NEW APPROACHES IN THE STUDY OF RADIATION-INDUCED AND CANCER-ASSOCIATED CHROMOSOMAL ABERRATIONS

An International Graduate Course

May 22-31, 2000
Instituto de Investigaciones Biológicas
Clemente Estable
Montevideo - Uruguay
Anti-topoisomerase drugs as potent inducers of chromosomal aberrations*

Loredana Bassi and Fabrizio Palitti

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.

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 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.
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 heterodimeric 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 (G0 or G1, treatment) or chromatid-type aberrations (S or G2, 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; Andersson 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...
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 G2 phases of the cell cycle, while G1 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 G2-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 G2 phase or to chromatin condensation. Bassi et al. (1998) investigated the localization of CPT-induced chromosome breakpoints in the G2 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 G2 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 G0-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 G2-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 topoisomerase and chromosomal damage.
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 topoisomerasers 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.

REFERENCES


Dillehay, E.L., Thompson, L.H., Minkler, J.L. and Carrano, A.V. (1983). The relationship between sister chromatid exchanges and perturba-


(Received November 23, 2000)