INVESTIGATION OF ANTITUMORAL EFFECT AND MECHANISM OF ACTION OF LQB-223 IN AML CELL LINES WITH DIFFERENT RESISTANCE PHENOTYPES

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INTRODUCTION

Acute myeloid leukemia (AML) is a clonal disease identified by the presence of myeloid cells that lost the ability to differentiate, presenting high proliferation rate. The standard treatment for AML patients involves the association of anthracyclines with cytarabine. Although this strategy had increased survival rate, patients often develop resistance to these drugs and relapse to a disease with a complex karyotype. For these reasons, is essencial the investigation of new drugs to bypass the intrinsic or acquired resistance to standard treatment. Thus, our objectives were to evaluate the antitumoral effect of LQB-223 in AML cell lines with different molecular and karyotype background and to investigate the mechanism of action of this compound in AML cell lines.

METHODS

AML cell lines were treated with 5 M of LQB-223 for 24 hours. Cell death in HL60, HL60R (resistant to 50 M of Cytarabine) and U937 cell line were accessed by DNA fragmentation analysis, Annexin V/PI assay and evaluation of cleaved caspase-3 by Western blotting. Cell cycle distribution was analyzed by flow cytometry after treatment with LQB-223. To further investigate the putative mechanisms of action of the compound in AML cell lines, Western blotting and Immunofluorence assays were conducted.

RESULTS AND CONCLUSION

Our results showed that LQB-223 increases the number of Anexin V positive cells, DNA fragmentation and induces an arrest of cells in G2/M cell cycle phase after treatment for 24 hour in all AML cell lines evaluated. LQB-223 also induces phosphorylation of γ-H2aX, decrease of Caspase-3 and increase of cleaved Caspase-3. Also, the profile of cells arrested in G2/M was observed after LQB-223 treatment by DAPI staining, corroborating the data of cell cycle analysis. Together our data suggests that LQB-223 induces apoptosis in AML cell lines with different response phenotypes to standard treatment. Also, our data suggests that LQB-223 mechanisms of action are related to DNA damage and other pathways involved in DNA repair and apoptosis.

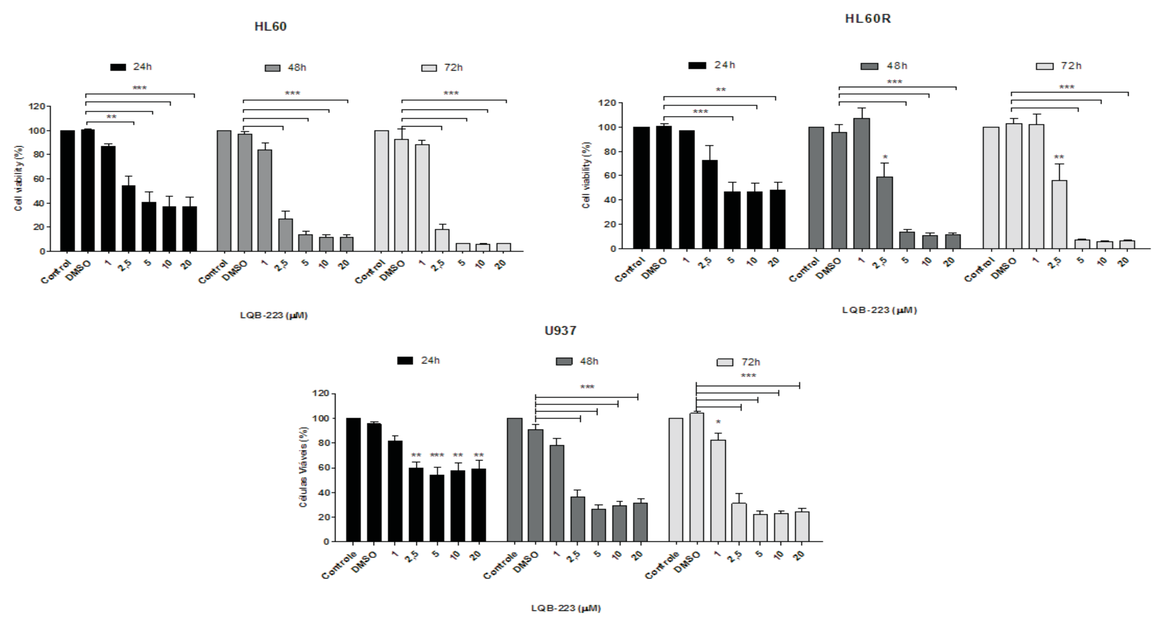

Figure 1: Viability assay (MTT) of AML cell lines HL60, HL60R and U937 after 24h, 48h and 72h of LQB-223 treatment.

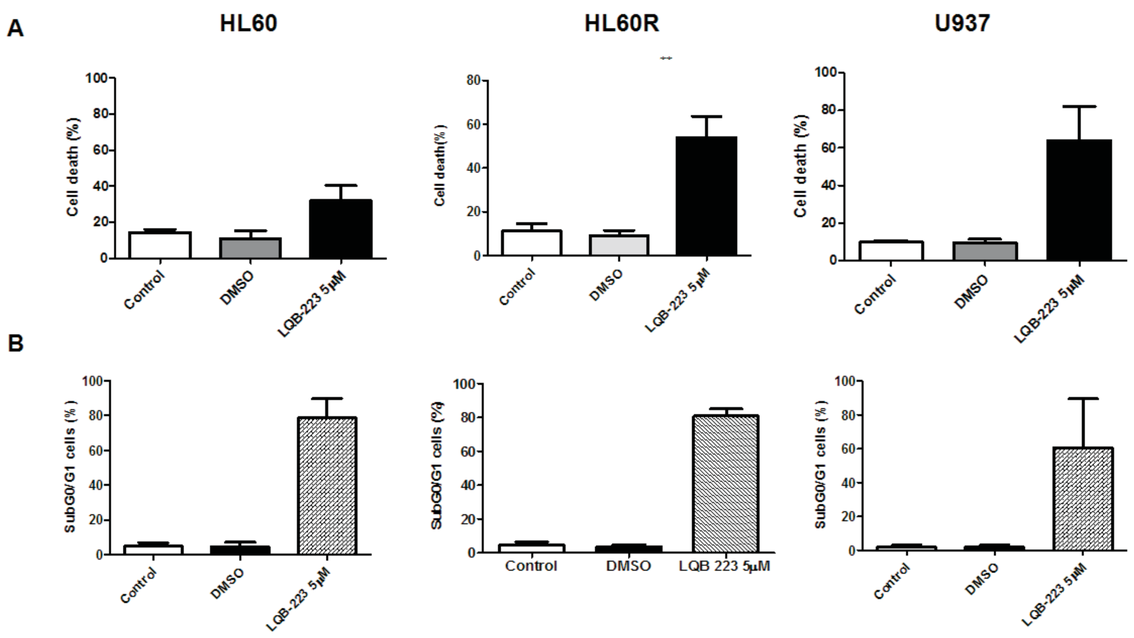

Figure 2: Cell death analysis of AML cell lines treated with LQB-223. **A** – Anexin V$^+$ and Anexin V$^+$ + PI cells of HL60, HL60R and U937 cell lines, after 24h of LQB-223 treatment. **B** – DNA fragmentation analysis after LQB-223 treatment (24h) in HL60, HL60R and U937 cell lines.

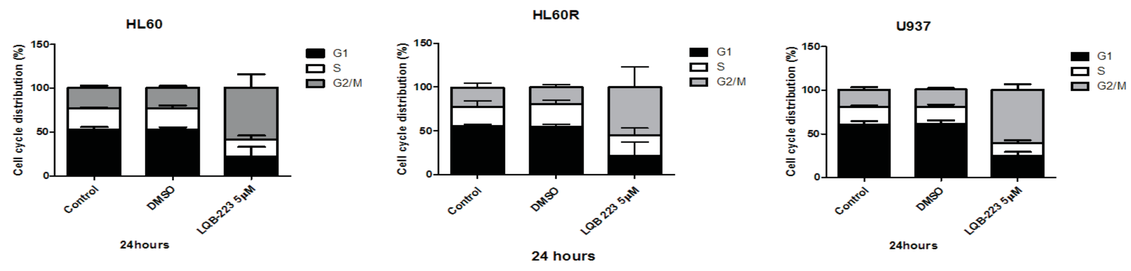

Figure 3: Cell cycle distribution of AML cell lines HL60, HL60R and U937 treated with LQB-223 for 24h.

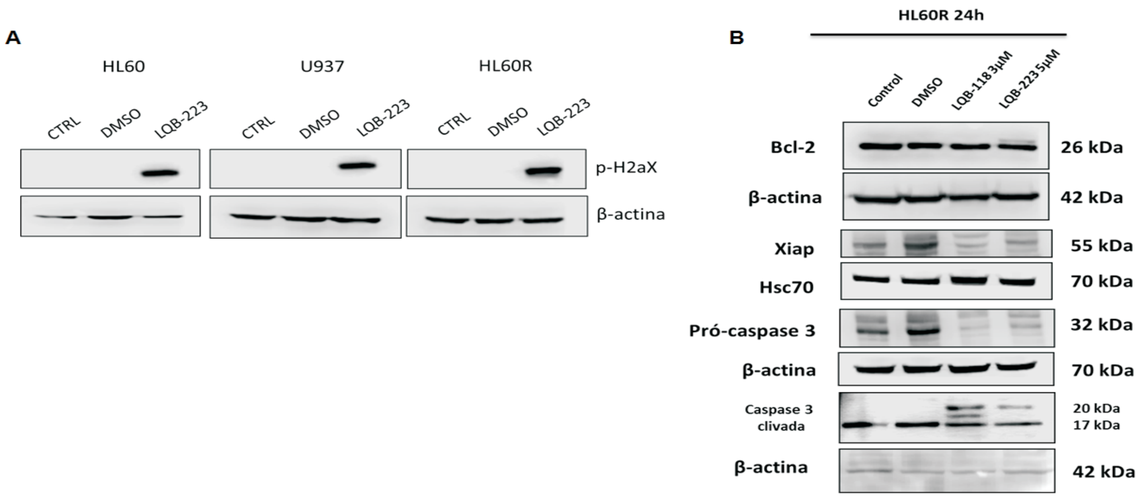

Figure 4: A - Western blot protein levels of phosphorilation of H2aX, in AML cell lines HL60, HL60R and U937, after 24h of LQB-223 treatment. **B** – Protein panel modulated by LQB-223 treatment (24h) in HL60R cell line.

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