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Genotoxic profile of inhibitors of topoisomerases I (camptothecin) and II (etoposide) in a mitotic recombination and sex-chromosome loss somatic eye assay of *Drosophila melanogaster*

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Abstract

Genotoxic carcinogens which interact with DNA may produce double-strand breaks as normal intermediates of homologous mitotic recombination, and may give rise to structural chromosome aberrations and inter-chromosomal deletion-recombination. The genotoxic profile of two inhibitors of DNA topoisomerases were evaluated using an in vivo somatic *w/w*$^+$ eye assay of *Drosophila melanogaster* for the detection of loss of heterozygosity (LOH) by homologous mitotic recombination, intra-chromosomal recombination and structural chromosomal aberrations. We studied camptothecin (CPT) as a topoisomerase-I-interactive agent and etoposide (ETOP) as a topoisomerase II inhibitor. These drugs act by stabilizing a ternary complex consisting of topoisomerases covalently linked to DNA at single-strand or at double-strand breaks, thereby preventing the relegation step of the breakage/rejoining reaction mediated by the enzyme. The genotoxic profiles were determined from the appearance of eye tissue in adult flies, in which LOH and expression of the reporter gene *white* produced light clones. The results demonstrated that both compounds were significantly genotoxic, with CPT being more effective than ETOP. Inter-chromosomal mitotic recombination was the major mechanism responsible for the induction of light spots by both compounds in XX females. Loss of the ring X chromosome (rX), was significantly enhanced by CPT, and this topoisomerase blocker also produced intra-chromosomal recombination (XY males).

Keywords: Clastogenicity; Loss of heterozygosity (LOH); Recombinogenicity; Somatic cells; Topoisomerase-interactive agents

1. Introduction

DNA topoisomerases I and II are essential nuclear enzymes that modulate DNA topology during multiple cellular processes, including DNA replication, recombination and transcription. They play a crucial role in chromosome structure, condensation/decondensation and segregation during mitosis [1]. There are a number of steps in the action of topoisomerases, all of which are potential targets for inhibition by drugs, for cellular and genetic toxicity, and for mutagenesis [2]. Topoisomerases are the principal intercellular targets for a number of clinically important anticancer drugs. Among the topoisomerase-interactive agents, the alkaloid camptothecin (CPT) and the non-intercalative epipodophyllotoxin derivative etoposide (ETOP) are used in chemotherapy. In vitro studies show that the clinically effective agent CPT stabilizes a ternary complex

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consisting of topoisomerase I covalently linked to DNA at single-strand breaks, thereby preventing the relegation step of the breakage/rejoining reaction mediated by the enzyme [3,4]. ETOP stabilizes a similar ternary complex, in which topoisomerase II is covalently linked to DNA at double-strand breaks [3]. This complex represents an intermediate in the topoisomerase-II catalyzed DNA-supercoil-relaxation reaction [5]. Short-term tests of genotoxicity confirm that topoisomerase-interactive agents are mutagenic, and suggest common mechanisms by which they induce mutation and selectively kill tumor cells. These agents induce sister-chromatid exchanges, chromosomal aberrations and mutations in specific mammalian genes [6,7]. Studies in bacterial mutation assays suggest that topoisomerase-interactive agents might also induce mutations, albeit at a lower rate, through simple DNA intercalation or via the generation of oxygen-free radicals [8]. As a result of their extensive use in the clinic and their association with secondary malignancies, inhibitors of topoisomerases I and II are of great interest with regard to their genotoxic activity in vivo [9,10].

This paper describes a series of experiments that were employed to determine the in vivo genotoxic profile of CPT and ETOP in a mitotic recombination and sex-chromosome loss assay of *Drosophila melanogaster*. We found that the genetic profiles of topoisomerase inhibitors seemed to differ: CPT produced loss of heterozygosity (LOH) by its recombinogenic and clastogenic effects, while ETOP was mainly recombinogenic.

2. Materials and methods

2.1. Chemical compounds and concentrations tested

Camptothecin (CPT, CAS # 7689-03-4) and etoposide (ETOP, CAS # 33419-42-0) were purchased from Sigma (St. Louis, MO, USA). CPT was dissolved in a mixture of 1% Tween-80 and 3% ethanol; the final concentration of the solvent mixture was 4%. ETOP was dissolved in dimethylsulfoxide (DMSO) at different concentrations. All solutions and dilutions of the compounds were prepared immediately before use. The higher exposure dose was defined as the LD_{50} for CPT and as the therapeutic concentration determined by solubility of the compound for ETOP; several additional lower doses were tested.

2.2. Somatic assay

Wild-type (Hague-79) female flies were mated with rX, R(1)2; *y*(yellow)*f*(forked)/y^+ Y males. Virgin F_1 females heterozygous for a ring-X and a rod-X chromosome were mated with *ywf* males (for a description of the genetic markers see reference [11]), generating four regular classes in the F_2: XX, $y^+w^+f^+/ywf$ ♀ (phenotype wild-type); XY, $y^+w^+f^+$/Y ♂ (wild-type); XrX, yw^+f/ywf ♀ (yellow, forked); and XrY, yw^+f/Y ♂ (yellow, forked). All four regular genotypes were heterozygous or hemizygous for the w^+ reporter gene and, thus, expressed the red wild-type eye color. Loss of the w^+ reporter gene in w/w^+ (XX or XrX) or in w^+ (XY or XrY) pre-ommatidia cells during larval development led to *white* clones, which became visible in the adult flies. Besides these four regular F_2 classes, five additional genotypes were expected from double crossovers in ring/rod heterozygous flies and owing to rX loss. These exceptional flies were excluded from the scoring procedure [12]. Visible light spots in the red compound eyes of the four regular F_1 phenotypes were produced by loss of heterozygosity (LOH) in XX ♀ by homologous inter-chromosomal recombination between the two rod X chromosomes. In addition, in all two genotypes carrying on their rod-X chromosomes the w^+ allele several genetic endpoints could be produced by unequal sister-strand recombination generating a w^+w^+ duplication and a w^- deletion (intra-chromosomal recombination), or by forward mutations and small deletions at the *white* locus. Thus, LOH in XX ♀ predominantly monitors homologous recombination while LOH in XrX represents rX loss from breakage events [12].

2.3. Test protocol

All crosses were set up as mass cultures, with 50 pairs of flies per bottle. Chemicals were administrated by acute treatment. Eggs derived from crosses were collected for 6 h in culture bottles containing standard medium enriched with live baker's yeast supplemented with sucrose. Three days later, larvae were collected by washing them out with an aqueous solution of 20% sucrose and seeded in bottles (500 larvae/bottle) containing 50 ml of standard medium and 2 ml of the solvent mixture with the test solution. Larvae were fed on the above medium during 6 h then were transferred to fresh medium till the end of development. Newly hatched adults, females and males, were scored 1–5 days later. Adult females are heterozygous for *white* and were inspected for the occurrence of *white* in their compound eyes. Spots separated from each other by at least four non-mutated ommatidia were counted as independent clones. Proliferation of pre-ommatidia cells in the imaginal discs of developing larvae increases the number of target cells from about 20 at the end of the first instar, to 100–150 cells in the second instar, and reaches a final number of 780–800 pre-ommatidia cells at the end of the third instar [13]. Thus primordial cells of the adult compound eye divide continuously throughout the larval period. Mosaic white clones induced in early larvae will be large but less frequent, whereas those produced later will successively be smaller and more frequent, as the number of potential target cells increases with larval age. The size of the white clone and their distribution among size classes provides therefore information of the time point of induction of a LOH event [14,12]. For each experiment a concurrent control was run, where larvae were treated with the solvent mixture alone.

2.4. Statistical evaluation

For evaluation of the genotoxic effects recorded, the frequencies of mosaic eyes of each treated series were compared to its concurrent negative control series. These statistical comparisons were done using the χ^2-test for proportions. Statistical analyses were done exclusively for the total number of spots recovered. To test the alternative hypothesis (H_A) the parameter $m = 2$ (multiplication factor) was used due to the relatively high spontaneous incidence of total spots [15].

3. Results

3.1. Non-regular progeny

The use of markers *yellow* (*y*) and *forked* (*f*), in addition to *white* (*w*) enabled the phenotypic identification of five additional non-regular classes that were expected from double crossovers in ring/rod heterozygous flies and owing to rX loss. These classes expressed *y* (♀ and ♂), *f* (♀ and ♂) and *ywf* (patroclinous males); although they were excluded from the scoring procedure, these classes had to be recorded. For unexposed cultures, the frequencies of the five additional F_2 class genotypes in the present study were as follows: 0.0140 exceptional females (i.e. 28 among a total female progeny of 1992 flies) and 0.01 exceptional males (i.e. 30 among a total male progeny of 3000 male flies). For exposed cultures, the respective frequencies were 0.02 for females (68/3397) and 0.0549 for males (148/2657).

3.2. Toxic effects

In order to determine the dose range to be used in the experiments, we performed acute-toxicity assays of the compounds to be tested. Approximately hundred 48-h-old larvae were treated, during 9 h, with different concentrations of each compound, and then transferred onto a regular diet until the adults emerged. The numbers of adults were recorded and the LD_{50} values determined. CPT showed the highest toxicity: at 0.05 mM 50% of the flies survived (therapeutic dose near 0.025 mM). We could not determine this parameter for ETOP due to solubility problems (50 mM was the solubility limit), although the highest concentration tested (15 mM) was the therapeutic dose employed in patients.

3.3. Frequency of spots and size of clones

Table 1 summarizes the results obtained in the eye-spot assay of *D. melanogaster*. The effects induced by the topoisomerase-interactive agents were mainly related to increases in the frequency of small spots (in which one or two ommatidia were affected). Large spots (in which more than two ommatidia were affected) were less frequent. The ratio between small and large spots varied between 1.86 and 12.5, with the exception of the lowest and highest concentrations tested for CPT, which showed ratios of between 0.46 and 1.46. The size classes were related to the number of cell divisions that occurred between the time of clone induction in the imaginal larval cells and the beginning of eye differentiation. In acute treatments, the numbers of cell divisions before pupation were two or three [13]. The majority of spots were of the following size classes: one or two, three or four and five to eight. The induction of small spots occurred at the end of the third larvae stage. The average clone size, which measured the genotoxicity, varied between 1.1 and 3.3 for ETOP and between 2.1 and 7.1 for CPT. The total spot frequencies were calculated assuming that the number of potential total target cells increased per cell division by a power of two, and that recombinogenic, clastogenic or mutagenic events occurred in pre-ommatidial eye cells at a constant rate per cell division. In unexposed flies total spot frequencies (S_F) showed that rX loss occurred more frequently than any other event and, furthermore, the majority of clones had small spots.

3.4. Genotoxic profile

Homologous inter-chromosomal mitotic recombination detected in XX females was induced in a dose–response manner for both topoisomerase-interactive agents. The total number of spots per 100 eyes did not significantly increase above the control level at the lowest concentration tested for ETOP, while CPT was positive at all concentrations used (Table 1). CPT generated more large spots than ETOP, an effect that could be related to the persistent damage induced by CPT and/or its chemical instability combined with a retardation of larval development. CPT showed the highest recombinogenic activity, inducing 363.45 mutant clones per 10^4 cells; about five-times fewer mutant clones (65.39/10^4 cells) were produced by ETOP (Table 1).

Clastogenic activities, measured according to the induction of X-chromosome loss in XrX females, were significantly increased above control levels at all concentrations tested for CPT and produced dose-dependent frequency increases (Fig. 1). The topoisomerase-II-inhibitor agent ETOP did not produce structural chromosome aberrations (Fig. 2). Again, CPT induced both small and large spots, while the majority of those produced by ETOP were of clone size one or two (Table 1). Loss of rX in males was significant for both compounds

Table 1
Number of eyes, distribution of spots (frequency of spots), clone size (ommatidia affected), average clone size, estimate of total spot frequencies and statistical diagnoses induced in the eyes of flies after acute treatment (6 h) with two topoisomerase-interactive drugs

Genotype	Compound and concentration (mM)	No. of eyes	Number of spots scored (frequency of spots per 100 eyes)				Total	Ratio (small/large spots[a])	Average clone size	Total spot frequency [b]	Statistical diagnoses[c]
			Clone (1 + 2)	Size (3 + 4)	Ommatidia 5–8	Affected >8					
Camptothecin (CPT)											
♀XX (+)	Control	500	47 (9.4)	5 (1)	1 (0.2)		53 (10.6)	7.83	2.3	6.095	
♀XX (+)	0.01	500	95 (19)	39 (7.8)	16 (3.2)	10 (2)	160 (32)	1.46	3.4	27.2	+
♀XX (+)	0.025	606	644 (106.27)	291 (48.02)	239 (39.48)	165 (27.23)	1339 (221)	9.09	4.9	270.89	+++
♀XX (+)	0.05	500	327 (65)	274 (55)	226 (45)	214 (43)	1041 (208)	0.46	7.1	369.55	+++
♀XrX (yf)	Control	492	75 (15)	6 (1)			81 (16)	19	2.1	8.64	
♀XrX (yf)	0.01	500	134 (27)	25 (5)	10 (2)	7 (1)	176 (35)	3.19	3	26.4	+
♀XrX (yf)	0.025	500	325 (65)	31 (6)	47 (9)	14 (3)	417 (83)	3.53	3.3	68.8	+++
♀XrX (yf)	0.05	500	406 (81)	103 (21)	37 (7)	29 (6)	575 (115)	2.4	3.2	206	+++
♂XY (+)	Control	500	44 (8.8)	3 (0.6)			47 (9.4)	14.6	2.1	4.93	
♂XY (+)	0.01	500	49 (10)	12 (2)			61 (12)	4.08	2.2	3.41	
♂XY (+)	0.025	520	67 (12.88)	8 (1.54)			75 (14.42)	8.42	2.2	7.93	
♂XY (+)	0.05	500	176 (35.2)	41 (8.2)	6 (1.2)	10 (2)	233 (46.6)	3.09	4	46.6	+++
♂XrY (yf)	Control	500	104 (20.8)	12 (2)	1 (0.2)		117 (23)	9.45	2.3	13.45	
♂XrY (yf)	0.01	500	118 (23.6)	29 (5.8)	15 (3)	2 (0.4)	164 (32.8)	2.57	2.8	22.96	
♂XrY (yf)	0.025	496	178 (36)	24 (5)			202 (41)	7.43	2.2	22.4	
♂XrY (yf)	0.05	500	351 (70)	88 (18)	45 (9)	19 (4)	503 (101)	2.31	3.1	77.96	+++
Etoposide (ETOP)											
♀XX (+)	C (4%)[d]	500	51 (10)	9 (1.8)	2 (0.2)		62 (12)	4.64	2.2	6.82	
♀XX (+)	0.35	500	73 (14.6)	9 (1.8)	2 (0.4)	1 (0.2)	85 (17)	5.62	2.4	10.2	
♀XX (+)	0.7	348	70 (20.15)	8 (2.3)	1 (0.29)		79 (22.74)	7.79	2.1	11.92	+
♀XX (+)	1.5	500	215 (43)	18 (4)	11 (2)	6 (1)	250 (50)	6.14	2.5	31.25	+
♀XX (+)	C (10%)[d]	250	32 (13)	11 (4)	3 (1)		46 (18)	2.29	2.6	11.96	
♀XX (+)	5	272	180 (66.18)	57 (20.96)	39 (14.34)	4 (1.47)	280 (102.95)	1.8	3.3	84.93	+++
♀XX (+)	C (40%)[d]	250	23 (9)	7 (3)	4 (2)		34 (14)	2.09	2.7	9.18	
♀XX (+)	15	262	492 (187.78)	90 (34.35)	100 (38.17)	6 (2.29)	688 (262.59)	2.54	1.1	72.21	+++
♀XrX (yf)	C (4%)[d]	500	56 (11)	4 (1)	3 (1)	5 (1)	68 (14)	3.67	3.3	11.22	
♀XrX (yf)	0.35	500	107 (21)	13 (2)	6 (1)	2 (1)	125 (25)	5.25	2.5	15.62	
♀XrX (yf)	0.7	306	70 (23)	9 (3)	3 (1)		82 (27)	5.75	2.2	14.74	
♀XrX (yf)	1.5	500	119 (24)	12 (2)	6 (1)		137 (27)	8	2.3	15.75	
♀XrX (yf)	C (10%)[d]	250	26 (11)	10 (4)	6 (2)		42 (17)	1.83	2.8	11.76	
♀XrX (yf)	5	250	29 (12)				29 (12)	0	2	5.8	

♀XrX (yf)	C (40%)[d]	250	43 (17)	8 (3)	3 (2)		54 (22)	3.4	2.4	12.96	
♀XrX (yf)	15	250	91 (36.2)	8 (3.2)	3 (1.2)		102 (40.8)	9	2.2	22.44	
♂XY (+)	C (4%)[d]	500	50 (10)	1 (0.2)	2 (0.4)	1 (0.2)	54 (10.8)	10	2.5	6.75	
♂XY (+)	0.35	500	102 (20)	2 (0.4)	2 (0.4)	1 (0.2)	107 (21)	6.67	2.1	11.23	
♂XY (+)	0.7	512	67 (13)	7 (1.4)	4 (0.6)		78 (15)	6.5	2.2	8.38	
♂XY (+)	1.5	500	104 (21)	12 (2.4)	7 (1.4)	1 (0.2)	124 (25)	5.25	2.5	15.5	
♂XY (+)	C (10%)[d]	250	37 (14.8)	11 (4.4)	1 (0.4)		49 (19.6)	3	2.5	12.25	
♂XY (+)	5	250	22 (9)	3 (1)			25 (10)	9	2.2	5.5	
♂XY (+)	C (40%)[d]	250	25 (10)	14 (6)			39 (16)	1.67	2.3	8.97	
♂XY (+)	15	250	45 (18)	4 (1.6)	1 (0.4)		50 (20)	6	2.3	11.5	
♂XrY (yf)	C (4%)[d]	500	65 (13)	4 (0.8)	3 (0.6)		72 (14.4)	6.5	2.1	7.56	
♂XrY (yf)	0.35	500	105 (21)	4 (1)	5 (1)		114 (23)	10.5	2.2	12.54	
♂XrY (yf)	0.7	356	88 (24.7)	3 (0.8)	1 (0.3)		92 (25.8)	12.5	2	12.92	
♂XrY (yf)	1.5	500	158 (32)	10 (2)	10 (2)		178 (36)	8	2.2	19.58	
♂XrY (yf)	C (10%)[d]	250	41 (16)	8 (3)	2 (1)		51 (20)	4	2.2	11.22	
♂XrY (yf)	5	250	22 (9)	3 (1)	2 (1)		27 (11)	4.5	2.7	7.29	
♂XrY (yf)	C (40%)[d]	250	32 (13)	7 (3)	5 (2)		44 (18)	2.6	2.7	11.88	
♂XrY (yf)	15	250	97 (38.8)	5 (2)	3 (1.2)	1 (0.4)	106 (42.4)	13	2.5	26.5	+

[a] Ratio between: small spots (1 + 2 ommatidia affected)/large spots (>2 ommatidia affected).
[b] $S_F = 2n_T m_T/NC \times 10^{-4}$ [12].
[c] Statistical diagnosis according to Frei and Wügler [15]: + (positive); − (negative); $^{+}P < 0.05$; $^{++}P < 0.01$; $^{+++}P < 0.001$.
[d] Control DMSO at different concentrations.

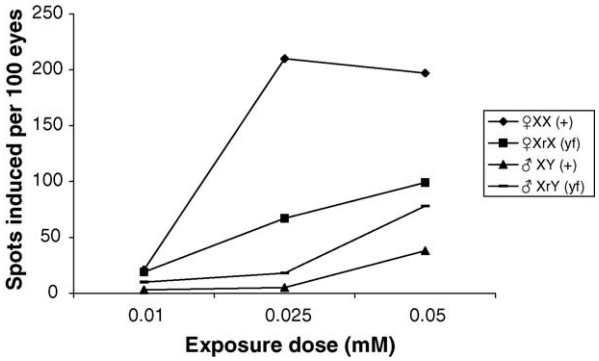

Fig. 1. Frequencies of total white spots induced by CPT.

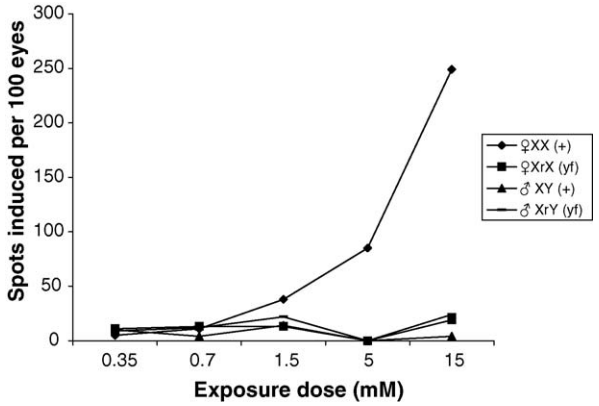

Fig. 2. Frequencies of total white spots induced by ETOP.

only at the highest concentration assayed, although CPT was much more effective than ETOP at this level (Figs. 1 and 2). CPT produced both types of spots (small and large), while ETOP mainly induced small spots (Table 1).

Intra-chromosomal recombination was significantly induced at the LD_{50} concentration assayed for CPT, while XY males were not significantly induced by ETOP. Small spots were by far the most abundant (Table 1).

Thus, the genetic profiles of topoisomerase inhibitors seemed to differ: CPT produced LOH by its recombinogenic and clastogenic effects, while ETOP was mainly recombinogenic.

4. Discussion

Somatic assays of *D. melanogaster* are versatile systems for detecting the LOH processes induced by several mechanisms, including mitotic recombination, mutations, small deletions and chromosome loss. LOH owing to mitotic recombination is an important mechanism that is involved in carcinogenesis. It can be detected in *Drosophila* by several in vivo systems, including the *multiple wing/flare* wing spot system and the *white/white+* eye assay [16,14]. The improvement of the w/w^+ system allows the detection not only of homologous mitotic recombination, but also of illegitimate somatic recombination and sex-chromosome loss; these end points are also cancer-prone mechanisms [17]. Thus, using this system, it is possible to detect genotoxic chemicals that bind covalently to DNA, as well as those that produce their effects by interactions with other cellular targets, such as enzymes that play a significant role in replication or in the segregation of chromosomes during cell division [18].

The present study aimed to elucidate the genotoxic profiles based on the mechanistically different processes induced by two topoisomerase-inhibitors, topoisomerase I (CPT) and topoisomerase II (ETOP), using an in vivo w/w^+ somatic assay of *D. melanogaster* for the parallel monitoring of three separate endpoints: homologous (inter-chromosomal) mitotic recombination, unequal sister-strand (intra-chromosomal) recombination and structural chromosomal aberrations [12]. The drugs are important agents that are used in chemotherapy, and topoisomerase-inhibiting drugs form the backbone of most chemotherapeutic strategies; however, like many other anti-cancer agents they are a 'double-edged sword', as they might cause mutations and cancer themselves [5]. Topoisomerase-interactive agents, like CPT and ETOP, bind to the cleavable complex formed between topoisomerase and DNA, and prevent it from reverting to the original DNA [19]. We have shown that, under similar experimental conditions, these drugs are capable of damaging the DNA of *D. melanogaster*, thus, both inhibitors are genotoxic, with CPT being more effective than ETOP.

Using the protocol described here, we were able to compare the recombinogenic and clastogenic efficiencies of these drugs on a quantitative basis. We found that the recombination frequencies in XX females were generally higher than those for ring-X-loss in XrX females. Both drugs induced LOH mainly by homologous mitotic recombination, thus the expected preponderance of interchanges is assumed to have occurred. Furthermore, CPT produced unequal sister-strand recombination and structural chromosomal aberrations. From these results, it appears that the improved w/w^+ system can simultaneously screen several processes and separate them from each other based on the DNA lesions that cause these effects [12]; thus, the genotoxic profile of chemical agents being studied can be determined [18].

Our results, suggesting that the induction of mitotic homologous recombination is the prevalent mechanism of action of CPT and ETOP, are in agreement with those previously obtained by other authors using the wing

somatic mutation and recombination test [20,21]. These findings are relevant as the drugs are topoisomerase poisons, and the structural and functional conservation of topoisomerases I and II are remarkable in eukaryotes [22,23]. DNA strand breakage and rejoining reactions catalyzed by DNA topoisomerases might be required for the formation of recombination intermediates [24].

The disruption of topoisomerase function once topocleaved DNA complexes are formed might have several different consequences, two of which might be associated with recombinogenicity: first, the blocking of DNA replication in one of the DNA strands by topoisomerase I and/or the attachment of topoisomerase II to both strands, leading to non-homologous recombination repair; and second, the unfavourable DNA topology related to the inhibition of topoisomerase function after replication, leading to the occurrence of homologous and non-homologous recombination events [25]. Our data are also in agreement with reports that suggest that topoisomerase-I-interactive agents might promote illegitimate, as well as, homologous recombination [5,26]. Thus, CPT, besides inducing large numbers of single-strand DNA breaks, is also able to induce double-strand damage [27–29], while topoisomerase-II-inhibitory agents enhance double-strand break levels [30] and bind exclusively to the enzymatic component of the cleavage complex [31]. The ultimate types of genetic damage induced by topoisomerase-interactive agents result from complex interactions of cell-cycle-specific variations in topoisomerase enzyme levels, the abilities of these drugs to interfere with the orderly DNA breakage/reunion associated with topoisomerase activity, and the processing of the damage resulting from these interactions [3].

The genetic changes induced by topoisomerase blockers in other bioassays were related to gene mutations and chromosome aberrations. In cytogenetic assays, CPT induced high frequencies of chromosomal aberrations and mutational processes, which mainly involved gene deletions [32,33]. ETOP was cytotoxic and induced micronuclei in male rat meiotic cells [7], and in in vivo and in vitro tests in mice [9,2,10]; however, it did not interfere with cell-cycle progression in mouse bone marrow [34].

In summary, the results obtained in this study showed that the genotoxic profiles of topoisomerase-interactive agents were markedly different. ETOP induced mainly recombinogenic events, while CPT promoted homologous and illegitimate recombination, as well as whole chromosome loss through clastogenicity. LOH is involved in the multi-step model of carcinogenesis [35]. Moreover, increases in homologous and illegitimate recombination, as well as in chromosomal aberrations and genome rearrangements, could act as the primary steps in carcinogenesis. Thus the *Drosophila* assay employed in this study seems suitable for determining the genotoxic profiles of anticancer agents. We therefore conclude that topoisomerase-interactive agents are potent inducers of genotoxicity, and that patients treated with these agents during cancer chemotherapy are at a risk of developing secondary malignancies after treatment.

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