Journal of Inorganic Biochemistry 104 (2010) 79–86

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

In vivo tumour and metastasis reduction and in vitro effects on invasion assays of the ruthenium RM175 and osmium AFAP51 organometallics in the mammary cancer model

A. Bergamo [a,*], A. Masi [a], A.F.A. Peacock [b], A. Habtemariam [c], P.J. Sadler [c], G. Sava [a,d]

[a] Callerio Foundation Onlus, Via A. Fleming 22-31, 34127 Trieste, Italy
[b] School of Chemistry, University of Edinburgh, West Mains Road, Edinburgh EH9 3JJ, UK
[c] Department of Chemistry, University of Warwick, Gibbet Hill Road, CV4 7AL, UK
[d] Department of Life Sciences, University of Trieste, Via L. Giorgieri 7, Trieste, Italy

ARTICLE INFO

Article history:
Received 8 May 2009
Received in revised form 21 September 2009
Accepted 7 October 2009
Available online 14 October 2009

Keywords:
Metastasis
Invasion
Mammary cancer
Ruthenium
Osmium
Organometallic

ABSTRACT

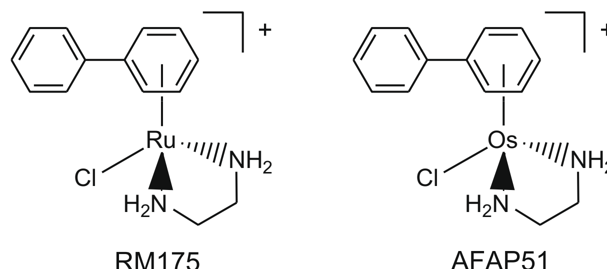
We have compared the organometallic arene complexes [(η^6-biphenyl)M(ethylenediamine)Cl]$^+$ RM175 (M = Ru^{II}) and its isostructural osmium(II) analogue AFAP51 (M = Os^{II}) for their ability to induce cell detachment resistance from fibronectin, collagen IV and poly-L-lysine, and cell re-adhesion after treatment, their effects on cell migration and cell viability, on matrix metalloproteinases production, and on primary tumour growth of MCa mammary carcinoma, the effect of human serum albumin on their cytotoxicity. There are differences between ruthenium and osmium. The Os complex is up to 6× more potent than RM175 towards highly-invasive breast MDA-MB-231, human breast MCF-7 and human epithelial HBL-100 cancer cells, but whereas RM175 was active against MCa mammary carcinoma in vivo and caused metastasis reduction, AFAP51 was not. Intriguingly the presence of human serum albumin in the growth medium enhanced the cytotoxicity of both compounds. RM175 increased the resistance of MDA-MB-231 cells to detachment from substrates and both compounds inhibited the production of MMP-2. These data confirm the key role of ruthenium itself in anti-metastatic activity. It will be interesting to explore the activity of osmium arene complexes in other tumour models and the possibility of changing the non-arene ligands to tune the anticancer activity of osmium in vivo.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Despite the progress in medicine, metastases cause 90% of deaths from solid tumours and display a remarkably diverse set of clinical manifestations. Research on drugs based on metal compounds offers promise in this fight [1,2]. Inorganic chemistry offers wide scope for the design of novel drugs based on the coordination and redox properties of metal ions [3], and the exploration of medicinal applications is driven by the necessity to fill the unmet needs of tumour chemotherapy. In particular these needs include the minimisation of undesirable side-effects, overcoming the resistance problem, enlarging the spectrum of activity to more tumour types and to metastatic (secondary) cancers.

Ruthenium compounds, as an alternative to platinum-based tumour inhibitors, are receiving a great attention [4,5] and two ruthenium(III) compounds have successfully concluded a phase I clinical trial [6,7]. KP1019 [8], known for its activity against cisplatin-resistant colorectal carcinoma and NAMI-A [9] a lead compound for its ability to combat the development of metastasis of solid tumours.



The hypothesis [10] that ruthenium(III) complexes are pro-drugs which are activated by reduction suggests that Ru^{II} may be an important component of the final reactive drug and this has stimulated investigations of the activity of Ru^{II} complexes themselves. Arene ligands stabilise Ru^{II} and various classes of organometallic half-sandwich Ru^{II} compounds have been found to be active both in vitro and in vivo [11–13]. Amongst these is the biphenyl ethy-

* Corresponding author. Tel./fax: +39 040 569933.
E-mail address: a.bergamo@callerio.org (A. Bergamo).

0162-0134/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jinorgbio.2009.10.005

lenediamine chlorido complex RM175. RM175 exhibits in vitro cytotoxic effects similar or greater than those of carboplatin [12], without cross resistance with other platinum drugs, induces in vitro G1 and G2 growth arrest and apoptosis [14], and a significant growth delay of tumours in vivo [12,15].

The aim of the present study was to evaluate the effects of RM175 in an in vitro model of tumour invasion and metastasis. For this purpose we investigated the role of the metal centre by comparing the ruthenium complex RM175 with its osmium analogue AFAP51. In general organometallic half-sandwich Ru^{II} and Os^{II} complexes have similar (often almost identical) structures but differ in their rates of reaction, often ca. 100× slower for Os^{II}, and with an increased acidity of its aqua adducts (by ca. 1.5 p*K*a units) [16,17]. Hydrolysis of the chlorido adducts appears to play a role in intracellular activation. Although isostructural with RM175, the Os^{II} complex AFAP51 hydrolyses more slowly and reacts more slowly with nucleobases [17] although like RM175 it is active towards human lung and ovarian cancer cells in vitro [18]. Interestingly, initial experiments have shown that the Os^{II} complex can induce unwinding of plasmid DNA to a greater extent than either the Ru^{II} analogue or cisplatin but causes little DNA bending [19,20].

Metastatic progression is mimicked in vitro by opportune experiments to study cell detachment from the primary tumour, extracellular matrix degradation, migratory ability, invasion and re-adherence to a substrate, using cell lines of the mammary gland with different degree of aggressiveness: MDA-MB-231, a highly-invasive breast cancer cell line, MCF-7 a tumorigenic but non-invasive cell line, and HBL-100 a non-tumorigenic cell line of the mammary epithelium. The in vitro study is compared with the anti-tumour and anti-metastatic effects of the same compounds in vivo in the mouse model of MCa mammary carcinoma.

2. Materials and methods

2.1. Drugs and reagents

AFAP51, $[(\eta^6\text{-biphenyl})Os(ethylenediamine)Cl]BF_4$, was prepared as described previously [18] by refluxing the chlorido-bridged dimer, $[(\eta^6\text{-biphenyl})OsCl_2]_2$, and ethylenediamine in methanol, followed by the addition of NH_4BF_4 so as to generate the BF_4^- salt. The complex was purified by Soxhlet extraction with dichloromethane, with purity confirmed by both ^1H NMR (>99%) and CHN analysis.

The ruthenium complex RM175 was prepared by a similar route [11,18] starting from the chlorido-bridged dimer $[(\eta^6\text{-biphenyl})RuCl_2]_2$ except as a PF_6^- salt and was purified similarly by Soxhlet extraction. The purity as determined by ^1H NMR spectroscopy was ca 99%, and the CHN elemental analysis again showed excellent agreement between the calculated and experimentally-determined values.

All reagents were purchased from Sigma–Aldrich (St. Louis, MO) unless otherwise indicated.

2.2. Tumour cell lines for in vitro tests

The MDA-MB-231 human highly-invasive breast cancer cell line was kindly supplied by Dr. P. Spessotto (Cro, Aviano, Italy), and maintained in Dulbecco's modified Eagle's medium (EuroClone®, Devon, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, Invitrogen™, Paisley, Scotland, UK), 2 mM L-glutamine (EuroClone®, Devon, UK), 1% non-essential aminoacids, and 100 IU/mL penicillin and 100 μg/mL streptomycin (EuroClone®, Devon, UK).

The MCF-7 human breast cancer cell line was obtained from the American Type Culture Collection (Manassas, VA; catalogue number HTB-22) and maintained in Dulbecco's modified Eagle's medium/F12 medium 1:1 v/v (EuroClone®, Devon, UK) supplemented with 10% FBS, 2 mM L-glutamine, and 100 IU/mL penicillin and 100 μg/mL streptomycin.

The HBL-100 human non-tumorigenic epithelial cell line was kindly supplied by Dr. G. Decorti (Department of Biomedical Sciences, University of Trieste, Italy), and maintained in McCoy's 5A medium supplemented with 10% FBS, 2 mM L-glutamine, and 100 IU/mL penicillin and 100 μg/mL streptomycin.

All cell lines were kept in a CO_2 incubator with 5% CO_2 and 100% relative humidity at 37 °C. Cells from a confluent monolayer were removed from flasks by a trypsin–EDTA solution. Cell viability was determined by the trypan blue dye exclusion test. For experimental purposes cells were sown in multiwell culture clusters.

2.3. Resistance to detachment assay

The ability of cells to resist detachment after treatment with RM175 and AFAP51 was measured by the following procedure. Ninety-six well plastic plates (Corning Costar, Milano, Italy) were coated with the following substrates: 10 μg/mL poly-L-lysine, 20 μg/mL fibronectin from human plasma, and 20 μg/mL collagen IV from human placenta, and left in a humidified cell-culture chamber at 37 °C for 4 h. Before cell seeding, plates were washed with CMF-DPBS (calcium and magnesium-free Dulbecco's phosphate buffered saline), then 6×10^3 cells in 0.2 mL complete medium were sown in each well. After 2 days at 37 °C, complete medium was replaced with serum-starved medium, containing 0.1% w/v BSA (bovine serum albumin). After 24 h the medium was removed and the plates washed with CMF-DPBS, before the treatment with 10^{-4} M RM175 or AFAP51, dissolved in DPBS, was added to the wells and incubated for 1 h. At the end of the treatment, the RM175- and AFAP51- containing solutions were removed, the plates were washed twice with CMF-DPBS, and a 0.008% w/v trypsin solution added to each well. Plates were kept in agitation for 30 min at room temperature then the trypsin solution was removed and wells washed with CMF-DPBS. Cells that were still adherent to the plates were detected by the sulforhodamine B (SRB) test. Resistance to detachment is expressed in arbitrary units, calculated by dividing the mean absorbance of treated cells by the mean absorbance of control cells. The resistance to detachment of controls is set equal to 1.

2.4. Re-adhesion assay

The effect on the ability of the cells to re-adhere after RM175 and AFAP51 treatment, was studied in cells maintained for 24 h in serum-starved medium, and then treated for 1 h with 10^{-4} M RM175 or AFAP51 in DPBS. At the end of the treatment cells were removed from flasks by a trypsin–EDTA solution, collected by centrifugation, re-suspended in serum-starved medium supplemented with 0.1% w/v BSA and kept for 30 min at room temperature to allow surface receptor reconstitution. The cells were then seeded at a density of 1×10^4 cells in 0.1 mL/well on 96-well plastic plates previously coated as described above with poly-L-lysine, fibronectin, collagen IV or 20 μg/mL Matrigel® (BD, Biosciences, San Josè, CA). Cells were left to adhere for 60 min at 37 °C with 5% CO_2 and 100% relative humidity, then the medium containing the non-adherent cells was removed and wells were gently washed with CMF-DPBS. Cells that had adhered to the substrates in 60 min were detected by the sulforhodamine B (SRB) test.

2.5. Sulforhodamine B assay

Adherent cells were detected with the SRB test described by Skehan et al. [21]. Briefly, adherent cells were fixed with 10% v/v

Download English Version:

https://daneshyari.com/en/article/1316774

Download Persian Version:

https://daneshyari.com/article/1316774

Daneshyari.com