LQB-118 COMPOUND REDUCES VIABILITY AND MIGRATION IN 3D CULTURES OF GLIOBLASTOMA

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ABSTRACT

Background: Glioblastoma (GB) is the most common and aggressive astrocytoma with a dismal overall survival. The median survival is 14 months and only 5.1% of patients survive in five years. The development of effective alternative therapies is essential to overcome treatment failure and improve patients' survival. The purpose of the study was to evaluate the antitumoral activity of the synthetic compound LQB-118 in two and three dimensional models (2D and 3D) of glioblastoma, *in vitro*.

Methods: Human GB cell lines were used to evaluate LQB-118 antitumoral effect. 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 and microRNAs expression were assessed by Western blotting and PCR, respectively. Multicellular Tumor Spheroids, 3D cell culture system, were used to evaluate cell viability and migration index as this culture model has been demonstrated to be a great toll for drug screening. Cell viability in 3D spheroids was assessed using APH assay.

Results: LQB-118 reduced cell viability and promoted apoptosis with an effect higher than temozolomide (TMZ), the first line chemotherapy, in monolayer cells. Moreover, LQB-118 reduced ERK1/2 and AKT expression and phosphorylation, while TMZ only slightly reduced ERK1/2 phosphorylation. As the microRNAs, miR-7 and miR-143 are regulators of Akt and ERK pathways, their expression after LQB-118 treatment were evaluated. However, LQB-118 did not modulate the miRNAs expression levels. LQB-118 also demonstrated a potentiated effect when associated with ionizing radiation and cisplatin. In 3D culture models, LQB-118 reduced cell viability and inhibited cell migration.

Conclusions: Our results demonstrated that LQB-118 effect is associated with Akt and ERK pathways, although further studies are necessary to fully understand LQB-118 mechanism of action. Our study suggests that the compound LQB-118 is a promising agent for GB treatment as monotherapy and associated with radiotherapy or cisplatin. Furthermore, this compound keeps its effectiveness in 3D cell conformation that mimics more accurately the patients' tumor than monolayer cultures.

RESULTS

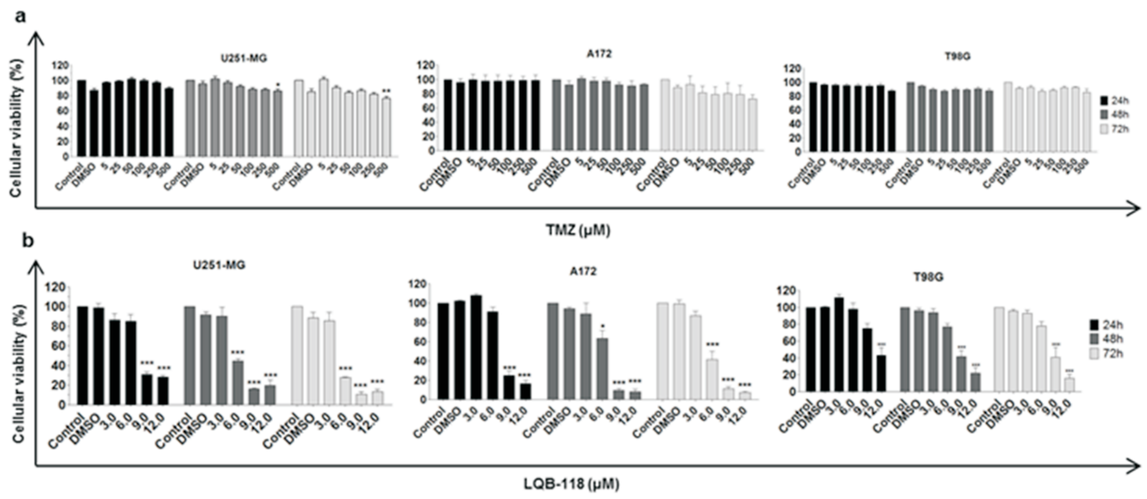

Fig. 1 Effect of LQB-118 and temozolomide (TMZ) on cell viability by MTT assay. Percentage of U251-MG, A172 and T98G viable cells after treatment with increasing concentrations of TMZ (a) and LQB-118 (b) evaluated for 24, 48 and 72 h. Mean of three independent experiments ± SEM.

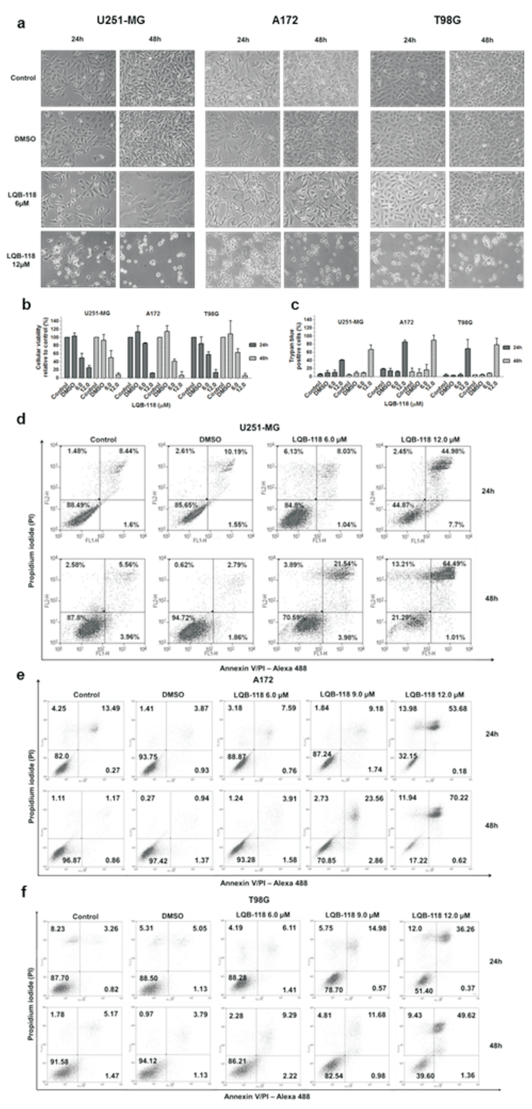

Fig. 2Effect of LQB-118 on cell detachment and apoptosis. LQB-118 cytotoxic effect evaluated inU251-MG, A172 and T98G cells after 24 and 48h. (a) Contrast phase photomicrography showing morphological features observed after treatment with LQB-118 obtained in 10 times magnification. (b) Percentage of cell viability in relation to control and (c) percentage of cell death inside each condition evaluated by trypan blue exclusion assay. (d-f) Representative dot plots of annexin V/PI assay after LQB-118 treatment in (d) U251-MG, (e) A172 and (f) T98G cells evaluated by flow cytometry.

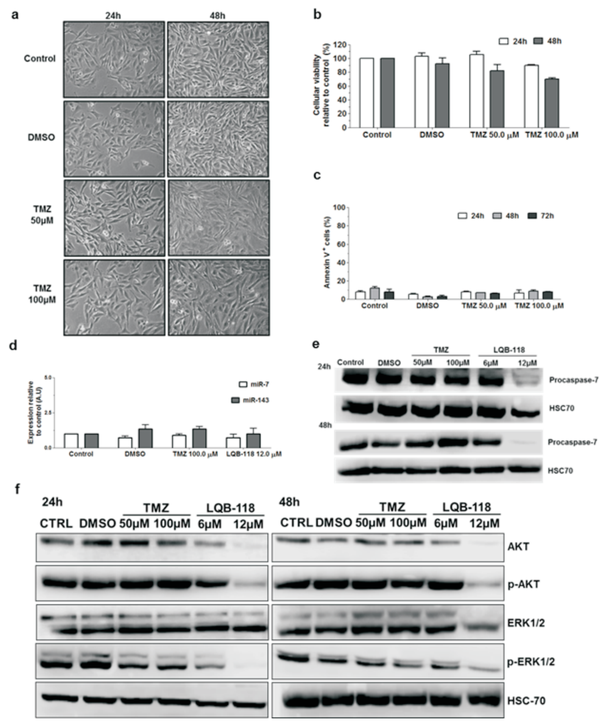

Fig. 3 Effect of TMZ and/or LQB-118 on cell death and signaling pathways' modulation in U251-MG cell line. (a) Contrast phase photomicrography showing morphological features and cell detachment from culture flasks after treatment with TMZ. (b) Percentage of cell viability in relation to control evaluated by trypan blue exclusion assay. (c) Percentage of annexin V positive cells (annexinV$^+$/ PI$^-$ + annexinV$^+$/ PI$^+$) after TMZ treatment evaluated by flow cytometry. (d) miR-7 and miR-143 expression evaluated after treatment with TMZ or LQB-118 for 24 h. microRNAs expression was normalized by the endogenous control, RNU6B. Mean of two independent experiments ± SD. (e) Procaspase-7 expression evaluated after treatment with LQB-118 or TMZ. (f) Akt, pAkt, ERK1/2 and pERK1/2 expressions were analyzed after 24 h and 48 h. HSC-70 (70kDa) expression was used as endogenous control.

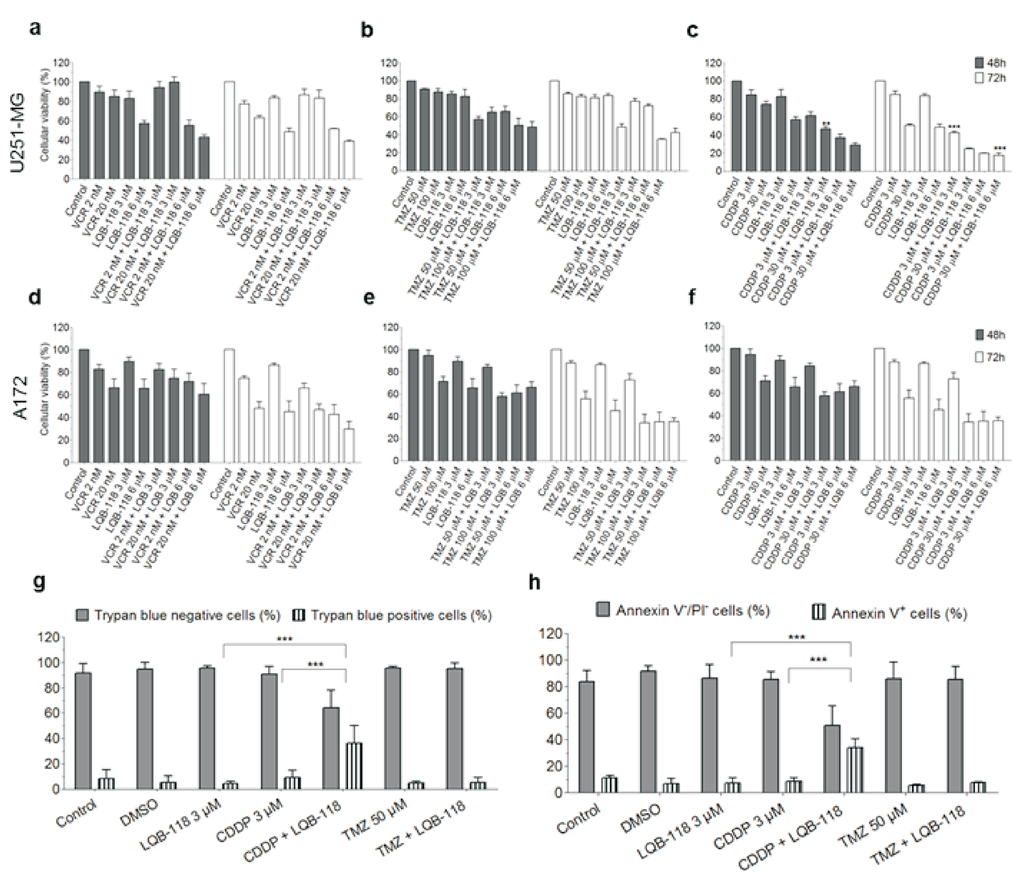

Fig. 4 Effect of LQB-118 associated with chemotherapeutic agents on cell viability and cell death. Percentage of U251-MG and A172 viable cells after treatment with LQB-118 associated with (a, b) vincristine (VCR), (c, d) cisplatin (CDDP) and (e, f) temozolomide (TMZ) evaluated by MTT for 24, 48 and 72 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.

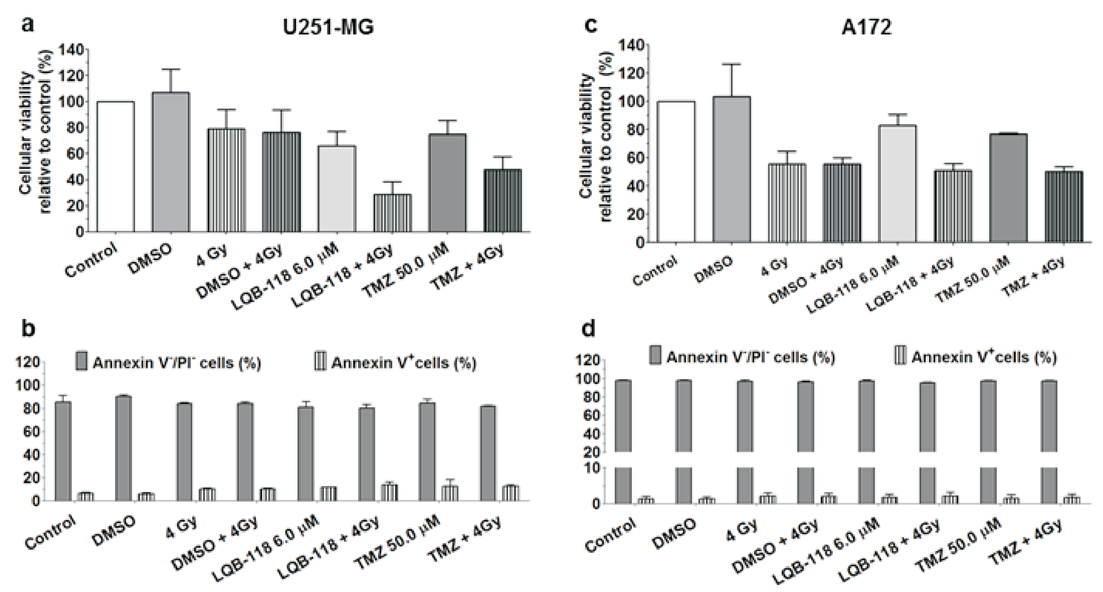

Fig.5 Effect of LQB-118 or TMZ associated with ionizing radiation on cell viability and cell death. Percentage of U251-MG and A172 viable cells in relation to control after treatment with LQB-118 or TMZ associated with ionizing radiation evaluated by trypan blue exclusion for 48h (a, c). Percentage of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

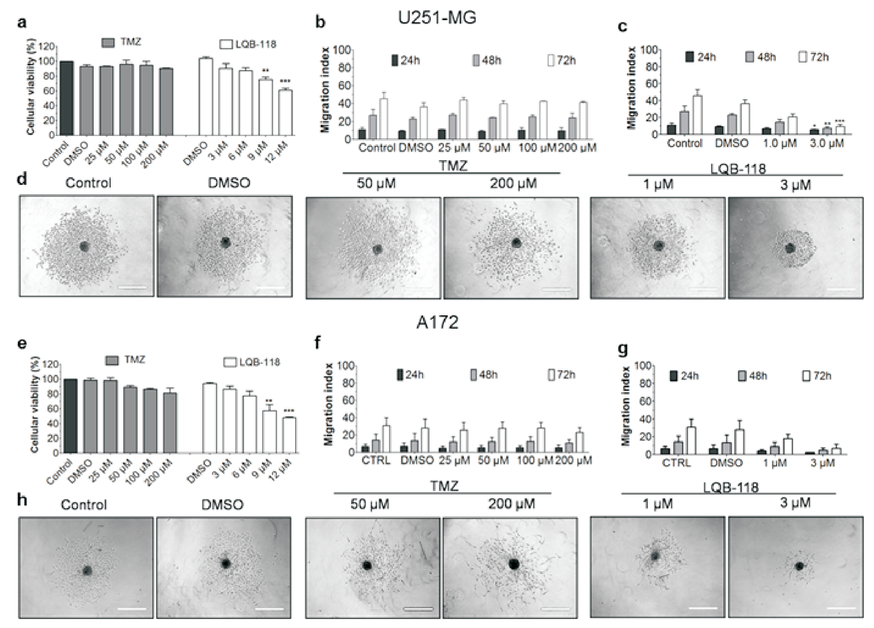

Fig.6 Effect of LQB-118 and TMZ on viability and migration index of U251-MG and A172 spheroids. Percentage of viable cells after treatment with LQB-118 or TMZ assessed by APH assay in U251-MG (a) and A172 (e) spheroids. Migration index evaluated after 24, 48 and 72h of (b) U251-MG cells treatment with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

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