ELSEVIER

Mutation Research 400308 (2001) 1–9

Genetic Toxicology and Environmental Mutagenesis

www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Testing the SCE mechanism with non-poisoning topoisomerase II inhibitors

Inmaculada Domínguez, Nuria Pastor, Santiago Mateos, Felipe Cortés*

Department of Cell Biology, Faculty of Biology, University of Sevilla, Avda. Reina Mercedes 6, 41012 Sevilla, Spain

Received 6 February 2001; received in revised form 29 May 2001; accepted 30 May 2001

Abstract

There are controversial theoretical models about a possible involvement of DNA topoisomerase II (topo II) in the molecular mechanism of sister chromatid exchanges (SCEs). In order to clarify the role of this enzyme, if any, in such recombinational event, CHO parental AA8 and mutant EM9 cells, which shows and extremely high baseline frequency of SCE, have been treated with different doses of the non-poisoning topoisomerase inhibitors, ICRF-193 and bufalin. The frequencies of SCEs after the treatments have been determined and the inhibitory effect of these compounds has been assessed using a topo II activity assay. The results indicate that ICRF-193 and bufalin effectively inhibit topo II activity in AA8 and EM9 cell lines. ICRF-193 induced a moderate increase in the frequency of SCEs in both types of cells, while bufalin did not modify the level of SCEs in any of them. The results are discussed taking into account the apparently unlike mechanisms of inhibition of topo II by ICRF-193 and bufalin. © 2001 Published by Elsevier Science B.V.

Keywords: SCEs; Topoisomerase II; ICRF-193; Bufalin

1. Introduction

DNA topoisomerases (topos) are conserved nuclear enzymes that catalyze a series of topological changes that take place in DNA during many fundamental metabolic processes such as replication, transcription and recombination. These changes depend basically upon the capacity of the enzymes to perform transient cleavage of DNA, strand passing and religation (for a review, see [1]). While topoisomerase I (topo I) (monomer) breaks and rejoins one DNA strand at a time, topo II (homodimer) is able to do so with the two strands that make up duplex DNA. As for the DNA substrates that they do resolve, both type I and II enzymes are proficient in relaxing supercoiled DNA in order to relieve torsional tension generated during replication and transcription, while only topo II can decatenate intertwined DNA molecules. This unique decatenating and unknotting activity of DNA topo II is essential to efficiently carry out segregation of daughter chromosomes after DNA replication [1].

Focusing on the possible role of topoisomerases in recombination, in all three types of recombination mechanisms, i.e. homologous conservative, homologous non-conservative and non-homologous (illegitimate), it is thought that the initial step is a double-strand break in one or both target sequences, and one obvious candidate for such an enzymatic activity, given its mechanism of action (see above), is topo II [2]. In a pioneer work, Ikeda et al. [3] first reported that bacterial gyrase (a prokaryotic topo II enzyme) directly participates in illegitimate recombi-

* Corresponding author. Tel.: +34-95-4557039;
fax: +34-95-4610261.
E-mail address: cortes@cica.es (F. Cortés).

1383-5718/01/$ – see front matter © 2001 Published by Elsevier Science B.V.
PII: S1383-5718(01)00241-8

nation in vitro. An association of the sites for recombination with the topoisomerase cleavage sites was further observed for bacteriophage T4 DNA topoisomerase [4]. Similarly, eukaryotic topo II has been shown to mediate illegitimate recombination in vitro [5,6]. As for the capacity of topo II to stimulate recombination, also in vivo, the addition of exogenous topo II resulted in an increase in the recombination frequency in mammalian cells [2].

Contrasting with this proposed role of topo II in promoting recombination, studies mainly carried out in yeast have shown results that seem to support that topoisomerases (both I and II) could also suppress recombination. Nitiss and Wang [7] reported that anti-tumor drugs camptothecin and mAMSA, that interfere with topo I and II, respectively induce high levels of homologous recombination. A strong suppression of mitotic recombination within the *Saccharomyces cerevisiae* rDNA cluster as a result of a combined action of DNA topo I and II has also been found [8].

Sister chromatid exchange (SCE) is the cytological manifestation of double-strand breakage of sister chromatids, at supposedly the same locus, and exchange and rejoining of the subunits [9]. This recombinational process, that can take place spontaneously to some extent but is highly sensitive to base damage in DNA, occurs through an as yet unknown molecular mechanism, though some favored models have proposed the possible participation of DNA topoisomerases [10–12].

The CHO mutant EM9 shows a defect in the repair of DNA strand breaks induced by either chemicals or ionizing radiation, and its main feature is an extremely high baseline SCE frequency compared to its parental line AA8 [13–15]. This extraordinarily high yield of SCEs in EM9 parallels that found in cells from the human hereditary disease Bloom's syndrome [16].

The classical topo II inhibitors exert their effects by stabilizing covalent complexes between topo II and DNA, the so-called "cleavable complex" thus "poisoning" the otherwise beneficial reaction and generating DNA double-strand breaks that lead to chromosome damage, SCE and cell death. In recent years, a diverse group of drugs has been reported which inhibit catalytic activity but, unlike the classical topo II poisons, do not stabilize cleavable complexes [17–21]. Thus, they are widely known as true "catalytic inhibitors". The bis-dioxopiperazine ICRF-193 is one of the topo II inhibitors that belongs to this "catalytic" type [19,20]. Bufalin, one of the components of the bufadienolides in the traditional Chinese medicine, has also been reported to inhibit topo II activity [22]. Although, its molecular mechanism of inhibition remains unclear, there is evidence that it is not a topo II poison [23].

With the aim of testing the hypothesis of a possible involvement of DNA topo II in the molecular mechanism leading to SCE, we have treated EM9 and AA8 Chinese hamster cultured cells with the topo II inhibitors ICRF-193 and bufalin. The frequencies of SCEs in EM9 and its parental cell line AA8 after treatment with these inhibitors has been assessed. The topo II inhibition assays were carried out in order to check the effects of these compounds on the catalytic activity of the enzyme.

2. Materials and methods

2.1. Culture conditions

The parental cell line AA8 and mutant EM9 were grown as monolayers in McCoy's 5A medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine and the antibiotics penicillin (50 U/ml) and streptomycin (50 μg/ml). Cells were grown in dark at 37°C in a 5% CO_2 atmosphere.

2.2. Cell treatments

Exponentially growing cells were cultured for two complete rounds of replication in a mixture made up of 1 μM 5-fluorodeoxyuridine (FdU, Sigma), 100 μM deoxycytidine (dC, Sigma) as well as bromodeoxyuridine (BrdU, Sigma) at 5 μM for AA8, and 1.25 μM for EM9. The topo II inhibitors were added to the cultures after the first round of replication (13 h for AA8 and 16 h for EM9) at concentrations ranging from 10^{-3} to 2.5×10^{-1} μM for ICRF-193 and from10^{-7} to 5×10^{-4} M for bufalin. Cell cultures which were treated with the halogenated mixture but did not receive any inhibitor treatment served as controls. The inhibitor treatments were given for 13 h in AA8 cells and 16 h in EM9 cells, that is one round of replication. The cultures were then washed and the medium plus the halogenated mixture was replaced. After 5 h

UNCORRECTED PROOF

of cell recovery, Colcemid (2×10^{-7} M) was added for the last 3 h of cell culture for metaphase arrest.

The flasks were shaken to dislodge the mitotic cells, which were collected by centrifugation, treated with 0.075 M KCl for 2 min, fixed in methanol:acetic acid (3:1) and dropped onto clean glass microscope slides. The slides were used for SCEs and chromosome aberrations (CAs) analysis. Two independent experiments were carried out for each inhibitor.

2.3. Analysis of SCEs and CAs

Differential staining of BrdU-substituted sister chromatids was obtained in one set of slides by the fluorescence-plus-Giemsa (FPG) method of Perry and Wolff [24] modified by Morgan et al. [25]. A number of 50 complete metaphases with well preserved chromosome morphology were scored for each treatment from two independent experiments.

Another set of slides was stained with 3% Giemsa in order to analyze CAs. A number of 100 metaphases were analyzed for each treatment from the two independent experiments.

2.4. Topoisomerase II activity assay

2.4.1. Preparation of nuclear extracts

Exponentially growing AA8 and EM9 cells were incubated for 22 h in the presence of different doses of the topo II inhibitors. ICRF-193 was added to the cultures at concentrations of 0.05–5 μM, while the doses used for bufalin were in the range of 10^{-6} to 5×10^{-4} M. After the treatment, the cells were processed to obtain extracts of nuclear proteins, while control (untreated) cells were also sampled in parallel for comparison. The procedure followed was basically that described by Heartlein et al. [26]. Approximately 10^7 cells were suspended in 1 ml of 0.32 M sucrose, 0.01 M Tris–HCl (pH 7.5), 0.05 M $MgCl_2$, 1% Triton X-100 and thoroughly vortexed to lyse the cells. Nuclear pellets were obtained by centrifugation at 2000 rpm (Eppendorf centrifuge), for 5 min at 4°C. Nuclei were then washed in 1 ml of nucleus wash buffer (5 mM potassium phosphate buffer, pH 7.5, 1 mM phenylmethyl sulfonyl fluoride (PMSF), 1 mM β-mercaptoethanol, and 0.5 mM dithiothreitol (DTT)). The nuclei were then pelleted as described above and resuspended in 50 μl of nucleus wash buffer, and 50 μl of 4 mM EDTA was added. Following incubation at 0°C for 15 min, the nuclei were lysed by adding 100 μl of 2 M NaCl, 20 mM Tris–HCl pH 7.5, 10 mM β-mercaptoethanol, 1 mM PMSF. Following a 15 min incubation at 0°C, 50 μl of 18% polyethylene glycol (PEG-6000) in 1 M NaCl, 50 mM Tris–HCl pH 7.5, 10 mM β-mercaptoethanol, and 1 mM PMSF was added. The suspension was incubated for a further 40 min period at 0°C. The supernatant from a 30 min centrifugation at 12 500 rpm at 4°C was then collected. Total protein concentration in each extract was determined in a Beckman DU-64 spectrophotometer by the Bradford [27] protein assay (Bio-Rad Laboratories) and extracts were kept at −80°C for no longer than a month.

2.4.2. Topoisomerase II activity in nuclear extracts

The topo II activity in nuclear extracts was assayed using TopoGen (Columbus, OH, USA) assay kits based upon decatenation of kinetoplast DNA (kDNA). The amount of nuclear extract protein from the different cell lines used in each assay was 100 ng. Reaction products were resolved using agarose gel electrophoresis of DNA. After 40 min incubation at 37°C the samples were loaded onto 1% agarose gels and subjected to electrophoresis for 2.5 h at 100 V. Finally, gels were stained with 0.5 μg/ml ethidium bromide, destained (30 min) in distilled water and photographed using a standard photodyne set.

3. Results

Fig. 1A shows the decatenation activity, assessed by the decatenation assay using kinetoplast DNA (kDNA) as a substrate, in nuclear extracts in AA8 and EM9 cell lines after treatments with different doses of the topo II catalytic inhibitor ICRF-193. As can be seen, topo II activity was clearly reduced in AA8 when this compound was used at 0.1 μM or higher. Nevertheless, in EM9 the inhibition of topo II activity was only clear when the dose of ICRF-193 was as high as 5 μM.

EM9 is highly sensitive to BrdU and has an extremely elevated frequency of SCEs [13–15] compared with its parental line AA8. In order to reduce the level of SCEs induced by BrdU alone thus making possible a more accurate scoring, a low dose of 1.25 μM BrdU was used in this mutant cell line, while

UNCORRECTED PROOF

% Decatenated DNA

	[BUFALIN]				
	Control	10^{-6} M	10^{-5} M	10^{-4} M	5×10^{-4} M
AA8	100%	84,69%	76,11%	21,40%	
EM9	100%		98,24%	52,03%	25,86%

ICRF-193					
Control	0,05µM	0,1µM	1µM	2µM	5µM
100%	87,33%	45,85%	36,81%		
100%			98,12%	89,21%	64,50%

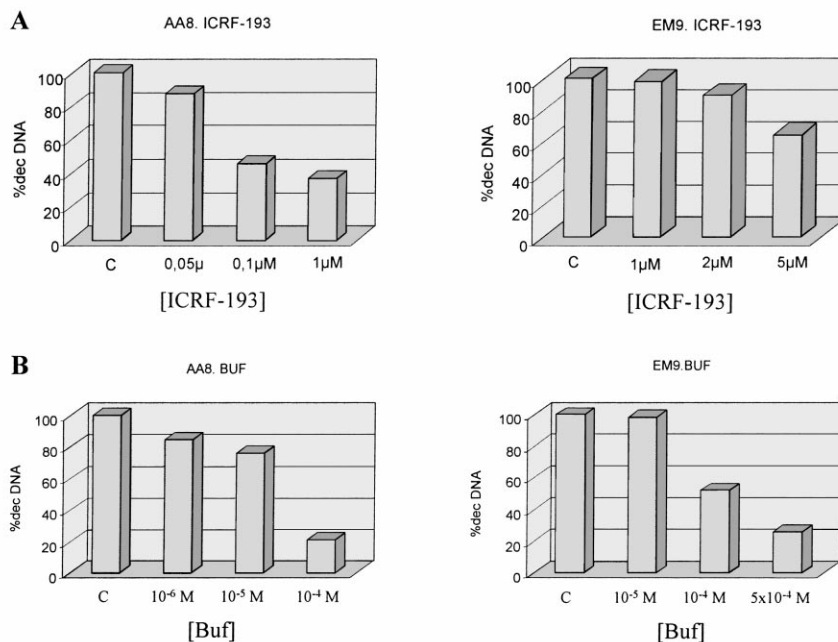

Fig. 1. Percentage of decatenated DNA, that shows the catalytic activity of topo II, in AA8 and EM9 cells treated with: (A) different doses of the enzyme catalytic inhibitor ICRF-193; and (B) different doses of bufalin.

the dose chosen for the parental line AA8 was 5 µM BrdU.

Table 1 shows the frequencies of SCEs induced by different doses of ICRF-193 in AA8 and EM9 cell lines, respectively. The results indicate that ICRF-193 did not clearly modify the frequency of SCEs in AA8 cells for inhibitor concentrations up to 10^{-2} µM, while a detectable enhancement in the frequency of SCEs in cells treated with non-cytotoxic higher doses of ICRF-193 was observed. The percentage of second and third mitosis was also analyzed for all the treatments so that any delay in the cell cycle could be detected and the results are shown in Table 1 as well. For all the doses tested up to 10^{-1} µM, the percentage of second and/or third mitosis was about 90%. Contrasting with this, the highest dose used, 2.5×10^{-1} µM ICRF-193, was shown as cytotoxic and to produce such a delay in the cell cycle that no proper scoring could be done.

As can be seen in Table 1, ICRF-193 also induced an increase in the frequency of SCEs in EM9 cell line as compared with that observed in cells treated with BrdU alone. Nevertheless, this increase was more moderated than that obtained in the parental cell line. The percentage of second mitosis, that is indicated in Table 1 as well, was about 90% for all the doses up to 5×10^{-2} µM of ICRF-193. The dose of 10^{-1} µM reduced this value to 65% and scoring was not possible due to bad morphology of chromosomes; no mitosis were obtained at the dose of 2.5×10^{-1} µM.

Table 1
Effect of different doses of ICRF-193 on the induction of SCEs and CAs in AA8 and EM9 cell lines[a]

Cell line	BrdU (μM)	ICRF-193 (μM)	SCEs per metaphase ± S.E.	CAs (%)	Proliferation rate[b]	
					Second mitosis (%)	Third mitosis (%)
AA8	5	–	7.84 ± 0.78	8.67	56	44
	5	10^{-3}	10.76 ± 0.91	9.15	67	33
	5	5×10^{-3}	9.42 ± 0.85	32.22	72	28
	5	10^{-2}	10.06 ± 0.88	34	72	27
	5	5×10^{-2}	12.24 ± 0.97	141.5	90	7
	5	10^{-1}	13.88 ± 1.03	80.5	93	0
	5	2.5×10^{-1}	nm[c]	–	–	
EM9	1.25	–	51.84 ± 1.99	21.33	99	0
	1.25	10^{-3}	58.24 ± 2.11	17.8	99	0
	1.25	5×10^{-3}	58.96 ± 2.13	43.15	94	0
	1.25	10^{-2}	57.52 ± 2.10	63.2	100	0
	1.25	5×10^{-2}	59.70 ± 2.14	187.03	86	0
	1.25	10^{-1}	–	–	65[d]	0
	1.25	2.5×10^{-1}	nm	–	–	

[a] A number of 100 metaphases were scored in each of two independent experiments and the mean of the values is shown.
[b] Second mitosis (%): chromosome staining pattern corresponding to cells with two rounds of replication; and third mitosis (%): cells with at least some chromosomes stained according to a pattern corresponding to more than two rounds of replication.
[c] nm: no mitosis.
[d] SCE scoring was not possible due to the bad morphology of chromosomes.

Table 2
Effect of different doses of bufalin on the induction of SCEs and CAs in AA8 and EM9 cell lines[a]

Cell line	BrdU (μM)	Bufalin (M)	SCEs per metaphase ± S.E.	CAs (%)	Proliferation rate[b]	
					Second mitosis (%)	Third mitosis (%)
AA8	5	–	9.16 ± 0.84	9	32	68
	5	10^{-7}	10.94 ± 0.92	8.5	20	80
	5	10^{-6}	11.48 ± 0.94	11	32	67
	5	10^{-5}	10.82 ± 0.91	11.24	23	75
	5	5×10^{-5}	10.32 ± 0.89	7.2	48	50
	5	10^{-4}	9.56 ± 0.86	6.8	99	0
	5	5×10^{-4}	–	–	2	0
EM9	1.25	–	52.8 ± 2.01	19.2	96	0
	1.25	10^{-7}	54.26 ± 2.04	22.4	95	0
	1.25	10^{-6}	51.46 ± 1.99	22.6	95	0
	1.25	10^{-5}	60.36 ± 2.15	26.78	99	0
	1.25	5×10^{-5}	55.80 ± 2.07	21.91	96	0
	1.25	10^{-4}	51.36 ± 1.99	20.45	87	0
	1.25	5×10^{-4}	nm[c]		–	

[a] A number of 100 metaphases were scored in each of two independent experiments and the mean of the values is shown.
[b] Second mitosis (%): chromosome staining pattern corresponding to cells with two rounds of replication; and third mitosis (%): cells with at least some chromosomes stained according to a pattern corresponding to more than two rounds of replication.
[c] nm: no mitosis.

UNCORRECTED PROOF

The frequencies of chromosome aberrations (see Table 1), on the other hand, indicated that ICRF-193 induced a significant level of damage in AA8 at concentrations of 5×10^{-3} μM or higher, while in EM9 this clastogenic effect was observed at concentrations of 10^{-2} μM or higher.

As previously shown for ICRF-193 (Fig. 1A), in AA8 bufalin caused a partial inhibition of topo II activity at the dose of 10^{-5} M (Fig. 1B) while the inhibition was more evident for the dose of 10^{-4} M. In EM9 cell line, on the other hand, a partial inhibition of topo II activity was demonstrated when bufalin treatment had been given at the dose of 10^{-4} M, whereas the inhibition was total when the dose used was 5×10^{-4} M.

Table 2 shows the results on the frequency of SCEs in AA8 and EM9 cell lines treated with BrdU plus different doses of the anti-topoisomerase agent bufalin. As can be seen, bufalin did not produce any significant modification in the frequency of SCEs induced by BrdU in AA8 and EM9 cell lines for all the doses tested up to 10^{-4} M, that turned out to be the highest non-cytotoxic. The results on cell cycle progression are also presented in Table 2, and indicate that about 90% of the cells had completed two rounds of replication in all the treatments up to 10^{-4} M of bufalin. When 5×10^{-4} M was used, no result could be obtained due to the low number of metaphases found in EM9, and the low percentage of metaphases in their second mitosis found in AA8 (2%).

Chromosome aberrations were also analyzed for this inhibitor. Contrasting with that found for ICRF-193 (see above) the observation was that bufalin did not induce chromosome aberrations at any of the doses tested (see Table 2).

4. Discussion

Although, SCE is a cytogenetic end-point now known for over 30 years, many features of its molecular mechanism still remain to be fully elucidated. As they occur during S, it is generally believed that SCE is a recombinational process that represents the interchange of DNA replication products at apparently homologous loci, involving DNA breakage and reunion [9,28]. There are two major models to explain SCE. According to the first model, SCE is mediated by homologous recombination [10,11,29]. The second model proposes that, topo II causes transient double-stranded DNA breaks during replication, and the proximity of DNA breaks on sister chromatids may result in incorrect rejoining, causing an SCE [12,26,30,31]. Concerning the second model, there are many reports which support that DNA topoisomerases are involved in recombination [32,33]. Such a possible role of topoisomerases in the SCE mechanism is based upon the ability of these nuclear enzymes to carry out a concerted breakage and rejoining of DNA [10–12,31,34].

In order to study the still open question of a possible involvement of topo II activity in the formation of SCEs, we have examined the effect of the topo II inhibitors ICRF-193 and bufalin on the production of SCEs in the CHO mutant EM9, which shows an extremely elevated baseline frequency of SCE after BrdU incorporation in DNA, and its parental line AA8. Both ICRF-193 and bufalin act on topo II activity without forming any cleavable complex [17,23].

ICRF-193 and related dioxopiperazines catalytically inhibit mammalian DNA topo II [17,18]. ICRF-193 stabilizes the closed clamp-form of the enzyme on DNA as a post-passage complex by inhibiting the intrinsic ATPase activity of the topo II, sequestering the enzyme from its normal turnover [19].

We have found that the treatment with ICRF-193 induced a moderate increase in the level of SCEs in the cell line AA8, while the induction of SCE was only slight for the mutant cell line. In good agreement with our results, it has been shown earlier that ICRF-193 only slightly elevated the frequency of SCEs in MR-6 cells [35]. The effect of ICRF-193 on the induction of chromosome damage has been recently reported. Ikushima et al. [36] showed that ICRF-193 causes both chromatid- and chromosome-type aberrations in Chinese V79 cells. Our results also indicated that ICRF-193 induced chromosome aberrations in both cell lines.

It has been suggested that SCE reflect a DNA repair process [31,34] and that it is intimately associated with DNA replication. In our opinion, the well established mechanism by which ICRF-193 inhibits topo II activity [19] could provide an explanation for our results. ICRF-193 produces stabilization of the closed-clamp form of the enzyme, which could represent an obstacle for the progression of the replication fork. A num-

UNCORRECTED PROOF

ber of recent reports point to the importance of replication fork arrest for DNA double-strand breaks [37] and recombination [38,39]. The closed-clamp structure, similarly to a bulky lesion, could recruit recombinatory repair enzymes which in turn would induce DNA breaks leading to the induction of chromosome aberrations and SCE. It is noteworthy that, according to recent data, SCE are mediated by homologous recombination in vertebrate cells [29].

The mechanism by which bufalin exerts its action against topo II, on the other hand, is not yet completely understood, but it has been demonstrated that it can induce a decrease in the level of ARNm for topo IIα that in turn leads to a decrease in the amount and activity of topo IIα [23].

Our results show that, bufalin was unable to modify the frequencies of SCEs in both, AA8 and EM9 cell lines. Since, bufalin causes a drastic decrease in topo II activity, this absence of any effect on the yield of SCE seems to indicate that this enzyme activity has not a direct role in the formation of SCEs, so contrasting with that proposed earlier by different authors in their models to explain the molecular mechanism of this recombinational event [10,11,31]. Nevertheless, in our opinion, caution has to be taken before reaching any conclusion on this controversial subject. A possible way by which bufalin could cause the decrease in the activity of the nuclear enzyme has been proposed. Hashimoto et al. [23] suggested that topo IIα in HL-60 cells that had been treated with bufalin might undergo post-translational modification, such as ubiquitination, and the modified topo IIα might be then easily degraded. Concerning the localization of topo II, however, it has been shown that matrix association regions (MARs) contain multiple topo II cleavage sites and it has been hypothesized that topo II could mediate recombination at these sites [40]. It is well known that protein ubiquitination occurs when a certain signal of the protein is shown, so that its conformation must be different in some way to that of the same protein that is not going to be ubiquitinated. It could, then, be proposed that maybe the conformation of topo II that is associated to MARs might be different from that of the enzyme which is not associated to DNA. While this is highly speculative, it could be proposed that bufalin might somehow induce ubiquitination specifically on topo II that is not associated to DNA. On this basis, bufalin would not be affecting the topoisomerase activity associated to DNA, thus maybe explaining the lack of any effect on SCEs. According to this explanation, in spite of our observations on a lack of effect of bufalin, it cannot be conclusively ruled out a possible role of topo II in the formation of SCEs.

While there are still many unanswered questions concerning the molecular mechanism of SCE, the role of other enzymes in the formation of SCEs has been demonstrated. Recently, it has been shown that DNA helicase activity of Sgs1 is required for suppression of SCE in yeast [41]. It has also been shown that RecA mediates homologous recombination between sister chromatids during S-phase in transformed plants expressing this protein [42]. In the same way, as stated above, there are evidences that eukaryotic homologues of RecA are involved in SCE formation in vertebrate cells [29].

Acknowledgements

We thank M.A. Ledesma for expert technical assistance. This work was supported by grants from Junta de Andalucía (P.A.I. CVI120) and the Spanish Ministry of Science and Technology (SAF 2000-0167).

References

[1] J.C. Wang, DNA topoisomerases, Annu. Rev. Biochem. 65 (1996) 635–692.
[2] P. Chartrand, DNA recombination in mammalian cells: potential role of topoisomerases, in: DNA Topoisomerases in Cancer, Oxford University Press, London, 1991, pp. 240–245.
[3] H. Ikeda, K. Aoki, A. Naito, Illegitimate recombination mediated in vitro by DNA gyrase of *Escherichia coli*: structure of recombinant DNA molecules, Proc. Natl. Acad. Sci. U.S.A. 79 (1982) 3724–3728.
[4] M. Chiba, H. Shimizu, A. Fujimoto, H. Nashimoto, H. Ikeda, Common sites for recombination and cleavage mediated by bacteriophage T4 DNA topoisomerase in vitro, J. Biol. Chem. 264 (1989) 12785–12790.
[5] Y.S. Bae, I. Kawasaki, H. Ikeda, L.F. Liu, Illegitimate recombination mediated by calf thymus DNA topoisomerase II in vitro, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 2076–2080.
[6] K.C. Gale, N. Osheroff, Intrinsic intermolecular DNA ligation activity of eukaryotic topoisomerase II. Potential roles in recombination, J. Biol. Chem. 267 (1992) 12090–12097.
[7] J. Nitiss, J.C. Wang, DNA topoisomerase-targeting anti-tumor drugs can be studied in yeast, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 7501–7505.

UNCORRECTED PROOF

[8] M.F. Christman, F.S. Dietrich, R.F. Gerald, Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II, Cell 55 (1988) 413–425.

[9] S. Wolff, Sister Chromatid Exchange, Wiley, New York, 1982.

[10] J.E. Cleaver, Correlations between sister chromatid exchange frequencies and replicon sizes, a model for the mechanism of SCE production, Exp. Cell Res. 136 (1981) 27–39.

[11] R.B. Painter, A replication model for sister chromatid exchange, Mutat. Res. 70 (1980) 337–341.

[12] Y. Pommier, L.A. Zwelling, C.S. Kao-Shan, J. Whang-Peng, M.O. Bradley, Correlations between intercalator-induced DNA strand breaks and sister chromatid exchanges, mutations, and cytotoxicity in Chinese hamster cells, Cancer Res. 45 (1985) 3143–3149.

[13] L.H. Thompson, K.W. Brookman, L.E. Dillehay, A.V. Carrano, J.A. Mazrimas, C.L. Mooney, J.L. Minkler, A CHO-cell strain having hypersensitivity to mutagens, a defect in DNA strand-break repair, and a extraordinary baseline frequency of sister chromatid exchange, Mutat. Res. 95 (1982) 427–440.

[14] F. Cortés, J. Piñero, F. Palitti, Cytogenetics effects of inhibition of topoisomerase I and II activities in the CHO mutant EM9 and its parental line AA8, Mutat. Res. 288 (1993) 281–289.

[15] J. Piñero, F. Cortés, Abnormally high incidence of SCE in three successive cell cycles in the CHO mutant EM9 as detected by a three-way immunoperoxidase differential staining, Mutat. Res. 292 (1993) 205–211.

[16] R.S.K. Chaganti, S. Schonberg, J. German, A manifold increase in sister chromatid exchanges in Bloom's syndrome lymphocytes, Proc. Natl. Acad. Sci. U.S.A. 71 (1974) 4508–4512.

[17] K. Tanabe, Y. Ikegami, R. Ishida, T. Andoh, Inhibition of topoisomerase II by anti-tumor agents bis(2,6-dioxopiperazine) derivatives, Cancer Res. 51 (1991) 4903–4908.

[18] R. Ishida, T. Miki, T. Narita, R. Yui, M. Sato, K.R. Utsumi, K. Tanabe, T. Andoh, Inhibition of intracellular topoisomerase II by anti-tumor bis-(2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors, Cancer Res. 51 (1991) 4909–4916.

[19] J. Roca, R. Ishida, J.M. Berger, T. Andoh, J.C. Wang, Anti-tumor bis-dioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 1781–1785.

[20] T. Andoh, R. Ishida, Catalytic inhibitors of DNA topoisomerase II, Biochim. Biophys. Acta 1400 (1998) 155–171.

[21] D.A. Burden, N. Osheroff, Mechanism of action of eukaryotic topoisomerase II and drugs targeted to the enzyme, Biochim. Biophys. Acta 1400 (1998) 139–154.

[22] M. Watabe, S. Nakajo, T. Yoshida, Y. Kuroiwa, K. Nakaya, Treatment of U937 cells with bufalin induces the translocation of casein kinase 2 and modulates the activity of topoisomerase II prior to the induction of apoptosis, Cell Growth Differentiation 8 (1997) 871–879.

[23] S. Hashimoto, Y. Jing, N. Kawazoe, Y. Masuda, S. Nakajo, T. Yoshida, Y. Kuroiwa, K. Nakaya, Bufalin reduces the level of topoisomerase II in human leukemia cells and affects the cytotoxicity of anti-cancer drugs, Leukemia Res. 21 (1997) 875–883.

[24] P. Perry, S. Wolff, New Giemsa method for the differential staining of sister chromatids, Nature 251 (1974) 156–158.

[25] W.F. Morgan, J.L. Schwartz, J.P. Murnane, S. Wolff, Effect of 3-aminobenzamide on sister chromatid exchange frequency in X-irradiated cells, Radiat. Res. 93 (1983) 567–571.

[26] M.W. Heartlein, H. Tsuji, S.A. Latt, 5-Bromodeoxyuridine-dependent increase in sister chromatid exchange formation in Bloom's syndrome is associated with reduction in topoisomerase II activity, Exp. Cell Res. 169 (1987) 245–254.

[27] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248–254.

[28] S.A. Latt, J. Allen, S.E. Bloom, A. Carrano, E. Falke, D. Kram, E. Schneider, R. Schreck, R. Tice, B. Whitfield, S. Wolff, Sister chromatid exchanges: a report of the Gene-Tox Program, Mutat. Res. 87 (1981) 17–62.

[29] E. Sonoda, M.S. Sasaki, C. Morrison, Y. Yamguchi-Iwai, M. Takata, S. Takeda, Sister chromatid exchanges are mediated by homologous recombination in vertebrate cells, Mol. Cell Biol. 19 (1999) 5166–5169.

[30] Y. Ishii, A. Bender, Effects of inhibitors of DNA synthesis on spontaneous and ultraviolet light-induced sister chromatid exchanges in Chinese hamster cells, Mutat. Res. 79 (1980) 19–32.

[31] L.E. Dillehay, D. Jacobson-Kram, J.R. Williams, DNA topoisomerases and models of sister chromatid exchange, Mutat. Res. 215 (1989) 15–23.

[32] J.C. Wang, P.R. Caron, R.A. Kim, The role of DNA topoisomerases in recombination and genome stability: a double-edged sword? Cell 62 (1990) 403–406.

[33] J.W. Wallis, G. Chrebet, G. Brodsky, M. Rolfe, R. Rothstein, A hyper-recombination mutation in *S. cerevisiae* identifies a novel eukaryotic topoisomerase, Cell 58 (1989) 409–419.

[34] L.F. Liu, C.C. Liu, B.M. Alberts, Type II DNA topoisomerases. Enzymes that can unknot a topologically knotted DNA molecule via a reversible double strand break, Cell 19 (1980) 697–707.

[35] M. Hamatake, T. Andoh, R. Ishida, Effects of ICRF-193, a catalytic inhibitor of DNA topoisomerase II, on sister chromatid exchange, Anti-Cancer Drugs 8 (1997) 637–642.

[36] T. Ikushima, Y. Shima, Y. Ishii, Effects of an inhibitor of topoisomerase II, ICRF-193 on the formation of ultraviolet-induced chromosomal aberrations, Mutat. Res. 404 (1998) 35–38.

[37] B. Michel, S.D. Ehrlich, M. Uzest, DNA double-strand breaks caused by replication arrest, EMBO J. 16 (1997) 430–438.

[38] B. Michel, Replication fork arrest and DNA recombination, TIBS 25 (2000) 173–178.

[39] G. Hong, K.N. Kreuzer, An anti-tumor drug-induced topoisomerase cleavage complex blocks a bacteriophage T4 replication fork in vivo, Mol. Cell Biol. 20 (2000) 594–603.

UNCORRECTED PROOF

563 [40] A.O. Sperry, V.C. Blasquez, W.T. Garrard, Disfunction chromosomal loop attachment sites: illegitimate recombination linked to matrix association regions and topoisomerase II, Proc. Natl. Acad. Sci. U.S.A. 86 (1989) 5497–5501.
564
565
566
567
568 [41] F. Onoda, M. Seki, A. Miyajima, T. Enomoto, Elevation of sister chromatid exchange in *Saccharomyces cerevisiae sgs I* disruptants and the relevance of the disruptants as a system to evaluate mutations in Bloom's syndrome gene, Mutat. Res. 459 (2000) 203–209.
569
570
571
572
573
574 [42] B. Reiss, I. Schubert, K. Köpchen, E. Wendeler, J. Schell, H. Puchta, RecA stimulates sister chromatid exchange and the fidelity of double-strand break repair, but not gene targeting, in plants transformed by *Agrobacterium*, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 3358–3363.
575
576
577
578
579

UNCORRECTED PROOF