

Comparison of camptothecin derivatives presently in clinical trials: genotoxic potency and mitotic recombination

Journal Article**Author(s):**

Cunha, Kênya Silva; Reguly, Maria Luíza; Graf, Ulrich; Rodrigues De Andrade, Heloisa Helena

Publication date:

2002

Permanent link:

<https://doi.org/10.3929/ethz-b-000422901>

Rights / license:

[In Copyright - Non-Commercial Use Permitted](#)

Originally published in:

Mutagenesis 17(2), <https://doi.org/10.1093/mutage/17.2.141>

Comparison of camptothecin derivatives presently in clinical trials: genotoxic potency and mitotic recombination

Kênya Silva Cunha, Maria Luíza Reguly¹, Ulrich Graf² and Heloisa Helena Rodrigues de Andrade^{1,3}

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

The genotoxicity of camptothecin (CPT) and its clinical antineoplastic analogues irinotecan (CPT-11) and topotecan (TPT) were evaluated using the wing somatic mutation and recombination test (SMART) in *Drosophila melanogaster*. These compounds stabilize and trap the topoisomerase I–DNA complex, preventing the religation step of the breakage/rejoining reaction mediated by the enzyme. The standard version of the wing SMART was used to evaluate the three compounds and to compare the wing spots induced in marker-heterozygous and balancer-heterozygous flies. The results demonstrate that all compounds tested have a significant genotoxic effect in both genotypes analysed. At the same time, a comparison of the clone induction frequencies in marker-heterozygous and balancer-heterozygous flies shows that mitotic recombination is the prevalent mechanism through which the three compounds induce all categories of wing spots (78–93% recombination). TPT was the most genotoxic compound, probably because substitutions of amino groups for the 9-carbon of the CPT A ring leads to compounds with greater *in vivo* activity. CPT and CPT-11 induced, respectively, about 7 and 28 times fewer mutant clones per millimolar exposure unit than TPT.

Introduction

Camptothecins define a new family of anticancer agents, which have eukaryotic DNA topoisomerase (top1) as the sole target. They are able to stabilize and trap top1 in a covalent linkage with DNA (Tanizawa *et al.*, 1995; Desai *et al.*, 1997), preventing the religation step of the breakage/rejoining reaction mediated by the enzyme. This inhibition is most specific for the rejoining step, resulting in accumulation of a reversible intermediate complex known as the cleavable complex. The net result is that these drugs cause fragmentation of chromosomal DNA, cell death and extensive sister chromatid exchange and chromosomal aberration (Hsiang *et al.*, 1989; Gromova *et al.*, 1993; Liu, 1995).

Currently, two camptothecin (CPT) derivatives, topotecan (TPT) and irinotecan (CPT-11), are under clinical trials and their ultimate role in cancer therapy appears promising (Slichenmyer *et al.*, 1993; Lynch, 1996). Studies focused on these two drugs have demonstrated mechanistic differences between them related to the cytotoxic potency and the stability of the

cleavable complexes (Tanizawa *et al.*, 1994, 1995; Wang *et al.*, 1998). Consequently, this differential action could influence not only formation of strand breaks during replication but also the likelihood of cell killing or genetic toxicity of these new anticancer agents.

In the present study we employed the wing somatic mutation and recombination test (SMART) in *Drosophila melanogaster* to assess the structure–activity relationships among this class of compounds with respect to their genotoxic potency. CPT was chosen for this study, together with two analogues, TPT and CPT-11, mainly focusing on their recombinogenic effects in two intervals of chromosome 3 of *D. melanogaster*.

Materials and methods

Chemicals

CPT (CAS no. 7689-03-4) was purchased from Sigma Chemical Co. (St Louis, MO). CPT-11 (CAS no. 97682-44-5) was obtained from Yakult Ltd, Japan. TPT (CAS no. 123948-87-8) was tested as the clinical preparation Hycamtin (SmithKline Beecham, NJ), containing 5 mg/ml topotecan HCl. The structural formulae of the three compounds are shown in Figure 1. All the solutions and dilutions of the compounds were prepared immediately before use. CPT was dissolved in a mixture of 4% ethanol plus 4% Tween 80, while CPT-11 and TPT were diluted in distilled water.

Wing spot test

Two *D. melanogaster* strains carrying markers on the left arm of chromosome 3 were used: (i) *flr³/TM3, ri p^p sep l(3)89Aa bx^{34c} e Bd^S* and (ii) *mwh/mwh*. For more detailed information on the genetic symbols and descriptions see Lindsley and Zimm (1992). All stock cultures and experimental populations were maintained at 25 ± 1°C and ~60% relative humidity.

Eggs derived from the standard cross (*flr³/In(3LR) TM3, ri p^p sep l(3)89Aa bx^{34c} e Bd^S* virgin females crossed with *mwh/mwh* males) were collected for 8 h on standard medium enriched with baker's yeast. Three days later the larvae were transferred to vials containing 1.5 g *Drosophila* Instant Medium (Carolina Biological Supply, Burlington, NC) rehydrated with 5 ml of the test solutions. Negative solvent controls were always included. The larvae were allowed to feed on this medium until pupation (~48 h).

Emerged adult flies of the two genotypes, namely marker-heterozygous (*mwh +/+ flr³*) and balancer-heterozygous (*mwh +/TM3, Bd^S*), were collected and stored in 70% ethanol. Their wings were mounted in Faure's solution and inspected under 400× magnification for the presence of mutant spots. The number of spots as well as their type and size were recorded. On marker-heterozygous wings two types of spots could be observed: (i) single spots, either *mwh* or *flr³*, which can be produced by somatic point mutation, chromosome aberration or mitotic recombination; (ii) twin spots consisting of both *mwh* and *flr³* subclones, which originated exclusively from mitotic recombination (Graf *et al.*, 1984). On balancer-heterozygous wings, *mwh* single spots reflect predominantly somatic point mutation and chromosome aberration, since products of mitotic recombination involving the multiple inverted balancer chromosome (*TM3*) and its structurally normal homologue are normally non-viable (Szabad *et al.*, 1983).

Statistical evaluation

To evaluate the genotoxic effects recorded, the frequencies of each type of spot per fly of a treated series were compared with its concurrent negative (solvent) control series. These statistical comparisons were done using the computer program SMART 2.0 (Zordan, unpublished), which employs the χ^2 test for proportions and follows a multiple decision procedure according to Frei and Würzler (1988). The compounds tested were compared with their respective concurrent control data to obtain the statistical diagnosis. The statistical test used was the χ^2 test for proportions and we tested two hypotheses

³To whom correspondence should be addressed. Tel: +55 51 33166741; Fax: +55 51 33167311; Email: heloisa.andrade@ufrgs.br

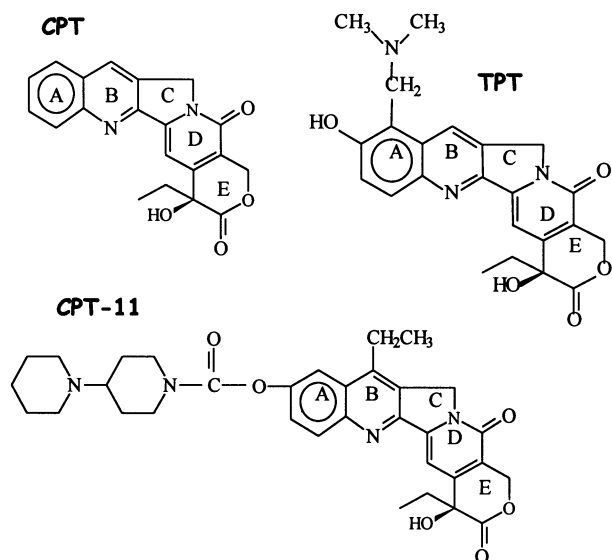


Fig. 1. Chemical structures of three top1 inhibitors according to Tanizawa *et al.* (1994).

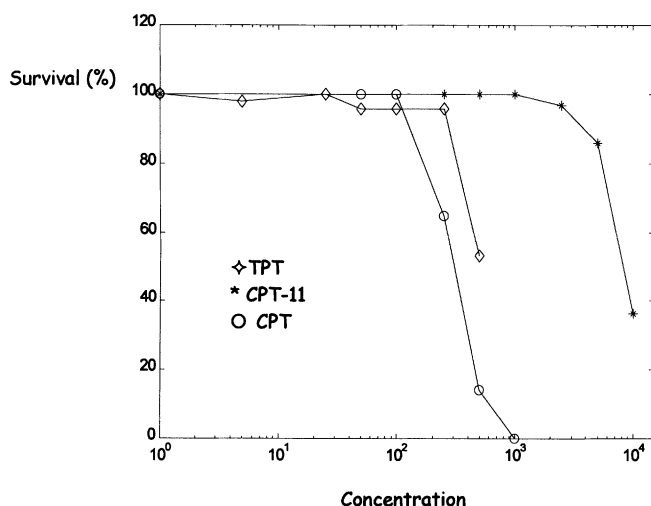


Fig. 2. Chronic toxicity of CPT, CPT-11 and TPT. Plot of log (10 000×exposure concentration) and survival frequency following 48 h exposure of third larval instars. ◇, TPT; ★, CPT-11; ○, CPT.

(according to Frei and Würgler, 1988): (i) the null hypothesis, which assumes that there is no difference in the mutation frequency between control and treated series; (ii) the alternative hypothesis, which postulates *a priori* that the treatment results in an increased mutation frequency that is m times the spontaneous frequency. The results allow four conclusions: (i) +, positive; (ii) -, negative; (iii) w+, weak positive; (iv) i, inconclusive (Frei and Würgler, 1988).

For the final statistical diagnosis of all positive outcomes, the non-parametric Mann-Whitney U -test with significance levels $\alpha = \beta = 0.05$ was used in order to exclude false positive diagnoses (Frei and Würgler, 1995).

Results

Toxic effects and genetic toxicity

In a pilot dose range finding experiment the chronic toxicity of the three compounds tested was determined quantitatively. For this purpose, batches of 100 larvae were counted and then treated for 48 h with a wide range of concentrations of each compound. After emergence of the adults, the numbers of surviving adult flies were recorded. The resulting survival curves are shown in Figure 2. All three compounds tested were toxic in 48 h larval feeding experiments. CPT showed

the highest toxicity: at 0.1 mM concentration no flies survived. CPT-11 was the drug with the lowest toxicity (Figure 2). Due to the different chronic toxicities of the three compounds, different ranges of exposure concentrations were then chosen for each compound for the genotoxicity experiments.

Table I shows a summary of the wing spot test results. In the marker-heterozygous genotype the three compounds showed positive genotoxic effects with clear dose-response effects over the whole range of concentrations used. Statistically significant increases above control levels were observed for all spot categories considered, except for the small single spots at the lowest concentration of CPT. More importantly, these data demonstrate that all top1 blockers significantly increased the frequencies of twin spots, which is evidence of their recombinogenic properties.

The data given in Table I and Figure 3 also show that the top1-targeting drugs were able to induce wing spots in the balancer-heterozygous genotype, although at much lower frequencies than in the marker-heterozygous genotype. In fact, only TPT yielded positive results at all exposure levels for both single (small and large) and total spots. The other two compounds were negative or inconclusive at lower exposure concentrations, especially with respect to the incidence of large single spots. However, the increments in frequencies of total spots are in general statistically higher than those observed for the concurrent negative controls.

Genotoxicity as a function of exposure

For quantitative comparisons between the frequencies of spots induced by the three compounds the data were unified to equimolar standardized values (Table II). The *mwh* clone frequencies observed at each exposure level (Table I) were corrected by subtracting the number of spontaneous clones, in such a manner that the corrected frequencies correspond to an estimate of the mutant clones induced by the three top1 poisons. The historical and pooled control frequencies and the numbers of flies analysed in the different treatment series served as a basis for this correction. As shown in Table II, approximated clone induction frequencies per unit of exposure (mM) were calculated through linear extrapolation for each compound (Frei and Würgler, 1996).

From these data we observe that TPT showed the highest genotoxicity, inducing ~2219 mutant clones/10⁵ cells/mM. CPT is the second most potent drug (338 mutant clones/10⁵ cells/mM), followed by CPT-11 (79 mutant clones/10⁵ cells/mM). This means that CPT and CPT-11 induced, respectively, about 7 and 28 times fewer mutant clones per exposure unit (mM) than TPT (Table II).

The same correction procedure was also used for the data shown in Figure 4, except for the fact that the spots were organized into size classes and the standardized frequencies are given as clone induction frequencies per fly. These spot size distributions show that the mean clone size induced by the compounds in the marker-heterozygous flies is larger than expected in the ideal situation ($2^{i-1} = 2$ cells) (Frei *et al.*, 1992) with mean *mwh* clone sizes of about 15, 6 and 3 cells, respectively, for CPT, TPT and CPT-11 (Table II). CPT also offered a larger mean clone size in the balancer-heterozygous flies, whereas CPT-11 and TPT induced clones of expected theoretical size (Table II). The comparatively large clones induced by both CPT and TPT could be related to their more pronounced chemical instability combined with retardation of larval development (Frei and Würgler, 1996).

Table I. Fly spot data obtained after exposure of marker-heterozygous and balancer-heterozygous larvae of *D.melanogaster* to CPT, CPT-11 and TPT

Controls and compounds Genotype	Conc. (mM)	No. of flies (N)	Spots per fly (no. of spots) statistical diagnosis ^a				Total <i>mwh</i> clones ^b (n)	
			Small single spots (1–2 cells) ^c (m = 2)	Large single spots (>2 cells) ^c (m = 5)	Twin spots (m = 5)	Total spots (m = 2)		
Water (historical and pooled controls)								
<i>mwh/flr</i> ³		135	0.56 (75)	0.10 (14)	0.03 (04)	0.69 (93)	93	
<i>mwh/TM3</i>		128	0.32 (41)	0.03 (04)	^d	0.35 (45)	45	
4% Ethanol + 4% Tween-80 (historical and pooled controls)								
<i>mwh/flr</i> ³		78	0.41 (32)	0.05 (04)	0.01 (01)	0.47 (37)	37	
<i>mwh/TM3</i>		80	0.23 (18)	0.06 (05)	^d	0.29 (23)	23	
CPT								
<i>mwh/flr</i> ³	0	39	0.28 (11)	0.00 (0)	0.00 (0)	0.28 (11)	11	
	0.005	20	0.15 (03) –	0.75 (15) +	0.15 (3) +	1.05 (21) +	20	
	0.01	20	0.80 (16) +	1.55 (31) +	0.30 (6) +	2.65 (53) +	51	
	0.025	20	1.30 (26) +	2.95 (59) +	0.75 (15) +	5.00 (100) +	94	
	0.05	20	1.75 (35) +	5.60 (112) +	1.65 (33) +	9.00 (180) +	170	
	<i>mwh/TM3</i>	0	40	0.18 (7)	0.08 (3)	^d	0.25 (10)	10
		0.005	20	0.30 (6) i	0.05 (1) –		0.35 (7) i	7
		0.01	19	0.16 (3)–	0.16 (3) i		0.32 (6) i	6
0.025		20	0.45 (9) +	0.30 (6) +		0.75 (15) +	15	
0.05	20	0.55 (11) +	0.20 (4) +		0.75 (15) +	15		
CPT-11								
<i>mwh/flr</i> ³	0	40	0.38 (15)	0.15 (6)	0.05 (2)	0.58 (23)	23	
	0.05	40	2.13 (85) +	1.15 (46) +	0.55 (22) +	3.83 (153) +	153	
	0.1	30	3.27 (98) +	1.70 (51) +	0.33 (10) +	5.30 (159) +	156	
	0.25	30	4.87 (146) +	3.70 (111) +	1.27 (38) +	9.83 (295) +	287	
	0.5	30	10.40 (312) +	6.47 (194) +	1.83 (55) +	18.70 (561) +	552	
	<i>mwh/TM3</i>	0	40	0.33 (13)	0.08 (3)	^d	0.40 (16)	16
0.05		30	0.87 (26) +	0.30 (9) +		1.17 (35) +	35	
0.1		29	1.24 (36) +	0.21 (6) i		1.45 (42) +	42	
0.25		30	2.07 (62) +	0.13 (4) i		2.20 (66) +	66	
0.5		30	3.67 (110) +	0.53 (16) +		4.20 (126) +	126	
TPT								
<i>mwh/flr</i> ³	0	30	0.67 (20)	0.07 (2)	0.00 (0)	0.73 (22)	22	
	0.0025	20	1.30 (26) +	1.05 (21) +	0.50 (10) +	2.85 (57) +	57	
	0.005	20	3.20 (64) +	2.55 (51) +	1.00 (20) +	6.75 (135) +	131	
	0.01	20	5.55 (111) +	6.75 (135) +	1.90 (38) +	14.20 (284) +	276	
	0.025	20	9.65 (193) +	13.65 (273) +	3.30 (66) +	26.60 (532) +	517	
	<i>mwh/TM3</i>	0	30	0.37 (11)	0.03 (1)	^d	0.40 (12)	12
0.0025		20	1.00 (20) +	0.40 (8) +		1.40 (28) +	28	
0.005		20	1.00 (20) +	0.40 (8) +		1.40 (28) +	28	
0.01		20	1.75 (35) +	0.70 (14) +		2.45 (49) +	49	
0.025		20	3.20 (64) +	1.00 (20) +		4.20 (84) +	84	

^aStatistical diagnoses according to Frei and Würzler (1988): +, positive; –, negative; i, inconclusive; m, multiplication factor for the assessment of significantly negative results. Significance levels $\alpha = \beta = 0.05$.

^bConsidering *mwh* clones from *mwh* single spots and from twin spots.

^cIncluding rare *flr*³ spots.

^dOnly *mwh* single spots can be observed in *mwh/TM3* heterozygotes as the balancer chromosome *TM3* does not carry a *flr* mutation.

Genotoxicity and recombinogenicity

The proportion of mitotic recombination versus somatic mutation was calculated based on the standardized frequencies (*mwh* clones/10⁵ cells/mM) obtained for the two genotypes as shown in Table II (see also Graf *et al.*, 1992; Graf and Würzler, 1996; Rodriguez-Arnaiz *et al.*, 1996). The comparison between the two genotypes demonstrates that mitotic recombination is the prevalent mechanism through which the three compounds induce all categories of wing spots. In fact, 78–93% of the spots induced by these drugs are due to recombinational events. CPT has the highest recombinogenic activity (93%). TPT (83%) and CPT-11 (78%) are also quite recombinogenic, but ~17–22% of the spots are still of mutational origin.

Discussion

Three points are remarkable with respect to the detailed genetic toxicity analysis of top1-interactive agents and their evaluation

in the *D.melanogaster* SMART. With increased clinical applications, the marked genotoxic effects of top1 inhibitors are becoming more apparent, demonstrating that, like many other anticancer agents, they are a double-edged sword and may themselves induce genetic toxicity (Baguley and Ferguson, 1998). There is extensive homology between fly DNA top1 and its mammalian counterpart, represented by highly conserved residues that are critical for its structure and function (Hsieh *et al.*, 1992). Finally, the development of SMART has provided a great asset for simultaneous screening of a wide range of end points available for genetic toxicological studies, including point mutations, deletions, mitotic recombination and, presumably, also chromosomal loss and non-disjunction (Graf *et al.*, 1984; Vogel *et al.*, 1999).

Genotoxicity

Our *in vivo* experiments revealed that all top1-interactive agents tested are genotoxic in the SMART. The major mechanism

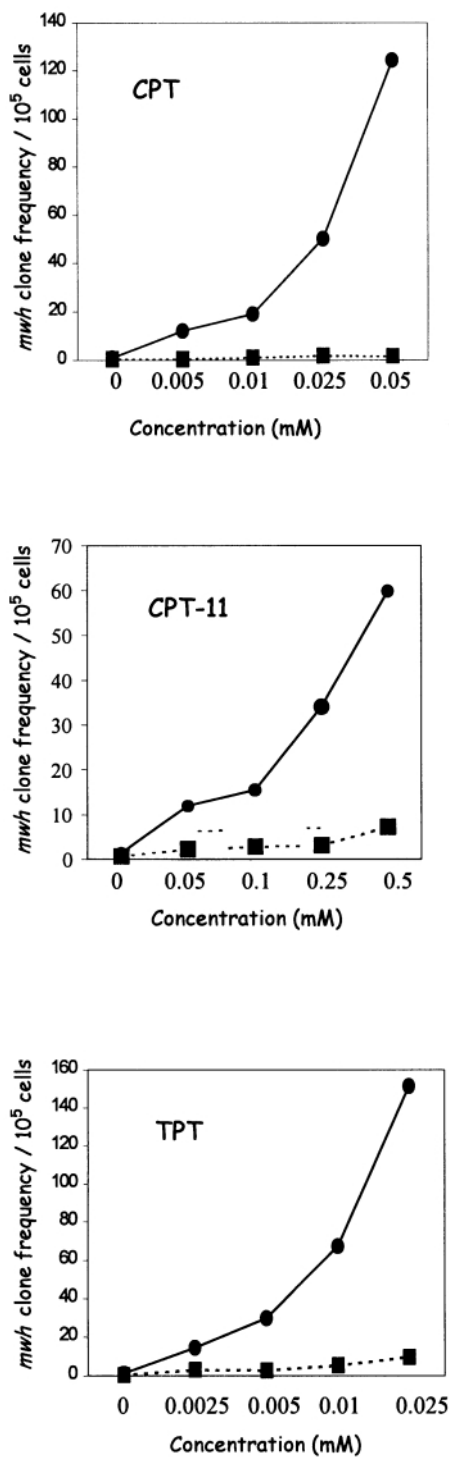


Fig. 3. Dose–response relationships for clone formation frequencies per 10⁵ cells recorded on marker-heterozygous (circles) and balancer-heterozygous (squares) flies after chronic feeding with different concentrations of CPT, CPT-11 and TPT.

responsible for the high genetic toxicity observed is the generation of DNA rearrangements, mainly associated with homologous mitotic recombination (Graf *et al.*, 1984; Vogel *et al.*, 1999). A similar response, represented by high increments in the incidence of this recombinational parameter, has already been demonstrated for CPT in the SMART (Frei and Würzler, 1996; Torres *et al.*, 1998). These observations become more relevant when one considers that disruption of topoisomerase function once cleavable complexes are formed may have two general consequences: (i) failure to replicate one of the DNA strands due to interference by the attached topoisomerase may lead to non-homologous recombinational repair; (ii) alternatively, inhibition of topoisomerase function may produce an unfavourable DNA topology leading to homologous or non-homologous recombination (Baguley and Ferguson, 1998). In fact, there are several lines of evidence suggesting that top1-interactive agents may promote illegitimate (Champoux and Bullock, 1988; Henningfeld and Hecht, 1995) as well as homologous recombination (Cortés *et al.*, 1993a,b; Palitti *et al.*, 1993; Anderson and Berger, 1994). The genetic changes produced in response to CPT and TPT inactivation of top1 lead to a high level of recombinational

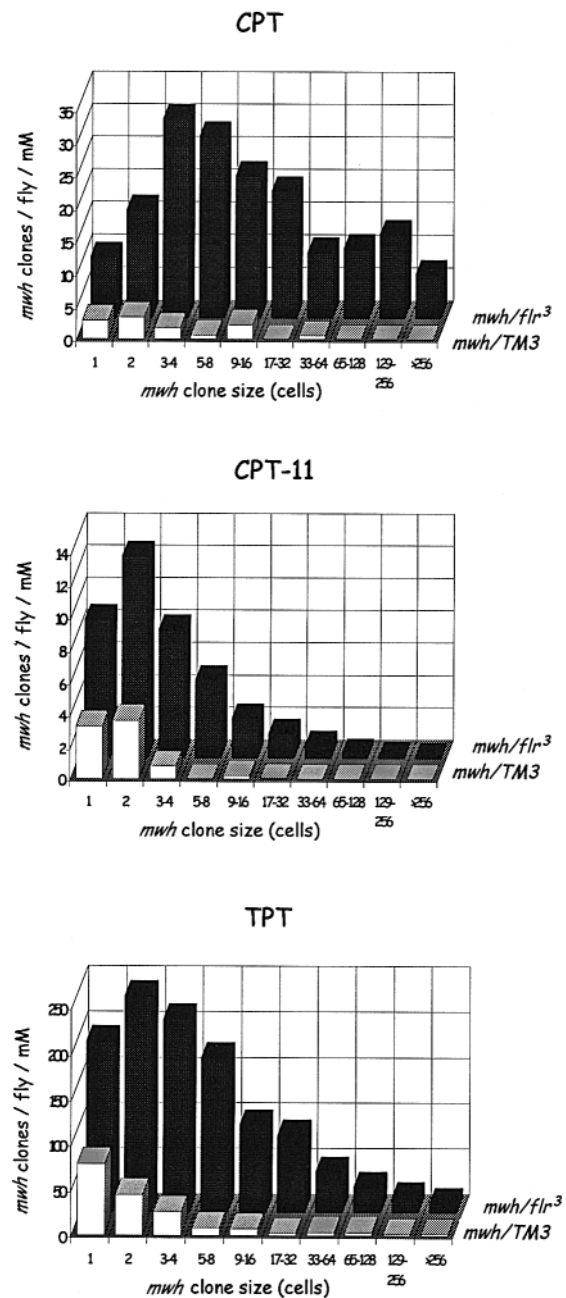


Fig. 4. Clone size distribution and *mwh* clone induction frequencies standardized to exposure concentration (mM) in marker-heterozygous and balancer-heterozygous flies. Frequencies are control corrected.

merase function once cleavable complexes are formed may have two general consequences: (i) failure to replicate one of the DNA strands due to interference by the attached topoisomerase may lead to non-homologous recombinational repair; (ii) alternatively, inhibition of topoisomerase function may produce an unfavourable DNA topology leading to homologous or non-homologous recombination (Baguley and Ferguson, 1998). In fact, there are several lines of evidence suggesting that top1-interactive agents may promote illegitimate (Champoux and Bullock, 1988; Henningfeld and Hecht, 1995) as well as homologous recombination (Cortés *et al.*, 1993a,b; Palitti *et al.*, 1993; Anderson and Berger, 1994). The genetic changes produced in response to CPT and TPT inactivation of top1 lead to a high level of recombinational

Table II. Standardized *mwh* clone induction frequencies per millimolar exposure concentration and the prevalence of recombination events^a

Compound	<i>mwh/ft³</i> marker <i>trans</i> -heterozygotes				<i>mwh/TM3</i> inversion heterozygotes				Recombination (%)
	Standardized frequency ^b (<i>mwh</i> clones/10 ⁵ cells/mM)	Mean clone size class (<i>i</i>)	Geometric mean clone size ^c (2^{i-1})	Standardized frequency per 10 ⁵ cells, corrected for clone size ^d ($f_t = 2^{i-2} \times f_i$)	Standardized frequency ^b (<i>mwh</i> clones/10 ⁵ cells/mM)	Mean clone size class (<i>i_h</i>)	Geometric mean clone size ^c (2^{i_h-1})	Standardized frequency per 10 ⁵ cells, corrected for clone size ^d ($f_h = 2^{i_h-2} \times f_{i_h}$)	
TPT	2218.62	3.47	5.54	6142.21	383.25	2.27	2.41	461.03	82.73
CPT	338.17	4.88	14.72	2492.92	23.22	2.83	3.56	41.39	93.13
CPT-11	78.87	2.75	3.35	132.27	17.30	1.80	1.74	15.04	78.06

^aAll values are control corrected. Frequencies in *mwh/ft³* marker-heterozygotes are calculated with and without clone size correction, accordingly, somewhat different estimates are obtained for the relative contributions of recombination to the totals of clone induction.

^bClone frequencies per fly divided by the number of cells examined per fly (48 800) estimate frequencies per cell and per cell division in chronic exposure experiments (Frei and Würigler, 1988).

^cGeometric mean calculated according to Frei *et al.* (1992).

^dCorrections calculated according to Frei *et al.* (1992).

events, as illustrated by marked increases in sister chromatid exchanges in cultured mammalian cells (Cortés *et al.*, 1993a,b; Piñero *et al.*, 1996; Ribas *et al.*, 1996). A high incidence of homologous recombination in *Saccharomyces cerevisiae* mutants, modified to increase CPT uptake through the cell wall, provides more evidence for the marked recombinogenic activity of these top1 blockers (Nitiss and Wang, 1988).

However, we must keep in mind that the genotoxic effects of top1 blockers are not restricted to recombinational events alone, as these drugs also induced significant enhancements in the frequencies of small and large single spots in balancer-heterozygous flies. This indicates that all three CPT derivatives also have mutagenic action, since in this genotype the spots originate exclusively from gene mutation or chromosome aberration (Frei *et al.*, 1992; Frei and Würigler, 1996). Our data are in agreement with two other reports demonstrating a low level of mutagenic activity of CPT in somatic cells of *Drosophila* (Frei and Würigler, 1996; Torres *et al.*, 1998). Accordingly, CPT derivatives tested in cytogenetic bioassays gave high frequencies of chromosomal aberrations, not only as a result of damage induced in S phase but also during G₂ phase (Andersson and Kihlman, 1992; Cortés *et al.*, 1993a,b; Palitti *et al.*, 1993; Piñero *et al.*, 1996). Studies at the hypoxanthine phosphoribosyltransferase locus also suggest that CPT and TPT induce a mutational process, frequently involving gene deletions (Hashimoto *et al.*, 1995).

Potency

The major aim of our study was to investigate the potency of camptothecin compounds as inducers of homologous recombination, trying to establish a relationship with their chemical structure. Our target was somatic cells of the *Drosophila* wing primordium (wing imaginal disc cells) that undergo approximately five or six rounds of mitotic division, beginning with ~780 cells at the start of the treatment and ending up with ~30 000 cells when division ceases at the onset of metamorphosis (Frei and Würigler, 1988). We determined that camptothecins induce mutational and recombinogenic events, using as an end point loss of heterozygosity (LOH) in somatic cells of *Drosophila*. By means of this experimental procedure, we determined that CPT was the drug with the highest recombinogenic activity (93%), the other two analogues having similar behaviour with respect to this genetic parameter (78–83%). However, in terms of genotoxic potency, TPT was the

most potent compound, followed by CPT, with a potency about 7 times lower. Another important observation was the fact that CPT-11 is the least active top1 blocker, inducing somatic genotoxic events at about a 4 times lower rate than its parental compound CPT.

All camptothecins have a basic five ring structure, which is essential for their chemotherapeutic efficacy. The structure–activity relationships of various camptothecin derivatives indicate that the two most distant rings of CPT (rings A and E) are critical for top1 inhibition (Fan *et al.*, 1998; O’Leary and Muggia, 1998). Substitution of amino groups for the 9-carbon of the CPT A ring leads to compounds with greater *in vivo* activity, because the cleavable complexes stabilized by these analogues are less reversible (Fan *et al.*, 1998). TPT [9-(dimethylaminomethyl)-10-hydroxycamptothecin] incorporates a stable basic side chain at position 9 and a hydroxy group at position 10. These replacements greatly increase the antitumour activity of TPT compared with the CPT parent molecule, and could be responsible for its higher genetic toxicity in the SMART. Nevertheless, previous structure–activity studies comparing cytotoxicity and induction of DNA damage, especially chromosomal aberrations, in human colon carcinoma cells and isolated nuclei showed that CPT is the most potent compound, followed by TPT, CPT-11 being inactive in this *in vitro* analysis (Tanizawa *et al.*, 1994). Alternatively, CPT-11 retains the lactone ring of CPT but has an additional piperidine side chain at position 10 and an ethyl group at C-7 of the B ring. As already observed, the presence of this bulky substitution at C-10 precludes significant induction of top1-mediated DNA cleavage (Rivory *et al.*, 1996b). In fact, CPT-11 itself has little, if any, activity *in vitro* and is thought to exert its anticancer action after biotransformation into SN-38 by carboxylesterases (Kawato *et al.*, 1991; Dodds *et al.*, 1998). Consequently, the lower genotoxic effect induced by CPT-11 in our system may be due to a low conversion to SN-38 by carboxylesterases, as already demonstrated in human liver (Rivory *et al.*, 1996a; Haaz *et al.*, 1997).

At least three arguments provide strong evidence that homologous recombination is one of the most important processes required for carcinogenesis: firstly, the demonstration that homologous recombination can be a major mechanism in the LOH required for the second step in the two-step model or for a later event in a multi-step model of carcinogenesis;

secondly, the elevated frequencies of homologous recombination and genome rearrangements observed in cells from human patients suffering from cancer-prone diseases. An increased frequency of homologous recombination may increase the likelihood of LOH occurring at an accelerated rate, but also raises the possibility that homologous recombination will cause aberrant genomic rearrangements that may act as the primary step in carcinogenesis. Finally, data from recent reports suggest that homologous recombination can act as an alternative mechanism of telomere maintenance (Bishop and Schiestl, 2001).

All in all, the high potency of the agents used in this study in inducing homologous recombination rather than mutational events demonstrates that they are a double-edged sword and may themselves contribute to the development of secondary cancer, especially TPT and to a lesser extent CPT-11, which are both in clinical trials.

Acknowledgements

We are grateful to Dr Gilberto Schwartzmann of the South American Office for Anticancer Drug Development (SOAD) for the samples of CPT-11 and Hycamtin[®]. Our sincere thanks go to Dr Hansjörg Frei for his help with the data evaluation. 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

- Anderson,R.D. and Berger,N.A. (1994) Mutagenicity and carcinogenicity of topoisomerase-interactive agents. *Mutat. Res.*, **309**, 109–142.
- Andersson,H.C. and Kihlman,B.A. (1992) Induction of chromosomal aberrations by camptothecin in root-tip cells of *Vicia faba*. *Mutat. Res.*, **268**, 167–181.
- Baguley,B.C. and Ferguson,L.R. (1998) Mutagenic properties of topoisomerase-targeted drugs. *Biochim. Biophys. Acta*, **1400**, 213–222.
- Bishop,A.J.R. and Schiestl,R.H. (2001) Homologous recombination as a mechanism of carcinogenesis. *Biochim. Biophys. Acta*, **1471**, M109–M121.
- Champoux,J.J. and Bullock,P.A. (1988) Possible role for the eukaryotic type I topoisomerase in illegitimate recombination. In Kucherlapati,R. and Smith,G.R. (eds), *Genetic Recombination*. American Association for Microbiology, Washington, DC, pp. 655–666.
- Cortés,F., Piñero,J. and Palitti,F. (1993a) Cytogenetic effects of inhibition of topoisomerase I or II activities in the CHO mutant EM9 and its parental line AA8. *Mutat. Res.*, **288**, 281–289.
- Cortés,F., Piñero,J. and Ortiz,T. (1993b) Importance of replication fork progression for the induction of chromosome damage and SCE by inhibitors of DNA topoisomerases. *Mutat. Res.*, **303**, 71–76.
- Desai,S., Liu,L., Vazquez-Abad,D. and D'Arpa,P. (1997) Ubiquitin-dependent destruction of topoisomerase I is stimulated by the antitumor drug camptothecin. *J. Biol. Chem.*, **27**, 24159–24164.
- Dodds,H.M., Haaz,M.-C., Riou,J.-F., Robert,J. and Rivory,L.P. (1998) Identification of a new metabolite of CPT-11 (Irinotecan): pharmacological properties and activation to SN-38. *J. Pharmacol. Exp. Ther.*, **286**, 578–583.
- Fan,Y., Weinstein,N., Kohn,K.W., Shi,L.M. and Pommier,Y. (1998) Molecular modeling studies of the DNA-topoisomerase I ternary cleavable complex with camptothecin. *J. Med. Chem.*, **41**, 2216–2226.
- Frei,H. and Würigler,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 Würigler,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 Würigler,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.
- Frei,H., Clements,J., Howe,D. and Würigler,F.E. (1992) The genotoxicity of the anti-cancer drug mitoxantrone in somatic and germ cells of *Drosophila melanogaster*. *Mutat. Res.*, **279**, 21–33.
- Graf,U. and Würigler,F.E. (1996) The somatic white-ivory eye spot test does not detect the same spectrum of genotoxic events as the wing somatic mutation and recombination test in *Drosophila melanogaster*. *Environ. Mol. Mutagen.*, **27**, 219–226.
- Graf,U., Würigler,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.
- Graf,U., Wild,D. and Würigler,F.E. (1992) Genotoxicity of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) and related compounds in *Drosophila melanogaster*. *Mutagenesis*, **7**, 145–149.
- Gromova,I.I., Kjeldsen,E., Svejstrup,J.Q., Alsner,J., Christiansen,K. and Westergaard,O. (1993) Characterization of an altered DNA catalysis of a camptothecin-resistant eukaryotic topoisomerase I. *Nucleic Acids Res.*, **21**, 593–600.
- Haaz,M.C., Rivory,L.P., Riché,C. and Robert,J. (1997) The transformation of irinotecan (CPT-11) to its active metabolite SN-38 by human liver microsomes. Differential hydrolysis by human liver microsomes. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **356**, 257–262.
- Hashimoto,H., Chatterjee,S. and Berger,N.A. (1995) Mutagenic activity of topoisomerase I inhibitors. *Clin. Cancer Res.*, **1**, 369–376.
- Henningfeld,K.A. and Hecht,S.M. (1995) A model for topoisomerase I-mediated insertions and deletions with duplex DNA substrates containing branches, nicks and gaps. *Biochemistry*, **34**, 6120–6129.
- Hsiang,Y.-H., Liu,L.F., Wall,M.E., Wani,M.C., Nicholas,A.W., Manikumar,G., Kirschenbaum,S., Silber,R. and Potmesil,M. (1989) DNA topoisomerase I-mediated DNA cleavage and cytotoxicity of camptothecin analogues. *Cancer Res.*, **49**, 4385–4389.
- Hsieh,T.-S., Brown,S.D., Huang,P. and Fostel,J. (1992) Isolation and characterization of a gene encoding DNA topoisomerase I in *Drosophila melanogaster*. *Nucleic Acids Res.*, **20**, 6177–6182.
- Kawato,Y., Furuta,T., Aonuma,M., Yasuoka,M., Tokokura,T. and Matsumoto,K. (1991) Antitumor activity of a camptothecin derivative, CPT-11, against human tumor xenografts in nude mice. *Cancer Chemother. Pharmacol.*, **28**, 192–198.
- Lindsley,D.L. and Zimm,G.G. (1992) *The Genome of Drosophila melanogaster*. Academic Press, New York, NY.
- Liu,L.F. (1995) Biochemistry of camptothecin. In Potmesil,M. and Pinedo,H. (eds), *Camptothecins: New Anticancer Agents*. CRC Press, Boca Raton, FL, pp. 11–14.
- Lynch,T. (1996) Topotecan today. *J. Clin. Oncol.*, **14**, 3053–3055.
- Nitiss,J. and Wang,J.C. (1988) DNA topoisomerase-targeting drugs can be studied in yeast. *Proc. Natl Acad. Sci. USA*, **85**, 7501–7505.
- O'Leary,J. and Muggia,F.M. (1998) Camptothecins: a review of their development and schedules of administration. *Eur. J. Cancer*, **34**, 1500–1508.
- Palitti,F., Cortés,F., Bassi,L., Di Chiara,D., Fiore,M. and Piñero,J. (1993) Higher G2 sensitivity in the CHO mutant EM9 than in its parental line AA8 to the induction of chromosomal damage by camptothecin, an inhibitor of DNA topoisomerase I. *Mutat. Res.*, **285**, 281–285.
- Piñero,J., Baena,M.L., Ortiz,T. and Cortés,F. (1996) Sister chromatid exchange induced by DNA topoisomerase poisons in late replicating heterochromatin: influence of inhibition of replication and transcription. *Mutat. Res.*, **354**, 195–201.
- Ribas,G., Xamena,M., Creus,A. and Marcos,R. (1996) Sister-chromatid exchanges (SCE) induced by inhibitors of DNA topoisomerases in cultured human lymphocytes. *Mutat. Res.*, **368**, 205–211.
- Rivory,L.P., Bowles,M.R., Robert,J. and Pond,S.M. (1996a) The conversion of irinotecan (CPT-11) to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38), by human liver carboxylesterase. *Biochem. Pharmacol.*, **52**, 1103–1111.
- Rivory,L.P., Riou,J.F., Haaz,M.C., Sable,S., Vuilhorgne,M., Commerçon,A., Pond,S.M. and Robert,J. (1996b) Identification of a major plasma metabolite of irinotecan (CPT-11) isolated from the plasma of patients. *Cancer Res.*, **56**, 3689–3694.
- Rodriguez-Arnaiz,R., Soto,P.O., Oyarzún,J.C.G. and Graf,U. (1996) Analysis of mitotic recombination induced by several mono- and bifunctional alkylating agents in the *Drosophila* wing-spot test. *Mutat. Res.*, **351**, 133–145.
- Slichenmyer,W.J., Rowinsky,E.K., Donehower,R.C. and Kaufmann,S.H. (1993) The current status of camptothecin analogues as antitumor agents. *J. Natl Cancer Inst.*, **85**, 271–291.
- Szabad,J., Soós,I., Polgár,G. and Brulag,D.L. (1983) Testing the mutagenicity of malondialdehyde and formaldehyde by the *Drosophila* mosaic and the sex-linked recessive lethal tests. *Mutat. Res.*, **113**, 117–133.
- Tanizawa,A., Fujimori,A., Fujimori,Y. and Pommier,Y. (1994) Comparison of topoisomerase I inhibition, DNA damage and cytotoxicity of camptothecin derivatives presently in clinical trials. *J. Natl Cancer Inst.*, **86**, 836–842.
- Tanizawa,A., Kohn,K.W., Kohlhagen,G., Leteurtre,F. and Pommier,Y. (1995) Differential stabilization of eukaryotic DNA topoisomerase I cleavable complexes by camptothecin derivatives. *Biochemistry*, **34**, 7200–7206

- Torres,C., Creus,A. and Marcos,R. (1998) Genotoxic activity of four inhibitors of DNA topoisomerases in larval cells of *Drosophila melanogaster*. *Mutat. Res.*, **413**, 191–203.
- Vogel,W., Graf,U., Frei,H.-J. and Nivard,M.M.J. (1999) The results of assays in *Drosophila* as indicators of exposure to carcinogens. In McGregor,D.B., Rice,J.M. and Venitt,S. (eds), *The Use of Short- and Medium-term Tests for Carcinogens and Data on Genetic Effects in Carcinogenic Hazard Evaluation*, IARC Scientific Publications no. 146. IARC, Lyon, pp. 427–470.
- Wang,X., Wang,L.-K., Kingsbury,W.D., Johnson,R.K. and Hecht,S.M. (1998) Differential effects of camptothecin derivatives on topoisomerase I-mediated DNA structure modification. *Biochemistry*, **37**, 9399–9408.

Received on June 15, 2001; revised on September 3, 2001;
accepted on October 19, 2001