Expression of multidrug resistance gene MDR1(ABCB1) in human alveolar carcinoma epithelial cell (A549) and human erythromyeloblastoid leukemia cell line (K562)

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1.0 INTRODUCTION

Multidrug resistance is a frequent cause of treatment failure in cancer patients. One mechanism of MDR is over-expression of ATP-binding cassette (ABC) transporter proteins, including P-glycoprotein (P-gp), that function as a drug efflux pump. Leukemia is a major type of cancer affecting a significant segment of the population, and especially children. In fact, leukemia is the most frequent childhood cancer, with 26% of all cases, and 20% mortality. Chemotherapy is a common treatment for leukemia (Ge et al., 2009). In general the therapy uses a number of different anticancer drugs, which destroy cancer cells by preventing them from growing and dividing rapidly. Unfortunately, a number of the body's normal, non-cancerous cells (e.g., hair cells, red and white blood cells, blood-clotting platelets, and cells of the gastrointestinal mucosa) also divide rapidly and are harmed by chemotherapy (Ge et al., 2009).

Lung cancer is one of the leading cause of cancer mortality in industrialized countries, with non-small cell lung cancer (NSCLC) accounting for nearly 80%. First-line treatment for patients with advanced NSCLC includes platinum compounds such cisplatin, carboplatin and oxaliplatin. Nevertheless, the clinical utility of these drugs has been proven limited due to the relatively narrow range of tumors affected and the development of acquired drug resistance (Karki et al., 2007).

The side effects of chemotherapy hamper many normal activities of patients undergoing treatment (Kim et al., 2002).Ruthenium complexes have shown potential utility in chemotherapy with lower toxicities compared to platinum-based chemoterapeutics attributed to their specific accumulation in cancer tissues. Ruthenium complexes have shown potential utility in chemotherapy with lower toxicities compared to cisplatin attributed to their specific accumulation in cancer

tissues (Silveira-Lacerda et al., 2010a). To our knowledge, the mechanism of multidrug resistance to chemotherapy remained largely unknown in A549 and K562 tumor cells. The present study aimed to investigate the relationship between the expression of MDR1 in human lung alveolar carcinoma epithelial cells (A549) and human erythromyeloblastoid leukemia cell line (K562) treated with two Ruthenium(III) compounds.

2.0 MATERIAL AND METHODS

2.1. Cell culture

The tumor cells A549 (ATCC number CCL-185^{TM}) and K562 (ATCC number CCL-243^{TM}) were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The A549 cell line were maintained at 37°C under 5% CO_2 in DMEM medium (pH 7.2-7.4) supplemented with 100 U mL^{-1} penicillin G, 100 μg mL^{-1} streptomycin, 2 mM L-glutamine, 1.5 g L^{-1} sodium bicarbonate, 4.5 g L^{-1} glucose, 10 mM HEPES, 1.0 m*M* sodium pyruvate and 10% fetal calf serum 1% (w/v) (all reagents were obtained from Gibco, Grand Island, NY). The K562 cell line was maintained at 37°C under 5% CO_2 in supplemented RPMI 1640 medium (pH 7.2-7.4), antibiotics and 10% fetal calf serum 1% (w/v) (all reagents were obtained from Gibco, Grand Island, NY). Both tumor cell lines were cultured in the presence or absence of two Ruthenium(III) Complexes CRu(III) and DRu(III).

The cells were disposed into 96 well plates (1×10^5 cells/well) and cultured in RPMI 1640 or DMEN media. Cells were harvested at specified intervals and the number of cells per well was determined by cell counting with a hemocytometer (Neubauer chamber). Briefly, tumor cells were aspirated, washed in sterile PBS and an aliquot of the cell suspension was put in Trypan Blue 1% (m/v) (Sigma-Aldrich, St. Louis, MO, USA) and counted. Only cell dilutions with > 95% of viable cells were included in the posteriors analysis.

2.2. Cell Viability (Trypan blue staining)

The viability of the A549 and K562 cells was evaluated by the trypan blue exclusion assay. The tumor cells were incubated for 24 h, 48 h and 72 h with different concentrations of the tested Ruthenium(III) complex at 37 °C. Additionally, Carboplatin (50 μM) and Paclitaxel (25 μM) were applied as positive control. After incubation, the cells were washed in PBS (pH 7.4) and suspended in a complete

RPMI 1640 medium. Then 40 μL of the trypan blue solution (0.4%, Sigma) and 10 μL of the cell suspension were mixed and after 5 min the percentage of viable K562 cells was evaluated under brightfield optical microscope using a newbauer chamber. The correlation between the viable cells (that excluded trypan blue dye) and dead cells (stained cells) were assessed. The results are presented as mean±S.D. (standard deviation) from three independent experiments.

2.4. Cytotoxicity assay.

Cytotoxic activity of ruthenium complexes on A549 and K562 was measured by modified MTT assay (Silveira-Lacerda et al., 2010b), which is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to dark-blue, insoluble formazan in mitochondria of the living cells. The MTT assay was performed in 96-well tissue culture plates (Nalge-Nunc, Rochester, NY, USA

2.3. Total RNA Isolation and Quantitative Real-Time RT-PCR

Total RNA was isolated from A549 cells with Trizol reagent (Invitrogen, USA). cDNA was prepared according to standard methods. For qPCR, 30 cycles of denaturation (94°C for 45s), annealing (60°C for 45s), and elongation (72°C for 1 min) was performed using the following primer pairs for *ABCB1* gene forward: 5'-CTATGCTGGATGTTTCCGGT-3', reverse: 5'-GCTTTGGCATAGTCAGGAGC-3', which yielded a 147-bp product. The *GAPDH* control PCR was performed using the following primer pairs: forward: 5'-ACAGTCAGCCGCATCTTCTT-3', reverse: 5'-GTTAAAAGCAGCCCTGGTGA-3', which yielded a 127-bp product. PCR reactions were performed using a LineGene K fluorescence quantitative PCR detection system (Hangzhou BIOER Tech Co., Tokyo, Japan).

3.0. RESULTS AND DISCUSSION

3.1. The ruthenium complexes CRu and DRu present cytotoxic activity towards A549 and K562 tumor cell lines

Measurement of the number of living cells using MTT or similar assays in drug-treated and control cultures is the most commonly used method in cell-based screening experiments. The results show that A549 and K562 tumor cells treated with a different concentration of ruthenium complexes CRu and DRu, Ruthenium-

based coordinated complexes, presented low significant cytotoxic and anti-proliferative activities (Figure 1).

3.2. MDR Expression on A549 and K562 tumor cell lines when treated with Ruthenium(III) complexes.

In this study, we described the expression of a protein associated with multidrug resistance in human lung carcinoma cell A549. The tested marker exhibited some changes in their expression pattern when tumor cells were treated with different concentrations of Ruthenium(III) compounds CRu(III) and DRu(III) and Carboplatin when compared with untreated A549 cells (Figure 1). Expression of MDR1 in tumor cells was detected by RT-PCR analysis and results showed a the the relative quantities of ABCB1 mRNA expression in A549 treated with DRu(III), CRu(III), and Carboplatin were 0.2850±0.0777, 5.4250±0.4313 and 8.9023±2.6162, respectively, when compared to untreated A549 cells (P = 0.011).

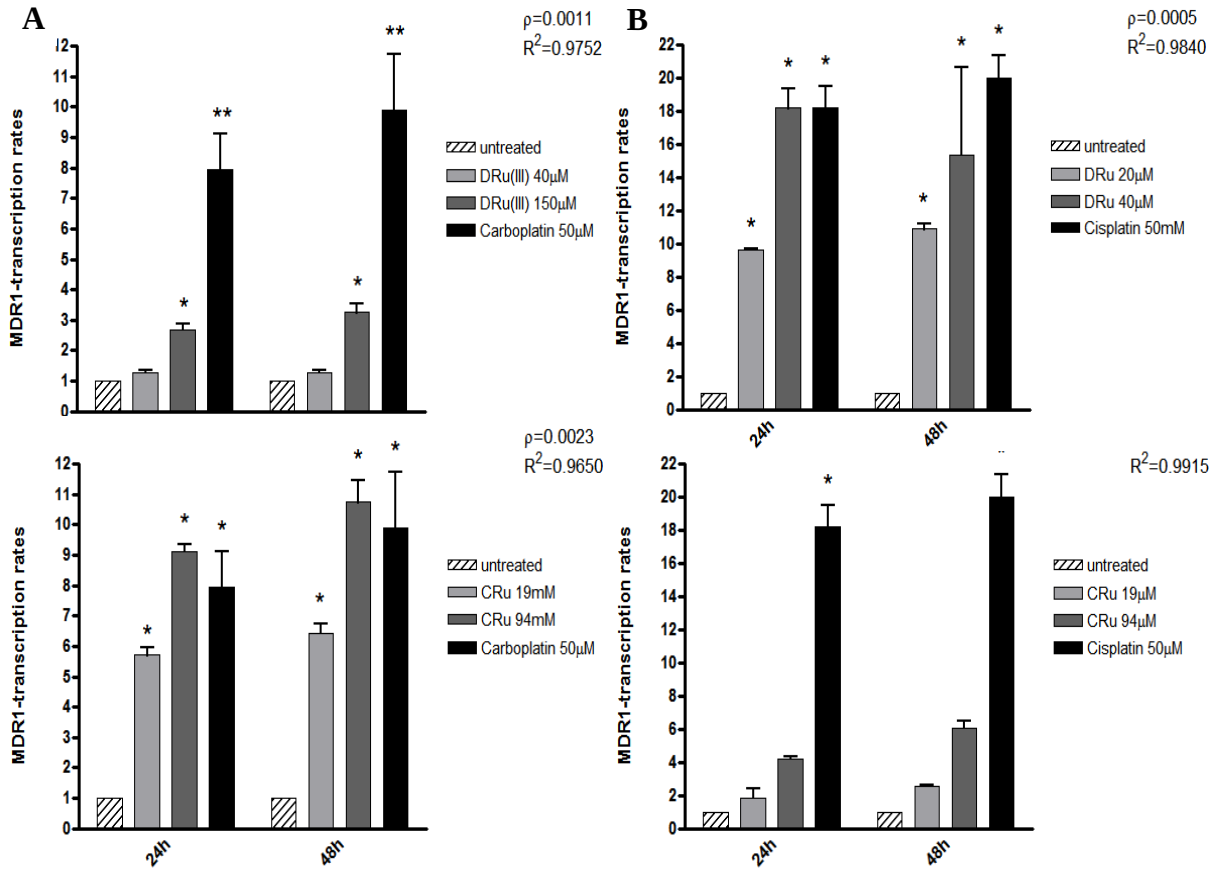

Figure 1. Alterations of MDR1-mRNA expression on A549(A) and K562(B) using RTQ-PCR after treatment with compound *cis*-Tetraammine(oxalato)Ruthenium(III) Dithionate, for 48 h. Samples are in relation to the untreated control cell line with a transcription rate set up to a value of one. The data are expressed as mean±SD of n=3. The statistical difference was determined by one-way ANOVA with Bonferroni multiple comparison test. An asterisk denotes $P < 0.01$

The tested molecular marker exhibited some changes in their expression pattern when tumor cells were treated with different concentrations of Ruthenium(III) compounds CRu(III) (19 and 94 µM) and DRu(III) (20 and 40 µM) and Cisplatin (50 µM) when compared to untreated K562 cells. Expression of MDR1 in K562 cells was also detected by RT-PCR analysis and results showed as the relative quantities of ABCB1 mRNA expression in K562 treated with DRu(III) (40 µM), CRu(III) (19 µM), and Cisplatin (50 µM) were 9.6±0.19; 1.8±0.82 and 18.19±1.86, respectively, when compared to untreated K562 cells (P=0.05) (Figure 2).

The current results showed that MDR1(ABCB1) was weakly expressed in the membranous and intracellular regions of K562 and A549 cells treated with Ruthenium(III) complexes, but highly expressed when tumor cells were treated with the antitumor agents Cisplatin or Carboplatin. The reduced induction of MDR1 in K562 and A549 tumor cells by CRu(III) and DRu(III) are particularly attractive attributes of these complexes and indicates that they are worthy of further studies as potential anti-tumor agents.

4.0. CONCLUSIONS

The reduced induction of MDR1 in A549 and K562 by CRu(III) and DRu(III) are particularly attractive attributes of these complexes and indicates that they are worthy of further studies as potential anti-tumor agents. The identification of new chemotherapeutics agents is critical for further progress in the treatment of NSCLC and leukemias.

5. REFERENCES

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