European Journal of Pharmacology 786 (2016) 60–71

Contents lists available at ScienceDirect

European Journal of Pharmacology

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

Pulmonary, gastrointestinal and urogenital pharmacology

Insights into anticancer activity and mechanism of action of a ruthenium(II) complex in human esophageal squamous carcinoma EC109 cells

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ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form
30 May 2016
Accepted 30 May 2016
Available online 1 June 2016

Keywords:
Ruthenium(II) complex
Anticancer activity
Mechanism of action
Human esophageal squamous carcinoma
Ec109

ABSTRACT

A ruthenium(II) complex [Ru(*p*-cymene)(NHC)Cl_2] (NHC = 1,3-bis(4-(tert-butyl)benzylimidazol-2-ylidene), referred to as **L-4**, has been designed and synthesized recently in order to look for new anticancer drugs with high efficacy and low side effects. The anticancer activity and mechanism of action of **L-4** in human esophageal squamous carcinoma EC109 cells were systematically investigated. The results revealed that **L-4** exerted strong inhibitory effect on the proliferation of EC109 cells, and it arrested EC109 cells at G2/M phase, accompanied with the up-regulation of p53 and p21 and the down-regulation of cyclin D1. The results also showed that the reactive oxygen species (ROS)-dependent apoptosis of EC109 can be induced by **L-4** via inhibiting the activity of glutathione reductase (GR), decreasing the ratio of glutathione to oxidized glutathione (GSH/GSSG), and leading to the generation of reactive oxygen species. The mitochondria-mediated apoptosis of EC109 induced by **L-4** was also observed from the increase of Bax/Bcl-2 ratio, overload of Ca^{2+}, disruption of mitochondrial membrane potential (MMP), redistribution of cytochrome *c*, and activation of caspase-3/-9. However, the effects of **L-4** on the cell viability, GR activity, GSH/GSSG ratio, reactive oxygen species level, mitochondria dysfunction and apoptosis induction were remarkably attenuated by adding the reactive oxygen species scavenger, NAC. Therefore, it was concluded that **L-4** can inhibit the proliferation of EC109 cells via blocking cell cycle progression and inducing reactive oxygen species-dependent and mitochondria-mediated apoptosis. These findings suggested that the ruthenium(II) complex might be a potential effective chemotherapeutic agent for human esophageal squamous carcinoma (ESCC) and worthy of further investigation.

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1. Introduction

Esophageal carcinoma (EC) is one of the most common cancer with the eighth incidence and the sixth most common cause of cancer-related death worldwide (Siegel et al., 2013; Tang et al., 2015). This type of cancer has distinct geographic feature all over the world and it is very common in China. Most EC belongs to esophageal squamous carcinoma (ESCC) in China, which are different from adenocarcinoma (DeSantis et al., 2014; Tang et al., 2015). Even though certain studies have demonstrated that the incidence of esophageal cancer is decreasing in Western countries (Wu et al., 2012), other studies have indicated that it has become one of the fastest-growing types of cancer in the Western world (Abnet et al., 2012). Consequently, it is urgent to find novel anticancer drugs to treat the prevalence of EC and increase survival rate.

Over the past decades, a variety of metal complexes have been identified in the anticancer therapy (Du et al., 2015; Markowska et al., 2015; Meng et al., 2009; Muhammad and Guo, 2014; Oehninger et al., 2013; Patil et al., 2015). Cisplatin has particularly become one of the most widely-used drugs and is highly effective in treating several cancers, such as ovarian, esophageal and testicular cancers (Kelland, 2007). However, serious side effects and intrinsic or acquired resistance of cisplatin are major therapeutic problems for cancer chemotherapy, which have limited its clinical applications (Bruijnincx and Sadler, 2008; Kannarkat et al., 2007; Kelland, 2007). Therefore, an increasing number of investigations have been carried out on various metal complexes. At present, much attention has focused on ruthenium compounds since they displayed significant antitumor activity, low toxicity to normal tissues, no cross-resistance with cisplatin, and easy absorption by tumor tissue as well as rapid excretion from the body (Meng et al.,

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http://dx.doi.org/10.1016/j.ejphar.2016.05.042
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2009; Saygideger et al., 2014). Encouragingly, two ruthenium complexes, NAMI-A and KP1019, are currently undergoing clinical trials (Brescacin et al., 2015; Depenbrock et al., 1997).

Recently, a series of Ru(II) complexes with N-heterocyclic carbenes (NHC) as the ligands have been reported by our group, including synthesis, characterization, and cytotoxic activity against various cancer cell lines (Lv et al., 2015). The preliminary biological evaluation has identified that the complex [Ru(p-cymene)(NHC)Cl_2] (NHC=1,3-bis(4-(tert-butyl)benzylimidazol-2-ylidene) (L-4, Fig. 1A) can inhibit the growth of human esophageal carcinoma cell lines EC109 strongly at a low concentration (Lv et al., 2015). In this continue work, the anticancer efficacy of **L-4** against EC109 cells and its mechanism of action was further examined.

2. Materials and methods

2.1. Chemicals and reagents

The complex **L-4** was dissolved in DMSO as a stock solution and further diluted with the culture medium before use. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT), DMSO, bicinchoninic acid (BCA) protein assay kit and bisbenzimide Hoechst 33258 were purchased from Beijing Solarbio Science & Technology (Beijing, China). Fluorescein isothiocyanate (FITC)-Annexin V/Propidium iodide (PI) apoptosis assay kit was purchased from Nanjing KeyGEN Biotech (Nanjing, Jiangsu, China). The antibodies against caspase-3/-9, p53, Bcl-2, Bax, survivin, PARP and β-actin were purchased from Cell Signaling Technology (Beverly, Massachusetts, USA). Sulforhodamine B (SRB) was procured from Sigma Chemicals (Saint Louis, Missouri, USA). Antibody against GAPDH was purchased from Bioworld Technology (Minneapolis, Minnesota, USA). Antibodies against Cyclin D1 and cytochrome *c*, pan-caspase inhibitor (z-VAD-fmk), the reactive oxygen species detection kit, the caspase-3/-9 activity kit, the glutathione reductase (GR) activity assay kit, the cell mitochondria isolation kit, N-acetyl-L-cysteine (NAC), Fluo-3 AM, PI and JC-1 fluorescence probe kit are the products of Beyotime Institute of Biotechnology (Shanghai, China). RPMI-1640 medium and fetal bovine serum (FBS) are the products of Biological Industries

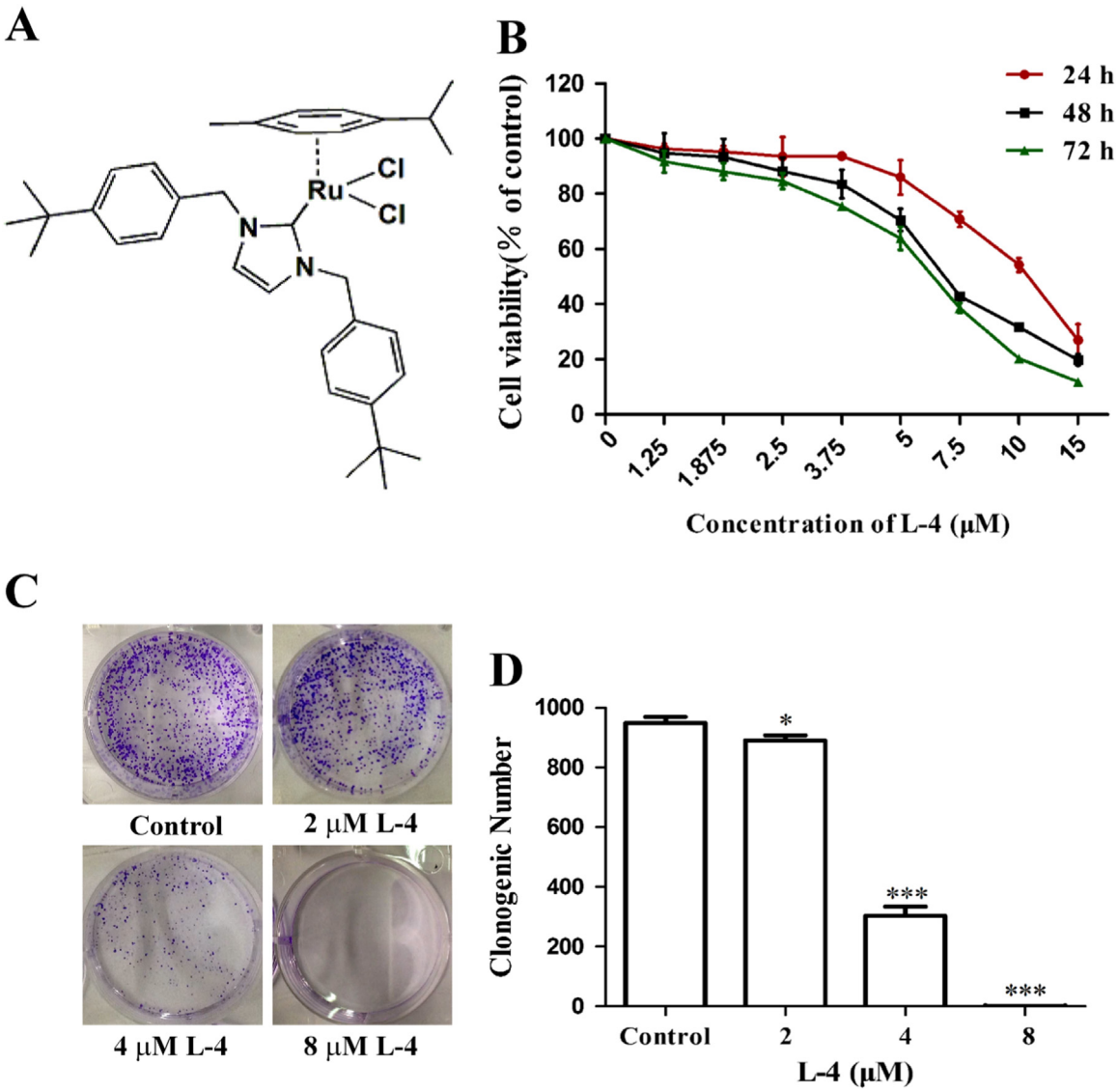

Fig. 1. Effects of **L-4** on the growth of EC109 cells *in vitro*. (A) Chemical structure of **L-4**. (B) Cells were incubated with **L-4** for 24, 48 and 72 h, respectively. MTT assay was used to assess the effect of **L-4** on the viability of EC109 cells. (C) The inhibition of colony formation in EC109 cells exposed to different **L-4** doses. (D) The results of colony formation assay were standardized to the clonogenic survival of control EC109 cells and expressed as % of control. Results were obtained from triplicate separate determinations and the bars represented mean ± S.D. $^{*}P < 0.05$, $^{***}P < 0.001$ *vs* untreated control.

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