

# Effect of taxol on chromosome aberrations induced by gamma radiation or by doxorubicin in Chinese hamster ovary cells

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## ABSTRACT

Combined therapy with radiation and chemotherapy has been increasingly used in cancer treatment. The effect of combinations of taxol (0.08 µg/ml) with doxorubicin (DXR, 0.5 or 1.0 µg/ml) or gamma radiation (20 or 40 cGy) was examined in two different treatment schedules (pretreatment or simultaneous treatment) using Chinese hamster ovary (CHO) cells treated at the G2 phase of the cell cycle. The results showed that taxol did not have a radiosensitizing effect on the chromosomal aberrations induced by gamma radiation nor did it have a potentiating effect on the chromosomal aberrations induced by DXR in CHO cells treated in the G2 phase of the cell cycle.

## INTRODUCTION

Antimicrotubule agents are among the most important anticancer drugs and have contributed to the therapy of most curable neoplasms. The antimitotic antitumor drug taxol, a diterpene derived from the yew tree *Taxus brevifolia*, has demonstrated significant activity in advanced and refractory ovarian epithelial neoplasms in phase II trials and activity in various malignancies, including melanoma, non-small cell lung cancer and breast cancer (Rowinsky *et al.*, 1990, 1991; Holmes *et al.*, 1991; Choy *et al.*, 1994; Choy and Browne; 1995, Gianni *et al.*, 1995).

The target for taxol appears to be microtubules, and in contrast to vinblastine, colchicine and other antimitotic compounds that can inhibit microtubule

polymerization both *in vitro* and *in vivo*, taxol can enhance microtubule polymerization (Schiff and Horwitz, 1980). Taxol can block mitosis, induces extensive formation of microtubule bundles in cells, and induces multinucleation of cells during interphase (Long and Fairchild, 1994). This drug seemed to be a good candidate for testing in combination with other chemotherapeutic agents because of its broad antitumor activity, its unique mechanism of action and its toxicity profile (Boros *et al.*, 1995).

Combined therapy modality with radiation and chemotherapy has been increasingly used in cancer treatment regimens. The study of the interaction between chemotherapeutic agents and radiation treatment is necessary to optimize clinical protocols using a combined radiation-chemotherapy approach (Stromberg *et al.*, 1995). The ability of taxol to arrest cells in the G2 and M phases of the cell cycle makes taxol a potential cell cycle radiosensitizer because G2 and M cells are more radiosensitive than cells in other phases (Sinclair, 1968).

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Combined chemotherapy and radiotherapy are superior to radiotherapy alone. Concurrent chemotherapy and radiotherapy may produce additive or synergistic interactions but increase toxicity (Aisner *et al.*, 1995). Reports by Tishler *et al.* (1992a,b) and Geard *et al.* (1993) using human astrocytoma and melanoma cell lines have shown increasing synergistic effects between taxol and radiation when higher taxol concentrations are used. It has also been reported that HL-60 cells treated with taxol show additive and possibly synergistic cytotoxic effects with radiation (Choy *et al.*, 1993). The ability of taxol to sensitize ovarian cell lines to radiation has also been described (Stereon *et al.*, 1993a,b). Lokeshwar *et al.* (1995) showed an enhancement of radiation toxicity associated with taxol-induced cell cycle phase arrest in G2/M. A role for radiation therapy and taxol, particularly in those tumors where taxol alone shows some promise, is thereby worthy of consideration.

In addition, as it enters more widespread clinical testing in combination with other chemotherapeutic agents and because of its activity as a single agent, taxol is being tested in breast cancer in combination with doxorubicin (DXR) given the very common use of anthracyclines in the treatment of different stages of this disease (Hahn *et al.*, 1993, O'Shaughnessy *et al.*, 1994, Sledge *et al.*, 1994, 1995). Clinical trials of cisplatin and taxol combination therapy also are very promising (Rowinsky *et al.*, 1991; Ozols, 1995; Cornelison *et al.*, 1995), and it appears that administration of taxol prior to cisplatin may be particularly useful in the treatment of solid tumors (Donaldson *et al.*, 1994). Boros *et al.* (1995) showed that taxol can be safely added to the iphosphamide, carboplatin and etoposide combination chemotherapy, although little is known about the effects of combining taxol with other active drugs.

In view of the fact that gamma radiation and doxorubicin induce chromosomal aberrations in mammalian cells (Mindek, 1986) and that taxol has an inhibitory action on cell division during the G2 phase of the cell cycle (Schiff and Horwitz, 1980), a possible combination of these agents may be useful for the treatment of human tumors.

## MATERIAL AND METHODS

### Cell line and culture conditions

Chinese hamster ovary cells (CHO-9) were kindly supplied by Prof. A.T. Natarajan (University of Leiden, The Netherlands). Cells were maintained as

monolayers growing at 37°C in 25-cm<sup>3</sup> flasks (Corning) containing HAM-F10 plus DEM (Sigma) medium, supplemented with 10% fetal calf serum (Cultilab), antibiotics (0.01 mg/ml streptomycin and 0.005 mg/ml penicillin) and 2.38 mg/ml Hepes (Sigma). Cells were subcultured two or three times a week, washed in phosphate buffered saline (PBS) and treated with ATV (0.2% trypsin and 0.02% versene; Instituto Adolfo Lutz) for removal from the inner surface of the culture flask. The number of passages from the same subculture was kept between 6-12. The mean doubling time cells were 12-16 h.

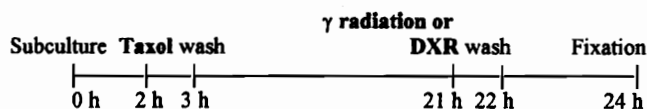
### Agents

Taxol (Paclitaxel) was obtained from Sigma. The stock solution was kept at  $5 \times 10^{-5}$  M in dimethyl sulfoxide (DMSO) at a final concentration of 0.08 µg/ml and stocked at -20°C. The final concentration of DMSO was not greater than 0.005%. DXR was a gift from Farmitalia Carlo Erba (Brazil) and was dissolved in distilled water before each experiment. The final DXR concentrations in cultures were 0.5 and 1.0 µg/ml. Cells were acutely irradiated at a dose rate of 84.386 cGy/min using a Gammatron S-80 Siemens source (Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto, USP, Brazil).

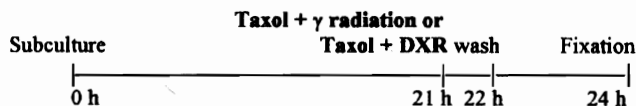
### Treatments

To determine taxol concentrations, 2 h after subculture, cells were treated with taxol (60 min) in different concentrations for one or two cell cycles. Exponentially growing CHO cells were seeded ( $1 \times 10^6$  cells/flask) and 2 h later incubated with taxol for 60 min at 37°C. After 18 h cells were exposed to a 60-min DXR pulse or were exposed to gamma radiation (20 or 40 cGy) during the G2 phase. After each treatment cells were washed twice with PBS and fed with fresh medium. For other experiments cells were exposed simultaneously to taxol (60 min) plus DXR (60 min) or gamma radiation during the G2 phase. According to Preston *et al.* (1981), cells can be fixed 3 h after the beginning of treatment. This method insures that cells analyzed were in the G2 phase of the cell cycle during the treatment. Controls without taxol were treated identically, with comparable medium changes. Colcemide (at a concentration of 0.1 µg/ml) was added to the culture medium 2 h before harvesting. Each experiment was repeated separately three times for each protocol.

## Pretreatment



## Simultaneous treatment



## Chromosome preparation and analysis

Cells were harvested by the method of Moorhead *et al.* (1960), modified (1% sodium citrate hypotonic solution, methanol/acetic acid 3:1 fixative). The air-dried chromosome preparations were stained with 1% Giemsa diluted in phosphate buffer. Only well-spread metaphases with  $21 \pm 2$  chromosomes were analyzed. Three hundred metaphases were analyzed per treatment in order to determine the frequencies of chromosomal aberrations (blind test). The mitotic index was obtained by counting the number of mitotic cells in a total of 6,000 cells analyzed per treatment.

## Statistical analysis

The differences in the number of cells with chromosomal aberrations and mitotic index between cells treated with taxol, DXR, radiation, and combinations were analyzed statistically by the Fischer exact test with  $\alpha < 0.05$ . Gaps were recorded but not included in the statistical analysis, since their cytogenetic significance is not well established (Swierenga *et al.*, 1991).

## RESULTS

In the two different protocols of taxol (pretreatment or simultaneous treatment) combination with gamma radiation or DXR the cells did not present statistically significant changes in the parameters analyzed compared to respective controls.

There was no significant increase of chromosomal aberrations induced by taxol when compared with the untreated control for the different times of treatment (12 or 24 h) (Table I).

The number of cells with chromosomal aberrations and the mitotic indices obtained in cultures treated with gamma radiation or DXR increased significantly ( $P < 0.05$ ) when compared to the negative control and to the culture treated with taxol alone (Table II). The reduction of mitotic index values was also significant ( $P < 0.05$ ) in cultures exposed to the higher concentration of DXR compared to negative control and to the taxol control in the pretreatment.

Table I - Distribution of the different types of chromosomal aberrations and MI observed in Chinese hamster ovary cells after treatment with taxol (60 min) for one or two cell cycles. One hundred cells were analyzed.

Treatments ( $\mu\text{g/ml}$ )	MI %	Chromosomal aberrations					Abnormal metaphases
		G	B'	B''	Tr	Int	
One cycle (12 h)							
0	3.2	1	1	0	0	0	2
DMSO 0.25 $\mu\text{l}$	4.1	3	0	0	0	0	3
0.004	3.5	2	0	0	0	0	3
0.008	4.3	1	1	0	0	0	1
0.08	2.9	5	2	0	0	0	6
0.4	2.3	2	0	0	0	0	2
0.8	2.7	2	3	1	1	0	7
Two cycles (24 h)							
0	2.4	2	2	0	0	1	5
DMSO	3.3	9	2	1	0	1	12
0.004	2.9	4	2	2	0	0	8
0.008	1.9	8	4	2	0	1	12
0.08	4.5	3	0	5	0	0	5
0.4	3.7	6	3	4	0	0	13
0.8	3.5	5	0	4	0	0	8

MI: Mitotic index; G: gaps; B': breaks, chromatid-type aberrations; B'': breaks, isochromatid-type aberrations; Tr: triradial figures; Int: interchanges. DMSO: Dimethyl sulfoxide.

Table II - Distribution of the different types of chromosomal aberrations and MI observed in Chinese hamster ovary cells after pretreatment with taxol for two hours after subculture and treatment with doxorubicin (DXR) for 60 min or gamma radiation during G2. Three hundred cells were analyzed for each treatment.

Treatments: drugs ( $\mu\text{g/ml}$ ) or radiation (cGy)	MI %	Chromosomal aberrations						Total of aberrations (%)	% Abnormal metaphases
		G	B'	B''	Tr	Qr	Int		
0	6.3	9	10	0	0	0	0	3.33	3.33
0.08 Taxol	7.7	3	12	4	0	2	2	6.66	6.33
20 cGy	9.8	8	24	6	1	0	0	10.33	9.66*
0.08 Taxol + 20 cGy	10.7	12	20	7	1	0	2	10.0	9.00
40 cGy	6.2	21	47	12	1	0	3	21.0	15.33*
0.08 Taxol + 40 cGy	9.7	16	41	7	1	0	3	17.33	14.66
DXR 0.5	4.3	22	15	8	0	1	2	8.66	8.33*
0.08 Taxol + DXR 0.5	6.3	10	16	6	0	0	2	8.00	6.33
DXR 1.0 $\mu\text{g}$	1.7*	24	44	8	2	3	2	21.00	17.33*
0.08 Taxol + DXR 1.0	3.4	30	56	10	4	1	7	26.00	18.00

MI: Mitotic index; G: gaps; B': breaks, chromatid-type aberrations; B'': breaks, isochromatid-type aberrations; Tr: triradial figures; Qr: quadriradial figures; Int: interchanges.

\*Significantly different from the control culture (without treatment) ( $P < 0.05$ ).

Gaps were not include in the number of abnormal metaphases.

When CHO cells were pretreated with taxol two hours after subculture and later submitted to combined treatment with DXR (0.5 or 1.0 µg/ml) or with gamma radiation (20 or 40 cGy) during the G2 phase, no effect of taxol was observed on the induction of chromosomal aberrations (Table II). No significant differences were observed between cultures treated with DXR or gamma radiation and cultures submitted to combined treatment ( $P > 0.05$ ). No significant changes in number of abnormal metaphases or mitotic indices were observed. In all treatments (Tables II and III) the chromosomal aberrations detected at the highest frequency were chromatid breaks, followed by chromatid gaps, chromosome breaks and other complex rearrangements such as interchanges, triradial and quadriradial figures.

When the cells were submitted to simultaneous treatment with taxol plus DXR or gamma radiation during the G2 phase of the cell cycle (Table III), no change in frequency of chromosomal aberrations was detected, with no significant difference between each treatment alone and combined treatments. A small reduction in the frequency of chromosomal aberrations was observed in cultures treated with gamma radiation or DXR plus taxol. These differences, however, were not statistically significant. The mitotic indices also showed no differences between treatments.

## DISCUSSION

Many cancer patients are submitted to combined radiotherapy and chemotherapy, either as part of a determined protocol or in cases of acquired

resistance to other treatment modalities (Choy *et al.*, 1993). Taxol, an antineoplastic agent with activity on the microtubules, has been extensively used in the treatment of patients with tumors refractory to other chemotherapeutic agents, alone or in combination with other antineoplastic agents. The aim of this study was to evaluate the effect of taxol on the chromosomal aberrations induced by DXR or by gamma radiation in CHO cells in the G2 phase. The results obtained showed that different concentrations of taxol did not induce chromosomal aberrations in CHO cells treated for one or two cell cycles, nor did they have a radiosensitizing effect when the cells were treated two hours after subculture and exposed to gamma radiation, and they had no significant effect on the chromosomal aberrations induced by DXR.

Treatment with taxol alone had no effect on the induction of chromosomal aberrations in CHO cell cultures compared to untreated control cultures. These results were expected since taxol acts on the microtubules (Schiff and Horwitz, 1980). In this study taxol, in the presence of colcemid in mature cultures, did not interfere in the mitotic index, since taxol binds preferentially and reversibly to microtubules, rather than tubulin dimers, at sites distinct from the binding sites of GTP (guanosine 5'-triphosphate), colchicine, vinblastine or podophyllotoxin (Schiff *et al.*, 1979).

The concentrations of taxol used in the present study were lower than those used by other investigators (Choy *et al.*, 1993) since Lopes *et al.* (1993) showed that long-term exposure to a low concentration of taxol can be more cytotoxic than short-term exposure to a high concentration. Jordan *et al.* (1993) also suggested that the mitotic block induced by low taxol concentrations is not

**Table III** - Distribution of the different types of chromosomal aberrations and MI observed in CHO cells after simultaneous treatments with taxol and DXR for 60 min or taxol 60 min plus gamma radiation during G2. Three hundred cells were analyzed.

Treatments: drugs (µg/ml) or radiation (cGy)	MI %	Chromosomal aberrations						Total of aberrations (%)	% Abnormal metaphases
		G	B'	B''	Tr	Qr	Int		
0	6.2	4	7	3	0	0	0	3.33	2.66
0.08 Taxol	8.5	3	5	2	0	0	0	2.33	2.33
20 cGy	5.9	11	31	14	0	1	6	17.33	12.66*
0.08 Taxol + 20 cGy	8.0	19	31	5	0	1	3	13.33	11.00
40 cGy	5.0	17	51	5	2	0	7	21.66	16.66*
0.08 Taxol + 40 cGy	4.5	19	31	22	0	1	5	19.66	18.00
DXR 0.5	5.0	27	35	10	1	2	6	18.00	15.00*
0.08 Taxol + DXR 0.5	4.2	18	36	3	1	0	8	16.00	12.66
DXR 1.0 µg	2.8	40	72	11	7	1	30	40.33	27.33*
0.08 Taxol + DXR 1.0	3.0	23	61	5	4	0	15	28.33	20.33

For abbreviations see Table II.

as sustained as at higher taxol concentrations. The cytotoxicity of taxol is also highly dependent on the time of exposure of cells to the drug. At a low concentration of taxol, 70% of the cells are accumulated in the G2/M phase after a 60-min drug exposure followed by incubation in a drug-free medium for at least 24 h (Choy *et al.*, 1993). Hennequin *et al.* (1996) showed that profound alteration of radiation survival in HeLa cells may occur in the presence of taxol or taxotere, ending in reduced or enhanced radiation response, depending on the drug concentration, the

cell line, the stage of growth, and the time scheduling of drugs and radiation.

The timing and duration of taxol treatment may also affect the degree of radiosensitization observed. Steren *et al.* (1993a) have shown a greater radiosensitizing effect in ovarian cancer cells with taxol 48 h or two cell cycles before irradiation than with treatment 24 h before irradiation. For this reason we treated CHO cells for two cell cycles instead of only one cell cycle. The enhanced radiosensitization may be due to the presence of a higher percentage of the cell population in G2/M at longer taxol exposure times. To intensify the block induced in the progression of the cell cycle, and on the basis of the studies cited above, in the present study CHO cells were treated with taxol two hours after subculture and in G2 after two cell cycles.

Hornback *et al.* (1994) reported that the decrease in viability and inhibition of proliferation of HeLa and B16 cells induced by irradiation were dose-dependent and significantly enhanced by pretreatment of the cells with taxol. The results obtained in the present study show that cells pretreated with taxol (Table II) and then exposed to radiation did not present an additive effect since no significant differences were observed between irradiated cultures and cultures submitted to combined treatment with taxol (40 or 20 cGy).

Cells submitted to simultaneous treatment with taxol and gamma radiation during the G2 phase of the cell cycle (Table III) also showed no additive, synergistic or antagonistic effect of taxol on the chromosomal aberrations induced by radiation, but there was a clear tendency to reduce the frequencies of chromosomal aberration when taxol was associated with gamma radiation or DXR. Taxol was not radiosensitive in CHO cells irradiated during G2, although survival curve analysis has shown that taxol acts as a radiosensitizer in human astrocytes (Tishler *et al.*, 1992a,b). It has also been reported that HL-60 cells treated with taxol show additive and possibly synergistic cytotoxic effects with radiation (Choy *et al.*, 1993). However, the failure of taxol radiosensitization in human cervical carcinoma cells and in a human lung adenocarcinoma line has also been noted in clonogenic assays (Liebmann *et al.*, 1994; Geard and Jones, 1994).

A supra-additivity or synergism is not suggested by the cell survival data obtained in a cell line derived from human cervical carcinoma treated with a combination of taxol and a low dose of gamma radiation (Minarik and Hall, 1994). Data of cellular survival determined by clonogenic assay and cell cycle distribution determined by flow cytometry also showed a lack of radiosensitization in cell lines derived from three common human carcinomas, MCF-7 (breast cancer),

DUT-145 (prostate cancer), and HT-29 (colon cancer), exposed to taxol (Stromberg *et al.*, 1995). In these cases, the absence of a significant G2/M block at any time-dose combination of taxol studied may explain the lack of radiosensitization observed because cells were not synchronized at the G2/M phase of the cell cycle before irradiation. Previous studies have shown varying magnitudes of G2/M block after taxol treatment (Rowinsk *et al.*, 1988).

Because of its activity as a single agent, taxol is being tested in breast cancer in combination with DXR (Hahn *et al.*, 1993). This benefit is based on different patterns of resistance and mechanisms of action. The two drugs appear to have distinctly different mechanisms of action; however, unexpected toxicities have been observed (Pestalozzi *et al.*, 1993), and it is not clear that the combination of taxol and DXR is more effective than either agent alone. No antagonistic action between taxol and DXR was detected in terms of cytotoxicity in the human cell lines MCF7, A549 e OVG1 (Hahn *et al.*, 1993) and preclinical *in vitro* and *in vivo* studies suggest that the combination of taxol and DXR is associated with minimal or no additive antitumor effects (O'Shaughnessy *et al.*, 1994).

Pretreatment and simultaneous treatment with taxol (Tables II and III) did not show any additive or synergistic effect on the chromosomal aberrations induced by DXR. The mitotic indices of cultures treated with DXR were slightly lower than those observed in control cultures and combined treatments also induced a reduction in MI values which, however, were significant only when cells were treated with taxol 2 h after subculture and exposed to DXR 1.0 µg/ml in G2. The combination with taxol (pretreatment or simultaneous treatment) had no significant effect on the chromosomal damage induced by DXR in CHO cells, although there was a small antagonist effect to reduce the frequencies of chromosomal aberration when taxol was associated in the simultaneous treatment in relation to pretreatment.

The chromosomal aberrations detected at the highest frequency in treatments with gamma radiation or with DXR combined or not with taxol were chromatid breaks, in agreement with the clastogenic effect on mammalian cells treated in the G2 phase of the cell cycle (Vig, 1971; Kusyk and Hsu, 1976; Natarajan and Obe, 1986). Other changes were also detected, such as gaps, chromosome breaks, quadriradial figures and others.

Under the present experimental conditions, pretreatment with taxol did not show a radiosensitizing effect on CHO cells exposed to gamma radiation in the G2 phase of the cell cycle and had no potentiating effect on the chromosomal aberrations by DXR. Additional

work is needed to elucidate the radiosensitizing ability of taxol and its effect on combined treatments of *in vitro* and *in vivo* systems.

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## RESUMO

A terapia combinada com radiação e agentes químicos tem aumentado nos regimes de tratamento do câncer. Nesse estudo foi investigado o efeito das combinações de taxol (0,08 µg/ml) com a doxorubicina (DXR 0,5 ou 1,0 µg/ml) ou com as radiações gama (20 ou 40 cGy) em dois diferentes protocolos de tratamento (pré-tratamento e simultâneo) em células CHO tratadas na fase G2 do ciclo celular. Os resultados mostraram que o taxol não teve efeito sensibilizador sobre as aberrações cromossômicas induzidas pelas radiações gama, nem teve efeito potenciador sobre as aberrações cromossômicas induzidas pela DXR em células CHO tratadas na fase G2 do ciclo celular.

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