CHARACTERIZATION OF RESISTANCE TO CYTARABINE IN ACUTE MYELOID LEUKEMIA CELL LINE HL60R AND THE ANTITUMOR EFFECT OF COMPOUNDS LQB-118 AND LQB-223

Thaís Hancio Pereira[1,4], Fernanda Costas Casal de Faria[1], Aline Rangel Pozzo[1], Luciano Mazzoccoli[1], Camilla Buarque[2], Paulo R. Costa[3], Raquel C. Maia[1]

[1]Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Pesquisa em Hemato-Oncologia Molecular, Instituto Nacional de Câncer (INCA), Rio de Janeiro, RJ.
[2]Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro, RJ. [3]Laboratório de Química Bio-orgânica (LQB), IPPN, CCS, UFRJ, RJ.
[4]Instituto de Biologia, Universidade Federal do Rio de Janeiro, RJ.

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. Previous data of our group showed that the compounds LQB-118 and LQB-223 induced cell death in several tumor cell lines and promoted low toxicity for lymphocytes isolated from healthy individuals. Thus, our objectives are: a) To characterize the molecular mechanisms associated with resistance to cytarabine in AML treatment, and b) To access cytotoxic activity of compounds LQB-118 and LQB-223 in HL60R (resistant to 50 µM cytarabine) cell line.

METHODS AND RESULTS

The HL60 cell line was treated with increasing concentrations of cytarabine until exhibit resistance to 50µM (HL60R). HL60R treated with cytarabine had a maximum reduction of cell viability of 15% with 200 µM of cytarabine in 72 hours, accessed via MTT. Cytarabine did not induce cell death in HL60R when cells were stained with Annexin V/PI. HL60R presented an increase of MYC, Bcl-2 and XIAP protein levels, but no alteration in pro-apoptotic proteins Bax and Bak-1 and in transcription factor NRF2 levels, analysed by Western blotting. The compounds LQB-118 and LQB-223 reduced HL60R cell viability from 3 µM and 5 µM, respectively, in 24 hours and induced cellular death in the same concentration and time. Also was observed that LQB-118 and LQB-223 changed the cell cycle profile in HL60R, evaluated by flow cytometry. The treatment with the compounds induces decrease of XIAP protein levels but not induce decrease of Bcl-2 protein levels.

CONCLUSION

Together, our data validates the resistance profile to cytarabine of HL60R cell line and suggest important signaling pathways associated with acquired resistance to this drug. Our data also show that LQB-118 and LQB-223 caused a potent antitumor effect in AML resistant cell line HL60R and this effect is possibly associated with the decrease of XIAP protein levels.

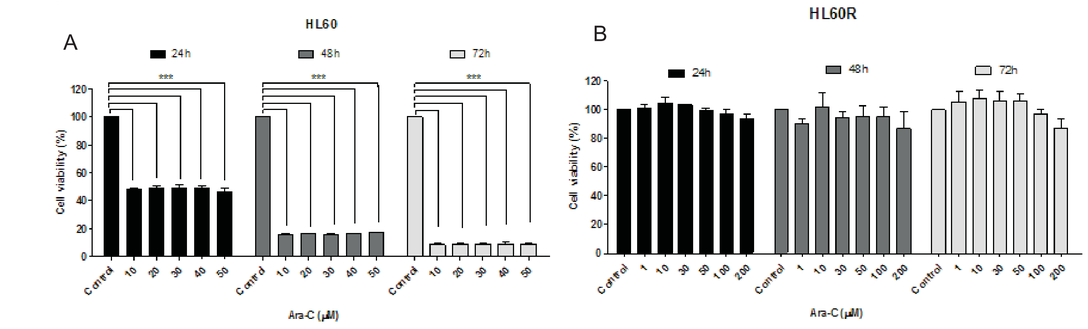

Figure 1: Evaluation of cell viability in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 24h, 48h and 72h. Cell lines were compared with their control (untreated cells). The graphs correspond to the mean ± standard deviation of three independent experiments. ANOVA with post-test Bonferroni * p <0.05, ** p <0.01, *** p <0.001

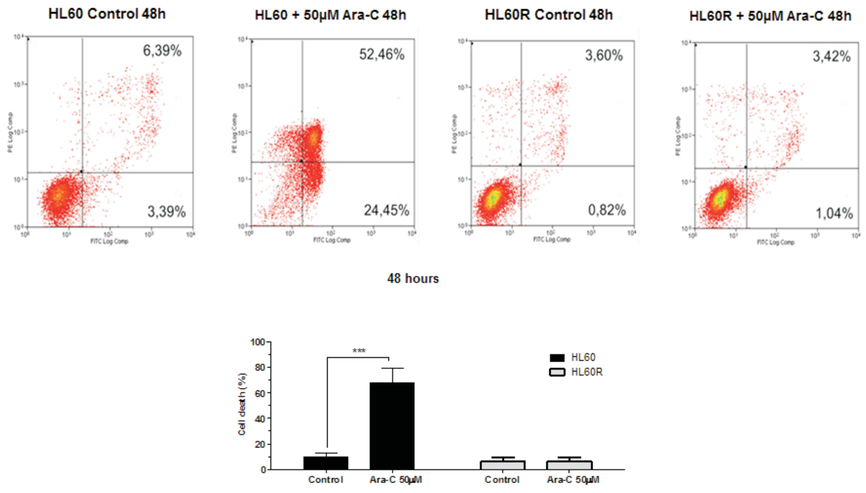

Figure 2: Evaluation of cell death in HL60 (A) and HL60R (B) cell lines treated with different concentrations of Ara-C after 48h. Statistical analyzes of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD. Student's t-test * p <0.05, ** p <0.01, *** p <0.001 compared the DMSO.

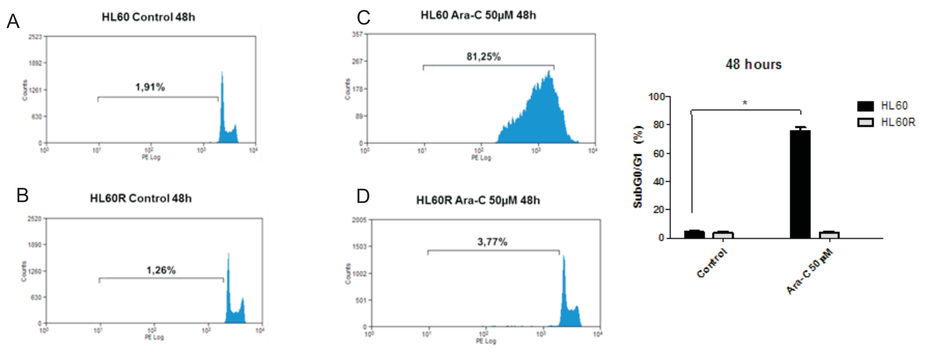

Figure 3: Evaluation of DNA fragmentation in HL60 (A and C) and HL60R (B and D) cell lines treated with different concentrations of Ara-C after 48h. For these results were considered two independent experiments. Percentage of subG0/G1 cells after treatment with 50µM of cytarabine (E).

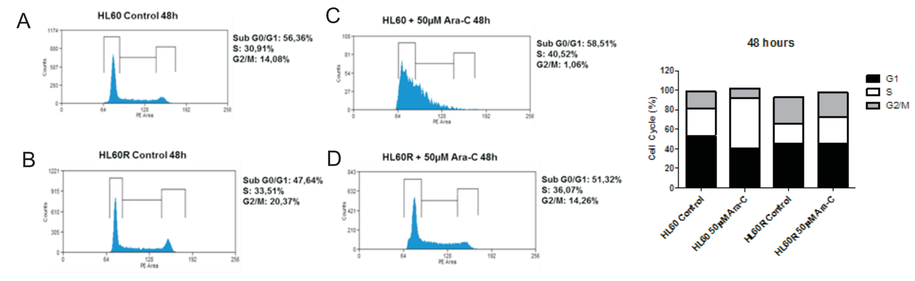

Figure 4: Evaluation of cell cycle profile in HL60 (A and C) and HL60R (B and D) cell lines treated with 50µM of Ara-C after 48h. For these results were considered two independent experiments.

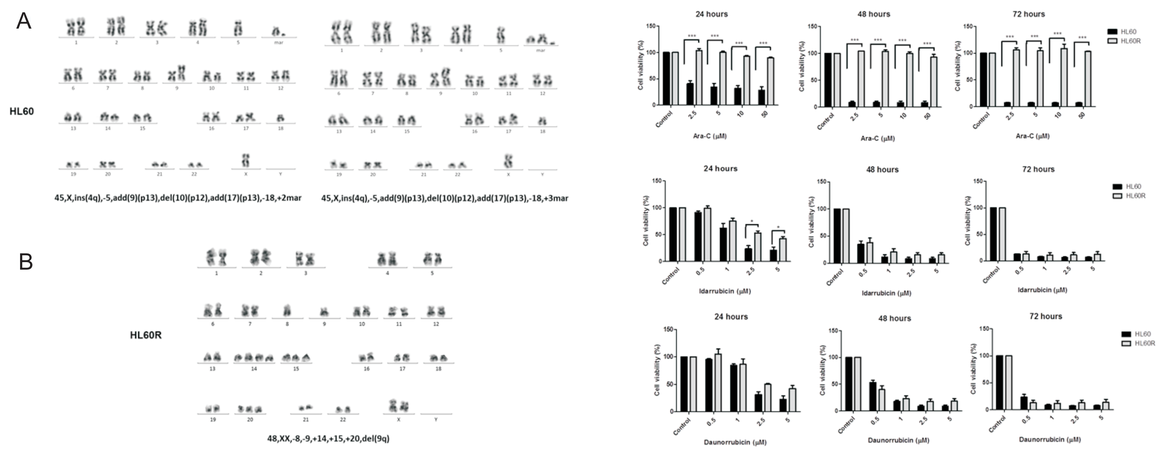

Figure 6: Evaluation of cross resistance after treatment with different concentrations of anthracycline in HL60R cell line. HL60 and HL60R cell lines were treated with idarrubicine and daunorrubicine for 24h, 48h and 72h. The graphs correspond to the mean ± standard deviation of three independent experiments. ANOVA with post-test Bonferroni * p <0.05, ** p <0.01, *** p <0.001

Figure 5: Analysis by classical cytogenetics (GTG banding) showing the chromosomal changes found in HL60 and HL60R cell lines.

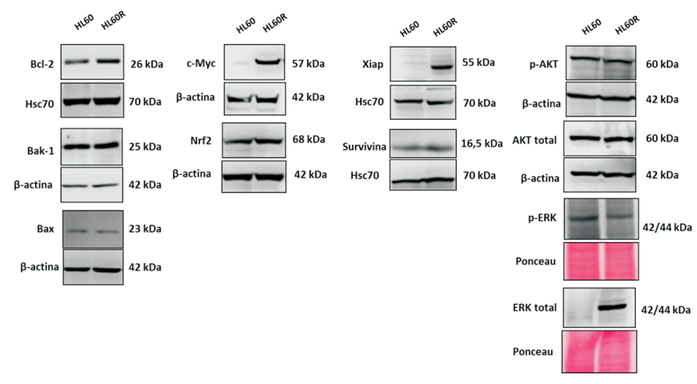

Figure 7: Protein expression in the cell lines HL60 and HL60R by Western blotting. The baseline evaluation of pro-apoptotic proteins (Bax and Bak-1), anti-apoptotic (Bcl-2, and XIAP), proteins involved with proliferation (Survivin and ERK), cell survival (AKT) and the transcription factors Nrf2 and MYC, in HL60 and HL60R cell lines. For this result were considered three independent experiments.

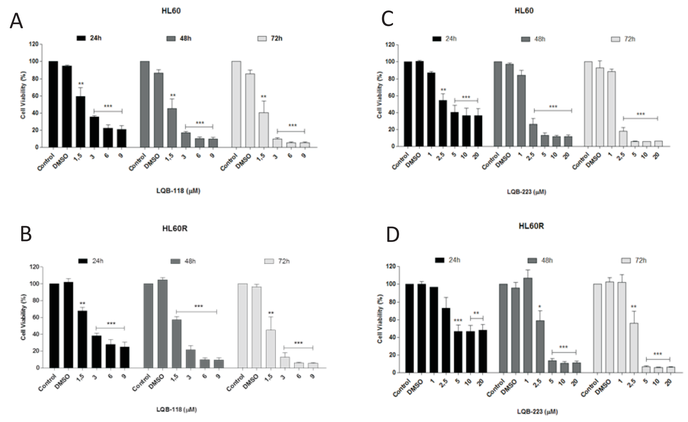

Figure 8: Evaluation of cell viability in HL60 (A and C) and HL60R (B and D) cell lines treated with different concentrations of LQB-118 and LQB-223 after 24h, 48h and 72h. Cell lines were compared with their control of cells trated with DMSO. The graphs correspond to the mean ± standard deviation of three independent experiments. ANOVA with post-test Bonferroni * p <0.05, ** p <0.01, *** p <0.001.

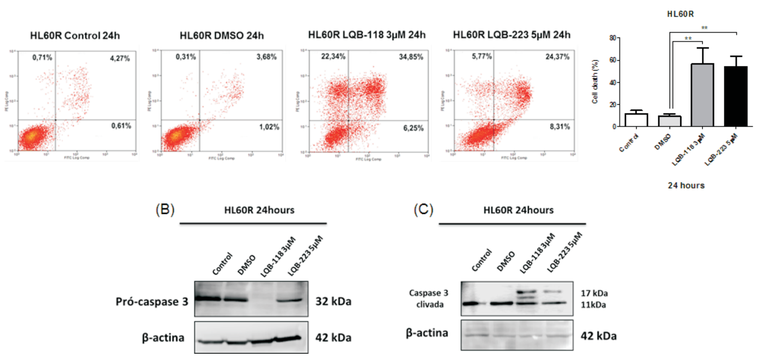

Figure 9: Evaluation of induction of apoptosis in HL60R cell line treated with new compouds LQB-118 and LQB-223 after 24h. Evaluation on cell death induced by the compounds analyzed by Anexin/PI assay (A). Evaluation of decrease of pro-caspase 3 (B) and cleavage of caspase 3 (C) by western blotting after treatment with compounds for 24 hours. Statistical analyzes of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD. Student's t-test * p <0.05, ** p <0.01, *** p <0.001 compared the DMSO.

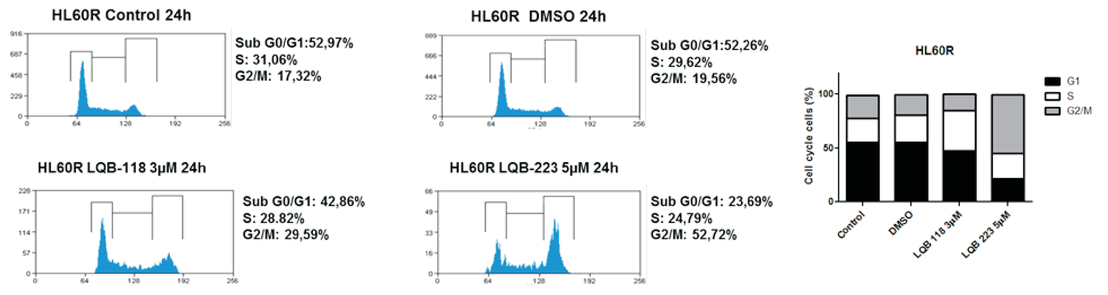

Figure 10: Evaluation of cell cycle profile in HL60R cell line treated with new compouds LQB-118 and LQB-223 after 24h. For these results were considered three independent experiments.

Financial Support: Programa de Oncobiologia (UFRJ/Fundação do Câncer), FAPERJ, INCT, CNPq, Ministério da Saúde INCA.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA