LQB-118 COMPOUND: POTENTIAL THERAPEUTIC AGENT TO OVERCOME GLIOBLASTOMA RESISTANCE

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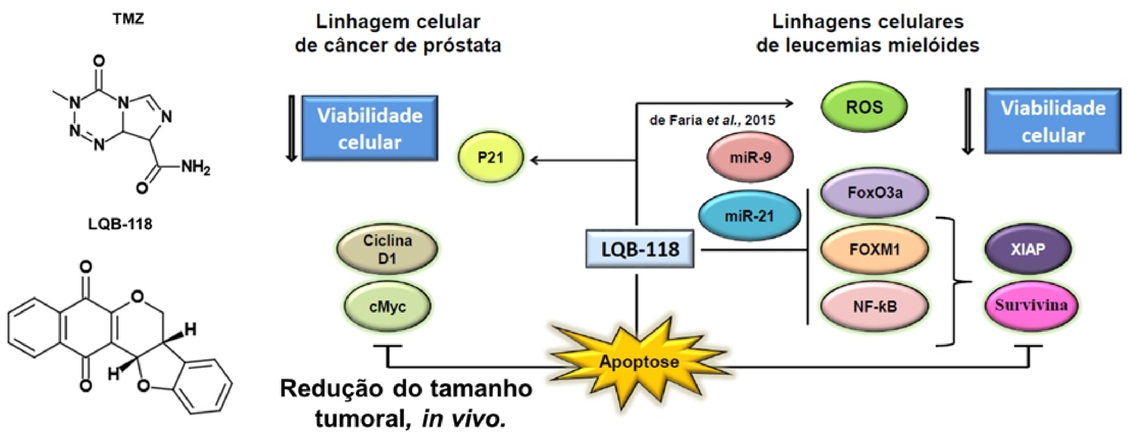
PURPOSE

Glioblastoma (GBM) is the most malignant primary brain tumor with an overall survival of only 14 months, remaining a therapeutic challenge. Treatment is based on surgery, radiotherapy and chemotherapy with temozolomide (TMZ). There is no standard of care for recurrence and the development of effective alternative therapies is essential to overcome treatment failure. In this context, synthetic compounds were developed by the Instituto de Pesquisa de Produtos Naturais from UFRJ and tested by our group and collaborators in several neoplasias. Among them, LQB-118 has antitumor activity in myeloid leukemia and prostate cancer cells with multidrug resistance (MDR) profile. The aim of the study was to evaluate the antitumoral activity of the compound LQB-118 in GBM, *in vitro*.



METHODS

Monolayer and three dimensional (3D) cell culture systems of human GBM – derived cell lines were used to evaluate LQB-118 effect on cell viability, cell death and migration. Cell viability was evaluated by MTT assay. Cell death and apoptosis were evaluated by trypan blue exclusion assay, annexin V/PI labeling and pro-caspase-7 expression. Protein expression was assessed by Western blotting. The 3D system was used to evaluate cell viability, using APH assay, and migration index.

RESULTS AND CONCLUSIONS

LQB-118 reduced cell viability and promoted apoptosis in monolayer cell lines that presented resistance to TMZ when evaluated by MTT and annexin V/PI assays. Moreover, LQB-118 reduced ERK1/2 and AKT expression and phosphorylation, while TMZ just slightly reduced ERK1/2 phosphorylation. LQB-118 also demonstrated an additional effect when associated with ionizing radiation and cisplatin, but not with TMZ. In 3D culture models, LQB-118 reduced cell viability and inhibited cell migration demonstrating its potential to impair the invasiveness of GBM tumors. In conclusion, LQB-118 is a promising agent for GBM treatment as monotherapy, and associated with radiotherapy or cisplatin, and its effect is associated with Akt and ERK pathways, in vitro. Furthermore, this compound keeps its effectiveness in 3D cell conformation that shares more similarities with the tumor mass. These results points out the potential of LQB-118 to overcome TMZ resistance.

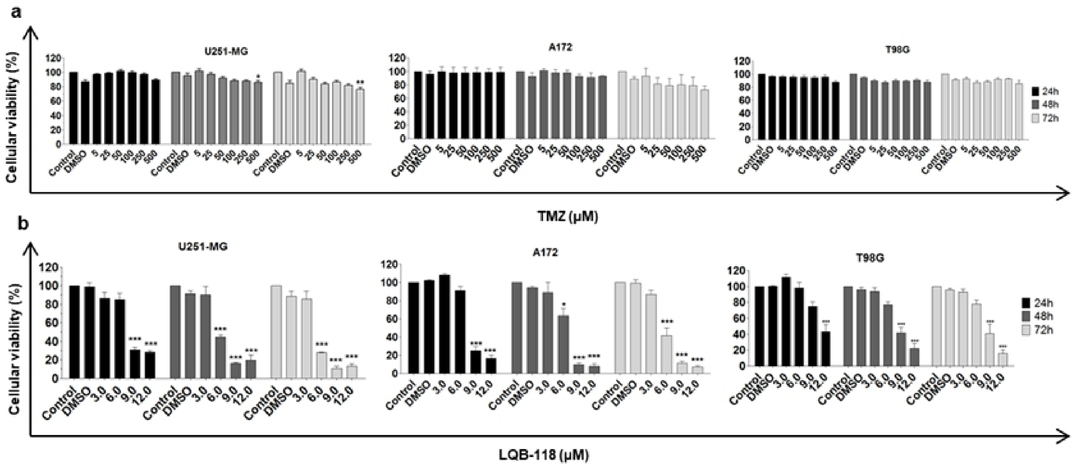


Fig. 1 Effect of LQB-118 and temozolomide (TMZ) on cell viability by MTT assay. Percentage of U251-MG, A172 and T98G viable cells after treatment with increasing concentrations of (a) TMZ and (b) LQB-118 evaluated for 24, 48 and 72 h. Mean of three independent experiments ± SEM

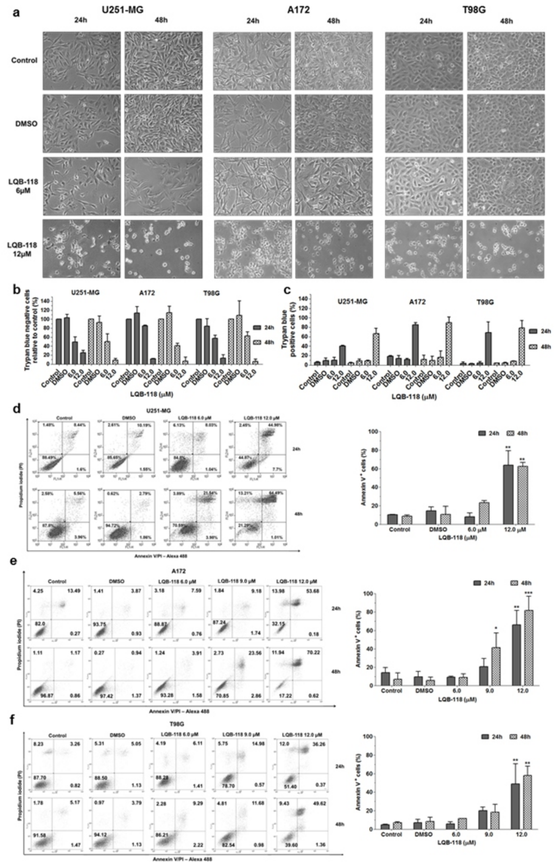


Fig. 2 Effect of LQB-118 on cell detachment and apoptosis. LQB-118 cytotoxic effect evaluated in U251-MG, A172 and T98G cells after 24 and 48h. (a) Contrast phase photomicrography from culture flasks after treatment. (b) Percentage of trypan blue negative cells in relation to control and (c) percentage of cell death inside each condition evaluated by trypan blue exclusion assay. (d-f) Percentage of annexin V positive cells (annexin V$^+$/PI$^+$ + annexinV$^+$/PI$^-$) after LQB-118 treatment in (d) U251-MG, (e) A172 and (f) T98G cells evaluated by flow cytometry. Graphics (in the right column) with mean of

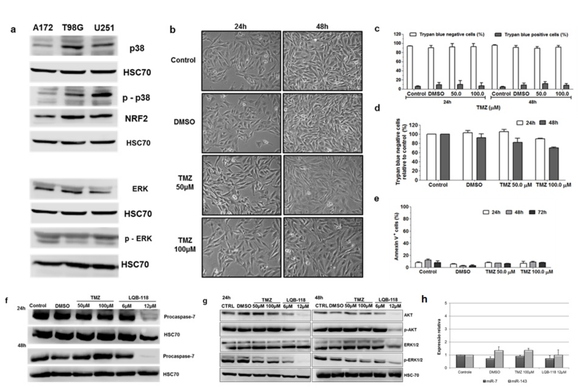


Fig. 3 Effect of TMZ and/or LQB-118 on cell death and signaling pathways' modulation. (a) Proteins basal expression levels in A172, T98G and U251 cells. After, U251-MG cells were treated with 50.0 and 100.0 μM of TMZ or 6.0 and 12.0μM of LQB-118. (b) Contrast phase photomicrography from culture flasks after treatment with TMZ. (c) Percentage of cell viability and cell death inside each condition by trypan blue exclusion assay and (d) percentage of trypan blue negative cells in relation to control. (e) Percentage of annexin V positive cells (annexinV$^+$/PI$^-$ + annexinV$^+$/PI$^+$) after TMZ treatment evaluated by flow cytometry. (f) Procaspase-7 expression and (g) Akt, pAkt, ERK1/2 and pERK1/2 expressions were analyzed after 24 h and 48 h of treatment. (h) miRNAs, miR-7 and miR-143 expression by PCR after treatment.

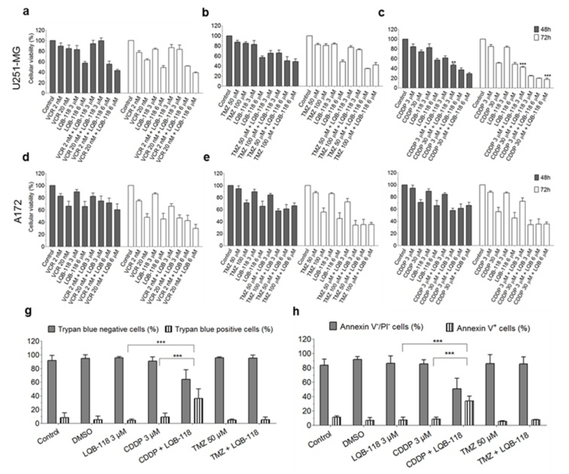


Fig. 4 Effect of LQB-118 associated with chemotherapeutic agents on cell viability and cell death. Percentage of U251-MG and A172 viable cells after treatment with LQB-118 associated with (a, d) vincristine (VCR), (b, e) temozolomide (TMZ) and (c, f) cisplatin (CDDP) and evaluated by MTT for 24, 48 and 72 h. Mean of three independent experiments ± SEM. (g) Percentage of cell viability and cell death inside each condition of U251-MG cells evaluated by trypan blue exclusion. (h) Percentage of annexin V and PI negative cells (viable cells) and annexin V positive cells (annexinV$^+$/PI$^-$ + annexinV$^+$/PI$^+$) in U251-MG cells evaluated by flow cytometry. Mean of 3 independent experiments ±SD

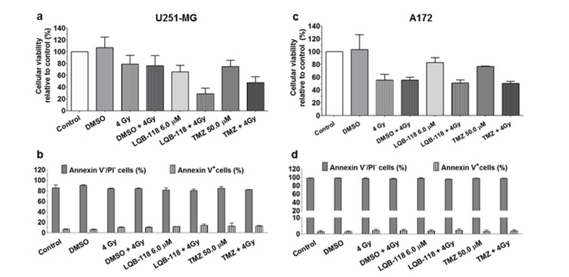


Fig.5 Effect of LQB-118 or TMZ associated with ionizing radiation on cell viability and cell death. (a, c) Percentage of U251-MG and A172 viable cells in relation to control after treatment with LQB-118 or TMZ associated with ionizing radiation evaluated by trypan blue exclusion for 48h. (b, d) Percentage of annexin V/PI negative cells (viable cells) and annexin V positive cells (annexinV$^+$/PI$^-$ + annexinV$^+$/PI$^+$) evaluated by flow cytometry. Mean of 3 independent experiments ±SD

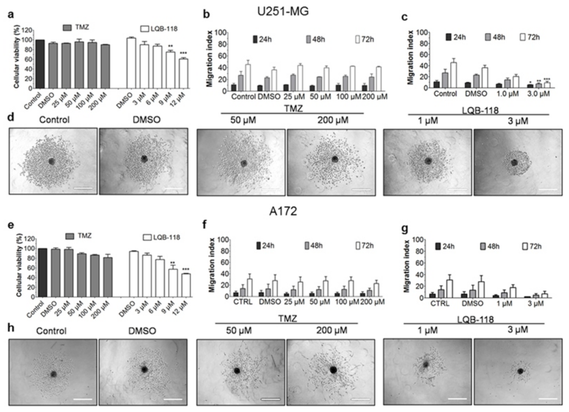
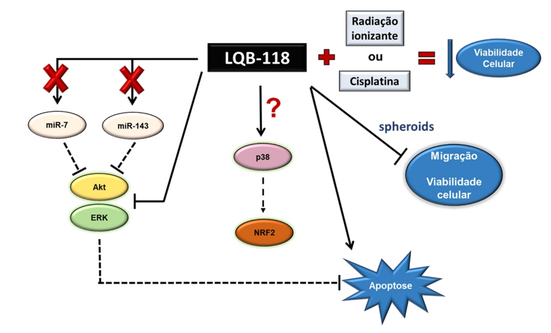


Fig.6 Effect of LQB-118 and TMZ on viability and migration index of U251-MG and A172 spheroids. Percentage of viable cells in relation to control after treatment with LQB-118 or TMZ assessed by APH assay in (a) U251-MG and (e) A172 spheroids. Migration index evaluated after 24, 48 and 72h of (b) U251-MG cells treatment with TMZ, (c) U251-MG cells treatment with LQB-118, (f) A172 cells treatment with TMZ and (g) A172 cells treatment with LQB-118. Migration index of spheroids calculated using the area at t=0h as reference. Mean of three experiments ±SEM. Representative figures of radial migration of spheroids after 72 hours of treatment in (d) U251-MG and (h) A172 spheroids. Each scale bar represents 300 μm



Projeto Gráfico: Área de edição e produção de materiais técnico-científicos / INCA

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