9.4: Survey of Metals Used for Diagnosis and Chemotherapy

Radiodiagnostic Agents[21,22]

Metal complexes having radioactive nuclei find many applications in medicine, such as in organ, and tissue imaging, Early detection of cancer, for example, by selective and imaging of the tumor using a radioactive metal compound can facilitate surgical removal or chemotherapeutic treatment before the disease reaches an advanced stage. radioisotopes used for diagnostic purposes be emit low-energy γ and no α or β particles. Table 9.2 lists the radionuclides most commonly employed for purpose in nuclear medicine. Among these, ^{99m}Tc is perhaps the most desirable,[23] for it gives off a 140-keV γ ray that is detected scintillation cameras and produces clear images. radionuclide is prepared from an alumina column loaded with $^{99}MoO_4^{2-}$, which decays to form $^{99m}TcO_4^-$, which in turn may be selectively eluted from the column with saline owing to its lower charge. treatment with a reducing agent in the presence of the appropriate ligands produces radiopharmaceuticals with desired water stability, and properties. Such complexes may be injected at concentrations of 10^{-6}-10^{-8} M. For example, isocyanide complexes such as $[Tc(CNR)_6]^+$= t-Bu, $CH_2CO_2Bu^t$, etc.) have been found to be taken up selectively into heart tissue and thus have the potential to be used as heart-imaging agents. Figure 9.2 displays bone as imaged a ^{99m}Tc bone agent. The dark correspond to surface areas of metabolic which can be used to diagnose or disease. One goal of research in this field is to images of myocardial infarcts or clogged arteries for physicians who can watch the patient's heart on a video surgery. Although chemical details responsible for the selective tissue of Tc isocyanide, and other complexes are largely synthetic modifications are and have many new compounds for evaluation.

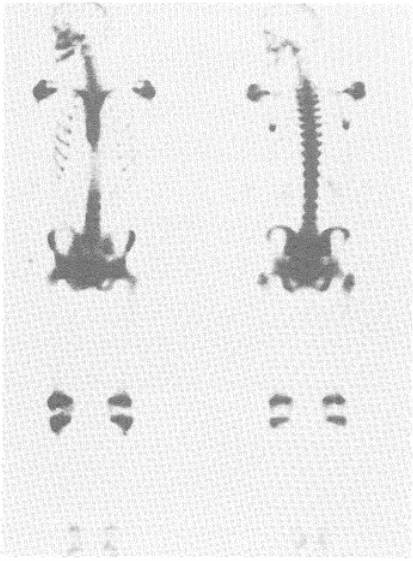

Figure 9.2 - Human skeleton (bone) imaged with ^{99m}Tc. Both anterior (left) and posterior (right) views are shown.

Table 9.2 - Radionuclides most commonly employed in diagnostic nuclear medicine.[a]

a) Data are from Table of the Isotopes in D. R. Lide, ed., *CRC Handbook of Chemistry and Physics*, CRC Press, 71 st ed., 1990-91, pp. 11-33 ff.

Radionuclide	Half-Life	Energy (keV)
^{57}Co	271 d	836
^{67}Ga	78 h	1,001
^{99m}Tc	6 h	140
^{111}In	67 h	172, 247
^{113m}In	104 m	392
^{123}I	13 h	1,230
^{169}Yb	32 d	207
^{197}Hg	64 h	159

Radionuclide	Half-Life	Energy (keV)
^{201}Tl	72 h	135, 167

Among the few known to be absorbed selectively tumor cells is the antitumor antibiotic bleomycin (BLM),[24,25] the structure of which is portrayed in Figure 9.3. binds most radioactive metal ions, but the $^{57}Co(III)$ complex has the best tumor-to-blood ratio. Unfortunately, the long ^{57}Co (Table 9.2) has limited its clinical utility. Attempts to prepare ^{99m}Tc complexes of BLM with selective uptake properties approaching that of the cobalt have not yet been successful, although the target molecule would be a most valuable radiodiagnostic agent. One imaginative solution[26] to this problem was achieved by covalent attachment of an EDTA moiety to the terminal thiazole ring of BLM (Figure 9.3). The resulting Co(III) BLM-EDTA molecule was radiolabeled with $^{111}In^{3+}$ and found to be useful for diagnosis of cancer in humans. Also used for tumor imaging are ^{99m}Tc and ^{67}Ga citrate complexes, the latter being the agent of choice for many applications. Again, there is little known at the molecular level about the mechanism of tumor-cell specificity

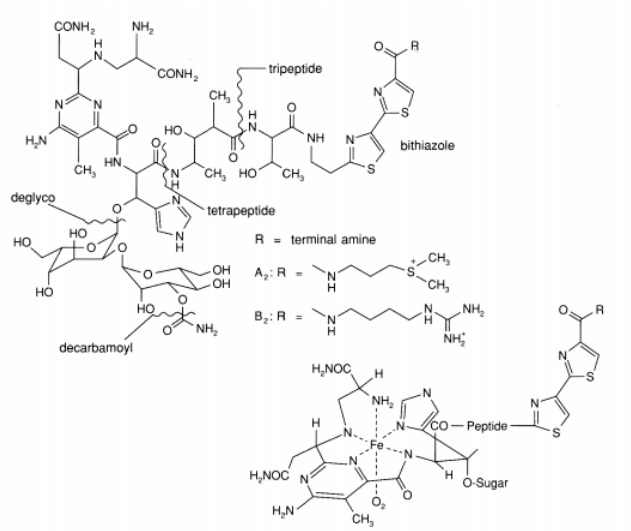

Figure 9.3 - Structure of bleomycin and its proposed iron complex (reproduced by permission from Reference 25).

An alternative approach to radionuclide-based tumor-imaging agents for diagnosis of disease is to modify, with metal chelating agents, antibodies raised against a biological substance, such as a tumor-cell antigen, hormone, or other target. Antibodies are proteins that are synthesized by specialized cells of the immune system in response to an external stimulant, or antigen. The high specificity and affinity of antibodies for the antigen can be used to target the antibody to a particular biological site, such as a site on the membrane of a particular cell type. Chelating agents are now routinely attached to antibodies and used to bind radioactive metal ions. The resulting radionuclide-labeled products are currently under extensive study in diagnostic medicine.[26]

Magnetic Resonance Imaging (MRI)[27]

Nuclear magnetic resonance (NMR) spectroscopy can be used to image specific tissues of biological specimens because of differences in the relaxation times of water proton resonances, usually brought about by paramagnetic metal ions. An early, pioneering example was the demonstration that Mn(lI) salts localize in normal heart-muscle tissue in dogs rather than in regions affected by blocked coronary arteries. Since the paramagnetism of the d^5 Mn(lI) ions alters the relaxation rate of nearby water protons, the normal and diseased tissue could be distinguished. Of the various metal ions surveyed in attempts to provide clinically useful NMR images in humans, Gd(III), Fe(III) , and Mn(lI) were found to give the best proton-relaxation enhancements. The gadolinium complex $[Gd(DTPA)(H_2O)]^{2-}$,an agent currently used in the clinic, has been successfully employed to image brain tumors. Ferric chloride improves gastrointestinal tract images in humans and, as already mentioned, manganous salts can be used for heart imaging. NMR imaging methodologies have advanced to the stage where increases as small as 10 to 20 percent in T_1^{-1}, the inverse nuclear-spin relaxation time, can be detected. As with radionuclide labeling, the complexes must be soluble and stable in biological fluids and relatively nontoxic, and are of greatest value when able to target a specific tissue. Even more important than targeting, however, is that proton relaxivity be maximally enhanced, an objective that depends not only upon the local binding constant but also upon large magnetic moments, long electron-spin relaxation (T_{le}) values, access to and the residence lifetime in the inner and outer coordination spheres by water molecules, and the rotational correlation time of the complex at its binding site.

An obvious advantage of paramagnetic NMR over radioisotopic imaging agents is that there is no possibility of radiation damage; on the other hand, the need for 10-100 μM concentrations at the site of imaging is a distinct drawback. Both methods are likely to continue to be used in the future, and both will benefit from the design of new stable chelates that are selectively absorbed by the tissue to be diagnosed.

Lithium and Mental Health[28-31]

One in every 1,000 people in the United States currently receives lithium, as Li_2CO_3, for the treatment and prophylaxis of manic-depressive behavior. Doses of 250 mg to 2 g per day are administered in order to maintain a 0.5 to 2.0 mM concentration window, outside of which the drug is either toxic or ineffective. The detailed molecular mechanism by which Li^+ ion brings about its remarkable chemotherapeutic effects is largely unknown, but there are various theories. One theory proposes that lithium binds to inositol phosphates, inhibiting their breakdown to inositol, and so reducing inositol-containing phospholipids. A consequence of this chain of events would be disruption of the neurotransmission pathway based on inositol 1,4,5-triphosphate and 1,2-diacylglycerol, reducing neuronal communication, which is most likely hyperactivated in the manic state. This theory does not account for the antidepressive action of the drug, however. An alternative explanation is that lithium inhibits cyclic adenosine monophosphate (AMP) formation, again interfering with neurotransmission by intercepting this key intracellular signaling molecule. Recent experiments indicate that lithium affects the activation of G-proteins, a class of guanosine triphosphate (GTP)-binding proteins involved in information transduction. Possibly these effects result from displacement by Li^+ of Mg^{2+} from GTP and/or from protein-binding sites normally required for activation. Use of ^{7}Li NMR spectroscopy to study lithium transport in human erythrocytes suggests that it might be possible to apply this method to unravel details of the bioinorganic chemistry of lithium associated with the management of manic depression.

Gold and Rheumatoid Arthritis[23,32,33]

Gold compounds have been used in medicine for centuries, an application known as chrysotherapy. Since 1940, however, complexes of gold have been used most successfully to treat arthritic disorders in humans and other animals. Au(I) compounds are currently the only class of pharmaceuticals known to halt the progression of rheumatoid arthritis.

Until recently, gold compounds used to treat arthritis were painfully administered as intramuscular injections. Included were colloidal gold metal, colloidal gold sulfides, $Na_3[Au(S_2O_3)_2]$ (Sanocrysin), gold thiomalate and its sodium and calcium salts (Myochrisin), and polymeric gold thioglucose (Solganol, approved by the FDA). It was discovered, however, that triethylphosphinegold(l) tetra-O-acetylthioglucose (auranofin, Figure 9.4, approved by the FDA) was equally effective against rheumatoid arthritis and could be orally administered. The availability of this compound has sparked many studies of its biodistribution, stability, and possible metabolism that lead to antiarthritic activity. The mode of action of antiarthritic gold drugs is largely unknown, but it may involve binding of Au(I) to protein thiol groups, a process that inhibits the formation of disulfide bonds, and could lead to denaturation and subsequent formation of macroglobulins.

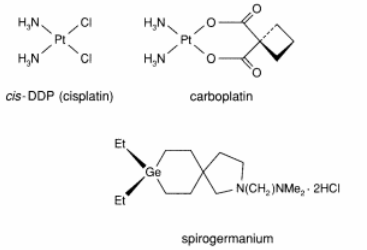

Figure 9.4 - Structures and trivial names of metal-based antitumor drugs.

Anticancer Drugs

1. Platinum Ammine Halides[34,35]

The discovery that *cis*-diamminedichloroplatinum(Il), *cis*-DDP or cisplatin (Figure 9.4), has anticancer activity in mice, and its subsequent clinical success in the treatment of genitourinary and head and neck tumors in humans, constitutes the most impressive contribution to the use of metals in medicine. Given in combination chemotherapy as an intravenous injection together with large amounts of saline solution to limit kidney toxicity, cisplatin treatment results in long-term (>5 yr) survival for more than 90 percent of testicular cancer patients. In a typical course, ~ 5 mg/kg body weight of the drug is administered once a week for four weeks.

Extensive studies of platinum ammine halide analogues led to a series of empirical rules governing their chemotherapeutic potential. Specifically, it was concluded that active compounds should:

1. be neutral, presumably to facilitate passive diffusion into cells;
2. have two leaving groups in a *cis* configuration;
3. contain nonleaving groups with poor trans-Iabilizing ability, similar to that of NH_3 or organic amines;
4. have leaving groups with a "window of lability" centered on chloride.

These early structure-activity relationships have had to be modified somewhat, however, since chelating dicarboxylate ligands such as 1,1-dicarboxylatocyclobutane can replace the two chloride ions, and since cationic complexes with only one labile ligand, specifically, *cis*-$[Pt(NH_3)_2CI(4\text{-}X\text{-}py)]^+$, where X = H, Br, CH_3, etc., showed activity in some tumor screens. The two compounds shown in Figure 9.4, cisplatin and carboplatin (Figure 9.4), were the first to be approved for clinical use. Of particular interest to the bioinorganic chemist is that complexes having a *trans* disposition of leaving groups are inactive *in vivo.* This difference suggests the presence of a specific cellular receptor that, when identified, should facilitate the design of new, metal-based anticancer drugs. Present evidence strongly points to DNA as being the relevant cellular target molecule. Section V of this chapter expands on this topic in considerable detail.

2. Metallocenes and Their Halides: Ti, V, Fe[36,37]

Several compounds in this category, including $[(C_5H_5)_2TiX_2]$ (X = CI, Br, O_2CCl_3), $[(C_5H_5)_2VCl_2]$, $[(C_5H_5)_2NbCl_2]$, $[(C_5H_5)_2MoCl_2]$, and $[(C_5H_5)_2Fe]^+$ salts, exhibit significant activity against experimental animal tumors. Higher quantities (200 mg/kg) of these compounds than of *cis*-DDP can be tolerated with fewer toxic side effects, but their failure in two mouse leukemia screens commonly used to predict the success of platinum anticancer agents appears to have delayed their introduction into human clinical trials. Studies of Ehrlich ascites tumor cells treated with $[(C_5H_5)_2VCl_2]$ *in vitro* revealed selective inhibition of incorporation of radiolabeled thymidine, versus uridine or leucine, indicating that the complex blocks DNA replication. Unlike cisplatin, however, metallocene halides undergo rapid hydrolysis reactions in aqueous media, forming oxobridged and aqua complexes that may have a higher affinity for phosphate oxygen atoms than the heterocyclic nitrogen atoms of the bases in DNA.[38] Exactly how the ferrocenium ion might bind to DNA is even more obscure, although partial metallointercalation and groove binding are more likely than covalent attachment of the chemically unmodified cation. From the limited information available, metallocenes and their halides appear to behave fundamentally differently from platinum antitumor compounds. As a class, they provide a promising new opportunity to expand the scope of metal complexes used in cancer chemotherapy.

3. Gold and Other Metal Phosphines[39]

Following the successful entry of the soluble gold-phosphine complex auranofin (Figure 9.4) into the metal-based pharmaceuticals industry, several gold-phosphine complexes were examined for possible anticancer activity. Although auranofin itself was active in only a small fraction of the mouse tumor models tested, biological activity approaching that of cisplatin was discovered for many analogues, most notably the diphosphine bridged complex $[ClAu(PPh_2CH_2CH_2\ PPh_2)AuCI]$. Attempts to replace the phosphine with As or S donor ligands, to increase or decrease the length of the 2-carbon bridge, or to replace the phenyl with alkyl groups all led to diminished activity. Most noteworthy is that the diphosphine ligands themselves have activity very similar to that of their gold complexes, and that Ag(I) and Cu(I) analogues are also effective. These results strongly imply that the phosphine ligands are the chemical agents responsible for the anticancer properties of these compounds. Coordination to a metal presumably serves to protect phosphines against oxidation to the phosphine oxides, which independent investigations have proved to be ineffective. A possible role for the metal in the cytotoxicity of the compounds cannot be ruled out, however.

4. Other Main Group and Transition-metal Compounds[36,40,41]

Several main group metal complexes exhibit anticancer activity. Gallium(III) nitrate is active against human lymphomas, but with dose-limiting side effects on the kidneys and gastrointestinal tract. Tin complexes of general formula $R_2L_2SnX_2$, where R = alkyl or phenyl, L_2 = py_2, bpy, or phen, and X_2 = two *cis*-oriented halide or pseudohalide leaving groups, are active against the mouse P388 leukemia tumor. The *cis* disposition of the leaving groups suggests a possible mechanism analogous to that of cisplatin (see below). Organo-gennanium compounds are also active, notably the derivative spirogermanium shown in Figure 9.4. Nothing is known about the mechanism of action of any of these compounds.

Following the discovery of activity for cisplatin, several thousand platinum and nearly 100 other transition-metal complexes have been screened in various tumor model systems in the hope of achieving better activity against a broader range of tumors. Among the classes of nonplatinum compounds showing some activity are ruthenium complexes *cis*-$[RuCl_2(DMSO)_4]$, $[Ru(NH_3)_5(Asc)](CF_3SO_3)$, where Asc is ascorbate dianion, and *fac*-$[Ru(NH_3)_2Cl_4]$, all of which are believed to bind to DNA; binuclear rhodium

complexes $[Rh_2(O_2CR)_4L_2]$; octahedral Pd(IV) complexes such as *cis*-$[Pd(NH_3)_2CI_4]$; and such miscellaneous molecules as the iron(II) complex of 2-formylpyridine thiosemicarbazone, the site of action of which is thought to be ribonucleotide reductase. These examples illustrate the broad scope encompassed by this field, which has a potential for developing fundamental infonnation about metal-biomolecule interactions as well as novel anticancer drugs. Much remains to be explored.

Miscellaneous Metals in Medicine

Numerous other anecdotal and some fairly elaborate studies have been reported for metal complexes as medicinal agents. The use of zinc applied topically to promote the healing of wounds dates back to around 1500 B.C., and silver is now commonly applied to prevent infection in bum patients.[42,43] Osmium carbohydrate polymers have been reported to have antiarthritic activity.[44] Transition-metal complexes have a long history of use as antibacterial and antiviral agents; for example, Zn^{2+} is used to treat herpes, possibly by inhibiting the viral DNA polymerase.[45] Early transition-metal (e.g., tungsten) polyoxoanions have been employed to treat AIDS patients.[46] Numerous reports have appeared detailing the anti-inflammatory, antiulcer, and analgesic activities of copper carboxylate complexes.[7] As in the previous section, these reports and others like them require more serious attention from bioinorganic chemists to elucidate the molecular events responsible for such a fascinating menu of biologically active metal complexes.

Summary and Prospectus

The clinical successes of platinum anticancer and gold antiarthritic drugs have changed the attitudes of many who doubted that heavy-metal compounds, notorious for their deleterious effects on human health, would ever playa serious role in chemotherapy. Indeed, we have seen that Hg^{2+}, Pb^{2+}, and Cd^{2+} are toxic elements. Even essential metals can be highly toxic if present in excess, either because of chronic or acute poisoning or because of metabolic defects that deregulate their control in the cell. An important common theme running throughout this discussion is selectivity. For a drug to be effective, it must be selectively toxic to diseased tissue while leaving nonnal tissue alone; or it must selectively kill harmful microorganisms at levels where it fails to deplete helpful ones. For a chelating agent to be useful in the toxic effects of metals, it must bind as selectively as possible to the deleterious ion while coordinating only weakly, if all, to others. For a diagnostic metal complex to be it must be taken up (or excluded) selectively from diseased cells relative to normal ones, or to one tissue type versus another. Rarely has such selectivity been designed in advance of the discovery of a useful metal-based pharmaceutical, although spectacular advances in biology, such as monoclonal antibodies, may be hastening the day when such an objective be common. Interestingly, the successes of such unlikely as *cis*-$[Pt(NH_3)_2Cl_2]$ and $[(Et_3P)Au(OAc_4$-thioglucose)] in chemotherapy were driven by the personal involvement of individuals like B. Rosenberg for the former and B. Sutton for the latter. Like Hollywood producers, these men mustered every conceivable resource to promote the compounds for testing, introduction into human clinical trials, and eventually approval by the FDA. Such zeal requires years, usually more than a decade, of sustained personal effort, and may be the reason why other metal complexes, such as those mentioned above, have not had the impact of a cisplatin or an auranofin. On average, only one of 7,000 such compounds makes it from the laboratory bench to the patient, at an average cost of 250 million dollars and a time interval of 13 years.

Another component of the evolving field of metals in medicine, however, is that, once a has proved its in the clinic, how does it work? This question is deceptively for coordination chemistry *in vivo*, and the of cells to respond to unnatural external stimuli such as metal complexes, are matters about which we are beginning to learn. As progress is made in this latter area, it should become possible to design drugs in a rational way to achieve the required selectivity.

The remainder of this chapter focuses on a case study where some progress in unraveling the molecular mechanism of a metal-based drug, cisplatin being made. If nothing else, this discussion will elucidate strategic guidelines that may be employed to attack similar questions about other chemotherapeutic metal compounds discussed earlier in this section. Unfortunately, there is very little information available about the molecular mechanisms of these other complexes. At this transition in our discussion, we move from general considerations to a specific, analysis. The reader must here take time to become familiar with the biological aspects of the new material.