Open Access Article

Ann. Acad. Med. Siles. (online) 2025; 79: 206–212
eISSN 1734-025X
DOI: 10.18794/aams/203580
www.annales.sum.edu.pl

PRACA ORYGINALNA
ORIGINAL PAPER

Comparative cytotoxicity of perphenazine on different human glioblastoma cells

Wpływ perfenazyny na przeżywalność różnych linii komórkowych glejaków

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ABSTRACT

INTRODUCTION: Despite medical advances glioblastoma multiforme (GBM) is still the most common malignant primary brain tumor. Additionally, the gold standard treatment possesses poor (only 12–15 months) survival median. Thus, drug repurposing may be a helpful strategy for discovering more effective GBM chemotherapeutic drugs. Interestingly, phenothiazine derivatives have been considered a promising candidate for drug repurposing for cancer therapy, since they possess several biological activities, such as anticancer, antibacterial, antifungal, and antiviral effects.
MATERIAL AND METHODS: We investigated the impact of perphenazine on the viability of several human glioblastoma (U-87 MG, A172, and T98G) cell lines after 24-, 48-, and 72-hour incubation using WST-1 assay.
RESULTS: Data showed that the tested phenothiazine derivative decrease glioblastoma viability in a time- and concentration-dependent manner.
CONCLUSIONS: Based on EC_{50} values, perphenazine is the most efficient against A172 human glioblastoma in all of the tested treatment time periods compared to T98G and U-87 MG cells. Based on previous research, which revealed that perphenazine does not affect normal human astrocytes, this drug is a promising candidate for glioblastoma treatment. Further studies are required to unravel the complete antitumor mechanism of these phenothiazine derivatives in GBM.

KEYWORDS

antitumor activity, cell viability, glioblastoma multiforme, perphenazine, DMSO, WST-1

Received: 20.03.2025 **Revised:** 30.03.2025 **Accepted:** 31.03.2025 **Published online:** 11.07.2025

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Publisher: Medical University of Silesia, Katowice, Poland

STRESZCZENIE

WSTĘP: Mimo postępu medycyny glejak wielopostaciowy (*glioblastoma multiforme* – GBM) jest nadal najczęstszym złośliwym pierwotnym guzem mózgu. Ponadto złoty standard leczenia charakteryzuje się niską (tylko 12–15 miesięcy) medianą przeżycia. Zatem ponowne wykorzystanie istniejących leków (*repurposing*) może być pomocną strategią w odkrywaniu skuteczniejszych leków chemioterapeutycznych w terapii GBM. Co ciekawe, pochodne fenotiazyny zostały uznane za obiecującego kandydata do ponownego wykorzystania leku w terapii nowotworowej, gdyż posiadają wiele istotnych aktywności biologicznych, takich jak działanie przeciwnowotworowe, przeciwbakteryjne, przeciwgrzybicze i przeciwwirusowe.

MATERIAŁ I METODY: Wpływ perfenazyny na przeżywalność różnych linii komórkowych ludzkiego glejaka wielopostaciowego (U-87 MG, A172 i T98G) po 24-, 48- i 72-godzinnej inkubacji zbadano z użyciem testu WST-1.

WYNIKI: wykazano, że testowana pochodna fenotiazyny zmniejsza żywotność glejaka wielopostaciowego w sposób zależny od czasu i stężenia.

WNIOSKI: Na podstawie uzyskanych wartości EC_{50} stwierdzono, że perfenazyna jest najskuteczniejsza przeciwko ludzkiemu glejakowi wielopostaciowemu A172 w porównaniu z komórkami T98G i U-87 MG. Na podstawie poprzednich badań, które wykazały, że perfenazyna nie wpływa na normalne ludzkie astrocyty, można stwierdzić, że lek ten jest obiecującym kandydatem w leczeniu glejaka wielopostaciowego. Konieczne są dalsze badania w celu odkrycia pełnego mechanizmu aktywności przeciwnowotworowej pochodnych fenotiazyny w terapii GBM.

SŁOWA KLUCZOWE

aktywność przeciwnowotworowa, przeżywalność, glejaki, perfenazyna, DMSO, WST-1

INTRODUCTION

Glioblastoma multiforme (GBM) is the most common malignant primary brain tumor [1]. The National Brain Tumor Society reported that GBM accounts for about 50.1% of all primary malignant brain tumors [2]. In 2024 in the USA, estimated new brain and other nervous system cancer cases and deaths are 25,400 and 18,760, respectively [3]. According to the data of Cancer Global Observatory, in 2022 in Europe, 67,559 cases of brain and other nervous system cancer incidents and 54,001 mortality cases were noticed. Noteworthy, the data from around the world were 321,731 incidents and 248,500 mortality cases in 2022 [4]. Despite the medical advances, the standard in GBM treatment is still "surgical resection followed by radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide" with a poor survival median (only 12 to 15 months) [1]. Therefore, the search for new and more effective GBM treatment methods is crucial.

Interestingly, in past years, phenothiazine derivatives have been of particular interest and are considered as potential drug repurposing for cancer therapy [5]. Perphenazine is piperazinyl phenothiazine [6] used to treat psychotic disorders (schizophrenia, mania in bipolar disorder, and psychosis), migraines, nausea, and vomiting [6,7]. Additionally, phenothiazine derivatives possess novel biological activities, such as antibacterial [8], antifungal [8], antiviral [8,9], and anticancer effect [8,10], even in multidrug resistance models of cancer [11].

Thus, in the present study, we evaluated the effects of perphenazine on the viability of human glioblastoma (U-87 MG, A172, and T98G) cell lines.

MATERIAL AND METHODS

Materials

Perphenazine, cell proliferation reagent WST-1, and human glioblastoma cell lines (U-87 MG, A172, and T98G) were purchased from Merck Life Science (Poland). The dimethyl sulfoxide (DMSO) analytical grade was purchased from Chempur (Poland). DMEM medium without sodium pyruvate, with 4.5 g/l glucose, L-glutamine, and 3.7 g/l $NaHCO_3$, fetal bovine serum (FBS), amphotericin B 250 µg/ml, penicillin/streptomycin solution, and trypsin/EDTA solution 0.25%/0.02% in PBS were obtained from PAN Biotech (Germany).

Cell treatment

The human glioblastoma cell lines were cultured in T-75 bottles in a growth medium DMEM medium supplemented with FBS (50 ml/500 ml of basal medium), amphotericin B (5 ml/500 ml of basal medium), and penicillin/streptomycin solution (5 ml/500 ml of basal medium) at 37°C in 5% CO_2.

Cell viability

Cell viability was measured using cell proliferation reagent WST-1 [12] with slight modification. U-87 MG, A172, or T98G were seeded 2500 cells/well and incubated with the supplemented growth medium for 24 hours. After 24 h incubation, the growth medium was changed into 150 µl medium containing perphenazine (0.5, 1.0, 5.0, 10.0, 25.0, and 50.0 µM), DMSO 1%, and the supplemented growth medium without DMSO. The concentration of DMSO in the analyzed samples of perphenazine was 1%. Cells were

incubated for 24, 48, and 72 hours at 37°C. Three hours before the end of incubation, 15 µl/well of WST-1 was added. The absorbance at 450 nm with a reference wavelength of 620 nm was measured by the microplate reader TRIAD LT microplate reader (Dynex Technologies, Chantilly, VA, USA). The results were expressed as the percentage of the control.

Statistical analysis

In the viability assay, mean values of at least three independent experiments (n = 3) performed in seven repetitions ± standard deviation (SD) were calculated. Statistical analysis was performed with one-way ANOVA with Dunnett's multiple comparison test and two-way ANOVA (the influence of incubation time and drug concentration), followed by the Tukey post--hoc test using GraphPad Prism 8 software. One-way ANOVA was also used to compare obtained EC_{50} values. The significance level was established at the value of $p < 0.05$ (*) or $p < 0.01$ (**).

RESULTS

The effect of DMSO on the human glioblastoma cell lines viability

First, we determined the effect of DMSO (1%) on the assay, since it was used as vehicle for phenothiazine derivatives. The results obtained after 24-, 48-, and 72-hour treatment with 1% DMSO for A172, T98G, and U-87 MG cells are presented in Figure 1 (A–C). As control, we used cells incubated in the supplemented growth medium but without DMSO (1%). No statistically significant differences were observed, excluding the possibility of the vehicle exhibit cytotoxicity at this concentration. Thus, DMSO was used as vehicle for the drugs in the assay.

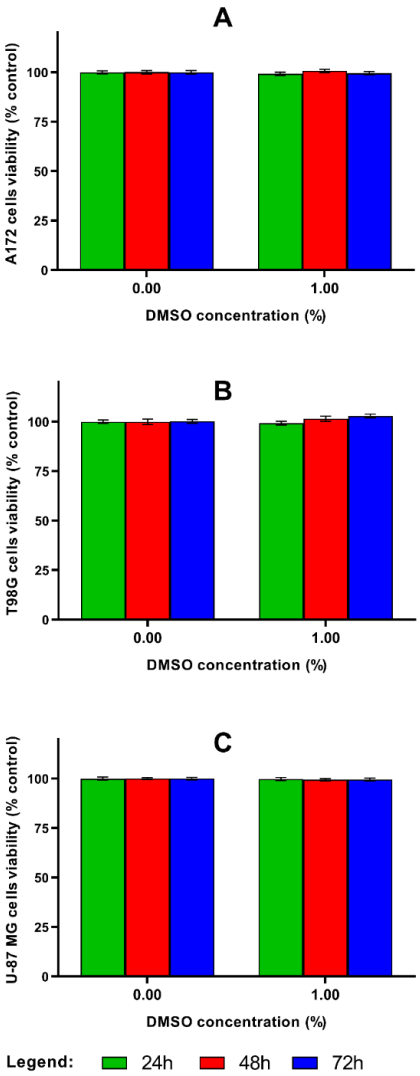

Fig. 1. Impact of DMSO (1%) after 24-, 48-, and 72-hour treatment on the viability of A172 (A), T98G (B), and U-87 MG (C). The cell proliferation reagent WST-1 was used to perform a viability assay. Mean values ± SD from three independent experiments (n = 3) performed in four repetitions are presented.

The effect of perphenazine on A172, T98G, and U-87 MG cells viability

Perphenazine in the concentration range from 0.5 to 50 μM decreased the viability of A172 cells concentration-dependently (Figure 2A). As a control, we used cells incubated in the supplemented growth medium without DMSO (1%). After 24-hour incubation of A172 cells perphenazine, we observed a significant decrease in viability by 9.6, 17.3, 28.3, 32.5, 72.7, and 90.7%, respectively, compared to the control. After 48-hour incubation of A172 cells perphenazine, we observed a significant decrease in viability by 19.1, 27.3, 50.5, 64.9, 94.5, and 95.1%, respectively, compared to the control. After 72-hour incubation of A172 cells perphenazine, we observed a significant decrease in viability by 18.2, 24.4, 44.3, 78.5, 97.4, and 96.8%, respectively, compared to the control. Moreover, we observed statistically significant differences between 24- and 72-hour and 24- and 48-hour perphenazine (0.5 to 25 μM) treatment. The statistically significant difference between 48- and 72-hour treatment was observed only after perphenazine (10 μM) treatment. Thus, we did not notice a time-dependent decrease in the viability of A172 cells caused by perphenazine (Figure 2A).

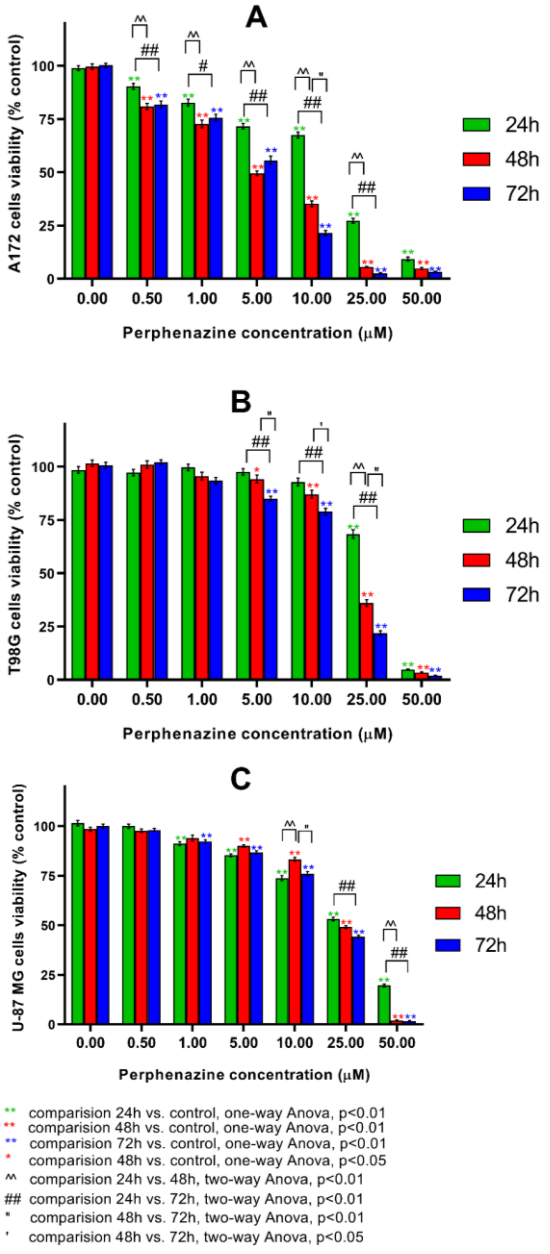

Fig. 2. Impact of perphenazine (0.5, 1.0, 5.0, 10.0, 25.0, and 50.0 μM) after 24-, 48-, and 72-hour treatment on the viability of A172 (A), T98G (B), and U-87 MG (C). The cell proliferation reagent WST-1 was used to perform a viability assay. Mean values ± SD from three independent experiments (n = 3) performed in four repetitions are presented. The values $p < 0.05$ and $p < 0.01$ were established as statistically significant.

In the case of T98G cell line, perphenazine in the concentration range from 5 to 50 μM caused a dose--dependently decrease in the viability (Figure 2B). As a control, we used cells incubated in the supplemented growth medium without DMSO (1%). 24-hour incubation of T98G cells with perphenazine (25 and 50 μM) decreased viability by 31.6 and 95.3% compared to the control. In the case of 48-hour incubation, perphenazine (5 to 50 μM) decreased viability by 5.7, 12.8, 63.9, and 96.5%, respectively, compared to the control. 72-hour incubation of T98G cells with perphenazine (5 to 50 μM) decreased viability by 15.0, 21.1, 78.0, and 98.0%, respectively, compared to control. Moreover, we observed statistically significant differences between 24- and 72-hour and 48- and 72-hour perphenazine (5 to 25 μM) treatment. A statistically significant difference between 24- and 48-hour treatment was observed after perphenazine (25 μM) treatment. Thus, we did not notice a time-dependent decrease in the viability of A172 cells caused by perphenazine (Figure 2B).

Perphenazine in the concentration range from 1.0 to 50 μM dose-dependently decreases the viability of U-87 MG cells (Figure 2C). As a control, we used cells incubated in the supplemented growth medium without DMSO (1%). After 24-hour incubation of U-87 MG cells perphenazine (1 to 50 μM), we observed a significant decrease in viability by 8.8, 14.7, 26.2, 46.9, and 80.3%, respectively, compared to the control. After 48-hour incubation of U-87 MG cells perphenazine (5 to 50 μM), we observed a significant decrease in viability by 9.9, 16.7, 50.9, and 98.0%, respectively, compared to the control. After 72-hour incubation of U-87 MG cells perphenazine (1 to 50 μM), we observed a significant decrease in viability by 7.7, 13.2, 23.9, 55.7, and 98.3%, respectively, compared to the control. Moreover, we observed statistically significant differences between 24- and 72-hour perphenazine (25 and 50 μM) and 24- and 48-hour perphenazine (10 and 50 μM) treatment. The statistically significant difference between 48- and 72-hour treatment was observed only after perphenazine (10 μM) treatment. Thus, we did not notice a time-dependent decrease in the viability of U-87 MG cells caused by perphenazine (Figure 2C).

The calculated EC_{50} values of perphenazine for tested cell lines after 24, 48, and 72 hour treatment are shown in Table I.

Table I. EC_{50} values were calculated for human glioblastoma incubated with perphenazine for 24, 48, and 72 hours

Cell line	Time of incubation with perphenazine (h)	EC_{50} ± SD (μM)
A172	24	12.28 ± 1.29
	48	4.46 ± 1.43
	72	3.94 ± 1.56
T98G	24	28.21 ± 2.26
	48	20.02 ± 2.44
	72	15.43 ± 1.03
U-87 MG	24	29.88 ± 4.95
	48	25.10 ± 1.44
	72	21.99 ± 1.48

DISCUSSION

In the presented manuscript, we evaluated the viability of human glioblastoma cell lines (A172, T98G, and U-87 MG) after 24, 48, and 72 hour treatment with perphenazine. First, we observed that DMSO could be utilized in the assays we performed since there were no statistically significant differences between the supplemented growth medium with DMSO (1%) and without DMSO (1%).

Gil-Ad et al. [13] analyzed the impact of perphenazine on the viability of rat glioma (C6) and human neuroblastoma (SHSY-5Y) after 24-hour exposure using neutral red and alamar blue staining. Perphenazine was dissolved in lactic acid (1%). The authors observed a dose-dependent decrease in viability in the 10 to 24 μM concentration range. The calculated IC_{50} were 15 ± 1.7 and 14 ± 1.9 μM for rat and human glioblastoma, respectively. Tzadok et al. [14] measured the viability of perphenazine dissolved in lactic acid (1%) on the viability of human glioblastoma U-87 MG using sulphorhodamine B staining. 1×10^4/ml cells were seeded on a 24-well plate. A dose-dependent decrease in U-87 MG cells viability was noticed after seven days of perphenazine (2 to 10 μM) treatment. The obtained LC_{50} value was 6.8 μM. Cheng et al. [15] tested the impact of perphenazine on human glioblastoma GBM8401 cells using MTT and clonogenic assay. For the MTT assay, 1500 cells/well in a 96-well plate were added, while 1000 cells/well in a 6-well plate were added for the

clonogenic assay. The study showed that the obtained IC_{50} differs depending on the method used. The obtained MTT IC_{50} were from 5 to 10 μM, while the clonogenic IC_{50} value was < 10 μM. Otręba and Buszman [16] measured the viability of U-87 MG cells after 24-hour treatment with perphenazine. Phenothiazines were solved in phosphate buffer pH 6.8. 2500 cells/well in a 96-well plate were seeded. The authors observed a dose-dependent decrease in viability after perphenazine (0.1 to 10 μM) treatment. The calculated IC_{50} value was 0.98 μM. Jacob et al. [17] analyzed the viability of human glioblastoma (U-87 MG, T98G, and LN18) cells, patient-derived human glioblastoma (OSU2, OSU61, ACPK1, ACPK4, and ACPK8), and normal human astrocytes after 24-hour perphenazine (5 to 25 μM) treatment using MTS viability assay. The authors noticed a dose--dependent decrease in patient-derived glioblastoma and commercially available human glioblastoma viability. In the case of normal human astrocytes, no changes in viability were observed up to 25 μM. Interestingly, all GBM cells showed sensitivity at a concentration range from 5 to 15 μM. A 50% decrease in viability was observed after perphenazine treatment (about 12 and 15 μM) for U-87 MG and T98G cells. The above assays align with our present results, showing that perphenazine can decrease the viability of human glioblastoma cells. It also suggests that the viability results depend not only on the cell line but also on the solvent, the method of analysis used, the equipment, and the cell number. Thus, it may explain the differences observed between calculated EC_{50} values in Otręba and Buszman [16] and the present study. Noteworthy, our previous study exploring the viability of normal human astrocytes after perphenazine treatment showed that perphenazine (0.1 to 10 μM) does not significantly decrease the viability of human astrocytes [18]. It is in line with Jacob et al. [17], showing no impact of perphenazine on normal human astrocyte viability after 24-hour treatment up to 25 μM.

Based on a statistical analysis of the obtained EC_{50} values (Table I) from the present study, we claimed that perphenazine is the most effective ($p < 0.01$) against A172 human glioblastoma after 24-, 48-, and 72-hour treatment compared to T98G and U-87 MG cells. No statistically significant differences were observed between T98G and U-87 MG cells, suggesting that anti-glioblastoma activity of perphenazine is present as follows:

$$A172 > T98G \sim U\text{-}87\ MG$$

Furthermore, the obtained EC_{50} value for A172 cells after 24-hour perphenazine treatment was 12.28 ± 1.29 μM. Thus, considering Jacob et al. [17], results about normal human astrocytes suggest that perphenazine may be potentially used in A172 human glioblastoma treatment since it does not decrease normal human astrocytes up to 25 μM.

CONCLUSIONS

The present study showed that perphenazine decreases glioblastoma viability dose-dependently. Based on EC_{50} values, the tested drug is the most effective against A172 human glioblastoma in all of the tested treatment time periods compared to T98G and U-87 MG cells. Based on our previous research, which revealed that perphenazine does not affect normal human astrocytes, this drug is a promising candidate to be used in the glioblastoma treatment. Of course, more studies are essential to explain the complete mechanism of phenothiazine's anti-glioblastoma activity. Thus, in further studies, we want to focus on the type of cell death (autophagy, necroptosis, and apoptosis) caused by perphenazine in human glioblastoma cells.

Funding

This study was funded by the Medical University of Silesia, Katowice, Poland (Grant number PCN-2-019/K/3/F).

Studies involving human and/or animals

Not applicable

Conflict of interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Authors' contribution

Study design – M. Otręba
Data collection – M. Otręba
Data interpretation – M. Otręba, A. Rzepecka-Stojko, T. Rodrigues
Statistical analysis – M. Otręba
Manuscript preparation – M. Otręba, A. Rzepecka-Stojko, T. Rodrigues, J. Stojko
Literature research – M. Otręba

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