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Coordination Chemistry Reviews
184 (1999) 67–89

COORDINATION CHEMISTRY REVIEWS

Coordination compounds in medicinal chemistry

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Received 1 May 1998

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PII: S0010-8545(98)00205-7

Abstract

In medicinal chemistry metal complexes as pharmaceuticals have received limited attention compared with organic compounds. Much of the attention has been given to Pt(II) complexes that inhibit tumor growth, but a widely applicable target for development of metal complexes as pharmaceuticals has emerged from new perspectives of enzymes in disease processes. In this review we look at work involving the development of Fe(II), Pt(II), and Ti(IV) complexes as pharmaceuticals, and what this work reveals about transport of metal compounds across cell membranes and future roles for *metallo-drugs*.

Keywords: Metallo-drugs; Fe(II); Pt(II); Ti(IV); Transport; Antineoplastic; Anti-infective; Enzyme inhibitors; Proteases; Telomerases; *Escherichia coli*; *Salmonella*; *Pseudomonas*; Tuberculosis

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1. Introduction

The essential role of transition metal ions in biological systems is well known [1–7]. In this paper we review issues that are important to development of transition metal complexes as pharmaceuticals. When designing metal complexes for therapeutic use the following events need to be considered: hydrolysis → protein binding → membrane transport → molecular target. Hydrolysis of metal complexes is important because of the aqueous milieu of biological systems, but the hydrophobic nature of cell membranes, vesicles and enzyme active sites requires consideration of lipophilic ligands in the design of complexes. Therefore, design of *metallo-drugs* requires bringing together organometallic chemistry with traditional aqueous coordination chemistry, a merger that is in its infancy [8–10]. Whether or not the ultimate target of a *metallo-drug* is a protein, protein binding is always a factor in the medicinal use of such compounds. For example. blood proteins such as albumin and transferrin have structural domains that strongly bind metal ions [11]. The greatest hurdle, however, is transport of metal complexes through cell membranes, which determines if metals enter cells with their ligands intact.

1.1. Transport of metal ions

Development of metal complexes as pharmaceuticals has been slow in coming due to a prior belief that metal ions are not transported across cell membranes but are incorporated at the formation stage of cells [12]. Evidence for transport in mature cells first appeared in 1939 when K^+ [13] and Na^+ [14] were shown to move across red cell membranes in opposition to concentration gradients (i.e. *active transport*). It took twenty years longer before *active transport* of Ca^{+2} [15] was reported, while for Mn^{+2} the *entry and exit* remained *a process of passive diffusion involving no carriers*, *transport*, *or metabolic linkage* [16,17]. In 1996 *active transport* of Mn^{+2} was reported [18], and in 1997 the *trans*-membrane protein *DCT1* which transports Mn^{+2} and other transition metal ions was reported [19]. *DCT1* is selective for divalent charge and is insensitive to electron configuration of the metal ions it transports. This suggests the protein provides a micro-environment where metal charge is balanced by charge on the protein positioned at or near optimum *Debye lengths* [20]. Movement of metal ions along the protein is coupled to H^+ transport and the electrochemical potential across the cell membrane [19]. To what extent the protein allows transport of metal bound ligands is not known.

2. Iron

Iron complexes were the earliest metal compounds to be studied in medicinal chemistry. They were and are used to treat hypochromic anemia caused by iron deficiency. Clinical experience has revealed Fe(II) to be better absorbed than Fe(III) when given orally for this disease [21].

The first mechanism identified for transport of Fe(II) through cell membranes was *endocytosis* [22]. In this process transferrin, an Fe(II)-protein complex, is internalized by cell membranes as the protein binds to specific receptors on the outside of cells [12,22–26]. Transport through the membrane results from Brownian motion of the receptors, with or without transferrin attached, as they continually move back and forth between the inner and outer membrane surfaces [22]. Endocytosis of Fe(II) is a highly organized and regulated process [11,22,27]; the time frame of which is no more than a few minutes, possibly a few seconds [22].

Recently another mechanism for Fe(II) transport was identified [28,29], which does not involve endocytosis. This mechanism appears to use the *trans*-membrane protein *DCT1* identified by Gunshin et al. [19]. Ideal conditions for Fe(II) transport by *DCT1* exist in the upper section of the small intestine (duodenum) closest to the stomach where pH increases from 2 to 7 as iron moves down the duodenum. (Little or no transferrin or apotransferrin is present in the duodenum to facilitate endocytosis of iron.) Under the pH conditions of the duodenum Fe(II) is free from hydrolysis and formation of insoluble hydroxides and oxides, while Fe(III) is not [30]. Thus, oral administration of Fe(II) complexes is more efficacious than those of Fe(III) in alleviating the iron deficiency of hypochromic anemia.

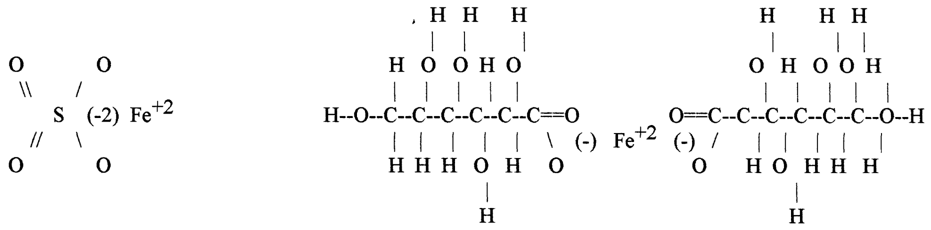
2.1. Iron(II) sulfate

The earliest formulation of Fe(II) still used for treatment of hypochromic anemia is $FeSO_4$ [31]. The complex has been formulated with a variety of excipients such as plant gums (disolved in glycerin or water), carbonate, citrate and tartrate. In the original formulation by Blaud [31] equal parts by weight of potassium carbonate and ferrous sulfate were added to a water based *mucilage adragant*. Although the Fe(II) complex was called ferrous carbonate, no mention was made of the characteristic gray color of $Fe(II)CO_3$. One interesting early formulation used honey as an excipient and was reported to yield a dark green ferrous complex [32]. For 100 years controversy surrounded the issue of what constituted stable and efficacious formulations of $FeSO_4$. Today, $FeSO_4$ is formulated either as dried $FeSO_4$ (tablets) or in water with sugar, ethyl alcohol and citric acid. The presence of SO_4^{-2} as a ligand stabilizes the +2 oxidation state of the metal [33].

2.2. Iron(II) carboxylates

The use of ferrous sulfate is not without problems [34], the most important of which is toxicity in young children [35]. Toxicity results from build-up of Fe(III) in the liver where it causes oxidative necrosis [36], presumably from free radical damage. In an effort to address this problem, various organic complexes of Fe(II) have been investigated [34,37]. Examples of such complexes licensed for clinical use are shown in Fig. 1 with their LD_{50} (in mice, given orally).

The toxicity of these compounds decrease with the increasing molecular character of the complex. For example, the electron distribution in the sulfate complex is the most localized, while in the fumarate complex it is delocalized over the entire complex. Since the principle mode of Fe(II) transport from the gut occurs through the mucosal cell membrane protein *DCT1* and not endocytosis, the differences in toxicity among the Fe(II) complexes shown must result from differences in their interaction with *DCT1*. It is expected that binding to *DCT1* is less favorable for *molecular* ferrous fumarate than for *ionic* ferrous sulfate, which results in different



Sulfate
LD_{50} = 230 mg/Kg

Gluconate
LD_{50} = 320 mg/Kg

Succinate
LD_{50} = 560 mg/Kg

Fumarate
LD_{50} = 630 mg/Kg

Fig. 1. Fe(II) drugs.

rates of delivery of iron across mucosal cell membranes. With iron carbonyl, $[Fe(CO)_5]$, the LD_{50} is $\sim 50\,000$ mg kg^{-1} body weight [10]. At pH achievable in the intestine, this complex is oxidized by O_2 to $[Fe(CO)_4]^{-2}$ [38], which is the source of Fe(II) presented to the *DCT1* transport protein.

Once inside mucosal cells Fe(II) is quickly oxidized to Fe(III) by ferritin [39,40] and stored as Fe(III) mineral particles. These particles have a variety of structures, with ferrihydrite $(5Fe_2O_3 \cdot 9(H_2O)$ being common, that are capable of growing to nano-scale size within the protein cage of ferritin [41]. Transport of iron from mucosal cells to blood for distribution to other cells results from reduction of ferritin bound Fe(III) at the inner surface of the mucosal cell membrane followed by Fe(II) transport out of the cell and ultimately to transferrin in blood [42,43]. Clearly, ligands used in the original formulation of iron are lost at this mineralization step and the original iron complexes given as pharmaceutical formulations do not make their way to biological targets such as developing red cells.

However, a report by Jonas and Riley [44] suggests iron transport can be influenced in other ways by the ligands bound to Fe(II). In their work, cultures of mammalian epithelial cells were exposed to various Fe(II) chelates. The ligands were low molecular weight *hydrophilic* or *lipophilic* molecules, or high molecular weight dextran. Fe(II) bound to hydrophilic ligands such as ethylendiaminetetraacetic acid was not taken-up by the cells, while Fe(II) bound to lipophlic ligands such as 8-hydroxyquinoline was taken-up in a manner consistent with diffusion through the membrane lipid bilayer. But it is not clear from available data if the binding constants of the hydrophilic ligands prevented *DCT1* from transporting Fe(II). Interestingly, Fe(II) bound to dextran appeared to be transported by endocytosis with ligand intact.

3. Platinum

Acceptance of metal complexes as drugs was dramatically advanced by the use of Pt(II) complexes to treat testicular and ovarian cancers. This began in1965 with a report by Rosenberg, Van Camp and Krigas [45] that *some Group VIIIb metal complexes inhibit cell division*, and quickly lead to study of Pt(II) complexes as agents capable of stopping tumor growth [46]. The first Pt(II) complex to be licensed for this purpose was *cisplatin*, *cis*-$[Pt(NH_3)_2Cl_2]$. For a history of cisplatin development see Rosenberg [47], and for an overview of platinum compounds as candidate drugs for treatment of tumors see Kelland [48]. The discussion here focuses on organizing and understanding the medicinal chemistry of Pt(II) chloroammine(amine) complexes.

In general such complexes are square-planar, and though *inert* with regard to the kinetics of ligand exchange [49,50] they do undergo reaction slowly. In Table 1 some data are shown for ligand exchange by cisplatin and its *trans*-isomer *transplatin*. The table is adapted from data provided by Banerjea, Basolo and Pearson [51]. In addition, it is known cisplatin slowly converts in aqueous solution to transplatin [52,53], and in general *trans*-Pt(II) ammine and amine complexes are more thermodynamically stable than their *cis*-isomers [54].

Table 1

Cis-Pt(II)	*Trans*-Pt(II)
$Pt(NH_3)_2Cl_2 + {}^{36}Cl^- \xrightarrow[k_1 = 2.3 \times 10-3]{H_2O} Pt(NH_3)_2(H_2O)Cl^- + Cl^-$	$Pt(NH_3)_2Cl_2 + {}^{36}Cl^- \xrightarrow[k_1 = 2.3 \times 10-3]{H_2O} Pt(NH_3)_2Cl({}^{36}Cl) + Cl^-$
$Pt(NH_3)_2Cl_2 + H_2O \xrightarrow[k_1 = 2.3 \times 10-3]{H_2O} Pt(NH_3)_2(H_2O)Cl^- + Cl^-$ (Complete substitution occurs; $t_{1/2} = 5$ h)	$Pt(NH_3)_2Cl_2 + H_2O \xrightarrow[k_1 = \sim 0]{H_2O} \text{No change}$ (No release of Cl^-, even after weeks)
$Pt(NH_3)_2Cl_2 + OH^- \xrightarrow[k_1 = 2.3 \times 10-3]{H_2O} Pt(NH_3)_2Cl(OH) + Cl^-$	$Pt(NH_3)_2Cl_2 + OH^- \xrightarrow[k_1 = 6.1 \times 10-3]{H_2O} Pt(NH_3)_2Cl(OH) + Cl^-$
$Pt(NH_3)_2Cl(H_2O)^+ + L \xrightarrow[k_1 = \text{fast}]{H_2O} Pt(NH_3)_2Cl(L) + H_2O$	(No equivalent reactant)
$Pt(NH_3)_2Cl(L) + L \xrightarrow[k_1 = \text{slow}]{H_2O} Pt(NH_3)_2L_2 + Cl^-$	$Pt(NH_3)_2Cl(OH) + L \xrightarrow[k_1 = \text{slow}]{H_2O} Pt(NH_3)_2(OH)L + Cl^-$
$Pt(NH_3)_2Cl(OH) + OH^- \xrightarrow[k_1 = 1.3 \times 10-3]{H_2O} Pt(NH_3)_2(OH)_2 + Cl^-$ (Completely reversible when H^+ added)	$Pt(NH_3)_2Cl(OH) + OH^- \xrightarrow[k_1 = 2.3 \times 10-3]{H_2O} Pt(NH_3)_2(OH)_2 + Cl^-$ (Not reversible when H^+ added; polymeric Pt(II) species)
$Pt(NH_3)_2(H_2O)Cl^+ + C_5H_5N \xrightarrow[k_1 = 1.5 \times 10-3]{H_2O} Pt(NH_3)_2Cl(C_5H_5N)^+ + H_2O$	$Pt(NH_3)_2Cl_2 + C_5H_5N \xrightarrow[k_1 = 5.7 \times 10-3]{H_2O} Pt(NH_3)_2Cl(C_5H_5N)^+ + Cl^-$
$Pt(NH_3)_2(H_2O)Cl^+ + NO_2^- \xrightarrow[k_1 = \sim 1 \times 10-2]{H_2O} Pt(NH_3)_2ClNO_2 + H_2O$ $[NO_2^-] = \sim 0.01$ M	$Pt(NH_3)_2Cl_2 + NO_2^- \xrightarrow[k_1 = \text{extremely rapid}]{H_2O} Pt(NH_3)_2ClNO_2 + Cl^-$

For historical clarification, the work of Reishus and Martin, Jr. [55] on *cis*-[Pt(NH_3)$_2$$Cl_2$] hydrolysis and Cl^- exchange has been directly [56] and indirectly [57,58] cited as the chemical basis of cisplatin pharmacology. Because of this it has been assumed the active species derived from cisplatin *in vivo* is $[Pt(NH_3)_2(H_2O)(OH)]^+$ [56], $[Pt(NH_3)_2(H_2O)Cl]^+$ [57] or $[Pt(NH_3)_2(H_2O)_2]^{+2}$ [58]. However, it is not clear what species was studied by Reishus and Martin, Jr. From the method they used to prepare *cis*-$[Pt(NH_3)_2Cl_2]$, the lack of structural analysis and the description of the product as a greenish–yellow precipitate, it appears the material studied was in part a *Magnus's green salt* [59].

The action of cisplatin as an anticancer drug is believed to result from binding of Pt(II) to cell DNA [60–63]. Correlation between Pt–DNA binding and cell death by apoptosis has been reported [61]. A variety of Pt(II)–DNA adducts have been identified in non-cellular systems [62,63]. The adducts include Pt(II) bridged intra-strand and inter-strand DNA complexes, with a preference for Pt(II) binding to N-7 in the guanine rings along the DNA chain [63]. It is not known which Pt(II)–DNA complex is critical to the mode of action, but *adenine*–Pt(II)–*guanine* adducts [64,65] and *guanine*–Pt(II)–*guanine* adducts of DNA have been proposed [66].

There are important biological differences in the behavior of cisplatin and transplatin, in that cisplatin is active against tumor cells while transplatin is not [67–69]. This difference in ID_{90} (inhibiting dose, 90% effective) is seen with other Pt(II) ammine and amine complexes [68] and does not correlate with isomer LD_{50} (lethal dose). These differences are seen as cell death by apoptosis versus cell death by toxicity. Cisplatin inhibition of tumor cell growth can be achieved at drug levels below those that cause animal death, while with transplatin inhibition of tumor cell growth does not occur until lethal concentrations of the compound are used. Thus, the clinical mode of action of cisplatin includes the specific ability to interfere with tumor cell replication, while transplatin has little or none of this ability.

3.1. $[PtCl_4]^{-2}$

Thousands of Pt(II) compounds have been investigated for their ability to stop tumor cell growth, but only a few of these compounds have made their way to clinical trials. Of these, few have yet to complete the licensing process. With so many compounds passing through the drug development pipeline how can they be rationally prioritized?

A useful framework in which the range of chemical and biological data on cisplatin and other Pt(II) chloro–ammine(amine) complexes can be placed is the molecular orbital diagram of $[PtCl_4]^{-2}$ in its ground state, Fig. 2. This representation of bonding in square planar Pt(II) chloro-complexes is adapted from that of Lever [70]. For application of this representation to other Pt(II) chloro-complexes it is helpful to note that the metal d_{xz} orbital interacts with the p_z ligand orbitals of the *trans*-chloride pair along the x-axis of the complex, while the metal d_{yz} orbital interacts with the p_z ligand orbitals of the *trans*-chloride pair along the y-axis of the complex [70]. To a first approximation, this permits the molecular orbitals for these two pairs to be treated independently when considering the affects of ligand

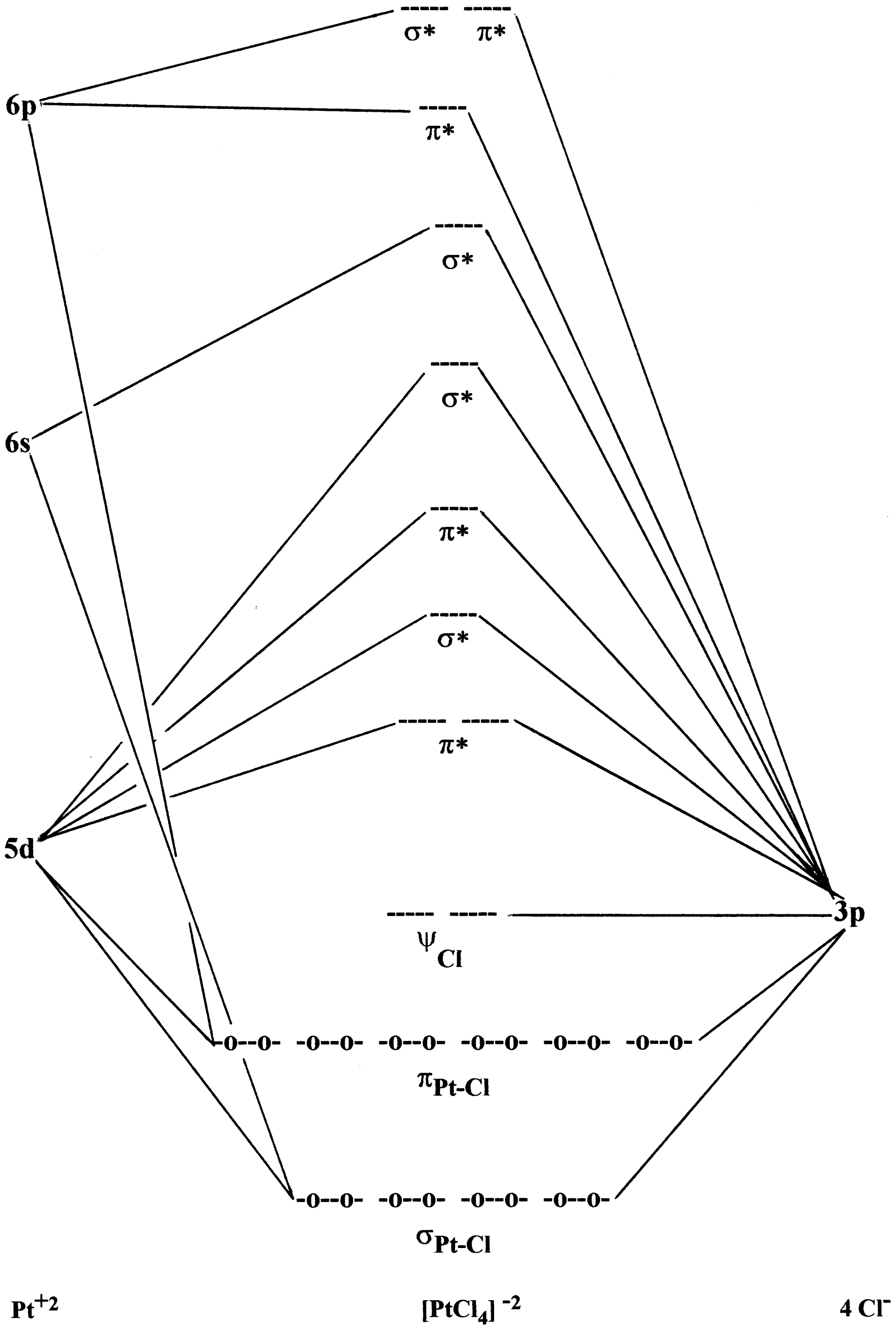


Fig. 2. Molecular orbital diagram of $[PtCl_4]^{-2}$.

substitution of the chlorides. With this in mind, the various Pt(II) chloro–ammine(amine) complexes reported in the literature can be organized in decreasing order of electron delocalization in the π molecular orbitals.

Fig. 2 shows that π-bonding in $[PtCl_4]^{-2}$ is a major contributor to molecular stability. This stability, for example, is reflected in the reaction $[PtCl_4]^{-2} + H_2O \rightarrow [PtCl_3\ (H_2O)]^- + Cl^-_{(aq.)}$, where $\Delta H = +21$ kcal mol^{-1} at 25°C [71]. The stability is also reflected in the kinetics of $[PtCl_4]^{-2}$ reactions. For example, $^{36}Cl^-$ exchange with $[PtCl_4]^{-2}$ is a first-order reaction with $t_{1/2} \approx 14$ h (rate constant $k_1 \approx 0.8 \times 10^{-3}$) [71].

To an incoming ligand, $[PtCl_4]^{-2}$ in its ground state appears to be a spherical electron cloud created by π molecular orbitals, which must be broken in order for the incoming ligand (electron donor) to approach the metal. Fig. 2 shows this can be accomplished by electron transfer from π-bonding orbitals between metal and ligands to ψ_{Cl} non-bonding orbitals on the ligands. Such transitions may be solvent assisted, ΔG°_{solv} for Cl^- in water is of the order of -100 kcal mol^{-1} [72]. Ligand exchange reactions for $[PtCl_4]^{-2}$, therefore, proceed by an S_N1 (D or I_d) mechanism. For more on π-bonding and solvent assisted substitution reactions of metal complexes see the discussion by Peter Lay [72].

3.2. Transplatin

Turning to $[Pt(NH_3)_2Cl_2]$ complexes, with *trans*-$[Pt(NH_3)_2Cl_2]$ the amount of π-bonding in Fig. 2 is reduced by half due to replacement of two Cl^- ligands with two NH_3 ligands. (The NH_3 ligands are not capable π-bonding.) Similarly, the number of ψ_{Cl} non-bonding orbitals must be reduced by half. In addition, the π molecular orbitals move to higher energy due to reduced molecular orbital delocalization. (With $[PtCl_4]^{-2}$, electrons in π-bonds are free to be anywhere in a sphere around the metal, while with *trans*-$[Pt(NH_3)_2Cl_2]$ they are not, due to loss of π-bonding from a pair of *trans*-chlorides.) The remaining π molecular orbitals are now closer in energy to the remaining ψ_{Cl} non-bonding orbital, thus it is easier for bonding electrons to access non-bonding orbitals and enter into S_N1 (D, I_d) reaction pathways. This is in agreement with observed $^{36}Cl^-$ substitution in *trans*-$[Pt(NH_3)_2Cl_2]$ being first order with a reaction rate 2.5 times ($t_{1/2} = 5.5$ h) [51] that observed for $[PtCl_4]^{-2}$ ($t_{1/2} \approx 14$ h) [71]. Further, since there is no ψ non-bonding orbital for NH_3 and the solvation energy of Cl^- in water is much larger than that of NH_3 ($\Delta G^\circ_{solv} = -2.4$ kcal/mole for NH_3 [73]), Cl^- and not NH_3 is the leaving group from $[Pt(NH_3)_2Cl_2]$.

3.3. Cisplatin

With *cis*-$[Pt(NH_3)_2Cl_2]$ the cooperative interaction of chlorides *trans* to each other is lost, resulting in further reduction of π electron delocalization. The degeneracy of the π molecular orbitals is reduced, with one π molecular orbital moving to higher energy. The energy of this orbital is closer to ψ_{Cl} than that of transplatin, thus cisplatin is expected to be more reactive toward ligand substitution than transplatin. This is in agreement with experimental observation. For example, the equilibrium of *cis*-$[Pt(NH_3)_2Cl_2] + H_2O \rightarrow [Pt(NH_3)_2Cl(H_2O)]^+ + Cl^-$ lies to the right, while with *trans*-$[Pt(NH_3)_2Cl_2]$ it does not (Table 1). Important for the medicinal chemistry of cisplatin, replacement of Cl^- by the weaker H_2O ligand opens *cis*-$[Pt(NH_3)_2Cl_2]$ to further reactivity [56].

3.4. *cis*-$[Pt(NH_3)(amine)Cl_2]$

With *cis*-$[Pt(NH_3)(amine)Cl_2]$ complexes the environments of the two chlorides are not identical, thus degeneracy of all π molecular orbitals is removed and these orbitals are energetically closer to the ψ_{Cl} non-bonding orbital. This will enhance the ease of ligand substitution compared to cisplatin. Measurements by Mellish, Barnard, Murrer and Kelland [74] (assuming biology is the consequence of chemistry) are in agreement with this prediction, in that *cis*-$[Pt(NH_3)(cyclohexylamine)Cl_2]$ was found to be twice as active as *cis*-$[Pt(NH_3)_2Cl_2]$ against growth of tumor cells.

In addition, amines and NH_3 differ as electron donors [75], therefore, the energy of π-bonds relative to ψ_{Cl} orbitals in *cis*-$[Pt(NH_3)(amine)Cl_2]$ and *cis*-$[Pt(amine)_2Cl_2]$ complexes are expected to vary with different amine ligands. Corresponding biological variation of *cis*-$[Pt(amine)_2Cl_2]$ reactivity with different amine ligands has been reported by Connors, Cleare and Harrap [68].

3.5. *cis*-$[Pt(NH_3)_2(amine)Cl]^+$ complexes

Coming finally to $[Pt(NH_3)_2(amine)Cl]^+$ complexes, π bonding is reduced further by fewer chlorides in the complex. These complexes should undergo Cl^- $S_N1(D, I_d)$ reactions faster than all other chloro–Pt(II) complexes. Hollis, Amundsen and Stern [76] prepared and studied $[Pt(NH_3)_2(aryl\text{-}amine)Cl]^+$ complexes and found, unlike $[Pt(Amine)_2Cl_2]$, that both the *cis*- and *trans*-isomers have anti-tumor activity. Although *cis*-isomer activity was greater than that of the *trans*-isomers.

3.6. Amines as leaving groups

The transition state associated with loss of Cl^- from $[Pt(NH_3)_2(amine)Cl]^+$ complexes is expected to occur more easily than one involving loss of amines, due to the existence of a ψ_{Cl} non-bonding orbital and the more favorable solvation energy of Cl^- [72,77]. It is not unreasonable, however, for weak ligands such as aryl-amines to undergo substitution during this transition state, because of the instability of transition states. Release of aryl-amines from *cis*-isomers of $[Pt(NH_3)_2(amine)Cl]^+$ complexes has been reported by Leng, Payet and Dalbies [78] when the complexes were allowed to react with DNA. The reported product was *cis*-$[Pt(NH_3]_2(dGuanine)(H_2O)]^{+2}$, where guanine is a much stronger ligand than aryl-amines.

3.7. Pt(II) thiols

An important clinical problem with the use of cisplatin is tumor resistance to the drug [79]. Such resistance is circumvented by complexes of the types [Pt(NH$_3$)(Amine)Cl$_2$] and [Pt(NH$_3$)$_2$(Amine)Cl]$^+$ [80,81]. Various reasons for tumor cell resistance to cisplatin and carboplatin (an analogue of cisplatin in which the chlorides are replaced by a dicarboxylic acid) have been identified [82]. Among them are reduced uptake of cisplatin by cells, enhanced DNA repair, sequestering of Pt(II) and transport of Pt(II) out of cells. The role of thiol and thioether binding to Pt(II) in these mechanisms of resistance has received the most attention [83–90]. The ability of thiols to sequester metals [84,91] and remove them from cells [90] has made them strong candidates for explaining resistance to cisplatin. But results of research on such effects has been varied. To understand these results Fig. 2 is again helpful.

The bonding orbital configuration in the thiols R–S–H (if R is a non-conjugated hydrocarbon) is revealed by its bond angle of $\sim 90°$, which means p-orbitals are used by sulfur to form S–R and S–H bonds. The two loan pairs of electrons on sulfur are then in orbitals with p and s symmetry [92]. If R–S:$^-$ replaces Cl$^-$ in cisplatin, the σ-bond formed between the thiol and Pt(II) utilizes one sulfur p-orbital while another sulfur p-orbital is used for the R–S bond. The third sulfur p-orbital is utilized by a lone-pair of electrons. The symmetry of this orbital is suitable for formation of a π-bond with Pt(II). Considerable empirical evidence indicates the sulfur 3 s-orbital forms a π-bond that accommodates charged groups bonded to the sulfur (see the discussion on carbonium ions and thiols in Price and Oae [94]). Therefore, if the chlorides in cisplatin are replaced by R–S:$^-$ there is no ψ non-bonding orbital. Thus, Pt(II)–thiol complexes are more stable than chloro–Pt(II) complexes. The ability of thiols to delocalize electron charge to positive charged centers (see Price and Oae [94]) such as Pt^{+2} creates a pathway by which amines can be displaced from Pt(II) complexes. Such displacement is more likely to occur when the amine is *trans* to the thiol in Pt(II) complex (see the discussion on the *trans effect* by Basolo and Johnson [93]). In this context the report by Goddard et al. [80] on [Pt(NH$_3$)(cyclohexylamine)Cl$_2$] is interesting, in that the *cis*-isomer was active against cisplatin resistant tumor cells and the *trans*-isomer was not, while both isomers were active against cisplatin sensitive tumor cells. In the *cis*-isomer thiols would end up *trans* to the amines and promote amines as leaving groups; while with the *trans*-isomer thiols would end up *trans* to each other and not promote amines as leaving groups. Thus, *cis*-[Pt(NH$_3$)(cyclohexylamine)Cl$_2$] would remain reactive toward DNA in the presence of thiols, while the *trans*-isomer would not.

3.8. Transport of Pt(II) complexes

The assumption made with studies of Pt(II) complexes is they retain their ligands long enough to account for the observable biological differences among the complexes. As seen with iron (Section 2.2), such biological differences do not necessarily mean the metal ion is transported with ligands still bound to it. Because concentrations of Pt(II)–DNA adducts in cells are low, direct observation of the ligands bound to Pt(II) in such adducts in cells has not been possible. Indirect evidence of ligands remaining bound to Pt(II) inside cells can, however, be found in the work of Pascoe and Roberts [96]. These investigators explored whether or not the differences in biological activity between cisplatin and transplatin *merely reflect differences in the relative ease of penetration of the two isomers into cells*. They found the amount of Pt(II) bound to cell DNA, RNA, nuclear proteins and cytoplasmic proteins is similar for cells treated with similar amounts of cisplatin and transplatin, while inhibition of cell growth was dramatically greater with cisplatin than with transplatin. Clearly, the same amount of Pt(II) enters the cells, but does the biological difference arise from different isomers inside the cells or from different isomers acting at the outer surface of the cells? Wang, Lu and Li [97] recently argued the case for *events on or in the plasma membrane*, but a good deal of data suggests the difference in cell growth arises from cisplatin (with NH_3 attached) forming bifunctional intrastrand DNA adducts in cells [60–68]. How Pt(II) with its ligands intact is transported across cell membranes is not known. It has been generally assumed that Pt(II) compounds enter cells by diffusion [96]. Such a process would preserve ligand attachment, and promote movement of unhydrolyzed neutral cisplatin through the lipid membrane barrier [12]. In support of diffusion as a mechanism of transport, intracellular accumulation of Pt(II) was found to increase linearly in response to extracellular cisplatin concentration [99–101]. This is consistent with diffusion through the lipid membrane, but does not rule out passive transport involving interaction with membrane proteins [12]. For transplatin and $[PtCl_4]^{-2}$, a nonlinear relationship between Pt(II) uptake by cells and extracellular concentration of the complexes has been reported [95], which can not be a diffusion controlled process [12]. As pointed out by Gately and Howell [96], the data paint a confusing picture of Pt(II) accumulation in cells. It is likely Pt(II) complexes enter cells by a variety of mechanisms that depend on the charge of the complex and the hydrophilic or lipophilic nature of the ligands. The complexes may even be self-assisted in that they modify membrane transport processes [97].

4. Titanium

The earliest reference to medicinal chemistry of Ti(IV) is by the German physician Julius Pick [98]. He found *Tiandisulfataufschwemmlosung* (hydrolyzed $Ti(SO_4)_2$) and Ti(IV) mono- and di-salicylates were effective topical and oral treatments for *Tuberkelbacillen* infections. Pick studied a variety of Ti(IV) complexes with organic acids and other organic compounds such as *Kresol*, *Thymol*, *α und β Naphtol*, which were tested in animals before settling on titanium sulfate and salicylates as being non-toxic. Topical application of hydrous Ti(IV) sulfate and Ti(IV) salicylates to healthy skin showed no irritation with rabbits or man. Injection of these compounds in water into mice and rabbits was *ohne jeden*

Schaden (without adverse effects), with tiny bumps appearing at the sites of injection that eventually disappeared. Oral daily administration of 0.2 g Ti(IV) sulfate in water or 10 g Ti(IV) salicylates in bread given to rabbits was also without adverse effects. Oral administration of similar quantities to himself for 14 day was without adverse effects. After oral administration, titanium was found in feces and urine, with evidence that salicylate remained attached to Ti(IV) in the urine. Because of his early observations that Ti(IV) compounds inhibit putrefaction of protein rich materials, Pick decided to study the affects of titanium sulfate and titanium salicylates on bacterial infections in humans (putrefaction—the formation of foul-smelling products by microbial decomposition of high-protein materials such as meat and eggs was known at the time). For treatment of tuberculosis infection of the skin, Pick used a 0.25–0.5% $Ti(SO_4)_2$ suspension in water (gel), or 1–3% Ti(IV) salicylate in Vaseline applied to the infected area. The time required for complete resolution of skin infections depended on the age of the infection, with older infections being stubborn. For infection of the eye, a 0.05–0.1 g of $Ti(SO_4)_2$ in 100 g of sterile, distilled cold water was filtered and two or three drops of the filtered solution applied 1 or 2 times daily. Improvement of the involved areas was seen within a week. For tuberculosis infection of the respiratory track (including lungs), a suspension of 0.25% $Ti(SO_4)_2$ and 0.25% Ti(IV) salicylate in water was sprayed in the throat and 4 teaspoons (1 to 2 mg Ti per teaspoon) of Ti(IV) salicylate given orally each day. After 6 weeks of treatment all symptoms were gone. Confirmation of tuberculosis before Ti(IV) treatment was made by identification of *Tuberkelbacillen im Sputum*.

With the onset of World War I attention turned to the use of hydrous TiO_2 by the French for treating mustard gas wounds [99]. No further work by Pick appeared in the literature, and presumably Pick and his work were casualties of a war that saw the death of 9 million people and the destruction of Germany.

The first American study of Ti(IV) as a therapeutic agent did not appear until 1929 [100]. In this study TiO_2 powder suspended in petroleum jelly was applied to wounds on laboratory animals. No benefit in preventing infection or promoting wound healing beyond that of petroleum jelly was observed. The next report of Ti(IV) and microbial growth appeared in 1963 when Buenemann, Klosteckoette and Ritzerfeld [101] reported TiO_2 powder inhibited *Staphylococcus aureus*, *Escherichia coli* and *Mycobacterium tuberculosis* growth in culture. This effect from TiO_2 is now known to result from photocatalyzed generation of oxygen free radicals that can kill bacteria growing in culture medium [102]. Presumably, the earlier study of Meredith and Christiansen [99] using TiO_2 powder in petroleum jelly failed for lack of oxygen penetration through the jelly.

As the Second World War started in Europe, the French physician Dinah Abragam was studying the affects of hydrous TiO_2 and Ti(IV) citrate on rats with Jensen sarcoma [103]. Ninety animals with tumors were divided into two equal groups, with one group receiving i.m. injections of hydrous TiO_2 suspended in olive oil and the other group receiving olive oil only. Within two weeks tumors in 25% of the Ti(IV) treated animals were noticeably smaller. The injections were continued to day 28, at which point usable injection sites on the animals had been exhausted.

The survival at this point was 24% for the Ti(IV) treated group, with half showing complete remission of their tumors. There were no survivors in the control group.

Likewise, 92 rats with Jensen sarcoma were divided into two equal groups, with one group receiving i.m. injections of Ti(IV) citrate (1 ml of 1 ppt Ti) in water and the other group receiving injections of Fe(II) citrate (1 ml of 1ppt Fe). Long term survivals were 88% for the Ti(IV) citrate treated group and 39% for the Fe(II) citrate treated group. After 3 weeks of injections the death rate in the control group was 5.5 times greater than the Ti(IV) treated group, with 12% of the Ti(IV) treated group dying and 61% of the control group dying from their tumors.

At the same time another French group reported using titanium ascorbate and titanium dehydroascorbate to treat various types of tumors in humans [104]. They found the *complexe titanicosodique rouge* prepared from ascorbic acid was toxic, presumably this was a Ti(III) complex. The *complexe titanicosodique jaune clair* prepared from dehydroascorbic acid was tolerated, and presumably this was a Ti(IV) complex. Ti(IV) dehydroascorbate given i.v. as 25 mg in 1.5 ml of sterile distilled water daily resulted in tumor mass reductions, particularly in the stomach and rectum. The results, however, were considered inferior to those seen with 25 mg daily injections of Cu(II) complexes and Fe(III) complexes.

After the war Henry Schroeder [105] and his colleagues at Dartmouth Medical School studied Ti(IV) in mice. They found $K_2[Ti(oxalate)_2]$ given in drinking water throughout the life-time of the animals resulted in fewer spontaneous tumors developing compared to control mice that did not receive $K_2[Ti(oxalate)_2]$. They also reported mice given $K_2[Ti(oxalate)_2]$ grew more rapidly during the first year of life and died slightly earlier than the controls. In a follow-up study, Schroeder and Mitchener [106] found $K_2[Ti(oxalate)_2]$ was slightly toxic to reproduction in rats.

By 1980 attention had turned to the biocompatability of Ti metal used in bone implants [107], and the bacteriostatic properties of the implants [108]. Bundy, Butler and Hochman [108] reported variable ability of Ti metal to inhibit *Streptococcus mutans* under aerobic and anaerobic conditions, and concluded *Bacteriostasis apparently is contingent upon the presence of a corrosion process*. By 1992 conflicting results from studies of titanium metal and bacteriostasis had emerged. Elagli et al. [109] found titanium metal powder had no effect on seven different bacteria studied in culture, and Leonhardt et al. [110] found titanium metal implants in dogs had no effect on bacteria growth around them. On the other hand, Berry et al. [111] found titanium metal did suppress the growth of various bacteria in culture. While Arbuthnott et al. [112] found titanium metal chambers implanted in rats did not prevent growth of bacteria in the chambers, but did significantly modify the protein profile of bacteria progeny.

4.1. Affect of Ti(IV) on proteases

The biological experience with Ti(IV) through the years has been varied, but recent discoveries clarify these experiences and provided insight to future directions for metal complexes as drugs.

In 1995, while searching for a suitable metal ion around which to design metal complexes that would *irreversibly* inhibit proteases, Duffy and McCue [113] found hydrolyzed $Ti(SO_4)_2$ inhibited the activity of trypsin. During the first 15 min after addition of $[Ti(SO_4)_2]_{aq.}$ to buffered enzyme/substrate mixtures, enzyme activity was *reversibly* inhibited. The inhibition of trypsin was not observed with freshly prepared $Ti(SO_4)_2$, but only after the sulfuric acid digest of metal had sat diluted in water for 2 weeks. Suspecting that hydrolysis had produced a titanyl sulfate, $[TiO(SO_4)]$, nitric acid was added during the sulfuric acid digest of titanium metal. The resultant, freshly prepared Ti(IV) inhibited trypsin activity. Duffy et al. [114] demonstrated that the $[TiO(SO_4)]_{aq.}$ complex binds to the active site of trypsin. Since $[TiO(SO_4)]_{aq.}$ exists in the two forms $[TiO(SO_4)(H_2O)_2]$ and $[TiO(SO_4)(H_2O)]$ [115,116] and since six-coordinate metal ions do not bind to the active site of trypsin [117–120], it was concluded the active form of $[TiO(SO_4)]_{aq.}$ is five-coordinate $[TiO(SO_4)(H_2O)]$. The five-coordinate geometry of $[TiO(SO_4)(H_2O)]$ is similar to the transition state geometry required for ligand substitution of octahedral metal complexes[121]. Presumably aqueous six-coordinate metal ions in the hydrophobic environment of the enzyme active site are unable to lose water and reach the required five-coordinate transition state needed to bind with the protein.

In addition, $[TiO(SO_4)]_{aq.}$ was found not to inhibit the action of chymotrypsin [114]. Since trypsin and chymotrypsin differ in trypsin having a free carboxyl group (Asp-189) at the bottom of its substrate binding site [122] and since carboxyl groups are good ligands for Ti(IV), Duffy et al. [114] concluded Ti(IV) binds to the carboxyl group of Asp-189.

Most important, after the initial 15 min contact between $[TiO(SO_4)(H_2O)]$ and trypsin *irreversible* inhibition of enzyme activity was observed [114]. This indicated Ti(IV) had become *bound so tightly that its dissociation from the enzyme is very slow* [123]. Such change eliminates competition from naturally occurring substrates, and makes development of viable resistance more difficult because it requires the organism to delete Asp-189. The development of irreversible inhibition indicates consolidation of Ti(IV) binding by the active site of the enzyme. Since trypsin has a serine hydroxyl group (Ser-190) near the carboxyl group of Asp-189, it was concluded the consolidation of Ti(IV) binding resulted from a chelate forming between Ti(IV) and the carboxyl group of Asp-190 and hydroxyl group of Ser-190. The bonding of Ti(IV) to Ser-190 may involve bridging by structural waters associated with Ser-190 and $[TiO(SO_4)(H_2O)]$.

4.2. Affect of Ti(IV) on bacteria

To test the biological significance of trypsin inhibition by Ti(IV), Duffy et al. [114] examined its affect on bacteria known to encode for *trypsin-like* enzymes. Among such bacteria are *E. coli* and *Salmonella typhimurium* [124]. When added to these organisms growing in buffered broth and protected from light, Ti(IV) prepared by digestion of the metal with HNO_3/H_2SO_4 inhibited growth of the organisms. Addition of HNO_3/H_2SO_4 (acid controls) to growing bacteria in buffered broth had no affect on the organisms. If Ti(IV) was prepared by digestion of the metal with H_2SO_4 only, its addition to growing bacteria in buffered broth had no affect on the organisms. However, if the Ti(IV) prepared by H_2SO_4 digestion sat for two weeks in water before addition to growing bacteria, the resultant Ti(IV) had the same affect on the bacteria as Ti(IV) prepared by HNO_3/H_2SO_4 digestion.

Microscope examination of bacteria exposed to $[TiO(SO_4)(H_2O)]$ revealed an absence of cell lysis, indicating the metal did not disrupt cell membranes. What could be seen was the inability of bacteria to complete cell division, with many multi-segmented daughter cells appearing. In addition, the motility of cells treated with $[TiO(SO_4)(H_2O)]$ was dramatically less than that of bacteria not exposed to Ti(IV). These observations were consistent with metabolic interference and, therefore, with enzyme inhibition. To test for such inhibition, trypsin activity of lysates from bacteria exposed to $[TiO(SO_4)(H_2O)]$ was compared with lysates from bacteria not exposed to Ti(IV). After exposure for 1 h to $[TiO(SO_4)(H_2O)]$ or HNO_3/H_2SO_4 acid controls, bacteria were centrifuged and washed with saline. The bacteria were then lysed by sonic disruption before adding buffered trypsin substrate. The color change of the substrate, L-BAPNA, was monitored for 24 h. The substrate color changed over time in a manner consistent with $[TiO(SO_4)(H_2O)]$ inhibition of trypsin [114].

4.3. Ti(IV)–alcohol complexes

Duffy et al. [114] also studied the affect of Ti(IV)–alcohol complexes on trypsin activity and bacterial growth. The complexes were prepared by adding $TiCl_4$ to an excess of anhydrous alcohol. This resulted in a vigorous reaction yielding clear, yellow solutions that remained free of precipitate. The addition of $TiCl_4$ to anhydrous alcohol had been shown previously by McCue and Kennedy [125] to result in formation of five-coordinate Ti(IV) complexes. Straight-chain alcohol complexes were found to inhibit trypsin with increasing ability as the alcohol carbon chain increased in length. With exception of octanol, the alcohols by themselves had no affect on the enzyme assays. Ti(IV)–alcohol complexes inhibited bacteria growth without cell lysis.

4.4. Affect of Ti(IV) on tumors

The affect of Ti(IV) citrate on tumors in rats [103], the use of Ti(IV) dehydroascorbate to treat tumors in humans [104] and the reduction in incidence of spontaneous tumors in mice resulting from ingestion of Ti(IV) oxalate [105] point to a role for Ti(IV) in the development of anticancer drugs. From the work of Schroeder, Balassa and Vinton [105] it appears the antitumor effect of Ti(IV) is different from that of Cu(II) given by injection. McCue and Pinkerton [126], for example, found Cu(II) acetate injected i.p. into C_3H brown mice carrying myeloma tumors resulted in the tumors being lost in $\sim 50\%$ of the animals, while shrinking in the remaining animals. This was in sharp contrast to death of all mice caused by tumor growth when the mice were not given Cu(II) injections. This affect of Cu(II) was due to severe wasting in the animals that starved the tumors. Schroeder et al. [105], on the other hand, found mice receiving Ti(IV) oxalate gained weight while developing fewer tumors, compared to mice not receiving Ti(IV). Clearly the mode of action of Cu(II) is toxic, while that of Ti(IV) is not. But what is the mode of action of Ti(IV)? The work of Duffy et al. [114] suggests it is enzyme inhibition. Inhibition of proteases in rapidly growing tumor cells would block growth in a manner similar to that seen with bacteria. In addition, the enzyme telomerase [127,128], which controls cell protein synthesis at its most fundamental point (the life and death of chromosomes) may be involved. Kohlstaedt et. al. [129] have identified carboxyl groups on two amino acids (Asp) in the active site of telomerases which bind metal ions and are highly conserved. An interesting point about telomerases, the terminal DNA sequence they repair in chromosomes is the nucleotide sequence TTAGGG [130], which is homologous with the critical target proposed for cisplatin [64–66].

4.5. Transport of Ti(IV)

A wide variety of data indicate Ti(IV) finds its way into cells. Schroeder et al. [132] studied the presence of titanium in man and found it accumulates in skin, lung, spleen, liver, kidney and heart. Accumulation in lung occurred in nearly all subjects studied and can be attributed to inhalation of TiO_2 containing particles. Accumulation in the spleen, kidney and liver may result from filtration of TiO_2 and related insoluble inorganic compounds, or filterable Ti(IV)-protein complexes. Accumulation in skin (which occurred in all subjects) and heart can not be explained by filtration. Geographical differences in accumulation and organ distribution of titanium show varying biological penetration. In New York City and Chicago, for example, 100% of the subjects studied had detectable levels of titanium in their spleen (0.31 ppm), kidney (0.14 ppm) and heart (0.13 ppm); while in Denver fewer subjects had detectable levels, but the levels were higher, except for heart tissues which had none. How much of this variation is due to the type of exposure (i.e. chemical form and route of exposure) and how much to genetic variation of the populations studied is not known. Variations in dietary intake must occur since certain raw foods such as black pepper, cloves, thyme, chili powder, lettuce, pork, chicken, and some modified food products such as margarine contain high levels of titanium compared to other foods [131]. In addition, the titanium content of water varies [131].

Laboratory animals given Ti(IV) oxalate in drinking water daily at a concentration of 5 ppm were found on average to have 6.5 times the amount of detectable titanium in their kidneys compared with control animals not given Ti(IV) oxalate [105]. The animals were found to have 7.4 times the amount of detectable titanium in their livers, 9.0 times the amount detectable titanium in their hearts and 5.2 times the amount detectable titanium in their spleens compared to control animals. But not all animals tested positive for titanium. In control animals 57% of kidneys tested positive compared to 79% in Ti(IV) oxalate treated animals, 56% of livers tested positive in controls compared to 73% in Ti(IV) oxalate treated animals and

67% of spleens from controls compare to 100% of treated animals. For the heart, however, there was no significant change between the control and Ti(IV) oxalate treated animals in terms of the percent of animals with detectable titanium. These numbers are consistent with accumulation in kidney, spleen and liver by filtration, and in heart by another mechanism. Unfortunately, the data do not allow determination of route of entry because detectable levels of titanium in lung increased 8.2 times in the Ti(IV) oxalate treated group compared to the control group. Thus, the animals may have been inhaling the Ti(IV) enriched water rather then experiencing migration of Ti(IV) from the gut to the lungs.

Merritt and Brown [132] injected hamsters i.p. with 100 μg of Ti(IV) as $(NH_4)_2[TiF_6]$ (incorrectly identified in the reference as $TiCl_4$) every week for 6 weeks and measured the amount of titanium found in various organs. In agreement with the data of Schroeder et al. [131], titanium levels in the kidney, liver, spleen and lung were elevated in the $(NH_4)_2[TiF_6]$ injected animals compared with the controls injected with saline. In addition, titanium concentrations in plasma, red cells and urine were greater in animals receiving $(NH_4)_2[TiF_6]$ than in controls. There were deposits of TiO_2 at the sites of injection, and no apparent harm to the animals.

Similar experiments were carried out using i.m. and i.p. injections of $TiOCl_2$ (structure based on information from the supplier). In these studies [133] the titanium in animals receiving $TiOCl_2$ by either i.m. or i.p. routes was not excreted in urine. Animals receiving $TiOCl_2$ by i.p. injection did not show elevated titanium levels in blood, while those receiving $TiOCl_2$ by i.m. injection did. Titanium levels in the kidney, liver, spleen and lung were, however, elevated in the animals receiving $TiOCl_2$ by either route.

Clearly, the biological fate of Ti(IV) in animals depends on the composition of the coordination complex and route of administration. In a physiologically simpler system than animals, Maurer et. al. [134] found mouse fibroblast cells in culture take up titanium from $(NH_4)_2[TiF_6]$ added to the growth medium in a linear fashion ($R^2 = 0.95$) as the amount of $(NH_4)_2[TiF_6]$ in the medium increased. In studies of corrosion products from titanium alloys used in implants it was found fibroblast cells took up most, but not all, of the titanium as membrane bound material, with 1.5% found in the cytosol [134]. Carroll and Tullis [135] reported that titanium and zinc are compartmentalized together in cells, which suggests that they are bound to the same proteins.

5. Conclusions

There is a large amount of data in the literature showing uptake of transition metal ions by mammalian cells, but little is known about the molecular mechanisms by which this happens [19]. Discovery of the *trans*-membrane protein *DCT1* [19] is an important step toward unraveling these mechanisms; but what are the structural components of this protein that permit transport and what type of ligands are transported with the metal ion? As for uptake of metal ions by endocytosis; are metal ion complexes with ligands intact transported by this mechanism and is endocytosis always a carrier/receptor mediated process? How effective is diffusion at getting metal complexes into cells and what are the restrictions imposed on ligands? Without further information rational design of *metallo-drugs* is difficult.

None the less, progress in understanding the medicinal chemistry of metal complexes can be achieved through application of molecular orbital theory to their structures. For example, application of molecular orbital theory to chloro–Pt(II) complexes predicts $[Pt(NH_3)_2(aryl\text{-}amine)Cl]^+$ complexes will be the lest likely to develop cell *resistance*, while substitution of Cl^- by $R-S^-$ will slow bio-availability of Pt(II) but not stop it. As with carboplatin, $[Pt(NH_3)_2(1,1\text{-cyclobutanedicarboxylate})_2]$, a slower release of reactive Pt(II) is expected to reduce renal toxicity [136]. Thus, *cis*-$[Pt(NH_3)_2(aryl\text{-}amine)Cl]^+$ and *cis*-$[Pt(NH_3)_2(aryl\text{-}amine)(thiol)]^+$ complexes should be placed first in line for clinical trials.

Most important, recognition of enzymes as targets for drug development against infectious diseases and cancers [137,138,128] and the ability to design metal complexes as enzyme inhibitors [114,139] identifies a major direction for development of *metallo-drugs*. For example: Ti(IV) inhibitors of hepatitis C virus appear possible because of a *trypsin-like* protease encoded by the virus that includes a Zn^{+2} binding site [140]; human immunodeficiency virus type-1 (HIV-1) which encodes for an *aspartic protease* [141] is another target, and telomerase which contains free aspartic groups at the active site [127] is another.

Thus far, targeting enzymes for development of *metallo-drugs* has helped to understand reported clinical affects of Ti(IV), and may lead to inexpensive approaches for controlling infections. For example, spread of bacterial infections and development of antibiotic resistance by bacteria is dependent on community based factors such as prevention, containment and treatment of infection in population groups [142], and on transmission of infections between animals and man [143]. Inexpensive compounds like $[TiO(SO_4)(H_2O)]$, Ti(IV)–alkoxides and Ti(IV)–salicylates provide methods for suppressing bacterial proliferation [98,114] that can be applied to human and animal populations. Suppression of bacterial proliferation supports and assists normal immune response and clearance mechanisms. Thus far, *E. coli*, *S. typhimurium*, *Pseudomonas aeruginosa* [114], *Staphylococcus epidermidis* [144], and *Tubercle bacillus* [98] have been found to be susceptible to Ti(IV) inhibition. Simple Ti(IV) compounds can be used as dietary supplements for farm animals to reduce the bacterial load in these animals and the food chain derived from them. In this regard, Schroeder et al. [105]b found Ti(IV) increased the growth of animals when it was given as the oxalate complex in drinking water. The effect they observed is similar to the one achieved when adding antibiotics to animal feed. It is interesting to note that Duffy and McCue [113] found Ti(IV)–oxalate does not inhibit the growth of bacteria in vitro, thus there appears to be a mechanism at play in vivo that releases Ti(IV) from the oxalate complex. The mechanism may be passage through the stomach where the pH ranges from 1 to 2, while pK_1 and pK_2 for oxalic acid are 1.23 and 4.19. This raises the possibility that raw mineral sources or crude industrial sources of Ti(IV) can be used to provide animals with bioactive Ti(IV). This would drop the cost of Ti(IV) supplements well below the cost of adding antibiotics to feed. Even if pure titanyl sulfate is needed, the cost would be significantly less than that of antibiotics, since titanyl sulfates are produced inexpensively in large quantities as intermediates in the production of TiO_2 [145]. Using commodity prices of TiO_2 to estimate the cost of intermediates and comparing this with commodity prices of antibiotics: The dosage cost of tetracycline to treat infections in an adult human was found to be ~ 14 times greater than that required for treatment with titanyl sulfate; while with ampicillin the cost is ~ 54 times greater than that of titanyl sulfate. As U.S.P. grade materials, the cost spreads between Ti(IV) compounds and traditional antibiotics is expected to be even greater. Thus, control of infections is expected to be less expensive using Ti(IV) than using conventional antibiotics.

Acknowledgements

Our work with Ti(IV) was supported by the Francis and Russell Lucas Family Trust. We would like to thank the Reference Departments at Francis A. Countway Library of Medicine and the Cabot Science Library at Harvard University for their help.

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