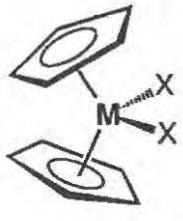
Early Transition Metal Metallocenes in Cancer Research

Cornelia Bauer Literature Seminar March 28, 1995

The accidental discovery of the antitumor properties of cisplatin by Rosenberg in 1969 led to a broad search of cytotoxic inorganic compounds. Aside from some platinum metal complexes, a variety of main group and transition metal compounds display such activity. One particularly promising group of compounds are based on early-transition-metal metallocene complexes, which are active against a wide range of experimental and human cancers but are less toxic than cisplatin. One of the most attractive features of those compounds is their activity against certain types of colon cancers which seldom respond to drugs. One important question in cancer research is the mechanism of the growth inhibition of cancerous cells. An understanding of the mechanism of drug action is critical to the rational design and improvement of new agents [1,2,3,4,5].

Systematic in *vitro* and in *vivo* studies have focused on the manipulation of the activity of metallocene(IV)- complexes by changing the metal ion, the substituents on the cyclopentadienyl (Cp) rings, and varying the metal bound ligands (e.g., halides, carboxylates, thiolates). Ti, V, Nb and Mo have been found to be the most active metals. Any kind of substitution on the Cp rings reduces the drug efficiency and the metal bound ligands exert only a slight effect on the activity of the drug [2,5,6].



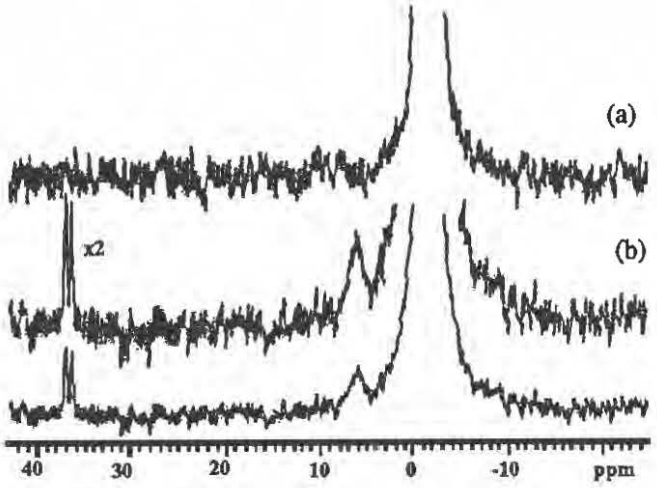
Ti	**V**	
Zr	**Nb**	**Mo**
Hf	*Ta*	*W*

Key: **M** = maximum activity
M = sporatic activity
M = no activity

Core level Electron Energy Loss Spectroscopy (EELS) on tumor cells treated with titanocene dichloride, reveals accumulation of Ti in DNA-rich cell areas, thus indicating DNA and RNA to be the primary cellular targets of metallocenes [7]. This is supported by Inductively Coupled Plasma (ICP) analyses on *calf thymus*-DNA, treated with various dichlorides [8]. The most studied anticancer drug, cisplatin, binds preferably to the nucleobases Guanine (N7) of the DNA disrupting the Watson-Crick base pairing binding [9]. Cisplatin and metallocene dihalides have similar bond angles, and therefore, similar binding to DNA was originally proposed [4].

The interactions of molybdocene dichloride with *calf thymus* DNA were detected by ^{31}P NMR. These findings are consistent with coordination of the Cp_2Mo^{2+} moiety to the DNA backbone *via* either one or two phosphato(O) bonds [10]. Though vanadocene dichloride is as active as titanocene dichloride, no DNA adducts were detected whereas some DNA-complexation was detected for the inactive zirconocene- and hafnocene dichlorides [8,11]. Experiments with tritium labeled Cp ($C_5H_nT_{5-n}$; T = ^3H; n = 0-5) indicates that the binding mode of titanocene dichloride depends on the pH: at low pH a $TiCp_2^{2+}$ moiety is bound to DNA, whereas at neutral pH, DNA is bound to a $TiCp^{3+}$ moiety [8]. UV-Vis, CD and fluorescence spectroscopic studies of interactions of titanocene with DNA, at physiological pH, indicate that titanocene can bind with DNA in a covalent, irreversible manner at high concentration [12].

^{31}P NMR of DNA-Cp_2MoCl_2 Complex



(a) *calf thymus* DNA
(b) DNA incubated with Cp_2MoCl_2

Early-transition-metal metallocene dihalide complexes, such as titanocene dichloride, are not stable in aqueous media. Under physiological conditions the chloride hydrolysis is more rapid and extensive than for cisplatin. Another difference between these two classes of compounds is the non-lability of amines at cisplatin whereas in the case of Cp_2MCl_2 the loss of Cp can become important depending on the nature of M and the solution pH. Zirconeocene and hafneocene complexes exchange Cp rapidly with H_2O which would explain their inactivity, but the Cp-Ti bond of the highly active titanocene dichloride is also hydrolytically unstable at neutral pH [13,14,15].

The solution and solid-state chemistry of molybdocene dichloride with various DNA constituents (nucleobases, nucleotides, phosphates) has provided some insight into metallocene-DNA coordination chemistry [14,16]. In absence of competing ligands, Cp_2MoCl_2 coordinates to both nucleobase (N) and phosphates in a non labile manner, causing major conformational changes without disrupting the Watson-Crick hydrogen bonding. In contrast, $Cp_2V(OH_2)^{2+}$ selectively binds to the phosphate group through hydrogen bonding [17]. The binding modes of metallocenes with DNA constituents in aqueous media are different from those of cisplatin. The exact structures of metallocene-DNA adducts as well as the mechanism of the tumor inhibition remain to be elucidated. It is still doubtful that DNA binding is the actual mode of the tumor inhibition. Inhibition of the angiogeneses (forming of blood vessels) [18] and inhibition by cyclopentadiene or dicyclopentadiene as the active agent [15] are other proposed mechanisms of the antitumor activity of metallocene dichlorides.

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