SYNTHETIC COMPOUNDS IN THE TREATMENT OF GLIOBLASTOMA AND COLORECTAL CANCER *IN VIVO*

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Introduction and Objective

Glioblastoma (GB) is a highly aggressive astrocytoma. Patients with GB present a mean overall survival (OS) of 14 months. Patients with GB are resistant to treatment with temozolomide (TMZ) and radiotherapy, what partially explains the poor OS. The estimates of GLOBOCAN indicate that colorectal cancer (CCR) is the third most commonly diagnosed cancer and the fourth highest cause of cancer death. First line treatment of CCR is based on resection of the tumor followed by treatment with 5-FU in combination with several drugs, such as oxaliplatin, irinotecan and others. This scenario justifies the development of new drugs to improve the outcome of GB and CCR patients. The antitumoral effect of LQB-118 and LQB-223 compounds was evaluated in GB and CCR cell lines by our group demonstrating remarkable antitumoral effect, *in vitro*. Therefore, to expand this work to an *in vivo* model, this project aims to evaluate the effect of compounds against subcutaneous xenografts of GB and CCRR tumors.

Materials and Methods

The experiments were approved by the Ethics Committee of Animal Experimentation from INCA (002/16). A172, U87, U251 GB cell lines and HT-29 CCR cell lines were subcutaneously inoculated in male BALB/c nude/nude mice. The drugs, Temozolamide (only GB), LQB-118, LQB-223 and vehicle were administered by oral gavage daily for 24 days. 5-FU (only CCR) was administered by intraperitoneal injection 3 days on week for 24 days. Tumor dimensions were measured every 3 days and tumor volume (mm^3) was calculated afterwards. In the endpoint of experiment, mice were euthanized by CO2 asphyxiation and tumors were weighted.

Results

Standardization of GB and CRC cell lines growth demonstrated that U87, U251 and HT-29 cells established subcutaneous tumor in nude mice, while A172 did not. 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. After 24 treatment days, we did not observe any significant difference in tumor volumes from vehicle (1855,24 mm^3) versus compounds, LQB-118 (1734,23 mm3) and LQB-223 (1818,36 mm^3). However, a significant decrease in tumor volume was observed for TMZ treatment (248,38 mm3). We also demonstrated that polyethylene glycol (PEG) is a better solvent when compared to the solvent used in the first assay (DMSO + PBS). Thus, the GB experiment *in vivo* was repeated using the new solvent, but the results remained the same. Experiments to evaluate the synthetic compounds effect in xenograft model of CCR are ongoing.

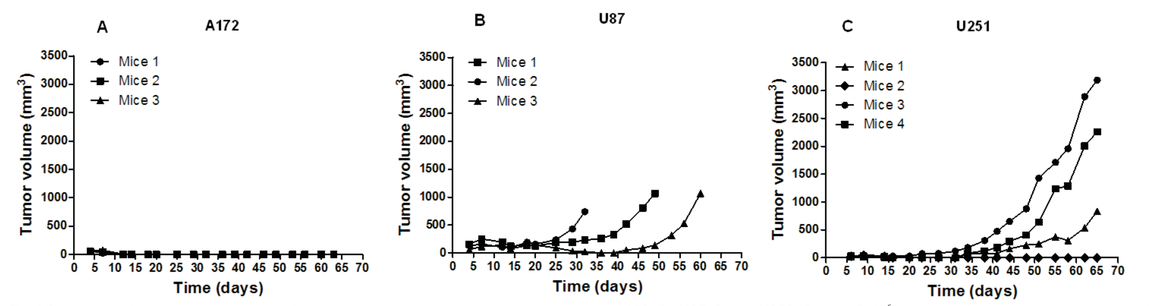

Fig. 1 Standardization of glioblastoma cell lines growth in a subcutaneous xenograft model. A172 (A), U87 (B) and U251 (C) cells ($5x10^6$ cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Tumor dimensions were measured every 3 days for 65 days using digital calipers. Tumor volume (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.

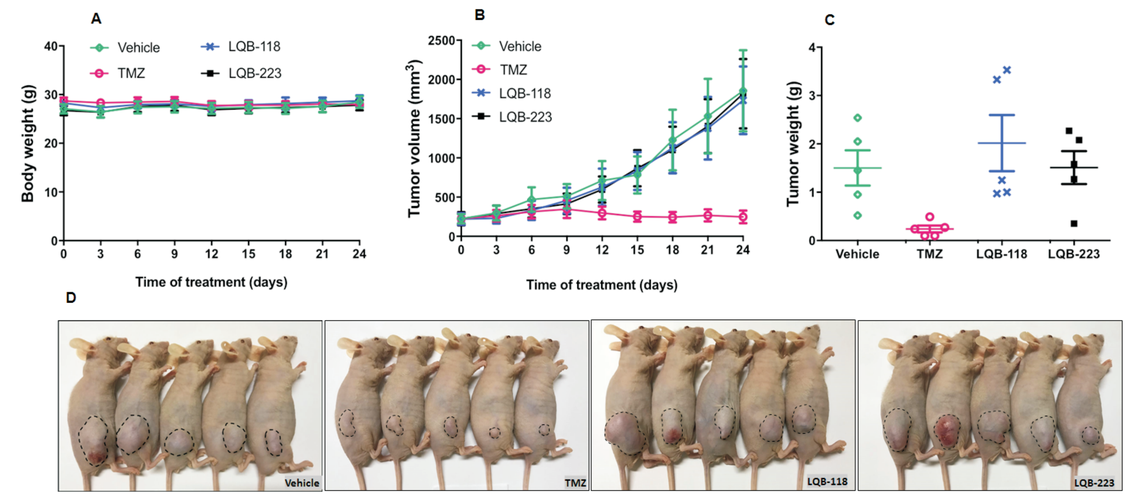

Fig. 2 Effect of LQB-118 and LQB-223 compounds against subcutaneous xenograft model of glioblastoma. U251 cells ($5x10^6$ cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Mice were randomized when tumors reached the mean of 220mm^3 in 4 experimental groups. Vehicle (10% DMSO + 90% 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.

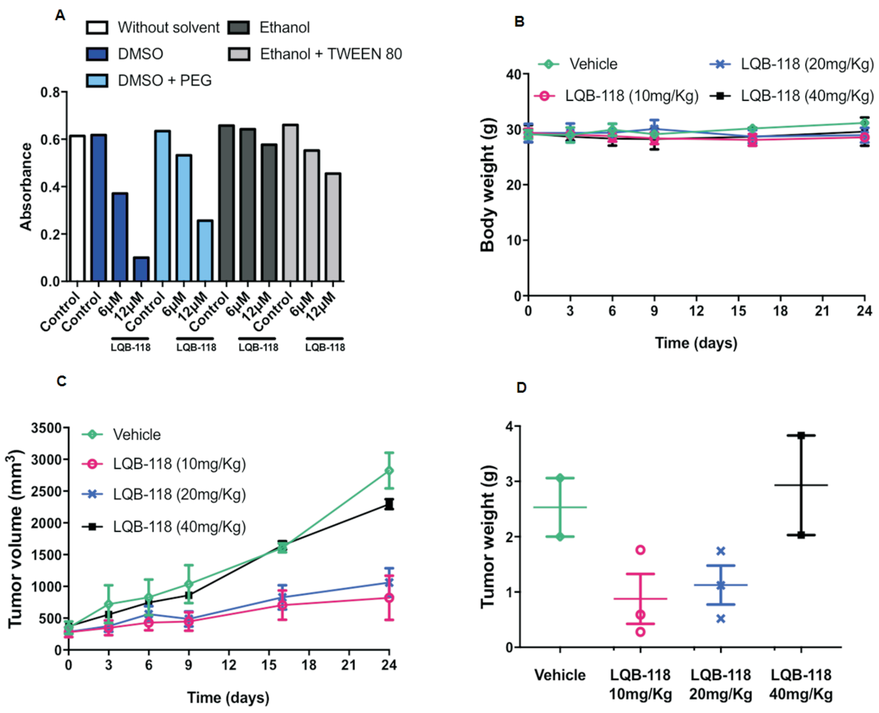

Fig. 3 Standardization of LQB-118 solubility enhancement using different solvents for *in vivo* experiments. U251 cells were treated with LQB-118 solubilized with four diverse solvents (DMSO, DMSO + PEG, Ethanol, Ethanol + TWEEN 80). Absorbance of viable cells (A) were measured by MTT assay. U251 cells ($5x10^6$ 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.

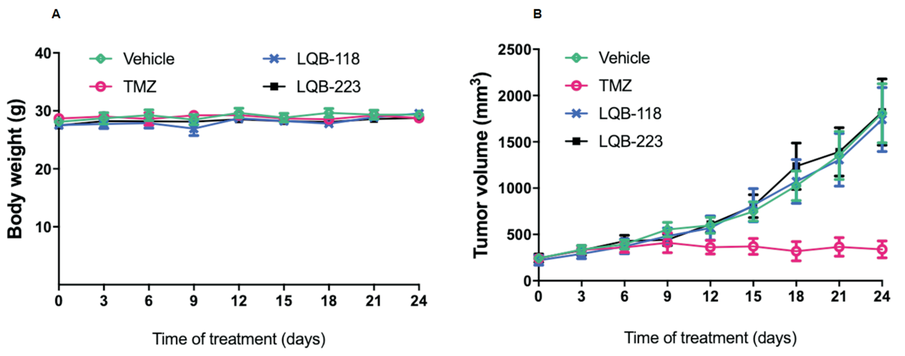

Fig. 4 Effect of LQB-118 and LQB-223 compounds against subcutaneous xenograft model of glioblastoma using the solvent DMSO + PEG. U251 cells ($5x10^6$ cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Mice were randomized when tumors reached the mean of 220mm^3 in 4 experimental groups. Vehicle (10% DMSO + 40% PEG 6000 + 50% H_2O), 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 graphs represent one experiment.

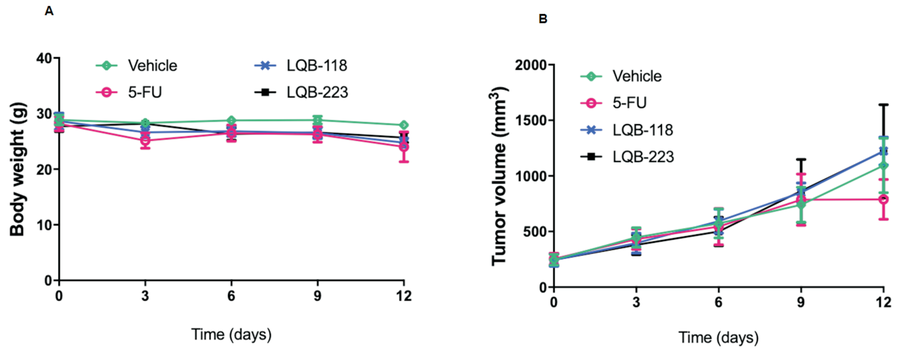

Fig. 5 Effect of LQB-118 and LQB-223 compounds against subcutaneous xenograft model of colorectal cancer using the solvent DMSO + PEG. HT-29 cells (5x106 cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Mice were randomized when tumors reached the mean of 220mm^3 in 4 experimental groups. Vehicle (10% DMSO + 40% PEG 6000 + 50% H_2O), LQB-118 (10mg/Kg) and LQB-223 (10mg/Kg) were orally administrated daily for the period of 12 days. 5-FU (50 mg/Kg) was administered via intraperitoneal in the day 0, 1, 2, 7, 8 and 9. The body weights (A) and tumor dimensions of all mice were measured every 3 days for 12 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 graphs represent one experiment.

Conclusion

Our data showed that U251, U87 and HT-29 develops tumor *in vivo*. However, synthetic compounds were not effective for treatment of subcutaneous GB xenotransplant, indicating the need for a better understanding of the pharmacokinetic processes in order to validate this result.

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