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Antimicrobial, DNA cleavage and antitumoral properties of some transition metal complexes of 1, 10 –phenanthroline and 2, 2′ – bipyridine: a review

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ABSTRACT

Transition metal ions coordinated to nitrogen containing ligands, such as 1, 10 – phenanthroline and/or 2, 2′ – bipyridine have found wide applications in chemotherapy. The ligands form very stable chelates with many first row transition metals and the ligands, as well as some of their derived complexes, do exhibit antimicrobial properties. The mechanism of action of these novel set of drugs was reviewed and compared to that of the conventional antifungal drugs (polyene and azoles). Transition metal complexes of 1, 10 – phenanthroline and/or 2, 2′ – bipyridine demonstrated a significantly different mode of action and thus could be used either in combination with existing antimicrobial drugs or in a situation where resistance to conventional antimicrobial drugs have emerged. However, the antimicrobial properties of the various transition metal complexes of phen and bpy are not uniform indicating a degree of metal – ion dependency on their mode of action. The DNA binding, DNA cleavage and antitumoral properties of the transition metal complexes of the chelating ligands were also reviewed. DNA cleavage by these metallonucleases is reported to be dependent on the presence of a biological reductant (e.g. L-ascorbic acid, gluthathione) and an oxidant (H_2O_2). The metal complexes were reported to control cancer cell division by significantly reducing DNA synthesis. However there is a significant difference in the mode of action of these novel sets of antitumoral drugs compared to the conventional antifungal drug cisplatin.

Introduction

Transition metal ions are known to play very important roles in biological processes in the human body. For example, Zn (II) and Cu (II) ions are the second and third most abundant transition metals in humans. They are found either at the active sites or as structural components of a good number of enzymes. Cobalt is present in vitamin B_{12}, a co-enzyme that plays significant roles in some biochemical processes.[5] There are numerous lists of transition metals which are effective therapeutic agents especially when coordinated to a ligand to form metal complexes. A list of metal containing compounds used in chemotherapy for treatment of diseases include platinum (anticancer), silver (antimicrobial), gold (antiarthritic), bismuth (antiulcer), antimony (antiprotozoal), vanadium (antidiabetic) and iron (antimalaria).[25]

Nitrogen containing chiral ligands has found wide applications in chemotherapy and asymmetric catalysis. Among them, bipyridines and 1, 10 – phenanthrolines are particularly attractive for their ability to coordinate several metal ions, and thus to generate different catalytic species involved in a great variety of reactions.[38] The ligands (1, 10 – phenanthroline and 2, 2′ – bipyridine) are strong field bidentate ligands that form very stable chelates with many first row transition metals.[28] 2, 2′ – bipyridine has been reported to be present in crude oil. It was first prepared in 1888 by the dry distillation of the copper salt of picolinic acid. Another old method involves the oxidation of 1, 10 – phenanthroline to 2, 2′ – bipyridine – 3, 3′ – dicarboxylic acid by alkaline permanganate followed by decarboxylation. High resolution x-ray diffraction analysis and other studies with 2, 2′ – bipyridine have shown that the rings are coplanar with the nitrogen atoms in an anti position with respect to the bond joining the rings.

1, 10-Phenanthroline (phen) is a rigid, planar, hydrophobic, electron-poor heteroaromatic system whose nitrogen atoms are beautifully placed to act cooperatively in cation binding. These structural features determine its coordination ability toward metal ions. Phen easily forms in aqueous solution octahedral complexes of the type $[M(phen)(H_2O)_4]^{2+}$, $[M(phen)_2(H_2O)_2]^{2+}$ and $[M(phen)_3]^{2+}$with first-row transition metal cations. The stability of the $[M(phen)]^{2+}$ complexes follows the Irving–Williams sequence, their formation constants spanning from 4.13 ($[Mn(phen)]^{2+}$) to 9.25 ($[Cu(phen)]^{2+}$) log units.[4]

Metal complexes containing diimine ligands such as 1,10 – phenanthroline and bipyridine have gained importance because of their versatile roles as binding blocks for the synthesis of metallo – dendrimers and as molecular scaffolding for supramolecular assemblies, and in analytical chemistry, catalysis, electrochemistry, ring opening metathesis polymerization and biochemistry.[2] The medicinal application of metal complexes has also been a subject of great interest recently.[22] For instance, to name among others, phen and its copper complexes have been reported to exert a range of biological activities, such as antitumour, anti-candida,[12] antimycobacterial[41] and anti-microbial effects.[47]

The interaction of transition metal complexes containing multidentate aromatic ligands, especially N-containing ligands, with DNA has gained a lot of attention in recent years. This is due to their possible application as therapeutic agents and

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photochemical properties which make them potential probes of DNA structure and conformation. There are three distinct modes of non-covalent interaction of these metal complexes with DNA - intercalative association, DNA groove binding and electrostatic attraction – the nature of which is determined by the characteristics of the metal complex. For instance, cationic metal complexes will exhibit electrostatic interactions with polyanionic DNA molecule while metal complexes of polyazine ligands with extended aromatic system may intercalate between the DNA base pairs.[33] DNA scission by artificial metallonucleases, (usually Cu (II) complexes) is dependent on the presence of a biological reductant and an oxidant (e.g H_2O_2).[34] The cleavage in the presence of oxidant; H_2O_2, may be attributed to the formation of hydroxyl free radicals which participate in the oxidation of the deoxyribose moiety followed by hydrolytic cleavage of a sugar phosphate backbone.[40] In the presence of a biological reductant and oxidant the supercoiled form of the DNA is relaxed to circular form and linear form.[26]

Numerous biological studies have demonstrated that DNA is the primary intracellular target of anticancer drugs. Metal complexes that interact with DNA have the potential to be exploited as anticancer agents.[33] 2,2′ – bipyridine and 1,10 – phenanthroline chelators also act as potential antitumor agents but they can display better antitumor activity if their hydrophobic groups are masked by metal ions that will form water soluble natural complexes. These natural complexes are expected to be more permeable than the cell membrane.[34] Cancer cells have been investigated for rates of metabolisms and amount of intracellular $O_2^{\bullet-}$present in them. Tumor cells have increased rate of metabolisms as well as a greater amount of intracellular $O_2^{\bullet-}$than normal cells. Hence the SOD activity in cancer cell is lower than the normal cell. SOD mimic enzymes affect tumor cell proliferation due to the generation of increase amount of H_2O_2 and its metabolite $OH^{\bullet}$ radical which crucially cause cytotoxicity in affected cell lines.[42] Fortunately there are transition metal complexes which are excellent SOD mimics that can either disproportionate the H_2O_2 to water and molecular oxygen or react with it to form the desirable cytotoxic hydroxyl.

Arguably the best known example of transition metal complexes used as anticancer agents is the drug cisplatin (cis – diaminedichloroplatinum (II)).[16] Upon entering the cell Cl^- dissociates from the drug molecule to leave a reactive complex which can react with water, which in turn react with DNA forming inter – and intra – strand and DNA crosslink. The reaction leads to the local denaturation of DNA chain. Furthermore, cisplatin has been shown to cause mitonchondrial damage and alters the cellular transport system leading to apoptosis, inflammation and necrosis.[10] However, acquired resistance and some side effects such as nausea, vomiting and severe nephrotoxicity has served to limit widespread use of cisplatin.[16]

Interestingly it was discovered that the transition metal complexes of 1, 10 – phenanthroline and 2, 2′ – bipyridine and their derivatives appear to have a mechanism of action significantly different to that of clinically used drug cisplatin. There seems to be three mechanistic pathways that can be adopted by various metal complexes that possess antitumoral property. The property may be effected through : the generation of reactive oxygen species (such as H_2O_2, $OH^{\bullet}$, and $O_2^{\bullet-}$) which have been shown to damage chromosomal DNA, or alteration of the metabolism and homeostasis of essential metal ions (such as Fe, Cu, and Zn), or the inhibition of DNA synthesis.[11,25,45]

Antimicrobial Property

Complexes formed through the coordination of bidentate ligands, 1, 10-phenanthroline and /or 2, 2′-bipyridine with transition metal ions have been shown to exhibit antifungal, antibacterial and antiviral activity. The ligands form very stable chelates with many first row transition metals and the ligands as well as some of their derived complexes, do exhibit antimicrobial properties.[1] The yeast *Candida albicans*, even though is a commensal of the human body, is an important fungal pathogen. Its infection can lead to the development of a disease called vagina candidosis (thrush), a condition from which over 75% of women suffer at some stages in their lifetime.[17] Fungal pathogens are also a serious cause of infection and death in patients immunocompromised as a result of disease or therapeutic procedures (e.g. broad spectrum antibiotics, immuno-suppression prior to organ transplantation).[12]

A list of metal containing compounds used in chemotherapy for treatment of diseases include platinum (anticancer), silver (antimicrobial), gold (antiarthritic), bismuth (antiulcer), antimony (antiprotozoal), vanadium (antidiabetic) and iron (antimalaria). While many metals are essential for all forms of life, their levels in normal homeostasis or therapeutic intervention must be strictly regulated because most are toxic in excess.[25]

There are substantial reports that 1, 10-phenanthroline and a number of transition metal complexes incorporating the chelating ligand are extremely active anti-fungal drugs.[12,19] The metal complexes of these two ligands (1, 10-phenanthroline and 2, 2′-bipyridine) also exhibit antibacterial property as their in vitro antibacterial action has been demonstrated on several species of bacteria. For example, bacteria such as *Enterobacterchoacae, P.pyocyanla, S.typhineurium, Staphylococcus aureus, Escherichia coli, Morganellamorganii, S.albas, P.avutgaris, Salmonella thyphi, Klebsella pneumonia, Shingella flexiniri, Citrobacterfreund and Pseudomonas aeruginosa*, among others, have all been examined with respect to the antibacterial action of the bidentate ligands metal complexes.[1,34] The various results obtained from the antifungal and antibacterial tests showed that the metal complexes were more active towards bacteria than fungi.[40] Whereas metal-phen complexes can be bacteristatic and bactericidal towards many gram positive bacteria, they are relatively ineffective against gram negative organisms.[12]

The conventional therapy for the control of fungal infections relies upon the use of polyene or azole drugs.[27] There are unique components in the fungal cell that are being exploited as antifungal targets. The mannans, glucans and chitins – the components of the fungal cell wall – and a few of the enzymes of the ergosterol biosynthetic pathway are unique to the fungal cells. Antifungal generation has therefore targeted enzymes involved in the synthesis of the above macromolecules of the cell wall. However in spite of the facts that there are many targets, mentioned above, with the exception of few, most of the currently used antifungal drugs are directed against the enzymes of the ergosterol biosynthetic pathways.[37]

Unfortunately, there is an emergence of *C.albicans*isolates resistant to these antifungal drugs.[12] Studies on the mechanism that confer antifungal drug resistance in yeast include an increase in the expression of drug efflux pumps which remove the drugs from the cell before a toxic concentration can be reached.[44] This has serious implications for the continued success of conventional antifungal therapy.[12]

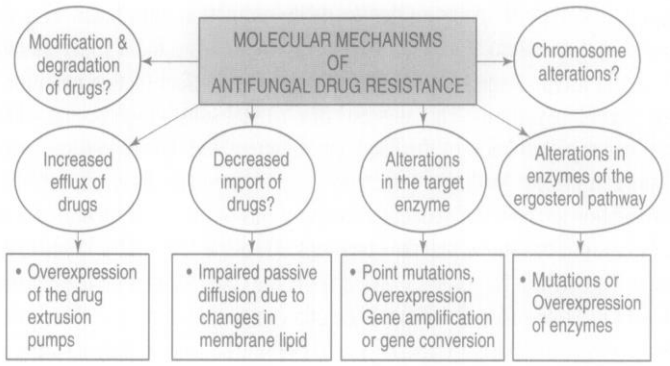


Fig 1: A schematic representation of the known molecular factors that contribute to antifungal drug resistance in C. albicans.[37]

1, 10-phenanthroline and metal-phen complexes represent a novel set of highly active anti-fungal agents whose mode of action is significantly different to that of the polyene and azole prescription drugs.[13] Consequently, they may be used either in combination with existing drugs or in cases where resistance to conventional drugs has emerged. Azoles (an antifungal) specifically inhibit P45014DM (Cytochrome P450 dependent 14 α – demethylase) enzyme of the ergosterol biosynthetic pathway. This inhibition results in the accumulation of 14 – methylated sterols that cause disruption of membrane structure and function. Azole derivatives interact with the haem of P45014DM where the unhindered nitrogen atom of the azole ring binds to the haem iron at its sixth coordinate position. The blocking of this position which is normally occupied by activated oxygen prevents initiation of the hydroxylation reaction.[13,37]

The mode of action of the metal complexes (drugs) has been examined using the pathogenic yeast *Candida albicans*as test organ. Their mechanism of action is significantly different to those of the polyene and azole drugs. Both the complexed and uncomplexed ligands have the potential to affect mitochondria function, retard the synthesis of cytochromes b and c and uncouple respiration. Some metal-phen complexes (e.g. Cu (II) and Mn(II) complexes) cause a dramatic increase in oxygen consumption when used to treat exponential and stationary phase yeast cells. It is believed that the oxygen taken up by the cells is not being utilized for normal respiration but is being used to induce oxidative stress in the organism through the formation of damaging oxygen free radicals.[12] Furthermore, the mechanisms of the antimicrobial activity of the metal complexes can be explained based on the Overtone's concept and the Tweedy chelation theory. According to the overtone's concept of cell permeability, the lipid membrane that surrounds the cell favours the passage of only lipid – soluble materials, due to which liposolubility is an important factor that controls antimicrobial activity.[40]

On chelation, the polarity of the metal ion (e.g copper ion) will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of the positive charge of the metal ion with donor groups. Further, it increases the delocalization of π -electrons over the whole chelate ring and enhances the lipophilicity of the complexes. The increased lipophilicity enhances the penetration of the complexes into lipid membranes and blocking of the metal binding sites in the enzymes of microorganisms.[8]

Previous kinetic studies[31] in which a clinical isolates of C. albicans was exposed to $[Mn(phen)_2(mal)].2H_2O$, showed that the cells have to be metabolically active before the administered metal complex has an inhibitory effect. It has already been established[20] that, in general, simple salts such as $MnCl_2.4H_2O$ and $CuCl.2H_20$, have limited activity against clinical isolates of *C. albicans* indicating the importance of the bidentate phen ligand in promoting the anti-fungal effect of the metals. It is noteworthy, however, that the antifungal properties of various metal-phen complexes are not uniform. For example, when phen, $[Cu(phen)_2(mal)].2H_2O$, $[Mn(phen)_2(mal)].2H_2O$ and $[Ag_2(phen)_3(mal)].2H_2O$ are tested against *C.albicans*, all of the drugs with the exception of the Ag(I) complex increase oxygen-uptake by the cells. Similarly, all of the drugs led to a decrease in cellular ergosterol content with the exception of $[Mn(phen)_2(mal)].2H_2O$. That the drugs are not uniformly active indicates a degree of metal-ion dependency on their mode of action.[13]

The coordination of some transition metal ions (eg Cu, Co and Zn) to phen and/or bpy brings about an increase in the antimicrobial activities of the metal ions.[20] However, there is a decrease in the activities of 1, 10-phenanthroline as well as an increase in the case of 2, 2′-bipyridine upon coordination. To justify this claim the antimicrobial activities of 1, 10-phenanthroline (phen), 2, 2′-bipyridine (bpy), Cobalt, Copper and Zinc salts [$Co(NO_3)_3.6H_2O$, $CuCl_2$, $ZnCl_2$], $[Co(bpy)(phen)_2](NO_3)_2.2H_2O$, $[Cu(bpy)(phen)(H_2O)_2]Cl_2.2H_2O$ and $[Zn(bpy)_2(phen)]Cl_2.6H_2O$ were all tested against nine species of bacteria, *Enterobacterchoacae, Staphylococcus aureus, Escherichia coli, Morganella morganii, Salmonella thyphi, Klebsiella pneumonia, Shigellaflexineri, Citrobacter freundi*and *Pseudomonas aeruginosa* and the results were recorded.[1]

Furthermore, when two mixed ligand copper (II) complexes $[Cu(dien)(phen)](ClO_4)_2$ and $[Cu(dien)(bpy)](BF_4)_2$ were tested for antimicrobial activity using *P.pyocyanla, S.typhineurium, S.albas, E.coli* and *P.vulgaris*as test organisms the results obtained revealed a higher antimicrobial activity of the bpy ligand coordinated to the copper (II) ion than the coordinated phen ligand.[37]

The 'metal-free' phen, however, has been reported to significantly show a greater antimicrobial activity than its metal complexes and its mixed-ligand complexes. Hence it is evident that for significant antimicrobial activity, the N, N′-donor ligand 1, 10-phenanthroline must be present in the growth medium, either as the metal-free molecule or as a water-soluble metal chelate. It is believed that the so called metal-free phen is probably coordinating to trace amounts of transition metals such as Cu and Fe, present in the growth media, which is then followed by transport of the metal complex formed into the cells.[12,17,19] The chemical structures of the metal complexes also affect their antimicrobial efficiency. The reduced antimicrobial activity of the metal complexes $[Cu (phen)_2(mal)].2H_2O$ and $[Mn(phen)_2(mal)].2H_2O$ compared to phen itself can be attributed to the chemical structures of the metal complexes. The x-ray crystal structures of closely related Cu (II) and Mn(II) dicarboxylate/phen complexes shows the phen ligands to be strongly chelated to the metal centers. As such, the equilibrium concentration of uncomplexed phen arising from the incorporation of the Cu (II) and Mn (II) complexes inside cells is expected to be relatively low thus minimizing the extent of coordination to cellular iron which was destined for cytochrome b and c synthesis.[13]

The reaction of the bidentate ligands, phen and bpy, with some ligand-metal complexes which initially demonstrated antimicrobial impotency may or may not improve the antimicrobial activity of the ligand-metal complexes. For example, fumaric acid, whether coordinated or uncoordinated to Manganese (Mn), does not possess any anticandidal properties. 1, 10-phenanthroline molecule is a potent anti-candida agent and upon reaction with the Manganese fumarate yields complexes with comparable fungi toxic activity. However, the presence of the N, N′-donor 2, 2′-bipyridine will not improve the activity of the complex.[17]

As with all drugs, the use of transition metals in drug development will depend largely on understanding their mechanism of action and selectively controlling their toxicity.[25]

DNA Binding

DNA plays a fundamental role in the storage and expression of genetic information in a cell. Studies on the interaction of transition metal complexes with DNA have been pursued in recent years. Of these studies, the interaction of transition metal complexes containing multidentate aromatic ligands, especially N-containing ligands, with DNA has gained much attention. This is due to their possible application as new therapeutic agents and their photochemical properties which make them potential probes of DNA structure and conformation.

Metal ions are electron deficient whereas most biological molecules (protein and DNA) are electron rich; consequently there is a general tendency for metal ions to bind and interact with many important biological molecules. In general, components have three distinct modes of non-covalent interaction with DNA i.e. intercalative association, DNA groove binding and electrostatic attraction.[6,14,25,36,46]

The nature of these interactions is determined by the characteristics of the metal complex. For instance, all cationic metal complexes exhibit electrostatic interactions with the polyanionic DNA molecule, metal complexes of polyazine ligands with extended aromatic systems may intercalate between the DNA base pairs while metal complexes with smaller polyazine ligands often bind to the major groove of DNA.[33] Although 2, 2′-bipyridine and 1, 10-phenanthroline are good intercalators due to the Π – stacking or hydrophobic interactions of the aromatic phenyl rings with the DNA base pairs, mixed – ligand metal complexes were found to be particularly useful because of their high potential to bind DNA via a multitude of interactions and to cleave the duplex by virtue of their intrinsic, chemical, electrochemical and photochemical reactivities.[40]

The ability to have stacking interactions with DNA bases dops markedly when passing from phen to bpyi.e when removing the protruding – CH=CH – group of the phenanthroline moiety. This was expressed in the comparison between [$Ru(bpy)_2phen]^{2+}$ which shows a binding geometry similar to $[Ru(phen)_3]^{2+}$, and $[Ru(bpy)_3]^{2+}$, which shows a more unordered or averaged binding geometry. However a certain similarity in the preferred angular geometries remains, suggesting that bpy also has a tendency to align itself parallel with the base pairs.[29]

DNA Cleavage

Chromosomal DNA fragmentation is caused by two types of DNA breaks viz; single strand break and double strand breaks. Single strand cleavage of DNA has been suggested to occur during apoptosis while double strand DNA breaks are generally thought to have a greater biological consequence because they can lead directly to chromosomal aberrations and more frequently to the loss of genetic information.[9] Double stand breaks are twenty times less frequent than single strand breaks and are more difficult to measure at physiological doses.[45]

DNA cleavage may be considered as an enzymatic reaction which comprises various biological processes as well as biotechnological manipulation of genetic material. It has wide applications in bioorganic chemistry, molecular biology and drug design.[39] Metal containing reagents that induce chemical DNA scission are often referred to as artificial metallonucleases [26]and the attention focused towards the development of new metallonucleases, which bind and cleave DNA at physiological conditions, have gained momentum.

DNA cleavage by metallonucleases (usually Cu (II) complexes) is dependent on the presence of a biological reductant (e.g. L-ascorbic acid, glutathione) and an oxidant (H_2O_2).[26,34,40] The requirement for a reductant for the cleavage of DNA by copper complexes suggests that Cu (II) ions are being reduced to Cu (I) ions which are susceptible to subsequent oxidation by dioxygen in air. The resulting reactive oxygen species diffuses into the double stranded DNA and cleave it.[34] The mechanistic pathway for the reaction is:

Cu^{2+} + reductant → Cu^{+1}

$Cu^{+1} + O_2 \rightarrow Cu^{2+} + O_2^{0-}$

O_2^{0-} + DNA → Non specific cleavage in DNA.

The cleavage of DNA strands in the presence of the oxidant (H_2O_2) may be attributed to the formation of hydroxyl free radicals. The hydroxyl radicals participate in the oxidation of the deoxyribose moiety, followed by hydrolytic cleavage of a sugar phosphate backbone.[40]

$(Ligand)Cu^{2+} + H_2O_2 \rightarrow (Ligand)Cu^{3+} + OH\bullet + OH^-$

The DNA cleavage properties of the first reported synthetic chemical nuclease $[Cu(phen)_2]^{2+}$ results from a cascade of redox reactions (I – IV) that ultimately lead to the formation of hydroxo (IV & V) and metaoxo(IV) radical species, which initiate DNA toxicity.[26]

I. $[Cu(phen)_2]^{2+} + Red \rightarrow [Cu(phen)_2]^{+} + Red\bullet$

II. $[Cu(phen)_2]^{+} + O_2 \rightarrow [Cu(phen)_2]^{2+} + O_2\bullet^-$

III. $2O_2\bullet^- + 2H^+ \rightarrow H_2O_2 + O_2$

IV. $[Cu(phen)_2]^{+} + O_2 + H^+ + e^- \rightarrow [\bullet O{-}Cu(phen)_2]^{2+} + \bullet OH$

V. $[Cu(phen)_2]^{+} + H_2O_2\text{-} \rightarrow [^-O{-}Cu(phen)_2]^{2+} + \bullet OH + H^+$

In the presence of the biological reductant (ascorbic acid) and the oxidant (H_2O_2), artificial metallonucleases relaxes the super coiled form of the DNA to the circular form and linear form.[26,34,39,40] The relaxation or nicking can be monitored using absorption spectroscopy, gel electrophoresis and viscosity measurements.

The metal ions play a crucial role in DNA binding by these complexes as the high binding nature of the metal complexes may be attributed to additional Π – Π interaction through the aromatic phenyl rings.[40] The presence of different substituents in the intercalative ligand could affect changes in space configuration and in the electron density distribution around

transition metal complexes, which not only influence their spectral properties but renders a clear understanding in evaluating the changes in SOD-like activity, as well as in the binding mechanism of transition metal complexes to DNA. The presence of coordination sites belonging to nitrogen heteroatomic rings such as imidazole, pyrazole, 1,10 phenanthroline, 2, 2′-bipyridine, pyridine etc are considered important for high SOD activity and posses remarkable DNA binding propensity. Among these coligands, phen (1, 10-

phenanthroline), is an important moiety which has attracted considerable attention for its versatility in exhibiting electronic properties and high cleavage efficiency.[42]

Another aspect for a complex to show SOD activity depends upon the availability of labile ligand in complex structure. It has been demonstrated that the axial site of Cu(II) complex that consists of solvent molecules, with little steric hindrance, undergoes a fast attachment of O_2^-.For instance, considering Cu(II) based SOD mimics **1-4** [CuLH(OAc)(H2O)Y)](LH=2-((E)-(1,3-dihydroxy-2-methylpropan-2-ylimino)methyl)-6-methoxyphenol,OAc = CH3COO, **1**: Y = H2O; **2**: Y= phen (1,10-phenanthroline), **3**: Y = tpimH(2,4,5-triphenylimidazole); **4**: Y= tfbimH (2-(trifluoromethyl)benzimidazole). Complexes **1**,**3**, and **4** possesses labile water molecules coordinated to the Cu(II) centre and could provides excellent locus for $O_2•^-$ attachment.[42]

Anticancer Property

Numerous biological studies have demonstrated that DNA is the primary intracellular target of anticancer drugs due to the interaction between small molecules and DNA which causes DNA damage in cancer cells, blocking the division of cancer cells and resulting in cell death.[32] Metal complexes that interact with DNA have the potential to be exploited as anticancer agents or used to modify or detect bio-molecules.[33] 2, 2'-bipyridine and 1, 10 –phenanthroline chelators also act as potential antitumor agents but they can be of better antitumor activity if their hydrophobic groups are masked by metal ions (e.g copper ions) that will form water soluble neutral complexes. These neutral complexes are expected to be more permeable through the cell membrane.[34]

It has been discovered that Reactive Oxygen Species (ROS) such as superoxide radical ($O_2•^-$) or hydrogen peroxide (H_2O_2) are important regulators of cell death.[16] Although the classification of cell death has proven difficult two distinct patterns of cell death have been identified based on the morphology of dying cells, and on the DNA fragmentation or damage. These have been termed necrosis and apoptosis. Apoptosis is characterized by chromatin condensation, activation of some caspases and fragmentation of DNA at internucleosomal linker sites. Necrosis, in contrast, is a passive process typified by cell and organelle swelling with spillage of the intracellular contents into the extracellular milieu.[45]

Many tumor cells have increased rate of metabolism compared with normal cells which would typically lead to increased number of reactive oxygen species (ROS). As a result, a large amount of intracellular $O_2•^-$ is found in most cancer cells and in the absence of significant SOD (Superoxide Dismutase) activity, this $O_2•^-$ can further metabolise to peroxynitrite ($OONO^-$) and perhydroxyl radical ($HO_2^{\cdot}$) which play a role in the tumor formation.[24] Cu (II) containing SOD enzyme, Cu_2,Zn_2SOD is the most efficient catalytic species found in the mammalian cell plasma and extracellular spaces. It catalyses the dismutation of superoxide radical ($O_2•^-$) and converts it into molecular oxygen and hydrogen peroxide via one electron redox cycle involving its Cu(II) centre. Since cancer cells have been investigated for rates of metabolism and generate large amount of intracellular $O_2•^-$as compared to normal cells, the SOD activity in cancer cell is lower than the normal cell. SOD mimic enzyme affects tumour cell proliferation, due to the generation of increased amount of H_2O_2 and its metabolite OH• radical (formed via Fenton's reaction), which crucially causes cytotoxicity in affected cell lines. Thus synthetic SOD mimics can be considered as a perfect tool inmediating apoptosis by implying oxidative stress induced by OH• radical.[42]

However there are transition metal complexes which are excellent SOD mimics that can either disproportionate the H_2O_2 to water and molecular oxygen or react with it to form the desirable cytotoxic hydroxyl radical (Fenton Chemistry).[16] Arguably, the best known example of transition metal complexes used as anticancer agents is the drug cisplatin.[30] It has been used widely in the treatment of a variety of cancer especially testicular cancer with 70-90% cure rate. When combined with other drugs, it has been used successfully to treat brain, ovarian, bladder and breast cancer. However, acquired resistance and some side effects such as nausea, vomiting and severe nephrotoxicity has served to limit the widespread use of cisplatin.[16]

Mechanism of action of anticancer drugs

The mode of action of cisplatin (Cis – diaminedichloroplatinum (II)) has been studied extensively. It has been shown that on entering the cell, Cl^- dissociates to leave a reactive complex which can react with water, which in turn reacts with DNA forming inter- and intra-strand DNA cross-links. It is the reaction which leads to the local denaturation of DNA chain. In addition, cisplatin has been shown to cause mitochondrial damage, and it alters the cellular transport system, eventually leading to apoptosis, inflammation and necrosis.[10]

Having demonstrated that the transition metal complexes of 1, 10-phenanthroline and 2,2′-bipyridine and their derivatives are potent anticancer agents, it is imperative to disclose, also, that they appear to have a mechanism of action significantly different to that of the clinically used drug cisplatin.

There seems to be three mechanistic pathways that can be adopted by the various metal complexes that possess antitumor property. The property may be effected through: the generation of Reactive oxygen species (such as H_2O_2, OH•, $O_2•^-$) which have been shown to damage chromosomal DNA, or alteration of the metabolism and homeostasis of essential metal ions (such as Fe, Cu and Zn), or the inhibition of DNA synthesis.[10,11,25,45]

However these mechanisms are not yet fully understood. Most agents producing ROS induce cell death including apoptosis by causing lipid peroxidation and DNA damage.

A secondary effect of transition metals is to catalyze the generation of these reactive oxygen species(ROS).The presence of these species play an important but poorly understood roles that can modulate drug-induced cytotoxic responses and affect cancer pathogenesis.[45] A large number of chemotherapeutic agents and cytokines imply potentially useful therapeutic strategies and elicit antitumor effect by inducing cancer cell apoptosis by generating large amount of noxious radicals into the cancer cells. SOD can putatively participate in such apoptotic events leading to tumor reduction and cell proliferation.[42]

Chelators have received substantial attention as therapeutic agents because of their capability to alter the metabolism and homeostasis of essential metals like iron, copper and zinc. 2,2′-bipyridine and 1, 10-phenanthroline are bidentate chelators with nitrogen donor ligands that have relatively high affinity for both copper and iron. Iron metabolism is altered in cancer; rapidly proliferating tumor cells show an increased requirement for iron. On the other hand, copper regulates growth factor production associated with angiogenesis and elevated copper levels have been observed in patients with several cancer types.

Table 1: Antimicrobial activities of the ligands, metal salts and metal mixed-ligand complexes. IZ = inhibition zone; 1 = 1,10-phenanthroline (phen); 2 = 2,2′-bipyridine (bpy); 3 = $Co(NO_3)_3.6H_2O$; 4 = $[Co(bpy)(phen)_2](NO_3)_2.2H_2O$; 5 = $CuCl_2.2H_2O$; 6 = $[Cu(bpy)(phen)]Cl_2.2H_2O$; 7 = $ZnCl_2$; 8 = $[Zn(bpy)_2(phen)]Cl_2.6H_2O$; RA = reference antibiotics (gentamycin).

Bacteria	Antimicrobial activity (IZ diameter in mm)								
	1	2	3	4	5	6	7	8	RA
Enterobacterchoacae	32	--	11	22	--	30	9	18	28
Staphylococcus aureus	31	9	10	24	7	30	8	20	30
Escherichia coli	31	14	13	22	8	29	9	22	22
Morganellamorganii	30	--	11	23	--	29	--	20	27
Salmonella thyphi	32	9	12	25	--	31	9	17	30
Klebsiellapneumoniae	28	--	11	20	7	26	10	17	29
Shigellaflexineri	31	13	13	22	8	30	10	20	22
Citrobacterfreundi	30	10	--	16	--	26	--	16	21
Pseudomonas aeruginosa	31	7	11	23	--	30	10	19	25

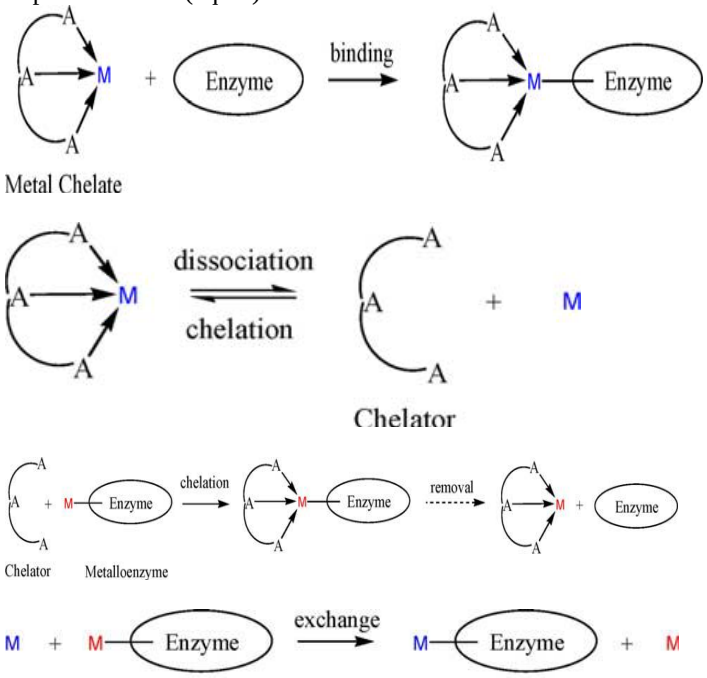
Table 2 : Anti-microbial activity of $[Cu(dien)(bipy)](BF_4)_2$ and $[Cu(dien)(phen)](ClO_4)_2$ against human pathogens

S. No.	Test organisms	Zone of inhibition (nm)	
		$[Cu(dien)(bipy)](BF4)_2$	$[Cu(dien)(phen)](ClO_4)_2$
1	*P. pyocyanla*	10.5	11.0
2	*S. typhineurium*	5.5	R
3	*S. albas*	R	R
4	*E. coli*	10.0	R
5	*P. vulgaris*	6.5	20.0

R:Resistant

Chelating agents could chelate (either bind to and/or remove) the functional metals in an enzyme or protein (eqn 2), or sequester the non-protein bound metal ions and thus deprive metalloenzymes of their metal supply (eqn 3).

Furthermore, the metallodrug could also bind to the enzymes metal-binding sites (eqn 4) or act as a carrier of the chelator by dissociating, after entering the biological system, into a free metal and a chelator (forward reaction in eqn 3) which is then available for chelation of other biologically important metals(eqn 4).[25]



M = Metal; A = Donor atom

It is widely recognized that any chemotherapeutic agents which can significantly reduce DNA synthesis is likely to be of particular value in controlling cancer cell division. Selected mechanistic studies showed that phendione and its metal complexes inhibited DNA syntheses which do not appear to be mediated through intercalation. Phendione, [Ag (phendione)$_2$]ClO_4 and particularly $[Cu(phendione)_3](ClO_4)_2.4H_2O$ are each capable of decreasing cancer cell viability through the inhibition of DNA synthesis. However the exact mechanism underlying the cytotoxicity of these compounds remains to be elucidated.[10]

Future Challenges

While many metals are essential for all forms of life there levels in normal homeostasis or therapeutic intervention must be strictly regulated because most are toxic in excess. As with all drugs, the use of metals in drug development will depend largely on understanding their mechanisms of action and selectively controlling their toxicity. With respect to antitumoral property the mechanism of action of the transition metal complexes of 2, 2′– bipyridine and/or 1,10 – phenanthroline are significantly different to that of the clinically used drug cisplatin. However, to our knowledge, these mechanisms are not yet fully understood. A secondary effect of transition metal is to catalyze the generation of reactive oxygen species (ROS) such as super oxide radical ($O_2^{\bullet-}$) or hydrogen peroxide H_2O_2 which are important regulators of cell death. The presence of these species plays important but poorly understood roles that can modulate drug – induced cytotoxic responses and affect cancer pathogenesis.

Finally many interesting antitumor active compounds have failed to reach clinical use due to poor physio-chemical properties, including insufficient water solubility, hydrolytic instability and the tendency to readily decompose when exposed to solvents, humidity, light or air. These difficulties in controlling selective toxicity or devising appropriate pharmacological properties have contributed to a general reluctance in the development of metal-based drugs. Furthermore, to our knowledge, little or no literature were provided on the kinetic studies of the antimicrobial and antitumoral properties of these novel therapeutic drugs.

Conclusion

The emergence of resistance to conventional chemotherapeutic drugs shown by some bacteria and fungi has serious implications for the continued success of conventional antibacterial and/or antifungal therapy. However some transition metal complexes of 1,10 – phenanthroline and 2,2′-bipyridine represent a novel set of highly active antimicrobial agents whose mode of action is significantly different to that of the prescription drugs (for example polyene and azole drugs). Hence, by controlling their selective toxicity or devising appropriate pharmacological properties, they may be used either in combination with existing drugs or where resistance to conventional drugs has emerged.

Although it is known that the DNA is a major target for cisplatin, only 5-10% intracellular concentration of cisplatin is found in DNA fraction while 75-85% binds to nucleophilic sites of intracellular constituents like thiol containing peptides,proteins,replication enzymes and RNA. This preferential binding to non DNA targets offers the explanation forcisplatin resistance as well as its high toxicity. Transition metal complexes of 1,10-phenanthroline or 2,2′-bipyridineaffords a mechanism of action significantly different to that of the clinically used drug cisplatin. These novel setof drugs have demonstrated therapeutic prowess in some cells that have shown resistance to conventional anticancer drugs like cisplatin.

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