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Growth suppression activity of bradykinin antagonists in glioma cells

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*Conventional glioblastoma treatment still gives very modest results, thus development of the new treatment modalities is desperately needed for these disease. The present study was aimed at analyzing the effect of bradykinin (BK) antagonists on proliferation of the human glioblastoma cells U373. **Methods.** MTT-based cell proliferation assay. **Results.** BKM-570 revealed a significant antiproliferative activity in the U373 cells with LC₅₀ 3,8 M. **Conclusions.** The antiproliferative properties of BK antagonists were shown in vitro using the glioma cells. Further investigations of the molecular mechanisms of their action and pre-clinical studies on animal models are needed for the evaluation of these compounds as new anti-cancer drugs.*

Keywords: bradykinin antagonists, glioma cells, antiproliferative activity.

Introduction. Glioblastoma is the most common and lethal form of the central nervous system tumors, and is known to be highly therapy resistant. Development of the new treatment modalities is desperately needed for these diseases.

Nonapeptide bradykinin (BK) is known to stimulate cancer cells growth [1] as well as their migration and invasion by inducing the activity of matrix metalloprotease (MMP) [2]. BK is linked to the formation of new blood vessels by stimulating the release of vascular endothelial growth factor (VEGF) [2]. These activities operate together to promote strongly the growth and invasive properties of certain tumor types, especially gliomas, thereby place BK in an ideal position for the generating of anti-cancer drugs.

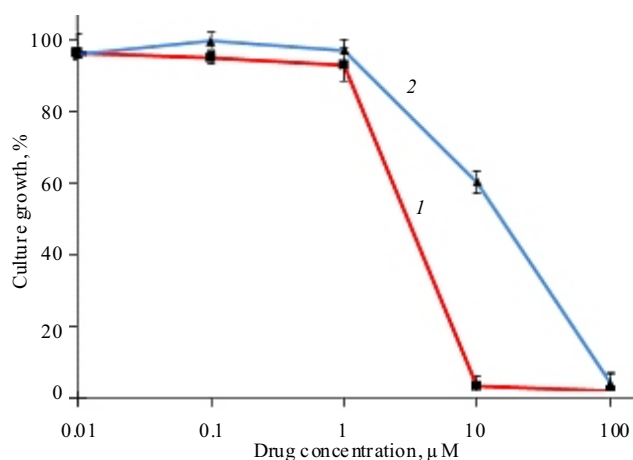
The BK antagonists with impressive inhibition of the human cancer cell lines and the mouse tumor xenograft growth have been developed [3]. Despite the inten-

sive study of these compounds, there are no published data about their testing on the glioblastoma cell lines. Recently, it has been shown that BK acts through a bradykinin receptor 2 (B2R) as a chemoattractant guiding the glioma cells toward blood vessels in the rat brain slices [4].

Thus, B2R antagonist emerges as an attractive therapeutic agent for glioma patients.

It has been reported that anti-angiogenesis and anti-matrix metalloproteases (MMP) activities are important components of the excellent *in vivo* anti-cancer activity of BK antagonists. These substances offer advantages of combination therapy in one compound. These promising multitargeted anti-cancer compounds selectively stimulate apoptosis in cancers and also inhibit both angiogenesis and MMP action in treated lung and prostate tumors in nude mice.

Here we present that the BK antagonist BKM-570 possesses a high antiproliferative activity in the human glioblastoma cells U373. The obtained results demonst-



Effect of BK antagonists on U373 cells growth. U373 cells were cultured in the absence (vehicle control) or in the presence of increasing doses of BKM-570 (1) or BKM-1800 (2) for 72 h. Cells viability was analyzed by MTT assay. Data are expressed as the percentage of growth compared to vehicle control (100 %). Each point represents the mean of three experiments performed in triplicate

rate that BKM-570 exhibits the anti-cancer activity. This work forms a basis for the pre-clinical studies using animal models for the evaluation of these compounds as new anti-cancer drugs.

Materials and methods. *Cell proliferation assay.* The U373 cells (obtained from R. E. Kavetskogo Institute of experimental pathology, oncology and radiobiology, NAS of UkraineU) were seeded in triplicates into 96 well plates at density 3×10^3 cells/well and were grown in complete DMEM («PAA», Germany) in an environment of 95 % air/5 % CO₂.

The drugs were applied at final concentration of 0.01, 0.1, 1, 10, and 100 µM, which were reached by a serial dilution method. The cell proliferation was measured using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) («Sigma», USA) after 72 h of cultivation.

Results and discussion. The cancer cells are characterized by an increased proliferation rate. To analyze the antiproliferative effect of the BK antagonists, we used the human glioblastoma cells U373. The MTT assay was employed to determine the growth-suppression effect of the tested compounds.

Based on the cytotoxicity test data, the LC₅₀ parameter was calculated (molar concentration of compound leading to 50 % cell death as compared to a control group treated with DMSO).

The non-peptide BK antagonist BKM-570 and its derivative BKM-1800 were enrolled in this study. It was found that BKM-570 revealed a significant growth suppressor activity in the U373 cells in dose-dependent manner after the addition to the culture medium (Figure, A). LC₅₀ of BKM-570 was 3,8 M, while LC₅₀ of BKM-1800 was 25,8 M.

The obtained results were in accordance with those previously described by Stewart *et al.* [5]: BKM-570 was the leader compound both *in vitro* (LC₅₀ 1,8 M on the small-cell lung cancer (SCLC) cells) and *in vivo* (91 % inhibition of the tumor xenograf growth in mice). Our assays revealed much lower activity of BKM-1800, in contrast to the data published previously by Gera *et al.* (LC₅₀ 1,7 M on the SCLC cells *in vitro* and 78 % of PC3 prostate cancer growth inhibition *in vivo*) [6].

Thus, the cytotoxic activity of these compounds seems to depend on the specificity of each cell line tested.

Conclusions. In summary, we have shown that BKM-570 represses the glioblastoma cells growth and is a promising compound for the *in vivo* studies as a potential anti-cancer agent.

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Антипроліферативна активність антагоністів брадикініну у клітинах гліоми

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Резюме

Стандартна терапія гліобластоми дає дуже незначні результати. Таким чином, розробка нових методів є вкрай необхідною для ефективного лікування цього захворювання. **Мета** представлено-го дослідження полягала в аналізі впливу антагоністів брадикініну на проліферацію клітин гліобластоми людини U373. **Методи.** МТТ аналіз проліферації клітин. **Результати.** ВКМ-570 продемонстрував значну антипроліферативну активність у клітинах U373 (LC₅₀ 3,8 мкМ). **Висновки.** Антипроліферативні властивості антагоністів брадикініну показано з використанням моделі гліоми *in vitro*. Подальші дослідження молекулярних механізмів дії, а також доклінічні випробування із застосуванням тваринних моделей необхідні для оцінювання зазначених сполук як нових протипракових препаратів.

Ключові слова: антагоністи брадикініну, клітини гліоми, антипроліферативна активність.

Антипроліферативная активность антагонистов брадикинина в клетках глиомы

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Резюме

Стандартная терапия глиобластомы дает очень незначительные результаты. Таким образом, разработка новых методов крайне необходима для эффективного лечения этого заболевания. **Цель** данного исследования состояла в анализе влияния антагонистов брадикинина на пролиферацию клеток глиобластомы человека U373. **Методы.** МТТ анализ пролиферации клеток. **Результаты.** ВКМ-570 продемонстрировал значительную антипролиферативную активность в клетках U373 (LC_{50} 3,8 мкМ). **Выводы.** Антипролиферативные свойства антагонистов брадикинина показаны с использованием модели глиомы *in vitro*. Дальнейшие исследования молекулярных механизмов действия, а также доклинические испытания с применением животных моделей необходимы для оценки этих соединений в качестве новых противораковых препаратов.

Ключевые слова: антагонисты брадикинина, клетки глиомы, антипролиферативная активность.

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