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ANTITUMORSKI POTENCIJAL HALOGENIRANOG BOROKSINA

ANTITUMOR POTENTIAL OF HALOGENATED BOROXINE

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SAŽETAK

Uvod: Potraga za novim terapeuticima u liječenju kancera je stalno u fokusu istraživanja. Dosadašnja istraživanja su pokazala da je halogenirani boroksin - K2(B3O3F4OH) efikasan antiproliferativni agens prema tumorskim ćelijskim linijama, sa blagim antiproliferativnim dejstvom prema netumorskim ćelijskim linijama, na koji inhibitorno djeluju visoke koncentracije kalcijumovih jona.

Cilj rada: Ovim istraživanjem se ispitao uticaj halogeniranog boroksina na indukciju autofagije, odnosno ekspresiju autofagno vezanih gena, kao potencijalnog citotoksičnog mehanizma halogeniranog boroksina prema tumorskim ćelijskim linijama, kao i metabolički fenotip. Studija je sprovedena na humanim tumorskim i netumorskim ćelijskim linijama.

Metode rada: U log fazi ćelije humanog karcinoma mokraćnog mjehura 5637, humanog melanoma GR-M i limfociti periferne krvi su pasažirani i nakon 24h inkubacije tretirani. Korištene su koncentracije halogeniranog boroksina od 0,05, 0,1, 0,2 0,4 i 0,8 mg/ml. Negativna kontrola je predstavljala ćelije u normalnom fiziološkom stanju sa kompletnim medijumom, dok je pozitivnoj kontroli izazvana autofagija gladovanjem, ćelije su uzgajane bez fetalnog goveđeg seruma. Za potrebe evaluacije citotoksičnosti halogeniranog boroksina Alamar blue test. Detekcija autofagije je rađena pomoću komercijalnog eseja za detekciju signala autofagozoma na epifluorescentnom mikroskopu. Glikoliza i oksidativna forsforilacija su mjerene komercijalnim The XFCell Energy Phenotype testom. Mjerenje relativne genske ekspresije BECN1, BCL-2, p62/SQSTM i DRAM1 gena je rađeno real-time PCR-om.

Rezultati: Halogenirani boroksin ima specifično citotoksično dejstvo prema tumorskim ćelijama i neznatno citotoksično dejstvo prema netumorskim ćelijama. Procenat inhibicije proliferacije ćelija raste povećanjem koncentracije halogeniranog boroksina, te ima dozno specifičan efekat. Eksperimenti potvrđuju da halogenirani boroksina utiče na stopu proliferacije tumorskih ćelija preko autofagije i modifikuje metabolički fenotip. Analiza genske ekspresije je pokazala da je efikasnost halogeniranog boroksina vezana za značajne promjene u ekspresiji autofagno specifičnih gena.

Zaključak: Halogenirani boroksin remeti bioenergetski metabolizam, mijenja ekspresiju gena i dovodi do povećanja osjetljivosti tumorskih ćelija ka apoptozi.

Ključne riječi: halogenirani boroksin, autofagija, citotoksičnost, tumorske ćelije

ABSTRACT

Introduction: The search for new therapeutic agents in cancer treatment remains a central focus of research. Previous studies have shown that halogenated boroxine – $K_2(B_3O_3F_4OH)$ – is an effective antiproliferative agent against tumor cell lines, with mild antiproliferative effects on non-tumor cell lines, which are inhibited by high concentrations of calcium ions.

Aim of the study: This research investigated the effect of halogenated boroxine on the induction of autophagy, specifically the expression of autophagy-related genes, as a potential cytotoxic mechanism of halogenated boroxine against tumor cell lines, as well as the metabolic phenotype. The study was conducted on human tumor and non-tumor cell lines.

Methods: After the initial 24 h, human bladder cancer 5637 cells, human melanoma GR-M cells, and peripheral blood lymphocytes, were treated with halogenated boroxine at final concentrations of 0.05, 0.1, 0.2, 0.4, and 0.8 mg/mL and incubated for an additional 24 h. The negative control consisted of cells in normal physiological conditions with complete medium, while the positive control was established by inducing autophagy through starvation, culturing the cells without fetal bovine serum. Cytotoxicity of halogenated boroxine was evaluated using the Alamar Blue assay. Autophagy detection was performed using a commercial assay for autophagosome signal detection under an epifluorescence microscope. Glycolysis and oxidative phosphorylation were measured using the commercial XF Cell Energy Phenotype Test. The relative gene expressions of BECN1, BCL-2, p62/SQSTM, and DRAM1 genes were measured using real-time PCR.

Results: Halogenated boroxine exhibits specific cytotoxic effects on tumor cells and minimal cytotoxic effects on non-tumor cells. The percentage of cell proliferation inhibition increases with higher concentrations of halogenated boroxine, demonstrating a dose-dependent effect. Experiments confirm that halogenated boroxine affects tumor cell proliferation via autophagy and modifies the metabolic phenotype. Gene expression analysis revealed that the efficacy of halogenated boroxine is associated with significant changes in the expression of autophagy-specific genes.

Conclusion: Halogenated boroxine disrupts bioenergetic metabolism, alters gene expression, and increases the sensitivity of tumor cells to apoptosis.

Keywords: halogenated boroxine, autophagy, cytotoxicity, tumor cells