Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 3, pp. 483-486, 1994

0960-894X/94 $6.00+0.00

0960-894X(93)E0109-E

ANTIPROLIFERATIVE ACTIVITY OF HALOOSMOCENIUM IONS AGAINST HUMAN AND MURINE CANCER CELL LINES

Yoshimitsu Yamazaki,* Midori Goto,† Kimiko Kobayashi,§ Yoshikatsu Ogawa, Takehiko Shimura, Shuichi Oka, and Hiroaki Okuno

National Institute of Bioscience and Human Technology, †National Institute of Materials and Chemical Research, Agency of Industrial Science and Technology, 1-1 Higashi, Tsukuba, Ibaraki 305, Japan; §The Institute of Physical and Chemical Research, 2-1 Hirosawa, Wako, Saitama 351-01, Japan

Abstract. Haloosmocenium salts, $[OsCp_2X]^+PF_6^-$ (X = Cl (**3**), Br (**4**) or I (**5**); Cp = η^5-2,4-cyclopentadienyl ring), have antiproliferative activity against 4 human and 2 murine cancer cell lines. The activity was highest in **3** and decreased in the order of **3**>**4**>**5**. This order was consistent with that of their redox potentials, inhibitory activity for cell respiration and NADH oxidation rates, implying that the antiproliferative activity of **3** might be due to interference with the intracellular redox or electron transfer systems.

Ferrocenium ion has a weak antineoplastic activity against several tumors *in vivo*[1a] and *in vitro*.[1b] Its structure implies that the cytotoxic mechanism is different from one involving DNA chelation as suggested for cisplatin[2] and other many *cis*-dihalometal antitumor complexes.[3] Interference with cellular redox systems has been assumed to be the antitumor mechanism for ferrocenium ion.[4] This dissimilarity in the mechanisms is interesting from the standpoint of drug design for new antitumor agents to suppress cisplatin-resistant cells which frequently appear in long-term therapy with cisplatin, thus stimulating investigation of a wider range of ferrocenium derivatives or, more generally, metallocene and metallocenium ion derivatives. Previous studies have primarily focused on dihalometallocenes of early transition metal elements (V, Ti, Nb, and Mo).[3,5] Here, we report the antiproliferative activity of metallocenium ions which contain Os or Ru, senior members of the Fe family, instead of Fe in ferrocenium ion.[6]

Six metallocenium salts (**1**,[7] **2**,[8] **3** ~ **6**[9]; listed in Table 1) were prepared by the methods in the literature[10] and tested for their antiproliferative effect against human and murine cell lines.[11] The IC_{50} values are summarized in Table 1, and the following common results can be pointed out for all cell lines. (1) The antiproliferative activity of osmocenium salt **2** was weaker than that of ferrocenium salt **1**,[12] but chloroosmocenium salt **3** showed remarkably stronger activity than **1**. (2) When the chlorine of **3** was replaced by bromine (**4**) or iodine (**5**), the antiproliferative activity decreased in the order of Cl (**3**) > Br (**4**) > I (**5**). (3) Chlororuthenocenium salt **6** was less active than the corresponding chloroosmocenium salt (**3**). In addition, $NH_4^+PF_6^-$ was inactive even at 100 μM. Thus, the PF_6^- ion is not responsible for the present activity of the haloosmocenium salts.

The above results indicate that the replacement of the center atom (Fe) for Os alone does not lead to an enhancement of antiproliferative activity of ferrocenium salt (**1**), but the replacement plus the introduction of a halogen atom, especially a chlorine atom, together were effective in increasing the activity. Although the activity of chloroosmocenium salt (**3**) was weaker than that of cisplatin, this compound was interesting in view of the relation between the structure and biochemical properties as described below.

Table 1. Biological and chemical properties of the metallocenium salts[a]

Compound	IC_{50} (μM) for cancer and embryonic cells				
	U937	HL60	Daudi	Colo320	S180
$[FeCp_2]^+PF_6^-$ (1)	62 ± 6	22 ± 2	20	62 ± 19	57 ± 25
$[OsCp_2]^+BF_4^-$ (2)	148 ± 56	90	53	180 ± 43	>200
$[OsCp_2Cl]^+PF_6^-$ (3)	14.8 ± 1.8	11.2 ± 2.3	7.9 ± 4.2	14.6 ± 1.6	19.3 ± 6.5
$[OsCp_2Br]^+PF_6^-$ (4)	60 ± 8	52 ± 11	24	48 ± 6	49 ± 7
$[OsCp_2I]^+PF_6^-$ (5)	167 ± 51	75 ± 12	79 ± 2	>200	120 ± 9
$[RuCp_2Cl]^+PF_6^-$ (6)	inactive	52 ± 14	70	111 ± 15	85 ± 8
cisplatin (CDDP)	5.2 ± 2.1	4.3 ± 1.3	1.7	6.6 ± 2.8	0.8 ± 0.1

Compound	IC_{50} (μM)		E_0	O_2 consumption rate[b]	Oxidation rate[c]	
	LX830	NIH/3T3	(V)	$\left(\frac{\text{nmol}}{\text{min}\cdot 10^7 \text{ cells}}\right)$	NADH (μM/min)	reduced cytochrome c (nM/min)
1	66 ± 20	20.2 ± 2.3	0.46	-	-	-
2	85	40	-	-	-	-
3	16.5 ± 3.1	16.1 ± 3.4	0.57	6.8 ± 0.4	13	96
4	63 ± 4	29 ± 6	0.54	7.6 ± 0.3	12	207
5	inactive	87 ± 12	0.46	10.5 ± 0.8	8	824
6	123 ± 27	44 ± 22	0.70	-	-	-
CDDP	13.2 ± 6.7	0.30 ± 0.06	-	13.4 ± 2.8 (14.5 ± 2.5)#	-	-

[a] Values are given in mean ± standard deviation (n = 3~15) or mean of two separate experiments. Origin of the cell : U937, human histiocytic lymphoma; HL60, human promyelocytic leukemia; Daudi, human Burkitt lymphoma; Colo320, human colon adenocarcinoma; S180, mouse sarcoma; LX830, mouse lymphoma; NIH/3T3, mouse embryo. [b] Of U937 cells with 200 μM **3** ~ **5**, 10 μM CDDP, or none of them # at 30 °C. The control value# indicates that the respiration of U937 cells was about 4 times that of normal human leucocytes.[18] [c] Measured with 0.24 mM NADH vs. 0.5 mM **3** ~ **5** and 0.049 mM reduced cytochrome c vs. 0.44 mM **3** ~ **5** at 30 °C. "-" indicates "not tested".

The structure of **3** was unknown and we determined it by X-ray crystallographic analysis.[13] The crystal structure shows that the chlorine atom is bound to the Os atom (Fig. 1). This Cl - Os structure caused us to wonder whether some nucleophilic group such as base nitrogen atoms or phosphate ions in DNA might substitute the chlorine atom as suggested for *cis*-dihalometal metallocenes by NMR.[3,14] We took ^1H- and ^{31}P-NMR spectra for mixtures of **3** with adenosine, guanosine, dAMP, or dGMP in D_2O. No significant change was found either for the base protons in the ^1H-NMR or for the signal of the 5'-phosphate in the ^{31}P-NMR spectra as compared with those of the pure nucleosides and

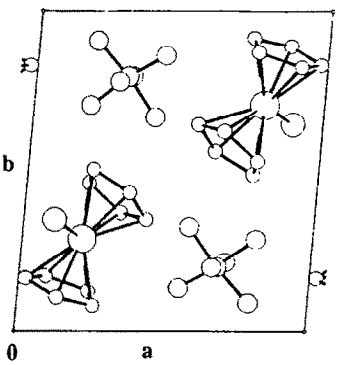

Fig. 1. Crystal structure of **3**

nucleotides, leaving little possibility of coordination binding of **3** to nucleic acid constituents. A recent study on the haloosmocenium salts by IR and NMR suggested that the interaction between the osmium and halogen atoms increases in the order of I<Br<Cl.[15] This means that substitution of the halogen atom by cellular components, if it were to occour, would be most difficult with the chloroosmocenium salt (**3**), contradicting the result in the antiproliferative activity. In conclusion, the Cl-Os structure seems not to directly connect with the cytotoxicity of **3** in such a way as ligand exchange reaction.

Since metallocenium ions have oxidation-reduction properties, redox potential (E_0) of the present compounds (**1**, **3** ~ **6**) was determined from their cyclic voltammogram (Table 1). The chloroosmocenium salt (**3**) showed the highest value among the three haloosmocenium salts, the bromo salt (**4**) the middle value, and the iodo salt (**5**) the lowest value. This order in E_0 (**3>4>5**) was the same as that found for the antiproliferative activity. The difference in E_0 would reflect difference in the interaction with cellular redox systems.

The respiration of U937 cells was then measured by the consumption of O_2 in the medium with or without the haloosmocenium salts.[16] All three compounds inhibited the respiration (Table 1) but **3** was most potent; inhibition rate was again in the order of **3>4>5**. These correlations between E_0, respiration inhibition and antiproliferative activity imply that interference with intracellular electron transfer might be the basis of the cytotoxicity of these haloosmocenium salts (**3** ~ **5**).

NADH and cyctochrome c are the early and the late constituent of the respiration chain, respectively. The haloosmocenium salts (**3** ~ **5**) chemically oxidized NADH as well as the reduced form of cytochrome c.[17] The NADH oxidation rates of the three compounds were aligned in the order **3>4>5**, while this order was reversed for the oxidation of reduced cytochrome c (Table 1). Although these rates were obtained under specific reaction conditions, comparison of the orders suggests that the main target of the chloroosmocenium salt (**3**) is not cytochrome c. It is not certain at present what is the true target for **3**, because living cells contain many oxidation-sensitive compounds, *e.g.*, cysteine, glutathione, proteins, ascorbic acid, metalloenzymes *etc.*, in addition to respiration chain components. The inhibition of respiration might be merely a consequence of metabolic disorder caused by the interaction with one of these compounds.

The present work suggests that a new drug design of antitumor agents is possible utilizing an interference with intracellular redox systems by organometallic moieties. *In vivo* evaluation of **3** and study of its activity against cisplatin-resistant cells are in progress.

Acknowledgement. We are grateful to Dr. Y. Nagawa (NIBH) for his ^{31}P-NMR measurement.

References and Notes

1. (a) Köpf-Maier, P.; Köpf, H.; Neuse, E. W. *J. Cancer Res. Clin. Oncol.* **1984**, *108*, 336; (b) Neuse, E. W.; Kanzawa, F. *Appl. Organomet. Chem.* **1990**, *4*, 19.
2. Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153.
3. Kuo, L. Y.; Kanatzidis, M. G.; Sabat, M.; Tipton, A. L.; Marks, T. J. *J. Am. Chem. Soc.* **1991**, *113*, 9027.
4. (a) Kovacic, P.; Popp, W. J.; Ames, J. R.; Ryan, M. D. *Anti-Cancer Drug Des.* **1988**, *3*, 205; (b) Joy, A. M.; Goodgame, D. M. L.; Stratford, I. J. *Int. J. Rad. Oncol. Biol. Phys.* **1989**, *16*, 1053.
5. Köpf-Maier, P.; Klapötke, T. *J. Cancer Res. Clin. Oncol.* **1992**, *118*, 216.

6. $[OsCp_2]^+$ ion was described as one example of metallocenium ions in an early publication of Köpf-Maier *et al.*,[1a] but no biological activity has been reported for osmocenium ion and its derivatives.

7. Martinez, R; Tiripicchio, A. *Acta Cryst.* **1990**, *C46*, 202.

8. Denisovich, L. I.; Zakurin, N. V.; Bezrukova, A. A.; Gubin, S. P. *J. Organomet. Chem.* **1974**, *81*, 207.

9. Smith, T. P.; Iverson, D. J.; Droege, M. W.; Kwan, K. S.; Taube, H. *Inorg. Chem.* **1987**, *26*, 2882.

10. Elemental analysis was satisfactory with every compound. Found for **1**: C, 36.11; H, 3.01 %; Calcd. for $C_{10}H_{10}F_6FeP$: C, 36.29; H, 3.05 %. Found for **2**: C, 29.12; H, 2.39 %; Calcd. for $C_{10}H_{10}BF_4Os$: C, 29.50; H, 2.48 %. Found for **3**: C, 24.01; H, 1.95; Cl, 6.96 %; Calcd. for $C_{10}H_{10}ClF_6OsP$: C, 23.98; H, 2.01; Cl, 7.08 %. Found for **4**: C, 22.27; H, 1.89; Br, 14.58 %; Calcd. for $C_{10}H_{10}BrF_6OsP$: C, 22.03; H, 1.85; Br, 14.66 %. Found for **5**: C, 19.84; H, 1.72; I, 21.74 %; Calcd. for $C_{10}H_{10}F_6IOsP$: C, 20.28; H, 1.70; I, 21.43 %. Found for **6**: C, 29.37; H, 2.48; Cl, 8.39 %; Calcd. for $C_{10}H_{10}ClF_6PRu$: C, 29.18; H, 2.45; Cl, 8.61 %. UV λ_{max} in H_2O, nm (ε, $M^{-1}cm^{-1}$): 310 (1.3×10^3), 397 (sh, 3.2×10^2), and 540 (4.0×10) for **3**; 340 (8.3×10^2), 406 (sh, 3.7×10^2), and 555 (3.4×10) for **4**; 314 (9.6×10^2), 413 (9.2×10^2), and 644 (5.3×10) for **5**. ^{1}H-NMR (D_2O) showed a singlet (10 equivalent ring protons) at δ 5.97, δ 6.01, and δ 6.08 for **3**, **4**, and **5**, respectively.

11. By counting cells with a Coulter counter after incubation with or without the sample (0.1~100 μM) for 2 (S180 cells) or 3 days (other cells) essentially according to the previous procedure: Okada, T.; Shimura, T.; Okuno, H. *Inorg. Chim. Acta* **1990**, *178*, 13. HL60, Colo320, and LX830 were grown in RPMI-1640 medium containing 10 % FBS and NIH/3T3 in DMEM medium containing 10 % CS.

12. The aqueous solution of osmocenium salt **2** was initially green, but it turned colorless within 1 hr at room temperature, indicating the instability of **2**. Chlororuthenocenium salt **6** was also unstable in aqueous solutions as Smith *et al.* (Smith, T. P.; Kwan, K. S.; Taube, H.; Bino, A.; Cohen, S. *Inorg. Chem.* **1984**, *23*, 1943) reported that it was slowly autooxidized in water to a ketone. For iodoosmocenium salt **5**, some decomposition (< 15 % in 12 hr) was found in the ^{1}H-NMR spectrum (D_2O).[10] The instability in aqueous solutions would be at least part of the cause of the weak antiproliferative activity of compounds **2**, **6**, and **5**. In contrast, the chloro- (**3**) and bromoosmocenium salts (**4**) were relatively stable and their UV(H_2O)[10] and ^{1}H-NMR (D_2O) spectra were unchanged at least for 24 hr.

13. Red needle-shaped single crystals of **3** were obtained from water: triclinic ($P\bar{1}$), a = 9.412(1) Å, b = 10.381(1), c = 6.600(3), α = 91.69(2)°, β = 92.27(2), γ = 84.72(2), V = 641.3(8) Å^3, Z = 2, R = 0.050 (Rw = 0.061, w = $1/\sigma(|F_0|)$). Data were collected by CAD4 (Enraf-Nonius) diffractometer with graphite-monochromatized MoKα radiation. The atomic co-ordinates are available on request from the Cambridge Crystallographic Data Centre.

14. Harding, M. M.; Harden, G. J.; Field, L. D. *FEBS Lett.* **1993**, *322*, 291.

15. Watanabe, M.; Sano, M. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 1455.

16. U937 cells (1×10^7) were suspended in 5 ml of the medium (RPMI-1640 +10 % FBS) containing 200 μM **3** ~ **5**, 10 μM cisplatin, or neither of them at 37 °C for 1 hr, collected by centrifugation, and then resuspended in 1 ml of the medium containing the corresponding above compound which had been placed in an oxygen electrode cell (1 cm^3) thermostated at 30 °C. The cell was tightly stoppered and the decrease of O_2 in the medium was automatically recorded.

17. Monitored by the absorption change at 340 nm (NADH) or at 550 nm (reduced cytochrome c) in 50 mM phosphate buffer, pH 7.5, at 30 °C. Reduced cytochrome c was prepared by the reduction with sodium dithionite and the complete oxidation of reduced cytochrome c was done with $K_3Fe(CN)_6$. Concentration of the substrates and compounds are given in the footnote of Table 1.

18. Kakinuma, K. *Biochim. Biophys. Acta* **1974**, *348*, 76.

(*Received in USA* 13 *September* 1993; *accepted* 23 *November* 1993)