Antitumor activity of Toluquinol

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Introduction

Colorectal cancer (CRC) is the third most common cause of cancer mortality worldwide, with more than 1.85 million cases and 850.000 deaths annually[1]. Currently, the primary methods for treating CRC are surgery, radiotherapy and chemotherapy, with targeted drugs playing an important role in the treatment of CRC. The most commonly used targeted drugs are directed against EGFR and VEGF[2], but limitations have been observed with these drugs due to problems arising from drug resistance. Therefore, it is an urgent need to explore alternative signaling pathways and discover novel effective compounds[2]. In this context, it has been shown that the occurrence of CRC is linked to the PI3K/AKT/mTOR signaling pathway, one of the main drivers and regulators of tumor cell proliferation, growth, migration, survival and metabolism. Additionally, 60-70% of colon cancer patients show an activation of the AKT pathway and a reduction of PTEN expression levels[2]. Therefore, inhibitors of the PI3K/Akt/mTOR signaling have been suggested as potential therapeutic agents in the treatment of colorectal cancer[1,2]. Our continuing efforts to identify new anti-tumor drugs led us to focus on Toluquinol, a natural compound present in both fungi and plants, which has been previously characterized by us as an angiogenesis and lymphangiogenesis inhibitor[3,4], as well as a compound displaying anti-inflammatory properties. In the present study, we have evaluated the anti-tumor effect of Toluquinol on a human colorectal cell line (HT29). Interestingly, we have observed that Toluquinol is able to modulate survival, proliferation, migration and apoptosis in HT29 cells, interfering with Akt/mTOR pathway. Altogether, out data suggests that Toluquinol is a promising drug candidate with potential for inhibiting colorectal cancer cell progression.

Methods

Cell survival Assay (MTT)

HT29 cells were treated during 72h with Toluquinol, and MTT colorimetric method was used to determinate the IC50 value.

Proliferation Assay (EdU)

HT29 cells were treated during 24 h with Toluquinol and labeled with 10 µM EdU for 2 h before fixation. Thereafter, cells were permeabilized, and analyzed by flow citometry acording to baseclick EdU Flow Cytometry Kit.

Cell migration (Boyden chamber)

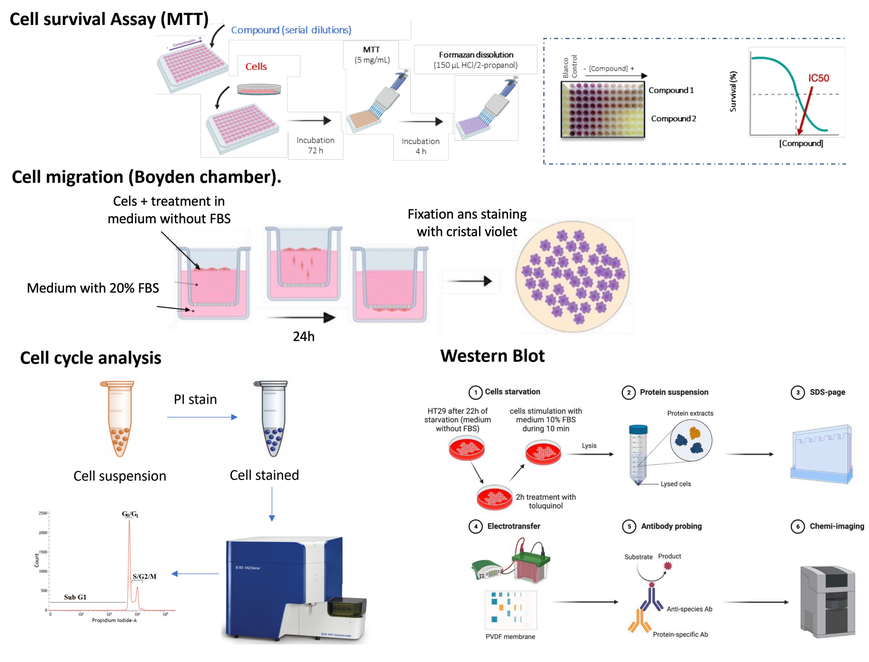
The migratory activity of HT29 cells was assessed using a Boyden chamber. Treated or untreated cells were seeded in a transwell insert with medium without FBS and with 20% FBS in lower well. After 24h cells were fixed and filters were stained with 1% of crystal violet.

Cell cycle analysis

Cell cycle analysis was assessed in untreated and treated HT29 for 24 h, after propidium iodide staining, and samples were analyzed by flow cytometry.

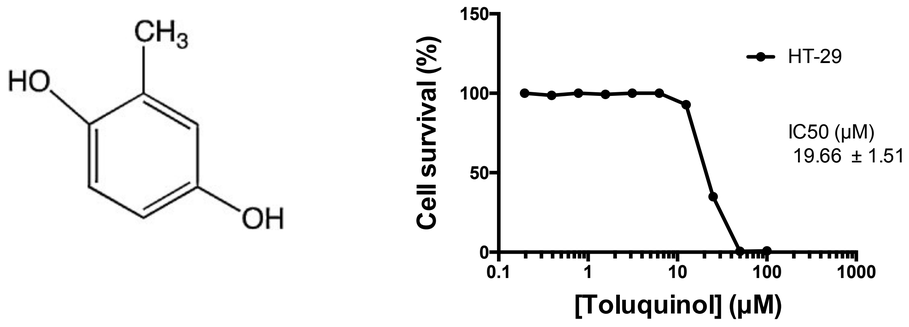
Western Blot

HT29 were starved during 24h in DMEM without FBS, and toluquinol was added during 2 h at the end of the starvation. Then, the cells were stimulated during 10 minutes with DMEM 10% FBS. Cells were lysated and subjected to SDS-PAGE electrophoresis, transferred to PVDF membranes. After being blocked and incubated with the corresponding antibodies, immunoreactive bands were detected using chemiluminescence systems.



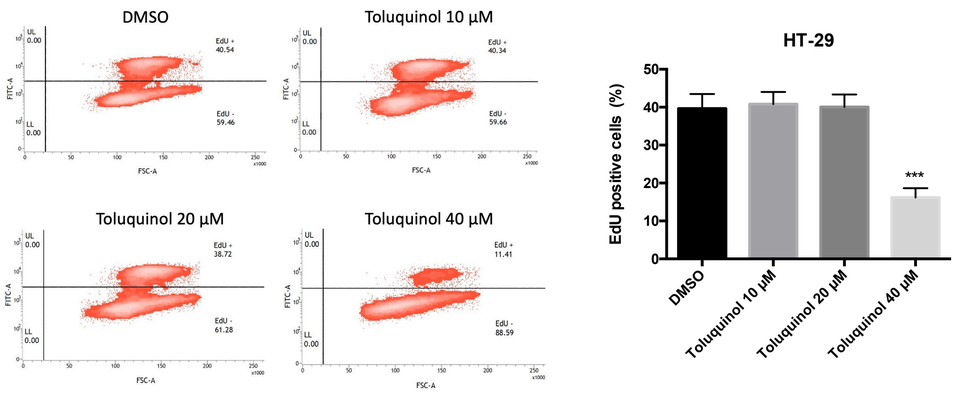
Results

Cell survival Assay (MTT)



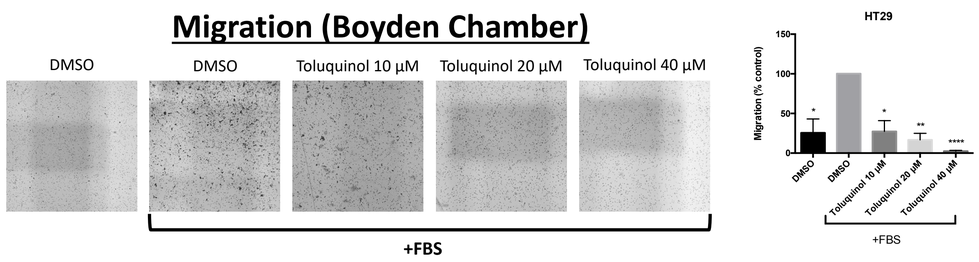
(Left) Chemical structure of Toluquinol. (Right) Representative survival curve of HT29 in presence of increasing concentrations of Toluquinol. Data are expressed in terms of percentage of cell survival with respect to control cells. The figure illustrates a representative experiment performed with quadruplicate samples; SD values were typically lower than 10% of the mean values and are omitted for clarity. Half-maximal inhibitory concentration (IC50) values of toluquinol which were calculated from dose-response curves as the concentration of compound yielding 50% of control cell survival.

Proliferation Assay (EdU)



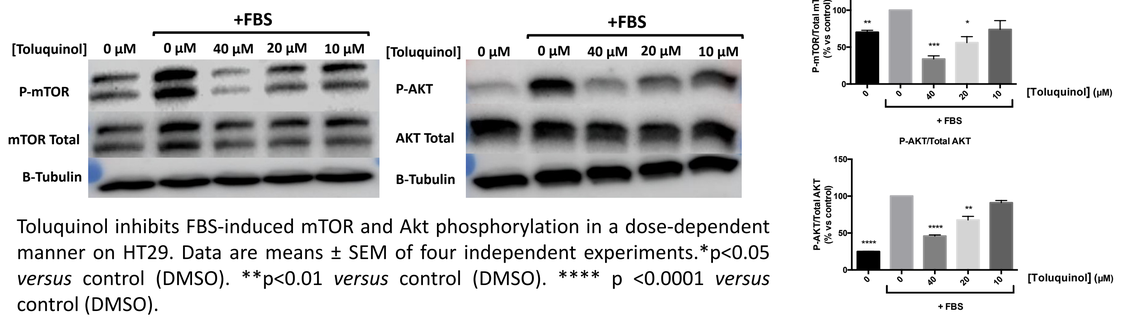
Toluquinol inhibits HT29 proliferation. (Left) Representative Fluorescence histograms of EdU incorporation by HT29 at the indicated concentrations of Toluquinol. (Right) Quantification of incorporated EdU for each treatment. Data are means ± SEM of at least three independent experiments (***p < 0.001).

Migration (Boyden Chamber)



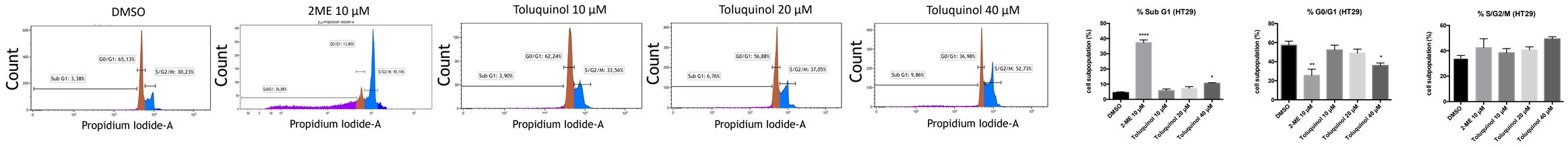
Toluquinol inhibits HT29 migration in a dose-dependent manner. Data are means ± SEM of four independent experiments.*p <0,05 *versus* (DMSO) control. ** p <0,01 *versus* control (DMSO). **** p <0.0001 *versus* control (DMSO).

Western Blotting



Toluquinol inhibits FBS-induced mTOR and Akt phosphorylation in a dose-dependent manner on HT29. Data are means ± SEM of four independent experiments.*p<0.05 *versus* control (DMSO). **p<0.01 *versus* control (DMSO). **** p <0.0001 *versus* control (DMSO).

Cell cycle analysis



Toluquinol cause an increase in subG1 population. These data suggest that toluquinol induce apoptosis in HT29. Data are means ± SEM of at least three independent experiments.*p <0.05 *versus* (DMSO) control. **p <0.01 *versus* control (DMSO). ****p <0.0001 *versus* control (DMSO).

Conclusion

Our results show that toluquinol is able to inhibit key steps of tumor progression, such as survival, proliferation and migration, as well as being able to induce apoptosis and inhibit the Akt/mTOR pathway in HT29 human colorectal cancer cells.

Therefore, although further studies are still required, toluquinol may be a potential therapeutic agent for the treatment of CRC.

References

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