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Studies on the anti-tumour activity of some iron sandwich compounds

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Abstract

Four types of water-soluble iron sandwich compounds, ferrocenes bearing charged side chains (Ia), ferrocenium salts (Ib), $(\eta\text{-arene})_2Fe^{2+}$ salts (II) and $(\eta\text{-arene})(\eta\text{-cyclopentadienyl})Fe^{+}$ salts (III), have been tested *in vitro* against experimental tumours, L1210, Walkers and Chinese hamster lung (V.79). Some activity was observed for all the types of complex; though that of only two, Ib and II, was good. Of special interest is that the iron(II) sandwich type II compounds display anti-tumour activity, since previous active complexes have contained type Ib or other known cytotoxic groups that would account for their activity. Examples of complexes of type Ib and II were further tested against improved cell lines L1210, PC6 and CH1. The results show the two types to be similar in their effect and specificity. An attempt is made to rationalise the data.

Introduction

In 1984 Köpf-Maier et al. [1] reported on the anti-tumour properties of ferrocenium salts against Ehrlich ascites tumour (EAT). Ferrocene, which is insoluble in water, did not show recognisable tumour inhibiting activity, whereas the water-soluble Fe^{III} ferrocenium salts showed cure rates as high as 100% [1]. This finding is of particular significance as these salts represent a new type of organometallic anti-tumour agent in that they do not contain the *cis*-dihalometal moiety, a feature long recognised for its role in the mode of action in platinum drugs [2] and metallocene dihalide complexes [3]. However, in the above report there was little mention of the possible mode of action for the ferrocenium species.

Many metallocene complexes show organ specificity, and this property is principally determined by the substituents on the cyclopentadienyl rings [4]. These facts have stimulated considerable interest in the search for ferrocene-based drugs [5–7]. Recently, both the synthesis and testing of potential tumour inhibiting ferrocenylmetal complexes have been reported [6,7]. Both of these utilised 1,1′bis(diphenylphosphino)ferrocene (Fdpp) as the ferrocenyl ligand [6,7]. However, despite the structural similarities with cisplatin and diphenylphosphinoethane, Scarcia et al. [6] demonstrated that the ferrocenyl analogues showed a marked decrease in activity.

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To further investigate the potential of iron sandwich compounds as anti-tumour agents, and to establish the generality of any such action, we have investigated four basic types of water-soluble derivatives; (Ia) ferrocenyl (Fc) and (Ib) ferrocenium salts (Fc^+); (II) $[(\eta^6\text{-arene})_2Fe]^{2+}$ salts; (III) $[(\eta^6\text{-arene})CpFe^+$ salts ($Cp = \eta^5\text{-}C_5H_5$) We present the results of this work here.

$$\underset{\text{(Ia)}}{CpFe\left(\eta^5\text{-}C_5H_4\overset{+}{N}RMe_2\right)X^-} \quad \underset{\text{(Ib)}}{[Cp_2Fe]^+[FeCl_4]^-} \quad \underset{\text{(II)}}{\left[\left(\eta^6\text{-}Me_6C_6\right)_2Fe\right]^{2+}[CoCl_4]^{2-}}$$

$$2\underset{\text{(III)}}{\left[\left(\eta^6\text{-}2,4,6\text{-}Me_3C_6H_3\right)FeCp\right]^+}[CoCl_4]^{2-}$$

Results

Table 1 presents the IC_{50} values for the compounds tested against three *in vitro* cell lines; the alkylating agent-sensitive Walkers tumour (WS), the antimetabolite-sensitive L1210 leukemia and the V.79 Chinese hamster lung cell.

Of the Ia type derivatives tested, only one, $[FcCH_2N^+HMe_2][Cl^-]$ showed any activity, and this was only against one cell line, L1210. The complex $[FcCH_2N^+Me_3]$ differs from $[FcCH_2N^+HMe_2]$ in that a methyl group replaces a proton. The fact that the former is completely inactive is difficult to rationalise. However, there is also a difference in the anion present (iodide in the former, chloride in the latter). As can be seen for type II complexes the influence of the anion can be considerable (see below). The anion effect is also to be seen in the data of Köpf-Maier et al. [1]. Qualitatively, the change in anion might be expected to effect the solubility and hence the cellular penetration and distributions and as a result would influence the activity.

The one type Ib complex, decamethylferrocenium tetrachloroferrate, $[DMFc]^+[FeCl_4]^-$ exhibits the second best single activity ($IC_{50} = 11\ \mu M$ against L1210) for the group. This supports the previous finding of Köpf-Maier et al. [1] and also demonstrates that extensive substitution can be made to the basic ferrocenium structure without destroying activity.

Table 1

IC_{50} (μm) values for iron sandwich compounds

	L1210	WS	V.79
$[(HMBz)_2Fe]^{2+}[CoCl_4]^{2-}$	25(10)	50(20)	4.6(1.8)
$[(HMBz)_2Fe]^{2+}[Cl]_2^-$	52(21)	>100	15(6)
$[(PMBz)_2Fe]^{2+}[Cl]_2^-$	>100	>100	72(29)
$[DMFc]^+[FeCl_4]^-$	11(4.5)	50(20)	>100
$[(Mes)FeCp]^+[CoCl_4]^{2-}_{1/2}$	60(24)	>100	48(20)
$[FcCH_2N^+HMe_2][Cl]^-$	40(16)	>100	>100
$CoCl_2 \cdot 6H_2O$	37(15)	>100	>100

Also $[FcCH_2N^+Me_3][I]^-$, $FcCH_2N(SO_3)Me_2$, $[FcSO_3^-][PyH]^+$, $[BzCpFe]^+[PF_6]^-$, were all tested and found to be inactive against all three cell lines (i.e. $IC_{50} > 100\ \mu M$). HMBz = hexamethyl benzene, PMBz = pentamethylbenzene, $DMFc^+$ = decamethylferrocenium, Mes = 1,3,5-trimethylbenzene, Cp = cyclopentadienyl, Fc = $(\eta\text{-}C_5H_5)Fe(\eta\text{-}C_5H_4)$, Py = C_5H_5N, Bz = benzene.

Table 2

Comparison of the IC_{50} values (μm) for $[(HMBz)_2Fe]^{2+}$ and $[Fc]^+$

	L1210	PC6	CH1
$[(HMBz)_2Fe]^{2+}[CoCl_4]^{2-}$	61(25)	12(5)	10(4)
$[Fc]^+[FeCl_4]^-$	22(9)	17.5(7)	10(4)

The most interesting results are those of type II complexes, the $[(\eta^6\text{-arene})_2Fe]^{2+}$ salts. Whilst a marked anion effect was evident, bis(η^6-hexamethylbenzene)Fe^{2+} ($[(HMBz)_2Fe]^{2+}$) showed the greatest activity of all the complexes tested (IC_{50} = 4.6 μM as its $[CoCl_4]^{2-}$ salt against V.79) and even some tissue selectivity against the V.79 cell line. However, replacement of two methyl groups for hydrogen almost inhibited the activity completely. Although only slight activity is seen for $[(PMBz)_2Fe]^{2+}$ (PMBz = pentamethylbenzene), it is perhaps significant that it is against V.79, demonstrating again some selectivity for this type of tumour.

Of the type III complexes tested only one showed any activity at all and this was poor.

In order to evaluate the effectiveness of the type II complex $[(HMBz)_2Fe]^{2+}[CoCl_4]^{2-}$ compared to the known activities of ferrocenium salts, a second series of tests were run. Ferrocenium tetrachloroferrate, a derivative previously investigated by Köpf-Maier et al. [1], was chosen for the comparison. These tests used a new L1210, PC6 (plasma cytoma) and CH1 (mammalian ovarian) cell lines. The results are presented in Table 2. From the IC_{50} values it can be established that $[(HMBz)_2Fe]^{2+}$ is as effective as ferrocenium.

Discussion

The metabolic fate of various metallocenes has been described by Dombrowski et al. [4]. They reported that metallocene complexes are metabolised and excreted from rats and mice in a manner similar to that of various aromatic compounds, that is, by hydroxylation of the aromatic moieties followed by glucuronide or sulphate conjugation, excretion being in the bile and urine. The detoxification occurs primarily in the liver microsomes via the cytochrome P-450 system. The hydroxylation of the metallocenes accounts for the instability of the normally stable metallocene (e.g. ferrocene) *in vivo*, since hydroxymetallocenes are unstable in air [4].

It is difficult to explain the differences in the activity observed for the four types of complexes studied on the basis of their ease of hydroxylation. However, while indeed all the compounds tested are iron sandwich compounds, they are also water soluble. This fact will ensure that their cellular distribution differs from that of a purely organic moiety, i.e. hydrophobic (e.g. ferrocene).

An attempt to rationalise the data establishes that complexes were only significantly active (i.e. $IC_{50} < 20$ μm) when they were cationic. However, it must be noted that this does not *a priori* ensure activity. It is interesting to compare this finding with those porphyrin systems that are reported to have anti-tumour properties [11]. Cationic porphyrins such as *p*-tetramethylpyridylporphyrin $[(p\text{-}TMPyPH_2)]^{4+}$ have a high affinity for DNA and are capable of intercalating into the DNA double helix whilst anionic porphyrins such as $[TPPSH_2]^{4-}$ or $[TCPPH_2]^{4-}$ do not interact with

Table 3

Selected physical properties

	Ring–ring distance (Å)	Reduction potential (V vs SCE)	charge/volume
$[(HMBz)_2Fe]^{2+}$	3.23–3.27 [a]	−0.50 [b]	0.098
$[HMBz)CpFe]^{+}$	3.21 [c]	–	0.056
$[BzCpFe]^{+}$	–	−1.4 [d]	0.056
$[Fc^{+}]$	3.36 [e]	−0.34 [f]	0.064
$[DMFc^{+}]$	3.412 [g]	–	0.064

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DNA under normal conditions [12]. It is expected that the mechanism of porphyrin–DNA binding is largely electrostatic in nature [11]. Of relevance to this work is the apparent influence of charge on the anti-tumour activity, cations being active in this respect, anions inactive.

With this in mind, in an attempt to correlate the order of activities observed with a physico-chemical feature we have calculated the charge/volume ratios for types Ib, II and III. In the calculation it was assumed that the charge did not delocalise over the substituent groups on the aromatic rings. In the case of types Ib and II, a charged cylinder approximated the molecular shape and for type III, a section of a cone was used. The molecular dimensions used and the (charge/volume) ratios are shown in Table 3. The (charge/volume) ratios decrease in the order:

$$[Bz_2Fe]^{2+} > [Fc]^{+} > [BzCpFe]^{+}$$

This reflects the order of overall activity for the three types of complexes (see Table 1). However, this crude model does not help to explain any specificity or allow the inclusion of type Ia complexes.

Also of interest are the ring–ring distances for the complexes. These range from 3.21 Å in $[(HMBz)CpFe]^{+}$ to 3.41 Å in $[DMFc]^{+}$ (see Table 3). This is close to the distance between adjacent bases in DNA at ~ 3.4 Å [4]. This coincidence may be of some importance, if these materials do indeed interact with DNA.

Finally a distinguishing feature of the four types of complexes can be found in their redox behaviours. Types Ib and II have relatively low (i.e. biologically feasible) reduction potentials. In contrast, types Ia and III cannot easily be reduced. It is possible that the activity observed for types Ib and II is attributable, in some way, to their potentially oxidizing nature.

Conclusions

The results presented here include the report of significant anti-tumour activity for an iron(II) sandwich compound that does not contain a previously known

cytotoxic substituent group. However, on closer examination $[(HMBz)_2Fe]^{2+}[CoCl_4]^{2-}$ is more comparable to the iron(III) ferrocenium derivatives than iron(II) ferrocenes. The two complexes, $[(HMBz)_2Fe]^{2+}[CoCl_4]^{2-}$ and $[Fc]^{+}[FeCl_4]^{-}$, demonstrated broadly similar activities against the cell lines tested, and in fact exhibited exactly the same IC_{50} value for their greatest activities, observed against the mammalian ovarian (CH1) line.

An attempt to rationalise the data presented in this paper has been made and the most pertinent features for anti-tumour activity in iron sandwich compounds appear to be: (i) cationic charge, (ii) a complementary anion, (iii) a biologically feasible reduction potential, (iv) correct molecular dimensions. We are currently undertaking work to try to enhance the activity of these sandwich compounds in accord with our findings.

Experimental

Dimethylaminomethylferrocene was purchased from Aldrich Chemical Co. Ltd. and converted to the methiodide by reaction with MeI in benzene. The hydrochloride was obtained by passing dry HCl through an ethereal solution of the amine. $FcCH_2N(SO_3)Me_2$ was prepared by mixing equimolar solutions of the amine and the pyridine-sulphur trioxide complex in DMSO at room temperature and filtering off the resultant precipitate.

Ferrocenium tetrachloroferrates were made by oxidising acetone solutions of the corresponding ferrocene with $FeCl_3$ and precipitation of the product with ether.

Bis(η^6-arene)iron(II) chlorides and hexafluorophosphates were prepared by the method developed by Helling [9]. The (η^6-arene)(η^5-C_5H_5)iron(II) chlorides were prepared by literature procedures [10].

Preparation of $[(C_6Me_6)_2Fe][CoCl_4]$

$CoCl_2$ (0.6 g, 4.6 mmol) was dissolved in hot acetone (60 ml) and the solution treated with $[(C_6Me_6)_2Fe][PF_6]_2$ (1.34 g, 2.0 mmol) in acetone (25 ml). The mixture was refluxed for 1 h, then set aside overnight. The resulting green precipitate was filtered off and air dried to give 0.71 g $[(C_6Me_6)_2Fe][CoCl_4]$ (61%). Found; C 49.2, H 6.1, Cl 23.6. $C_{24}H_{36}Cl_4CoFe$ calc.: C 49.6, H 6.2, Cl 24.4%. Other $CoCl_4^{2-}$ salts were prepared in a similar fashion.

Anti-tumour activity testing

Testing of the compounds was carried out at the Department of Biochemical Pharmacology, The Royal Marsden Hospital. Walker tumour (WS) and L1210 cell lines were set up as suspension cultures and after 48 hours contact with the drug over a range of concentrations, the cells were counted. The V.79 cells were grown as colonies and exposed for 6 days to the range of drug concentrations, after which the number of colonies were counted and compared to solvent controls. The second set of tests on $[(HMBZ)_2Fe]^{2+}[CoCl_4]^{2-}$ and $[Fc]^{+}[FeCl_4]^{-}$ were conducted using a second L1210 leukemia cell line, again exposing the cells to the drug for 48 hours. Also ADJ/PC6 plasmacytoma and CH1 human ovarian cell lines were used. The former was treated with the drug for 72 hours and the latter for the duration of the experiment. The index of activity was the IC_{50}, which is the dose which inhibits cell growth by 50% compared to controls. These tests were preliminary *in vitro* cyto-

toxicity tests and individual errors on the data are unavailable. These experiments typically give errors of $\pm 40\%$ on individual readings and such errors have been added to data in the tables [13].

$CoCl_2$ was tested against all three lines of cells to establish if the Co^{2+} ion, used as an anion in some of the salts, showed activity. Little activity was observed. Similarly, it has been reported elsewhere that $[FeCl_4^-]$ does not exhibit anti-tumour activity [1].

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