Anti-Cancer Drugs 1997, **6**, pp. 87–92

The novel use of Rh(I) complexes with naphthyridine ligands and poly(oxyethylene) as antitumorals

Rebeca Sartori, Guillermo Rencoret, Gustavo Rencoret, Alex Mora, Cristián Pérez, Rubén Pastene[1], Renato Sariego[1] and Sergio A Moya[1]

Departamento de Química y Bioquímica, Facultad de Medicina Norte, Universidad de Chile, Casilla 70086-7, Santiago, Chile. Fax: (+56) 2 7355580. [1] Departamento de Química Aplicada, Facultad de Química y Biología, Universidad de Santiago de Chile, Casilla 307-2, Santiago, Chile

Rh(I) complexes adsorbed on polymers, as a way to improve their transport and solubility properties, were studied as antitumor agents. The binding constants of the complexes to the polymer were evaluated in order to determine the conditions for maximum association to the vehicle. The toxicity of the pure complexes and those bound were determined *in vivo* using female mice. [Rh(NBD)(2,4N)]ClO_4, complex A; where NBD = norbornadiene, (2,4N) = 3,3′-dimethylene-2,2′-di-1,8-naphthyridine, was investigated on primary solid tumors and ascitic tumors. [Rh(NBD)(3,4N)]ClO_4, complex B; where (3,4N) = 3,3′-trimethylene-2,2′-di-1,8-naphthyridine, was investigated on ascitic tumors. These Rh(I) complexes appear to be promising drugs because of their solubility in aqueous polymer, which make them easier to handle in comparison with the neutral species. These complexes show a similarity to cisplatin by reducing tumor growth and by increasing the survival life span of mice. Poly (oxyethylene) was used to solubilize these poorly water-soluble compounds and to stabilize the compounds in the solution before injection. These studies suggest that both complexes, A and B, are good candidates for tumor control growth and increase the survival time.

Key words: Ascitic tumors, anti-metastatic, anti-tumor, poly(oxyethylene), rhodium complexes.

Introduction

The binding of electrolyte to poly (oxyethylene) (POE) is a well known phenomenon[1–3] that has been studied principally in non-aqueous solution.[4–18] Although this association is driven by primary cation–POE interactions,[8–12] the anion is also concentrated in the vicinity of the POE.[15] In aqueous solution the interaction of POE with electrolytes ranges from only a salting-out effect[5] to an association larger than that found in crown ethers.[19]

A prolongation of the activity of some drugs is obtained when they are bound to synthetic polymers[20] which are able to increase the solubility of the complexes in the plasma. The binding of the complexes to transferrin has been investigated.[21] POE has been used as a transport polymer bound to penicillin, aspirin, amphetamines, etc.[22,23] When these drugs have modifiable terminal groups, like insulin or anti-cancer drugs, they can form a structure with excellent solubility in water. The binding of alkali-metal cations to POE has been reported and a series of different anions, ranging from hydrophilic (e.g. fluoride, chloride, bromide, etc.) to those with hydrophobic alkyl chains (e.g. pentanoate, xanthate, etc.) have shown the ability of POE to absorb ions and decrease their bulk concentration in solution, stressing the importance of hydrophobic interactions in the binding.[24] Furthermore, POE is not metabolized and acts as an intestinal transport medium, easily eliminated by urine.[25,26]

During the last years cumulative evidence indicate the existence of complexes of Pt, Ru, Rh or Re, with ligands containing non-coordinated nucleophilic groups that show increased anti-tumor activity because of its interaction with DNA *in vitro* or *in vivo*.[27–31]

The main aim of research in this area in the last decade has been centered on inorganic drugs like cisplatin and its derivatives,[32,33] which can play a role in effective anti-tumor activity.[34,35] The more promising complexes are those containing Ru and Rh,[36] which, having potential anti-cancer properties, present covalent bonding with DNA bases and help to decrease the proliferative activity of cancer tissue.[37–40] It has been demonstrated that the affinity with DNA depends on the structure and the stereochemistry of the complex.[41] Heterocyclic compounds of Ru(III), which have been recently described,[42] can be characterized according to the following features: (i) having ligands as imidazole, indazole or their methyl substituted derivatives,

This work was supported by grants from DICYT (Universidad de Santiago de Chile), FONDECYT-Chile.

Correspondence to R Sartori

(ii) being water soluble, (iii) having or including ionic structures with one or more negative charges, (iv) being symmetrical, (v) having twisted molecules and not being planar, (vi) having a large ligand, which produces better antitumoral activity.[43]

In this work we studied the effect of the complexes [Rh(NBD)(2,4N)]ClO_4, complex **A**, and [Rh(NBD)(3,4N)]ClO_4, complex **B**, on ascitic tumor TA-3 growth, and on the survival time of CF-1 and AJ mice. Some aspects related to the role of POE in the vehicle used for injection, as well as the influence of the expansion of host extracellular volume on the anti-tumor effects of the compounds, have also been studied.

Materials and methods

POE (PM: 2×10^4) was supplied by Merck. [Rh(NBD)(3,4N)]ClO_4, complex **B**, was prepared, purified and characterized according to procedures described in detail elsewhere.[44] [Rh(NBD)(2,4N)]ClO_4, complex **A**, was prepared in a similar way.

Complex **B** shows a characteristic band at $\lambda_{max} = 498$ nm and complex **A** at $\lambda_{max} = 487$ nm in aqueous solution. These bands were used to evaluate the concentration of free complexes in the determination of the binding constants.

The complexes adsorbed on POE, 1.5×10^{-3} M in complex and 3.0×10^{-4} M in POE, were injected i.p. 3 days after tumor cell implantation. The doses used in AJ mice were 1 and 0.1 ml of this solution per 20–23 g body weight. The doses used in CF-1 mice were 1 and 0.1 ml of this solution per 30–33 g body weight. For each dose one group of mice (seven to 10 individuals) was injected every day for five consecutive days and another group (seven to 10 individuals) was injected on days 3, 7, 10, 14 and 17 after tumor implantation. MCa mammary carcinoma and TA-3 ascitic tumors were originally obtained from Dr A Guerrero (Experimental Laboratory, Medical School, University of Chile).

TA-3 tumor cells were locally maintained in female mice 6–8 weeks old, weighing 30 ± 3 g, by serial weekly passage in CF-1, and 20 ± 3 g, by serial weekly passage in AJ mice. TA-3 cells were inoculated i.p., observing an increase in the size of the abdomen of the mice.

Total increase in tumor size was measured by total mice weight increase. After the experiment, the mice were weighed, sacrificed by cervical dislocation and the following organs were separated: brain, liver, muscle, intestine, stomach, kidney, ovary and lung. These organs were placed in 9% formalin for histological analysis and for detection of Rh by atomic absorption spectroscopy (Perkin-Elmer 2380).

For the urine test, the mice were placed individually in metabolic cages for 3 days. About 10 ml of urine was collected, diluted to 30 ml and separated in three portions to carry on the following analysis. An Urotron RL-9 Analyzer with a Combur-9 tapes (Boehniger-Mannhein) was used to determinate qualitatively: leukocytes, nitrites, pH, total proteins, glucose, ketonic bodies, urobilinogen, bilirubin and hemoglobin. An Hitachi Analyzer was used to determine quantitatively: glucose, BUN, creatinine, uric acid and albumin. The spectra of urine solutions were obtained using a Shimadtzu 160 UV-visible Spectrophotometer.

The statistical evaluation was done according to the Mantel–Haezel test.

Results

The binding constants for the interaction of the complexes with POE

$$K_b = \frac{[\text{Complex–POE}]}{[\text{Complex}]_{free} \times [\text{POE}]_{free}}$$

were determined for complexes **A** and **B**. Figure 1 shows their values as a function of initial complex

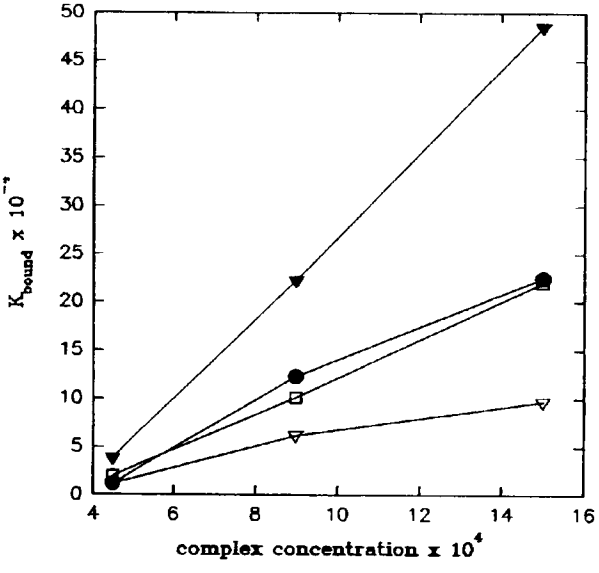

Figure 1. Effect of the concentration of the complexes and POE on the binding constants of complex to POE. ●, Complex **A** POE concentration $2{,}5 \times 10^{-4}$ M; ▽, complex **A** POE concentration 5×10^{-4} M; ▼, complex **B** POE concentration 2.5×10^{-4} M; □, complex **B** POE concentration 5×10^{-4} M.

and POE concentrations. The binding constants are high at large complex concentration and low POE concentration.

The effect of pure complexes, pure POE and the complexes bound to POE on CF-1 and AJ mice lethality are reported in Table 1.

The dose of complex used in the experiments was that which gave the best association with the vehicle, allowing for 100% survival in normal mice (mice without tumors) and which produced the same host toxicity. When the quantities of complex administered to the mice were too large, they died earlier and the size of the tumors was not reduced as much as when they were treated with a smaller amount. Antitumor effectiveness of complex **A** was studied on primary tumor growth and the survival time of mice bearing solid MCa tumor is shown in Figure 2(A). Antitumor effectiveness (TA-3 ascitic tumor) of complex **A** administered as a suspension in aqueous solution of POE is shown in Figure 2(B and C). The same activity of complex **B** is shown in Figure 2(D and E). The effects on primary tumor growth in AJ and CF-1 mice of complex **A** and **B** with POE is shown in Figure 3. This was more pronounced with treatment at 3 or 4 days intervals than daily. In both cases it was better with 0.1 ml than with 1 ml. The same effects were observed for survival time and body weight. Fractional or daily i.p. administration did not present any significant effect, neither in the increase of urinary volume nor in the observed agility of the mice. The effect of the administration of complex–POE solutions to mice bearing tumors was observed in a slight reduction (in CF-1 mice) and increase (in AJ mice) of the level of glucose, BUN and uric acid. Spectrophotometric analysis of the urine of the mice under study does not show any change after administration of the complexes.

Rh atomic absorption spectroscopy of the homogenized whole body and individual organs of the mice showed accumulation of the complex in all the organs studied.

Discussion

Addition of POE (0.1–0.5 mM) to solutions of complexes (1–5 mM) decreases the free complex concentration, as measured directly from the charge transfer band in aqueous solution. These results indicate an interaction between the macromolecule and the complexes. K_b values for **A** are greater than for **B**, but in every case it increases as the complex concentration increases and decreases with increasing concentrations of POE. These results indicate that the small difference in conformation of the naphthyridine ligand (because of an extra methylene), can play a role in the ability of the complexes to associate to POE. Furthermore, changes in POE concentration affect the viscosity of the solution producing changes in the conformation of the macromolecule[9] and in its association to the complexes.

Table 1. Lethality (L) of the complexes with ligands 3,4N and 2,4N, POE and the complexes bound to POE on CF-1 and AJ mice at different doses (D)[a]

Molar concentrations	CF-1 MICE[b]				AJ MICE[c]			
	3,4N		2,4N		3,4N		2,4N	
	D (th)	L (%)	D (th)	L (%)	D (th)	L (%)	D (th)	L (%)
Complex 3.38×10^{-3}	4	50	5	50	3	50	3	50
	5	100	6	100	4	100	5	100
Complex 2.00×10^{-3}	8	25	8	0	8	30	8	0
	9	50	9	0	9	60	9	0
	11	100	11	0	10	100	10	0
Complex 1.50×10^{-3}	9	0	9	0	9	0	9	0
	11	50	11	0	10	50	10	0
	12	100	12	0	12	100	12	0
POE 3.0×10^{-4}	9	0	9	0	9	0	9	0
Complex–POE $2.5–0.3 \times 10^{-3}$	6	50	6	0	6	50	6	0
	7	100	9	0	7	100	9	0
Complex–POE $1.5–0.3 \times 10^{-3}$	9	0	9	0	9	0	9	0

[a] 1 ml of solution i.p. daily.
[b] Weight 31–33 g.
[c] Weight 21–25 g.

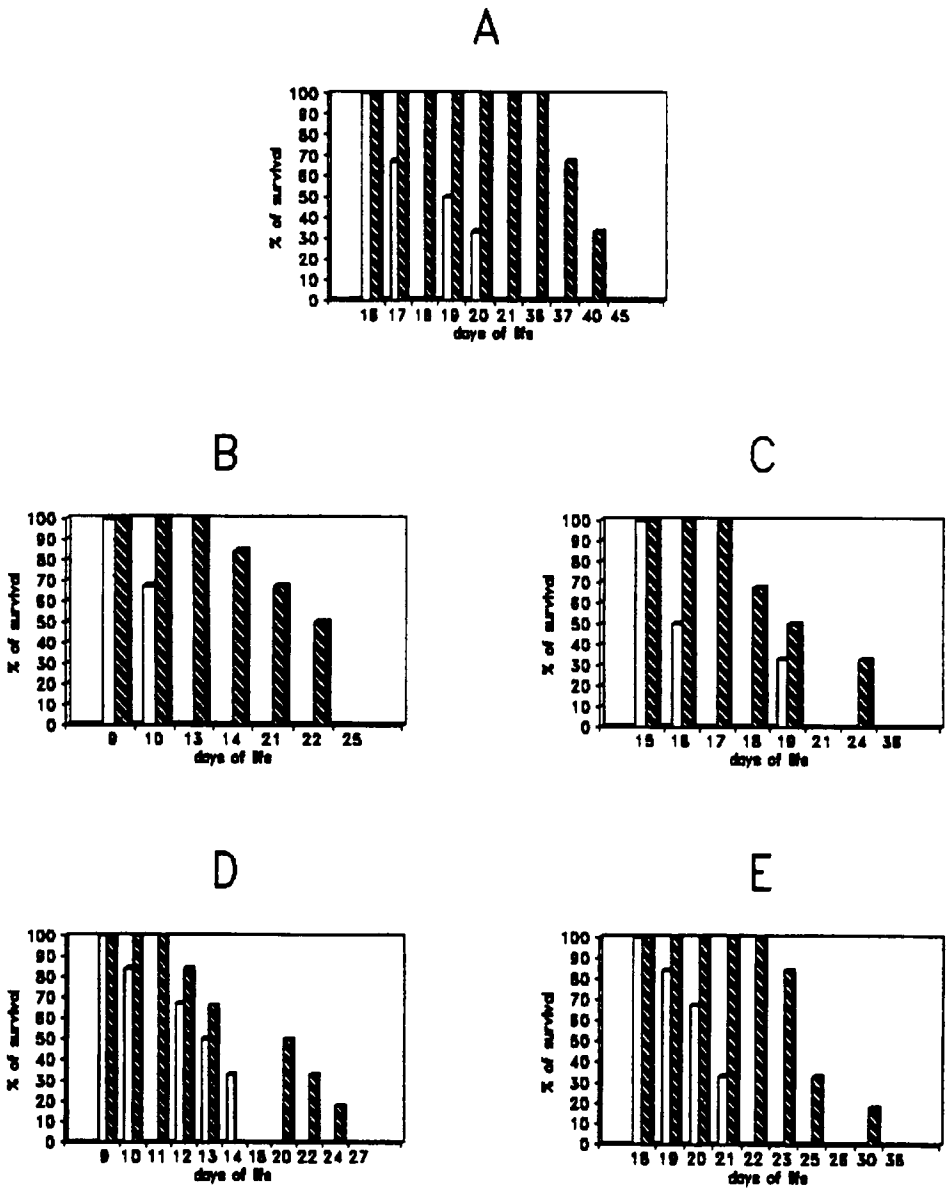

Figure 2. Percentage of survival time versus days of life. Empty columns; control mice. (A) Complex **A**. Nude female mice. Tumor MCa mammary carcinoma. (B) Complex **A**. AJ mice. TA-3 ascitic tumor. (C) Complex **A**. CF-1 mice. TA-3 ascitic tumor. (D) Complex **B**. AJ mice. TA-3 ascitic tumor. (E) Complex **B**. CF-1 mice. TA-3 ascitic tumor.

Association of the complexes with solutions of POE in water makes their transport through the body of the mice easier because of the considerable increase in their solubility. Free POE does not show any decrease in the survival time and neither does it affect the biochemical panel of the mice as shown in the urine analysis. These results suggest that the unique function of POE is to increase the solubility of the complexes, therefore behaving as a vehicle for them.

Mice bearing tumors injected with too large a concentration of complex show a decrease in their survival time. On the other hand, a low concentration does not produce any observed change in it.

Examination of the antineoplastic properties of the new Rh(I) complexes, characterized by the presence of POE and polypyridinic ligand, show an important reduction in tumor growth, both in AJ and CF-1 mice. Complex **A** is slightly better. On the other hand, both complexes increase dramatically the life span (from 10–20 to more than 45 days). The spectra of urine do not show the metal ligand bands, suggesting that the total complex is accumulated in the body and it is not excreted by urine. Rh atomic

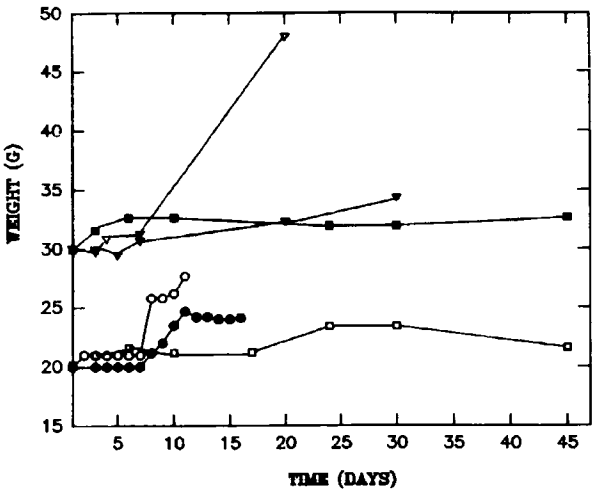

Figure 3. Weight versus days. AJ mice: ○, control; ●, treated with complex **A**; □, treated with complex **B**. CF-1 mice; inverted triangle, control; ▼, treated with complex **A**; ■, treated with complex **B**.

absorption spectroscopy shows distribution of the Rh in all the organs and tissue studied. These results imply that it can be possible, by the use of these complexes, to decrease the size of tumors or metastasis in any organ.

Conclusions

The association of POE to recently synthesized Rh(I) complexes of olefine and nitrogen donor ligands, [Rh(NBD)(3,4N)]ClO_4 and [Rh(NBD)(2,4N)]ClO_4, has some advantages, such as water solubilization, preserving practically the same activity in the other parameters on the tumor growth studied, including the prolongation of the host survival time with ascitic tumor. This opens the possibility of investigating new approaches for the treatment of liquid tumors and metastases.

Acknowledgments

We are grateful to A Guerrero for the gift of tumor cells and The Central Laboratory of the Hospital JJ Aguirre for their collaboration in the urine analysis.

References

1. Bailey FE Jr, Koleski JV. *Poly(ethylene oxide)*. New York: Academic Press 1976: 87.
2. Izatt RM, Christensen JJ *Synthetic multidental macrocyclic compounds*. New York: Academic Press 1978.
3. Balasubramanian D, Chandami BJ. Poly(ethylene glycol). A poor chemist's crown. *J Chem Educ* 1983; **60**: 77.
4. Lundberg RD, Bailey FE Jr, Callard RW. Interactions of inorganic salts with poly(ethylene oxide). *J Polym Sci, Part A-I 1966; 4: 1563.*
5. Bailey FE Jr, Callard RW. Some properties of poly (ethylene oxide) in aqueous solution. *J Appl Polym Sci* 1959; **56**: 1.
6. Amantaramen AV. Thermodynamic of solvent mixture. I. Density and viscosity of binary mixture of *n*-ethyl pyrrolidine—tetrahydrofuran and propylene carbonate acenonitrile. *Can J Chem* 1986; **64**: 46.
7. (a) Sears PG, Holmes R, Dawson LR. Conductances, viscosities, and densities of solutions of potassium thiocyanate in methanol at temperatures within the range 25° to − 50°. *J Electrochem Soc* 1955; **102**: 145. (b) Sears PG, McNeer RL, Dawson LR. Conductance of lithium bromide at low concentrations in methanol within the temperature range 20° to − 50°C. *J Electrochem Soc* 1955; **102**: 269. (c) Dawson LR, Hagstrom RA, Sears PG. Conductimetric and viscometric behaviour of lithium bromide in 50–50 weight per cent methanol–acetone at temperatures within the range 20° to 50°C. *J Electrochem Soc* 1955; **102**: 341.
8. Ono K, Konami H, Murakami K. Conductimetric studies of ion binding to poly(oxyethylene) in methanol. *J Phys Chem* 1979; **83**: 1665.
9. Awano H, Ono K, Murakami K. The interaction of a neutral polymer with small ions in solution. I. A. method for the analysis of ion binding to a neutral polymer. *Bull Chem Soc Jpn* 1982; **55**: 2525.
10. Awano H, Ono K, Murakami K. The interaction of a neutral polymer with small ions in solution. II. The binding of alkali metal ions to poly(oxyethylene) in several organic solvents. *Bull Chem Soc Jpn* 1982; **55**: 2530.
11. Awano H, Ono K, Murakami K. The interaction of a neutral polymer with small ions in solution. III. Ionic association on a poly(oxyethylene) chain. *Bull Chem Soc Jpn* 1983; **56**: 1715.
12. Awano H, Ono K, and Murakani K. Ionic association on a polyoxyethylene chain. *Chem Lett* 1982, 149.
13. Buschmann HJ. Complexation of alkali- and alkaline-earth cations by poly(oxyethylene)s in methanol. *Macromol Chem* 1986; **187**: 423.
14. Yanagida S, Takashashi K, and Okahara M. Metal-ion complexation of noncyclic poly(oxyethylene) derivatives. I. Solvent extraction of alkali and alkaline earth metal thiocyanates and iodides. *Bull Chem Soc Jpn* 1977; **50**: 1386.
15. Quina F, Sepúlveda L, Sartori R, *et al.* Binding of electrolytes to poly (ethylene oxide) in methanol. *Macromolecules* 1986; **19**: 990.
16. Liu KI. *Macromolecules* 1986; **1**: 308.
17. Panayotov JM, Dimov DK, Tsvetanov Ch B, *et al.* Complex formation between poly (ethylene oxide) and alkali picrates. *J Polym Sci Polym Chem Ed* 1980; **18**: 3059.
18. Chan LL, Wong KH, Smid J. Complexation of lithium, sodium, and potassium carbanion pairs with polyglycol dimethyl ethers (glymes). Effect of chain length and temperature. *J Am Chem Soc* 1970; **92**: 1955.
19. Sartori R, Lissi E. Asociación de etil y hexil xantato de potasio sobre poli(oxietileno) y eter corona. *Abstr XIX Chilean Meeting of Chemistry* 1991; 64.

20. Batz HG. Polymeric drugs. *Adv Polym Sci* 1975; **23**: 25.
21. Scrivastava SC, Mausner LF, Clarke MJ. Radioruthenium-labeled compounds for diagnostic tumor imaging. *Prog Clin Biochem Med* 1989; **10**: 112.
22. Poroell GM. Polyethylene Glycol. In: Davison RI, ed. *Handbook of water soluble gums & resins.* New York: McGraw-Hill 1980.
23. Williams RJP. Coordination compounds and genes In: Williams AF, Floriani C, Herbach AE, eds. *Perspectives in coordination chemistry.* Basel: Verlag Helvetica Chimica Acta 1992: 381.
24. (a) Sartori R, Sepúlveda L, Quina F, *et al.* Binding of electrolytes to poly(ethylene oxide) in aqueous solutions. *Macromolecules* 1990; **23**: 3878. (b) Sartori R, Sepúlveda L, Aedo P, *et al.* Binding of potassium alkyl xanthate to poly(oxyethylene) in water solution. *Abstr 1989 Int Chemical Congress of Pacific Basin Societies.* (c) Bordi F, Cannetti C, DiBiasio A. *J Phys Chem* 1988; **92**: 4772.
25. Hollander D, Koyama S, Dadufalsa V, *et al.* Polyethylene glycol 900 permeability of rat intestinal and colonic segments *in vivo* and brush border membrane vesicles *in vitro. J Lab Clin Med* 1989; **113**: 505.
26. Sava G, Pacor S, Mestroni G, *et al.* Na[*trans*-$RuCl_4$(DMSO)Im], a metal complex of ruthenium with antimetastatic properties. *Clin Exp Mestastasis* 1992; **10**: 273.
27. Hill JM, Speer RJ. Organo-platinum complexes as antitumor agents. *Anticancer Res* 1982; **2**: 173.
28. Cleare MJ, and Hydef PC. Antitumor properties of metal complexes. In: Siegel H, ed., *Metal ions in biological systems, Vol II. Metal complexes as anticancer agents.* New York: Dekker 1980; 1–56.
29. Williams DR. Metals, ligands, and cancer. *Chem Rev* 1972; **72**: 203.
30. Köpf-Maier P, Köpf-Hartmut. ?. Non-platinum-group metal antitumor agents: history, current status, and perspectives. *Chem Rev* 1987; **87**: 1137.
31. Sherman SE, Lippard SJ. Structural aspect of platinum anticancer drug interactions with DNA. *Chem Rev* 1987; **87**: 1153.
32. Cleare MJ, Hydes PC In: Siegal H, ed. *Metal ions in biological systems.* New York: Dekker, 1979: 1.
33. (a)Clarke MJ, and Taube H. Pentaammine ruthenium guanine complexes. *J Am Chem Soc.* 1974; **96**: 5413. (b) Clarke MJ, Buchinder M, Kelman AD. Binding of penta amine ruthenium(III) to double-helical and single-stranded DNA. *Inorg Chim Acta* 1978; **27**: L-87. (c) Clarke MJ. Electrochemistry, synthesis, and spectra of penta amine ruthenium(III) complexes of cytidine, adenosine, and related ligands. *J Am Chem Soc.* 1978; **100**: 5068. (d) Clarke MJ, Jansen B, Marx KA, *et al.* Biochemical effects of binding $[(H_2O)(NH_3)_5Ru^{II}]^{2+}$ to DNA and oxidation to $[(NH_3)_5Ru^{III}]_n$–DNA. *Inorg Chim Acta 1986; **124**: 13.*
34. Pyle AM, Rehmann JP, Meshoyrer R, *et al.* Mixed-ligand complexes of ruthenium(II): factors governing binding to DNA. *J Am Chem Soc* 1989, **111**: 3051.
35. Alessio E, Attia WM, Callagaris M, *et al.* In: Nicoli M, ed. *Platinum and other metal coordination compounds in cancer chemotherapy* Boston: Martinus Nijhoff 1988: 617.
36. Sava G, Pacor S, Zorzet S, *et al.* Antitumor properties of dimethyl sulphoxide ruthenium(II) complexes in the Lewis lung carcinoma system. *Pharma Res* 1989; **21**: 617.
37. Pacor S, Sava G, Mestroni G, *et al.* Tumor Inhibition and effects on host survival time by ruthenium^{III} and rhodium^{III} complexes with dimethyl sulphoxide ligands. *Pharmac Res* 1992; **25**: 73.
38. Cauci S, Alessio E, Mestroni G, *et al.* Reaction of *cis*-$Ra^{II}(DMSO)_4Cl_2$ with DNA and with some of its bases in aqueous solution. *Inorg Chim Acta.* 1987, **137**: 19.
39. Satyanarayama S, Dabrowiak JC, Chaires JB. Neither Δ nor Λ *tris* (phenanthroline) ruthenium(II) binds to DNA by classical intercalation. *Biochemistry* 1992; **31**: 9319.
40. Esposito G, Cauci S, Fogolari F, *et al.* NMR structural characterization of the reaction product between d(GpG) and the octahedral antitumor complex *trans*-$RuCl_2(DMSO)_4$. *Biochemistry* 1992; **31**: 7094.
41. Jenkins Y, Friedman AE, Turro NJ, *et al.* Characterization of dipyridophenazine complexes of ruthenium(II): the light switch effect as a function of nucleic acid sequences and conformation. *Biochemistry.* 1992; **31**: 10809.
42. (a) Clarke MJ. In: Siegal H, ed. *Metal ions in biological systems.* New York: Dekker 1979; **11**: 231. (b) Keppler BK, Wehe D, Endres H, *et al.* Synthesis, antitumor activity, and X-ray structure of bis(imidazolium)-(imidazole)-pentachloro ruthenate(III), $(ImH)_2(RulmCl_5)$. *Inorg Chem* 1987; **26**: 844. (c) Keppler BK, Rupp W, Juhl UM, *et al.*, Synthesis, molecular structure, and tumor-inhibit properties in imidazolium *trans*-bis (imidazole) tetrachloro ruthenate (III) and its methyl-submitted derivaties. *Inorg Chem* 1987; **26**: 4366.
43. Maeders U, von Zelewsky A. Complexes of rhodium(III) with two chelating C N ligands and one diimine ligand. *Helv Chim Acta* 1992, **75**: 1320.
44. Moya SA, Pastene R, Sartori R, *et al.* Complexes with heterocycle nitrogen ligands—Part III: cationic rhodium I derivatives and applications in catalysis. *Polyhedron*, in press.

(Received 25 January 1995; received in revised form 22 September 1995; accepted 26 September 1995)