MBC.

Using a translational approach from laboratory bench to clinic, in this study we showed that each taxanes have different property for the action. Docetaxel is more potent than paclitaxel for the cytotoxicity when used as nonfractionated. Fractionation of total cytotoxic dose and usage of sequentially of paclitaxel augments the cytotoxicity and this phenomenon is not true for Docetaxel. This observation suggests that, paclitaxel may have different effects on tumor cells rather than direct cytotoxicity. These may related to is known effects on different gene expression and other biological pathways. These kinds of effects are not known for docetaxel.

This study is the first one that shows the difference between the fractionated, sequential usage of PAC and DOC in vitro. Demonstrating of these differences is very important to reach the correct and optimal treatment modalities in clinical. Any drug in cancer medicine should be used in patients without any underestimation. Based on these in vitro findings, usage of PAC in weekly schedule would more effective and with combination of our results and Phase 2 clinical studies would less toxic in patients with MBC. This is not true for DOC, and should be used as standard schedule. Using DOC in weekly schedule, based on this study results would decrease the clinical activity. Of course randomized clinical studies comparing to two different schedules are needed.

Translational studies are required for the best results in patients and clinical studies should be planned based on in vitro studies rather than empirical results.

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