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Ferricenium Complexes: A New Type of Water-Soluble Antitumor Agent *

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Summary. The antitumor activity of a series of iron complexes, i.e., of ferrocene [Cp_2Fe], of tetrachloroferrates(III) $[R_4N]^+[FeCl_4]^-$, and of ferricenium complexes $[Cp_2Fe]^+X^-$ ($X^- = [FeCl_4]^-$, $\frac{1}{2}[Cl_3FeOFeCl_3]^{2-}$, $[H_5Mo_7O_{24}]^- \cdot 2H_2O$, $[2,4,6-(NO_2)_3C_6H_2O]^-$, or $[CCl_3COO]^- \cdot 2\,CCl_3COOH$) was investigated against EAT in CF1 mice. Whereas ferrocene and the ammonium tetrachloroferrates(III) did not show recognizable tumor-inhibiting activity, such activity was exhibited by the water-soluble, salt-like ferricenium complexes; the best antineoplastic properties, with optimum cure rates of 100%, were found for ferricenium picrate and ferricenium trichloroacetate.

The ferricenium compounds are the first iron complexes for which antineoplastic activity has now been shown. They represent a new type of antitumor agent insofar as they differ fundamentally from known inorganic and organometallic antitumor agents (a) by their ionic, salt-like character, which is responsible for their high water solubility, and (b) by the absence of a *cis*-dihalometal moiety; this moiety has been recognized as important for the intracellular action of other known inorganic cytostatics.

Key words: Iron compounds – Ferricenium complexes – Antitumor activity – Ehrlich ascites tumor

Introduction

The unexpected discovery of the antitumor activity of *cis*-diammine-dichloroplatinum(II) (*cis*-platinum; Rosenberg et al. 1969) has opened up the 'era of inorganic cytostatics'. It has stimulated a broad search for other inorganic or organometallic compounds with antitumor activity and initiated a series of developments.

Thus, it was shown that, in addition to *cis*-platinum, numerous other inorganic compounds containing platinum or other platinum metals (Cleare and Hoeschele 1973; Cleare et al. 1978) are potent antitumor agents; the 'platinum complexes of the second generation' (Cleare et al. 1980) in particular, e.g., bis(isopropylamine)-*cis*-dichloro-*trans*-dihydroxoplatinum(IV) (CHIP) or diammine(cyclobutane-1,1-dicarboxylato)platinum(II) (CBDCA), are promising candidates for clinical use because of their increased water solubility and reduced toxicity compared with *cis*-platinum.

Antitumor activity was also demonstrated for various organometallic compounds: for metallocene dihalides that, in contrast to the platinum compounds, contain early transition metals such as titanium or vanadium (Köpf and Köpf-Maier 1979, 1983) and for other titanium compounds (Keller et al. 1983). Another group of organometallic antitumor agents, the octahedral *cis*-dihaloorganotin(IV) complexes (Crowe et al. 1980), contains the main group element tin as the central atom.

Searching for further organometallic compounds with antitumor properties, we recently tested a number of inorganic and organic iron complexes against experimental tumor systems; some of the results obtained are described in the present paper.

Materials and Methods

a. Substances

Ferrocene [Cp_2Fe] (Ventron, Karlsruhe), the tetraalkylammonium *tetrachloroferrates(III)* $[(CH_3)_4N]^+[FeCl_4]^-$ and $[C_6H_5CH_2(C_2H_5)_3N]^+[FeCl_4]^-$ (Neuse and Meirim 1984), and the *ferricenium complexes* $[Cp_2Fe]^+[FeCl_4]^-$ (tetrachloroferrate(III);

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Abbreviations: $Cp = C_5H_5$, cyclopentadienyl ring ligand; EAT, Ehrlich ascites tumor; IP = intraperitoneal(ly); p. t. t., post transplantationem tumoris

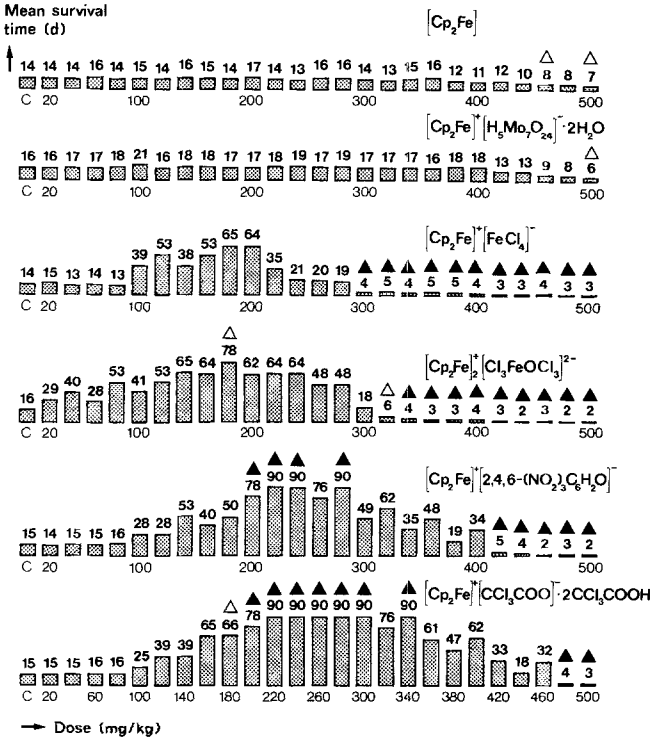

Fig. 1. Dose-dependent influence of ferrocene $[Cp_2Fe]$ and ferricenium complexes $[Cp_2Fe]^+X^-$ on the mean survival time of mice bearing EAT. *Numbers* on top of *columns* indicate values of mean survival time of each dose group; ▲, highly significant; △, significant differences compared with untreated controls

Nesmejanow et al. 1960); $[Cp_2Fe]_2^+$ $[Cl_3FeOFeCl_3]^{2-}$ (μ-oxo-bis[trichloroferrate(III)]; Boeyens et al. 1984), $[Cp_2Fe]^+[H_5Mo_7O_{24}]^- \cdot 2\,H_2O$ (heptamolybdate; Pavlik and Klikorka 1965), $[Cp_2Fe]^+$ $[2,4,6-(NO_2)_3C_6H_2O]^-$ (picrate; Wilkinson et al. 1952), $[Cp_2Fe]^+$ $[CCl_3COO]^- \cdot 2\ CCl_3COOH$ (trichloroacetate; Hendrickson et al. 1973) were prepared by the methods described in the literature and purified (Neuse, 1984) as required.

For testing purposes, ferrocene and the five ferricenium complexes were given in doses rising by increments of 20 mg/kg from 20 to 500 mg/kg, whereas the two ammonium tetrachloroferrates were given in doses of 4–200 mg/kg, with increments of 4 mg/kg up to 20 mg/kg and then of 20 mg/kg. The ferrocene samples were dissolved or suspended in propylene glycol (Ferak, Berlin); a maximum volume of 0.1 ml propylene glycol was given per mouse. The samples of ferricenium heptamolybdate were suspended in Cremophor EL (BASF, Ludwigshafen) by heating to 70 °C and ultrasonic treatment; then, ninefold volumes of saline were added and total volumes of 0.4–0.5 ml per animal were injected. All other ferricenium compounds and the ammonium tetrachloroferrates were highly water-soluble; they were dissolved in pure saline, the concentrations being so selected that each mouse received a total volume of 0.4–0.5 ml (0.02 ml/g body weight). The preparations were administered IP within 30 min after dissolution.

b. Animals and Antitumor Bioassay

The antitumor activity of the substances was tested against EAT growing as fluid tumor in the peritoneal cavitiy of female CF1 mice (20–25 g; Winkelmann, Paderborn). Details of the experimental procedure were as described before (Köpf-Maier et al. 1980 a, b). The substances were injected as single doses at 24 h p.t.t.; every dose group consisted of six animals. Additional groups of six animals served as untreated tumor-bearing control animals; these received only (a) 0.1 ml propylene glycol, *or* (b) 0.5 ml Cremophor/saline mixture (1:9 = v:v), *or* (c) 0.5 ml pure saline.

The number of deaths was recorded daily; deaths within 8 days p.t.t. were defined as toxic and those occurring later, as tumor deaths. The key-date for determining the survival rate was day 90 p.t.t.; survival times of the control animals ranged from 11 to 19 (mean value 15.2 ± 2.4) days p.t.t. Animals that were still alive on the key-date and had no recognizable signs of tumor were considered as cured. For every dose group, the mean survival times were calculated and statistically evaluated in comparison with the untreated control groups with the use of the Wilcoxon–Mann–Whitney U-test (significant differences: $2P \leq 0.05$; highly significant differences: $2P \leq 0.01$).

Results

The salient results of the present study, summarized in Figs. 1 and 2, show the influence of a treatment with ferrocene $[Cp_2Fe]$ or with ferricenium complexes $[Cp_2Fe]^+X^-$ on the survival time of mice bearing EAT (Fig. 1), and on the occurrence of tumor deaths, toxic deaths, and cures (Fig. 2). The main pharmacological and toxicological data are given in Table 1.

Treatment with Ferrocene

Treatment of mice bearing EAT with the uncharged ferrocene complex $[Cp_2Fe]$, which is characterized by pronounced hydrophobic properties and which was therefore administered in propylene glycol, neither induced a significant increase in mean survival time at any given dose level nor caused survival of animals until the key-date. This suggests that the neutral ferrocene complex lacks antitumor efficacy against EAT.

Treatment with Ammonium Tetrachloroferrates

In parallel with ferrocene behavior, the two tetrachloroferrates, $[(CH_3)_4N]^+[FeCl_4]^-$ and $[C_6H_5CH_2(C_2H_5)_3N]^+[FeCl_4]^-$ also failed to show antitumor activity against EAT. All the animals died, following lower doses as a result of tumor development, and after higher doses due to acute toxicity. The following characteristic values for acute toxicity were found: LD_{50}, 80 and 120 mg/kg, respectively; LD_{100}, 110 or 150 mg/kg, respectively.

Treatment with Ferricenium Complexes

The ferricenium complexes are salt-like compounds containing the ferricenium cation $[Cp_2Fe]^+$ and an anion X^-; with the exception of the ferricenium heptamolybdate, all tested ferricenium compounds are distinguished by excellent solubility in water. In these cases, all doses could be applied in pure saline.

Whereas treatment with the poorly water-soluble ferricenium heptamolybdate did not induce tumor inhibition at any dose level, administration of the

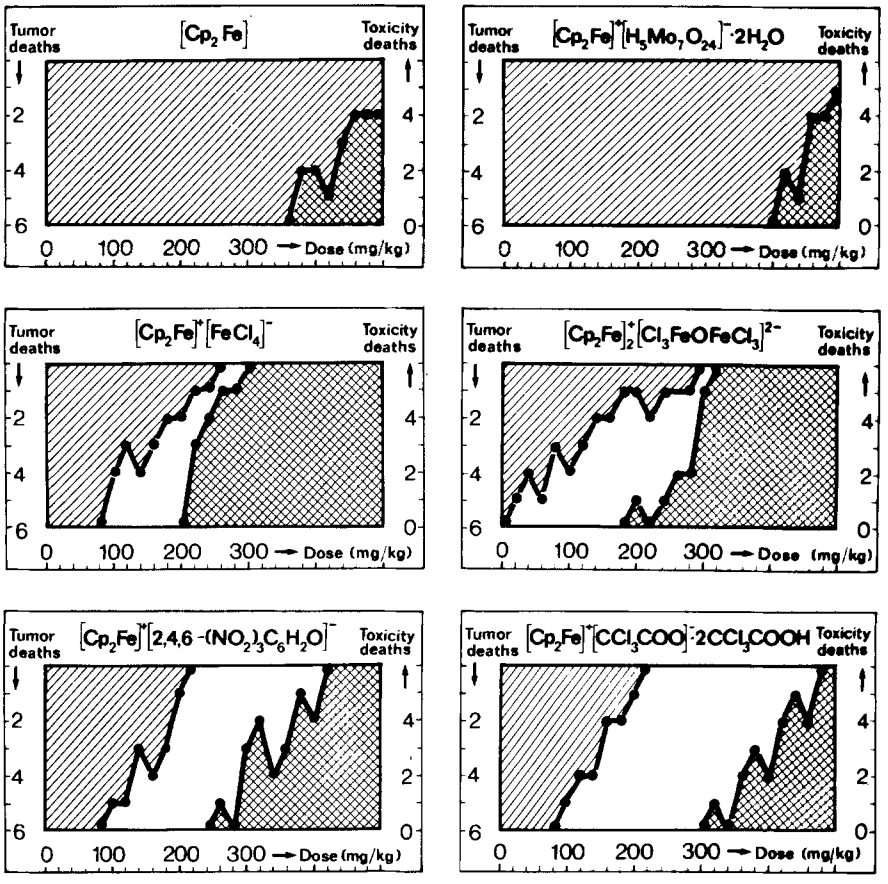

Fig. 2. Dose-dependent influence of ferrocene $[Cp_2Fe]$ and ferricenium complexes $[Cp_2Fe]^+X^-$ on the number of tumor deaths ▨, of toxic deaths ▩, and of cures □ observed within 90 days p.t.t.

Table 1. Pharmacological and toxicological data of ferrocene $[Cp_2Fe]$ and ferricenium salts $[Cp_2Fe]^+X^-$

Compound	Maximum cure rate (%)	Optimum dose range (mg/kg)	LD_{50} (mg/kg)	LD_{100} (mg/kg)	T.I.[a]
$[Cp_2Fe]$	0	–	440	>500	–
$[Cp_2Fe]^+[H_5Mo_7O_{24}]^- \cdot 2H_2O$	0	–	450	>500	–
$[Cp_2Fe]^+[FeCl_4]^-$	67	180–200	240	300	1.3
$[Cp_2Fe]_2^+[Cl_3FeOFeCl_3]^{2-}$	83	180	290	320	1.3
$[Cp_2Fe]^+[2,4,6\text{-}(NO_2)_3C_6H_2O]^-$	100	220–240	340	420	1.7
$[Cp_2Fe]^+[CCl_3COO]^- \cdot 2CCl_3COOH$	100	220–300	400	480	2.0

[a] Defined as LD_{50}/ED_{90}. T.I. values of other cytostatic metal complexes for comparison purposes: *cis*-platinum, 8.1; titanocene dichloride, 3.3; titanocene dibromide, 4.5; cf. Cleare 1974; Köpf-Maier et al. 1980b

ferricenium chloroferrates $[Cp_2Fe]^+[FeCl_4]^-$ or $[Cp_2Fe]_2^+[Cl_3FeOFeCl_3]^{2-}$ caused survival of 67% or 83% of the animals after treatment with optimum doses (Table 1). In consequence, the corresponding mean survival times were extended to 65 or 78 days, leading to increases in life-span of 350% or 380% compared with the untreated controls.

Even higher cure rates were observed after treatment of EAT-bearing mice with ferricenium picrate, which at doses of 220, 240, or 280 mg/kg effected the survival of all animals, or with ferricenium trichloroacetate, which also induced cure rates of 100% over the relatively broad dose range of 220–300 mg/kg. Thus, ferricenium picrate and trichloroacetate are both characterized by marked tumor-inhibiting activity against EAT; they are able to prolong survival until the key-date and to cure all animals that have received optimum doses (increase in life-span 490% compared with untreated controls). As the optimum therapeutic and the toxic ranges come close together, the therapeutic indices (T.I.) are restricted to lower values than with other cytostatic metal complexes.

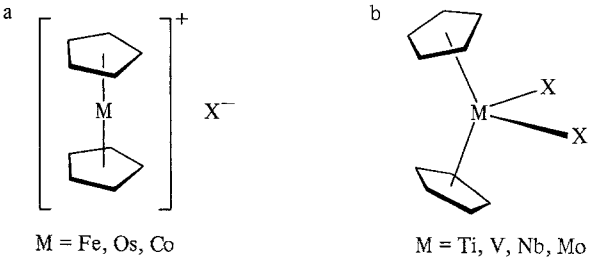

Fig. 3a, b. Structural formulae of metallicenium salts **a** and of metallocene dihalides **b**

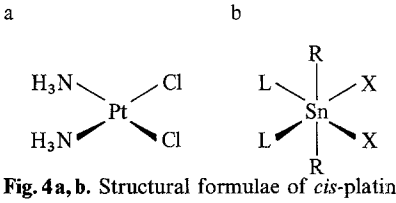

Fig. 4a, b. Structural formulae of *cis*-platinum **a** and of *cis*-dihaloorganotin complexes **b**

The toxic symptoms of the animals treated with high doses of ferricenium salts consisted in diminished activity, shagginess of the fur, hyposthenia and neuromuscular disorder. Studies concerning organ-specific toxic symptoms have not yet been performed.

Discussion

The ferricenium complexes are the first iron compounds to exhibit antitumor properties by themselves. Ferrocene derivatives, e.g., *N*-ferrocenoyl-*p*-bis(β-chloroethyl)amino-phenylalanine methyl ester or ferrocenealdehyde *N*-methyl-*N*-β-chloroethyl-hydrazone, for which antineoplastic activity has been reported earlier (Yashchenko et al. 1978; Wenzel et al. 1979), always consist of a cytostatically active organic function, such as an alkylating agent, which is covalently bound to one of the two cyclopentadienyl rings of the ferrocene molecule. Because ferrocene itself, as shown in the present study, does not exhibit antitumor activity, the cytostatic properties of the ferrocene derivatives mentioned are obviously caused by the organic residues and not by the ferrocene nucleus.

In view of this, it is a surprising result of the present study that oxidation of ferrocene [Cp_2Fe] to the ferricenium cation $[Cp_2Fe]^+$, accomplished by transition of the iron(II) into an iron(III) central atom, apparently yields a cytostatically active moiety, which shows antitumor activity in combination with a variety of anions X^-, e.g., chloroferrates, picrate, or trichloroacetate. To exclude, the possibility that the anions are the cytostatically active components, on the other hand, we also tested the tetrachloroferrate(III) anion combined with ammonium cations and were unable to detect any tumor-inhibiting properties of these species.

The ferricenium compounds, although belonging to the group of metallocenes (Fig. 3a) differ distinctly from the metallocene dihalides (Fig. 3b) for some of which antitumor activity has already been described (Köpf and Köpf-Maier 1979, 1983): (a) The metallicenium compounds contain medium or late transition metals such as iron, osmium, or cobalt, in contrast to the early transition metals in the metallocene dihalides; (b) the cyclopentadienyl ring planes in metallicenium salts are arranged parallel to each other (Paulus and Schäfer 1978; Churchill et al. 1981; other literature cited by Churchill et al.), in contrast to the tilted arrangement in the metallocene dihalides (Green et al. 1972; Clearfield et al. 1975); and (c) the metallicenium cations and the anions X^- form a salt-like crystal lattice (Paulus and Schäfer 1978; Churchill et al. 1981) and are not linked together by covalent bonds; in contrast, the metallocene dihalides are neutral complexes with the halide ligands covalently bound to the central metal atom.

The metallocene dihalides are able to dissociate both halide ligands X in aqueous solutions and to vacate two coordination sites suitable for interaction with intracellular molecules (Köpf-Maier et al. 1980a, b). This structural feature of a dissociable *cis*-dihalometal moiety is also present in other inorganic and organometallic cytostatics, such as platinum or organotin compounds.

Therefore, it has been argued that the *cis*-dihalometal moiety is responsible for the antitumor action of these substances (Cleare et al. 1978; Köpf-Maier et al. 1980a, b; Crowe et al. 1980). Detailed studies with *cis*-platinum have shown that after dissociation of the Cl ligands, the formation of intrastrand cross-links within the DNA is probably the critical molecular step (Rosenberg 1978).

This step, however, which may also be performed by the metallocene dihalides and organotin compounds, is not a possible pathway with the ferricenium compounds, for both stereo- and coordination-chemical reasons. Other processes must therefore be postulated for the intracellular action of ferricenium compounds: the formation of charge-transfer complexes of the ferricenium cyclopentadienyl rings with intracellular aromatic systems, which are present, for example, in DNA, RNA, or protein molecules, or the incorporation of iron ions into macromolecules after cleavage of the Fe-Cp bonds, might be conceivable mechanisms.

A further important difference between ferricenium complexes and other inorganic and organometallic cytostatics is that the ferricenium salts are ionic compounds; some of these are therefore distinguished by excellent solubility in water – an obvious advantage for their use in biological systems. Ready

dissolution and distribution of the complexes in the aqueous compartments of the organism appear to be important prerequisites for their antitumor action; this is confirmed by the cytostatic ineffectivity of ferricenium heptamolybdate, which is only poorly soluble in water. On the other hand, the two cyclopentadienyl rings present in all ferricenium salts are apparently sufficiently lipophilic to ensure penetration of biological membranes.

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