Review Article

Topoisomerase II Inhibitors in Cancer Treatment

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ABSTRACT: Topoisomerase II constitutes a family of nuclear enzymes essential to all living cells. These enzymes are capable of transferring one DNA double helix through a transient break in another DNA double helix. Type II topoisomerases play important roles in DNA metabolic processes, in which they are involved in DNA replication, transcription, chromosome condensation and de-condensation. Topoisomerase II is also the cellular target for a number of widely used anticancer agents currently in clinical use, such as the anthracyclines (daunorubicin and doxorubicin), the epipodophyllotoxins (etoposide and teniposide), and the aminoacridines. These agents stimulate the topoisomerase II-cleavable complex, which is a transient configuration of topoisomerase II on DNA in which topoisomerase II is covalently attached to DNA. This causes the accumulation of cytotoxic nonreversible DNA double-strand breaks generated by the processing of such complexes by DNA metabolic processes. As of present, the clinical use of catalytic topoisomerase inhibitors as antineoplastic agents is limited to aclacinomycin and MST-16. Both of these compounds are preferentially active toward hematological malignancies and show limited activity toward solid tumors. This review explains the role of topoisomerase inhibitors in cancer therapy.

KEYWORDS: DNA topoisomerase; Topoisomerase II inhibitors; Epipodophyllotoxins; Merbarone

Introduction

DNA Topoisomerase

Topoisomerase II constitutes a family of nuclear enzymes essential to all living cells. These enzymes are capable of transferring one DNA double helix through a transient break in another DNA double helix. Type II topoisomerases play important roles in DNA metabolic processes, in which they are involved in DNA replication, transcription, chromosome condensation and de-condensation, DNA recombination, and untangling of replicated chromosomes. Topoisomerase II is also the cellular target for a number of widely used anticancer agents currently in clinical use, such as the anthracyclines (daunorubicin and doxorubicin), the epipodophyllotoxins (etoposide and teniposide). These agents stimulate the topoisomerase II-cleavable complex, which is a transient configuration of topoisomerase II on DNA in which topoisomerase II is covalently attached to DNA. This causes the accumulation of cytotoxic nonreversible DNA double-strand breaks generated by the processing of such complexes by DNA metabolic processes (Liu., 1989; Chen and Liu., 1994).

DNA topoisomerases are nuclear enzymes that make transient strand breaks in DNA to allow a cell to manipulate its topology. Topoisomerase I makes single-strand breaks while Topoisomerase II makes double-strand breaks and passes double-stranded DNA through the nick to allow relaxation of over-coiled DNA (Kellner U et al., 2002). Topoisomerases are highly conserved enzymes essential for the survival of all eukaryotic organisms. There is little sequence homology between topoisomerase I and II. Topoisomerases function in DNA replication, chromosome condensation, and chromosome segregation. Several currently approved chemotherapeutic drugs interfere with the action of topoisomerases. Currently available topoisomerase I inhibitors are: irinotecan (CPT-11) and topotecan. FDA-approved topoisomerase II inhibitors are: etoposide, teniposide, doxorubicin, idarubicin, epirubicin, and mitoxantrone.
Summary of DNA topoisomerases

<table>
<thead>
<tr>
<th>Topoisomerase I</th>
<th>Topoisomerase II</th>
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<tr>
<td>Makes single-strand DNA breaks</td>
<td>Makes double-strand DNA breaks</td>
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<tr>
<td>ATP independent</td>
<td>ATP dependent</td>
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<td>Genes located on chromosome 20q12</td>
<td>Genes located on chromosomes 17q21 and 3p24</td>
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<td>Two types, alpha and beta</td>
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Topoisomerase II inhibitors

(a) Mitoxantrone

Mitoxantrone is an anthracenedione that targets topoisomerase II. It is the only agent of its class approved for clinical purpose (Faullas D et al., 1991). Mitoxantrone lacks the ability to form the quinone-type free radicals thought to account for anthracycline cardiotoxicity.

Mechanism of action

Mitoxantrone binds to topoisomerase II resulting in cleavable complexes that induce DNA strand breaks. Mitoxantrone's poisoning of topoisomerase II, with resultant DNA damage, is a signal for NF-kappa B activation and induction of apoptosis (Boland MP et al., 2000). These induction requires the integrity of functional DNA-damage response genes (Ferrer et al., 2004).

Pharmacology

Mitoxantrone is highly protein bound (78%) with a large volume of distribution 1000–4000 l/m² (Alberts DS et al., 1985). Hepatic metabolism is the principal mechanism for clearance (Ehninger G et al., 1990). With 6–11% of mitoxantrone being cleared by the kidney. No adjustment in dosage is necessary for mild to moderate renal dysfunction. Hepatic dysfunction likely leads to increased AUC due to decreased drug elimination but firm data are lacking.

Clinical uses

Mitoxantrone is used primarily in therapy for breast cancer, leukemia, lymphoma and prostate cancer. Because of the anticipated reduced toxicity with mitoxantrone as compared to doxorubicin, mitoxantrone has been incorporated into selected chemotherapy regimens for patients with a poor performance status who are believed to be at significant risk for doxorubicin toxicity. However, mitoxantrone offers no advantage over other anthracyclines (Anderson J et al., 2002).

High dose of mitoxantrone has no more effect than standard drug doses in therapy of breast cancer (Wells RJ et al., 2003). Mitoxantrone and prednisone for hormone-refractory prostate cancer delays disease progression and improves quality of life without altering survival. Mitoxantrone has been used for therapy of multiple sclerosis (Neuhaus V et al., 2006).

Epipodophyllotoxins

Teniposide

Mechanism of action

Both Teniposide and Etoposide damage DNA by interaction with topoisomerase II to form cleavable complexes that prevent religation of DNA leading to double-strand DNA breaks. It has been proposed that the topoisomerase II –DNA covalent complex arrests transcription and triggers 26S proteasome-mediated degradation of topoisomerase II beta. Xiao et al., 2003 found that the proteosomal degradation of topoisomerase II beta induced by formation of a topo II –DNA complex but not DNA damage. Teniposide prefers to form stabilized cleavable complexes at DNA sites bound to the nuclear matrix (Lambert JM et al., 2000). The rate of topoisomerase II –DNA complex formation with teniposide correlates with DNA damage but not with cytotoxicity.

Toxicity

Teniposide's toxicities are like those of etoposide: myelosuppression, alopecia, mucositis, nausea, and vomiting. Acute myelogenous leukemia with 11q23 chromosome changes occur following teniposide therapy (Felix CA et al., 2001). Hypersensitivity reactions appear more frequently with teniposide infusions than etoposide infusions.

Pharmacology

In vitro, teniposide is about 10-fold more potent than etoposide in killing malignant cells. Since both agents have relatively similar abilities to inhibit topoisomerase II, the greater in vitro cytotoxicity is likely due to greater cellular uptake (Stahelin HF et al., 1991). Teniposide has less water solubility, a lower renal clearance (10%), and is more tightly bound to plasma proteins than Certain drugs, such as cyclosporine, increase the unbound fraction of teniposide resulting in increased toxicity. Teniposide also has a longer drug half-life and greater biliary clearance than does etoposide. Anticonvulsants, such as phenobarbital and phenytoin, increase teniposide clearance, presumable by increasing hepatic metabolism. This increased clearance results in a lower efficacy of teniposide chemotherapy in children with ALL who are receiving seizure medications (Relling MV et al., 2000).
Clinical use

Teniposide has been used as a component of therapy for pediatric patients with poor prognosis acute lymphocytic leukemia. Although teniposide is not a major component of therapy for any adult neoplasms, it has antitumor activity in small cell lung cancer, Kaposi's sarcoma, bladder cancer, leukemias, and lymphomas (Muggia FM et al., 1994; Ettinger DS et al., 2002).

Etoposide

Mechanism of action

Etoposide and Teniposide work via different mechanisms by inhibiting the enzyme topoisomerase II thus preventing DNA synthesis and hence replication. The difference in mechanism is attributed to the presence of small changes in the stereochemistry of the molecules. Etoposide is now marketed as Vepesid for small cell lung cancer, testicular cancer and lymphomas while Teniposide is also used in treating brain tumours.

Topoisomerase II is a multi-subunit enzyme which uses ATP to pass an intact helix through a transient double-stranded break in DNA to modulate DNA topology. After strand passage, the DNA backbone is religated and DNA structure restored. Etoposide prevents topoisomerase II from religating cleaved DNA (Fortune JM et al., 2000). Etoposide thus converts topoisomerase II into a poison that introduces high levels of transient protein-associated breaks in the genome of treated cells.

Topoisomerase II exists as two highly homologous isoforms, alpha and beta, which differ in their production during the cell cycle. The alpha isoform concentration increases 2–3-fold during G2/M, and orders of magnitude is higher in rapidly proliferating cells than in quiescent cell populations. The alpha isoform appears to be the target of etoposid (Gatto B et al., 2003). The beta enzyme does not change significantly during the cell cycle and could potentially be a target in slow growing cancers. Two scissile bonds are formed per every topoisomerase II-mediated double-stranded DNA break. Results of DNA cleavage and ligation assay studies indicate a two-site model for the action of etoposide against human topoisomerase II alpha. This model suggests that drug interactions at both scissile bonds are required in order to increase enzyme-mediated double-stranded DNA breaks.

There does not appear to be a single DNA binding site for etoposide- topoisomerase II targeted breaks. The cell-signaling pathways that lead to apoptosis following topoisomerase II-induced DNA damage are not completely understood. Current research is attempting to elucidate the mechanisms involved (Montecucco A et al., 2007). Caspases are a group of cysteine proteases that orchestrate apoptosis. Robertson et al. have identified caspase 2 as an important link between etoposide-induced DNA damage and the engagement of the mitochondrial apoptotic pathway (Robertson JD et al., 2002). Caspase 2 activates caspase 8 resulting in mitochondrial damage and subsequent downstream caspase 9 and 3 activation (Lin CF et al., 2004). Caspase 3 appears critical for apoptosis-associated chromatin margination, DNA fragmentation, and nuclear collapse. Cells lacking caspase 3 are resistant to etoposide (Yang XH et al., 2001). Caspase 10 appears to trigger a feedback amplification loop that amplifies caspases 9 and 3 (Filomenko R et al., 2006). Tumor necrosis factor-related apoptosis inducing ligand (TRAIL) augments the expression of caspases induced by etoposide.

Toxicity

Common toxicities from etoposide include bone marrow suppression, nausea, vomiting, and alopecia. At very high doses, such as those used with bone marrow transplantation regimens, mucositis becomes the dose-limiting toxicity. Liver toxicity, fever, and chills may also occur with high-dose therapy. Palmar-plantar eruptions and irritation of the anal canal have been associated with etoposide use (Marigny K et al., 2005). The most serious adverse event associated with etoposide is the development of acute myelogenous leukemia (Pui CH., 1991; Smith MA et al., 1999).

Clinical use

Etoposide has been used for treatment of a wide variety of malignancies, including lung cancer, germ-cell malignancies, leukemias, non-Hodgkin's lymphoma, Kaposi's sarcoma, soft tissue sarcomas, and neuroblastoma (Hande KR., 1998).
Novel topoisomerase II inhibitors

Merbarone

Merbarone, 5-(N-phenylcarboxamido)-2-thiobarbituric acid (NSC 336628), is a derivative of barbituric acid and represents a unique class of antineoplastic agents. Merbarone is a conjugate of thiobarbituric acid and aniline, joined by an amide linkage. Of near about 700 barbituric acid analogues that were tested in the National Cancer Institute (Bethesda, MD, USA) screening program, only merbarone was active. Merbarone shows curative activity against L1210 leukemia and also possess important activity against some other murine tumors (Glover A et al., 1987). A brief study of the different steps in the catalytic cycle shows that merbarone has no effect on either DNA binding or ATP hydrolysis but it is a potent inhibitor of enzyme-mediated DNA cleavage (Fortune JM and Osheroff N., 1998). Furthermore, merbarone is able to compete with etoposide, suggesting that the two agents might be competing for similar binding sites on topoisomerase II. Interaction of living cells to merbarone leads to formation of DNA single-strand breaks, S phase retardation, and G2 arrest (Chen M and Beck WT., 1993). Mitotic chromosomes are elongated and intertwined, consistent with inhibition of topoisomerase IIα (Chen M and Beck WT., 1993; Anderson H and Roberge M., 1996; Kallio and Lahdetie., 1997).

More recently, colony formation assays show that both inhibitors, merbarone and ICRF-187 are able to significantly potentiate the cytotoxicity of the alkylationing agent melphalan (Hirot a H et al., 2002). Merbarone has been tested clinically toward many different tumor types. However, these studies were discontinued due to nephrotoxicity as well as to a general lack of antitumor activity (E. Sausville, personal communication).

Aclarubicin (acclinomycin A)

An oligosaccharide anthracycline antineoplastic antibiotic isolated from the bacterium Streptomyces galilaeus. Aclarubicin intercalates into DNA and interacts with topoisomerases I and II, thereby inhibiting DNA replication and repair and RNA and protein synthesis.

Aclarubicin is used clinically in the treatment of acute myelocytic leukemia. Aclarubicin is a strong DNA intercalating agent that inhibit the binding of topoisomerase II to DNA (Sorensen B et al., 1992). As a result, aclarubicin is antagonistic to classical topoisomerase II poisons, such as etoposide, teniposide, and anasmazine. High level of aclarubicin results stimulation of the formation of covalent DNA topoisomerase I complexes (Nitsis JL et al., 1997). But, at biologically relevant concentrations, aclarubicin prevents the binding of topoisomerase I to DNA and is therefore antagonistic to camptothecin (Sorensen BS et al., 1994; Bridewell DJ et al., 1997). Upon consistent studies showing that strong intercalating agents may bind differently to DNA, dependent on the drug structure and the drug to-DNA ratio. Cellular exposure to aclarubicin is accompanied by the occurrence of DNA damage as determined by the single-cell microgel assay (“comet assay”). Interestingly, only 25–40% of the exposed cells (3-hr drug exposure time) show visible DNA damage compared with almost 100% of cells exposed to an isotoxic dose of etoposide (Gieseler F et al., 1999). These results suggest that aclarubicin-induced DNA damage is likely to occur during S phase, where topoisomerase activity is needed to relax supercoils ahead of the replication fork. Interestingly, the morphology of aclarubicin-treated cells is clearly different from that of cells treated with topoisomerase II poisons. Etoposide and daunorubicin both induce small apoptotic nuclei, whereas aclarubicin exposure results in swelling of the nuclei and a more granular pattern of the DNA tail (Gieseler F et al., 1999).

Physicochemical Characteristics

Stability

Aclarubicin was most stable in sodium chloride injection, with a pH of 6.2, and any increase or decrease in pH appeared to affect stability adversely (Poochikian GK et al., 1981).

Pharmacokinetics

Aclarubicin is rapidly distributed into tissues after intravenous injection. Clearance is triphasic, with a terminal elimination half-life of about 3 hours; the principal active metabolite has a terminal half-life of about 13 hours. Aclarubicin is extensively metabolised and only about 1% of the total dose is eliminated unchanged. It is excreted in urine, chiefly as metabolites; some is also eliminated in bile.
Suramin

Suramin is a polyanionic compound contains antitrypanosomal and antifilarial properties (Eisenberger MA and Reyno LM., 1994). Suramin inhibits the catalytic activity of purified topoisomerase II with an IC$_{50}$ value near about 5 µM by preventing the binding capability of the enzyme to DNA. Suramin also inhibits the formation of amsacrine-induced cleavable complexes in both in vitro and living cells and protects cells from the cytotoxic action of amsacrine. Suramin is used to treat patients with hormone refractory prostate cancer and, more recently, high-grade gliomas (Calvo E et al., 2001; Grossman SA et al., 2001; Knox and Moore., 2001). However, suramin exposure may lead to serious side effects, such as neuropathy and lymphopenia. Recently a number of studies are based on the ability of suramin to inhibit the binding of certain growth factors, including basic fibroblast growth factor and insulin-like growth factor I, to their respective cell surface receptors. This is important because binding of both basic fibroblast growth factor and insulin-like growth factor I are able to provide broad spectrum resistance to anticancer drugs (Song S et al., 2000). Low-dose of suramin has proven successful in xenograft models and is currently under clinical development (Qu G et al., 2002).

Novobiocin

Novobiocin is one of aminocoumarin antibiotics that were produced by the actinomycete Streptomyces nivens. It inhibits bacterial DNA synthesis by targeting the enzyme DNA gyrase; and the related enzyme DNA topoisomerase IV is a secondary target of these compounds. Novobiocin inhibits bacterial gyrase B and mammalian topoisomerase II by blocking the ATP binding site (Gormley NA et al., 1996). Novobiocin-resistant leukemia cells show important levels of cross resistance to etoposide (VP-16) and teniposide (VM-26) and to a lesser extent to amsacrine and doxorubicin. It is also a potent inhibitor of arginine-specific mono-ADP-ribosyltransferases (Lodhi IJ et al., 2001). Novobiocin has been used extensively to modulate the cellular response to both alkylating agents and other topoisomerase inhibitors. Novobiocin is also able to potentiate the cytotoxic activity of etoposide and teniposide in several tumor cell lines. The increased cytotoxicity is not due to an additive effect of these agents on topoisomerase II but to inhibition of etoposide and teniposide efflux, leading to higher intracellular drug concentrations and hence increasing the formation of covalent DNA topoisomerase complexes (Rappa G et al., 1993).
Fostriecin

Fostriecin (CI-920) inhibits the catalytic activity of topoisomerase II in vitro without inducing cleavable complexes. It is also a very potent inhibitor of different protein phosphatases and its cellular effects are more similar to other protein phosphatase inhibitors than to catalytic topoisomerase inhibitors.

Fostriecin is a structurally unique polyene lactone phosphate ester. Both the lactone and the phosphate group are necessary for cytotoxic activity in vitro as well as for antitumor activity (Leopold WR et al., 1984; Jackson RC et al., 1985). Antiantibacterial effects have not found in Fostriecin but is inhibit to many species of yeast (Mamber SW et al., 1986). It is very active in mice toward different types of leukemias but is generally less active against solid tumors (Jackson RC et al., 1985). Less activity toward some solid tumors may be due to transport deficiencies similar to what has been observed for methotrexate (Leopold WR et al., 1984). This is probably because both fostriecin and methotrexate enter the cells by the reduced folate carrier system. In addition, fostriecin-resistant cells are highly cross-resistant to methotrexate due to reduced methotrexate influx (Fry DW et al., 1984).

It is a good inhibitor of purified type II topoisomerase from Ehrlich ascites carcinoma, with a 50% inhibitory concentration of 40 µM (Boritzki TJ et al., 1988). Fostriecin is a very potent inhibitor of serine/threonine protein phosphatase PP2A and PP4 and a weaker inhibitor of PP1, with a 50% inhibitory concentration between 1 and 40 nM for PP2A and PP4 and 4–130 µM for PP1. In contrast, fostriecin has no apparent effect on PP2B.

Quinolone derivatives

Quinolone derivatives are widely used antibiotics that target DNA gyrase, a bacterial form of topoisomerase II. Quinolones or fluoroquinolones, including related aryl-fused pyridone carboxylates (e.g., pyridones and naphthyridines; act by binding to the quinolone-resistance-determining region (QRDR) in the catalytic domain of the topoisomerase II. A few quinolone derivatives also show activity toward the eukaryotic form of the enzyme. These include A-62176 and its produg A-74932, which are both members of a new family of quinolones, the quinobenoxazines (Chu DT et al., 1992, 1994). Both drugs show good cytotoxic activity toward tumor cell lines, such as HT-29 human colon carcinoma and A546 human breast carcinoma cells, with a 50% inhibitory concentration between 0.1 and 0.6 µg/mL (Permana et al., 1994). Furthermore, A-74932 also possesses good activity in vivo against both systemic tumors and subcutaneously implanted murine solid tumors as well as human tumor xenographs (Chu DT et al., 1992). A-62176 and A-74932 are DNA-interacting agents that inhibit the catalytic activity of purified calf thymus topoisomerase II with a 50% inhibitory concentration of 0.5–2 µg/mL without induction of cleavable complexes.

Development of new quinolones continues more than 40 years after the first quinolone class antibacterial agent, nalidixic acid, was introduced into clinical
practice in 1962 (Lesher GY et al., 1962). A number of quinolones have been actively developed over the past five years. They include garenoxacin, ozenoxacin (T-3912), DC-159a, DX-619, DQ-113, DW-224a, WCK-771, ABT-492 (WQ-3034), nemonoxacin (TG-873870), and besifloxacin (BOL-303224-A).

A-74932 has been under clinical development as an antitumor agent but was discontinued. No recent development has been reported for quinobenoxazines or their analogues.

**Conclusion**

In conclusion, catalytic topoisomerase inhibitors have demonstrated a number of clinically interesting features that deserve further development. However, such efforts are only likely to succeed if based on a solid understanding of the cellular pharmacology of these drugs and their dependence on tumor-associated genetic and epigenetic alteration.

**References**


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