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Effects of the Ru(III) complexes [*mer*-$RuCl_3(DMSO)_2Im]^\circ$ and Na[*trans*-$RuCl_4(DMSO)Im$] on solid mouse tumors

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The effects of two new Ru(III) complexes, [*mer*-$RuCl_3(DMSO)_2Im]^\circ$ and Na[*trans*-$RuCl_4(DMSO)Im$], were investigated on primary tumor growth and on the survival time using three solid metastasizing tumors of the mouse: Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma. Na[*trans*-$RuCl_4(DMSO)Im$] appears to be the most promising compound, in that: (1) it is soluble in water and therefore easy to handle in comparison with the neutral species [*mer*-$RuCl_3(DMSO)_2Im]^\circ$ or to the already described BBR2382; (2) similarly to cisplatin, though at a lower level, it reduces tumor growth in its primary site in each tumor model employed; (3) unlike cisplatin, it increases the life span of tumor-bearing hosts in all tumors used, independently of the effects on primary tumor growth; and (4) it is also effective in reducing spontaneous metastasis formation when the effects on primary tumor growth are completely absent. Dimethylsulfoxide (DMSO), used for solubilizing poorly water-soluble compounds (i.e. [*mer*-$RuCl_3(DMSO)_2Im]^\circ$) or for stabilizing the compound in the solution before injection (i.e. Na[*trans*-$RuCl_4(DMSO)Im$]), reduces the anti-tumor potency. Conversely, the anti-tumor effects of Na[*trans*-$RuCl_4(DMSO)Im$] are more pronounced in mice hydrated with isotonic saline. We conclude that Na[*trans*-$RuCl_4(DMSO)Im$] is a good candidate for further investigations aimed at ascertaining the mechanism of the anti-metastatic activity and of the positive effects on survival time of mice bearing solid metastasizing tumors.

Key words: Anti-metastatic, anti-tumor, ruthenium complexes, solid mouse tumors.

Introduction

The most recent advances in the anti-neoplastic properties of metal ions and metal complexes have pointed out the role played by metal-based ruthenium complexes.[1,2] Experimental evidence showed that several of the ruthenium compounds studied for anti-tumor activity can interact with DNA *in vitro* and also in *in vivo* models, as expected for anti-tumor cytostatics.[3–8] Furthermore, DNA interaction of ruthenium compounds makes some of them effective, in a similar way to *cis*-dichlorodiammine-Pt(II) (cisplatin), in spite of their octahedral structure.[8,9] $RuCl_3.H_2O$, but also some ruthenium complexes, accumulate in tumors where they can be delivered and concentrated by transferrin more efficiently than in normal tissues such as blood, muscle and liver.[10–12]

The fact that ruthenium-based compounds can represent a tool for investigating anti-cancer drugs of a new generation should not hide the fact that the chemical–biological interactions of these substances are still only poorly known. In our laboratory, we have investigated several aspects of the nature of the anti-neoplastic action of some Ru(II) derivatives, characterized by the presence of dimethylsulphoxide (DMSO) ligands, for many years.[13–16] More recently, Keppler *et al.* have shown anti-tumor properties in a new series of Ru(III) derivatives containing heterocycle ligands, such as $ImH[RuCl_4Im_2]$,[17,18] with particular activity in a model of spontaneous colo-rectal tumor in carcinogen-treated rats.[17,19] Based on these data, we have undertaken a program for studying a new series of Ru(III) complexes, still characterized by the presence of DMSO ligands, of the type Na[*trans*-$RuCl_4(DMSO)L$] or [*mer*-$RuCl_3(DMSO)_2L]^-$, where L = nitrogen donor ligand (Italian Patent 20385 A/89). One of these complexes (i.e. BBR2382; L = NH_3) turned out to be particularly effective in the Lewis lung carcinoma model,[20] being at least as active as cisplatin but superior to the

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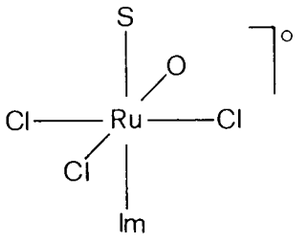
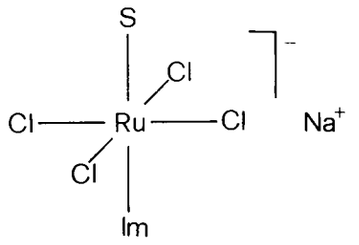


mer-trichlorobis(dimethylsulphoxide)imidazoleruthenium(III)
[*mer*-$RuCl_3(DMSO)_2Im$]°



Sodium *trans*-dimethylsulphoxideimidazoletetrachlororuthenate(III)
Na[*trans*-$RuCl_4(DMSO)Im$]

Figure 1. Chemical structure, denomination and abbreviations used for the ruthenium compounds tested. S = sulphur-bonded DMSO; O = oxygen-bonded DMSO.

reference Ru(III) complex $ImH[RuCl_4Im_2]$. See Figure 1.

In the present investigation we present new data on this series of Ru(III) complexes, by studying the effects on primary tumor growth and on the survival time of [*mer*-$RuCl_3(DMSO)_2Im$]° and of Na[*trans*-$RuCl_4(DMSO)Im$] (Im = imidazole) using three solid metastasizing tumors of the mouse: Lewis lung carcinoma, B16 melanoma and MCa mammary carcinoma of the CBA mouse. Some aspects related to the role of the presence of DMSO in the vehicle used for injections, as well as the influence of the expansion of host's extracellular volume on the anti-tumor effects of the two compounds, have also been studied.

Materials and methods

Compounds

[*mer*-$RuCl_3(DMSO)_2Im$]° and Na[*trans*-$RuCl_4(DMSO)Im$] were prepared, purified and characterized according to procedures described in detail elsewhere.[21] Cisplatin was kindly supplied by the Drug Synthesis and Chemistry Branch, Division of Cancer treatment, NCI, NIH, Bethesda, MD. Na[*trans*-$RuCl_4(DMSO)Im$] and cisplatin were administered in solutions of 0.9% NaCl, in volumes of 0.1 ml/10 g body weight (0.05 ml for i.v. injections); [*mer*-$RuCl_3(DMSO)_2Im$]° was given either as a suspension, made by sonication in the cold for 5 s, in double distilled water or dissolved in DMSO, then diluted with 0.9% NaCl to the desired concentration, and administered in volumes of 0.1 ml/10 g body weight.

Tumor lines

The Lewis lung carcinoma and B16 melanoma lines used, originally obtained from the Tumor Repository Bank, NCI, NIH, Bethesda, MD, were locally maintained in C57B1/6 mice by serial biweekly passages according to NCI protocols.[22] For experimental purposes, $BD2F_1$ female mice of 20 ± 1 g purchased from Charles River, Calco, Como, Italy, were inoculated s.c. in the flank with 50 mm^3 tumor fragments obtained from donors similarly inoculated 2 weeks before. MCa mammary carcinoma of CBA mouse was obtained from Rudjer Boskovich Institute, Zagreb, Yugoslavia, and was maintained as described for Lewis lung carcinoma, by biweekly passages of 10^6 viable tumor cells into the calf of the left hind leg of CBA inbred female mice of 20 ± 2 g obtained from a locally established breeding colony. Tumor propagation for experimental purposes was similarly made using female mice 6–8 weeks old.

Primary tumor and lung metastasis evaluation

Primary tumors were measured using a caliper and their weight estimated by the following formula: $(\pi/6) \times a^2 \times b$ where a and b are two perpendicular axis ($a < b$) and assuming tumor density equal to 1. The evaluation of the number and weight of lung metastases, spontaneously formed from the i.m. administration of MCa mammary carcinoma, was performed on day 26 from tumor implantation, after killing of the animals by cervical dislocation. The number of lung colonies on the surface of the freshly removed lungs was counted by means of a low-power stereo microscope equipped with a calibrated grid. The weight of the metastatic tumor per mouse was calculated by determining the volume of each metastatic nodule by the formula of primary tumors reported above.

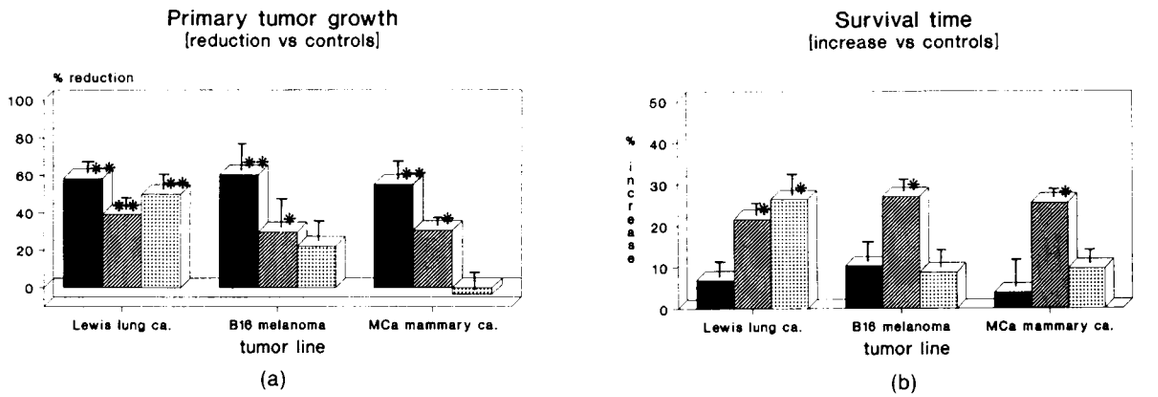


Figure 2. Effects on (a) primary tumor growth and (b) survival time of [*mer*-$RuCl_3(DMSO)_2Im$] (anionic-Ru(III); ▦), Na[*trans*-$RuCl_4(DMSO)Im$] (ionic-Ru(III); ▨) and cisplatin (■) in mice bearing solid metastasizing tumors. Groups of 10–13 mice, inoculated s.c. with the relevant tumors on day 0, were given i.p. the test compounds on days 1, 5, 9 and 13; [*mer*-$RuCl_3(DMSO)_2Im$] was dissolved in 0.9% NaCl containing 30% DMSO. $^*p < 0.05$ and $^{**}p < 0.01$ versus controls.

Statistical analysis

Experimental data were subjected to computerized statistical analysis with the Student–Newmann–Keuls test.

Results

The effects of Na[*trans*-$RuCl_4(DMSO)Im$] and [*mer*-$RuCl_3(DMSO)_2Im$] compared with that of cisplatin on primary tumor growth and on the survival time of mice bearing s.c. implants of Lewis lung carcinoma, B16 melanoma or MCa mammary carcinoma are reported in Figure 2. The dosages used for each compound are the maximum tolerated doses and have been chosen so as to give similar host toxicity in terms of loss of body weight gain at the end of treatment (reduction by 8–10% versus untreated tumor bearing controls). On primary tumor growth (Figure 2a), Na[*trans*-$RuCl_4(DMSO)Im$] caused a similar and statistically significant reduction, smaller than that of cisplatin, with each tumor model used. The effects of [*mer*-$RuCl_3(DMSO)_2Im$] decreased from a marked inhibition on Lewis lung carcinoma (inhibition by 60%, $p < 0.01$) to a statistically, not significant, mild reduction on B16 melanoma (inhibition by 22%) to a complete inactivity on MCa mammary carcinoma. With regard to survival time, Na[*trans*-

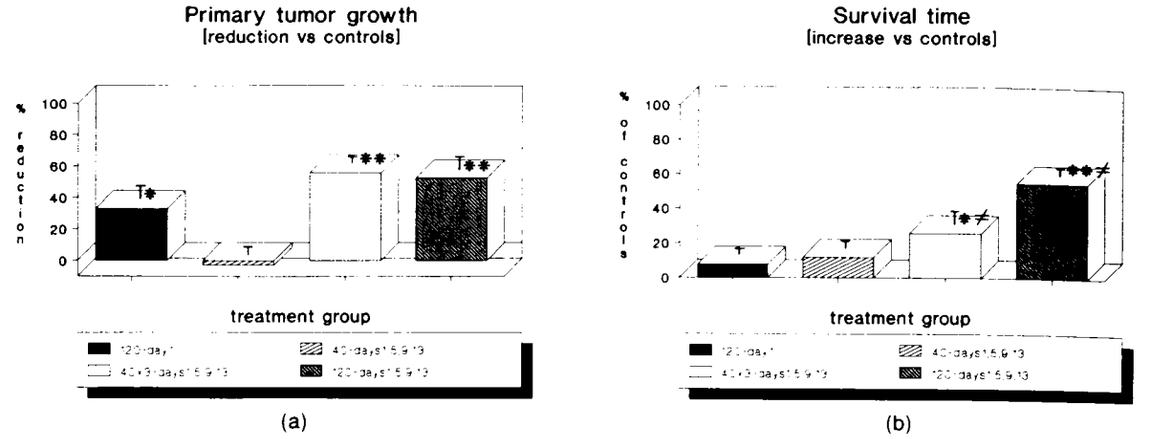


Figure 3. Effects of different schedules of administration of [*mer*-$RuCl_3(DMSO)_2Im$] suspended in double-distilled water on (a) primary tumor growth and (b) survival time of mice bearing Lewis lung carcinoma. Groups of 10 mice, inoculated s.c. with Lewis lung carcinoma on day 0, were given i.p. [*mer*-$RuCl_3(DMSO)_2Im$] daily on days 1, 5, 9 and 13 at the doses of 40, 40 × 3 (with 1 h intervals between two adjacent administrations) and 120 mg/kg/day or with 120 mg/kg on day 1 only. $^*p < 0.05$ and $^{**}p < 0.01$ versus untreated controls; groups marked with ≠ are statistically different, $p < 0.05$.

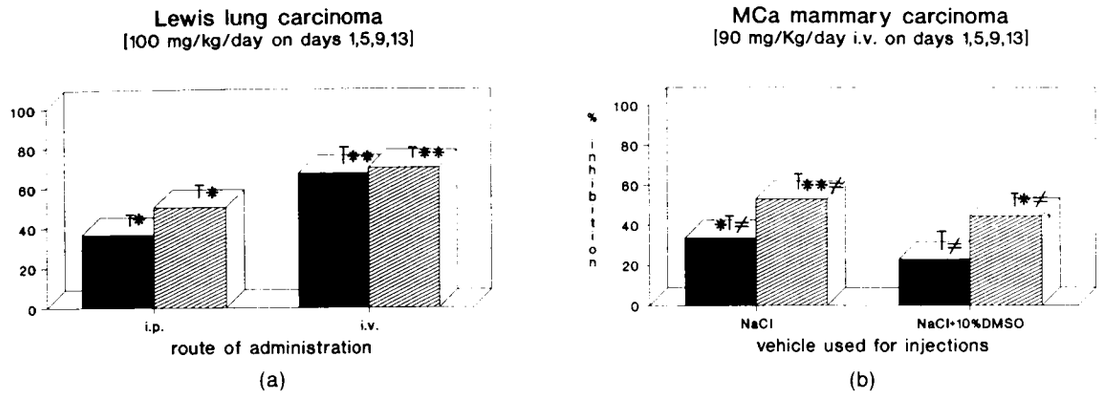


Figure 4. Effects of hydration (■ = without hydration; □ = with hydration), route of administration and of DMSO on the anti-tumor action of Na[*trans*-$RuCl_4$(DMSO)Im] in mice bearing MCa mammary carcinoma or Lewis lung carcinoma. Groups of eight mice, inoculated s.c. with Lewis lung carcinoma (or i.m. with MCa mammary carcinoma) on day 0, were given i.p. or i.v. Na[*trans*-$RuCl_4$(DMSO)Im], dissolved in 0.9% NaCl alone or containing 10% DMSO, on days 1, 5, 9 and 13. Hydration was performed by injecting i.p. 2.0 ml/animal 0.9% NaCl at 1 and 24 h after each treatment. $^*p < 0.05$ and $^{**}p < 0.01$ versus controls; groups marked with $\neq$ are statistically different, $p < 0.05$.

$RuCl_4$(DMSO)Im] was statistically effective in all tumor models used; [*mer*-$RuCl_3(DMSO)_2Im]^\circ$ was effective only on Lewis lung carcinoma, and cisplatin was always devoid of effects (Figure 2b).

The anti-tumor effectiveness of [*mer*-$RuCl_3(DMSO)_2Im]^\circ$ was further studied using different schedules of administration with the compound administered as a suspension in double distilled water (Figure 3). The effects on primary tumor growth (Figure 3a) were statistically more pronounced after treatment on days 1, 5, 9 and 13 with 120 mg/kg/day given in bolus (inhibition by 52.6%, $p < 0.01$) or fractionated (inhibition by 56%, $p < 0.01$) than with a single dose on day 1 (inhibition by 33%, $p < 0.05$) or with the treatment on days 1, 5, 9 and 13 with 40 mg/kg/day (inactive). On survival time, the effects of 120 mg/kg on days 1, 5, 9 and 13 (in bolus or fractionated) were statistically significant, treatment in bolus being statistically more effective than fractionation into three administrations ($p < 0.05$).

The role of DMSO and of the *in vivo* dilution of the compound was further studied by examining the anti-tumor effects of Na[*trans*-$RuCl_4$(DMSO)Im], administered i.p. or i.v. on days 1, 5, 9 and 13 at the daily doses of 90–100 mg/kg, dissolved

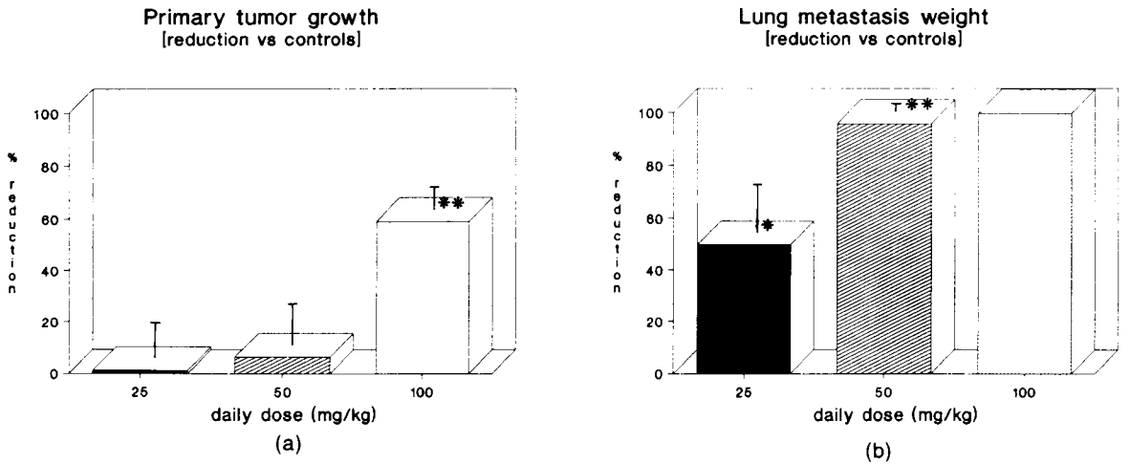


Figure 5. Effects of different doses of Na[*trans*-$RuCl_4$(DMSO)Im] on primary tumor growth and on spontaneous metastasis formation in mice bearing MCa mammary carcinoma. Groups of five mice, inoculated i.m. with MCa mammary carcinoma on day 0, were given Na[*trans*-$RuCl_4$(DMSO)Im] i.p. daily on days 1, 5, 9 and 13. Lung metastases were counted on freshly removed lungs, after animal killing by cervical dislocation, on day 26. $^*p < 0.05$ and $^{**}p < 0.01$ versus controls.

in 0.9% NaCl or in 0.9% NaCl containing 10% DMSO, in mice bearing Lewis lung carcinoma or MCa mammary carcinoma and hydrated with 0.9% NaCl (Figure 4). Hydration, performed by injecting i.p. 2 ml/mouse 0.9% NaCl at 1 and 24 h after each treatment with the ruthenium complex, expanded the extracellular volume. There was a significant increase of urinary volume in the following 4 h (0.20 versus 0.09 ml/h in non-hydrated controls), with a peak of 0.4 ml/h between 3 and 4 h, obtained from separate studies performed using metabolic cages. On Lewis lung carcinoma, the i.v. administration of 100 mg/kg/day of Na[*trans*-$RuCl_4$(DMSO)Im] was statistically more effective than the corresponding dose given by i.p. route. No significant difference was recorded between hydrated and non-hydrated mice.

The i.v. treatment with 100 mg/kg/day, however, proved particularly toxic, giving an average loss of body weight gain at the end of treatment of about 21-25% with no appreciable increase of survival time. A similar toxicity was observed on MCa mammary carcinoma: the i.v. administration of 100 mg/kg/day on days 1, 5, 9 and 13, either given in 0.9% NaCl or even buffered with 0.1M phosphate buffer (pH 7.52 versus 5.5), proved lethal for tumor bearing hosts. Interestingly, the better tolerated i.v. doses of 25 and 50 mg/kg/day, though devoid of significant effects on primary tumor growth, gave significant prolongations of hosts life span by 37.7 and 29.4%, respectively. The effect of i.v. treatment with Na[*trans*-$RuCl_4$(DMSO)Im] on MCa mammary carcinoma, reported in Figure 4, was therefore studied at the daily dose of 90 mg/kg/day, a dose which gave an host toxicity comparable with that of i.p. treatments (reduction of body weight gain by 13%). In these conditions, Na[*trans*-$RuCl_4$(DMSO)Im], given in 0.9% NaCl, was more effective as compared with the same dosage given in 0.9% NaCl containing 10% DMSO.

The anti-tumor effects of Na[*trans*-$RuCl_4$(DMSO)Im] given i.p. to mice bearing i.m. implants effective as compared with the same dosage given in 0.9% NaCl containing 10% DMSO.

The anti-tumor effects of Na[*trans*-$RuCl_4$(DMSO)Im] given i.p. to mice bearing i.m. implants of MCa mammary carcinoma depended on the daily dose used and are statistically significant only at 100 mg kg day (Figure 5a). Conversely, the reduction of lung metastasis formation in the same animals was also much greater and statistically significant at the lower dosages used (Figure 5b), although the number of animals with macroscopically countable lung colonies at sacrifice was lower than for controls only in the group treated with the maximum tolerated dose (1/5 versus 3/5).

Discussion

The examination of some of the anti-neoplastic properties of two new Ru(III) complexes, characterized by the presence of DMSO and imidazole ligands, widens our knowledge of this class of ruthenium containing compounds. The anti-neoplastic effects of the neutral compound [*mer*-$RuCl_3(DMSO)_2Im]^0$, which differs from the already studied BBR2382[20] by the presence of an imidazole moiety in place of the NH_3 group, are generally lower than those of the ionic compound Na[*trans*-$RuCl_4$(DMSO)Im]. Compound $[mer\text{-}RuCl_3(DMSO)_2Im]^0$, which proved active only in the Lewis lung carcinoma model, gave the same problems of administration encountered for BBR2382, i.e. rather poor solubility in aqueous solutions. Unexpectedly, the anti-tumor activity is greater when the compound is given as a suspension in double distilled water than when it is dissolved in a DMSO–water vehicle. It is again surprising that the fractionated administration of the water suspension of this compound, which allowed the use of a 3 times larger volume of injection and therefore the dissolution of a larger amount of compound, did not show any increase of toxicity (measured by body weight reduction at the end of treatment, 7.1 versus 11.1%, respectively) or of anti-tumor activity. However, the complete solubilization by a DMSO-water vehicle, though devoid of significant modifications on host toxicity, decreased the anti-tumor effects: besides the lower effect on survival time in mice bearing Lewis lung carcinoma (55.6 versus 26.3%); the reduction of primary tumor growth on MCa mammary carcinoma by the suspension in water was also higher (inhibition by 42.1% versus inactivity).

Indeed, the presence of even smaller amounts of DMSO in the solution for treatments also reduces the anti-tumor activity of Na[*trans*-$RuCl_4$(DMSO)Im], as already stated for BBR2382.[20] In practice, this procedure, which should avoid substitution reactions in the molecule, possible by using other solvents (e.g. dimethylformamide) should stabilize the molecule in the solution prior to injection.

Interestingly, Na[*trans*-$RuCl_4$(DMSO)Im] is even more effective when used in combination with the expansion of extracellular host volume. This observation is consistent with that of Keppler *et*

al.[17] who found a significantly better tolerability of their ruthenium complexes when administered in volumes of 8 ml instead of 2 ml per animal, and indicates that an *in vivo* dilution and/or a faster renal elimination can render anti-tumor treatment with this compound more efficient.

Among the compounds so far studied, including the reference doses of cisplatin, only the derivative Na[*trans*-$RuCl_4$(DMSO)Im] exhibited an interesting relationship between inhibition of primary tumor growth and increase of survival time of the treated hosts, with a global efficacy independent of the tumor line being used. In this context, it must be emphasized that cisplatin, even when capable of reducing i.m. tumor growth of MCa mammary carcinoma by more than 90% (from the results of a separate experiment designed to provide primary tumors at 2 weeks of less than 0.5 g) was practically inactive on host survival time (T/C = 121 ± 20%).

Data on the effects of Na[*trans*-$RuCl_4$(DMSO)Im] on survival time are consistent with a significant anti-metastatic action, in addition to the anti-tumor effect on primary tumor growth. Nevertheless, it is not clear whether this latter effect is the result of a higher concentration of the compound in the lung tissue or, alternatively, whether Na[*trans*-$RuCl_4$(DMSO)Im] behaves as already shown with BBR2382[20] and with a Ru(II) complex,[14] involving host resistance against tumor and particularly metastatic growth.

Conclusions

Of the recently synthesized Ru(III) complexes characterized by the presence of DMSO and nitrogen donor ligands, Na[*trans*-$RuCl_4$(DMSO)Im] has some advantages such as water solubility and the same activity on all parameters of tumor growth studied, including the prolongation of host survival time, independently of the tumor line used. Thus, Na[*trans*-$RuCl_4$(DMSO)Im] could represent a standard, within this group of compounds, for the development of analogs endowed with better directed cytotoxicity and tumor targeting. In particular, the anti-metastatic activity of Na[*trans*-$RuCl_4$(DMSO)Im] and its effects on survival time, always better than those of cisplatin, shed new light in the activity of this class of compounds on solid metastasizing tumors. This opens the possibility of investigating new approaches in the treatment of solid tumor metastases.

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