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Review paper

Metal complexes of ruthenium: antineoplastic properties and perspectives

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Octahedral ruthenium(III) and ruthenium(II) complexes show antineoplastic properties on a number of experimental tumors. Tetraammine-, pentaammine-, heterocycle-, and dimethylsulfoxide-coordinated ruthenium complexes have shown high affinity for nitrogen donor ligands *in vitro* and as a result exhibit various degrees of biological activities including antitumor action *in vivo*.

The chemical behavior of ruthenium(III) complexes indicates the possibility of opening a window of selective toxicity, in practice lacking in the chemotherapeutic approach to neoplastic diseases. Ruthenium ions may accumulate in tumor tissues via a mechanism mediated by transferrin transport. Moreover, binding of ruthenium to DNA is several times higher in its reduced ruthenium(II) form and the reduction from ruthenium(III) prodrugs to the more toxic ruthenium(II) compounds is particularly efficient in tumor hypoxic environments. Correspondingly, solid tumors appear to be more susceptible than those of the lymphoproliferative type. In particular, tumors of the colorectal region and lung tumors (primary or metastatic), which are generally associated with a bad prognosis, have given interesting responses in experimental models, indicating these tumors as preferential targets for the development of ruthenium anticancer drugs.

Key words: Antitumor action, experimental models, ruthenium complexes.

Introduction

Transition metal complexes have been subjected to evaluation for biological activity with the aim of obtaining active drugs. Compounds not only of group VIII (the platinum group) but also from other groups were synthesized and tested on a number of systems. As well as some investigations on antiinflammatory properties stimulating the studies of rhodium and iridium compounds,[1] the main research involved the neoplastic diseases.[2–9]

Following a classical procedure, the antineoplastic activity of metal complexes has been tested to find alternatives to platinum anticancer derivatives and particularly to *cis*-dichlorodiammineplatinum(II) (generic name cisplatin). This is a result of the concept that new derivatives to be introduced into clinical practice should have a therapeutic index better than that of cisplatin either by reducing the side-effects or by increasing the antitumor potency. However, this research could be restrictive and misleading in that test compounds have often been studied only in conditions in which cisplatin is active, thereby hampering the possibility of exploring therapeutic activities different from those of the reference compound.

Evidence accumulated so far seems to indicate that this could be the case for ruthenium complexes. The rather poor knowledge of the biological features of metal ions and of transition metal complexes of ruthenium in particular still favors this hypothesis and suggests the need for wider investigations.

Biological features of ruthenium complexes

Ruthenium ions have been subjected to a number of studies concerning their chemical behavior and

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their potential role in medical applications. Particular emphasis has been given to the examination of the possible antineoplastic properties of ruthenium complexes with a number of biologically active ligands.[10–12]

In agreement with the above considerations, data on the antitumor activity of ruthenium complexes with ligands ranging from NH_3 and Cl to imidazole, purines, pyrimidines and dimethylsulfoxide indicate some therapeutic potentiality and suggest the possibility of acquiring new compounds of potential value in the therapy of neoplastic diseases.

Chemical interactions and chemical reactivity of ruthenium ions have been studied under different conditions. This work, reviewed by Clarke in several papers,[9,10,13,14] suggests ruthenium to be a metal ion endowed with a rather high propensity to bind DNA. In a series of "phenanthroline–ruthenium(II) complexes", binding affinity appears to depend on the shape of the complex and on how that shape matches the DNA, rather than on the introduction of hydrogen-bonding functionalities.[15] Intercalation and surface bindings occur as well. The intercalating ability seems to depend on the nature of the ligands employed. Using a series of mixed-ligand complexes, which highlights variation in geometry, size and hydrophobicity, the highest affinity was observed with 1,10-phenanthroline.[16] DNA binding was observed also with a number of ammineruthenium(II) complexes[17] and with a ruthenium(II) complex containing dimethylsulfoxide, namely Ru(II) $(DMSO)_4Cl_2$;[18,19] concerning the latter compound, DNA interaction is higher for the *trans* isomer.[19]

Further evidence favors ruthenium as an interesting antitumor agent. In particular, a series of results indicate the propensity of ruthenium to bind to tumor cells. ^{97}Ru and ^{103}Ru distribute in tumor tissue with levels higher than those of normal tissue (for instance, 5-fold that of muscle).[20–22] This selective distribution could be facilitated by transferrin transport, as suggested by Srivastava *et al.*[22] who based their theory on the concept that tumor cells have a higher requirement for iron and therefore a larger number of transferrin receptors than normal tissues (Figure 1). Binding to transferrin should be favored by the similarity of ruthenium to iron, which belongs to group VIII of the transition metals. It is therefore conceivable that ruthenium, once it has reached plasma level, binds to transferrin being transported to tissues as a ruthenium–transferrin complex.

The combination of preferential distribution in

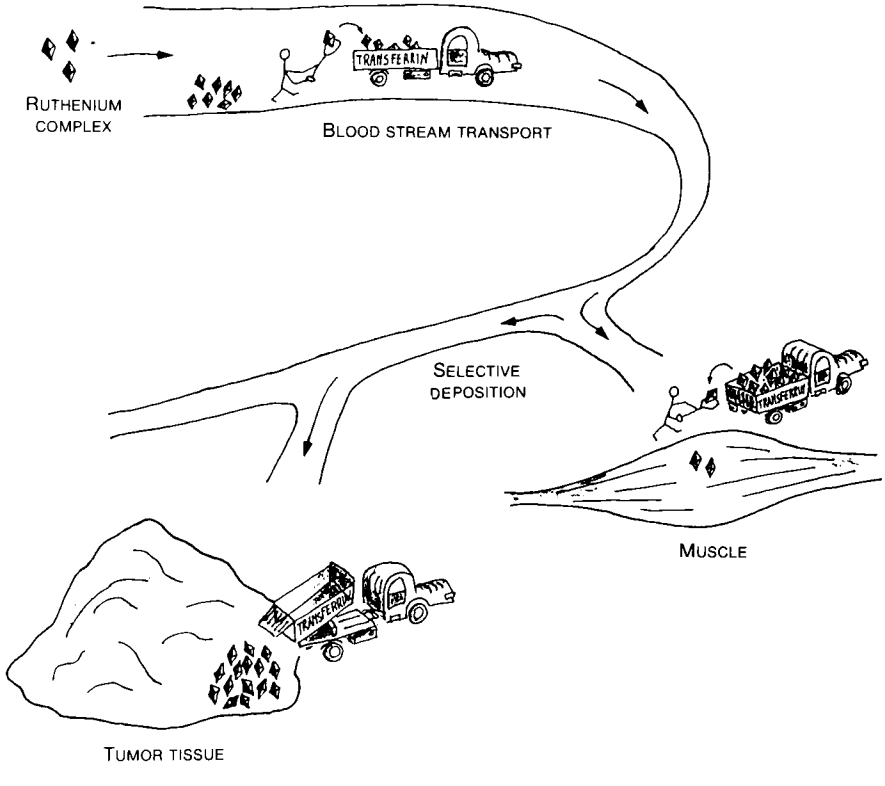


Figure 1. Preferential selective deposition of ruthenium ions by transferrin transport.

tumor tissue and DNA affinity binding of ruthenium was further stressed by Farrell *et al.* who demonstrated the peculiar radiosensitizing properties of $RuCl_2$ $(DMSO)_2$(4-nitroimidazole)$_2$ for hypoxic cells.[23–26]

The interaction of ruthenium ions with DNA is emphasized by studies on mutagenicity in bacteria.[27–29] Although mutagenicity tests, performed according to the Ames test, indicate that ruthenium complexes are in general endowed with weak mutagenic properties, evidence was provided that compounds such as *cis*-$RuCl_2(DMSO)_4$ induce chromatid aberrations,[25] are mutagenic for eukaryotic cells and show a concomitant binding to DNA of the same cells.[12] Interestingly, in these conditions the *trans* isomer exhibited a 20-fold higher affinity for DNA.[12]

Antineoplastic activity of ruthenium complexes

Since the main goal of antitumor chemotherapy is to kill cells by inhibiting their replication, it appears that ruthenium compounds, on the basis of the above considerations, could be of value. In this respect, some families of ruthenium compounds were investigated for antitumor activity and the data and discussions in the following sections are in agreement with this suggestion. Although stressing the need for additional chemical interventions to improve the targeting properties of ruthenium ions to DNA of tumor cells, in amounts sufficient to realize the most favorable selectivity, the evidence uniformly suggests ruthenium as a tool for investigating selective anticancer drugs of a new generation.

Ammine–ruthenium(III) complexes

cis-$Ru(NH_3)_3Cl_3$ induces filamentous growth in *Escherichia coli*: filamentation occurs to the same extent as observed with cisplatin.[29] This effect, in the case of cisplatin,[30] correlates with the antitumor activity in experimental systems[31] and suggests interactions with mechanisms of cellular replication.[32]

The biological activities of $Ru(NH_3)_3Cl_3$ consist also of the inhibition of DNA synthesis in a cell line derived from rat kidney, as evaluated by a significant reduction of uptake of ^{3}H-thymidine into the DNA (Table 1). Correspondingly, this compound inhibits P388 lymphocytic leukemia in mice.

Table 1. Biological activities of *fac*-Ru(III)-$(NH_3)_3Cl_3$

Induction of filamentation in *E. coli* (induction of 95% of long filaments)
Inhibition of DNA synthesis in RK cells (86% reduction of ^{3}H-thymidine uptake)
Inhibition of P388 lymphocytic leukemia (89% life-span increase in leukemic hosts)

The observed inhibition is the highest observed among the ruthenium ammine derivatives tested.[9] Surprisingly, this compound, because of its rather poor water solubility, did not enter further experimental design with other types of tumors. Conversely, more emphasis was given to pentaammineruthenium(III) complexes.[9,10,13,14,17,33,34] A large number of pentaammineruthenium(III) complexes were synthesized with ligands such as halides, purines and pyrimidines, methylxanthines, carboxylic acids and others with the aim of improving the targeting to DNA (Table 2).

The presence of labile ligands, however, gave rise to a variety of biological activities but not

Table 2. Summary of the biological activities of some Ru(III)$(NH_3)_5X$ complexes

X = Cl

(1) Inhibition of DNA synthesis on RK cells
(2) Borderline mutagenicity on TA98 *Salmonella typhimurium* strain
(3) Weak induction of transformation on *Bacillus subtilis* strain
(4) Mild cytotoxicity on *B. subtilis* strain
(5) No cytotoxicity on RK cells.
(6) No antitumor activity in mice with P388 lymphocytic leukemia

X = NH_3

(1) Weak mutagenicity on TA98 *S. typhimurium* strain
(2) Mild cytotoxicity on *B. subtilis* strain
(3) Moderate cytotoxicity for L1210 leukemic cells *in vitro*
(4) No antitumor activity in mice with P388 lymphocytic leukemia

X = purine, pyrimidine, methylxanthine

(1) Mild to pronounced inhibition of DNA synthesis
(2) Marked mutagenicity on TA98 *S. typhimurium* strain
(3) No antitumor activity in mice with P388 lymphocytic leukemia

X = carboxylic acids

(1) Moderate to no inhibition of DNA synthesis (formic acid > acetic acid > propionic acid)
(2) Moderate to good activity on P388 lymphocytic leukemia (propionic acid > acetic acid > formic acid)

necessarily to antitumor effects. In fact [Ru(III)-$(NH_3)_5Cl]^{2+}$, a complex which rapidly replaces Cl with H_2O, increasing its reactivity, is rather poor in cytotoxic effects and in mutagenicity *in vivo* and *in vitro*, although inhibition of DNA synthesis occurred *in vitro* on RK cells.[27,33] The lack of correlation between cytotoxicity–mutagenicity and DNA synthesis inhibition could be explained by assuming that two separate mechanisms are operative and considering that bacteria have more efficient DNA-repairing systems.

A discrepancy between antitumor effects *in vivo* and mutagenicity *in vitro* is also reported for purine and pyrimidine derivatives which were much more mutagenic than other pentaammineruthenium complexes tested.[9,13,14,27,33] The only group of these complexes endowed with antitumor activity in the P388 system is that of pentaammineruthenium carboxylates.[9] In this group a double dependency is shown: (a) the antitumor activity increases with the increasing size of the carboxylic acid; (b) the inhibition of DNA synthesis decreases with the increasing size of the carboxylic acid.

Again, a pentaammineruthenium bleomycin complex is able to potentiate the *in vitro* cytotoxicity of the free ligand bleomycin in an L1210 leukemic line *in vitro*, with a mechanism apparently unrelated to DNA strand scission.[34]

These data do not completely account for the supposed antitumor action hypothesized for these molecules. Detailed physicochemical studies revealed the marked propensity of ammineruthenium complexes to interact with DNA; the interaction is of the same order as that observed with cisplatin.[10] Indeed, a large portion of the literature on ammineruthenium complexes concerns the examination of ruthenium activation as a function of the redox potential of the environment.[9,10,13,14,17] The relevance of the redox potential in activating ruthenium complexes is based on the observations that (a) tumor tissues have a reducing environment owing to the biological characteristics of tumor growth and (b) ruthenium(II), obtained by reduction from ruthenium(III), is several times more reactive than ruthenium(III) itself.[9] Connecting these two observations, it can be assumed that ruthenium(III) complexes should exhibit a selective toxicity for tumor cells rather than for normal tissues (Clarke's hypothesis) (see Figures 2 and 3).

The approach used for investigating the antitumor activity of ammineruthenium(III) complexes is in some ways criticizable. Tests performed and reported to date in the literature were carried out using tumors of a leukemic type that do not

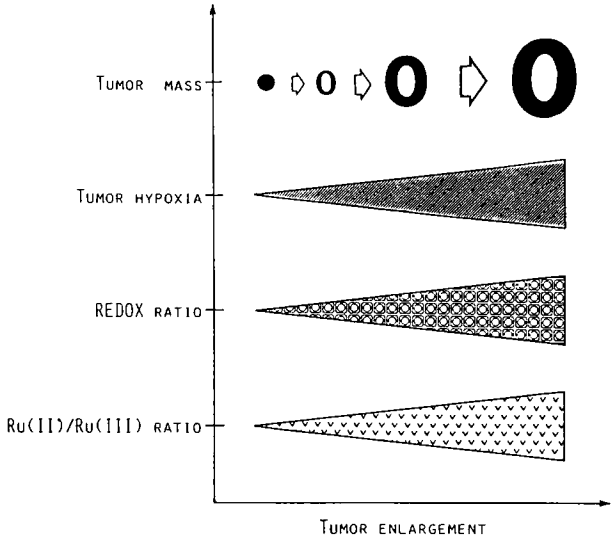


Figure 2. Relationships between the increasing mass of tumors and redox potential of tumor tissue.

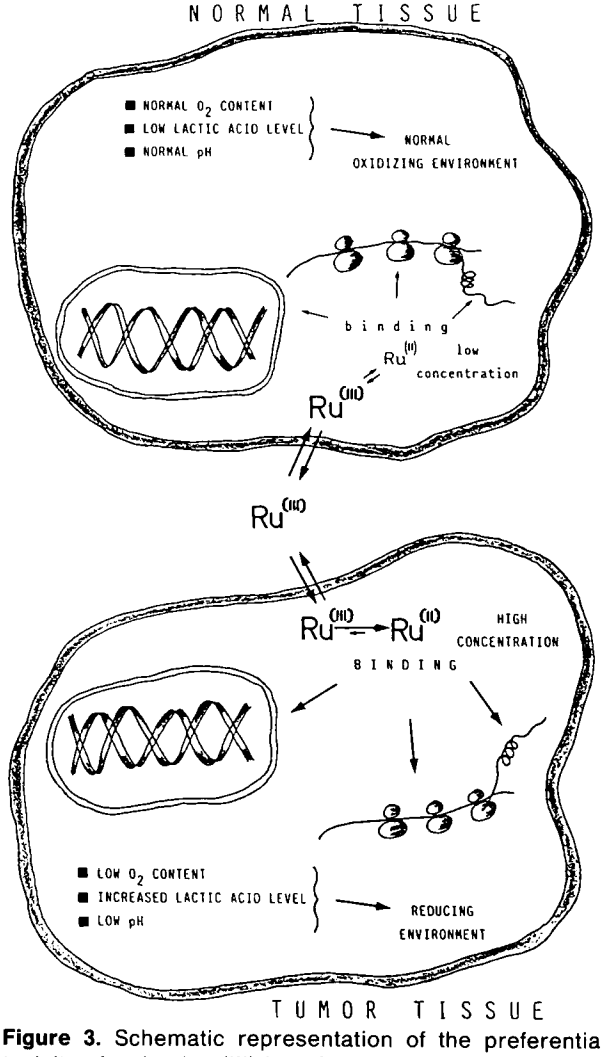
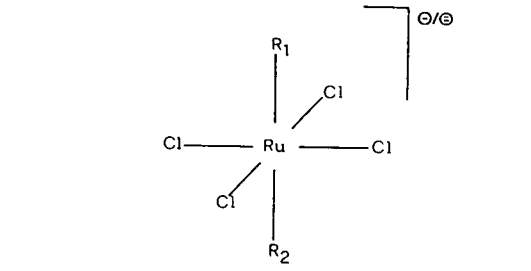


Figure 3. Schematic representation of the preferential toxicity of ruthenium(III) ions for tumor cells.



2 SERIES { A) $R_1=R_2$= HETEROCYCLE LIGAND*
B) R_1= HETEROCYCLE LIGAND*; R_2= Cl

*: IMIDAZOLE, PYRAZOLE, INDAZOLE, 1-METHYLIMIDAZOLE, BENZIMIDAZOLE, CHINOLINE, 2-AMINOTHIAZOLE, 1,2,4-TRIAZOLE.

Figure 4. Chemical structure of heterocycle ruthenium(III) complexes.

necessarily reflect the above consideration of the role played by redox potential in selectively activating ruthenium(III) prodrugs to the more reactive ruthenium(II) forms in tumor tissues. In order to establish their real effectiveness, a solid growing tumor could be a model more suitable for ascertaining the selectivity of antitumor activity of pentaammineruthenium complexes.

Heterocycle–ruthenium(III) complexes

Among "ruthenium heterocycles", a series of compounds synthesized and studied by Keppler *et al.* at Heidelberg (Germany) and characterized by the presence of one or two heterocyclic ligands are included (Figure 4).[11,35–39] Chronologically these compounds belong to the most recent group of ruthenium complexes to appear in the literature.

These complexes are derivatives of ruthenium(III) and are characterized by (a) the presence of heterocyclic ligands such as imidazole, pyrazole and indazole or their methyl-substituted derivatives,[36,38,39] (b) a generally good hydrosolubility, and (c) an ionic structure with one or two negative charges. These compounds are also characterized by the presence of the heterocyclic ligands in the *trans* position, and are symmetrical.

Compared with ammineruthenium derivatives, the antitumor potential of these complexes appears to be better investigated. Overall, it can be stressed that the tetrachloro derivatives with two heterocyclic ligands are more active than the corresponding pentachloro analogs with one heterocycle.[38,39] According to the potential predictivity given by Kepper *et al.* to the model of spontaneous colorectal tumors, the bis(indazole) derivative appears to be the most active compound yet studied (Figure 5).[11]

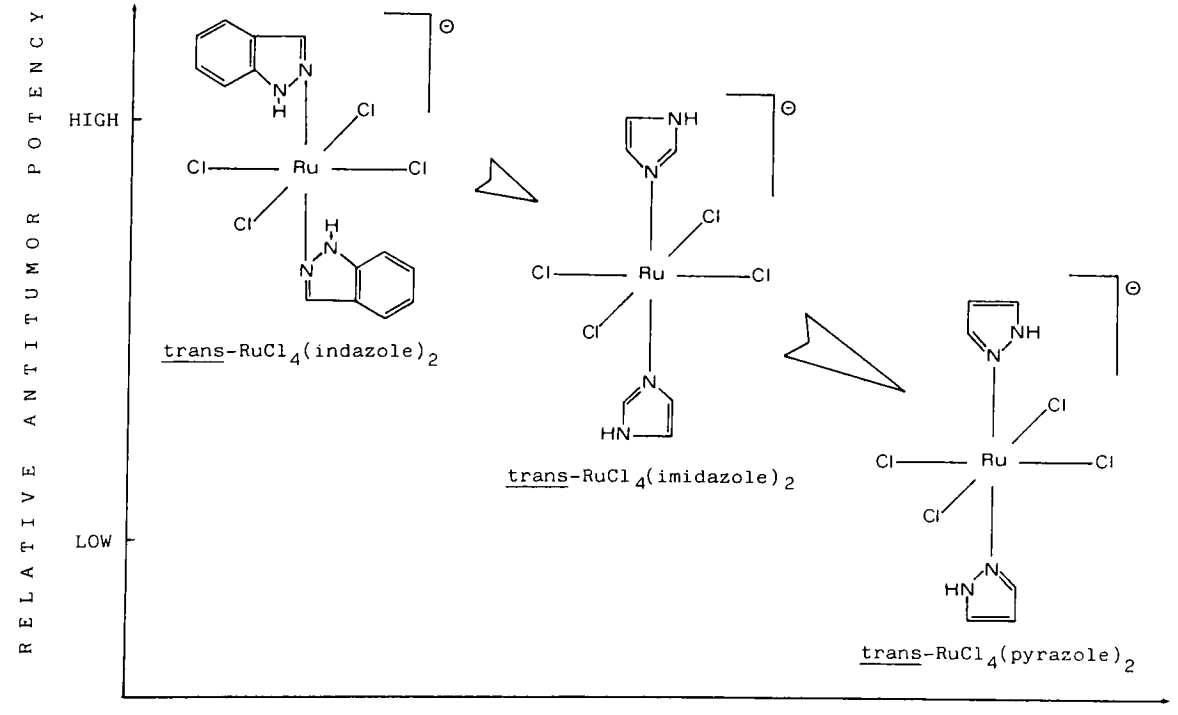


Figure 5. Grading of antitumor "potency" of bis-heterocycle-coordinated ruthenium(III) complexes.

Therefore, ruthenium heterocycle complexes could be indicated for treating colorectal tumors for which cisplatin and drug chemotherapy in general are completely ineffective.

On screening tumors, such as P388 lymphocytic leukemia or B16 melanoma, the complex imidazolium bis(imidazole)tetrachlororuthenate(III) is, at maximum tolerated doses, as active as medium dosages of conventional cytotoxic drugs such as cyclophosphamide, cisplatin and 5-fluorouracil.[35,39]

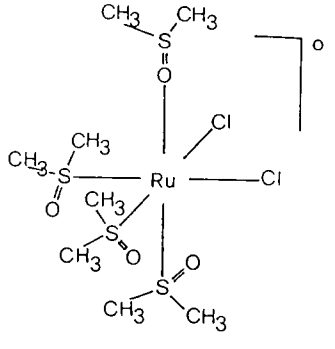
Indeed, some aspects of toxicity and antitumor activity are not yet fully elucidated. Renal toxicity is rather pronounced as in cisplatin-treated hosts, and is characterized by necrosis, evident also on liver cell parenchyma.[11] Interestingly, the 4-fold increase of the volume of administration significantly reduces the lethality of the given dose.[11] Presumably this effect can be attributed to a reduced renal damage due to increased urinary flux. However, the antitumor activity in these conditions has not yet been proven.

The pronounced antitumor activity observed on colorectal tumors seems to indicate a particular propensity of these ruthenium complexes to reach the colorectal region.[11,37] In favor of this hypothesis, data on the tumor distribution of ruthenium trichloride and nitrosyl complexes show that, after oral administration, ruthenium appears to be rapidly adsorbed through the intestine (for a detailed review of data see Ref. 9); furthermore, investigations on ruthenium bound to DNA seem to indicate a slow but preferential deposition of ruthenium ions on intestinal mucosa (unpublished results). However, the histological analysis of intestinal mucosa, following treatment with ruthenium imidazoles or indazoles, indicated no toxicity at this level.[11] It could be hypothesized that this situation represents the theory presented by Clarke on the role of the redox potential of tumor tissue but not of normal intestinal mucosa in activating ruthenium(III) compounds to the more toxic ruthenium(II) derivatives.

This explanation should be confirmed by a comparative histological analysis of colorectal tumor cells and of normal intestinal mucosa, possibly carried out in the same animal. It would also be interesting to evaluate the biological characteristics of asymmetric ruthenium(III) mixed-heterocycle complexes (for example the complex imidazolium- (or indazolium-) [(indazole-imidazole)tetrachlororuthenate]) which include the characteristics of the compounds found to be more active in the test systems examined.

Dimethylsulfoxide–ruthenium(II) complexes

To this class of compounds belong a small series of ruthenium(II) complexes whose parent compounds are *cis-* and *trans*-rutheniumdichlorotetrakisdimethylsulfoxide ($RuCl_2(DMSO)_4$).[12,19] These complexes differ from the previously discussed compounds in that they are not influenced by the redox potential of the environment (Clarke's hypothesis), and are rather stable in their +2 oxidation state. Nevertheless, they exhibit different toxicities comparable with those shown by heterocycle complexes: marked histopathological alterations at the renal level, a lesser degree of damage on intestinal mucosa and spleen[40] (not resulting from DNA synthesis evaluation[41]) while having no effect on erythrocyte, leukocyte and platelet counts.[41] The former complex studied, namely *cis*-$RuCl_2(DMSO)_4$, was initially chosen because of its similarity with cisplatin. Although having an octahedral structure, it is neutral, is very stable in the +2 oxidation state, has two chloride ligands in a planar *cis* position, has a pronounced affinity for nitrogen donor ligands and is rather freely soluble in aqueous solutions (Figure 6). This complex has been extensively studied in a number of biological systems and on experimental tumors in particular. Similarly to cisplatin, *cis*-$RuCl_2(DMSO)_4$ induces lambda profage, produces filamentous growth in *E. coli* and is selectively toxic



cis-Dichlorotetrakisdimethylsulfoxideruthenium (II)

Characteristics:

- octahedral structure
- neutrality
- stability at +2 oxidation state
- 2 *cis*-chloride ligands
- high affinity for nitrogen-donor ligands
- elevated water solubility

Figure 6. Chemical structure, denomination and characteristics of *cis*-$RuCl_2(DMSO)_4$.

Table 3. Spectrum of the antineoplastic activity of *cis*-$RuCl_2(DMSO)_4$

Tumor system	Antineoplastic action
L1210 lymphoid leukemia	Mild increase of host life-span
P388 lymphocytic leukemia	Inactive[a] or moderately active[b]
Ehrlich ascites carcinoma	Mild to marked inhibition of tumor growth
Lewis lung carcinoma, B16 melanoma, MCa mammary carcinoma	Inhibition of primary tumor growth and of lung metastasis formation; increase of the post-treatment survival time

[a] Data from NCI screening.
[b] M. Coluccia, personal communication.

for a strain defective in deoxyribonucleic acid repairing systems.[28,42] Correspondingly, it was active in a number of animal tumors (Table 3). Apart from some controversial effects observed in ascitic (leukemic type) tumors,[40] the antitumor activity of *cis*-$RuCl_2(DMSO)_4$ is better expressed on a panel of solid tumors: Lewis lung carcinoma, B16 melanoma and the MCa mammary carcinoma of CBA mouse.[41,43] It does seem that solid metastasizing tumors are a better target than tumors of the lymphoproliferative type. This consideration is valid also for the *trans* analog: *trans*-$RuCl_2(DMSO)_4$. This compound behaved quite differently from the *cis* isomer. Its host toxicity is 10–20-fold higher than that of the *cis* isomer, and this effect is paralleled by a concomitantly higher affinity for DNA binding[12,44] (Table 4). *trans*-$RuCl_2(DMSO)_4$ appears more easy to handle than the *cis* isomer; it has approximately the same water solubility but is administered at repeated optimal doses about 20-fold lower than the 700 (mg/kg)/day or even greater necessary for *cis*-$RuCl_2(DMSO)_4$.[12,19,44]

The chemical aspects pertaining to biological interactions stress the higher toxicity of *trans*- compared with *cis*-ruthenium complexes.[12] The former rapidly replaces two adjacent equatorial DMSO ligands with molecules of water, immediately acquiring two reactive sites but still remaining a neutral species. In contrast *cis*-$RuCl_2(DMSO)_4$ has the possibility of immediately replacing the O-linked DMSO ligand only, therefore acquiring one reactive site. The second phase of substitution is similar for both complexes, depends on Cl^- concentration and gives rise to a charged species with all the possible consequent implications for its mobility across cell membranes (Figure 7). However, to the latter species is ascribed the higher affinity binding to DNA, studied with *in vitro* calf thymus DNA[12,18,19] or by means of restriction enzyme tests (M. Coluccia, Bari, personal communication).

The examination of the differential effects of *cis*- and *trans*-$RuCl_2(DMSO)_4$ on primary tumor and on metastasis development has revealed antimetastatic activities superior to the effects on primary tumor growth.[12,44,45] The comparison between the antitumor effect of *cis*- and *trans*-$RuCl_2(DMSO)_4$ indicates the superiority of the latter, whose antimetastatic activity is comparable with or even greater than that of cisplatin.[44] Replacement of Cl with other halides such as bromide or iodide does

Table 4. Comparison of the biological activities of *cis*- and *trans*-$RuCl_2(DMSO)_4$

Biological activity	*cis*-$RuCl_2(DMSO)_4$	*trans*-$RuCl_2(DMSO)_4$
Toxicity (LD_{50} in mice)	+	+ + + +
Effects on bacteria:		
mutagenicity (Ames test)	+ + +	+
cytotoxicity	+	+ + +
DNA binding:		
in vitro with calf thymus DNA	+ +	+ + + +
in vitro with V79 cells	+ +	+ + + +
Antitumor activity:		
on lung metastasis formation	+ + +	+ + + +
on survival time	+ + +	+ + + +

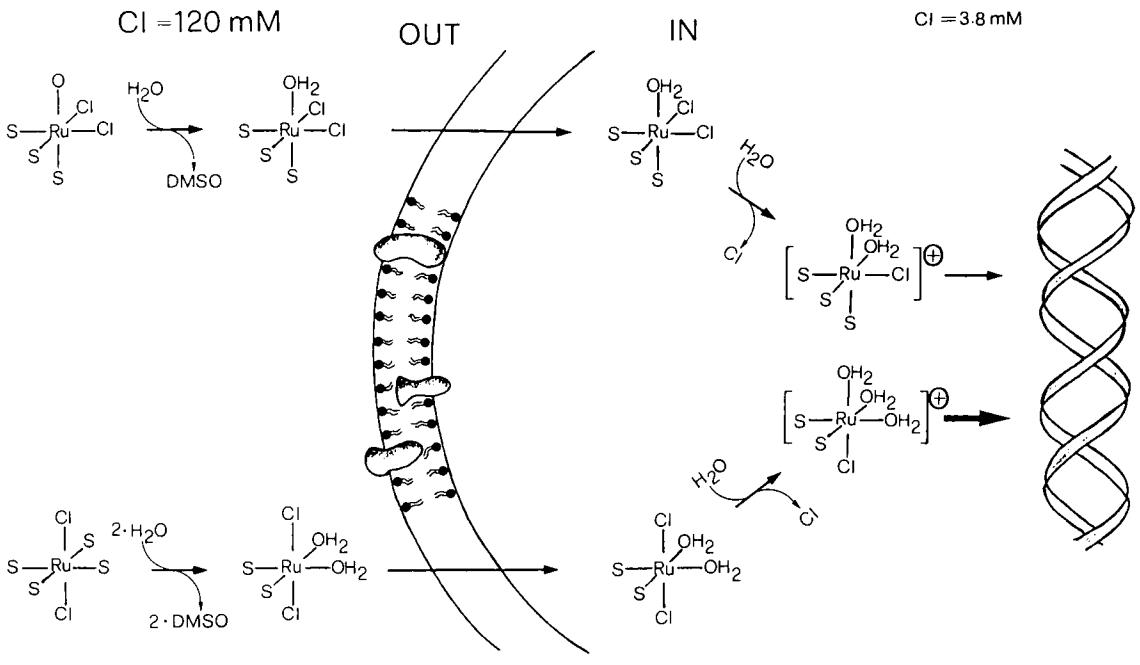


Figure 7. Activation of *cis*- and *trans*-$RuCl_2(DMSO)_4$ across membranes and binding to DNA. O, O-bonded DMSO; S, S-bonded DMSO.

not increase the overall effect. For example, *trans*-$RuI_2(DMSO)_4$ is more active on primary tumor growth but loses its antimetastatic effects.[45] The antimetastatic effects of *trans*-$RuCl_2(DMSO)_4$ can be ascribed to a selective lung deposition of ruthenium ions and/or to a higher vulnerability of tumor cells in the lungs: 24 h after treatment, the concentration of ruthenium bound to DNA of lung tissue is higher than in subcutaneous tumor tissue (unpublished results). However, the antitumor activity of *trans*-ruthenium seems not to be simply attributable to a direct cytotoxicity for tumor cells. It is possible that a role can be played by the integrity of host immune reactivity whose alteration hampers most of the antitumor effects of *trans*-$RuCl_2(DMSO)_4$.[46] This observation, at least partially, explains the low toxicity of *trans*-$RuCl_2(DMSO)_4$ in *in vitro* tests. It can be hypothesized that non-lethal DNA interactions in tumor cells can *in vivo* lead to alterations of the antigenicity of tumor cells which become more susceptible to being recognized by host defence mechanisms.

Conclusions

The available data indicate the potential usefulness of ruthenium complexes for the control of cancer growth. It appears that the number of complexes studied should be enlarged, mainly by studying the possibility of better selective delivery of the active drug to the tumor tissue. Nevertheless, it also appears that ruthenium ions can exhibit antitumor effects different from those exhibited by cisplatin. On the basis of the few compounds so far studied, it appears that colon tumors and lung tumors could be two preferential targets for these drugs which will help in treating cancers rather resistant to chemotherapy.

One can come to this conclusion either on the basis of the response of the experimental tumors tested or even by considering the characteristics of ruthenium distribution in the body. Although fascinating, the hypothesis proposed by Clarke on the selective activation of ruthenium(II) toxic complexes from ruthenium(III) inert prodrugs is far from being fully elucidated. It is conceivable, however, that, if proven it could be of help in preparing new compounds which will be delivered to the tumor tissue at the expense of a reduced or even absent host toxicity.

With regard to this, it would be interesting to explore the group of ruthenium(III) complexes with dimethylsulfoxide ligands. The relatively high antitumor activity of the corresponding analogs of ruthenium(II), reported in the present paper, suggests that compounds could be achieved that

will have a reduced host toxicity without any loss of antitumor activity.

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