Mini-Review

DOI: 10.5582/ddt.2012.v6.5.230

Topoisomerase IIα, rather than IIβ, is a promising target in development of anti-cancer drugs

Wang Chen[1], Jin Qiu[1,2], Yuemao Shen[1,*]

[1] *School of Pharmaceutical Sciences, Shandong University, Ji'nan, Shandong, China;*
[2] *School of Pharmaceutical Sciences, Shandong University of Traditional Chinese Medicine, Ji'nan, Shandong, China.*

ABSTRACT: DNA topoisomerase II (TOP2) is a well-known anticancer target. Its inhibitors are among the most effective anticancer drugs currently in clinical use. TOP2-targeting agents fall into two major classes of "Topo poisons" and "Topo inhibitors" based on their mechanisms of action. Mammalian cells possess two genetically distinct TOP2 isoforms, TOP2α and TOP2β, that are differentially regulated and play different roles in living cells. Compared to TOP2β, TOP2α may be an efficacious and safe chemotherapeutic target for cancer treatment. This review discusses the advantage of targeting TOP2α over TOP2β and action of various agents on TOP2α.

Keywords: Topoisomerase IIα, topoisomerase IIβ, therapeutic target, inhibitors, cancer therapy

1. Introduction

Cancer has become one of the leading causes of death in many countries such as China and Japan that are witnessing the aging of society. Screening for effective anticancer agents is an important part of oncotherapy. Alkylating agents have numerous drawbacks such as greater toxicity and less selectivity. In contrast, anticancer reagents that target topoisomerases are more selective, so they restrain the DNA replication of tumor cells in the proliferative phase. Among topoisomerases, DNA topoisomerase II (TOP2) is a well-known anticancer target. Agents that target TOP2 are among the most effective anticancer drugs currently available for the treatment of human cancers. Nevertheless, TOP2-based chemotherapy remains associated with incidences of life-threatening toxic side effects, *e.g.* drug-induced secondary malignancies (*1*). Intriguingly, previous studies suggested that suppressing TOP2β, a subtype of TOP2, was responsible for the development of secondary malignancy associated with etoposide treatment (*1*). However, etoposide and doxorubicin may have potent anti-cancer activity because they target another subtype of TOP2, *i.e.* TOP2α (*2*). Hence, agents specifically targeting TOP2α may potentially be efficacious and safe chemotherapeutic drugs with a reduced risk of treatment related to secondary malignancies (*2*). The current paper discusses the advantage of targeting TOP2α over TOP2β and the therapeutic effects of various agents on TOP2α.

******Address correspondence to:*
Dr. Yuemao Shen, School of Pharmaceutical Sciences, Shandong University, No. 44, Wenhuaxi Road, Ji'nan 250012, Shandong, China.
E-mail: yshen@sdu.edu.cn

2. DNA topoisomerase

DNA topoisomerases are enzymes that regulate the overwinding or underwinding of DNA and are required for the survival of all organisms. They play an important role in regulating cellular processes such as replication, transcription, and chromosomal segregation by altering DNA topology. The main families of mammalian DNA topoisomerases are topoisomerase I (TOP1) and topoisomerase II (TOP2). Most type I DNA topoisomerases modify DNA topology in an ATP-independent fashion by creating single strand breaks in DNA whereas type II DNA topoisomerases do so in an ATP-dependent fashion by creating double strand breaks in DNA (*3*). That said, the classification of the topoisomerase family is less unambiguous. There are some similarities between the families, both in functions and amino acid sequences. For example, TOPVI β subunit (Spo11) shares GHKL sequence motifs with type IIA topoisomerases, indicating the evolutionary relationship between these enzymes (*4*).

2.1. *Activity of topoisomerases and its role in cancer pathology*

Despite sequence homology and functional motifs, the mechanisms of action of TOP1 and TOP2 differ. TOP1, the so-called ω protein from *Escherichia coli*, was the first DNA topoisomerase and was discovered by James Wang in 1971 (*5*). TOP1 is an enzyme that relaxes supercoiled DNA through a cycle of cleavage and

religation steps involving the active site residue Tyr723 (*6*). This residue attacks the phosphodiester backbone, breaking the single strand and forming a covalent 'cleavage complex' in which the unbroken strand undergoes 'controlled rotation' and relaxes the DNA. After relaxation, the scissile strand is religated and the enzyme is released (*7*). TOP2 is a ubiquitous ribozyme that alters the instantaneous cleavage of double-stranded DNA and the chromosomal topological structure, facilitating subsequent double-strand break (DSB) religation (*2,8*). These enzymes are involved in many aspects of DNA metabolism, including DNA replication, transcription, repair, and chromosome condensation/ segregation (*2,9*).

Because of the high level of TOP2 expression in cancer cells, TOP2 represents an important target for cancer chemotherapy (*10*). Several widely prescribed anticancer drugs induce TOP2-mediated chromosome DNA breakage and death of cancer cells by increasing the population of TOP2 cleavage complex (*11*). There are two major classes of TOP2, TOP2α and TOP2β, in mammalian cells, and they share catalytic mechanisms and have a high degree of amino acid similarity (~70% identity at the amino acid level) (*12,13*). Despite their similar structural features and biological properties, the two isoforms are differentially regulated and are involved in different cellular processes.

2.2. *Advantage of targeting TOP2α over TOP2β in the development of anti-cancer drugs*

TOP2α is essential for cell growth and is typically expressed at high levels in rapidly growing cancer cells. Its expression is cell cycle-regulated, peaking in G2/ M, whereas TOP2β is expressed in quiescent cells in virtually all tissues throughout the whole cell cycle and is dispensable for cell survival (*14*). The drawbacks of targeting TOP2β include the induction of cardiotoxicity and the potential development of secondary malignancies. Etoposide (**1**), teniposide (**2**), and doxorubicin (**3**) all target both isoforms of TOP2 and are among the most effective anticancer drugs in clinical use; however, these drugs often cause serious side effects, such as secondary malignancies (Table 1). These facts, together with the evidence that specific inhibition of TOP2α resulted in significant anti-tumor action (*1*), suggest that TOP2α-targeted therapy may be a valuable approach for cancer chemotherapy.

Podophyllotoxin is a well-known naturally occurring antitumor lignan lactone isolated from the genus *Podophyllum*. The semisynthetic podophyllotoxin derivative etoposide (VP-16) is in clinical use as antineoplastic agent because of its ability to inhibit TOP2 (*15*). Studies indicated that VP-16-induced carcinogenesis involves mainly the β isoform rather than the α isoform of TOP2. In a mouse skin carcinogenesis model, the incidence of VP-16-induced melanomas in the skin of 7, 12-dimethylbenz[*a*] anthracene-treated mice was found to be significantly higher in *TOP2β(+)* than in skin-specific *TOP2β*-knockout mice (*1*). Furthermore, VP-16-induced DNA sequence rearrangements and DSB were found to be TOP2β-dependent and preventable by co-treatment with a proteasome inhibitor, suggesting the importance of proteasomal degradation of the TOP2β-DNA cleavage complexes in VP-16-induced DNA sequence rearrangements. The anticancer activity of VP-16 on transformed cells expressing both TOP2 isozymes was, however, found to be primarily TOP2β-dependent. These results indicate the importance of developing TOP2α-specific anticancer drugs for effective chemotherapy without causing the development of treatment-related secondary malignancies (*1*).

Anthracyclines such as doxorubicin (adriamycin, **3**) and daunomycin (**4**) are TOP2-targeting drugs and are some of the most effective anticancer drugs in clinical use. However, doxorubicin-based chemotherapy could result in, among other toxic side effects, life-threatening cardiotoxicity (*17-20*). Doxorubicin is known to potentially cause damage to the heart in some individuals. In addition, other anthracyclines (like epirubicin and mitoxantrone) may also cause heart damage. Doxorubicin induced less DNA damage in *TOP2β(-/-)* mouse embryonic fibroblasts (MEF) than in *TOP2β(+/+)* MEFs, and the damage was reduced by the proteasome inhibitors bortezomib and MG132. These findings suggest the specific involvement of proteasome and TOP2β in doxorubicin-induced DNA damage. This supposition is compatible with a model in which proteasome processing of doxorubicin-induced TOP2β-DNA covalent complexes exposed TOP2β-concealed DNA DSB (*21*).

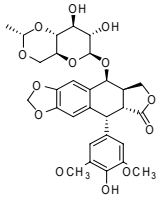
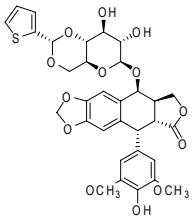
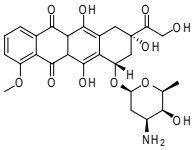
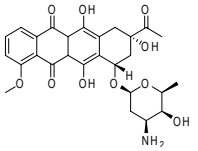
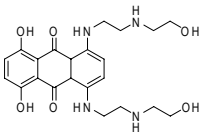
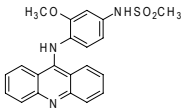
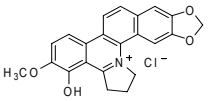
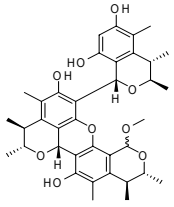
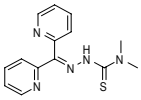
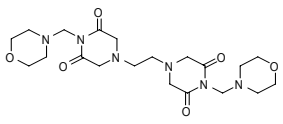
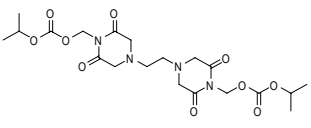
Mitoxantrone (**5**) is a synthetic antibiotic widely used as a chemotherapeutic drug for the treatment of solid tumors, leukemia, and lymphoma (*22,23*). It was approved by the US Food and Drug Administration (FDA) in 2000 for the treatment of neurologic disability and/or reduction of the frequency of clinical relapses in patients with secondary progressive, progressive relapsing, or worsening relapsing-remitting multiple sclerosis. Numerous studies on the interaction of mitoxantrone and DNA have been undertaken and all indicate that the drug functions by intercalating into DNA double strands (*24,25*). It has often been referred to as a TOP2 poison, but mitoxantrone also belongs to the class of TOP2 inhibitors (*26,27*). Mitoxantrone is not a specific TOP2α poison (or inhibitor) and it has marked myelosuppression, heart damage, and hepatotoxicity due to its inhibition of TOP2β.

Although the mechanisms of these side effects have not been fully studied, there might be a considerable benefit to developing TOP2-targeting drugs that are specific for the TOP2α isoform.

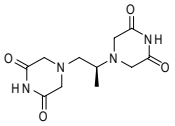
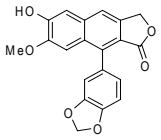
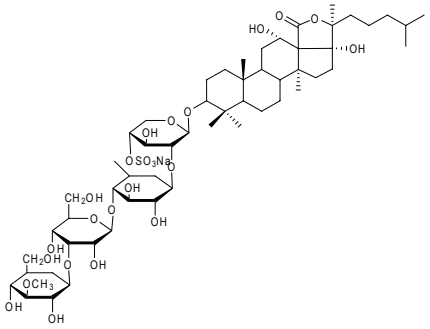
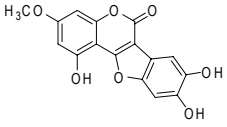
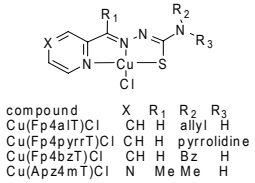
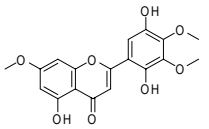
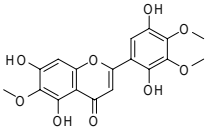
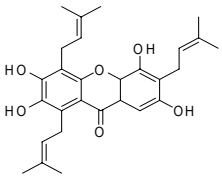
3. TOP2α-targeting agents

TOP2 is known to play important roles in cell cycle events, such as DNA replication, chromosome

Table 1. Structure, origin and activity of TOP2 targeting agents

Compound	Origin	Chemical structure	Target	Mechanism and activity (IC_{50})	References
Etoposide (**1**) (VP-16)	*Sinopodophyllum emodi* (Wall.)		TOP2α,β	Topo poison (2.6 ± 0.8 μM)	(*19*)
Teniposide (**2**) (VM-26)	Semisynthesis		TOP2α,β	Topo poison	(*19*)
Doxorubicin (**3**) (Adriamycin)	Semisynthesis		TOP2α,β	Topo poison (0.1 – 65 μM)	(*20-24*)
Daunomycin (**4**)	*Streptomyces peucetius*		TOP2α,β	Topo poison (9.4 – 100μM)	(*20-24*)
Mitoxantrone (**5**)	Semisynthesis		TOP2α,β	Topo poison	(*32,33*)
Amsacrine (**6**)	Synthesis		TOP2α,β	Topo poison (0.2 – 2.0μM)	(*38*)
NK314 (**7**)	Synthesis		TOP2α	Topo poison (5.3 – 12.9 μM)	(*25*)
Tricitrinol B (**8**)	*Penicillium citrinum*		TOP2α	Topo poison (1 – 10 μM)	(*26*)
Dp44mT (**9**)	Synthesis		TOP2α	Topo poison (0.1 μM)	(*27,28*)
Bimolane (**10**)	Synthesis		TOP2α	Topo inhibitor	(*29,30*)
MST-16 (**11**)	Synthesis		TOP2α	Topo inhibitor	(*29,30*)

Continued

Dexrazoxane (**12**) (ICRF-187)	Synthesis		TOP2α	Topo inhibitor	(*29,30*)
Daurinol (**13**)	*Haplophyllum dauricum*		TOP2α	Topo inhibitor	(*48*)
Echinoside A (**14**)	*Holothuria nobilis* Selenka		TOP2α	Topo inhibitor (2.39 μM)	(*41*)
Wedelolactone (**15**)	*Wedelia calandulaceae*		TOP2α	Topo inhibitor (10 – 20 μM)	(*42*)
TSC (**16**)	Synthesis	 compound X R_1 R_2 R_3 Cu(Fp4alT)Cl CH H allyl H Cu(Fp4pyrrT)Cl CH H pyrrolidine Cu(Fp4bzT)Cl CH H Bz H Cu(Apz4mT)Cl N Me Me H	TOP2α	Topo inhibitor (0.3 – 1.6 μM)	(*43*)
Flavone (**17**)	*Gardenia carinata* (Rubiaceae)		TOP2α	Undetermined (3.4 μM)	(*48*)
Flavone (**18**)	*Gardenia carinata* (Rubiaceae)		TOP2α	Undetermined	(*48*)
Garcinone E (**19**)	*Garcinia mangostana* L.		TOP2α	Undetermined (0.5 – 5.4 μM)	(*49,50*)

condensation/decondensation, and sister chromatid segregation (*9*). TOP2-targeting agents, involving etoposide, doxorubicin, and mitoxantrone, are the most effective and most widely used anti-cancer drugs in cancer chemotherapy (Table 1) (28). Distinct from "TOP2 inhibitors", these agents are referred to as "TOP2 poisons". TOP2 poisons convert the essential enzyme into a highly cytotoxic DNA-damaging agent through the formation of 'cleavage complex'. There, a TOP2-linked DNA strand-passing intermediate is stabilized, allowing the generation of double strand breaks (*29*). TOP2 poisons come in two types, intercalating and non-intercalating agents. Intercalators may interact with DNA through covalent binding, electrostatic binding, or intercalating (*30*). When agents of an appropriate size and chemical structure fit themselves between base pairs of DNA, intercalation occurs. These agents are mainly polycyclic, aromatic, and planar. Non-intercalating agents may lead to protein-drug interactions involving TOP2 poisons and trapped TOP2 covalent complexes because they do not interact strongly with DNA. "TOP2 inhibitors" interrupt the binding of TOP2 and DNA by

binding enzymes to inhibit TOP2 catalytic activity but do not generate increases in the levels of TOP2 covalent complexes (Table 1). In cell culture experiments, TOP2 inhibitors antagonize the toxicity of TOP2 poisons, indicating that TOP2 inhibitors act by separate mechanisms (*31*). These agents may competitively or non-competitively inhibit TOP2 ATPase activity or trap TOP2 and thus interrupt the binding of TOP2 and DNA. These drugs encompass a diverse group of natural and synthetic compounds that are commonly used to treat a variety of human malignancies (*32-34*).

3.1. *TOP2α poisons*

Di-2-pyridylketone-4,4-dimethyl-3-thiosemicarbazone (Dp44mT, **6**), an iron chelator, is presumed to have selective antitumor activity *in vitro* and *in vivo* (*35*). In previous studies, Dp44mT alone was capable of inducing apoptosis in neuroepithelioma, melanoma, and breast cancer cells in contrast to healthy fibroblasts. It also had activity in VP-16-resistant clones of MCF-7 breast tumor and KB3-1 epidermoid carcinoma cells. Short-term studies with low-dose Dp44mT inhibited lung, melanoma, and neuroepithelioma tumor growth in nude mice without systemic iron depletion, changes in organ weights, or differences in serum biochemical parameters, in contrast to triapine. Dp44mT inhibits TOP2α activity at low concentrations (*36*) that are 10- to 100-fold lower than those used to show TOP2 inhibition by ICRF-187 (*21*). Dp44mT is able to induce the formation of stable cellular TOP2α-DNA complexes, indicative of TOP2α poisoning in cancer cells.

NK314 (**7**) is a novel synthetic benzo[c]phenanthridine alkaloid that has strong antitumor activity. The compound was previously reported to stabilize TOP2 cleavage complexes and induce rapid DSBs, causing G2 arrest in tumor cells. Subsequently, NK314 was found to be an α isoform-specific TOP2 poison in living mammalian cells that induces TOP2-DNA complexes and chromosomal DSBs in a TOP2α-dependent manner (*37*). NK-314 can also inhibit DNA-dependent protein kinase (DNA-PK) and induce ataxia telangiectasia, mutated (ATM), contributing to cell survival (*38*). This perhaps is the reason why NK314 had an antitumor activity on etoposide and doxorubicin-resistant cell lines.

Tricitrinol B (**8**), a citrinin trimer derivative, was extracted from a volcano ash isolate of the fungus Penicillium citrinum HGY1-5 (*39*). It had comprehensive cytotoxicity in 17 tumor cell lines (IC_{50}: 1-10 μM) and was equally cytotoxic toward two multidrug-resistant cell lines overexpressing Pgp compared to the parental cell lines. Tricitrinol B induced cell cycle arrest in the G2/M phase and apoptosis by inducing DNA fragmentation and caspase activation. Molecular modeling and topoisomerase-mediated DNA relaxation experiments suggested that it is an intercalating TOP2α poison. It inhibited TOP2α isomer activity by interfering with the TOP2α-mediated poststrand-passage cleavage/religation equilibrium in comparison to the prestrand-passage cleavage/religation equilibrium and increased the amount of broken DNA in cells.

Amsacrine (**9**) is a chemotherapy drug that is usually given in combination with other chemotherapy drugs to treat types of adult and childhood leukemia (*40*). Its major and minor groove proportions can be altered because its planar fused ring system can intercalate into the DNA of tumor cells. Amsacrine inhibits DNA replication and transcription by reducing the association between the affected DNA and DNA polymerase, RNA polymerase, and transcription factors. Like the better-known agent etoposide, amsacrine also inhibits topoisomerases (*41*). That said, intercalation of the molecule in the structurally similar *o*-amsacrine is insufficient to trap TOP2 as a covalent complex on DNA because of the different position of the methoxy substituent group on the anilino-ring. This precludes TOP2 poisons despite amsacrine's intercalative behavior (*42*).

3.2. *TOP2α inhibitors*

Bisdioxopiperazines (Biz) such as ICRF-154 (**10**), MST-16 (**11**), and ICRF-187 (**12**) both non-competitively inhibit TOP2 ATPase activity (*43*) and trap the TOP2 structure as a closed clamp. Biz have been the most commonly used specific catalytic inhibitors of type II topoisomerases in mammalian cells (*44*). They are catalytic noncleavable complex-forming inhibitors of DNA TOP2 that do not produce protein-linked DNA strand breaks. Biz have been widely used as a cardioprotectant against anthracycline-induced cardiomyopathy (*31,45*). Their mechanism of cardioprotection is through anthracycline-induced generation of reactive oxygen species (*46*). Biz could lead to degradation of TOP2β, a toxic source of anthracycline.

Wedelolactone (**13**), a coumestan isolated in 1956, is one of the active polyphenolic compounds in extracts of *Wedelia calandulaceae* and *Eclipta prostrata*. Wedelolactone has been found to have a wide range of biological effects. Wedelolactone was previously thought to attenuate the NF-κB transcription factor and/or androgen receptor activity and orthotopic growth of prostate cancer in nude mice. However, wedelolactone was cytotoxic to breast cancer MDA-MB-231 cells at concentrations that did not inhibit NF-κB activity. Wedelolactone induced S and G2/M phase cell cycle arrest and led to DNA damage signaling (*47*). Wedelolactone was found to inhibit DNA relaxation and TOP2α-mediated decatenation. Inhibition of growth and promotion of apoptosis by wedelolactone did not result from deregulation of NF-κB but were more likely attributable to its ability to bind dsDNA, inhibit TOP2α, and block DNA synthesis.

Daurinol (**14**), a novel arylnaphthalene lignan isolated from the ethnopharmacological plant *Haplophyllum dauricum*, has potent catalytic inhibition of human TOP2α and induces S phase cell cycle arrest through the enhanced expression of cyclins E and A and by activation of the ATM/Chk/Cdc25A pathway in HCT116 cells (*48*). Unlike etoposide, daurinol did not cause DNA damage or nuclear enlargement *in vitro*. It has potential antitumor action in mice, with fewer side effects than etoposide, although it has same chemical structure backbone as etoposide.

Echinoside A (**15**), isolated from the sea cucumber *Holothuria nobilis* Selenka, has potent anticancer activity. It is a new nonintercalative TOP2α inhibitor because of its inhibition of the noncovalent binding of TOP2α to DNA rather than the ATPase activity of TOP2. Echinoside A interferes with the binding of TOP2 to DNA, thus impairing the prestrand passage cleavage/religation equilibrium. In addition, echinoside A results in TOP2α-dependent DNA DSBs. These facts make echinoside A the first marine-derived triterpene glycoside TOP2α inhibitor (*49*). Echinoside A reduces the noncovalent binding of TOP2 to DNA. Echinoside imitates DNA action and competitively inhibits the binding of the enzyme to DNA. This concept may lead to novel strategies for designing new TOP2 inhibitors, and more importantly, echinoside A can be used to implement such a strategy.

Thiosemicarbazone (CuII(thiosemicarbazonato)Cl complexes, TSC, **16**), condensed heterocyclic carboxaldehyde moieties, have been found to have marked antibacterial, antiviral, antifungal, and, most intriguingly, antineoplastic activity (*50*). Recent studies indicated that the thiosemicarbazones and their metal complexes had the ability to inhibit TOP2α These studies both reinforced the significant potential of metal-thiosemicarbazonato complexes in cancer research and they also expanded the array of potential biochemical targets for those molecules (*51-54*). *R*-Heterocyclic thiosemicarbazones and their Cu(II) complexes are capable of *in vivo* and *in vitro* inhibition of TOP2α at an IC_{50} below that of the widely employed TOP2α poison etoposide (VP-16). Given the great potential of TSCs and their metal complexes in the development of chemotherapeutic agents and the importance of TOP2α in many forms of cancer, they may be a promising avenue for study (*51-53*).

3.3. *TOP2α inhibitors with undetermined mechanisms*

Two novel flavones, 5,2',5'-trihydroxy-7,3',4'-trimethoxyflavone (**17**), and 5,7,2',5'-tetrahydroxy-6,3',4'-trimethoxyflavone (**18**), were isolated from the leaves and twigs of Gardenia carinata (Rubiaceae). Flavone **16** had cytotoxic activity against P-388 and MCF-7 cell lines, while **17** acted only on the P-388 cell line. All active compounds were found to inhibit DNA TOP2α activity, which may be responsible for the observed cytotoxicity (*55*). Active compounds were evaluated in terms of their DNA TOP2α inhibition in a relaxation assay. All compounds had similar inhibition of the TOP2α enzyme at a low concentration. This indicates that all of the tested compounds effectively inhibited the relaxation activity of TOP2α like the positive control etoposide did at the same concentration. This inhibitory activity may be responsible for their observed cytotoxicity.

Xanthones (for example, garcinone E, **19**) are hetero-tricyclic planar compounds originally isolated as secondary metabolites from plants and microorganisms (*56*). Xanthones have a diverse biological profile including antihypertensive, anticonvulsant, anti-thrombotic, anticholinesterase, and anti-cancer activity. This activity depends on differing structures modified by substituents on the xanthone ring. Recently, epoxide ring-opened xanthone derivatives were synthesized and tested for their topoisomerase inhibitory activity and cytotoxicity. Most of the compounds had TOP2α-specific inhibitory activity (*57*).

3.4. *Prospects for development of TOP2α-targeting agents in the future*

Classifying all of these TOP2α-targeting compounds would be difficult due to their various chemical structures, origins, and mechanisms. Additionally, drugs in clinical use have several flaws (*e.g.* drug resistance, myelosuppression, cardiotoxicity, hepatotoxicity, and poor water solubility) that need to be remedied. Nowadays, Biz is a promising TOP2α inhibitor that could be developed into a drug (*31,44,45*). Natural products are still the main source of TOP2α-targeting agents since more effective novel etoposide-related natural products continue to be isolated (*48,58*). Podophyllotoxin derivatives might be a safer anticancer drug, but the majority of TOP II-targeting compounds are (semi)synthetic products, so the design and synthesis of podophyllotoxin derivatives may have good prospects.

4. Conclusion and perspectives

TOP2-targeting agents encompass a diverse group of natural and synthetic compounds that are commonly used to treat a variety of human malignancies (*32-34*). Although TOP2 is the cytotoxic target of the drugs, the relative contributions of TOP2α and TOP2β to the chemotherapeutic effects of these agents have yet to be elucidated. Until now, no truly 'isoform-specific' agents have been identified. However, some drugs appear to favor one isoform or the other. TOP2α-favoring drugs may be a valuable novel approach for cancer treatment, so more active compounds targeting TOP2α need to be sought. The discussion herein should contribute to the development of more potent and effective TOP2α inhibitors and an enhanced understanding of their mechanisms of action.

Acknowledgements

This work was financially supported as a Key Project of the Chinese Ministry of Education (306010).

References

1. Azarova AM, Lyu YL, Lin CP, Tsai YC, Lau JY, Wang JC, Liu LF. Roles of DNA topoisomerase II isozymes in chemotherapy and secondary malignancies. Proc Natl Acad Sci U S A. 2007; 104:11014-11019.
2. Toyoda E, Kagaya S, Cowell IG, Kurosawa A, Kamoshita K, Nishikawa K, Iiizumi S, Koyama H, Austin CA, Adachi N. NK314, a topoisomerase II inhibitor that specifically targets the α isoform. J Biol Chem. 2008; M803936200.
3. Schoeffler AJ, Berger JM. DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. Q Rev Biophys. 2008; 41:41-101.
4. Corbett KD, Berger JM. Structure of the topoisomerase VI-B subunit: Implications for type II topoisomerase mechanism and evolution. EMBO J. 2003; 22:151-163.
5. Wang JC. Interaction between DNA and an *Escherichia coli* protein omega. J Mol Biol. 1971; 55:523-533.
6. Tesauro C, Fiorani P, D'Annessa I, Chillemi G, Turchi G, Desideri A. Erybraedin C, a natural compound from the plant Bituminaria bituminosa, inhibits both the cleavage and religation activities of human topoisomerase I. Biochem J. 2010; 425:531-539.
7. Peterson KE, Cinelli MA, Morrell AE, Mehta A, Dexheimer TS, Agama K, Antony S, Pommier Y, Cushman M. Alcohol-, diol-, and carbohydrate-substituted indenoisoquinolines as topoisomerase I inhibitors: Investigating the relationships involving stereochemistry, hydrogen bonding, and biological activity. J Med Chem. 2011; 54:4937-4953.
8. Dong KC, Berger JM. Structural basis for gate-DNA recognition and bending by type IIA topoisomerases. Nature. 2007; 450:1201-1205.
9. Wang JC. Cellular roles of DNA topoisomerases: A molecular perspective. Nat Rev Mol Cell Biol. 2002; 3:430-440.
10. Li TK, Liu LF. Tumor cell death induced by topoisomerase-targeting drugs. Annu Rev Pharmacol Toxicol. 2001; 41:53-77.
11. Wu CC, Li TK, Farh L, Lin LY, Lin TS, Yu YJ, Yen TJ, Chiang CW, Chan NL. Structural basis of type II topoisomerase inhibition by the anticancer drug etoposide. Science. 2011; 333:459-462.
12. Austin CA, Sng JH, Patel S, Fisher LM. Novel HeLa topoisomerase II is the II β isoform: Complete coding sequence and homology with other type II topoisomerases. Biochim Biophys Acta. 1993; 1172:283-291.
13. Jenkins JR, Ayton P, Jones T, Davies SL, Simmons DL, Harris AL, Sheer D, Hickson ID. Isolation of cDNA clones encoding the β isozyme of human DNA topoisomerase II and localisation of the gene to chromosome 3p24. Nucleic Acids Res. 1992; 20:5587-5592.
14. Woessner RD, Mattern MR, Mirabelli CK, Johnson RK, Drake FH. Proliferation- and cell cycle-dependent differences in expression of the 170 kilodalton and 180 kilodalton forms of topoisomerase II in NIH-3T3 cells. Cell Growth Differ. 1991; 2:209-214.
15. Meresse P, Dechaux E, Monneret C, Bertounesque E. Etoposide: Discovery and medicinal chemistry. Curr Med Chem. 2004; 11:2443-2466.
16. Long BH. Mechanisms of action of teniposide (VM-26) and comparison with etoposide (VP-16). Semin Oncol. 1992; 19:3-19.
17. De Beer EL, Bottone AE, Voest EE. Doxorubicin and mechanical performance of cardiac trabeculae after acute and chronic treatment: A review. Eur J Pharmacol. 2001; 415:1-11.
18. Wallace KB. Doxorubicin-induced cardiac mitochondrionopathy. Pharmacol Toxicol. 2003; 93:105-115.
19. Zucchi R,Danesi R. Cardiac toxicity of antineoplastic anthracyclines. Curr Med Chem Anticancer Agents. 2003; 3:151-171.
20. Xu X, Persson HL, Richardson DR. Molecular pharmacology of the interaction of anthracyclines with iron. Mol Pharmacol. 2005; 68:261-271.
21. Lyu YL, Kerrigan JE, Lin CP, Azarova AM, Tsai YC, Ban Y, Liu LF. Topoisomerase II mediated DNA double-strand breaks: Implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. Cancer Res. 2007; 67:8839-8846.
22. Holmes FA, Yap HY, Esparza L, Buzdar AU, Hortobagyi GN, Blumenschein GR. Mitoxantrone, cyclophosphamide, and 5-fluorouracil in the treatment of hormonally unresponsive metastatic breast cancer. Semin Oncol. 1984; 11:28-31.
23. Velasquez WS, Lew D, Grogan TM, Spiridonidis CH, Balcerzak SP, Dakhil SR, Miller TP, Lanier KS, Chapman RA, Fisher RI. Combination of fludarabine and mitoxantrone in untreated stages III and IV low-grade lymphoma: S9501. J Clin Oncol. 2003; 21:1996-2010.
24. Lown JW, Morgan AR, Yen SF, Wang YH, Wilson WD. Characteristics of the binding of the anticancer agents mitoxantrone and ametantrone and related structures to deoxyribonucleic acids. Biochemistry. 1985; 24:4028-4035.
25. Krishnamoorthy CR, Yen SF, Smith JC, Lown JW, Wilson WD. Stopped-flow kinetic analysis of the interaction of anthraquinone anticancer drugs with calf thymus DNA, poly[d(G-C)].poly[d(G-C)], and poly[d(A-T)]. poly[d(A-T)]. Biochemistry. 1986; 25:5933-5940.
26. Fortune JM, Osheroff N. Topoisomerase II as a target for anticancer drugs: When enzymes stop being nice. Prog Nucleic Acid Res Mol Biol. 2000; 64:221-253.
27. Giles GI, Sharma RP. Topoisomerase enzymes as therapeutic targets for cancer chemotherapy. Med Chem. 2005; 1:383-394.
28. Osheroff N. Effect of antineoplastic agents on the DNA cleavage/religation reaction of eukaryotic topoisomerase II: inhibition of DNA religation by etoposide. Biochemistry. 1989; 28:6157-6160.
29. Nitiss JL, Wang JC. Mechanisms of cell killing by drugs that trap covalent complexes between DNA topoisomerases and DNA. Mol Pharmacol. 1996; 50:1095-1102.
30. Richards AD, Rodger A. Synthetic metallomolecules as agents for the control of DNA structure. Chem Soc Rev. 2007; 36:471-483.
31. Speyer JL, Green MD, Kramer E, Rey M, Sanger J, Ward C, Dubin N, Ferrans V, Stecy P, Zeleniuch-Jacquotte A. Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med. 1988; 319:745-752.

32. McClendon AK, Osheroff N. DNA topoisomerase II, genotoxicity, and cancer. Mutat Res. 2007; 623:83-97.
33. Bender RP, Osheroff N. DNA Topoisomerases as targets for the chemotherapeutic treatment of cancer. In: Cancer Drug Discovery and Development. Checkpoint Responses in Cancer Therapy (Dai W, ed.). Humana Press, New York, NY, USA, 2008; pp. 57-91.
34. Martincic D, Hande KR. Topoisomerase II inhibitors. Cancer Chemother Biol Response Modif. 2005; 22:101-121.
35. Whitnall M, Howard J, Ponka P, Richardson DR. A class of iron chelators with a wide spectrum of potent antitumor activity that overcomes resistance to chemotherapeutics. Proc Natl Acad Sci U S A. 2006; 103:14901-14906.
36. Yuan J, Lovejoy DB, Richardson DR. Novel di-2-pyridyl-derived iron chelators with marked and selective antitumor activity: *In vitro* and *in vivo* assessment. Proc Natl Acad Sci U S A. 2006; 103:14901-14906.
37. Toyoda E, Kagaya S, Cowell IG, Kurosawa A, Kamoshita K, Nishikawa K, Iiizumi S, Koyama H, Austin CA, Adachi N. NK314, a topoisomerase II inhibitor that specifically targets the isoform. Blood. 2004; 104:1450-1458.
38. Guo L, Liu X, Jiang Y, Nishikawa K, Plunkett W. DNA dependent protein kinase and ataxia telangiectasia mutated (ATM) promote cell survival in response to NK314, a topoisomerase IIα inhibitor. Mol Pharmacol. 2011; 80:321-327.
39. Du L, Liu HC, Fu W, Li DH, Pan QM, Zhu TJ, Geng MY, Gu QQ. Unprecedented citrinin trimer tricitinol B functions as a novel topoisomerase IIα inhibitor. J Med Chem. 2011; 54:5796-5810.
40. Horstmann MA, Hassenpflug WA, zur Stadt U, Escherich G, Janka G, Kabisch H. Amsacrine combined with etoposide and high-dose methylprednisolone as salvage therapy in acute lymphoblastic leukemia in children. Haematologica. 2005; 90:1701-1703.
41. Wadkins RM, Graves DE. Thermodynamics of the interactions of *m*-AMSA and *o*-AMSA with nucleic acids: Influence of ionic strength and DNA base composition. Nucleic Acids Res. 1989; 17:9933-9946.
42. Nitiss JL. Targeting DNA topoisomerase II in cancer chemotherapy. Nat Rev Cancer. 2009; 9:338-350.
43. Roca J, Ishida R, Berger JM, Andoh T, Wang JC. Antitumor bisdioxopiperazines inhibit yeast DNA topoisomerase II by trapping the enzyme in the form of a closed protein clamp. Proc Natl Acad Sci U S A. 1994; 91:1781-1785.
44. Andoh T, Ishida R. Catalytic inhibitors of DNA topoisomerase II. Biochim Biophys Acta. 1998; 1400:155-171.
45. Shan K, Lincoff AM, Young JB. Anthracycline-induced cardiotoxicity. Ann Intern Med. 1996; 125:47-58.
46. Menna P, Salvatorelli E, Minotti G. Cardiotoxicity of antitumor drugs. Chem Res Toxicol. 2008; 21:978-989.
47. Benes P, Knopfova L, Trcka F, Nemajerova A, Pinheiro D, Soucek K, Fojta M, Smarda J. Inhibition of topoisomerase IIα: Novel function of wedelolactone. Cancer Lett. 2011; 303:29-38.
48. Kang K, Oh SH, Yun JH, Jho EH, Kang JH, Batsuren D, Tunsag J, Park KH, Kim M, Nho CW. A novel topoisomerase inhibitor, daurinol, suppresses growth of HCT116 cells with low hematological toxicity compared to etoposide. Neoplasia. 2011; 13:1043-1057.
49. Li M, Miao ZH, Chen Z, Chen Q, Gui M, Lin LP, Sun P, Yi YH, Ding J. Echinoside A, a new marine-derived anticancer saponin, targets topoisomerase II by unique interference with its DNA binding and catalytic cycle. Ann Oncol. 2009; 21:597-607.
50. Yu Y, Kalinowski DS, Kovacevic Z, Siafakas AR, Jansson PJ, Stefani C, Lovejoy DB, Sharpe PC, Bernhardt PV, Richardson DR. Thiosemicarbazones from the old to new: iron chelators that are more than just ribonucleotide reductase inhibitors. J Med Chem. 2009; 52:5271-5294.
51. Miller MC, 3rd, Bastow KF, Stineman CN, Vance JR, Song SC, West DX, Hall IH. The cytotoxicity of 2-formyl and 2-acetyl-(6-picolyl)-4N-substituted thiosemicarbazones and their copper(II) complexes. Arch Pharm (Weinheim). 1998; 331:121-127.
52. Miller MC, 3rd, Stineman CN, Vance JR, West DX, Hall IH. The cytotoxicity of copper(II) complexes of 2-acetylpyridyl-4N-substituted thiosemicarbazones. Anticancer Res. 1998; 18:4131-4139.
53. Miller MC, Stineman CN, Vance JR, West DX, Hall IH. Multiple mechanisms for cytotoxicity induced by copper(II) complexes of 2-acetylpyrazine-N-substituted thiosemicarbazones. Applied Organometallic Chemistry. 1999; 13:9-19.
54. Zeglis BM, Divilov V, Lewis JS. Role of metalation in the topoisomerase IIα inhibition and antiproliferation activity of a series of α-heterocyclic-N4-substituted thiosemicarbazones and their Cu(II) complexes. J Med Chem. 2011; 54:2391-2398.
55. Kongkum N, Tuchinda P, Pohmakotr M, Reutrakul V, Piyachaturawat P, Jariyawat S, Suksen K, Yoosook C, Kasisit J, Napaswad C. DNA topoisomerase IIα inhibitory and anti-HIV-1 flavones from leaves and twigs of Gardenia carinata. Fitoterapia. 2011; 83:368-372.
56. Pinto MMM, Sousa ME, Nascimento MSJ. Xanthone derivatives: New insights in biological activities. Curr Med Chem. 2005; 12:2517-2538.
57. Jun KY, Lee EY, Jung MJ, Lee OH, Lee ES, Choo HYP, Na Y, Kwon Y. Synthesis, biological evaluation, and molecular docking study of 3-(3'-heteroatom substituted-2'-hydroxy-1'-propyloxy) xanthone analogues as novel topoisomerase IIα catalytic inhibitor. Eur J Med Chem. 2011; 46:1964-1971.
58. Liu Y, Zhao C, Li H, Yu M, Gao J, Wang L, Zhai Y. Cytotoxicity and apoptosis induced by a new podophyllotoxin glucoside in human hepatoma (HepG2) cells. Can J Physiol Pharmacol. 2010; 88:472-479.

(Received December 10, 2011; Revised April 15, 2012; Re-revised August 17, 2012; Accepted October 26, 2012)