Therapeutic effect of LQB-118 and LQB-223 compounds in vivo: Standardization of glioblastoma cell lines growth in a subcutaneous xenograft model

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INTRODUCTION AND OBJECTIVE

Glioblastoma (GB) is a highly aggressive grade IV 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 adjuvant chemotherapy with temozolomide. Patients with GB are resistance to treatment, which explains the low OS rate. This justifies our interest in the development of new drugs to improve the outcome of GB patients. To this end, LQB-118 and LQB-223 compounds were synthesized by collaborative groups, and the *in vitro* antitumoral effect was evaluated in GB cell lines by our group. Our results demonstrated that LQBs have a great antitumor potential by inhibiting cell viability and proliferation (Figures 1 and 2), and inducing apoptotic cell death (Figures 3 and 4). Therefore, to expand this work to an *in vivo* model, this project aims to evaluate the therapeutic effect of compounds against subcutaneous xenografts of GB tumors in nude mice.

PREVIOUS RESULTS

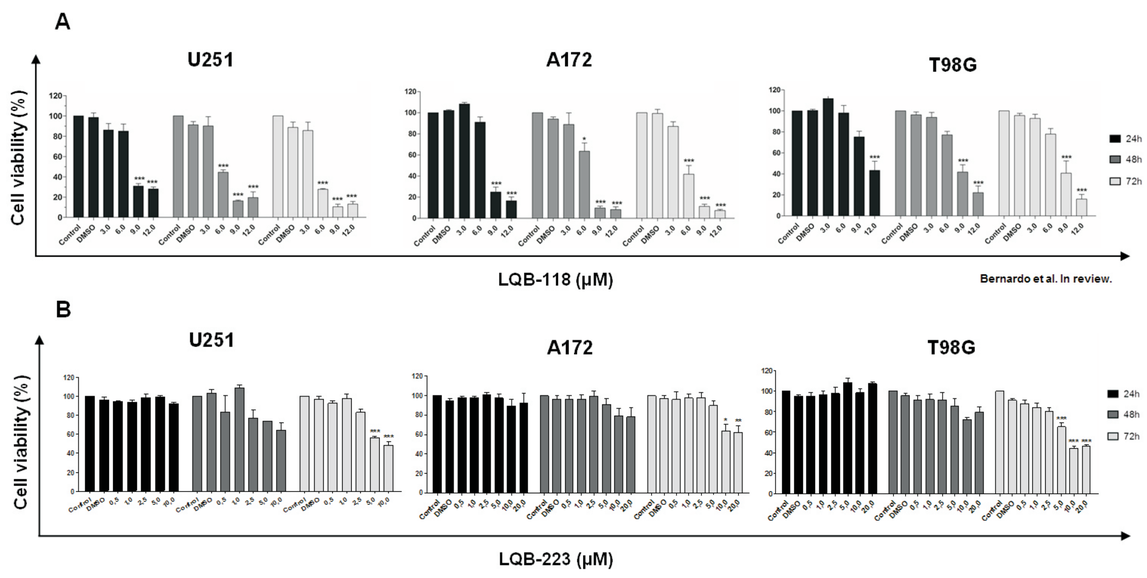

Fig. 1 LQB-118 and LQB-223 effect on cell viability. Percentage of U251, T98G and A172 viable cells after treatment with increasing concentrations of LQB-118 (A) and LQB-223 (B) for 24, 48 and 72h. The graphs represent the mean of three independent experiments ± standard error. **p*<0.05, p<0.01, ****p*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

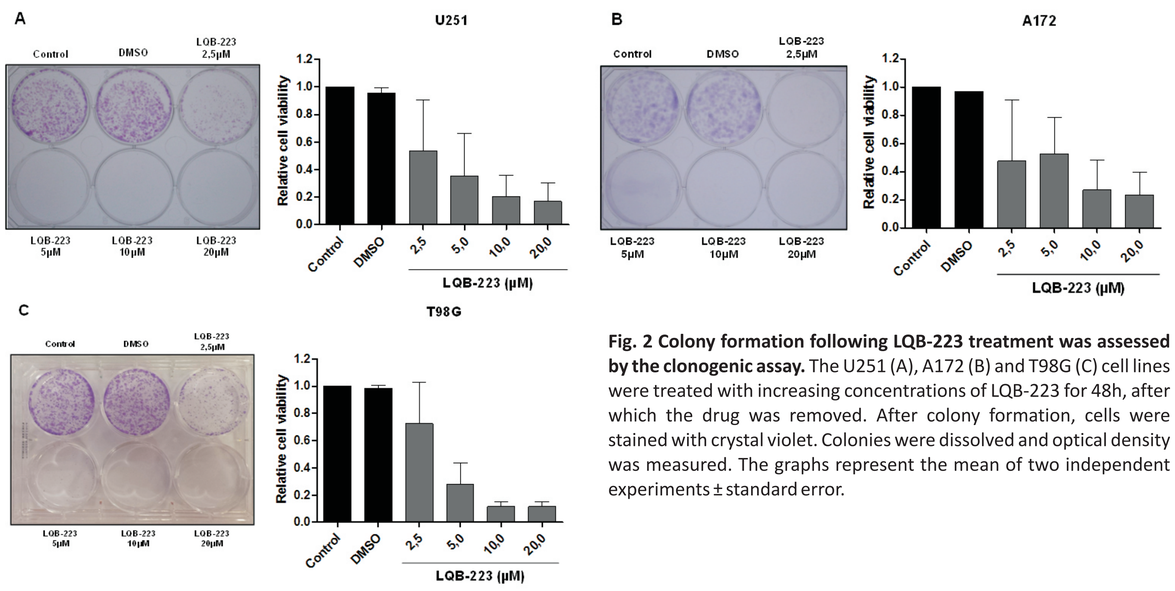

Fig. 2 Colony formation following LQB-223 treatment was assessed by the clonogenic assay. The U251 (A), A172 (B) and T98G (C) cell lines were treated with increasing concentrations of LQB-223 for 48h, after which the drug was removed. After colony formation, cells were stained with crystal violet. Colonies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

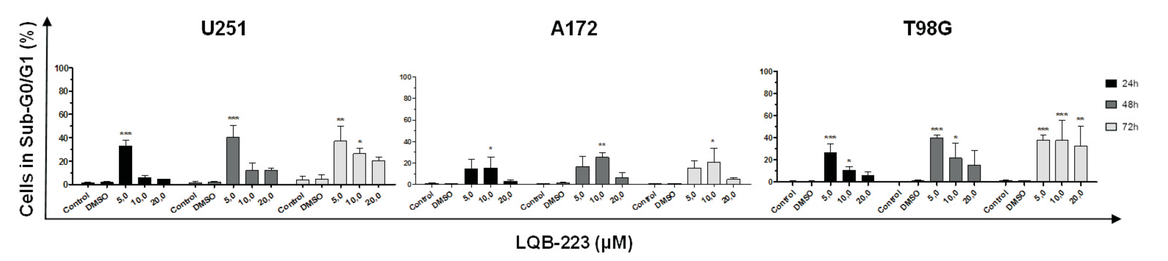

Fig. 3 DNA fragmentation evaluated by flow cytometry after LQB-223 treatment. Graphic showing percentage of U251, A172 and T98G cells in Sub-G0/G1 phase of cell cycle after exposure to 5, 10 and 20μM of LQB-223 for 24, 48 and 72h. Mean of three independent experiments ± standard error. **p*<0.05, p<0.01,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

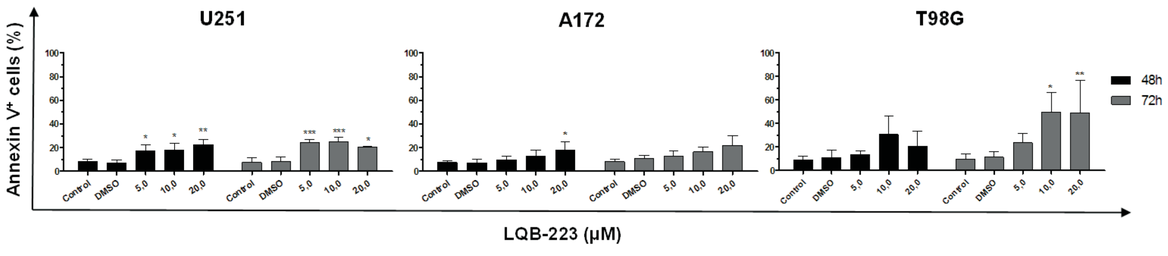

Fig. 4 Apoptosis evaluated by annexin V/PI assay after LQB-223 treatment. The U251, A172, and T98G cells were treated with 5, 10 and 20μM of LQB-223 for 48 and 72h. Graphic showing percentage of annexin positive cells (annexin V$^+$ cells = annexinV$^+$/ PI$^-$ + annexinV$^+$/ PI$^+$) after LQB-223 treatment 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.

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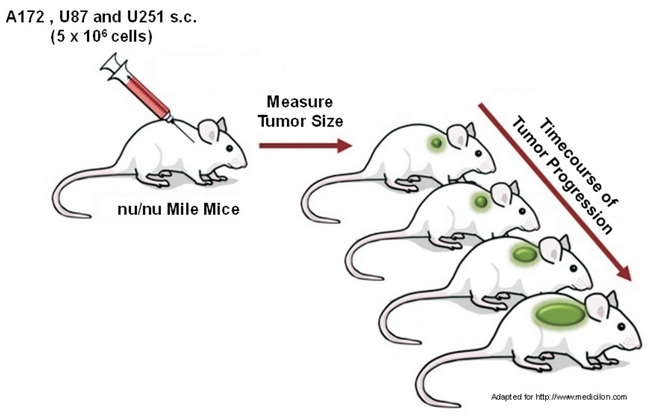
MATERIALS AND METHODS

- 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:



RESULTS

We began the study with standardization experiment of GB cell lines growth in vivo. U87 and U251 cell lines (Figures 5 B and C) established subcutaneous tumor in nude mice, while the A172 cell line (Figure 5C) did not establish until sixty days after inoculation. Tumor growth was observed in all mice inoculated with U87 cell line. However, U251 cell line presented tumor growth in three of four inoculated mice. The subcutaneous tumors growth originated from U251 cells were more uniform than those observed from U87 cells. In addition, among the four nude mice inoculated with U87 cells, two mice showed signals of tumor necrosis before the tumor has reached its maximum permissible size. The tumor growth of U251 cell line remained undetectable until thirty days after inoculation and grew exponentially until sixty-five days.

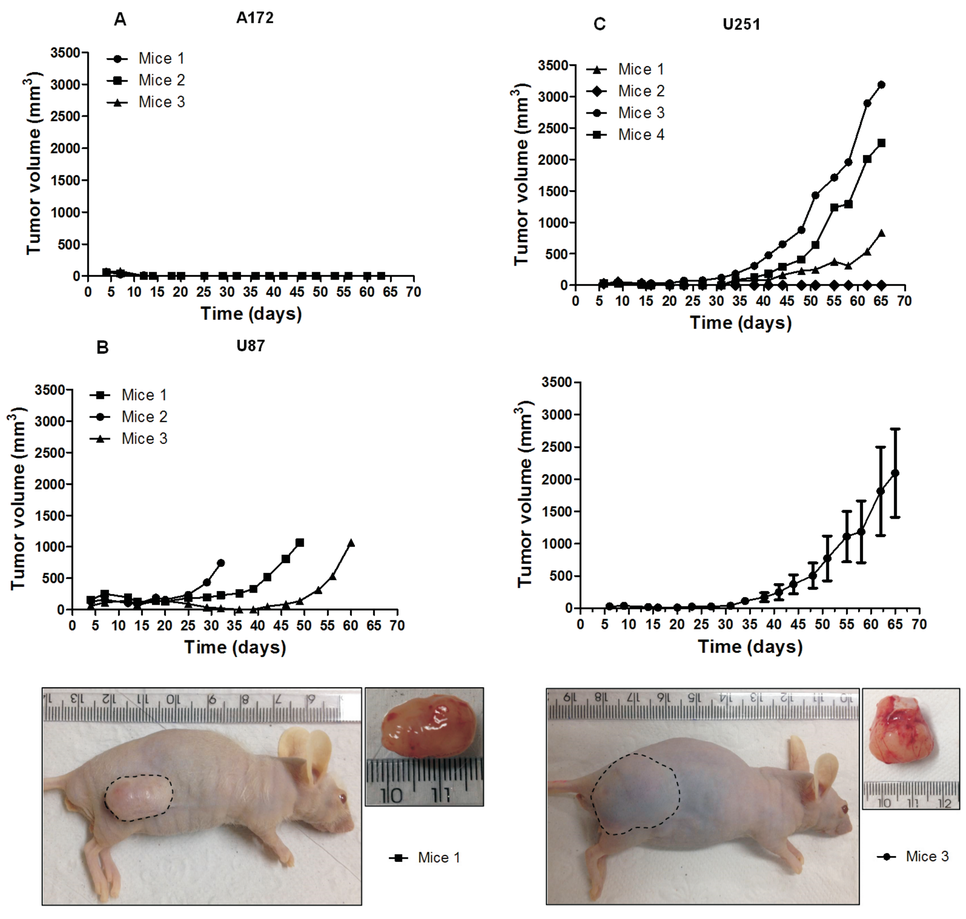


Fig. 5 **Standardization of glioblastoma cell lines growth in a subcutaneous xenograft model.** A172 (A), U87 (B) and U251 (C) cells (5x10^6 cells) were inoculated subcutaneously in male BALB/c nude/nude mice. Tumor dimensions were measured every 3 days for 65 days using digital calipers. Tumor volume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

CONCLUSION

Taken together, our data showed that U251 cell line develops better tumor in vivo than the U87 cell line. Therefore, we have chosen the U251 cell line to continue our studies.

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