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Medicine

Amphicezine + AgNPs (Silver Nanoparticles): Antiproliferative and Cytotoxic Effects on Human Breast Adenocarcinoma MDA-MB-231

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Abstract. The present study investigates and quantitatively evaluates the cytotoxic and antiproliferative effects of Amphicezine on the human breast adenocarcinoma cell line MDA-MB-231, a triple-negative breast ductal carcinoma characterized by high invasive and migratory potential. The MDA-MB-231 cell line serves as an established model of aggressive, hormone-independent breast cancer. Experimental evaluation has revealed that Amphicezine's biological activity demonstrated a pronounced cytotoxic effect on MDA-MB-231 breast adenocarcinoma cells. In addition, Amphicezine displays significant antiproliferative activity at concentrations lower than those required to induce cytotoxicity. Importantly, this antiproliferative effect appears to be largely independent of direct cytotoxic mechanisms and is instead associated with Amphicezine's modulation of cellular pathways involved in cell-cycle regulation and proliferation control. These findings suggest that Amphicezine may interfere with key molecular targets governing tumor cell growth and survival, underscoring its potential as a promising candidate for further preclinical evaluation in breast cancer therapy. © *2026 Bull. Natl. Acad. Sci. Georg.*

Keywords: amphicezine, MDA-MB-231 adenocarcinoma, antiproliferative, citotoxic activity

Introduction

Amphicezine is the first fundamentally novel, non-traditional antitumor agent whose mechanism of action is based on the pathogenetic principles of the Machabeli syndrome, specifically involving the use of negative electric charge to suppress its pathological reactions (Nadiradze & Chigogidze, 2019; Nadiradze & Chigogidze, 2021).

During surgical intervention for any malignant tumor, there is a substantially increased risk of shedding cancer cells into the bloodstream. Additionally, surgical trauma triggers local and systemic inflammatory responses which may also contribute to the accelerated growth of the residual tumors and micrometastatic disease (Nadiradze & Chigogidze, 2021). The cancer cell can travel through the blood-

stream and lymphatic system, and its sedimentation occurs in the microcirculatory bed of any distant organs by means of a fibrin membrane. Fibrin plays an important role in promoting tumor cell migration by providing a supportive matrix for tumor cell migration and by interacting with adhesive molecules and integrins (Cereceda et al., 2024).

Vascular endothelial growth factor (VEGF) bound to fibrin promotes angiogenesis. Fibrin interacts with platelets and leukocytes, thereby enhancing their respective carcinogenic properties. Amphicezine is a state-of-the-art formulation consisting of a multiply-charged anion-active compound containing negative, multi-charged, longchain organic ions and alkali metals such as cesium (Cs) and rubidium (Rb). The compound inhibits interactions between fibrin and tumor cells and as a result, prevents protective fibrin enveloping of cancer cells detached from the primary tumor lesion during surgery. Thus, cesium (Cs) and rubidium (Rb) cations are absorbed by tumor cells, penetrate inside and induce alkalization of the intracellular environment, leading to the inhibition and death of metastatic cells.

Materials and Methods

Reagents. Trypsin, Dulbecco's Modified Eagle Medium (DMEM), acridine orange (AO), and ethidium bromide (EB) were purchased from Sigma-Aldrich (Germany). Isotonic phosphate buffer (IPB; pH 7.4) was obtained from Lonza *(*Belgium). Antibiotics and L-glutamine were purchased from Gibco (USA), fetal bovine serum (FBS) from Capricorn (Poland), and PrestoBlue™ reagent from Invitrogen (USA).

Test compound. Basic 20% aqueous solution of the test sample "Amphicezine".

Amphicezine compound name	Solvent	Concentration
Amphicezine + AgNP (silver nanoparticles)	Distilled water	200 mg/ml

Solution of Amphicezine was used to determine its cytotoxic and antiproliferative activity.

Cell culture. The human breast adenocarcinoma cell line MDA-MB-231 (breast ductal carcinoma) was used as the object of the study. The cell line was obtained from the University of Western Ontario (London, Canada). Cells were cultured under standard conditions (37°C, 5% CO_2) in 96- or 24-well plates in modified Eagle (DMEM) medium containing 10% heat-inactivated fetal bovine serum, 50 μg/ml streptomycin, 50 U/ml penicillin and 2 mM L-glutamine.

General scheme of experiments. To determine the cytotoxic activity, Amphicezine solutions were added to serum-free incubation medium to achieve the required concentrations. Exposure to the test Amphicezine compound was carried out in 96-well plates for 24 hours. The number of viable cells of the MDA-MB-231 line was determined using the PrestoBlue™ reagent (*Invitrogen*, USA), which contains the non-fluorescent blue component resazurin, modified by viable cells into a highly fluorescent red connection. PrestoBlue™ reagent was diluted in culture medium at a ration of 1:9 and added to the cells in an amount of 100 μl/well. Wells containing culture medium without cells were left on each plate to determine the baseline level of fluorescence. The fluorescence intensity was measured after 1.5-2 hours of incubation at 37°C using an Ex (560 ± 25) nm, Em (590 ± 10) nm filters. The average fluorescence intensity of wells containing control cells was taken as 100%.

To determine antiproliferative activity, Amphicezine solutions were added to the serum-free containing incubation medium to obtain the required concentrations. Exposure to the test Amphicezine compound was carried out in 96-well plates for 24 hours. The viability of cultured MDA-MB-231 cells was determined using the PrestoBlue™ reagent (*Invitrogen*, USA) as described above. The average fluorescence intensity of wells containing control cells cultured in complete medium (containing EBS), minus the fluorescence of wells containing control cells cultured in incomplete medium (without EBS), was taken as 100%.

Live and dead cell test was performed using dual fluorescent staining with acridine orange (AO)

and ethidium bromide (EB). Cells of the MDA-MB-231 line were cultured in modified Eagle-DMEM medium containing 10% heat-inactivated fetal bovine serum under standard conditions (37°C, 5% CO_2) . Exposure to study Amphicezine was carried out in 24-well plates. 24 hours after the addition of the Amphicezine compound, the attached cells were stained with AO/EB. Cells were visualized and photographed using an Axiovert 25 inverted fluorescence microscope (Zeiss, Germany).

Statistical analysis. Statistical processing of the results was carried out using the standard software program Excel. The obtained data were tabulated and presented as Xsr ± SD, where Xsr is the arithmetic mean and SD is the standard deviation. Student's t-test was used to evaluate the difference between experimental groups, and *P* values <0.05 were considered statistically significant.

Results and Discussion

In this work, the cytotoxic and antiproliferative activities of Amphicezine were evaluated against cultured human breast adenocarcinoma cells MDA-MB-231 (breast ductal carcinoma), which exhibit high invasive and migratory ability. MDA-MB-231 cells serve as a model of aggressive, hormone-independent breast cancer.

Results of a study of the cytotoxic activity of Amphicezine against human breast adenocarcinoma MDA-MB-231. To study the cytotoxic activity of Amphicezine, cancer cells of the MDA-MB-231 line were cultured in complete medium until reaching 70-80% confluence, then the growth medium was removed and serum-free medium containing the test compounds was added to the cells and incubated for 24 hours, after which the number of viable cells in control and experimental wells was assessed. Since incubation in a culture medium without serum causes the cells to enter the G_0 phase and stop proliferation, the results obtained under these experimental conditions characterize predominantly the cytotoxic activity of the compound under study. The data obtained are summarized in Table 1, which served as the basis for the constructing a dose-response curve demonstrating the cytotoxic effect of Amphicezine compound (% cell damage) relative to its concentration in the culture medium (Fig. 1). From this dose-effect relationship, the concentration of Amphicezine causing 50% reduction in the number of cultured cells (ED Cyt_{50}) was determined. The obtained data are presented in Table 2.

Table 1. Cytotoxic effect of Amphicezine against MDA - MB -231 cells after 24 h of incubation

Amphicezine	Final concentration of the Amphicezine compound in the medium (mg/ml)			
	1.25	2.5	5.0	100 _ _
Damage, %	17.4±11.0* _	33.0±9.1**	54.7±6.0**	87.6±7.6**

* - P ≤ 0.00 02 ; ** - P ≤ 0.000001 *vs* control

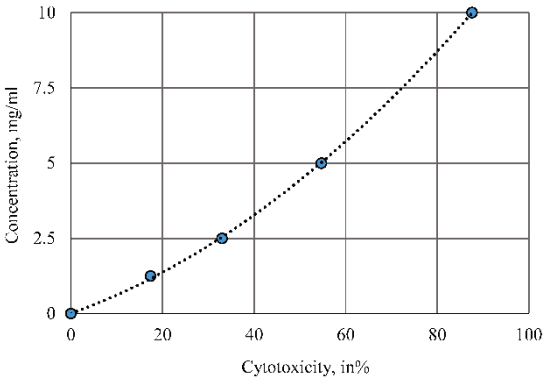

Fig 1. Dependence of cytotoxicity on the concentration of Amphicezine.

Table 2. The value of ED Cyt $_{50}$ is the effective dose of Amphicezine, leading to 50% death of MDA-MB-231 cells after 24 hours of incubation

Amphicezine	ED Cyt_{50} (mg/ml)
	4.5

The detected cytotoxic effect of Amphicezine was confirmed by a standard test for live and dead cells using fluorescent dyes – Acridine orange and ethidium bromide (Fig. 2).

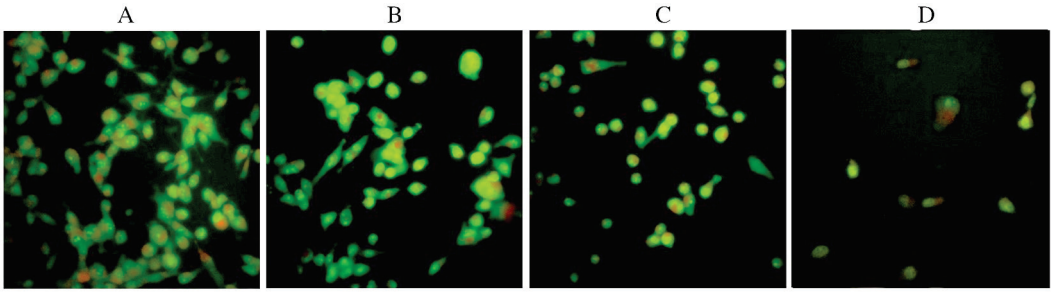

Fig. 2. Representative fluorescent micrographs of MDA - MB -231 cells in the presence and without Amphicezine 24 hours after exposure. AO and EB staining; A – control cells, B, C and D – cells after 24 hours of incubation with Amphicezine 2.5 mg/ml, 5.0 mg/ml and 10.0 mg/ml, respectively.

Living cells absorb AO, which has intense green fluorescence (Fig. 2 A). Necrotic and late apoptotic cells with impaired membrane integrity become permeable to EB and, when bound to nuclear DNA, the nuclei of such cells have intense red fluorescence. It was found that incubation of MDA - MB -231 cells with Amphicezine leads to a dose-dependent decrease in the number of living cells (Fig. 2 B, C, D); the absence in this case of cells with red fluorescence is apparently due to low adhesion ability damaged cancer cells, which leads to their loss during the staining procedure.

In this work, the antiproliferative activity of Amphicezine against human breast adenocarcinoma cells (MDA-MB-231) was investigated and quantitatively evaluated. The cells were cultured in complete medium until reaching 50-60% confluence, then the growth medium was removed and replaced with serum-containing medium supplemented with the compounds under study. The plates were incubated for 24 hours, after which the number of viable cells in control and experimental wells was assessed. The level of proliferation was determined as the difference between the number of viable cells cultured in complete medium (containing EBS) and the number of viable cells cultured in incomplete medium (without EBS).

The obtained data are summarized in Table 3, on the basis of which a graph was constructed showing the dependence of the antiproliferative effect of Amphicezine (in %) on its concentration in the culture medium (Fig. 3). For Amphicezine, using a graph reflecting the dose-effect relationship, the concentration causing a decrease in proliferation by 50% (ED Pr $_{50}$) was calculated. The obtained data are presented in Table 4.

Table 3. Antiproliferative activity of Amphicezine after 24 h of incubation with cells of the MDA line - MB -231 (%)

Amphicezine	Final concentration of Amphicezine compounds in the medium (mg/ml)		
	1.25	2.5	5.0
Decrease in proliferation, %	38.6±29.5_	52.1±13.8_ _ _	97.8±18.8

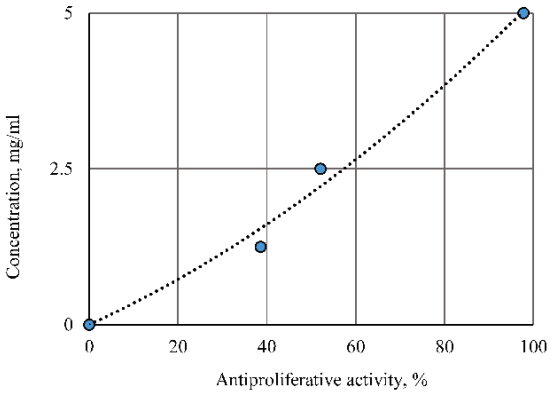

Fig. 3. Dependence of antiproliferative activity on of Amphicezine concentration.

Table 4. The ED value Pr $_{50}$ is the effective dose of Amphicezine, leading to a 50% suppression of proliferation of MDA - MB - 231 cells after 24 h of incubation

Amphicezine	ED Pr $_{50}$ (mg/ml)
	2.1

Conclusions

Amphicezine demonstrates a pronounced cytotoxic effect on human breast adenocarcinoma MDA-MB-231 cells, with a median effective cytotoxic dose (ED_{50}) of 4.5 mg/mL. Notably, the compound also exhibits significant antiproliferative activity at concentrations lower than those required to induce cytotoxicity, with a median effective antiproliferative dose (ED_{50}) of 2.1 mg/mL. This indicates that Amphicezine's ability to suppress cell proliferation is not solely a consequence of cell death but likely involves the modulation of key cellular pathways regulating the cell cycle and proliferation. Thus, its antiproliferative effect appears to be mechanistically distinct from its cytotoxic activity, suggesting potential utility in targeting tumor growth through non-lethal inhibition of cancer cell proliferation.

მედიცინა

ამფიცეზინის + AgNPs (ვერცხლის ნანონაწილაკები) ნაერთის ანტიპროლიფერაციული და ციტოტოქსიკური აქტივობა ადამიანის ძუძუს ჰორმონდამოუკიდებელი MDA-MB-231-ადენოკარცინომის მიმართებაში

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(წარმოდგენილია აკადემიის წევრის რ. ხეცურიანის მიერ)

ნაშრომში წარმოდგენილია კვლევა, რის საფუძველზეც შესწავლილი და რაოდენობრივად განსაზღვრულია ,,ამფიცეზინის“ ციტოტოქსიკური და ანტიპროლიფერაციული აქტივობა ადამიანის ძუძუს ადენოკარცინომის MDA-MB-231-უჯრედებთან (ძუძუს სადინრის კარცინომა) მიმართებაში, რომლებსაც ახასიათებს მაღალი ინვაზიური და მიგრაციული თვისებები. MDA-MB-231-უჯრედები წარმოადგენს ძუძუს აგრესიული, ჰორმონდამოუკიდებელი კიბოს მოდელს. ,,ამფიცეზინის“ ციტოტოქსიკური და ანტიპროლიფერაციული აქტივობის კვლევამ გვიჩვენა, რომ მედიკამენტს გააჩნია გამოხატული ციტოტოქსიკური მოქმედება ადამიანის

ძუძუს MDA-MB-231-ადენოკარცინომის მიმართ. პრეპარატს ასევე აქვს ანტიპროლიფერაციული მოქმედება, მაგრამ შედარებით უფრო დაბალ კონცენტრაციებში, ციტოტოქსიკურთან შედარებით. ანტიპროლიფერაციული აქტივობა მნიშვნელოვნად განპირობებულია არა პრეპარატის ციტოტოქსიკურობით, არამედ მისი ზემოქმედებით პროლიფერაციის უჯრედულ მექანიზმებზე.

REFERENCES

Cereceda, L., Cardenas, JC., Khoury, M., Silva-Pavez, E., Hidalgo, Y. (2024). Impact of platelet-derived mitochondria transfer in the metabolic profiling and progression of metastatic MDA-MB-231 human triple-negative breast cancer cells. Front. Cell. Dev. Biol., 11, 1324158. doi: 10.3389/fcell.2023.1324158. PMID: 38283990; PMCID:PMC10811077.

Nadiradze, I., Chigogidze, N. (17 July, 2019). Creation of new metastasis inhibitors and oncological diseases chemotherapy. Georgian patent: P20197005 B.

Nadiradze, I., Chigogidze, N. (2021). Amphicezine. New approaches to fighting cancer preliminary theoretical and experimental (in vitro) message. Georgian Medical News, 9(318), 156-159.

Nadiradze, I., Chigogidze, N. (2021). Essentially new inhibitors of metastasis of malignant tumors for chemotherapy-sparing treatment//Global J. of Cancer Therapy, 7(1), 016-030. USA.

Otulakowski, L., Trzebicka, B. (2023). Aggregation of thermoresponsive polymethacrylates in a dulbecco's modified eagle medium and its salts. Polymers (Basel). 15(17), 3587. doi: 10.3390/polym15173587. PMID: 37688213; PMCID:PMC10489804.

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