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EFFECTS OF TAXOTERE ON MURINE AND HUMAN TUMOR CELL LINES

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Taxotere (RP 56976, NSC 628503), an analog of taxol, is an inhibitor of depolymerisation of microtubules and is currently in Phase I clinical trials. Comparisons of the cytotoxicities of Taxotere and taxol have been studied on several murine (P388, SVras) and human cell lines (Calc18, HCT116, T24, N417, KB). Taxotere was found more potent than taxol (1.3-12 fold), a result which could be explained by its higher affinity than taxol for microtubules. In agreement with its postulated mechanism of action, Taxotere is more cytotoxic on proliferating than on non proliferating N417 cells and does not inhibit cellular DNA, RNA and protein synthesis. Taxotere gives partial cross resistance on P-glycoprotein resistant P388/DOX cell line, in contrast to taxol which gives a complete cross resistance. On the other hand, no cross resistances were observed on Calc18/AM and P388/CPT5 cell lines, bearing modified activities of topoisomerase II and topoisomerase I, respectively. These results underline the higher cytotoxic activity of Taxotere compared to taxol, and the lack of cross resistance of that class of agent with the topoisomerase I and II-related multidrug resistance phenotypes.

Taxotere (RP 56976, NSC 628503) is a new anticancer agent related to taxol (Figure 1). Taxol, isolated from the bark of the pacific yew, *Taxus brevifolia,* was selected for its antitumor properties on leukemias (1). Taxol is a mitotic spindle poison that increases the rate of microtubules assembly and inhibits the depolymerisation of microtubules (2). Taxotere [N-debenzoyl-N-tert-butoxycarbonyl-10-deacetyl taxol) was obtained by hemisynthesis from 10-deacetyl baccatin III, a precursor isolated from the needles of *Taxus baccata*, a renewable source of biological material (3, 4, 5). Initial reports establish that Taxotere inhibits the depolymerisation of microtubules more efficiently than taxol and has *in vitro* and *in vivo* antitumor properties (6, 7, 8). Taxotere has a good spectrum of efficacy against a wide variety of transplantable tumors in mice, and can give complete regressions of advanced stage tumors (9). Taxotere is currently undergoing clinical evaluation in phase I trials in the United States of America and in Europe.

We present in this report an analysis of the cytotoxic properties of Taxotere, comparatively to taxol on seven murine and human cell lines. The effect of Taxotere was examined on cell lines bearing P-glycoprotein- or topoisomerases- related multidrug resistance phenotypes.

MATERIALS AND METHODS

Drugs: Taxol was obtained from the National Cancer Institute (Bethesda, USA). Taxotere (RP 56976, NSC 628503) prepared as previously described (3). Stock solutions were prepared in ethanol at 10 mg/ml and stored at -20°C. Further dilutions were made in water.

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Cell lines and culture conditions: Murine leukemia P388 and P388/DOX cell lines were obtained from the tumor bank of the National Cancer Institute. These cell lines were grown in RPMI 1640 medium containing 10 μM 2-mercaptoethanol, 2mM L-glutamine, 200 U/ml penicillin, 200 μg/ml streptomycin and supplemented with 10 % (v/v) foetal calf serum. Doxorubicin (1 μg/ml) was added to the medium of P388/DOX. P388/CPT5 is a stable camptothecin resistant subclone isolated in soft agar, which derived from the P388CPT0.3 cell line (10). Cells were grown in the same medium than P388 cells. N417 human small cell lung carcinoma cell line was a gift from Dr. J. Minna (National Cancer Institute, USA) and grown as previously described (11). KB human epidermoid carcinoma cells were obtained from the American Type Culture Cell Collection and grown in Dulbecco's Modified Eagle Medium, with 2 mM L-glutamine and 10 % foetal calf serum. SV1Ras4 cell line was obtained after transformation of DBA2 mouse embryonic cells with SV40 large T antigen and VHa-ras oncogene (12). T24 human bladder carcinoma cell line was obtained from the American Type Culture Cell Collection and grown in McCoy's 5a medium with 2mM L-glutamine and 10%v/v foetal calf serum. Cells were grown as monolayers in Dulbecco's Modified Eagle Medium containing 2 mM L-glutamine and 10 % v/v foetal calf serum and antibiotics. Calc18 human breast carcinoma and Calc18/AM with acquired resistance to mAMSA were a gift from Dr. G. Riou (Institut Gustave Roussy, France). Cells were grown as monolayers in Dulbecco's Modified Eagle Medium with 10 % foetal calf serum (13). HCT116 human colon adenocarcinoma cell line (14) was obtained from the American Type Culture Collection through Dr. M.C. Bissery and was grown in CMRL 1066/Fisher (50/50 v/v) medium with 2mM L-glutamine, 4mM CaCl2, 20 μg/ml gentamycin and supplemented with 10% (v/v) foetal calf serum.

Measurement of DNA, RNA and protein synthesis: DNA, RNA and protein synthesis were determined on P388 cells in exponential growth by measuring the incorporation of radiolabelled precursors (15). Treatments with drugs were applied for 3 hours, each point in quadruplicate. Results are expressed in percent incorporated radioactivity relatively to controls and represent the mean of at least two independent determinations.

Evaluation of the antiproliferative properties: The concentration of drugs giving 50 % of growth inhibition (IC_{50}) were determined from two or three separate experiments in 96 well microculture plates.Cell lines seeded at 3.10^4-3.10^5 cells/ml (0.2 ml/well) were grown for 96-120 h in the presence of various drug concentrations (each point in quadruplicate). Cells were then incubated for 16 h with 0.02 % neutral red. The cells were washed and lyzed with 1 % SDS. The incorporation of the dye reflecting cellular growth and viability was evaluated by the measurement of the optical density for each well at 540 and 346 nm, using a Titertek multiwell spectrophotometer. For cloning experiments, KB cells in exponential growth or N417 cells in exponential or plateau phase were treated for 1 hour in liquid medium, then washed and poured with Noble Difco Agar into Petri dishes (4 dishes/concentration) as previously described (15). The number of cellular clones (> 60 cells) were measured after 15 days of incubation at 37°C under 5 % CO_2 in a humidified atmosphere.

RESULTS

Effect on DNA, RNA and protein synthesis

The effects of Taxotere and taxol (Figure 1) were compared on DNA, RNA and protein synthesis. After 3 hours of incubation, Taxotere (100 μg/ml) reduced by 50 % the biosynthesis of DNA, RNA and protein in P388 cells as judged by the decreased incorporation of radiolabelled precursors in the cellular DNA, RNA and proteins (Table I). At lower concentration of Taxotere (50, 25 and 10 μg/ml), only marginal inhibition was observed. These results indicate that Taxotere is not a potent inhibitor of DNA, RNA or protein synthesis. Taxol (200 μg/ml) was found two times less potent than Taxotere on DNA and RNA synthesis and presented a similar inhibition of protein synthesis (Table I).

Effect on proliferative and plateau-phase N417 cells

The cytotoxic effects of Taxotere and taxol were examined on the human small cell lung carcinoma N417 cell line under different conditions of proliferation. The cell line in exponential growth was incubated in liquid medium with various concentrations of drugs for 1 hour. The IC_{50} of

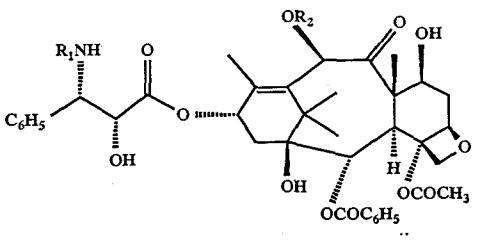

Figure 1 : Chemical structure.
Taxotere: R_1 = -COOC$(CH_3)_3$; R_2 = H.
Taxol : R_1 = -COC_6H_5 ; R_2 = -$COCH_3$.

Taxotere and taxol were respectively equal to 150 and 1700 ng/ml, indicating that Taxotere is 11.3-fold more cytotoxic than taxol on proliferating N417 cells (Table II).

Similar experiments were done on N417 cells from plateau-phase culture. As presented in Table II, IC_{50} increased to a value of 2000 ng/ml for Taxotere and only a marginal cytotoxic effect was observed for taxol (16.8 % inhibition of cloning efficiency at 10000 ng/ml). Taxotere is 13.3-fold less cytotoxic on non proliferating cells than on proliferating cells. Similar properties were observed for taxol.

Cellular effects of Taxotere and taxol

The antiproliferative effects of taxol and Taxotere were compared on murine P388 (leukemia), SV1 ras (ras transformed embryonic cells), human Calc18 (breast adenocarcinoma), KB (epidermoid carcinoma), HCT116 (colon adenocarcinoma) and T24 (bladder carcinoma) cell lines. The IC_{50} obtained after 96-120 hours of drug exposure in liquid medium range from 4 to 35 ng/ml (Table III). Taxotere was found 1.3 to 9.3-fold more potent than taxol. We have also compared the cytotoxicities of taxol and Taxotere against human epidermoid carcinoma KB cells by soft agar cloning efficiency, after 1 hour of contact with the drugs (Table III). The IC_{50} of Taxotere and taxol were respectively equal to 1500 and 18000 ng/ml. The results indicated that the differences of

Table I : Effect of taxol and Taxotere on DNA, RNA and protein synthesis

Drug (3 h treatment)	Percent incorporated radioactivity[1] Mean (lowest and highest values) DNA	RNA	Protein	N[2]
Taxol 200 µg/ml	76 (66- 86)	73 (64- 80)	59 (49- 68)	2
100	83 (72- 89)	86 (73-103)	65 (50- 81)	3
10	93 (85-102)	104 (97-112)	75 (71- 77)	2
Taxotere 100 µg/ml	55 (36- 93)	59 (48- 87)	52 (25-114)	7
50	89 (77- 96)	79 (63- 93)	91 (74- 99)	4
25	95 (71-120)	92 (72-113)	97 (93-101)	2
10	96 (86-109)	110 (92-140)	88 (69-114)	5

[1] Results are expressed in percent of radioactivity incorporated relatively to control untreated cells and represent the mean of at least two independent determinations, each point in quadruplicate. Lowest and highest determinations are indicated in brackets.

[2] N : number of independent determinations.

Table II : Effects of Taxotere on clonogenic properties of N417 cells

	% inhibition of clonogenicity[a]							
N417 cells :	Proliferating cells				Non Proliferating cells			
drug (ng/ml) :	10000	1000	100	IC_{50}[b]	10000	1000	100	IC_{50}[b]
Taxol	67	46	27	1700	16.8	12.4	0	>10000
Taxotere	94	80	37	150	99.5	11.7	11.7	2000

[a] 1 hour of drug exposure, mean value of 3 experiments, each point in triplicate.

[b] IC_{50} : concentration giving 50 % inhibition of clonogenicity.

activities between these two compounds on KB are the same whatever the experimental conditions (liquid medium or cloning in soft agar) which were used.

Effect on resistant cell lines

We have investigated the effect of Taxotere on cell lines presenting various resistance phenotypes. P388/DOX cell line was previously characterized and exhibited a complex multidrug resistance phenotype associated both with a mdr gene overexpression and with an alteration of the topoisomerase II activity (16, 17). As reported in Table IV (see also Table III for the IC50 values), the P388/DOX cell line presented a 187-fold resistance to doxorubicin and a partial cross-resistance to Taxotere corresponding to a relative resistance index equal to 26. Comparison of taxol and Taxotere cytotoxicities on P388/DOX cells showed that Taxotere is >21.9-fold more active than taxol (see Table III). This result also indicated that Taxotere is 4-fold less cross-resistant than taxol on P388/DOX cells.

Calc18/AM is a cell line resistant to the topoisomerase II inhibitor mAMSA which presents an altered expression of topoisomerase II and cross resistances to topoisomerases II inhibitors only (13). Such multidrug resistance, without P-glycoprotein (mdr1) gene expression was also known as "atypical mdr" (18). We did not detect any significative differences of cytotoxicity for Taxotere on Calc18 and Calc18/AM cell lines (Table IV), indicating that topoisomerase II alterations did not modify the activity of Taxotere.

Table III : Comparative cytotoxicity of taxol and Taxotere on murine and human cell lines

Cell line	Drug exposure (hours)	IC_{50} (ng/ml) Taxol	Taxotere	R[c]
[a] P388	96	180	35	5.1
P388/DOX	96	>20000	910	>21.9
SV1Ras4	96	300	35	8.6
Calc18	120	30	5	6.0
KB	96	75	8	9.3
HCT116	96	9	7	1.3
T24	96	8	4	2.0
[b] KB	1	18000	1500	12
N417	1	1700	150	11.3

[a] Incubation in liquid medium, IC_{50} = median toxic concentration that inhibits cell growth by 50 %, determined from 2-3 separate experiment, each concentration of drug in quadruplicate.

[b] Cells were incubated with drugs at different concentrations for 1h at 37°C. Drugs were removed and the cell viability was determined by cloning in soft agar.

[c] R is the IC_{50} ratio between taxol and Taxotere.

Table IV : Relative resistance index to Taxotere of different cell line resistant to other antitumor agents

cell line		Relative resistance index to		Characteristic of the resistant cell line
sensitive	resistant			
P388	P388/DOX	Doxorubicin 187	Taxotere[a] 26	- P-glycoprotein overexpression - Altered topoisomerase II
P388	P388/CPT5	Camptothecin 80	Taxotere 0.75	- Mutant topoisomerase I
Calc18	Calc18/AM	mAMSA 18.5	Taxotere 1.1	- Decreased topoisomerase II activity : "AT-mdr"

[a] Relative resistance index to taxol is > 111.

P388/CPT5 is a cell line resistant to the topoisomerase I inhibitor camptothecin and bearing a mutant topoisomerase I , but no P-glycoprotein (mdr1) gene expression (10). The cell line presents cross resistances to other topoisomerase I inhibitors, such as Fagaronin (RIOU J.F., unpublished result). As reported in Table IV, the P388/CPT5 cell line was found slightly more sensitive to Taxotere than the P388 parental cells.

DISCUSSION

Taxotere inhibits cell replication : its mechanism of action is not related to an specific inhibition of DNA, RNA or protein synthesis as predicted by the previous studies on tubulin depolymerisation (5, 8). Similar results have been reported for taxol which inhibits cell division, without grossly affecting DNA,RNA or protein synthesis (19). Taxotere and taxol present dose dependent cytotoxic effects on proliferating cells which are considerably decreased on non proliferating cells. These results are in agreement with those obtained with all the chemotherapeutic agents which are cell cycle dependent (20).

An initial report have shown that Taxotere was 2.5 times more potent than taxol on J774.2 and P388 murine cell lines (8). This result was confirmed on P388 and SV1Ras murine cell lines and extended on several human cell lines including: Calc18, KB, HCT116, T24 and N417. Our data demonstrate that Taxotere is 1.3-12 fold more active than taxol. Pharmakocinetic studies in mice indicated that plasma concentrations of Taxotere (peak plasma concentration and area under the curve) are higher than the concentrations necessary to search the cytotoxicity *in vitro* on the different cell lines which have been tested (21). *In vivo* activities of Taxotere and taxol were compared against early stage B16 melanoma : the maximum tolerated dose of Taxotere was found 1.6 time lower than the maximum tolerated dose of taxol and this was associated with an improved antitumor activity for Taxotere (9). These results suggest that Taxotere might possess a better therapeutic index than taxol. Finally, Taxotere is more active as a promoter of stable microtubules assembly *in vitro* (7, 8). Taken together, all these data, indicate a possible correlation between the mechanism of action of taxol and Taxotere on tubulin and the cytotoxic or antitumoral properties.

The cytotoxic activity of Taxotere was examined on cell lines given resistant by various mechanisms. We have found that Taxotere presented partial cross resistance towards the multidrug resistance phenotype of P388/DOX cells. Interestingly, Taxotere is 4-fold less resistant

than taxol in these cells, suggesting that Taxotere could be less recognized by the P-glycoprotein. Since the multidrug resistance phenotype of P388/DOX is also mediated by a topoisomerase II alteration (17), we have also investigated the activity of Taxotere on Calc18/AM and P388/CPT5 cell lines which presented topoisomerases-related resistant phenotype without P-glycoprotein expression (10, 13). Taxotere is fully effective against these cell lines, indicating that an alteration of the topoisomerases functions in cell does not alter the activity of Taxotere. This data may contribute to a therapeutic advantage in cancer relapse from treatment with topoisomerases inhibitors.

These results underline the specific cytotoxic properties of Taxotere related to its unique mechanism of action on tubulin.

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