

## Review Article

Indian J Med Res 123, March 2006, pp. 221-232

# Topoisomerase research of kinetoplastid parasite *Leishmania*, with special reference to development of therapeutics

Benu Brata Das, Nilkantha Sen, Somdeb Bose Dasgupta, Agneyo Ganguly, Rakhee Das & Hemanta K. Majumder

*Department of Molecular Parasitology, Indian Institute of Chemical Biology, Kolkata, India*

Received June 27, 2005

**Protozoan parasites of the order Kinetoplastida cause severe diseases primarily in the tropical and subtropical areas. The enormous development of molecular and cellular biology in recent times have provided opportunities for discovering newer molecular targets for drug designing, which now form a rational basis for the development of improved anti-parasitic therapy. DNA topoisomerases play a key role in cellular processes affecting the topology and organization of intracellular DNA. Recently, emergence of the bi-subunit topoisomerase I in the kinetoplastid family has brought a new twist in topoisomerase research related to evolution, functional conservation and as a potential target that can be exploited in drug designing and development of new intervention strategies. This review summarizes the biology of kinetoplastid topoisomerases, which are the key molecular targets in antileishmanial chemotherapy.**

**Key words** DNA topoisomerase - kinetoplastids - *Leishmania* - therapeutic targets

Leishmaniasis constitutes an array of human diseases caused by obligate intracellular protozoan parasites of genus *Leishmania*. Natural transmission of *Leishmania* is carried out by certain species of sandfly vectors of the genus *Phlebotomus* (Old World) or *Lutzomyia* (New World). The disease leishmaniasis is present in four clinical forms: (i) visceral leishmaniasis (VL), (ii) cutaneous leishmaniasis (CL), (iii) mucocutaneous leishmaniasis (MCL), and (iv) diffuse cutaneous leishmaniasis (DCL). Visceral leishmaniasis or kala-azar (KA) is primarily caused by parasites belonging to the *Leishmania donovani* complex and is widely prevalent in many tropical and subtropical regions of the world including the eastern part of India<sup>1,2</sup>.

Since the first epidemic, management of VL has been a major health problem in India. It is the most severe form of the disease and if left untreated, is usually fatal. Although confirmed cases of VL have been reported from 66 countries, 90 per cent of world's VL burden occur in Indian subcontinent and Sudan<sup>3</sup>. After recovery some patients (50% in Sudan and 1-3% in India) develop post kala-azar dermal leishmaniasis (PKDL), which requires prolong and extensive treatment. PKDL patients also play a vital role in VL transmission. With the spread of human immunodeficiency virus (HIV) and general low standard of public hygiene, the parasites pose a much greater threat than before particularly in developing countries. To make the situation even worse, some

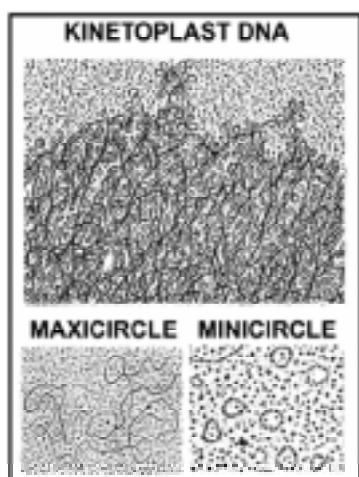
parasite strains have also developed resistance against the classical antimonial drugs, like sodium stibogluconate and megalumine antimonite. The second line of drug, amphotericin B and pentamidines, although used clinically are very toxic. Therefore, improved chemotherapy of leishmanial infection is still desirable and the need for new molecular targets on which to base the future treatment strategies is clearly justified. In search for such strategies DNA topoisomerases of *Leishmania* offer most attractive targets. The aim of this review is to provide an insight into the target based therapeutic approach against leishmaniasis.

### The unusual DNA metabolism of the kinetoplasts

*Trypanosoma* and *Leishmania* are ancient eukaryotes. The distinctive features include structurally and metabolically unusual kinetoplast DNA (kDNA) within the single mitochondrion of the organism. Unlike any other DNA in nature, kDNA is a network containing thousands of DNA minicircles topologically interlocked into an elliptical shape of about 10-15  $\mu\text{m}$  in size and arranged in a planar array. The isolated networks can be easily viewed by electron microscopy (EM) (Fig. 1). It appears to have a fairly regular structure and accounts for 5-20 per cent of the total cellular DNA. It has a relatively high AT content

with a molecular weight up to  $4 \times 10^{10}$  daltons. Each kDNA network comprises two types of DNA molecules, minicircles and maxicircles. About 90 per cent of the kDNA is made up of minicircles. Each minicircle is joined to three other minicircles<sup>4</sup> and a single interlock links the minicircles to one another within the network. They are generally heterogenous in sequence, but are usually homogenous in size (commonly 0.5-2.5 kb, depending on the species). In *Leishmania*, they are about 830 bp. Minicircles do not appear to encode information for protein but replicate very well. The genetic function of the minicircles are now known to encode small guide RNAs (gRNAs), of about 70 nt in size with a short oligo-U tail at the 3' end, that governs the specificity of mRNA editing<sup>5</sup> in these parasites. Although the kDNA network structure has no counterpart in nature, there is functional similarity with conventional mitochondrial DNA from higher eukaryotes. Maxicircles resemble mitochondrial DNA of other eukaryotes in size (20-40 kb, depending on the species) and in their transcripts, including rRNAs and mRNAs for several subunits of respiratory complexes. Unlike other mitochondrial DNAs, the maxicircles do not encode tRNAs which therefore must be imported into the mitochondrion<sup>6</sup>. One of the most striking properties of maxicircles is that their transcripts undergo post-transcriptional uridine insertion and deletion to create functional open reading frames, a process termed as RNA editing. The kDNA network is a highly dynamic structure with topological interlocks between the DNA circles, which are continuously broken and re-attached.

During kDNA replication the DNA content of the network doubles and the progeny networks partition into the two daughter cells. kDNA replication occurs in approximate synchrony with nuclear DNA synthesis<sup>7,8</sup>, unlike mitochondrial DNA of other species whose replication occurs throughout the cell cycle<sup>9</sup>. It is clear that the minicircles actually detach from the network for replication and there is significant network remodeling during and after replication. Free minicircles, replicate unidirectionally as theta ( $\theta$ ) structure. A vital feature of kDNA replication is that the minicircles can replicate only when they are released from their network. DNA topoisomerases from the kinetoplast parasites plays a key role in many aspects of nucleic



**Fig. 1.** Electron microscopy of *Leishmania donovani* (MHOM/IN/1978/UR6) kDNA networks (left panel). kDNA network treated with the topoisomerase II enzyme under standard decatenation assay condition as described by Das *et al*<sup>45</sup>. Decatenated maxicircles and minicircles are indicated respectively.

acid metabolism. DNA topoisomerase II has been implicated in this process, where it decatenates the kDNA network into minicircles prior to replication<sup>10</sup>. Recently, it has been reported that the *L. donovani* topoisomerase I enzyme is located both in the nucleus and the kinetoplast<sup>11</sup>, thus revealing a probable role of the enzyme in kDNA metabolism.

### DNA topoisomerases: the cell's magician

DNA topoisomerases are ubiquitous enzymes that play a pivotal role in modulating the dynamic nature of DNA secondary and higher order structures and thus carrying out essential functions inside the cell. These functions relate mainly to nucleic acid metabolism namely replication, transcription, recombination and repair<sup>12,13</sup>. All known topoisomerases share two characteristics: (i) their ability to cleave and resealed the phosphodiester backbone of DNA in two successive transesterification reactions; and (ii) once a topoisomerase cleaved DNA intermediate is formed, the enzyme allows the severed DNA ends to come apart, opening a gate for the passage of another single- or double-stranded DNA segment.

### Classification of DNA topoisomerases

DNA topoisomerases can be classified into three evolutionary independent families: type IA, type IB and type II. Those that cleave one strand of DNA and allow single step changes in the linking number of circular DNA are defined as type I DNA topoisomerases. The *Escherichia coli* topoisomerase I and topoisomerase III, *Saccharomyces cerevisiae* topoisomerase III and reverse gyrase belong to the type IA or type I-5' subfamily as the protein link is to a 5' phosphate in the DNA. The prototype of type IB or I-3' enzymes are found in all eukaryotes and also in vaccinia virus topoisomerase I where the protein is attached to a 3' phosphate<sup>14</sup>. Though essentially similar in their action, these enzymes have a broader specificity than that of *E. coli* enzyme. Despite the differences in the mechanism and specificity between the bacterial and eukaryotic enzymes, the yeast DNA topoisomerase I has been shown to functionally complement a bacteria mutant in DNA topoisomerase I<sup>15</sup>. A certain degree of

divergence also exists in the substrate preference, co-factor requirement and subunit composition of different topoisomerase families. Type IA topoisomerases are able to relax only negatively supercoiled DNA and require magnesium and single-stranded stretch of DNA for their function. Topoisomerases IB, however, are able to relax both positively and negatively supercoiled DNA with equal efficiency and do not require a single-stranded region of DNA or metal ions for function<sup>16</sup>.

The type II family includes *E. coli* DNA gyrase, *E. coli* topoisomerase IV (par E), all known eukaryotic type II topoisomerases and archaic topoisomerase VI. Type II enzymes are homo dimeric (eukaryotic topoisomerase II) or tetrameric (gyrase), cleaving both strands of a duplex that changes in linking number in steps of two. The current mechanistic model for topoisomerase II catalysed reactions involves the binding of two segments of DNA: a G (gate) segment, which is cleaved in both strands by the enzyme with the formation of an ester bond between active tyrosines and 5'-phosphates in the DNA and a T (transport) segment, which is captured by an ATP operated clamp that passes through the enzyme-stabilized break in the G segment<sup>17,18</sup>.

The discovery of several new DNA topoisomerases has brought a deeper understanding of their important roles in living cells. The biological functions of DNA topoisomerases are deeply rooted in the double helical structure of DNA and the selection of double stranded DNA as substrate has set the stage for their entrance<sup>19</sup>. Broad classifications of the different types of topoisomerases in different organisms are represented in the Table.

Because DNA topoisomerases play key roles in cellular processes, affecting the topology and organization of intracellular DNA, it is important to define the physiological functions and understand the molecular basis of their action. Moreover, beyond their normal cellular activities, these enzymes are proven molecular targets for clinically useful antitumor<sup>20-22</sup> and antimicrobial drugs<sup>23-25</sup>. In this context work on topoisomerases from the parasites has been a growing focus of interest.

**Table.** Classification of type I and type II DNA topoisomerases from different species

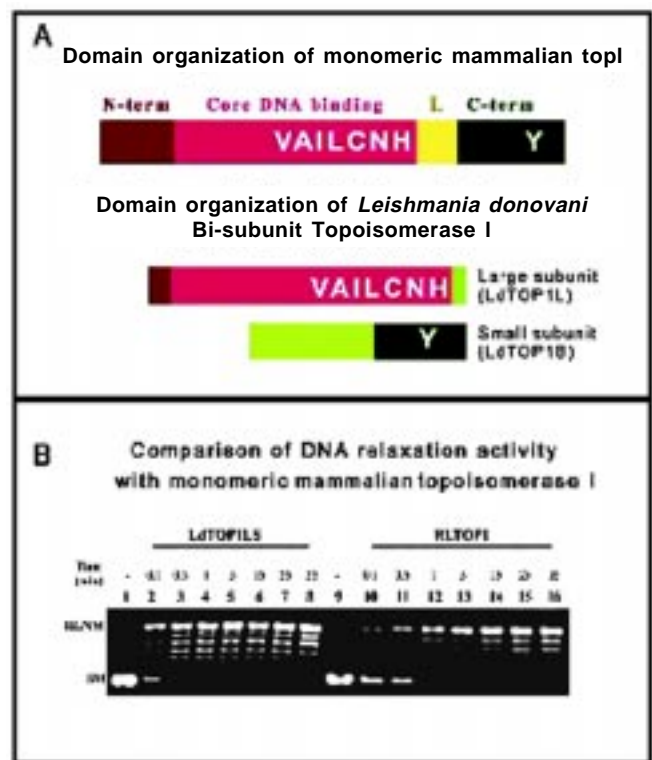
Subfamily	Representative members
1A	Bacterial DNA topoisomerase I & II, Yeast DNA topoisomerase III, Drosophila DNA topoisomerase III $\alpha$ and III $\beta$ Mammalian DNA topoisomerase III $\alpha$ and III $\beta$
1B	Eukaryotic monomeric topoisomerase I, Mammalian mitochondrial DNA topoisomerase I, Kinetoplastida Bi-subunit DNA topoisomerase I, Pox virus topoisomerase I
IIA	Bacterial gyrase, DNA topoisomerase IV, Phage T4, DNA topoisomerase, Yeast DNA topoisomerase II, Drosophila DNA topoisomerase II, Mammalian DNA topoisomerase II $\alpha$ and II $\beta$
IIB	<i>Sulfolobus shibate</i> DNA topoisomerase VI (subunit A homologous to yeast SPO11)

Toxic chemotherapy, increasing drug resistance of some parasite strains to classical drugs along with co-infection of *Leishmania* with HIV, have made them a severe threat to public health in developing countries. Development of vaccines is still under trial and improved therapy desirable.

### DNA topoisomerases of the kinetoplastid parasites

**Type I DNA topoisomerase:** All eukaryotic type IB topoisomerase is monomeric and consists of four domains<sup>26</sup>. The unconserved amino terminal domain contains putative signals for nuclear localization of the enzyme and is highly sensitive to proteolysis and dispensable for *in vitro* activity<sup>27</sup>. The largest core domain is essential for enzyme activity and shows high phylogenetic conservation, particularly in the amino acid residues interacting closely with DNA. The third domain is known as the linker, which is poorly conserved and is variable in length. Finally, the carboxy terminal domain is highly conserved and contains the SKINYL motif. Cleavage occurs by trans-esterification reaction involving nucleophilic attack by an active site tyrosine (Tyr 723 in human Topo I) on a DNA phosphodiester bond resulting in the formation of a covalent DNA 3' phosphotyrosyl linkage. In relegation, phase, a similar trans-esterification reaction involves attack by the free DNA 5' hydroxyl that releases the enzyme from DNA<sup>27,28</sup>.

DNA topoisomerase I of kinetoplastid protozoan parasite *L. donovani* is distinct from other eukaryotic counterparts with respect to its biological properties and preferential sensitivity to many therapeutic agents<sup>29</sup>. The first DNA sequence of topoisomerase IB like gene from kinetoplastid *L. donovani* was reported by Broccoli *et al*<sup>30</sup>, but the overexpressed protein in *E. coli* failed to show any relaxation activity *in vitro* or complement a mutant deficient in topoisomerase I activity. The consensus SKXXY motif harbouring the active site tyrosine was absent in this protein (Fig. 2A).

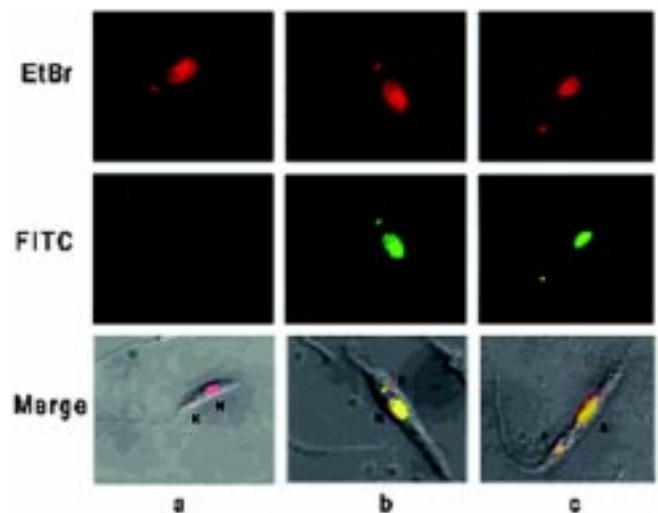


**Fig. 2 A.** Schematic representation of the domain organization of monomeric mammalian topoisomerase I and *Leishmania donovani* bi-subunit topoisomerase I. The enzymes contain a central DNA binding core domain and a C-terminal catalytic domain harbouring SKINYL motif. The linker domain (L), which is poorly conserved and variable in length, links the two domains. But, in *L. donovani* topo I the core DNA binding domain (LdTOP1L) and the catalytic domain (LdTOP1S) harbouring the consensus SKXXY motif lies in separate subunits. **B.** Under standard plasmid relaxation assay condition reconstituted enzyme (LdTOP1LS) showed reduced processivity as well as 2 fold reduced affinity for DNA compared to eukaryotic monomeric rat liver topoisomerase I (RLTOP1) as described by Das *et al*<sup>11</sup>.

Recently, emergence of the bi-subunit topoisomerase I of *Leishmania*<sup>31</sup> in the kinetoplastid family has brought a new twist in topoisomerase research related to evolution and functional conservation of type IB family. The core DNA binding domain, LdTOP1L (73 kDa) and the catalytic domain LdTOP1S, (29 kDa) harbouring the consensus SKXXY motif lies in separate subunits. The two subunits are synthesized by two different genes, which associate with each other through protein-protein interaction to form an active heterodimeric topoisomerase I within the parasite. This unusual structure of DNA topoisomerase I may provide a missing link in the evolution of type IB enzyme. Another report<sup>32</sup> from *Trypanosoma brucei* also shows that the topoisomerase I activity is comprised of two subunits encoded by two different genes, one for the 90 kDa DNA binding domain and the second for the 36 kDa C-terminal catalytic domain harbouring the conserved pentad with the nucleophilic tyrosine<sup>32</sup>. However, both the groups were unsuccessful in the *in vitro* reconstitution of the enzyme.

Das *et al*<sup>11</sup> has shown for the first time the *in vitro* reconstitution of bi-subunit topoisomerase I of *L. donovani*. The reconstituted enzyme (LdTOP1LS) is characterized by a direct 1:1 molar interaction between the large (LdTOP1L) and the small (LdTOP1S) subunits. Under standard relaxation assay condition reconstituted enzyme showed reduced processivity as well as 2-fold reduced affinity for DNA compared to eukaryotic monomeric rat liver topoisomerase I (Fig. 2B). The enzyme is conventional in its Mg<sup>+2</sup> independence, and binds with eukaryotic type 1B specific recognition site. Camptothecin (CPT) enhanced the formation of 'cleavable complex' at low salt<sup>11</sup>. A precise insight into the intracellular location of the expressed bi-subunit topoisomerase I proteins in *L. donovani* was gained by indirect immunofluorescence experiments. A polyclonal antiserum raised against a conserved portion of LdTOP1L and full length LdTOP1S showed nuclear and kinetoplast localizations for *L. donovani* topoisomerase I protein (Fig. 3). This laboratory has shown the importance of the large subunit which resembles the core DNA binding domain with an

identity score of 37 per cent and a similarity score of 49 per cent with human topoisomerase I. Two N-terminal truncation constructs, LdTOP1Δ39L (lacking 1-39 amino acids) and LdTOP1Δ99L (lacking 1-99 amino acids) of the large subunit were generated and mixed with intact small subunit (LdTOP1S). The results suggest that 1-39 amino acid residues of the large subunit (LdTOP1L) of the unusual bi-subunit enzyme regulates DNA dynamics during relaxation by controlling non-covalent DNA binding or by co-ordinating DNA contacts by other parts of the enzyme and camptothecin (CPT) sensitivity. The residues within 40-99 amino acid region of LdTOP1L appear to be important in relation to interaction with LdTOP1S<sup>33</sup>. CPT induces cellular dysfunction in *L. donovani* promastigotes and amastigotes with features that are well characterized by several cytoplasmic and nuclear events of apoptosis<sup>34,35</sup>.



**Fig. 3.** Immunocytochemical localisation with anti-LdTOP1L and LdTOP1S antiserum using fluorescent detection methods. Late log phase *L. donovani* promastigotes were fixed. No fluorescence was observed when pre-immune serum was used as primary antibody and FITC-tagged secondary antibody (Panel a) as described by Das *et al*<sup>11</sup>. Panel b, same as Panel a, but probed with anti-LdTOP1L. Panel c, same as panel a, but probed with anti-LdTOP1S primary antibody. Parasite cells were also stained with ethidium bromide to locate the nucleus and kinetoplast and the area of the overlapping fluorescein isothiocyanate (FITC) and ethidium bromide (EtBr) stain are shown in merged pictures. Cells were viewed at an original magnification of 100 X under a Leica DM IRB inverted microscope. The nucleus (N) and kinetoplast (K) are indicated.

*Type II DNA topoisomerase:* Active topoisomerase II has been purified from *Crithidia fasciculata*<sup>36-38</sup>, *T. cruzi*, *T. equiperdum*<sup>39</sup> and *L. donovani*<sup>40,41</sup>. Intriguingly *L. donovani* and *T. cruzi* are reported to have both ATP-dependent and ATP independent decatenating activities, though the artifactual occurrence of ATP independence on the basis of partial proteolysis remains a possibility<sup>42</sup>. Two apparently distinct mitochondrial topoisomerases have been isolated from *C. fasciculata*, one consisting of four 60 kDa subunits<sup>38</sup> and the other a dimer of 132 kDa<sup>43</sup>. Polyclonal antibody to the dimer does not recognize the enzyme with four 60 kDa subunits.

Genes for topoisomerase II have been isolated and sequenced from kinetoplastid organisms, *C. fasciculata*<sup>44</sup>, *L. donovani*<sup>45</sup>, *T. brucei*<sup>46</sup>, *T. cruzi*<sup>47</sup> and *Bodo saltans*<sup>48</sup>. Although smaller than the genes reported from other eukaryotes, they share the expected functional domains and are more homologous to eukaryotic than prokaryotic type II enzymes with 30-35 per cent identity and 45-65 per cent similarity to human topoisomerase II. The predicted proteins are 137-160 kDa in size. The recombinant *L. donovani* enzyme showed ATP-dependent decatenating activity and could complement a temperature sensitive topoisomerase II mutant of *Saccharomyces cerevisiae*<sup>49</sup>, just like the protein from a related parasite, *L. infantum*<sup>50</sup>. A precise insight into the intracellular location of the expressed topoisomerase II protein in *L. donovani* was gained by indirect immunofluorescence. A polyclonal antiserum raised against a conserved portion of LdTOP2 showed nuclear and kinetoplast localizations for *L. donovani* topoisomerase II protein<sup>45</sup>. Recently topoisomerase II from primitive kinetoplastid *B. saltans* was also found to localize both in the nucleus and kinetoplast<sup>48</sup>. Evidences demonstrate a nuclear localization for *T. cruzi* as well as *C. fasciculata* type II enzymes<sup>51</sup>. On the contrary, a type II enzyme of 132 kDa has been found to be immunolocalized at the antipodal sites of kinetoplast of *C. fasciculata*<sup>43</sup> which also colocalizes with DNA polymerase  $\beta$ <sup>52</sup>. This type II enzyme probably facilitates minicircle replication or could be involved in the attachment of the newly replicated minicircles to the network<sup>52</sup>. It was shown that monoclonal antibodies raised against *C. fasciculata* mitochondrial

topoisomerase II (CfTOP2mt) recognized only the mitochondrial or kinetoplast topoisomerase II counterparts in both *C. fasciculata*<sup>43</sup> and *T. cruzi* epimastigotes<sup>47</sup>. It has been hypothesized that the enzyme recognized by the anti CfTOP2mt antibodies in the kinetoplast of *T. cruzi* is the same as recognized by antiTcTOP2 serum in the nuclei of *T. cruzi* and *C. fasciculata* and differences in cellular localization might be explained in terms of which epitopes were available for the recognition by the two different antisera<sup>47</sup>. RNA interference of topoisomerase II of *T. brucei* has been shown to cause dyskinetoplasty and the major cause of progressive kDNA loss was the inefficient attachment of newly replicated minicircles to the network<sup>52</sup>.

### **DNA topoisomerases as a 'key molecular targets' for anti parasitic agents**

The dramatic advances in molecular and cellular biology have provided opportunities for discovering and evaluating molecular targets for drug designing, which now form a rational basis for development of improved antiparasitic therapy. Topoisomerases are fundamentally dualistic in nature, catalyzing essential cellular reactions and possessing an inherent dark side capable of inflicting great harm to the genome of an organism. For these reasons DNA topoisomerases have been recognized as potential chemotherapeutic targets for anti tumor and anti-parasitic agents<sup>51,52</sup>. Studies show that the parasite topoisomerases are sufficiently distinct from their human counterparts so as to allow differential chemical targeting and will therefore make good cellular targets.

### **Classification of topoisomerase inhibitors**

The known DNA topoisomerase drugs can be divided into two classes. The class I drugs act by stabilizing the covalent topoisomerase-DNA complexes and are also referred to as 'topoisomerase poisons'<sup>53-55</sup>. They include the bacterial gyrase inhibitors *e.g.*, quinolones, the DNA topoisomerase I drug camptothecin<sup>55</sup> and the DNA topoisomerase II drugs doxorubicin, amsacrine, etoposide, teniposide, quercetin<sup>56</sup> and related flavonoids<sup>56</sup>.

The stabilization of cleavable complexes by these class I poisons subsequently leads to cell death, but the process leading to it is a myriad spectra of complex pathways, namely, (i) initiation of damage induced signaling, (ii) cell cycle alterations with subsequent arrest, and (iii) engagement of the enzymatic machinery that results in apoptosis. However, a fraction of the cells treated with topoisomerase poisons survive the treatment due to efficient DNA repair mechanisms and other processes, which are still to be explored. Nonetheless, they contain an increased frequency of chromosomal aberrations and mutations. Programmed cell death after treatment of topoisomerase directed agents are brought about by (i) stress activated kinases and death receptor pathways; and (ii) caspase activation resulting in caspase mediated cleavages<sup>57-59</sup>.

The class II drugs interfere with the catalytic functions of DNA topoisomerases without trapping the covalent complexes, and are referred to as 'catalytic inhibitors'. This class of drugs includes the coumermycin family of antibiotics that act on bacterial gyrase<sup>54</sup> and the eukaryotic topoisomerase II inhibitors suramin<sup>60</sup>, fostriecin<sup>61</sup>, merbarone<sup>62</sup> and bis (2,6-dioxopiparazine)<sup>63,64</sup>. Several Class II inhibitors of eukaryotic topoisomerase I have also been reported<sup>65,66</sup>. All class II drugs mediate their action by either binding to the enzyme, which prevents them to sit on the substrate DNA, *e.g.*, merbarone<sup>62</sup> and acetyl boswellic acids<sup>67</sup> or by intercalating/binding to DNA and making it inaccessible for the enzyme, *e.g.*, aclarubicin and chloroquine<sup>68,69</sup>. Coumermycins interact with the ATPase domain of gyrase and thus interfere with the ATPase activity<sup>70,71</sup>.

To begin with, several anti-tumor drugs were used as starting compounds in preliminary screens for antiprotozoal activity. Camptothecin, a plant alkaloid, a specific inhibitor of eukaryotic topoisomerase I, that promotes cleavable complex in mammalian cells have been shown to be cytotoxic to three pathogenic kinetoplastids, *T. brucei*, *T. cruzi* and *L. donovani*. The cytotoxicity of 9-substituted-10, 11-methylenedioxy analogs of camptothecin correlates well with cleavable complex formation in the nucleus and kinetoplast, and structural motifs

have been identified that disproportionately increase toxicity to parasites, compared with mammalian cells<sup>24</sup>. Sen *et al*<sup>34</sup> has demonstrated that CPT induces programmed cell death (PCD) both in the amastigotes and promastigotes form of *L. donovani* parasites. CPT-induced cellular dysfunction in *L. donovani* promastigotes is characterized by several cytoplasmic and nuclear features of apoptosis. CPT inhibits cellular respiration that results in mitochondrial hyperpolarization taking place by oligomycin-sensitive F0-F1 ATPase-like protein in leishmanial cells. During the early phase of activation, there is an increase in reactive oxygen species (ROS) inside cells, which causes subsequent elevation in the level of lipid peroxidation and decrease in reducing equivalents like reduced glutathione (GSH). Endogenous ROS formation and lipid peroxidation cause eventual loss of mitochondrial membrane potential. Further, cytochrome c is released into the cytosol in a manner independent of involvement of CED3/CPP32 group of proteases and unlike mammalian cells it is insensitive to cyclosporin A. These events are followed by activation of both CED3/CPP32 and ICE group of proteases in PCD of *Leishmania*. These studies provide information that could be exploited to develop newer potential therapeutic targets<sup>34</sup>.

Other inhibitors of type IB topoisomerase activity include the antileishmanial compounds such as pentavalent antimonials, sodium stibogluconate and urea stibamine, a secoirdoid glycoside, amarogentin<sup>72</sup> and a bisnaphthoquinone, diospyrin<sup>73</sup>. Diospyrin appears to be a class I inhibitor as it stabilizes topoisomerase I cleavable complex formation in *L. donovani*. One of the earliest anti-tumor compounds, the 9-anilinoacridines and other acridine derivatives that act by topoisomerase II poisoning was shown to possess potent antileishmanial and antitrypanosomal activities<sup>74</sup>. These compounds stabilized cleavable complexes with both nuclear and kinetoplast topoisomerase II in *L. chagasi*, however, they also exhibited cytotoxicity against the human enzyme and were not selective agents<sup>75</sup>. Selective targeting of leishmanial topoisomerase II and human topoisomerase II was shown to be achieved by some mitonafide analogs<sup>76</sup>. Incubation of *T. equiperdum*

cells in presence of mammalian topoisomerase II inhibitors like epipodophyllotoxins (etoposide and teniposide), 2-methyl, 9-hydroxy-ellipticinium, acriflavine and m-AMSA resulted in release of sufficient amounts of kDNA minicircles with the enzyme linked to 5'-end of the cleaved minicircle catenane<sup>77</sup>. These results strongly implicate the kinetoplast topoisomerase of these parasites to be the intracellular target of these compounds. Classical antitrypanosomal drugs such as pentamidine, berenil and samorin were found to promote linearization of *T. equiperdum* minicircles from the kDNA networks<sup>78</sup>. These drugs were shown to act at minimum therapeutic concentrations and their selective inhibition of kinetoplast type II topoisomerase might explain why they preferentially disrupted kDNA structure and generated dyskinetoplastic trypanosomes.

Some drugs that poison bacterial type II enzymes by promoting cleavable complex formation have also been tested against trypanosomes. Among them, some tetracyclic analogs of fluoroquinolones<sup>79,80</sup> were found to exhibit two-fold greater toxicity *in vitro* towards *T. brucei* than towards L1210 leukaemia cells. The toxicity was measured by determining the extent of protein-DNA covalent complexes formed between topoisomerase II and DNA in presence of the drug<sup>79</sup>. Recently fluoroquinolones with pyrrolidinyl substitutions were found to show increased antitrypanosomal activity<sup>81</sup>. These compounds promoted trapping of protein-DNA covalent complexes, and inhibited nucleic acid biosynthesis in trypanosomes, indicating that they target the parasitic topoisomerase II enzyme. The gyrase inhibitors coumarmycin A1 and chlorobiocin were found to be effective against epimastigote and amastigote forms of *T. cruzi* but exhibited no effect against the trypomastigote forms of the parasite<sup>82</sup>. The mechanism of action of these drugs might involve antagonism of *T. cruzi* topoisomerase II, although the possibility of other molecular targets cannot be ruled out. Bacterial topoisomerase II inhibitors such as ofloxacin and its commercial derivatives were also found to block *T. cruzi* differentiation and proliferation<sup>83</sup>, and the kinetoplast organelle of these parasites might be a target of these drugs.

Oxocarboxylic acid, acridines and other gyrase inhibiting derivatives showed moderate activity towards African trypanosomes *in vitro*, that could be correlated with the parasitic topoisomerase inhibition, however no significant trypanocidal activities could be observed *in vivo*<sup>84,85</sup>.

Flavonoids that promoted topoisomerase-mediated site-specific DNA cleavage in mammalian cells *in vitro*<sup>86</sup> were recently tested for antileishmanial activity. Luteolin and quercetin were found to inhibit growth of *L. donovani* promastigotes and amastigotes and induce cell cycle arrest leading to apoptosis. These compounds also promoted topoisomerase II mediated kDNA cleavage and the cleavable complex formation induced by these compounds could be correlated with their cytotoxicity towards the parasites *in vitro*<sup>87</sup>. There are also reports of dual inhibition of the relaxation and decatenating activities of *Leishmania* topoisomerase I and II by indolylquinolene compounds at least sensitive doses from their host counterparts<sup>88</sup>. Betulinic acid, a pentacyclic triterpenoid with a wide spectrum of pharmacological properties was reported to be a potent inhibitor of DNA topoisomerase I and II<sup>89</sup>. More recently, it was reported that dihydrobetulinic acid (DHBA), a derivative of betulinic acid that exhibits anti-HIV activity<sup>90</sup>, is another excellent inhibitor of *Leishmania* DNA topoisomerase I and II<sup>91</sup> with the potential to become a lead therapeutic compound. DHBA is a potent antileishmanial agent that induces apoptosis by primarily targeting parasitic topoisomerases.

## Summary

DNA topoisomerases within the kinetoplastid parasites are primarily involved in kDNA replication, an essential event for parasitic survival. In addition, interactions between topoisomerases and other proteins which are related to mechanisms of parasite DNA replication and share common immunolocalizations, e.g., LACK antigen (*Leishmania* analogue of receptor for activated PKC), DNA polymerase  $\beta$ , and the mechanistic and functional consequence of these interactions, might be further explored to shed light on the many unanswered queries regarding the parasite biochemistry.

Topoisomerase genes and proteins characterized from these lower eukaryotes appear to share many characteristics associated with their human homologues, but certain striking differences, including different enzyme activity requirements, and different sensitivities to topoisomerase poisons provide insight for the development of topoisomerase-directed antiparasitic therapeutics. It has been established by several studies that the inhibitors of topoisomerases convert these essential enzymes into intracellular proliferating cell toxins and thereby provide a good tool for preferentially killing of the highly replicative parasite cells within the host. The interaction of the enzyme with specific inhibitors and poisons screened from natural or synthetic sources will help in the quest to selectively target the topoisomerase-based replication apparatus as a means to therapeutically control the parasitic menace in the foreseeable future.

### Acknowledgment

Authors thank Director, Indian Institute of Chemical Biology, Kolkata for constructive criticisms and interest. This work was supported by grants from the Department of Science & Technology, Government of India to HKM, University Grant Commission, Government of India to BBD with a Senior Research Fellowship.

### References

1. Tropical diseases research-A partnership. In: Eighth programme report of UNDP/World Bank/WHO for research and training in tropical diseases. Geneva: World Health Organization; 1987; p. 99-112.
2. Moadabber F. Leishmaniasis. In: TDR 1991-1992. Eleventh programme report, UNDP/ World Bank/WHO for research and training in tropical diseases. Geneva: World Health Organization; 1992 p. 77-86.
3. Ramesh V, Mukherjee A. Post-kala-azar dermal leishmaniasis. *Int J Dermatol* 1995; 34 : 85-91.
4. Chen J, Rauch CA, White JH, Englund PT, Cozzarelli NR. The topology of the kinetoplast DNA network. *Cell* 1995; 80 : 61-9.
5. Sturm NR, Simpson L. Partially edited mRNAs for cytochrome b and subunit III of cytochrome oxidase from *Leishmania tarentolae* mitochondria: RNA editing intermediates. *Cell* 1990; 61 : 878-9.
6. Schneider A, Marechal-Drouard L. Mitochondrial tRNA import: are there distinct mechanisms? *Trends Cell Biol* 2000; 10 : 509-13.
7. Cosgrove WB, Skeen MJ. The cell cycle in *Crithidia fasciculata*. Temporal relationships between synthesis of deoxyribonucleic acid in the nucleus and in the kinetoplast. *J Protozool* 1970; 17: 172-7.
8. Simpson L, Braly P. Synchronization of *Leishmania tarentolae* by hydroxyurea. *J Protozool* 1970; 17 : 511-7.
9. Clayton DA. Replication of animal mitochondrial DNA. *Cell* 1982; 28 : 693-705.
10. Shapiro TA. Mitochondrial topoisomerase II activity is essential for kinetoplast DNA minicircle segregation. *Mol Cell Biol* 1994; 14 : 3660-7.
11. Das BB, Sen N, Ganguly A, Majumder HK. Reconstitution and functional characterization of the unusual bi-subunit type I DNA topoisomerase from *Leishmania donovani*. *FEBS Lett* 2004; 565 : 81-8.
12. Wang JC. Cellular roles of DNA topoisomerases: a molecular perspective. *Nat Rev Mol Cell Biol* 2002; 3 : 430-40.
13. Cortes F, Cortes F, Pastor N, Mateos S, Dominguez I. Roles of DNA topoisomerases in chromosome segregation and mitosis. *Mutation Res* 2003; 543 : 59-66.
14. Champoux JJ. DNA topoisomerases: structure, function and mechanism. *Annu Rev Biochem* 2001; 70 : 369-413.
15. Bjornsti MA, Wang JC. Expression of yeast DNA topoisomerase I can complement a conditional-lethal DNA topoisomerase I mutation in *Escherichia coli*. *Proc Natl Acad Sci USA* 1987; 84 : 8971-5.
16. Stewart L, Ireton GC, Champoux JJ. Reconstitution of human topoisomerase I by fragment complementation. *J Mol Biol* 1997; 269 : 355-72.
17. Berger JM. Structure of DNA topoisomerases. *Biochim Biophys Acta* 1998; 1400 : 3-18.
18. Bakshi RP, Gelende S, Muniyappa K. Functional and regulatory characteristics of eukaryotic type II DNA topoisomerases. *Crit Rev Biochem Mol Biol* 2001; 36 : 1-37.
19. Wang JC. DNA topoisomerases: why so many? *J Biol Chem* 1991; 266 : 6659-62.
20. Broxterman HJ, Georgopapadakou N. Cancer research 2001: Drug resistance, new targets and drug combinations. *Drug Resist Updat* 2001; 4 : 197-209.

21. Nitiss JL. DNA topoisomerases in cancer chemotherapy: using enzymes to generate selective DNA damage. *Curr Opin Investig Drugs* 2002; 3 : 1512-6.
22. Denny WA, Baguley BC. Dual topoisomerase I/II Inhibitors in cancer therapy. *Curr Top Med Chem* 2003; 3 : 339-53.
23. Shapiro TA. Inhibition of topoisomerases in African trypanosomes. *Acta Trop* 1993; 54 : 251-60.
24. Bodley AL, Wani MC, Wall ME, Shapiro TA. Antitrypanosomal activity of camptothecin analogs. Structure-activity correlations. *Biochem Pharmacol* 1995; 50 : 937-42.
25. Bearden DT, Danziger LH. Mechanism of action of and resistance to quinolones. *Pharmacotherapy* 2001; 21 : 224S-32.
26. Stewart L, Ireton,GC, Champoux JJ. The domain organization of human topoisomerase I. *J Biol Chem* 1996; 271 : 7602-8.
27. D'Arpa P, Machlin PS, Ratrie H III, Rothfield NF, Cleveland DW, Earnshaw WC. cDNA cloning of human DNA Topoisomerase I: catalytic activities of a 67.7kDa carboxy-terminal fragment. *Proc Natl Acad Sci USA* 1988; 85 : 2543-7.
28. Liu LF, Miller KG. Eukaryotic DNA topoisomerases: two forms of type I DNA topoisomerases from HeLa cell nuclei. *Proc Natl Acad Sci USA* 1981; 78 : 3487-91.
29. Cheesman SJ. The topoisomerases of protozoan parasites. *Parasitol Today* 2000; 7 : 277-81.
30. Broccoli S, Marquis JF, Papadopoulou B, Olivier M, Drolet M. Characterization of a *Leishmania donovani* gene encoding a protein that resembles a type IB topoisomerase. *Nucleic Acids Res* 1999; 27 : 2745-52.
31. Villa H, OteroMarcos AR, Reguera RM, Balana-Fouce R, Garcia-Estrada C, Perez-Pertejo Y, et al. A novel active DNA topoisomerase I in *Leishmania donovani*. *J Biol Chem* 2003; 278 : 3521-6.
32. Bodley AL, Chakraborty AK, Xie S, Burri C, Shapiro TA. An unusual type IB topoisomerase from Africa trypanosomes. *Proc Natl Acad Sci USA* 2003; 100 : 7539-44.
33. Das BB, Sen N, Dasgupta SB, Ganguly A, Majumder HK. N-terminal region of the large subunit of *Leishmania donovani* bisubunit topoisomerase I is involved in DNA relaxation and interaction with smaller subunit. *J Biol Chem* 2005; 280 : 16335-44.
34. Sen N, Das BB, Ganguly A, Mukherjee T, Tripathi G, Bandyopadhyay S, et al. Camptothecin induced mitochondrial dysfunction leading to programmed cell death in unicellular hemoflagellate *Leishmania donovani*. *Cell Death Differ* 2004; 11 : 924-36.
35. Sen N, Das BB, Ganguly A, Mukherjee T, Bandyopadhyay S, Majumder HK. Camptothecin-induced imbalance in intracellular cation homeostasis regulates programmed cell death in unicellular hemoflagellate *Leishmania donovani*. *J Biol Chem* 2004; 279 : 52366-75.
36. Melendy T, Ray DS. Novobiocin affinity purification of a mitochondrial type II topoisomerase from the trypanosomatid *Crithidia fasciculata*. *J Biol Chem* 1989; 264 : 1870-6.
37. Shlomai J, Zadok A. Reversible decatenation of kinetoplast DNA by a DNA topoisomerase from trypanosomatids. *Nucleic Acids Res* 1983; 11 : 4019-34.
38. Shlomai J, Zadok A, Frank D. A unique ATP-dependent DNA topoisomerase from trypanosomatids. *Adv Exp Med Biol* 1984; 179 : 409-22.
39. Douc-Rasy S, Kayser A, Riou JF, Riou G. ATP independent type II topoisomerase from trypanosomes. *Proc Natl Acad Sci USA* 1986; 83 : 7152-6.
40. Chakraborty AK, Majumder HK. Decatenation of kinetoplast DNA by an ATP-dependent DNA topoisomerase from the kinetoplast hemoflagellate *Leishmania donovani*. *Mol Biochem Parasitol* 1987; 26 : 215-24.
41. Chakraborty AK, Majumder HK. An ATP independent catenating enzyme from the kinetoplast hemoflagellate *Leishmania donovani*. *Biochem Biophys Res Commun* 1991; 180 : 279-85.
42. Wang JC. DNA topoisomerases. *Annu Rev Biochem* 1985; 54 : 665-97.
43. Melendy T, Shelina C, Ray DS. Localization of a type II DNA topoisomerase to two sites at the periphery of the kinetoplast DNA of *Crithidia fasciculata*. *Cell* 1988; 55 : 1083-8.
44. Pasion SG, Hines JC, Aebersold R, Ray DS. Molecular cloning and expression of the gene encoding the kinetoplast-associated type II DNA topoisomerase of *Crithidia fasciculata*. *Mol Biochem Parasitol* 1992; 50 : 57-68.
45. Das A, Dasgupta A, Sharma S, Ghosh M, Sengupta T, Bandyopadhyay S, et al. Characterisation of the gene encoding type II DNA topoisomerase from *Leishmania donovani*: a key molecular target in antileishmanial therapy. *Nucleic Acids Res* 2001; 29 : 1844-51.
46. Strauss PR, Wang JC. The *TOP2* gene of *Trypanosoma brucei*: a single copy gene that shares extensive homology with other *TOP2* genes encoding eukaryotic DNA topoisomerase II. *Mol Biochem Parasitol* 1990; 38 : 141-50.

47. Fragoso SP, Goldenberg S. Cloning and characterization of the gene encoding *Trypanosoma cruzi* DNA topoisomerase II. *Mol Biochem Parasitol* 1992; 55 : 127-34.
48. Gaziova I, Lukes J. Mitochondrial and nuclear localization of topoisomerase II in the flagellate *Bodo saltans* (Kinetoplastida), a species with non-catenated kinetoplast DNA. *J Biol Chem* 2003; 278 : 10900-7.
49. Sengupta T, Mukherjee M, Mandal C, Das A, Majumder HK. Functional dissection of the C-terminal domain of the type II topoisomerase of the kinetoplastid hemoflagellate *Leishmania donovani*. *Nucleic Acids Res* 2003; 31 : 5305-16.
50. Hanke T, Raviro MJ, Trigueros S, Roca J, Larraga V. Cloning, functional analysis and post-transcriptional regulation of a type II DNA drugs. *Nucleic Acids Res* 2003; 31 : 4917-28.
51. Fragoso SP, Mattei D, Hines JC, Ray D, Goldenberg S. Expression and cellular localization of *Trypanosoma cruzi* type II DNA topoisomerase. *Mol Biochem Parasitol* 1998; 94 : 197-204.
52. Johnson CE, Englund PT. Changes in organization of *Crithidia fasciculata* kinetoplast DNA replication proteins during the cell cycle. *J Cell Biol* 1998; 143 : 911-9.
53. Liu LF. DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 1989; 58 : 351-75.
54. Drlica K, Franco RJ. Inhibitors of DNA topoisomerases. *Biochemistry* 1988; 27 : 2252-9.
55. Kreuzer KN, Cozzarelli NR. *Escherichia coli* mutants thermosensitive for deoxyribonucleic acid gyrase subunit A: effects on deoxyribonucleic acid replication, transcription, and bacteriophage growth. *J Bacteriol* 1979; 140 : 424-35.
56. Mitra B, Saha A, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S, *et al.* Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Mol Med* 2000; 6 : 527-41.
57. Kaufmann SH. Cell death induced by topoisomerase-targeted drugs: more questions than answers. *Biochim Biophys Acta* 1998; 1400 : 195-211.
58. Drlica K, Franco RJ. Inhibitors of DNA topoisomerases. *Biochemistry* 1988; 27 : 2252-9.
59. Newton BA. The chemotherapy of *Trypanosomiasis* and *Leishmaniasis*: towards a more rational approach. In: *Trypanosomiasis and Leishmaniasis with special reference to Chagas disease*. Amsterdam, North Holland: Elsevier Excerpta Medica; 1974 p. 285-307.
60. Bojanowski K, Lelievre S, Markovits J, Coupne J, Jacquemin-Sablon A, Larsen AK. Suramin is an inhibitor of DNA topoisomerase II *in vitro* and in Chinese hamster fibrosarcoma cells *Proc Natl Acad Sci USA* 1992; 89 : 3025-29.
61. Boritzki TJ, Wolfard TS, Besser JA, Jackson RC, Fry DW. Inhibition of type II topoisomerase by fostriecin. *Biochem Pharmacol* 1988; 37 : 4063-8.
62. Drake FH, Hoffmann GA, Bartus HF, Hettzberg RP, Johnson RK, Mattern MR. *In vitro* and intracellular inhibition of topoisomerase II by the antitumor agent merbarone. *Cancer Res* 1989; 49 : 2578-83.
63. Ishida R, Miki T, Narita T, Yui R, Sato M, Utsumi KR, *et al.* Inhibition of intracellular topoisomerase II by antitumor bis(2,6-dioxopiperazine) derivatives: mode of cell growth inhibition distinct from that of cleavable complex-forming type inhibitors. *Cancer Res* 1991; 51 : 4909-16.
64. Tanabe K, Ikegami Y, Ishida R, Andoh T. Inhibition of topoisomerase II by antitumor agents bis (2,6-dioxopiperazine) derivatives. *Cancer Res* 1991; 51 : 4903-8.
65. Hecht SM, Berry DE, Mackenzie LJ, Busby RW, Nasuti CA. A strategy for identifying novel, mechanistically unique inhibitors of topoisomerase I. *J Nat Prod* 1992; 55 : 401-13.
66. Li CJ, Averboukh L, Pardee AB. Beta-Lapachone, a novel DNA topoisomerase I inhibitor with a mode of action different from camptothecin. *J Biol Chem* 1993; 268 : 22463-8.
67. Syrovets T, Buchele B, Gedig E, Slupsky JR, Simmet T. Acetyl-boswellic acids are novel catalytic inhibitors of human topoisomerases I and II alpha *Mol Pharmacol* 2000; 58 : 71-81.
68. Bridewell DJ, Finlay GJ, Baguley BC. Differential actions of aclarubicin and doxorubicin: the role of topoisomerase I. *Oncol Res* 1997; 9 : 535-42.
69. O'Brien RL, Allison JL, Hahn FE. Evidence for intercalation of chloroquine into DNA. *Biochim Biophys Acta* 1966; 129 : 622-4.
70. DelCastillo I, Vizan JL, Rodriguez-Sainz MC, Moreno F. An unusual mechanism for resistance to the antibiotic coumermycin A1. *Proc Natl Acad Sci USA* 1991; 88 : 8860-4.
71. Contreras A, Maxwell A. GyrB mutations which confer coumarin resistance also affect DNA supercoiling and ATP hydrolysis by *Escherichia coli* DNA gyrase. *Mol Microbiol* 1992; 6 : 1617-24.
72. Ray S, Majumder HK, Chakravarty AK, Mukhopadhyay S, Gil RR, Cordell GA. Amarogentin, a naturally occurring secoiridoid glycoside and a newly recognized inhibitor of

- topoisomerase I from *Leishmania donovani*. *J Natl Prod* 1996; 59 : 27-9.
73. Ray S, Hazra B, Mitra B, Das A, Majumder HK. Diospyrin, a bisnaphthoquinone: a novel inhibitor of type I DNA topoisomerase of *Leishmania donovani*. *Mol Pharmacol* 1998; 54 : 994-9.
74. Figgitt D, Deny W, Chavalitsheiwinkoon P, Walairat P, Ralph R. *In vitro* study of anticancer acridines as potential antitrypanosomal and antimalarial agents. *Antimicrob Agents Chemother* 1992; 36 : 1644-7.
75. Werbovets KA, Spoors PG, Pearson RD, Macdonald TL. Cleavable complex formation in *Leishmania chagasi* treated with anilinoacridines. *Mol Biochem Parasitol* 1994; 65 : 1-10.
76. Slunt KM, Grace JM, Macdonald TL, Pearson RD. Effect of mitonafide analogs on topoisomerase II of *Leishmania chagasi*. *Antimicrob Agents Chemother* 1996; 40 : 706-9.
77. Shapiro TA, Englund PT. Selective cleavage of kinetoplast DNA minicircles promoted by antitrypanosomal drugs. *Proc Natl Acad Sci USA* 1990; 87 : 950-4.
78. Shapiro TA, Klein VA, Englund PT. Drug-promoted cleavage of kinetoplast DNA minicircles. Evidence for type II topoisomerase activity in trypanosome mitochondria. *J Biol Chem* 1989; 264 : 4173-8.
79. Nenortas E, Burri C, Shapiro TA. Antitrypanosomal activity of fluoroquinolones. *Antimicrob Agents Chemother* 1999; 43 : 2066-8.
80. Keiser J, Burri C. Evaluation of quinolone derivatives for antitrypanosomal activity. *Trop Med Int Health* 2001; 6 : 369-89.
81. Nenortas E, Kulikowicz T, Burri C, Shapiro TA. Antitrypanosomal activities of fluoroquinolones with pyrrolidinyl substitutions. *Antimicrob Agents Chemother* 2003; 47 : 3015-7.
82. Pate PG, Wolfson JS, McHugh GL, Pan SC, Swartz MN. Novobiocin antagonism of amastigotes of *Trypanosoma cruzi* growing in cell-free medium. *Antimicrob Agents Chemother* 1986; 29 : 426-31.
83. Gonzales-Perdomo M, de Castro SL, Meirelles MN, Goldenberg S. *Trypanosoma cruzi* proliferation and differentiation are blocked by topoisomerase II inhibitors. *Antimicrob Agents Chemother* 1990; 34 : 1707-14.
84. Loiseau PM. Action of DNA-gyrase inhibiting derivatives of 4-oxo-1,4-dihydro-3-pyridinecarboxylic acid against *Trypanosoma brucei*. *Int J Parasitol* 1995; 25 : 403-5.
85. Obexer W, Schmid C, Barbe J, Galy JP, Brun R. Activity and structure relationship of acridine derivatives against African trypanosomes. *Trop Med Parasitol* 1995; 46 : 49-53.
86. Yamashita Y, Kawada S, Nakano H. Induction of mammalian topoisomerase II dependent DNA cleavage by non-intercalative flavonoids, genistein and orobol. *Biochem Pharmacol* 1990; 39 : 737-44.
87. Mitra B, Saha H, Chowdhury AR, Pal C, Mandal S, Mukhopadhyay S, *et al.* Luteolin, an abundant dietary component is a potent anti-leishmanial agent that acts by inducing topoisomerase II-mediated kinetoplast DNA cleavage leading to apoptosis. *Mol Med* 2000; 6 : 527-41.
88. Ray S, Sadhukhan PK, Mandal NB, Mahato SB, Majumder HK. Dual inhibition of DNA topoisomerases of *Leishmania donovani* by novel indolylquinolines. *Biochem Biophys Res Commun* 1997; 230 : 171-5.
89. Chowdhury AR, Mandal S, Mitra B, Sharma S, Mukhopadhyay S, Majumder HK. Betulinic acid, a potent inhibitor of eukaryotic topoisomerase I: identification of the inhibitory step, the major functional group responsible and development of more potent derivatives. *Med Sci Monit* 2002; 8 : BR254-65.
90. Kashiwada Y, Hashimoto F, Cosentino LM, Chen CH, Gorret PE, Lee KH. Betulinic acid and dihydrobetulinic acid derivatives as potent anti-HIV agents. *J Med Chem* 1996; 39 : 1016-17.
91. Chowdhury AR, Mandal S, Goswami A, Ghosh M, Mandal L, Chakraborty D, *et al.* Dihydrobetulinic acid induces apoptosis in *Leishmania donovani* by primarily targeting DNA topoisomerase I and II: implications in antileishmanial therapy. *Mol Med* 2003; 9 : 26-36.

Reprint requests: Dr H.K. Majumder, Molecular Parasitology Laboratory, Indian Institute of Chemical Biology  
4 Raja S.C Mullick Road, Kolkata 700032, India  
e-mail: hkmajumder@iicb.res.in