Short Communication

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Cytotoxic evaluation of 5-nitrothiophene-thiosemicarbazone derivative against HL-60

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Abstract

Studies with 5-nitrothiophene-thiosemicarbazone derivatives has shown antiproliferative activity against several cancer cells lines. Therefore, the main goal of this work was to evaluate the cytotoxicity activity of the new derivative LQIT/LNN8 against acute promyelocytic leukemia cancer cells by MMT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] method. It was observed that the compound inhibited the cell growth more than 90%, with an IC50 of 1.2 µg/mL against HL-60 cells. In human peripheral blood mononuclear cells (PBMC), the derivative had IC50 of 14.4 µg/mL. Regarding of morphological analysis, the alterations indicated death by apoptosis. In our results we have found that the LQIT/LNN08 derivative has cytotoxic activity against HL-60 cancer cells. However, future studies are necessary to help elucidate the mechanism of action of this derivative.

Introduction

Cancer is a group of more than 100 different types of diseases that has in common disorderly cell growth and invasive potential. Cancer is associated with several conditions that might act together or in sequence to initiate or promote carcinogenesis (INCA, 2015).

Considering that chemotherapy, radiotherapy, and surgery are the main therapeutic approaches, cancer treatment is extremely complex. Therefore, the search for new drugs with anticancer activity is necessary (VIDEIRA; REIS; BRITO, 2014). In this context, 5-nitrothiophene-thiosemicarbazone derivatives are important molecules for the development of new anticancer drugs due to the simplicity of its structure and broad-spectrum of chemical and biological activities (KUPADHYAY, MISHRA; 2015).

Material and Methods

Compounds: The new derivative LQIT/LNN08 was synthetized at the Chemistry and Therapeutic Innovation Laboratory - LQIT/UFPE. The control drug doxorubicin was purchased from Sigma-Aldrich. Stock solutions (5 mg/mL) were solved in pure and sterile DMSO.

Cytotoxicity against HL-60 cancer cells and peripheral blood mononuclear human cells: HL-60 cancer cells were plated at 0.3x106 per well on 96-well plates. The cancer cells were treated with doxorubicin or with LQIT/LNN08 at the single dose of 25 µg/mL (inhibitory potential), and serial concentrations of 0.19 – 25.0 µg/mL (tested derivative) or 0.009 – 5.0 µg/mL (reference drug) following incubation for 72 h. Following, 25 µL of MTT was added and cells were reincubated for 3 h. After incubation, the supernatant was aspirated and 100 µL of DMSO was added on each well, and absorbance was obtained in spectrophotometer at 560 nm. To assess cytotoxicity in PBMC, the colorimetric assay using alamar blue was performed as described by Collins & Franzblau (1997).

Morphological Analysis: HL-60 cancer cells were plated on 24-well plates and incubated with LQIT/LNN08 for 72 h. To analyse cell morphology, slices were made using a centrifuge followed by fixation with methanol for 1 min, and hematoxylin/eosin staining.

Results

LQIT/LNN08 inhibited cell growth in more than 90% against HL-60 cancer cells, and had an IC50 of 1.2 µg/mL. In PBMC, the CI50 was 14.4 µg/mL, which was less cytotoxic when compared to the reference drug doxorubicin (CI50 = 0.22) (Table 1). In the morphological analysis of the treated cells (1.2 e 2.4 µg/mL of LQIT/LNN08) was observed that the alterations indicated death by apoptosis (Figure 1).

Table 1 - IC50 (µg/mL) values of 5-nitrothiophene-thiosemicarbazone (LQIT/LNN08) derivative against HL-60 cancer cells and PBMC.

Compounds	HL-60	PBMC
LQIT/LNN08	1.2	14.4
Doxorubicin	0.06	0.22

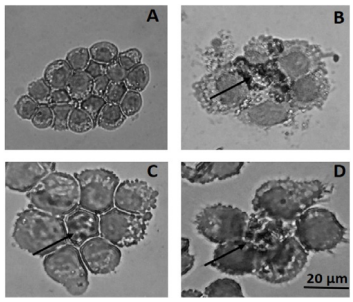

Figure 1 – Morphological analysis of HL-60 cancer cells by May-Grunwald-Giemsa staining after 72 h of incubation with LQIT/LNN08. A: Negative control, B: Doxorubicin, C: LQIT/LNN08 (1,2 µg/mL) and D: LQIT/LNN08 (2,4 µg/mL). The arrows indicate apoptotic bodies.

Discussion and Conclusion

The cytotoxic effect of the nitro-heterocyclic derivatives is related to the nitro group reduction into amino, and the oxygen reactive species interaction with the intracellular components (PAULAI; SERRANO; TAVARES, 2009). However, we believe that the cytotoxic activity of the LQIT/LNN08 is associated with substitute's changes in the phenyl ring structure. The presence of thiosemicarbazones (TSC) in the structure of the studied derivative might also have contributed to its cytotoxic activity, since the biological activity of these molecules is due to their ability to inhibit the ribonucleotide reductase (RR) enzyme, which is responsible for the biosynthesis and catalytic activity of deoxynucleotide triphosphates (dNTPs) for DNA synthesis through the reduction of the ribonucleotides components. For this reason, dNTPs are important targets for therapeutic intervention against several human diseases, such as cancer (AYE et al., 2012).

Regarding of the morphological analysis, the cells treated with LQIT/LNN08 indicated apoptosis as the possible mechanism of cellular death. According to Kroemer et al. (2009), characteristics such as, chromatin condensation and DNA fragmentation are needed to indicate death by apoptosis. In our study, these characteristics were observed. The presented results highlighted the importance of new 5-nitrothiophene-thiosemicarbazone derivatives as promissory anticancer agents.

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