Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 150 (2015) 127–134

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Anticancer activity studies of a ruthenium(II) polypyridyl complex against human hepatocellular (BEL-7402) cells

Wei Li [a], Bing-Jie Han [a], Jun-Hua Yao [b], Guang-Bin Jiang [a], Gan-Jian Lin [a], Yang-Yin Xie [a], Hong-Liang Huang [c,*], Yun-Jun Liu [a,*]

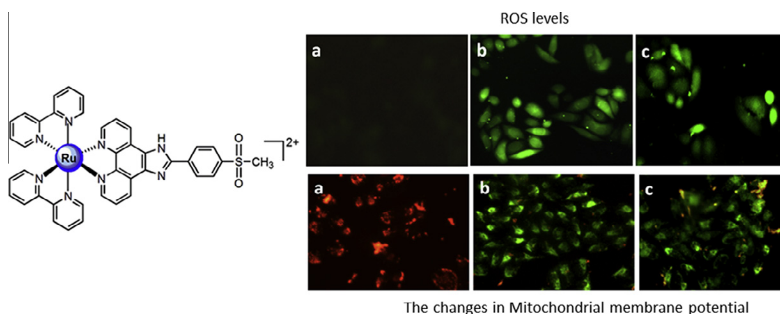
[a] School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou 510006, PR China
[b] Instrumentation Analysis and Research Center, Sun Yat-Sen University, Guangzhou 510275, PR China
[c] School of Life Science and Biopharmaceutical, Guangdong Pharmaceutical University, Guangzhou 510006, PR China

HIGHLIGHTS

- The cytotoxicity of complex **1** against BEL-7402, MG-63, A549, SK-BR-3 cells was studied.
- The apoptosis, scratch assay and cellular uptake in BEL-7402 cells were investigated.
- The levels of ROS and the changes of mitochondrial membrane potential were assayed.
- The comet assay and cell cycle arrest was investigated by flow cytometry.
- The expression of caspases and Bcl-1 family proteins were studied.

GRAPHICAL ABSTRACT

The cytotoxicity in vitro, apoptosis, comet assay, scratch assay, ROS, mitochondrial membrane potential, cell cycle arrest and the expression of proteins induced by the Ru(II) complex were investigated.



ARTICLE INFO

Article history:
Received 5 December 2014
Received in revised form 18 March 2015
Accepted 5 May 2015
Available online 25 May 2015

Keywords:
Ru(II) polypyridyl complex
Scratch assay
Cytotoxicity in vitro
Reactive oxygen species
Mitochondrial membrane potential
Western blot analysis

ABSTRACT

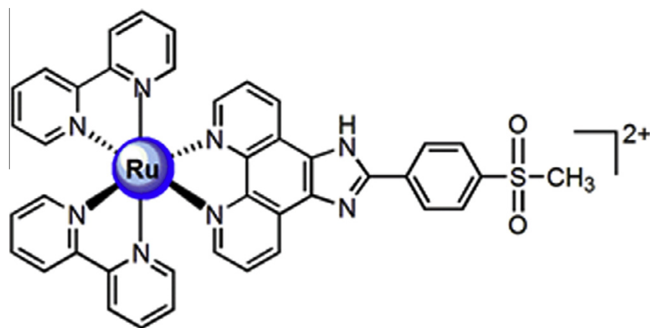
A Ru(II) polypyridyl complex $[Ru(bpy)_2(HMSPIP)](ClO_4)_2$ (**1**) (bpy = 2,2′-bipyridine, HMSPIP = 2-(4-methylsulfonyl)phenyl-1H-imidazo[4,5-*f*][1,10] phenanthroline) was synthesized. The IC_{50} value of the complex against human hepatocellular cell BEL-7402 is 21.6 ± 2.7 μM. The complex shows no cytotoxic activity toward human lung adenocarcinoma cell A549, human osteosarcoma cell MG-63 and human breast cancer cell SK-BR-3 cells. It is easily for complex **1** to be taken up by BEL-7402 cells. The complex can enhance the reactive oxygen species (ROS) levels and induce the decrease in the mitochondrial membrane potential. The complex inhibits the cell growth in BEL-7402 cells at G2/M phase. Complex **1** can regulate the expression of Bcl-2 family proteins. The results show that the complex induces apoptosis of BEL-7402 cells through a ROS-mediated mitochondrial dysfunction pathway.
© 2015 Elsevier B.V. All rights reserved.

Introduction

Cisplatin has been widely used as antimetastatic drug for treating ovarian and testicular cancers [1]. However, the serious side effects such as neurotoxicity, nephrotoxicity and dose-limitation of cisplatin have limited its clinical application [2,3]. These therapeutic drawbacks have fostered a growing number of studies on

* Corresponding authors. Tel.: +86 20 39352122; fax: +86 20 39352128.
E-mail addresses: hhongliang@163.com (H.-L. Huang), lyjche@163.com (Y.-J. Liu).

http://dx.doi.org/10.1016/j.saa.2015.05.032
1386-1425/© 2015 Elsevier B.V. All rights reserved.



Scheme 1. The structures of complex **1**.

diverse classes of metal complexes and their biological effects. The studies of ruthenium complex as anticancer drug have been received great attention [4–16]. Two Ru(II) complexes have successfully entered clinical trials, namely, NAMI-A ([ImH][trans-$RuCl_4$(DMSO)(Im)], Im = imidazole and DMSO = dimethylsulfoxide) [17] and KP1019 ([IndH][trans-$RuCl_4$(Ind_2)], Ind = indazole) [18]. $[Ru(hdpa)_2(7\text{-F-dppz})]^{2+}$ (hdpa = 2,2′-dipyridylamine, 7-F-dppz = 7-fluorodipyrido[3,2-*a*:2′,3′-*c*]phenazine) shows high antiproliferative activity toward HeLa [19]. The $[Ru(3,4,7,8\text{-tmp})_2(dmdppz)]^{2+}$ complex shows high cytotoxicity against the non-small lung carcinoma cell line (NCI-H460) (IC_{50} = 7.0 μM) [20]. The polypyridyl Ru(II) complex $[Ru(dip)_2(1\text{-Py-}\beta C)]^{2+}$ displays a high inhibition of cell growth against HeLa (IC_{50} = 1.9 ± 0.2 μM) [21]. $[Ru(dip)_2(dcdppz)]^{2+}$ (dcdppz = 7,8-dichlorodipyrido[3,2-*a*:2′,3′-*c*]phenanzine) exhibits higher cytotoxicity (IC_{50} = 12.3 ± 1.4 μM) on HeLa cells [22]. Complex $[Ru(bpy)_2(2,9\text{-dimethyldpq})]^{2+}$ has no cytotoxicity toward A549 cells in the dark (IC_{50} = 250 ± 5 μM), but irradiated with >450 nm light for 3 min, the complex shows highly inhibitory effect on the cell growth (IC_{50} = 1.2 ± 0.1 μM) [23]. In order to obtain more insight into cytotoxic activity of ruthenium (II) complex, based on our previous work [24], a Ru(II) polypyridyl complex $[Ru(bpy)_2(HSMPIP)](ClO_4)_2$ **(1)** was synthesized [25] (Scheme 1). The cytotoxicity of the complex toward BEL-7402, MG-63, A549 and SK-BR-3 cell lines was tested through MTT (3-(4,5-dimethylthiazole)-2,5-diphenyltetraazolium bromide) assays. The apoptosis in BEL-7402 cells was investigated by the acridine orange (AO)/ethidium bromide (EB) staining method. The cellular uptake, comet assay, wound healing assay, reactive oxygen species (ROS) and mitochondrial membrane potential were investigated by fluorescence microscopy. The cell cycle arrest of BEL-7402 was studied by flow cytometry. The expression of Bcl-2 family proteins was investigated by the western blot analysis.

Experimental

Materials and methods

All reagents and solvents were purchased commercially and used without further purification unless otherwise noted. Ultrapure MilliQ water was used in all experiments. DMSO and RPMI (Roswell Park Memorial Institute) 1640 were purchased from Sigma. Cell lines BEL-7402 (Hepatocellular), MG-63 (Human osteosarcoma), A549 (Human lung adenocarcinoma cell line) and SK-BR-3 (human breast cancer) were purchased from the American Type Culture Collection. $RuCl_3{\cdot}3H_2O$ was purchased from the Kunming Institution of Precious Metals. 1,10-phenanthroline was obtained from the Guangzhou Chemical Reagent Factory.

Cytotoxicity assay in vitro

MTT assay procedures were used [26]. Cells were placed in 96-well microassay culture plates (8×10^3 cells per well) and grown overnight at 37 °C in a 5% CO_2 incubator. The tested compound was then added to the wells to achieve final concentrations ranging from 10^{-6} to 10^{-4} M. Control wells were prepared by addition of culture medium (100 μL). The plates were incubated at 37 °C in a 5% CO_2 incubator for 48 h. Upon completion of the incubation, stock MTT dye solution (20 μL, 5 mg/mL^{-1}) was added to each well. After 4 h, buffer (100 μL) containing dimethylformamide (50%) and sodium dodecyl sulfate (20%) was added to solubilize the MTT formazan. The optical density of each well was measured with a microplate spectrophotometer at 490 nm. The IC_{50} values were determined by plotting the percentage viability versus concentration on a logarithmic graph and reading off the concentration at which 50% of cells remained viable relative to the control. Each experiment was repeated at least three times to obtain the mean values.

Comet assay

DNA damage was investigated by means of comet assay. BEL-7402 cells in culture medium were incubated with 12.5 or 25 μM of complex **1** for 24 h at 37 °C. The control cells were also incubated in the same time. The cells were harvested by a trypsinization process at 24 h. A total of 100 μL of 0.5% normal agarose in phosphate buffer saline (PBS) was dropped gently onto a fully frosted microslide, covered immediately with a coverslip, and then placed at 4 °C for 10 min. The coverslip was removed after the gel had set. 50 μL of the cell suspension (200 cells/μL) was mixed with 50 μL of 1% low melting agarose preserved at 37 °C. A total of 100 μL of this mixture was applied quickly on top of the gel, coated over the microslide, covered immediately with a coverslip, and then placed at 4 °C for 10 min. The coverslip was again removed after the gel had set. A third coating of 50 μL of 0.5% low melting agarose was placed on the gel and allowed to set at 4 °C for 15 min. After solidification of the agarose, the coverslips were removed, and the slides were immersed in an ice-cold lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris (tris(hydro xymethylaminomethane)-HCl), 90 mM sodium sarcosinate, NaOH, pH 10, 1% Triton X-100 and 10% DMSO) and placed in a refrigerator at 4 °C for 2 h. All of the above operations were performed under low lighting conditions to avoid additional DNA damage. The slides, after removal from the lysis solution, were placed horizontally in an electrophoresis chamber. The reservoirs were filled with an electrophoresis buffer (300 mM NaOH, 1.2 mM EDTA) until the slides were just immersed in it, and the DNA was allowed to unwind for 30 min in electrophoresis solution. Then the electrophoresis was carried out at 25 V and 300 mA for 20 min. After electrophoresis, the slides were removed, washed thrice in a neutralization buffer (400 mM Tris, HCl, pH 7.5). Nuclear DNA was stained with 20 μL of EtBr (20 μg/mL) in the dark for 20 min. The slides were washed in chilled distilled water for 10 min to neutralize the excess alkali, air-dried and scored for comets by fluorescence microscopy.

Scratch assay

BEL-7402 cells (4×10^5 cells per mL) were plated in six well tissue culture plates and grown to 90–95% confluence. After aspirating the medium, the center of the cell monolayers was scraped with a pipette tip to create a denuded zone (gap) of constant width. Then cellular debris was washed with PBS twice and the RMPI 1640 (containing 1% FBS) was added, and BEL-7402 cells were exposed to 12.5 μM of complex **1**. The wound closure was monitored and photographed at different time intervals with an Olympus inverted microscope and camera.

Download English Version:

https://daneshyari.com/en/article/1231598

Download Persian Version:

https://daneshyari.com/article/1231598

Daneshyari.com