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Review Article

DNA Topoisomerases: As target for anti-cancer drugs

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ABSTRACT

Topoisomerase inhibitors are agents designed to interfere with the action of topoisomerase enzymes I and II. Topoisomerases are enzymes that control the changes in DNA tridimensional structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. DNA Topoisomerases control the conformational changes in DNA topology by breaking and resealing DNA strands during normal cellular growth, that's why these are essential enzymes. A major class of anticancer drugs acts as inhibitors of DNA Topoisomerases. This paper gives a brief review on Topoisomerase targeting drugs which shows anticancer activities. The mechanism of action of these drugs by inhibiting type I and type II DNA Topoisomerases are discussed. DNA topoisomerases, especially type II A topoisomerases, are proved therapeutic targets of anticancer and antibacterial drugs. Clinically successful topoisomerase-targeting anticancer drugs act through topoisomerase poisoning, which leads to replication fork arrest and double-strand break formation. Unfortunately, this unique mode of action is associated with the development of secondary cancers and cardiotoxicity. Structures of topoisomerase–drug–DNA ternary complexes have revealed the exact binding sites and mechanisms of topoisomerase poisons. It may also be possible to design catalytic inhibitors of topoisomerases by targeting certain inactive conformations of these enzymes. Furthermore, identification of various new bacterial topoisomerase inhibitors and regulatory proteins may inspire the discovery of novel human topoisomerase inhibitors. Thus, topoisomerases remain as important therapeutic targets of anticancer agents.

1. Introduction

DNA Topoisomerases control DNA topology. It participates in all events of DNA metabolism including replication, transcription, and chromosome segregation.[1] These Topoisomerases enzymes covalently bind to DNA phosphorous group then splits the DNA strand or strands and finally rejoin. These enzymes show their activity by removing or introducing DNA torsional tensions, tying, or untying DNA knots.[2–6] There are two types of DNA Topoisomerases that have been derived from prokaryotes and eukaryotes. Topoisomerases I (topoisomerase I) show activity by making single-strand breaks of DNA, whereas Topoisomerases II (topoisomerase II) act by making a transient double-strand break. Other enzymes like resolvase and integrase proteins which are not originally identified as Topoisomerases also perform topoisomerization reactions. Topoisomerase I and Topoisomerase II relaxes positive and negative supercoils of DNA strand. supercoiling. No supercoiling activity. Covalently links to the 3' end of DNA. Covalently binds to 5'end of DNA. Divalent metal ions stimulate activity It also requires ATP and divalent metal but not necessary for ion activity. The cytostatic agents inhibit enzyme activity that leads to irreversible

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interruption of DNA strands, which causes cell death. Camptothecin and Topotecan are DNA Topoisomerases I inhibitors and VM (teniposide) is DNA Topoisomerases II inhibitor. These drugs act by breakage and rejoin reaction of DNA Topoisomerases by binding to Topoisomerases-DNA cleavable complex. Upon its discovery,[7] DNA gyrase was identified as the cellular target of the coumarin and the quinolone antibacterial drugs.[8–10] Since then, DNA topoisomerases, especially type IIA topoisomerases, are recognized as therapeutic targets of many anticancer and antibacterial drugs.

1.1. Classification of topoisomerase targeting anti-cancer drug

The anticancer drugs that targets DNA topoisomerase are divided into two classes that vary in their mechanism of action. Class I drugs contains acridine (e.g.m AMSA), Anthracyclines (e.g. Aldriamycin, daunomycin), Actinomycines (e.g. actinomycin D), Ellipticine (e.g. 2-methyl-9-OH-ellipticinium acetate), Alkaloids(e.g.Camptothecin, irinotecan, Topotecan), Isoflavodins (e.g.Genistein), Quinolones (e.g.Oxolinic acid, norfloxacin). Class I drugs are also called as 'topoisomerase poisons' because it converts the enzyme into a potent cellular toxin. In contrast of class I drugs, class II drugs interfere with catalytic function of enzyme without binding to covalent complex.

Class II drugs are called as 'topoisomerase I inhibitors'. It includes Coumarins (e.g. Nivobiocin, Chlorobiocin), Forstriecin analogues (Forstriecin). Some of these

drugs have intercalative properties in which it can bind to hydrophobic surfaces of DNA and inhibit protein synthesis, RNA and DNA synthesis. DNA binding property is not an important requirement as teniposide and camptothecin also act by inhibiting Topoisomerases without binding to DNA.

2. Topoisomerase- I Inhibitors

Camptothecin is a cytotoxic Alkaloid derived from *Camptotheca acuminata.* It is inhibitor of eukaryotic Topoisomerases-I. Camptothecin shows activity against leukemia and lung cancer. It causes dose limiting haemorrhagic cystitis and myelosuppression. Structure activity relationship for improving the anticancer activity and reducing the toxicity of camptothecin shown that the six membered lactone ring was necessary for anticancer activity. Irinotecan and Topotecan are the derivatives of Camptothecin. They are synthesized by modifying the A-ring of the parent compound.[11–13]

3. Irinotecan Topotecan

Irinotecan shows its activity through conversion to the active compound 7-ethyl 10-hydroxycamptothecin-beta glucoronide by the enzyme caboxylesterase. Irinotecan

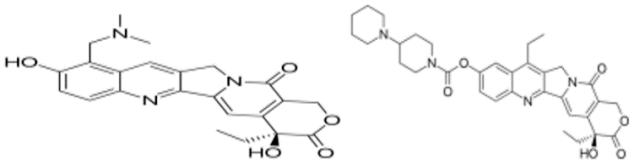

Fig. 1:

is active against breast, colon, gastric, and small cell lung cancers as well as leukemia. The major adverse effects of this drug are Neutropenia and diarrhoea.[14] Topotecan is another derivative of Camptothecin which is positively charged with dimethyl-amino side chains. Topotecan is active against cancers of head and neck, refractory colorectal cancer, and malignant glioma. The side effects of Topotecan includes myelosuppression, Neutropenia and thrombocytopenia. Both Irinotecan and Topotecan are currently used with variable optimum doses via Intraventricular or oral administration. The two alkaloids extracted from *Ocotea leucoxylon* (ie Dicentrinone and dicentrine) shows selective activities against Topoisomerases-I.

The mechanism of action of Camptothecin on Topoisomerases-I involves the binding of enzyme to supercoiled DNA and it leads to a nick in one strand. This single strand break is stabilized due to formation of covalent DNA- protein bond. The single strand break relieves torsional strain and allow DNA replication to proceed. Camptothecin binds to Topoisomerase-I-DNA complex and prevents the religation of the DNA strands. This results in double strand DNA breakage and cell death.

Topoisomerase- II Inhibitors Variety of drugs targets the DNA Topoisomerases-II enzyme which is categorised into two types: DNA intercalators and non- intercalators. Type II enzyme shows its activity by inducing double strand breaks which involves a covalent attachment at the 5'-phosphate end. Translocation of DNA through both a protein complex and double strand break requires in strand passege which involves type II Topoisomerase. The position of the 3'-OH end of DNA shifts to 5' topoisomerase II-linked DNA after binding of an intercalative drug, it leads to the formation of cleavable complex. Hence, unwinding of helix generated by intercalation leads to break down in DNA strands and misalignment of cleavable complex. Due to absence of the structure which is necessary for binding to the enzyme, some of intercalators are not able to induce the formation of the cleavable complex[7–10].

Acridine derivatives from the Topoisomerase II targeting intercalative drugs have been used for the treatment of different types of leukemia. M-AMSA shows activity against acute myelogenous leukemia but it is a toxic drug. Anthracyclines antibiotics are active against a wide variety of experimental tumors, with significant activities against

breast, bone marrow and ovarian Cancers. Anthracyclines stimulate production of hydroxyl radical (.OH) and hydrogen peroxide (H2O2) by microsomal, mitochondrial and nuclear preparations in cancer cells. This shows that metabolism of oxygen free radical plays important role in the anticancer action of these drugs. Ellipticine is an effective drug against breast cancer. It's adverse effects includes xerostomia, body weight loss, renal toxicity and haemolysis.[2,15–17] Non-intercalators Topoisomerase II Inhibitors involves Epipodophyllotoxins (e.g.teniposide) and Isoflavodins (e.g.genistein). These drugs are composed of DNA-binding domain and an enzyme binding domain. Epipodophyllotoxins shows its activity by binding directly and specifically to Topoisomerases II enzyme and inhibit religation of DNA strands. Epipodophyllotoxins are used to treat testicular cancer, malignant lymphoma, central nervous system tumors and small cell lung cancer.

3.1. Manipulation of enzyme activities to achieve therapeutic effects

The enzyme activities are suppressed by catalytic inhibitors and also activates enzyme activities toward the bona fide or a surrogate substrate. Therefore, turning the enzyme into a poisonous agent that is toxic to cells. Interestingly, many clinically successful inhibitors of hTop 1 and hTop 2, as well as bacterial type IIA topoisomerase inhibitors, utilize mechanism 3, which is often referred to as 'topoisomerase poisoning'[6,24-31][18–24]. These topoisomerase poisons convert a topoisomerase into a cellular poison by trapping a covalent topoisomerase–DNA catalytic intermediate as a topoisomerase–drug–DNA ternary complex[21,22]

Topoisomerase poisoning is often caused by the inhibition of the religation reaction,[9,10,19–21] which is also supported by the structural studies of topoisomerase–drug–DNA ternary complexesfluoroquinolones, have reported to stimulate the strand breakage reaction.[35-38]target.Ternary. Thus, catalytic inhibitors that are capable of inhibiting multiple human topoisomerases may be developed as potent anticancer drugs

4. Table 1 Eukaryotic Topoisomerases

The DNA topoisomerase drugs in current clinical use influence these enzymes in a very selective manner. These agents—including the eukaryotic DNA topoisomerase I drugs, camptothecin, irinotecan, and topotecan, and the eukaryotic DNA topoisomerase II drugs, doxorubicin and etoposide—convert their target topoisomerases to DNA-damaging agents. Normally, topoisomerases bind to and cleave DNA by forming an enzyme, DNA covalent intermediate. The DNA is cut in one or both strands depending upon whether DNA topoisomerase I or II is involved. By forming a drug-enzyme-DNA complex, these

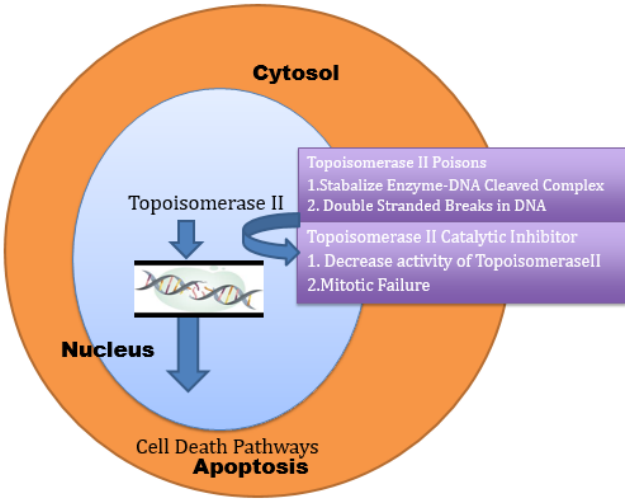

Fig. 2: DNA topoisomeraseII : Promising target for anti cancer drugs

chemotherapeutic agents prevent the subsequent DNA-resealing step normally catalyzed by topoisomerases. Such drugs are referred to as "topoisomerase poisons," and are mechanistically similar to the bactericidal quinolones, which act on DNA gyrase and DNA topoisomerase IV, the bacterial counterparts of eukaryotic DNA topoisomerase II.[23]

Table 1:

S. No.	Enzyme	Type	Inhibitor
1.	Topoisomerase I	IB	Camptothecin, topotecan, irinotecan, actinomycin Db, aclarubicinb
2.	Topoisomerase II	II	Doxorubicin,-daunomycin, etoposide, mitoxantrone
3.	Topoisomerase III	IA	No known inhibitors

5. HTop 1-Targetting Anticancer Drugs

5.1. Camptothecins

5.1.1. Topotecan

Topotecan (Hycamtin) is a semi-synthetic water-soluble derivative of camptothecin. It was the first hTop 1 inhibitor approved for oral administration.[11] It is often used to treat ovarian and small cell lung cancer.

5.1.2. Irinotecan

Irinotecan (CPT-11, Campostar) is another water-soluble derivative of camptothecin.[11,12] It is a prodrug and is

converted to a biologically active metabolite, ethyl-10-hydroxy-camptothecin (SN-38), by a carboxylesterase.[12] Irinotecan, together with fluorouracil, is often used for the treatment of advanced colorectal cancer.[6,11,12]

5.2. Non-camptothecins

Camptothecins are the only hTop1 inhibitors approved for clinical use. Despite their effectiveness, these drugs have limitations due to their instability and severe side effects, as well as drug resistance caused by P-glycoprotein[5,6]. To overcome these limitations, non-camptothecin hTop 1 inhibitors have been developed and investigated[12–14]. Among them, indolocarbazoles (NB-506)[3–5], indenoisoquinolines[6,11,12], and dibenzonaphthyridinones (ARC-111) [30] are under clinical development.

6. Indolocarbazoles

Indolocarbozoles are synthetic analogs of antibiotics isolated from several actinomycetes.[13] NB-506 is a DNA intercaltor that can poison hTop 1.[3,4] NB-506 and camptothecins share the binding site on hTop 1, but hTop 1 is not the only target of NB-506. Edotecarin (J-107088) is a derivative of NB-506 that does not intercalate into DNA.[19] Similar to NB-506, it poisons hTop 1, but its effect on an unidentified target(s) also contributes to its promising anticancer activity.[5]

7. Indenoisoquinolines

Indenoisoquinolines are synthetic non-camptothecin analogs that poison hTop 1. Since the first indenoisoquinline, NSC 314622, was reported in 1978, many derivatives have been synthesized and tested for anticancer activity.[24] A Phase 1 clinical study of indotecan (LMP400) and indimitecan (LMP776) in adults with relapsed solid tumors and lymphomas was recently completed, although study results have not been published.[11]

8. Dibenzonaphthyridinones

ARC-111 was selected among dibenzonaphthyridinones for preclinical studies of its anticancer activity.

9. hTop 2-Targetting Anticancer Drugs

hTop 2 poisons are successful anticancer drugs used in the treatments of various cancers. However, two serious side effects, therapy-related cancer,[7–10,19–21] and cardiotoxicity[21,22] associated with these drugs limit their use. Etoposide and other hTop 2 poisons cause the development of secondary malignancies, especially therapy-related acute myeloid leukemia (t-AML)[8–10,19–22] and therapy-related acute promyelocytic leukemia (t-APL),[10,19–22] t-AML is caused by hTop 2-mediated, and more specifically hTop 2β-mediated [200], DSBs and chromosome rearrangements in the mixed lineage leukemia gene,[22,23] and t-APL is caused by the translocation between the promyelocytic leukemia gene and

9.1. The retinoic receptor α gene

Doxorubicin- is a potent anticancer drug that can be used to treat many cancers. However, its full potential has not been realized due to the cardiotoxicity associated with its use. The primary mechanism of doxorubicin-associated cardiotoxicity appears to be oxidative stress, due to increased levels of reactive oxygen species (ROS) and lipid peroxidation.

1. *Epirubicin (Ellence)* is an active isomer of doxorubicin [318]. Although epirubicin has similar therapeutic effect to doxorubicin, epirubicin is preferred over doxorubicin in certain chemotherapy regimens because it has fewer side effects.
2. *Mitoxantrone (Novantrone)* is a synthetic anthracenedione developed as an alternative to anthracyclines to reduce cardiotoxicity while retaining antineoplastic activity,[24] It is used for the treatment of multiple sclerosis and AML.

10. Source of Funding

None.

11. Conflict of Interest

The Authors declare that there are no competing interests associated with the manuscript.

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