ETH zürich

ETH Library

Taxanes: the genetic toxicity of paclitaxel and docetaxel in somatic cells of Drosophila melanogaster

Journal Article

Author(s):
Cunha, Kênya Silva; Reguly, Maria Luíza; Graf, Ulrich; De Andrade, Heloisa Helena Rodrigues

Publication date:
2001

Permanent link:
https://doi.org/10.3929/ethz-b-000422900

Rights / license:
In Copyright - Non-Commercial Use Permitted

Originally published in:
Mutagenesis 16(1), https://doi.org/10.1093/mutage/16.1.79

This page was generated automatically upon download from the ETH Zurich Research Collection.
For more information, please consult the Terms of use.

Mutagenesis vol.16 no.1 pp.79–84, 2001

Taxanes: the genetic toxicity of paclitaxel and docetaxel in somatic cells of *Drosophila melanogaster*

Kênya Silva Cunha[1], Maria Luíza Reguly[2], Ulrich Graf[3] and Heloisa Helena Rodrigues de Andrade[2,4]

[1]Departamento de Ciências Fisiológicas, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia, GO, Brasil, [2]Laboratório de Mutagênese, Departamento de Genética, Universidade Federal do Rio Grande do Sul, CP 15053, 91501-970 Porto Alegre, RS, Brazil and [3]Institute of Toxicology, Swiss Federal Institute of Technology (ETH) Zürich, CH-8603 Schwerzenbach, Switzerland

In this study, the taxanes, paclitaxel and docetaxel were investigated for genotoxicity in the wing spot test of *Drosophila melanogaster.* These relatively new drugs are used in cancer therapy and show great promise in the treatment of a variety of cancers. Their major cellular target is the α,β-tubulin dimer but, unlike other spindle poisons, they stabilize microtubules by a shift towards assembly, producing nonfunctional microtubule bundles. The *Drosophila* wing Somatic Mutation and Recombination Test (SMART) provides a rapid means to evaluate agents able to induce gene mutations and chromosome aberrations, as well as rearrangements related to mitotic recombination. We applied the standard version of SMART (with normal bioactivation) and a variant version with increased cytochrome P450-dependent biotransformation capacity. In the standard assay, docetaxel was found to be aneuploidogenic; this was effectively abolished by a high cytochrome P450-dependent detoxification capacity. This suggests, as previously reported, the involvement of this family of enzymes in the detoxification of docetaxel rather than in its activation. In contrast, paclitaxel was clearly non-genotoxic at the same (millimolar) concentrations as used for docetaxel in both crosses. The weak responsiveness of SMART assays to aneugenic compounds, the weaker ligand and assembly action of paclitaxel and the more rapid reversibility of the microtubules formed with this compound, may have caused the negative response observed in the present study.

Introduction

Taxanes comprise a relatively new class of anticancer drugs which have shown promise against breast, ovarian, non-small cell lung, and head and neck cancers (Rowinsky, 1997; von Hoff, 1997). The discovery of paclitaxel (Taxol; Bristol-Myers Squibb) in the early 1970s was followed by the development of docetaxel (Taxotere; Rhône–Poulenc Rorer), a semisynthetic taxoid, which is considered to show an even more positive clinical response. The major cellular target for taxanes is the α,β-tubulin dimer. They block the cell cycle during mitosis in the transition from prometaphase to metaphase, as has been observed with numerous spindle poison agents (Jordan and Wilson, 1998). Many of these agents inhibit the polymerization of tubulin into micrutubules, but taxanes stabilize microtubules by promoting assembly, rather than disassembly, leading to the formation of nonfunctional microtubule bundles (Kumar, 1981; Ringel and Horwitz, 1991). Although they share the same mode of action, docetaxel is about twice as potent as paclitaxel as an inhibitor of spindle depolymerization and can alter certain classes of microtubules (Tinwell and Ashby, 1994) in keeping with its affinity for specific binding sites (von Hoff, 1997).

Chemicals able to induce genotoxic effects by mechanisms other than covalent binding to DNA are of special interest. A great deal of work is being devoted to the validation of genotoxicity test procedures able to detect drugs that cause genetic damage by interaction with other cellular targets such as enzymes and microtubules, particularly because they play a critical role in DNA replication or in the segregation of chromosomes during cell division. Interest in taxanes, perhaps the most successful drugs used in the treatment of various cancers, has led to exhaustive research on their therapeutic effects, although to our knowledge only one previous paper has addressed their genetic toxicity (Tinwell and Ashby, 1994).

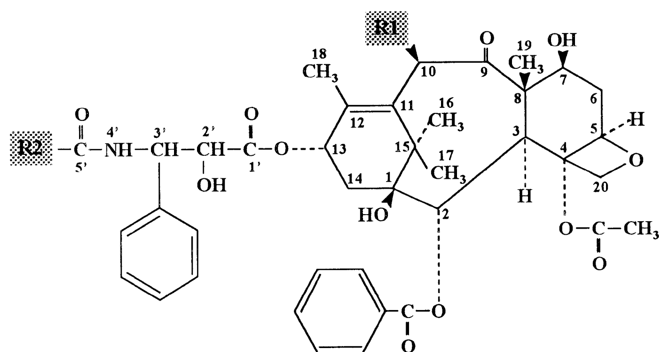
As taxanes affect microtubules, it might be expected that they would induce numerical chromosome aberrations (aneuploidy). Although we know that compounds that interfere with microtubule assembly—such as chloral hydrate (Zordan *et al*., 1994), vincristine (Vogel and Nivard, 1993), vinorelbine (Tiburi,M,F., Reguly,M.L., Schwartsmann,G. and Andrade,H.H.R., in preparation) and vinblastine (Graf *et al*., 1984; Vogel and Nivard, 1993) are genotoxic in *Drosophila*, there have been no experimental data proving the aneugenicity of drugs that inhibit tubulin disassembly in this insect.

To understand more about the risk–benefit of these cancer chemotherapeutic agents, we set out to test the genotoxicity of paclitaxel and docetaxel in the *Drosophila* Somatic Mutation And Recombination Test (SMART), which was developed to detect loss of heterozygosity of suitable marker genes that are expressed phenotypically on the wings of the flies. This bioassay provides a rapid means to assess the ability of candidate genotoxic agents to induce gene mutations or rearrangements related to mitotic recombination, as well as to break chromosomes or to cause their loss during cell division (Graf *et al*., 1984; Guzmán-Rincón and Graf, 1995; Vogel *et al*., 1999). There is a high level of conservation between *Drosophila* and humans in many respects, not only in individual domains and proteins but also in entire complexes and multistep pathways (St John and Xu, 1997), so genotoxicity testing in fruit flies can be valuable.

We used two versions of the wing SMART: a standard version with normal bioactivation and a version characterized by an increased cytochrome P (CYP)6A2-dependent biotransformation capacity (Frölich and Würgler, 1989, 1990a,b; Graf and van Schaik, 1992; Saner *et al*., 1996).

[4]To whom correspondence should be addressed Email: heloisa@if.ufrgs.br

© UK Environmental Mutagen Society/Oxford University Press 2000



Compound	R1	R2
Paclitaxel	$OCOCH_3$	C_6H_5
Docetaxel	OH	$OC(CH_3)_3$

Fig. 1. Chemical structure of paclitaxel and docetaxel according to Ringel and Horwitz (1991) and Chazard *et al.* (1994).

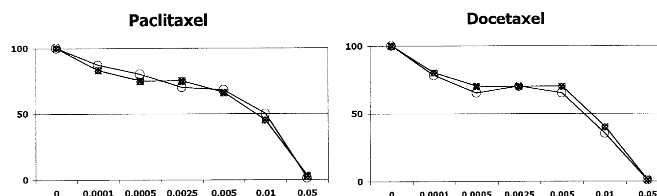


Fig. 2. General toxicity of paclitaxel and docetaxel. Exposure concentrations and survival following 48 h of exposure during the third larval instar. ■, ST cross; ○, HB cross.

Materials and methods

Chemical compounds

Paclitaxel (Taxol; CAS no. 33069-62-4) was purchased from Bristol-Myers Squibb (New York, NY, USA) and docetaxel (Taxotere; CAS no. 114977-28-5) from Rhône-Poulenc Rorer (Antony, France). The chemical structures of these compounds are given in Figure 1. Paclitaxel was dissolved in 52.7% Cremofor (Sigma, Saint Louis, MO, USA; CAS no. 61791-12-6) and then diluted with double-distilled water to give, for all paclitaxel doses, a final Cremofor concentration of 0.75%. This solvent mixture alone was not visibly toxic towards 3 day old larvae. Docetaxel was dissolved in ethanol (Merck, Darmstadt, Germany) and then diluted with double-distilled water to obtain a final concentration of 4% ethanol. Vincristine sulfate (2068-78-2; Eli Lilly do Brasil Ltda, São Paulo, SP, Brazil) dissolved in double-distilled water was used as a positive control. Controls with the corresponding solvents were always conducted in parallel.

Drosophila tester strains and crosses for the SMART

The following two crosses of flies carrying visible markers for the shape of wing hairs were set up: (i) Standard cross (ST)—*flr^3/In (3LR) TM3, ri p^p sep l(3)89Aa bx^{34e} e Bd^S* females mated to *mwh/mwh* males and (ii) High Bioactivation cross (HB)—*ORR/ORR; flr^3/In (3LR) TM3, ri p^p sep l(3)89Aa bx^{34e} e Bd^S* females crossed to *mwh/mwh* males. The ORR strain has chromosomes 1 and 2 from a DDT-resistant Oregon R(R) line, which are responsible for a high constitutive level of CYP450. Information on these crosses are available in Graf and van Schaik (1992). More details about the genetic markers are given in Lindsley and Zimm (1992).

Experimental procedures

Eggs from both ST and HB crosses were collected for 8 h in culture bottles containing a solid agar base [5% (w/v) agar in water] covered with a 5 mm layer of live baker's yeast supplemented with sucrose. Three days later, larvae were washed out of the culture bottles with tap water and seeded in plastic vials (50 larvae/vial) containing 1.5 g of Instant Medium (Formula 4-24; Carolina Biological Supply, Burlington, NC, USA) wetted with 5 ml of the test solutions. Double-distilled water, 4% ethanol and 0.75% Cremofor were used as negative controls. All experiments were carried out at 25°C and 65% relative humidity.

Larvae were fed on the above medium for the rest of their development (48 h). After eclosion, adult flies were collected from treatment vials and stored in 70% ethanol. Wings of the marker-heterozygous (*mwh +/+ flr^3*) flies were mounted on slides and scored under 400× magnification for the presence of cell clones showing malformed wing hairs. Such spots appeared as single spots expressing either the multiple wing hair (mwh) or flare (flr) phenotypes or as twin spots with adjacent mwh and flr areas. We recorded whether there were (i) small single spots (one or two cells affected); (ii) large single spots (more than two cells affected) or (iii) twin spots.

Data evaluation and statistical analysis

In order to evaluate the genotoxic effects recorded, the frequencies of spot per fly in each treated series were compared with those in the concurrent negative control series. In the first instance, statistical comparisons were done using a computer program written by Zordan (unpublished data), which employs the chi-square test for proportions and follows a multiple-decision procedure according to Frei and Würgler (1988). Positive diagnoses were confirmed by the non-parametric *U*-test of Wilcoxon, Mann and Whitney (Frei and Würgler, 1995). For maximum power, statistical analyses were done exclusively for the total number of spots recovered.

Results

The direct or indirect genotoxic potential of paclitaxel and docetaxel was assayed in at least two chronic and independent experimental proceedings. There were no significant statistical differences between the results of individual experiments, so the data were pooled. Larvae from the ST crosses and those from the HB crosses were studied in parallel, so the larvae derived from these two crosses were treated under identical conditions. The concentrations chosen to assess the possible genotoxicity of these compounds ranged from 0.0001 to 0.01 mM. Both paclitaxel and docetaxel are very toxic and at concentrations of >0.01 mM no flies survived. In one experiment, batches of 100 larvae were treated with one of five concentrations of each compound. The number of surviving flies was counted (Figure 2). Even at the concentrations used, some flies were visibly smaller than control flies, suggesting that the compounds tested were toxic and/or that larvae had avoided food. There was no noticeable difference in survival rates between flies from the two crosses.

Table I. Frequency and number of spots induced in the wings of standard (ST) flies after chronic treatment with paclitaxel and docetaxel (exposure: 48 h)

Group/dose (mM)	Number of flies	Frequency of spots per fly (number of spots)				Statistical diagnosis[a] (m = 2)
		small single spots (one or two cells)	large single spots (more than two cells)	twin spots	total spots	
Controls						
water	80	0.50 (40)	0.09 (07)	0.04 (03)	0.63 (50)	
vincristine	10	1.60 (16)	0.10 (01)	0.01 (01)	1.80 (18)	+[c]
Paclitaxel (Taxol)						
0[d]	100	0.53 (53)	0.11 (11)	0.05 (05)	0.69 (69)	
0.0001	60	0.42 (25)	0.07 (04)	0.05 (03)	0.53 (32)	–
0.0005	60	0.37 (22)	0.08 (05)	0.07 (04)	0.52 (31)	–
0.0025	60	0.37 (22)	0.12 (07)	0.02 (01)	0.50 (30)	–
0.005	60	0.50 (30)	0.10 (06)	0.03 (02)	0.63 (38)	–
0.01	60	0.32 (19)	0.02 (01)	0.03 (02)	0.37 (22)	–
Docetaxel (Taxotere)						
0[e]	100	0.40 (40)	0.04 (04)	0.02 (02)	0.46 (46)	
0.0001	60	0.58 (35)	0.05 (03)	0.05 (03)	0.68 (41)	+[b]
0.0005	60	0.87 (52)	0.05 (03)	0.02 (01)	0.93 (56)	+[c]
0.0025	60	0.62 (37)	0.12 (07)	0.02 (01)	0.75 (45)	+[c]
0.005	60	0.72 (43)	0.05 (03)	0.02 (01)	0.79 (47)	+[c]
0.01	60	0.45 (27)	0.07 (04)	0.03 (02)	0.55 (33)	–

[a]Statistical diagnosis according to Frei and Würgler (1988): +, positive; w, weakly positive; –, negative; i, inconclusive; m = multiplication factor for the assessment of negative results; χ^2 test for proportions, significance level $\alpha = \beta = 0.05$ (one-sided tests).
[b,c]Positive diagnosis confirmed by the U-test: [b]$P < 0.05$, [c]$P < 0.01$.
[d]Solvent alone (0.75% cremophor).
[e]Solvent alone (4% ethanol).

Table II. Frequency and number of spots induced in the wings of high bioactivation (HB) flies after chronic treatment with paclitaxel and docetaxel (exposure: 48 h)

Group/dose (mM)	Number of flies	Frequency of spots per fly (number of spots)				Statistical diagnosis[a] (m = 2)
		small single spots (one or two cells)	large single spots (more than two cells)	twin spots	total spots	
Controls						
water	80	0.66 (53)	0.18 (14)	0.04 (03)	0.88 (70)	
vincristine	10	1.30 (13)	0.20 (02)	0.10 (01)	1.60 (16)	+[b]
Paclitaxel (Taxol)						
0[b]	100	0.49 (49)	0.08 (08)	0.05 (05)	0.62 (62)	
0.0001	60	0.55 (33)	0.10 (06)	0.00 (00)	0.65 (39)	–
0.0005	60	0.53 (32)	0.12 (07)	0.02 (01)	0.67 (40)	–
0.0025	60	0.35 (21)	0.05 (03)	0.03 (02)	0.43 (26)	–
0.005	60	0.45 (27)	0.12 (07)	0.00 (00)	0.57 (34)	–
0.01	60	0.43 (26)	0.08 (05)	0.02 (01)	0.53 (32)	–
Docetaxel (Taxotere)						
0[c]	100	0.56 (56)	0.08 (08)	0.04 (04)	0.68 (68)	
0.0001	60	0.63 (38)	0.07 (04)	0.02 (01)	0.72 (43)	–
0.0005	60	0.62 (37)	0.08 (05)	0.03 (02)	0.73 (44)	–
0.0025	60	0.58 (35)	0.12 (07)	0.02 (01)	0.72 (43)	–
0.005	60	0.62 (37)	0.10 (06)	0.00 (00)	0.72 (43)	–
0.01	60	0.72 (43)	0.15 (09)	0.03 (02)	0.90 (54)	–

[a]Statistical diagnosis according to Frei and Würgler (1988): +, positive; w, weakly positive; –, negative; i, inconclusive; m = multiplication factor for the assessment of negative results; χ^2 test for proportions, significance level $\alpha = \beta = 0.05$ (one-sided tests).
[b]Solvent alone (0.75% cremophor).
[c]Solvent alone (4% ethanol).

Controls

The spontaneous frequencies of spots per fly recorded in the solvent series [Cremofor (ST, 0.69 spots/fly; HB, 0.62 spots/fly) and ethanol 4% (ST, 0.46 spots/fly; HB, 0.68 spots/fly)] were comparable to those in the negative water controls (ST, 0.63 spots/fly; HB, 0.88 spots/fly) and within the usual range reported previously (Frei and Würgler, 1996; Campesato *et al.*, 1997). The similarity between the values found in the present study in both crosses also indicates that the genetic background of the strains used in the ST and HB crosses does not interfere with the spontaneous level of mutant clones (Tables I and II). The positive controls, treated with vincristine sulfate (0.05 mM), behaved in the expected way, as the responses to this aneugenic mutagen were mainly related to increases in the frequencies of small single spots (Tiburi,M,F., Reguly,M.L., Schwartsmann,G. and Andrade,H.H.R., in preparation).

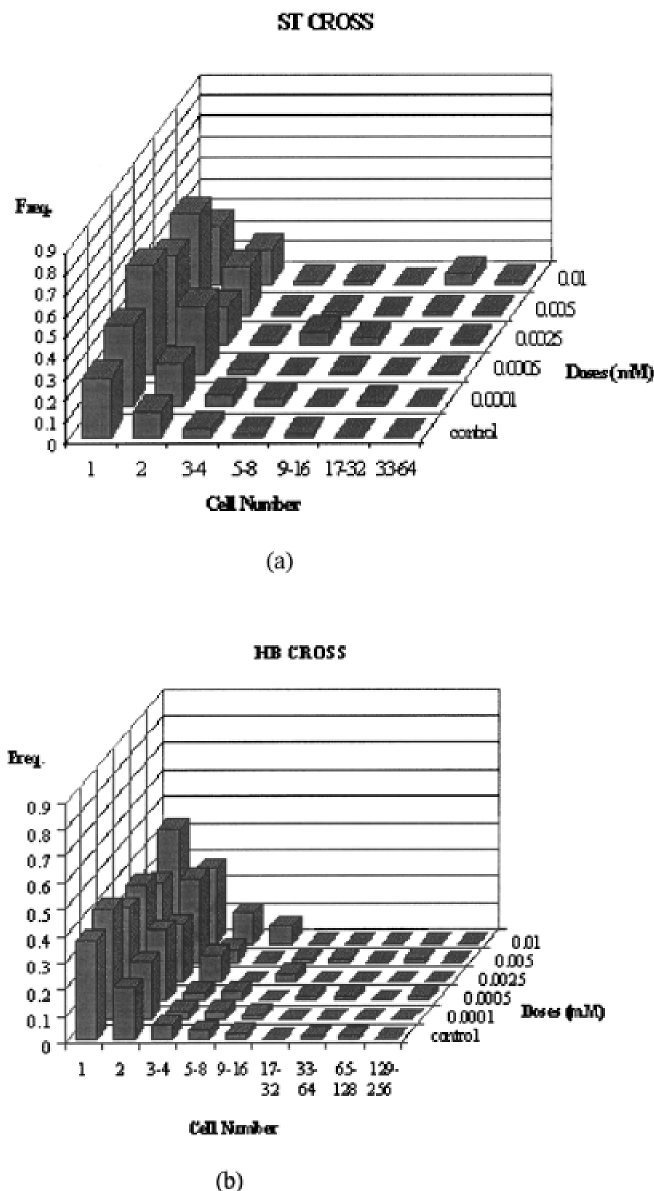


Fig. 3. Size distributions of the clones recorded in the flies treated with docetaxel: (a) ST cross; (b) HB cross.

Taxanes

The results from analysis of the wings obtained with the ST cross and HB cross are given in Tables I and II, respectively. In both cases, five concentrations of each taxoid were assayed in parallel together with the concurrent water and solvent controls. For significance testing, the spot scores in treated groups were always compared with the corresponding solvent controls. For each of the anti-microtubule agents tested, 600 flies of each cross were analysed, i.e. a total of 1200 flies were scored. The total numbers of spots per fly were not significantly above solvent control levels for any of the paclitaxel concentrations in ST (Table I) and HB flies (Table II), so it was concluded that paclitaxel is non-genotoxic in the *Drosophila* wing spot test.

In contrast, in the two experiments with the ST cross, the results obtained with docetaxel were positive, except at the highest concentration used (0.01mM). No dose-dependent increase was observed for the frequency of total spots throughout the exposure range of 0.0001–0.005 mM. The reduction at the highest concentration could be due to the toxicity of the compound since 70% of the larvae did not reach the adult stage at the 0.01 mM exposure level (Table I). This positive response was restricted to the ST cross: docetaxel was not genotoxic in the HB cross (Table II).

Figure 3 shows the distribution of spot sizes as a function of docetaxel exposure in the two crosses. It can be seen from this figure that the majority of spots observed in ST flies consisted of one or two cells, while in HB flies the frequencies and sizes of these spots were no different from those in solvent controls.

Discussion

While SMART assays seem to be good predictors of the genotoxicity of spindle poisons, the aneuploidogenic compounds found positive in these bioassays have typically shown weak effects. This behaviour was found with the microtubule antagonists chloral hydrate (Zordan *et al*., 1994) and vincristine (Vogel and Nivard, 1993), which induce significant increases only in the frequency of small (one- or two-cell) single spots. Such a weak response of the SMART assay to induce somatic monosomy has also been observed for vinblastine (Graf *et al*., 1984; Vogel and Nivard, 1993) and vinorelbine (Tiburi,M,F., Reguly,M.L., Schwartsmann,G. and Andrade,H.H.R., in preparation). In addition, these compounds also produced a significant increase in the frequency of large single clones (Graf *et al*., 1984; Tiburi,M,F., Reguly,M.L., Schwartsmann,G. and Andrade,H.H.R., in preparation). This last point is relevant when considering the effects of vinblastine and vinorelbine in the micronucleus assay using immunofluorescence. Recent studies have shown that both compounds induced micronuclei containing whole chromosomes and acentric fragments, which are the consequence of their aneugenic and clastogenic actions, respectively (Satya-Prakash *et al*., 1986; Grawe *et al*., 1997a,b; González-Cid *et al*., 1999). Consequently, we infer that the pattern exhibited by these drugs in the SMART could express genetic changes related to different endpoints: aneugenesis (small single clones) and clastogenesis (large single spots). Although we cannot distinguish the clones of chromosomal origin from those induced by point mutation, it is well known that compounds interfering with the spindle apparatus are inactive as inducers of point mutation (Ferguson, 1995).

Considering the aneugenic and/or clastogenic action of spindle poisons acting on microtubule assembly, it is evident that docetaxel—which inhibits tubulin disassembly—induced a marked increase solely in small single spots in ST larvae. Since it induced predominantly small single spots and since the frequencies of the other types of clone were not different from those observed in the solvent control, docetaxel appears to be a pure aneugen. In fact, the small single spots induced by the exposure of ST larvae to docetaxel (Figure 3) may be expected if they were produced through non-disjunction of homologues and represent monosomic clones for recessively marked chromosomes. Such cells probably have greatly reduced reproductive rates and, if they remain viable, would be expected to yield small single spots (Graf *et al*., 1984; Frei and Würgler, 1996). Consequently, this enhancement could be taken as evidence that docetaxel induced monosomic cells as a result of its interaction with microtubules.

In contrast to the aneugenic action of docetaxel, its analogue paclitaxel induced a clone frequency similar to that obtained

in the solvent control in both test crosses with normal (ST) or increased P450 bioactivation (HB). Thus paclitaxel was not genotoxic in the wing spot assay. To our knowledge, there are no published genotoxicity data for docetaxel in the literature, and there is only one study indicating that paclitaxel has genotoxic activity. Paclitaxel gives a strong positive response in the mouse bone marrow micronucleus assay. Some of the micronuclei induced by paclitaxel are either large or of aberrant morphology (Tinwell and Ashby, 1994) and may be associated with metaphase spindle disturbances and consequently with an aneugenic action of this inhibitor of tubulin disassembly.

Paclitaxel has been shown to undergo two pathways of metabolism: the major one leads to its 6-hydroxylation and is catalysed by CYP2C (Cresteil *et al*., 1994; Rahman *et al*., 1994), whereas a minor human metabolite results from the hydroxylation of the lateral chain by CYP3A (Royer *et al*., 1996). The biotransformation of docetaxel in humans is mediated mainly by the liver CYP450 isoenzymes of the CYP3A subfamily (Marre *et al*., 1996). It is interesting to note that the main metabolizing enzyme of docetaxel is different from that of paclitaxel, which is transformed mainly by CYP2C8/9 in human liver microsomes (Cresteil *et al*., 1994; Harris *et al*., 1994; Ferguson, 1995). Since inhibitors of CYP450 decrease clearance of both taxoids and overcome their anti-neoplastic effect, it has been suggested that their biotransformation leads to detoxification instead of generating active metabolites (Rahman *et al*., 1994; Sonnichsen *et al*., 1995; Royer *et al*., 1996). In this connection, the main difference between the two *Drosophila* crosses applied in the present study is the higher level of CYP6A2 expressed in the high bioactivation ORR cross (Saner *et al*., 1996), which is similar to the CYP3A subfamily of humans (Aoyama *et al*., 1989) and mouse CYP3A16 (Itoh *et al*., 1994).

Our data indicate that the genotoxicity of docetaxel is due to its aneugenic effect, a behaviour expected for a compound that has microtubule stabilization activity and is a potent inhibitor of mitotic cellular division. Nevertheless, no genotoxic activity was observed for its analogue paclitaxel, although it was tested at the same (millimolar) concentrations as docetaxel. The reason why docetaxel was aneuploidogenic in our experimental model, while paclitaxel was negative, are not clear. However, we must keep in mind that in spite of the obvious similarities in the general mechanism of action and metabolic detoxification of both taxoids (Gelmon, 1994; Rahman *et al*., 1994; von Hoff, 1997), there are some differences between them. Docetaxel is about twice as potent as paclitaxel as an inhibitor of spindle depolymerization and the two drugs have affinity for classes of microtubules. The weaker ligand and assembly action of paclitaxel, as well as the more rapid reversibility of the microtubules formed with this compound (Ringel and Horwitz, 1991; von Hoff, 1997) may explain its negative response in the present study.

Microtubules play an important role in cell proliferation, and inhibition of microtubule dynamics appears to be the mechanistic basis underlying the anti-tumour effects of most anti-mitotic compounds. Coupled to their clinical efficacy in cancer chemotherapy, spindle poisons seem to disturb the integrity of the genome, mainly inducing loss of whole chromosomes. Accordingly, it is not possible, at this stage, to conclude that the unresponsiveness of paclitaxel in the wing SMART could be a result of its ineffectiveness as an aneuploidogen. Considering the importance of aneugenic events in the expression of recessive deleterious genes, as well as in the development of cancer (Tinwell and Ashby, 1994), further genotoxicity studies dealing with paclitaxel and related compounds are required in order to provide a deeper understanding of the possible risks that could be associated with the clinical use of taxanes.

Acknowledgments

We are grateful to Dr Gilberto Schwartsmann from the South American Office for Anticancer Drug Development (SOAD) for the samples of Taxol and Taxotere. This study was supported by the following Brazilian agencies: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Financiadora de Estudos e Projetos (FINEP) and Fundação e Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References

Aoyama,T., Yamano,S., Waxman,D.J., Lapenson,D.P., Meyer,V.A., Fischer,V., Tyndale,R., Inaba,T., Kalow,W., Gelbion,H.V. and Gonzalez,F.J. (1989) Cytochrome P450 hPCN3, a novel cytochrome P450 IIIA gene product that is differentially expressed in adult human liver. *J. Biol. Chem.*, **264**, 10388–10395.

Campesato,V.R., Graf,U., Reguly,M.L. and de Andrade,H.H.R. (1997) Recombinagenic activity of integerrimine, a pyrrolizidine alkaloid from *Senecio brasiliensis*, in somatic cells of *Drosophila melanogaster. Environ. Mol. Mutagen.*, **29**, 91–97.

Chazard,M., Pellae-Cosset,B., Garet,F., Soares,J.A., Lucidi,B., Lavail,Y. and Lenaz,L. (1994) Taxol (paclitaxel), première molécule d'une nouvelle classe d'agent cytotoxiques: les taxanes. *Bull Cancer*, **81**, 173–181.

Cresteil,T., Monsarrat,B., Alvinerie,P., Tréluyer,J.M., Vieira,I. and Wright,M. (1994) Taxol metabolism by human liver microsomes: identification of cytochrome P450 isozymes involved in its biotransformation. *Cancer Res.*, **54**, 386–392.

Ferguson,L.R. (1995) Mutagenic properties of anticancer drugs. In Ponder, B.A.J. and Waring, M.J. (eds), *The Genetics of Cancer.* Kluwer Scientific Publishers, Lancaster, pp. 177–216.

Frei,H. and Würgler,F.E. (1988) Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative or inconclusive result. *Mutat. Res.*, **203**, 97–308.

Frei,H. and Würgler,F.E. (1995) Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in *Drosophila*. *Mutat. Res.*, **334**, 247–258.

Frei,H. and Würgler,F.E. (1996) Induction of somatic mutation and recombination by four inhibitors of eukaryotic topoisomerases assayed in the wing spot test of *Drosophila melanogaster. Mutagenesis*, **11**, 315–325.

Frölich,A. and Würgler,F.E. (1989) New tester strains with improved bioactivation capacity for the *Drosophila* wing spot test. *Mutat. Res.*, **216**, 179–187.

Frölich,A. and Würgler,F.E. (1990a) *Drosophila* wing-spot test: improved detectability of genotoxicity of polycyclic aromatic hydrocarbons. *Mutat. Res.*, **234**, 71–80.

Frölich,A. and Würgler,F.E. (1990b) Genotoxicity of ethyl carbamate in the *Drosophila* wing spot test: dependence on genotype-controlled metabolic capacity. *Mutat. Res.*, **244**, 201–208.

Gelmon,K. (1994) The taxoids: paclitaxel and docetaxel. *Lancet*, **344**, 1267–1272.

González-Cid,M., Cuello,M.T. and Larripa,I. (1999) Comparison of the aneugenic effect of vinorelbine and vincristine in cultured human lymphocytes. *Mutagenesis*, **14**, 63–66.

Graf,U. and van Schaik,N. (1992) Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster. Mutat. Res.*, **271**, 59–67.

Graf,U., Würgler,F.E., Katz,A.J., Frei,H., Juon,H., Hall,C.B. and Kale,P.G. (1984) Somatic mutation and recombination test in *Drosophila melanogaster. Mutat. Res.*, **271**, 59–67.

Grawe,J., Adler,I.D. and Nusse,M. (1997a) Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. II. Analysis of micronuclei of aneugenic and clastogenic origin by dual-colour FISH on populations of bone marrow PCEs flow sorted on the basis of their relative DNA content. *Mutagenesis*, **12**, 9–16.

Grawe,J., Nusse,M. and Adler,I.D. (1997b) Quantitative and qualitative studies of micronucleus induction in mouse erythrocytes using flow cytometry. I. Measurement of micronucleus induction in peripheral blood polychromatic erythrocytes by chemicals with known and suspected genotoxicity. *Mutagenesis*, **12**, 1–8.

Guzmán-Rincón,J. and Graf,U. (1995) *Drosophila melanogaster* somatic mutation and recombination test as a biomonitor. In Butterworth,F.M. (ed.), *Biomonitors and Biomarkers of Environmental Change*. Plenum Press, New York, pp. 169–181.

Harris,J.W., Rahman,A., Kim,B.R., Guengerich,F.P. and Collins,M. (1994) Metabolism of taxol by human hepatic microsomes and liver slices: participation of cytochrome P450 3A4 and an unknown P450 enzyme. *Cancer Res.*, **54**, 4026–4035.

Itoh,S., Satoh,M., Abe,Y., Hashimoto,H., Yanagimoto,T. and Kamataki,T. (1994) A novel form of mouse cytochrome–P450 3A(cyp 3A–16). Its cDNA cloning and expression in fetal liver. *Eur. J. Biochem.*, **226**, 877–882.

Jordan,M.A. and Wilson,L. (1998) Microtubules and actin filaments: dynamic targets for cancer chemotherapy. *Curr. Opin. Cell Biol.*, **10**, 123–130.

Kumar,N. (1981) Taxol-induced polymerization of purified tubulin. *J. Biol. Chem.*, **256**, 10435–10441.

Lindsley,D.L. and Zimm,G.G. (1992) *The Genome of Drosophila melanogaster.* Academic Press, New York.

Marre,F., Sanderink,G.-J., de Sousa,G., Gaillard,C., Martinet,M.M. and Rahmani,R. (1996) Hepatic biotransformation of docetaxel (taxotere) *in vitro*: involvement of the CYP3A subfamily in humans. *Cancer Res.*, **56**, 1296–1302.

Rahman,A., Korzekwa,K.R., Grogan,J., Gonzalez,F.J. and Harris,J.W. (1994) Selective biotransformation of taxol to 6α-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res.*, **54**, 5543–5546.

Ringel,I. and Horwitz,S.B. (1991) Studies with RP 56976 (taxotere): a semisynthetic analogue of taxol. *J. Natl Cancer Inst.*, **83**, 288–291.

Rowinsky,E.K. (1997) The development and clinical utility of the taxane class of antimicrotubule chemotherapy agents. *Annu. Rev. Med.*, **48**, 353–374.

Royer,I., Monsarrat,B., Sonnier,M., Wright,M. and Cresteil,T. (1996) Metabolism of docetaxel by human cytochromes P450: interactions with paclitaxel and other antineoplasic drugs. *Cancer Res.*, **56**, 58–65.

St John,M.A.R. and Xu,T. (1997) Insights from model systems. Understanding human cancer in a fly. *Am. J. Genet.*, **61**, 1006–1010.

Saner,C., Weibel,B., Würgler,F.E. and Sengstag,C. (1996) Metabolism of promutagens catalyzed by *Drosophila melanogaster* CYP6A2 enzyme in *Saccharomyces cerevisiae. Environ. Mol. Mutagen.*, **27**, 46–58.

Satya-Prakash,K.L., Liang,J.C., Hsu,T.C. and Johnston,D.A. (1986) Chromosome aberrations in mouse bone marrow cells following treatment *in vivo* with vinblastine and colcemid. *Environ. Mutagen.*, **8**, 273–282.

Sonnichsen,D.S., Liu,Q., Schuetz,E.G., Schuetz,J.D., Pappo,A. and Relling,M.V. (1995) Variability in human cytochrome P450 paclitaxel metabolism. *J. Pharmacol. Exp. Ther.*, **275**, 566–575.

Tinwell,H. and Ashby,J. (1994) Genetic toxicity and potential carcinogenicity of taxol. *Carcinogenesis,* **15**, 1499–1501.

Vogel,E.W., Graf,V., Frei,H.J. and Nivard,M.M. (1999) The results of assays in *Drosophila* as indicators of exposure to carcinogens. *IARC Sci. Publi.*, **146**, 427–470.

Vogel,E.W. and Nivard,M.J.M. (1993) Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination. *Mutagenesis*, **8**, 57–81.

von Hoff,D.D. (1997) The taxoids: same roots, different drugs. *Semin. Oncol.*, **2**, S13-3–S13-10.

Zordan,M.A., Osti,M., Pesce,M. and Costa,R. (1994) Chloral hydrate is recombinogenic in the wing spot test in *Drosophila melanogaster. Mutat. Res.*, **322**, 111–116.

Received on May 11, 2000; accepted on July 26, 2000