EFFECT OF LQB-118 AND LQB-223 COMPOUNDS AGAINST SUBCUTANEOUS XENOGRAFT MODEL OF GLIOBLASTOMA

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INTRODUCTION AND OBJECTIVES

Glioblastoma (GB) is a highly aggressive astrocytoma. Patients with GB present a mean overall survival (OS) of 14 months, despite treatment, which is based on maximal surgical resection, followed by radiotherapy and chemotherapy with temozolomide (TMZ). Patients with GB are resistant to treatment, which partially explains the poor OS. The development of new drugs to improve the outcome of GB patients is justified by this scenario. The antitumoral effect of LQB-118 and LQB-223 compounds was evaluated in GB cell lines by our group demonstrating remarkable antitumoral effect (Figure 1, 2, 3 and 4). Therefore, to expand this work to an *in vivo* model, this project aims to evaluate the effect of compounds against subcutaneous xenografts of GB tumors.

MATERIAL AND METHODS

- Human GB cell lines: U251, A172, T98G and U87;
- Cell viability was evaluated by MTT assay;
- Cell proliferation for colony formation assay;
- DNA fragmentation by PI labeling and apoptosis by annexin V/PI labeling was evaluated by flow cytometry;
- Subcutaneous xenograft model:

PREVIOUS RESULTS

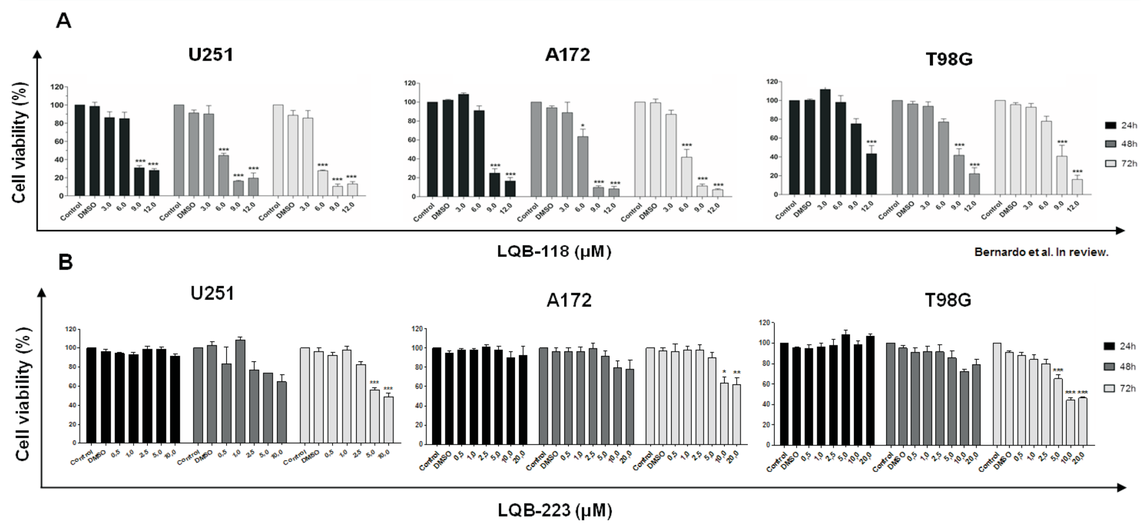

Fig. 1 LQB-118 and LQB-223 effect on cell viability. Percentage of U251, T98G and A172 viable cells after treatment with increasing concentrations of LQB-118 (A) and LQB-223 (B) for 24, 48 and 72h. The graphs represent the mean of three independent experiments ± standard error. **p*<0.05, p<0.01, ****p*<0.001 compared to DMSO.

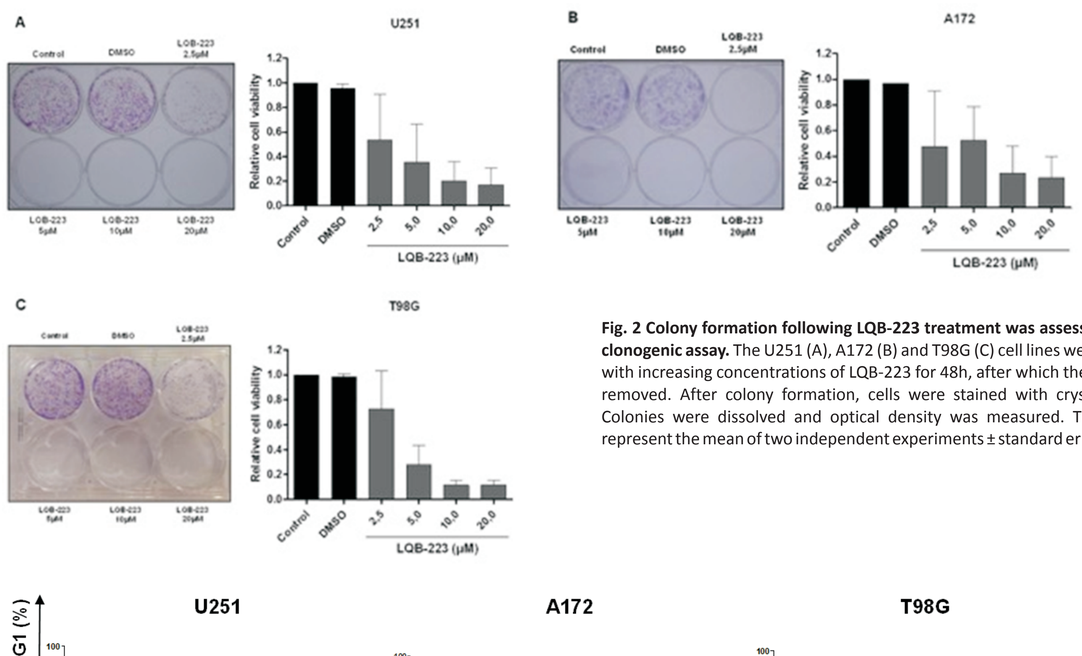

Fig. 2 Colony formation following LQB-223 treatment was assessed by the clonogenic assay. The U251 (A), A172 (B) and T98G (C) cell lines were treated with increasing concentrations of LQB-223 for 48h, after which the drug was removed. After colony formation, cells were stained with crystal violet. Colonies were dissolved and optical density was measured. The graphs represent the mean of two independent experiments ± standard error.

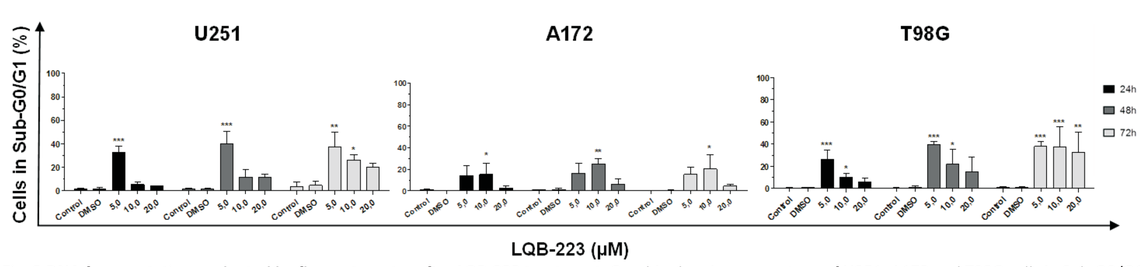

Fig. 3 DNA fragmentation evaluated by flow cytometry after LQB-223 treatment. Graphic showing percentage of U251, A172 and T98G cells in Sub-G0/G1 phase of cell cycle after exposure to 5, 10 and 20μM of LQB-223 for 24, 48 and 72h. Mean of three independent experiments ± standard error. **p*<0.05, p<0.01, ****p*<0.001 compared to DMSO

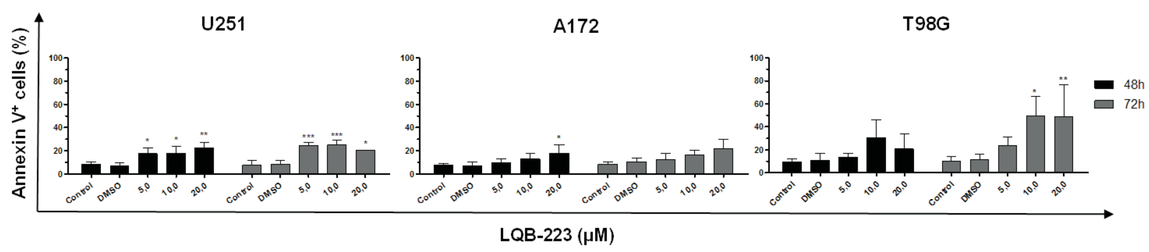

Fig. 4 Apoptosis evaluated by annexin V/PI assay after LQB-223 treatment. The U251, A172, and T98G cells were treated with 5, 10 and 20μM of LQB-223 for 48 and 72h. Graphic showing percentage of annexin positive cells (annexin V^+ cells = annexinV^+/ PI^- + annexinV^+/ PI^+) after LQB-223 treatment in U251, A172 and T98G cells evaluated by flow cytometry. Mean of three independent experiments ± standard error. **p*<0.05, ***p*<0.01, ****p*<0.001 compared to DMSO.

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

RESULTS

The experiments were approved by the Ethics Committee of Animal Experimentation from INCA (002/16). A172, U87 and U251 GB cell lines were inoculated subcutaneously in male BALB/c nude/nude mice. Standardization of GB cell lines growth demonstrated that U87 and U251 cells established subcutaneous tumor in nude mice, while A172 did not (Figure 5). The tumors from U251 cells grew more uniformly than those originated from U87 cells. For those reasons, we have chosen U251 subcutaneous xenograft model for next steps. The drugs (5mg/kg TMZ, 10mg/kg LQB-118 and 10mg/kg LQB-223) and DMSO plus saline (vehicle) were administered by oral gavage daily for 24 days (Figure 6). Tumor dimensions were measured every 3 days and tumor volume (mm^3) was calculated afterwards. Mice were euthanized by CO_2 asphyxiation and tumors were weighted. When we evaluated the mean of vehicle (1855,24 mm^3) *versus* compounds, LQB-118 (1734,23 mm^3) and LQB-223 (1818,36 mm^3), we did not observe any significant difference in tumor volumes after 24 treatment days. However, a significant decrease in mean tumor volume was observed during TMZ treatment (248,38 mm^3). Reports demonstrated that LQB-118 is well tolerated until 40mg/Kg *in vivo*. Preliminary results demonstrated that PEG (phospho ethylene glycol) is a better solvent than DMSO with saline, *in vitro* (Figure 7A). Therefore, experiments to improve compounds solubility and evaluating higher concentrations, *in vivo*, were performed (Figure 7).

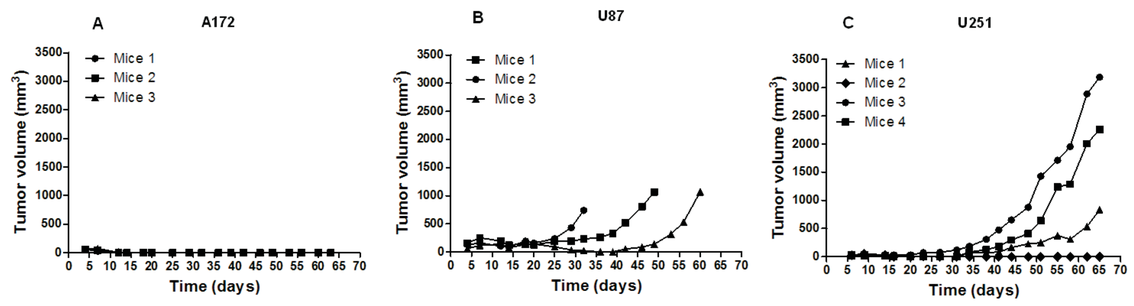

Fig. 5 Standardization of glioblastoma cell lines growth in a subcutaneous xenograft model. A172 (A), U87 (B) and U251 (C) cells (5x10^6 cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Tumor dimensions were measured every 3 days for 65 days using digital calipers. Tumor volume (mm^3) was calculated using the following formula: 0.52 x (d^2x D), where d and D are the shortest and longest diameter in mm, respectively. The graphs represent one single experiment.

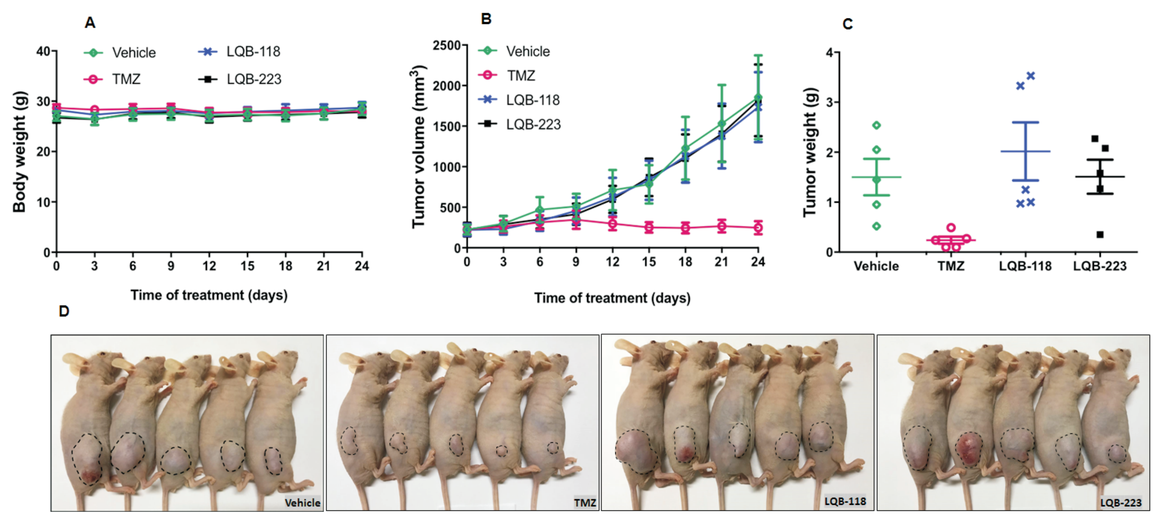

Fig. 6 Effect of LQB-118 and LQB-223 compounds against subcutaneous xenograft model of glioblastoma. U251 cells (5x10^6 cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Mice were randomized when tumors reached the mean of 220mm^3 in 4 experimental groups. Vehicle (10% DMSO + 90% PBS), TMZ (5mg/Kg), LQB-118 (10mg/Kg) and LQB-223 (10mg/Kg) were orally administrated daily for the period of 24 days. The body weights (A) and tumor dimensions of all mice were measured every 3 days for 24 days. Tumor volume (mm^3) (B) was calculated using the following formula: 0.52 x (d^2x D), where d and D are the shortest and longest diameter in mm, respectively. The mice were euthanized after 24 days of treatment, photographed (D) and their tumor were weighed (C). The graphs represent one experiment.

Fig. 7 Standardization of LQB-118 solubility enhancement using different solvents for *in vivo* experiments. U251 cells were treated with LQB-118 solubilized with four diverse solvents (DMSO, DMSO + PEG, Ethanol, Ethanol + TWEEN 80). Absorbance of viable cells (A) were measured by MTT assay. U251 cells (5x10^6 cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Mice were randomized when tumors reached the mean of 270mm^3 in 4 experimental groups. Vehicle (10% DMSO + 40% Polyethylene Glycol 80 (PEG 80) + 50% H_2O), LQB-118 (10mg/Kg, 20mg/Kg and 40mg/Kg) were orally administrated for the period of 14 days non consecutive. The body weights (B) and tumor volume (mm^3) (C) of all mice were measured every 3 days for 24 days. The mice were euthanized after 24 days of treatment and their tumor were weighed (D). The graphs represent one experiment.

CONCLUSION

Our data showed that U251 develops better tumor in vivo than U87 and A172 cell lines. LQB-118 and LQB-223 were not effective for treatment of subcutaneous xenografts when solubilized in DMSO + PBS, while TMZ demonstrated the best effect in reducing GB tumor growth, in vivo. The effect of LQB-118 against xenograft GB were improved using DMSO + PEG + H_2O as solvent.

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

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