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Review Article

Topoisomerase Inhibitors and Types of Them

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ABSTRACT

Objective: In this paper, we have introduced topoisomerase inhibitors, mechanism of action and types of them. DNA topoisomerases are ubiquitous enzymes that catalyze essential enzymes to solve the topological problems accompanying key nuclear processes such as DNA replication, transcription, repair and chromatin assembly by introducing temporary single or double strand breaks in the DNA. **Results:** There are two types of DNA topoisomerase: I and II, which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. Topoisomerases are crucial for the several DNA functions (e.g. replication and transcription) that require the DNA to be unravelled, a process that generates tension and entanglement in DNA. Topoisomerase I breaks a single DNA strand while topoisomerase II breaks both strands and requires ATP for full activity. In both cases, the enzyme is covalently attached to the DNA through tyrosine residues in the active site.

INTRODUCTION

Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes (topoisomerase I and II), which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. DNA topoisomerases are ubiquitous enzymes that catalyze essential enzymes to solve the topological problems accompanying key nuclear processes such as DNA replication, transcription, repair and chromatin assembly by introducing temporary single or double strand breaks in the DNA (Wang et al, 1996; Champoux et al, 2001; Jaxel et al, 1989). In addition, these enzymes finetune the steady-state level of DNA supercoiling to facilitate protein interactions with DNA and to prevent excessive supercoiling. Identical loops of DNA having different numbers of twists are topoisomers, that is, molecules with the same formula but different topologies, and their interconversion requires the breaking of DNA strands. DNA topoisomerases are enzymes that regulate the three-dimensional geometry (topology) of DNA, leading to the interconversion of its topological isomers and to its relaxation. This is related to the regulation of DNA supercoiling, which is essential to DNA transcription and replication, when the DNA helix must unwind to permit the proper function of the enzymatic machinery involved in these processes. In recent years, topoisomerases have become popular targets for cancer chemotherapy treatments. It is thought that topoisomerase inhibitors block the ligation step of the cell cycle, generating single and double stranded breaks that harm the integrity of the genome. Introduction of these breaks subsequently leads to apoptosis and cell death. Topoisomerase I breaks a single DNA strand while topoisomerase II breaks both

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strands and requires ATP for full activity. In both cases, the enzyme is covalently attached to the DNA through tyrosine residues in the active site. These are transient, easily reversible linkages, and for this reason this covalently bound structure is known as the 'cleavable complex'. Afterwards, another DNA strand passes through the transient break in the DNA, and finally the DNA break is resealed. The end result of the reaction is a DNA molecule which is chemically unchanged, but closed in a different topology. The normal catalytic cycle of both types of topoisomerases involves two transesterification steps, one for the cleavage and other for the religation process. In the cleavage reaction, nucleo- philic attack of an active site tyrosine forms a covalent bond with DNA by nucleophilic attack of its hydroxy group to a phosphate group of the phosphodiester DNA backbone. In the religation step, the 5'-hydroxyl group from deoxyribose attacks the previously formed tyrosine phosphate.

Topoisomerases are crucial for the several DNA functions (e.g. replication and transcription) that require the DNA to be unravelled, a process that generates tension and entanglement in DNA. Drugs that inhibit the topoisomerases include some of the most widely used anticancer drugs (Denny et al, 2004). On the other hand, topoisomerase poisons may trigger chromatid breakage to inactivate the ataxia telangiectasia (AT) gene function, disable cell cycle control, and induce genetic instability (Kaufmann et al, 1998). In this connection, some alarming studies have been published, suggesting that maternal exposure to low doses of dietary topoisomerase II poisons, including bioflavonoids such as genistein or quercetin, may contribute to the development of infant leukaemia (Hengstler et al, 2002).

Topoisomerase I mechanism

In the case of eukaryotic topoisomerase I, a single strand is attacked and a 3-phosphotyrosyl linkage is formed. Religation takes place through attack of the 5'-hydroxyl to the previously formed phosphate group (Fig. 1).

Topoisomerase I acts by making a transient break (nick) of a single strand of DNA, catalyzing the passage of DNA strands through one another and allowing release of the superhelical tension. Topoisomerase I enzymes have been subdivided into type IA and type IB sub-families based on their reaction mechanism. Type I topoisomerases of the type IA sub-family form covalent linkages to the 50 end of the DNA break, while type IB sub-family enzymes form covalent linkages to the 30 end of DNA break (Fig. 2). Eukaryotic DNA topoisomerase I is attached to the 30 DNA end of the break site, and is therefore a type IB topoisomerase. This enzyme is located in areas of active RNA transcription to release superhelical stress generated during mRNA synthesis.

Topoisomerase II mechanism

Eukaryotic topoisomerase II is a homodimeric enzyme that requires ATP to function. It makes a transient double strand break, where the tyrosines from the active sites of both monomers attack the phosphodiester bond to the 50-side of the phosphate, leading to a covalent 50-phosphotyrosyl linkage in each strand (Fig. 3). After binding of the enzyme to DNA, a double strand DNA break is produced by nucleophilic attack of both tyrosine residues. These breaks between the strands are not directly opposite to each other; instead, they are separated by a four base pair overhang, generating a space through which another region of intact DNA can be passed. The final steps involve religation of the DNA break, dissociation, and release of DNA from the topoisomerase (Fig. 4). Several of these steps require the binding and hydrolysis of ATP. The catalytic cycle of topoisomerase II is complex and is summarized in Fig. 5, together with the names of some drugs that have steps of this cycle as (Burden et al, 1998) targets. The enzyme assumes two different conformations, resembling an open clamp in the absence of ATP and a closed clamp in the presence of ATP. The open conformation can bind two segments of DNA, forming the pre-cleavage complex. One of these segments will be nicked by the enzyme (G segment) and another that will be transported (T segment). Afterwards, two ATP molecules are bound, leading to the dimerization of the ATPase domains and hence to a conformational change from the open- to the closed-clamp structure. The nucleophilic reactions that break both strands of the G segment of DNA then take place, generating the post-cleavage complex. This allows the passage of the T segment through the gap thus produced, which requires the hydrolysis of one molecule of ATP. The broken ends of the G segment are then ligated and the remaining ATP molecule is hydrolyzed. Upon dissociation of the two ADP molecules from ATP hydrolysis, the T segment is transported through the opening at the C-terminal part of the enzyme, which is then closed. Finally, the enzyme returns to the open clamp conformation, liberating the G segment.

Some antitumor drugs acting at the topoisomerase level have inhibition of enzymatic activity as their primary mode of action, and are known as 'catalytic topoisomerase inhibitors (Holden et al, 2001; Larsen et al, 2003; Andoh et al, 1998). Other drugs targeting the topoisomerases, including intercalating drugs, interfere with the enzyme's cleavage and rejoining activities by trapping the cleavable complex and thereby increasing the half-life of the transient topoisomerase catalyzed DNA break. Some of the most clinically useful anticancer drugs are of the latter type and are normally referred to as 'topoisomerase poisons' because they convert the topoisomerase enzyme into a DNA-damaging agent. Because the level and time-course of expression of these enzymes vary in different cell types, and the development of resistance to one type of inhibitor is often accompanied by a

concomitant rise in the level of the other enzyme, there is an increasing interest in drugs that can act as dual topoisomerase I and II poisons (Denny et al, 1997; Denny et al, 2003). Finally, it is important to mention that topoisomerase inhibitors are among the most efficient inducers of apoptosis (Sordet et al, 2003).

CONCLUSION

Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes (topoisomerase I and II), which are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle.

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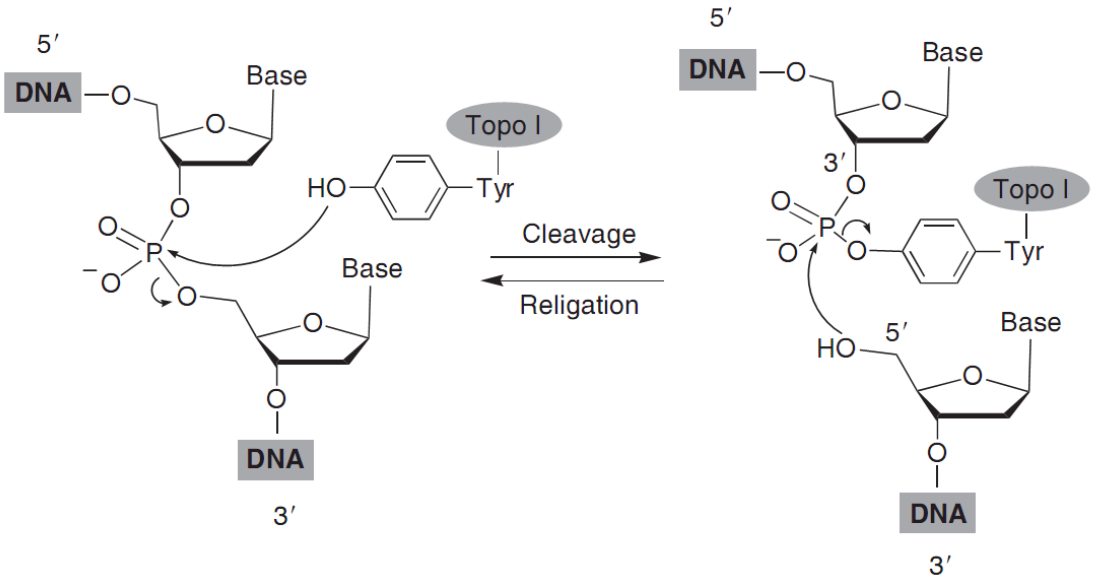

Figure 1. Transesterification reactions involved in topoisomerase I (topo I) activity.

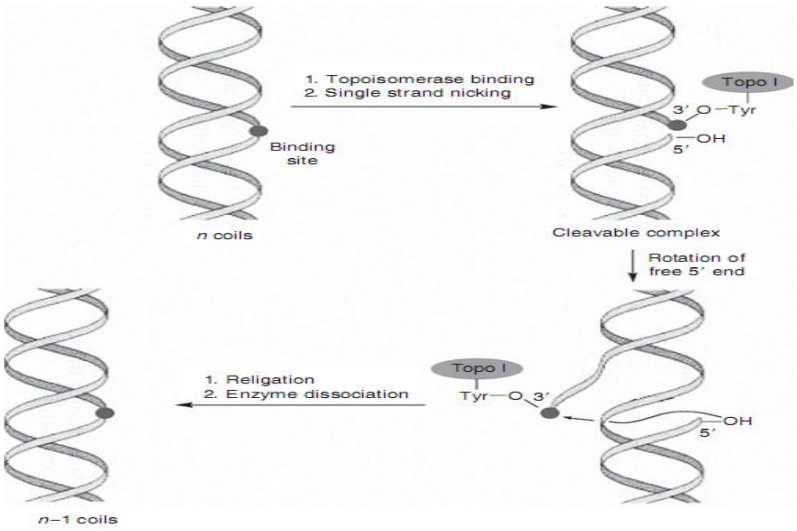

Figure 2. Mechanism of DNA unwinding by topoisomerase I.

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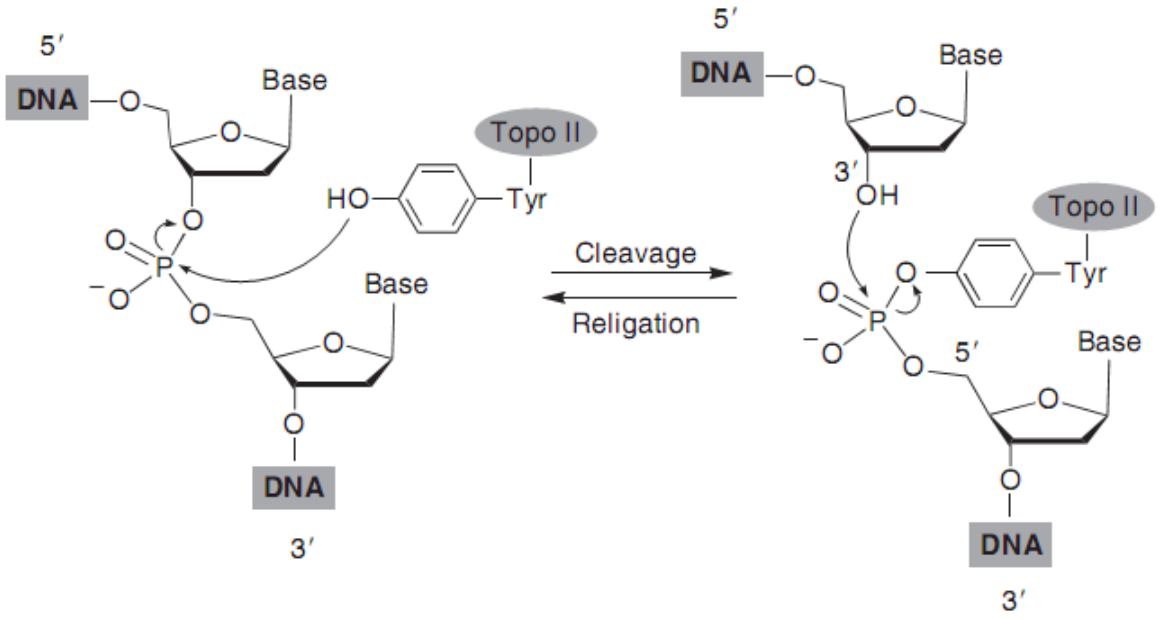

Figure 3. Transesterification reactions involved in topoisomerase II (topo II) activity

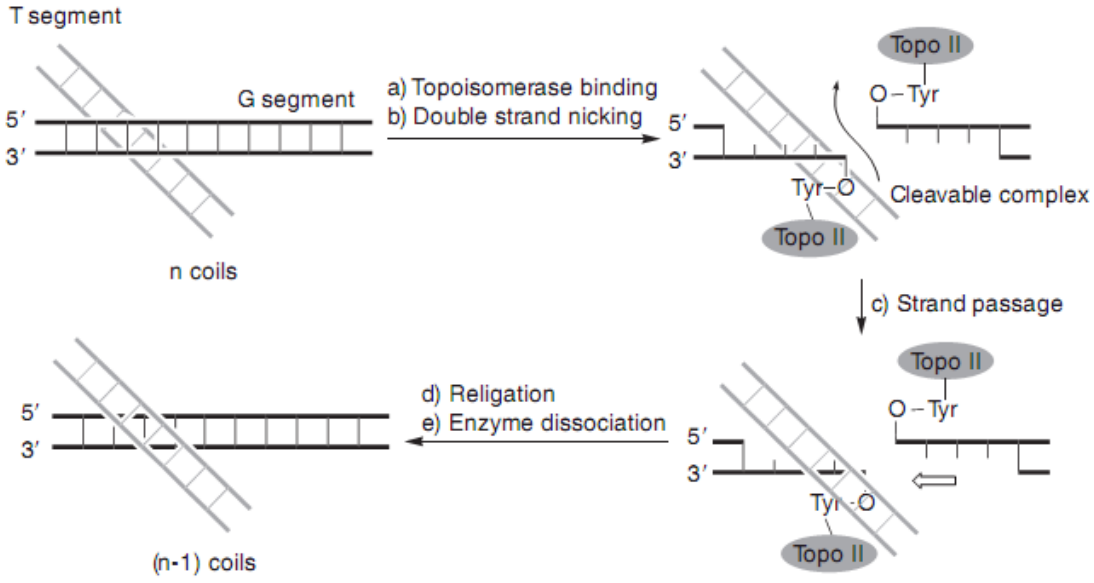

Figure 4. Mechanism of DNA unwinding by topoisomerase II.

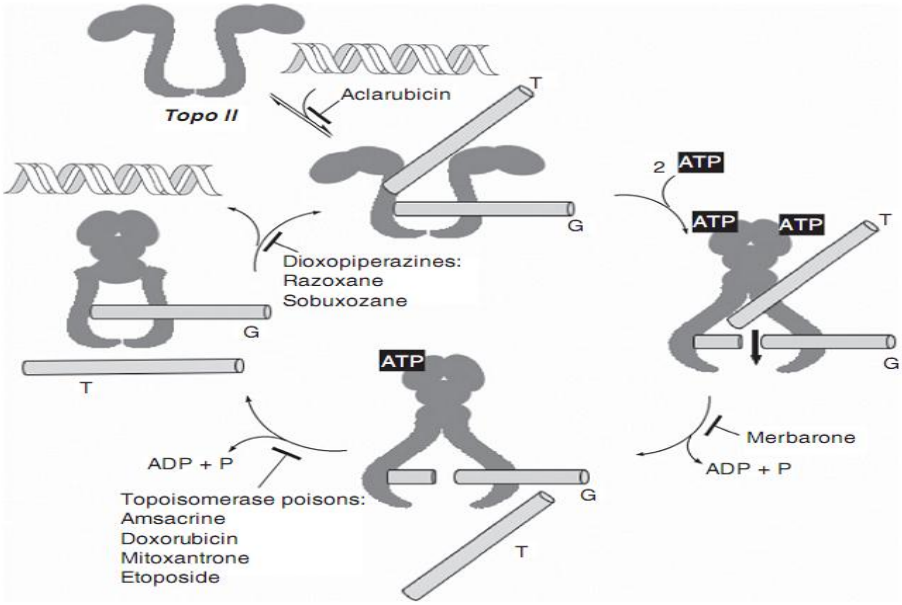

Figure 5. Catalytic cyclic of topoisomerase II and its main inhibitors.