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Anti-topoisomerase drugs as potent inducers of chromosomal aberrations*

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Abstract

DNA topoisomerases catalyze topological changes in DNA that are essential for normal cell cycle progression and therefore they are a preferential target for the development of anticancer drugs. Anti-topoisomerase drugs can be divided into two main classes: "cleavable complex" poisons and catalytic inhibitors. The "cleavable complex" poisons are very effective as anticancer drugs but are also potent inducers of chromosome aberrations so they can cause secondary malignancies. Catalytic inhibitors are cytotoxic but they do not induce chromosome aberrations. Knowledge about the mechanism of action of topoisomerase inhibitors is important to determine the best anti-topoisomerase combinations, with a reduced risk of induction of secondary malignancies.

INTRODUCTION

The induction of chromosomal aberrations is the result of an extremely complex series of biochemical events depending upon the peculiar mechanism of action of mutagen agents and the cellular metabolism. In fact, chromosomal aberrations are believed to be the consequence of DNA lesions induced by chemical and physical mutagens that when misrepaired or misreplicated can lead to the formation of chromosomal aberrations (Evans, 1968; Kihlman, 1971; Bender *et al.*, 1974; Natarajan *et al.*, 1986).

Direct and indirect evidence suggests that DNA is the main target of mutagenic agents responsible for the induction of chromosomal aberrations. However, protein-DNA cross links and the inhibition of protein and RNA synthesis may also have clastogenic consequences through an indirect mechanism of action.

Anti-topoisomerase (Topo) drugs, distinguishable in "cleavable complex" trappers and catalytic inhibitors, are an example of potentially genotoxic agents with an indirect mechanism of action. This class of drugs comprises very effective, though toxic, compounds for cancer chemotherapy. Moreover, the "cleavable complex" trappers are also potent inducers of chromosomal aberrations.

DNA TOPOISOMERASES

DNA topoisomerases are ubiquitous enzymes playing an essential role in maintaining the integrity of the DNA molecule. In fact, the extraordinary length of DNA and its double helical structure represent serious problems during the processes of transcription, replication, recombination and chromatid segregation (Wang, 1985; D'Arpa and Liu, 1989). DNA topoisomerases act by opening transient, protein-bridged, single- or double-stranded breaks through which the DNA strand can be passed in order to solve the topological problems and to relieve the torsion stress accumulated in all cellular transactions of the DNA molecule.

In regard to their ability to cleave single- or double-strand DNA molecules these enzymes have been classified as type I and type II DNA topoisomerases (Topo I and II), respectively. Moreover, on the basis of common enzymatic properties and protein sequence analysis, DNA-topoisomerases can be classified into three evolutionary independent families: type I-5', type I-3' and type II (Caron and Wang, 1994).

Type I-5' topoisomerases

Type I-5' topoisomerases preferentially bind single-strand DNA, introduce a transient gap and catalyze a partial relaxation of negatively supercoiled DNA. In the DNA-cleavage stage a protein-DNA covalent intermediate is formed between a tyrosyl residue and the 5'-phosphate at the DNA break site. The type I-5' topoisomerases act as monomers and are ATP independent so each reaction proceeds in the direction which gives a decrease of the free energy of the involved DNA segment.

Type I-3' topoisomerases

Type I-3' topoisomerases are monomeric and ATP-independent enzymes. They bind preferentially to double-stranded DNA and cut only one of the DNA strands, then

they form a protein-DNA covalent intermediate between a tyrosyl residue and the 3'-phosphate at the break site. The type I-3' topoisomerases can completely relax both overwound and underwound DNA duplexes.

The main prototype of this family is the eukaryotic DNA Topo I. This enzyme recognizes specific consensus sequences (Thomsen *et al.*, 1987; Camilloni *et al.*, 1991; Bugrew *et al.*, 1997) and binds double-stranded DNA covering a region of about 20 bp. Eukaryotic DNA Topo I binds preferentially to supercoiled DNA.

Type II topoisomerases

Type II topoisomerases are highly conserved proteins working as dimeric and ATP-dependent enzymes. In eukaryotes DNA Topo II are homodimeric proteins while in prokaryotes and phages they consist of heterodymeric structures. The prokaryotic DNA Topo II are defined DNA-gyrases: they are the only type of topoisomerases capable of introducing negative supercoiling in DNA coupled to ATP hydrolysis.

Type II topoisomerases have preferential cleavage sites (Spitzner and Muller, 1988; Spitzner *et al.*, 1989). They bind to a duplex DNA segment and cleave DNA with a 4-bp stagger, a protruding 5' end and a 3' recessed end with free hydroxyl groups (Morrison and Cozzarelli, 1979; Liu *et al.*, 1983; Sander and Hsieh, 1983). The two resulting free 5'phosphoryl groups are covalently linked to a pair of tyrosyl groups, one in each half of the enzyme. Moreover, free rotation of the 3' ends of DNA at the cleaved sites is avoided by additional interactions between DNA and the Topo II molecule. A second duplex DNA helix is transported through this transient open gate producing topological interconversion of DNA molecules leading to elimination of DNA supercoils, to formation and resolution of catenanes and knotting or unknotting of circular DNA (Gellert *et al.*, 1983; Liu *et al.*, 1983). A further and essential role of Topo II is to contribute to regulate the three-dimensional organization of DNA in interphase, mitotic and meiotic chromosomes; in fact Topo II are fundamental components of nuclear matrix and scaffold (Gasser *et al.*, 1986; Heck and Earnshaw, 1986).

DNA TOPOISOMERASE INHIBITORS

DNA Topo I and II catalyze topological changes in DNA that are essential for normal cell cycle progression and, therefore, this class of enzymes represents a preferential target for the development of anticancer drugs (Corbett and Osheroff, 1993; Chen and Liu, 1994). A summary of agents which have anti-topoisomerase effects is given in Table I.

Topoisomerases-targeting drugs can be classified into two main classes:

1) "cleavable complex" poisons and 2) catalytic inhibitors.

"Cleavable complex" poisons

This class of inhibitors includes some compounds which act by trapping the intermediate of the reaction catalyzed by Topo I or II, the so-called "cleavable complex", and inhibit the resealing of DNA breaks introduced physiologically by the enzymes (Hsiang *et al.*, 1985; Tewey *et al.*, 1985). As a consequence of such a stabilization of DNA cleavage sites, topoisomerase poisons can induce chromosomal abnormalities (Negrini *et al.*, 1993; Shibaya *et al.*, 1994) and therapy-related secondary malignancies (Ratain and Rowley, 1992; Harousseau, 1999).

The best known inhibitors of Topo I are camptothecin (CPT) and its derivatives. Among Topo II-targeting drugs we can find some intercalative drugs such as acridines, actinomycins, anthracenediones, anthracyclines, ellipticines and some non-intercalative drugs such as epipodophyllotoxins and isoflavonoids (Table I) (D'Arpa and Liu, 1989).

Catalytic inhibitors

The second class of topoisomerase inhibitors is composed of catalytic inhibitors which do not trap the "cleavable complex" but act as inhibitors of enzyme catalytic activity (Boritzki *et al.*, 1988; Tanabe *et al.*, 1991). Among these agents the most studied are the bisdioxopiperazines (ICRF-159 and ICRF-193) which inhibit DNA Topo II by trapping the enzyme in the form of a closed protein clamp (Roca and Wang, 1992).

EFFECTS OF TOPO II AND I "CLEAVABLE COMPLEX" TRAPPING

Inhibitors of DNA topoisomerase which act by trapping "cleavable complexes" (Ross, 1985) give rise to DNA SSB and DSB. DNA DSB is considered the ultimate lesion leading to chromosomal aberrations (Natarajan and Obe, 1978; Bryant, 1984). Consequently, as expected, treatments with Topo II inhibitors, depending on the phase of the cell cycle in which they are performed, induce chromosome-type aberrations (G_0 or G_1 treatment) or chromatid-type aberrations (S or G_2 treatment) (Andersson and Kihlman, 1989; Palitti *et al.*, 1990, 1994). Therefore, apparently they could be considered to have a mechanism of induction of chromosomal damage similar to X-rays, i.e., an S-independent mechanism: the DNA lesions can give rise to chromosomal aberrations without intervening DNA synthesis. Topo II inhibitors are also able to induce sister chromatid exchanges (SCE), provided that the treatment is performed in the S phase of the cell cycle (Dillehay *et al.*, 1983; Andersons and Kihlman, 1989; Palitti *et al.*, 1990). This type of effect resembles that obtained with restriction endonucleases (Natarajan *et al.*, 1985) pointing out that DNA DSB induced in the S phase of the cell cycle is able to induce SCE. The induction of chromosomal damage by these agents has been attributed

Table I - Anti-topoisomerase drugs.

Drug class	Example	Topoisomerase inhibited	Effects	References
Acridines	*Amsacrina (m-AMSA)*	II	stabilize cleavable complex	Louie and Issel (1985)
Actinomycins	*Actinomycin D*	main effect on Topo II but inhibits also Topo I	stabilize cleavable complex	
Anthracenediones	*Mitoxantrone*	II	stabilize cleavable complex	Harker *et al.* (1991)
Anthracyclines	*Doxorubicin*	II	stabilize cleavable complex	Au *et al.* (1981)
Ellipticines	*2-methyl-9-OH-ellipticinicum acetate*	II	stabilize cleavable complex	
Coumarine	*Novobiocin*	bacterial gyrase (sub B)	interferes with ATPase activity of Topo II	Sugino *et al.* (1978)
Isoflavonoids	*Genistein*	II	PTK inhibitor and cleavable-complex blocker	Markovits *et al.* (1989) Adlerkreutz (1995)
	Quercetin	I and II	PTK inhibitor	Boege *et al.* (1996)
Benzophenazine	*NC190*	main effect on Topo II but inhibits also Topo I	stabilize cleavable complex	Yamagishi *et al.* (1996)
Epipodophyllotoxins	*Etoposide, Teniposide*	II	stabilize cleavable complex	Ross *et al.* (1984)
	F 11782	I and II	inhibits Topo I and II binding to DNA	Perrin *et al.* (2000)
Quinolones	*Nalidixic acid, ciprofloxocin, oxolinic acid*	bacterial gyrase (sub A)	stabilize cleavable complex	Bredberg *et al.* (1989)
Alkaloid	*Camptothecin and its derivatives*	I	stabilize cleavable complex	Hsiang *et al.* (1985)
Indolocarbazole	*NB-506*	I	stabilize cleavable complex	Bailly *et al.* (1999)
	J-107088	I	stabilize cleavable complex	Yoshinari *et al.* (1999)
bis-piperazinediones	*ICRF-159, 193*	II	inhibits DNA relaxation and cleavable complex formation	Jensen *et al.* (2000)
Anthracenyl peptides	*Merbarone*	II	inhibits cleavable complex formation	Khelifa and Beck (1999)

to the trapping of the "cleavable complex" for a period of time, otherwise no DNA damage would be formed.

While there are several inhibitors of DNA Topo II, only CPT and its derivatives are known, for sure, to inhibit Topo I (D'Arpa and Liu, 1989). The trapping of the "cleavable complex" DNA Topo I by CPT gives rise only to DNA SSB. This type of lesion is not expected to result in chromosomal aberrations.

It has been found that CPT gives rise to chromatid aberrations only when the drug is present in the S or G_2 phases of the cell cycle, while G_1 treatment has no effect (Degrassi *et al.*, 1989). A high induction of SCE is yielded, provided that the CPT treatment is performed during the S phase. It has been proposed that the induction of chromatid-type aberrations in the S-phase is a consequence of the collision of the trapped "cleavable complex" with the replication fork, resulting in replication arrest and fork breakage with the production of DNA DSB (Hsiang *et al.*, 1985; D'Arpa and Liu, 1989). The induction of chromatid aberrations in G_2 phase-CPT-treated cells, found by several investigators (Degrassi *et al.*, 1989; Andersson and Kihlman, 1992; Palitti *et al.*, 1993; Palitti, 1993), has been attributed to a residual DNA synthesis still present in the G_2 phase or to chromatin condensation. Bassi *et al.* (1998) investigated the localization of CPT-induced chromosome breakpoints in the G_2 phase of a primary Chinese hamster cell line between euchromatic and heterochromatic regions of chromosomes. The results obtained indicated that CPT-induced breakpoints were not localized in the late replicating regions, suggesting that CPT-induced chromatid aberrations arise in the G_2 phase by a mechanism, which possibly does not involve DNA replication. Mosesso *et al.* (1999) studied the possible role of chromatin condensation in converting mechanically CPT-induced SSB into DSB to generate chromosomal aberrations and chromosome breaks detected in prematurely condensed G_0-CPT-treated human lymphocytes. Recently, Barrows *et al.* (1998), from an analysis of *in vitro* transcription of DNA templates containing Topo I "cleavable complexes", demonstrated the production of DSB dependent on transcription of RNA and cytotoxic and lethal damage independent of DNA synthesis. Recently, Mosesso *et al.* (2000) found that pretreatment with α-amanitin, an inhibitor of RNA transcription, reduced the G_2-CPT-induced chromosomal damage, demonstrating indirectly that the conversion of SSB into DSB at the cleavable complex is induced by CPT spaced closely on opposite strands by the action of traversing RNA polymerase.

EFFECT OF CATALYTIC INHIBITORS OF TOPOISOMERASES

The most effective catalytic inhibitors of topoisomerases known at the moment target the type II topoiso-

merase even though some synthetic flavone substitutes have also been recognized to selectively inhibit Topo I (Boege *et al.*, 1996). Bisdioxopiperazines are the most studied catalytic inhibitors of Topo II and they act by stabilizing, in the presence of ATP, eukaryotic Topo II in a closed clamp form and preventing it from opening again. Studies with mammalian cell lines treated with bisdioxopiperazines show that they could affect chromosomal condensation and decondensation and cause an inhibition of cell cycle progression at G_2-M (Ishida *et al.*, 1993) and prevention of chromosome segregation during anaphase (Clarke *et al.*, 1993). Furthermore, an accumulation of closed clamp conformation of human Topo II induced by ICRF 193 might interfere with transcription or other metabolic processes, resulting in cell death (Jensen *et al.*, 2000).

CONCLUSIONS

Advancement of our knowledge about the mechanism of action of topoisomerase inhibitors is important because it may enable the design of rational combinations of "cleavable complex" trappers and catalytic inhibitors which can target topoisomerases at various levels of their catalytic cycle. Bisdioxopiperazines, for example, are known to circumvent the cytotoxicity of etoposide by interfering with etoposide-induced formation of covalent Topo II-DNA complexes (Ishida *et al.*, 1991).

Further studies are needed, however, to optimize anti-topoisomerase combination in order to enhance the efficacy of anticancer therapy and to reduce the risk of secondary malignancies.

RESUMO

As topoisomerases de DNA catalisam alterações topológicas no DNA que são essenciais para a progressão do ciclo celular normal e, portanto, são um alvo preferencial para o desenvolvimento de drogas anticâncer. Drogas anti-topoisomerases podem ser divididas em duas classes principais: drogas anti-"complexos cliváveis" e inibidores catalíticos. As drogas anti-"complexos cliváveis" são muito eficazes como drogas anticancerígenas, mas são também potentes indutores de aberrações cromossômicas, podendo causar neoplasias malignas secundárias. Inibidores catalíticos são citotóxicos mas não induzem aberrações cromossômicas. Conhecimento a respeito do mecanismo de ação de inibidores de topoisomerases é importante para determinar as melhores combinações anti-topoisomerases, com um reduzido risco de indução de neoplasias malignas secundárias.

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