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The DNA-topoisomerase Inhibitors in Cancer Therapy

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DNA-topoisomerases are ubiquitous enzymes essential for major cellular processes. In recent years, interest in DNA-topoisomerases has increased not only because of their crucial role in promoting DNA replication and transcription processes, but also because they are the target of numerous active ingredients. The possibility of exploiting for therapeutic purposes the blocking of the activity of these enzymes has led to the development of a new class of anticancer agents capable of inducing apoptosis of tumor cells following DNA damage and its failure to repair.

Keywords: Campothecins; Inhibitors; Topoisomerases.

The recent knowledge of the mechanisms involved in the process of tumor transformation and progression and the recognition of the proteins involved in the regulation of these processes, has opened a new era in the formulation and clinical evaluation of new drugs giving more and more importance to those drugs that act with a mechanism involving nucleic acids, in particular DNA.

DNA-topoisomerases are ubiquitous enzymes that exhibit both nuclease and ligase activity. In fact, these proteins are essential for major cellular processes, such as replication, transcription, DNA duplication, chromatin assembly, chromosome segregation, and are also able to modify the topological properties of DNA by regulating, for example, the level of supercoiling of the double helix[1,2].

The three-dimensional structure of DNA in space is in fact controlled and regulated during the processes of coiling, linearization and supercoiling by DNA-topoisomerases.

After the first DNA-topoisomerase was purified in 1971 from *Escherichia coli*[3]. Since then, these enzymes have been identified in all eukaryotic and prokaryotic cells and in some viruses and bacteriophages[4-7]. The known topoisomerases have been grouped according to their mechanism of action and chemical/physical properties essentially into two classes: class I enzymes and class II enzymes. Within these classes, further subfamilies defined on the basis of structural considerations are distinguished[8-13].

Class I enzymes generally consist of a monomer and are capable of giving a break on a single strand of the DNA double helix, relaxing the DNA one turn at a time. This reaction is catalysed by the enzyme, by trans-esterification with a tyrosine and does not require an energy input in the form of ATP but uses the torsional energy of the supercoiled nucleic acid.

Class II enzymes, consisting of two or more subunits are capable of introducing cuts on both strands of the DNA double helix to unwind

it. The cutting of the two strands occurs by trans-esterification between a pair of tyrosines and two phosphodiester bonds facing each other; these two reactions occur in concert. The tyrosine residues result covalently bound to the 5' ends of the cleaved filaments, leaving the hydroxyls of the 3' positions free. Subsequently, conformational changes in the enzyme cause the 5' ends (bound) to move away from the 3' ends (free), thus opening a gate in the cut double helix. At this point, the enzyme transports an intact double strand through the opening created in the double helix, which is then closed again [9,10]. Finally, phosphodiester bonds are reformed by further trans-esterification. The result is a two-unit change in the DNA binding number.

In this case, the trans-esterification reaction proceeds only in the presence of ATP because the energy for this reaction is provided by the cleavage of a phosphodiester bond of an ATP molecule that binds as a cofactor to the inactive form of the protein[9].

In recent years, interest in DNA-topoisomerases has increased not only because of their crucial role in maintaining the topological state of DNA 1, which consists mainly in promoting the processes of replication and transcription (by relaxing the supercoils of the chains of this nucleic acid), but above all because they are the target of numerous active ingredients[14]. The possibility of exploiting these characteristics for therapeutic purposes has led to the development of a new class of anticancer agents capable of interfering with or inhibiting at least one of the phases of the catalytic cycle of these enzymes by means of two main mechanisms: a) stabilization of the covalent topoisomerase-DNA complex ("cleavage complex") with the formation of a ternary drug-topoisomerase-DNA complex, and consequent inhibition of DNA double helix reunification; b) inhibition of the catalytic cycle of the enzyme without direct intervention on the covalent topoisomerase-DNA complex.

The formation of the topoisomerase-DNA covalent complex and the consequent inhibition of topoisomerases lead to dramatic changes in vital mechanisms by triggering apoptosis. In fact, the enzyme-DNA complex interferes with the metabolism of nucleic acids and leads to irreversible DNA lesions, which constitute an activation signal for the production of the oncosuppressor gene p53[14-19].

This gene is normally capable of blocking growth in cells where DNA damage has occurred, encouraging repair. If DNA repair is successful the cycle can resume, otherwise the process of programmed death is initiated. A further classification of topoisomerase inhibitors is made on the basis of the target enzyme on which these drugs act, so they are usually referred to as topoisomerase I inhibitors, topoisomerase II inhibitors and gyrase (bacterial topoisomerase) inhibitors.

Topoisomerase I inhibitors

Camptothecins

The progenitor of topoisomerase I inhibitors is the natural pentacyclic alkaloid camptothecin (CPT), isolated from the *Camptotheca acuminata* tree[20-22]. Although its discovery dates back to the 1960s, the identification of this molecule as an anticancer agent is much more recent. Camptothecin is a non-competitive inhibitor of topoisomerase I, which acts by intercalating in the covalent topoisomerase I-DNA complex in a reversible manner.

In fact, the antitumor activity of this molecule is due to its ability to intercalate in the cleavage complex and to stabilize it by forming the ternary complex camptothecin-topoisomerase I-DNA, thus preventing the re-welding reaction of DNA strands and inducing the accumulation of "cleavage complex"[23-26].

Immediately after the cutting step performed by the enzyme, the camptothecin molecule intercalates between the DNA bases, so the enzyme can no longer proceed with the binding step, and remains locked around the DNA itself[27]. The cleavage of a DNA strand, previously carried out by topoisomerase, thus becomes permanent, resulting in premature termination of replication and inhibition of transcription.

Despite camptothecin's efficacy as an anticancer agent, its chemical instability at physiological pH, due to rapid conversion from the lactone form with activity to a more soluble but inactive carboxylated form, as well as its poor solubility and high dose-limiting toxicity (DTL), have greatly limited its clinical use[28]. In an attempt, therefore, to improve the pharmacokinetic profile

in particular and to broaden or even diversify the spectrum of activity with respect to tumor type, numerous structural modifications of this drug have been made[29-33]. Recent studies have shown that substitutions made at the C-7 and C-9 position do not alter the activity of the drug, just as the addition of an ethyl group at the C-7 position or a hydroxyl group at the C-10 position increases the inhibitory capacity of this compound[34-36]. On the other hand, substitutions in C-11, C-12 and on the E ring eliminate drug activity, which allows us to hypothesize their involvement in the interaction with the cleavage complex[37-40].

These results have allowed the synthesis and development of new camptothecin-like semi-synthetic derivatives that are more water-soluble and have fewer side effects.

Among the semi-synthetic derivatives currently used in the treatment of human carcinomas, topotecan and irinotecan (CPT-11) are of great interest. In particular, topotecan is characterized by the presence of a dimethylaminomethyl substitution in the C-9 position and a hydroxyl substitution in the C-10 position, which make it more water-soluble without altering its therapeutic efficacy[41,42]. In contrast, irinotecan (CPT-11) is a semisynthetic analogue containing an o-carbonyl-1-(4-piperidino)-piperidine side group in the C-10 position and an ethyl group in the C-7 position[43-47]. From irinotecan, side-chain cleavage by endogenous carboxylesterases results in the formation of its active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), which is approximately one thousand times more potent[48-52].

Indolocarbazole derivatives

Indolocarbazoles derived from the antibiotic *Rebeccamycin* represent an important group of anticancer agents. In fact, several indolocarbazoles are currently in clinical trials[53,54]. These compounds inhibit topoisomerase I causing DNA breaks that are responsible for cell death. Unlike camptothecin, glycosyl-indolocarbazoles can form stable complexes with DNA even in the absence of the enzyme.

Among these derivatives, edotecarin is a drug that showed good activity.

It is an inhibitor of the enzyme topoisomerase I, which induces single-stranded DNA cleavage leading to the formation of topoisomerase I-DNA complexes that are more stable than those induced by camptothecin (CPT) or other synthetic indolocarbazole derivatives such as NB-506[55-58].

Naphthoquinone derivatives

Recently, experimental data have shown that some naphthoquinone derivatives, both synthetic and natural, are also potent inhibitors of topoisomerase I. This activity seems to be due to the presence of phenolic hydroxyls considered indispensable for the inhibitory capacity against topoisomerase I.

Among these, the natural naphthoquinone shikonin and some of its esters exhibit interesting in *vitro* anticancer activity, when compared to that of camptothecin.

Although the information in the literature is not sufficient to define the mechanism of action of shikonin and its analogues, it appears from studies conducted so far that they inhibit the topoisomerase I enzyme not by intercalation with DNA but by direct interaction of the active ingredient with the enzyme itself[59,60].

New Topoisomerase I inhibitors

Among the new topisomerase I inhibitors active through a mechanism of intercalation in DNA, a relevant role belongs to nemorubicin (MMDX), a third-generation anthracycline derivative[61]. Nemorubicin has been shown to be effective on a broad spectrum of tumor models, significantly different from those on which other anthracyclines are active.

Unlike, for example, doxorubicin, nemorubicin is also highly cytotoxic to a variety of tumor cell lines that exhibit a multi-resistant phenotype both in *vitro* and in *vivo* to the aforementioned anthracycline and is not cardiotoxic at therapeutic doses[62-65].

Further clinical studies (Phase I/II studies) are currently underway to confirm its clinical efficacy.

Topoisomerase II Inhibitors

These inhibitors are divided, based on their mechanism of action, into intercalating agents (e.g., doxorubicin) and non-intercalating agents, such as epipodophyllotoxins (e.g., etoposide and teniposide).

DNA topoisomerases II compared to topoisomerases I are targets of a broader and more diverse class of antineoplastic compounds. Examples of inhibitors of such enzymes are:

the amsacrines in particular the *m-amsacrine;* actinomycins such as *actinomycin D*, an anticancer antibiotic used mainly for sarcomas anthracyclines, in particular *adriamycin* (*doxorubicin*), one of the most widely used anticancer drugs in chemotherapy for both solid and hematological tumors; *mitoxantrone*, an anthraquinone effective in malignant haemopathies and sensitizing to the effects of radiation; non-intercalating derivatives of epipodophyllotoxin: *etoposide* and *teniposide*.

The Amsacrines

Amsacrine and *m-amsacrine* are acridine derivatives that can inhibit topoisomerase II by intercalation at the major and minor DNA grooves, with which they form a sufficiently stable complex to resist until the DNA enters the topoisomerase II enzyme pocket[66].

The *m-amsacrine*, intercalates parallel to the axis of the DNA skeleton with the acridine nitrogen in the center of the major groove and the methanesulfonyl chain located in the minor groove. The DNA helix axis passes directly through the center of the acridine ring, so the tricyclic chromophore is surrounded by bases on either side. In this region, *m-amsacrine* allows the DNA to be cut but not rinsed, thus interrupting the catalytic cycle[67].

This drug, administered intravenously, has activity and toxic effects similar to those of doxorubicin. It is mainly used in acute myeloid leukemia. Toxic effects include: myelodepression and mucositis; there have also been cases of fatal arrhythmias due to hypokalemia[68].

Actinomycins

Actinomycins are chromopeptides: most of them contain the same chromophore, the planar phenoxazone actinocin, which is responsible for the yellow-red colour of these compounds. The differences between natural and synthetic actinomycins are limited to the peptide side chains and in particular to the structure of their amino acids.

Among the actinomycins, the antibiotic actinomycin *D* is important. This is a chemotherapeutic agent produced by three types of bacteria belonging to the *Streptomyces* species, which has a remarkable cytotoxic action. In fact, it inhibits all rapidly proliferating cells, and for these reasons it is among the most powerful substances with antitumor activity[69].

The biological activity and cytotoxicity of actinomycins are due to multiple mechanisms of action such as: formation of fragments of single DNA strands due to inhibition of the enzyme topoisomerase II or probably due to the formation of an intermediate free radical; ability to bind to the DNA double helix by intercalation of the planar phenoxazone ring between adjacent guanine-cytosine pairs; inhibitory capacity against topoisomerase I enzyme[70]. Due to the binding of dactinomycin to the DNA double helix, transcription of DNA by RNA-polymerase is blocked. The main clinical use of dactinomycin is in the treatment of paediatric neoplasms, in particular, rhabdomyosarcoma and Wilms' tumour, where it is curative in combination with primary surgery, radiotherapy and other drugs such as vincristine and cyclophosphamide. A neoplastic activity was observed in Ewing's tumor, tissue sarcomas may be effective in women with advanced choriocarcinomas, in patients with Metastatic carcinoma of the testis in combination with chlorambucil and methotrexate; is poorly effective in other adult malignancies[71].

Anthracyclines

Anthracyclines are considered among the most effective anticancer drugs belonging, like dactinomycin, to the category of cytotoxic antibiotics. This is a group of drugs, isolated from *Streptomyces peucetius* cultures, whose antineoplastic and cytotoxic actions derive from the superimposition of multiple mechanisms of cellular damage with the final result of apoptosis[72]. Anthracyclines act mainly as intercalants,63 by sliding their cyclic planar structure perpendicularly between two nucleotide pairs of the DNA helix. The result is a partial unwinding of the DNA double helix with subsequent blockade of DNA, RNA, and protein synthesis or all three[73].

The generation of free radicals by this drug may contribute, but is not the primary cause, of the antineoplastic effect. However, this process has been shown to play a role in the cardiac toxicity caused by these drugs. The first anthracycline derivatives to be discovered and used in therapy were *doxorubicin* (or adriamycin) and *daunorubicin* (or daunomycin).

The main mechanism of action by which anthracyclines exert their cytotoxic action is their intercalation activity. The presence of an

intercalating agent in the DNA also disrupts the action of topoisomerases, thus preventing the two chains from coiling. After intercalating in the double helix, anthracyclines are located at the interface between the active site of topoisomerase II and the DNA cleavage site, interacting with both. Doxorubicin, for example, interacts with topoisomerase II, which is trapped on the DNA by covalent bonding, thus forming a stable ternary complex: drug-enzyme-DNA, which makes it more difficult to reunite the strands. Other anthracycline derivatives used in clinical practice are: *epirubicin* and *idarubicin*[74]. Epirubicin is a structural derivative of doxorubicin; clinical studies suggest that as such it is equally effective in the treatment of breast cancer. Idarubicin, a synthetic derivative of daunorubicin, is, together with daunorubicin, among the most effective chemotherapeutic agents in the treatment of acute leukemia[75-79].

Mitoxantrone

Mitoxantrone is a synthetic compound having a tricyclic structure with two side chains.

Being a structural analogue of doxorubicin, like anthracyclines, it intercalates in DNA interfering with the function of topoisomerase II.

In more detail, *in vivo,* mitoxantrone accumulates in the cell nucleus and acts as a classical intercalating agent by inserting itself perpendicular to the major axis of DNA base pairs due to its planar molecular portion.

Experimental evidence shows that this also acts as a poison for topoisomerase II by stabilizing the topoisomerase II-DNA complex and uncoupling the catalytic activity of such enzyme[80,81].

Unlike anthracyclines, it does not have the ability to produce free radicals and was found to be much less cardiotoxic than doxorubicin.

It is used for the treatment of certain forms of acute non-lymphocytic leukemia, in lymphomas and breast cancer as well as hormone-resistant prostate cancer[82].

Non-intercalating agents such as topoisomerase II inhibitors

Epipodophyllotoxin derivatives: etoposide and teniposide

Etoposide (VP-16) and teniposide (VM-26) are semisynthetic derivatives of podophyllotoxins extracted from the herbaceous plant *Podophyllum peltatum* growing in the southern United States[83-86].

Unlike podophyllotoxins, which like vinca alkaloids bind to tubulin, epipodophyllotoxins are potent non-intercalating inhibitors of topoisomerase II. Specifically, etoposide exerts its antitumor effect by causing irreversible DNA damage through inhibition of the topoisomerase II enzyme[87].

Inhibition of this enzyme, which normally during the replication phase promotes the uncoiling/ rewinding of the double helix, thereby reducing the stresses caused by the uncoiling of the molecule itself by its temporary breakage, leads to a non-repairable breakage of cellular DNA, consequently preventing the reunification of the two cut strands[88].

Etoposide is an antineoplastic drug to be used alone or in combination with other antineoplastic drugs. According to currently available data, this drug is indicated in the treatment of small cell lung cancer and testicular cancer.

Teniposide, in proportion to the dosage administered, causes single- or double-strand breaks in DNA as well as the formation of "cross-links" between DNA and proteins. Teniposide, like etoposide, also acts by directly inhibiting the topoisomerase II enzyme because it neither intercalates in nor binds firmly to DNA. Its cytotoxic effects are commensurate with the number of double-strand breaks produced in the cells; each break corresponds to an interruption in the action of topoisomerase II upon formation of the DNA-topoisomerase II intermediate. Teniposide is used primarily in the treatment of pediatric leukemia[89-91].

The Genistein

Genistein is an isoflavone first isolated in 1899 from the plant *Genista tinctoria*[92].

This active ingredient, present in many plants (beans, soybeans, etc..) in addition to acting as an antioxidant and anthelmintic also has an antineoplastic activity expressed through different mechanisms.

Ginestein is primarily an inhibitor of protein-tyrosine kinase. It binds and inhibits this enzyme by disrupting signal transduction and inducing cell differentiation[93]. It is also able to block the uncontrolled growth of tumor cells, both by inhibiting the activity of growth factors, which in the body regulate cell division and survival, and

by inhibiting topoisomerase-II, resulting in DNA fragmentation and apoptosis by arresting the G2/M phase of the cell cycle.

Several studies have shown that moderate doses of genistein may have inhibitory effects on prostate, cervical, breast and colon cancers. It also appears to be able to make some tumor cells more sensitive to radiotherapy[94].

CONCLUSIONS

Research conducted so far has shown that the first event in the action of many anticancer drugs is the binding, reversible or irreversible, to DNA. This binding can be intercalative: the drug molecule is inserted between the base pairs of the double helix, or the drug can bind a major or minor groove in the DNA, or even alkylate one or more nitrogenous bases.

This knowledge on selected "targets" through experimental models both *in vitro* and in *vivo* has allowed the synthesis of molecules with cytotoxic activity, as well as to deepen the study of their mechanism of action in order to make them selective against tumor cells only.

It is therefore important to know the mechanisms of action of anticancer drugs in order to allow their proper use in different oncological pathologies.

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Authors' contributions

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Conflict of interest

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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