








Effect of perphenazine and prochlorperazine on viability of human astrocytes

Wpływ perfenazyny i prochlorperazyny na przeżywalność ludzkich astrocytów

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ABSTRACT

Phenothiazine derivatives are well-known anti-psychotic drugs that possess several biological activities including anticancer activity. Much research provides data about its anticancer activity against human glioblastoma cell lines. Unfortunately, to date *in vitro* studies analyzing the impact of phenothiazines on the viability of human astrocytes have not been performed. However, it is possible to find one study about the viability of neurons and neurons with glia after incubation with perphenazine. Thus, in this study we measured the viability of human astrocytes after 24-, 48-, and 72-hour incubation with perphenazine and prochlorperazine dimaleate using the WST-1 assay. The obtained results suggest that perphenazine is safer for human astrocytes than prochlorperazine dimaleate. Moreover, 24-hour incubation with perphenazine or prochlorperazine did not significantly reduce cellular viability. It is a very important finding since previously we proved that in similar concentrations both drugs reduce the viability of the human glioblastoma U-87 MG cell line by approximately 50%. Therefore, the results suggest that phenothiazines can be used in glioblastoma treatment in concentrations that do not impact human astrocyte viability.

KEYWORDS

perphenazine, prochlorperazine, DMSO, human astrocytes, viability, WST-1

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STRESZCZENIE

Pochodne fenotiazyny należą do dobrze znanej grupy leków przeciwpsychotycznych, posiadających wiele nowych aktywności biologicznych, w tym działanie przeciwnowotworowe. Istnieje wiele doniesień naukowych na temat działania przeciwnowotworowego wobec ludzkich linii komórkowych glejaka wielopostaciowego. Niestety, jak dotąd nie przeprowadzono badań *in vitro* oceniających wpływ fenotiazyn na żywotność ludzkich astrocytów. Można jednak znaleźć badanie dotyczące żywotności neuronów i neuronów z komórkami glejowymi po inkubacji z perfenazyną. Dlatego też w niniejszym badaniu z użyciem testu WST-1 oznaczono żywotność ludzkich astrocytów po 24-, 48- i 72-godzinnej inkubacji z perfenazyną lub dimaleinianem prochlorperazyny. Uzyskane wyniki sugerują, że perfenazyna jest bezpieczniejsza dla ludzkich astrocytów niż dimaleinian prochlorperazyny. Co więcej, 24-godzinna inkubacja z perfenazyną lub prochlorperazyną nie zmniejszyła znacząco żywotności komórek. Jest to o tyle istotne, że już wcześniej wykazano, iż oba leki w podobnym stężeniu zmniejszają żywotność ludzkiego glejaka wielopostaciowego U-87 MG o około 50%. Wyniki sugerują zatem, że fenotiazyny mogą mieć zastosowanie w leczeniu glejaka wielopostaciowego w stężeniu, które nie wpływa na żywotność ludzkich astrocytów.

SŁOWA KLUCZOWE

perfenazyna, prochlorperazyna, DMSO, ludzkie astrocyty, przeżywalność, WST-1

INTRODUCTION

Perphenazine and prochlorperazine belong to the phenothiazine derivatives class of drugs. The mentioned drugs are used in the treatment of hallucinations and delusions associated with psychosis [1]. Interestingly, prochlorperazine is also effective in the treatment of nausea and vomiting [2], which could be very helpful in other disease treatments such as cancer therapy. Phenothiazines are still being analyzed by researchers to find new biological activities. Recently, anticancer [3], antimicrobial [4], and antiviral [5] activity as well as efflux inhibitory properties [4] have been found. Thus, now it is taken into consideration to repurpose phenothiazines since the impact on multidrug resistance (MDR) pumps may lead to extruding antimicrobial or anticancer agents into the cell or bacteria. Potential candidates from already existing drugs in cancer or microbial infection treatment could save a great deal of time and costs in comparison to drug development from the beginning [6].

In 2018, we found that perphenazine and prochlorperazine decrease the viability of the human glioblastoma cell line U-87 MG. The obtained EC_{50} values were 0.97 and 0.98 μ M, respectively. Noteworthy is the fact that the obtained values were related to toxic (not lethal) concentrations in human plasma [7]. Now we wish to analyze the viability of normal astrocytes treated with perphenazine or prochlorperazine. Thus, in the present study we used normal human astrocytes.

MATERIAL AND METHODS

Cell proliferation reagent WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulphonate) was purchased from Merck Life Science (Poland).

The cell line, astrocyte medium, poly-L-lysine, fetal bovine serum, astrocyte growth supplement, penicillin/streptomycin solution, and trypsin/EDTA solution 0.05% were obtained from ScienCell Research Laboratories (USA). Perphenazine, prochlorperazine dimaleate, and dimethyl sulphoxide (DMSO; analytical grade) were purchased from Sigma-Aldrich.

Cell treatment

The human astrocytes isolated from the human brain (cerebral cortex) were characterized by immunofluorescence with antibodies specific to GFAP by the manufacturer – Scien Cell Research Laboratories (USA). The cells were cultured in poly-L-lysine-coated T-75 bottles in an astrocyte basal medium supplemented with fetal bovine serum (FBS, 10 ml/500 ml of basal medium), astrocyte growth supplement (AGS, 5 ml/500 ml of basal medium), and a penicillin/streptomycin solution (5 ml/500 ml of basal medium) at 37°C in 5% CO_2 . In the assay, cultured astrocytes from passages 3 to 5 were used.

Cell viability

The viability of astrocytes was analyzed using the colorimetric test WST-1 (4-(3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio)-1,3-benzene disulphonate) [7] with slight modification. 5000 normal human astrocytes/well were seeded in 96-well plates, which were previously coated with poly-L-lysine following the manufacturer's protocol.

The cells were incubated for 24 hours with a growth medium, then the medium was removed, and 200 μ l of medium containing perphenazine or prochlorperazine dimaleate (0.1, 0.5, 1.0, 5.0, and 10.0 μ M), growth medium with DMSO 1%, and growth medium without DMSO were added. The concentration of DMSO in the analyzed samples of perphenazine and prochlorperazine was 1%. Next, the human astrocytes were cultured for 24, 48, and 72 hours at 37°C. 20 μ l



of WST-1 was added to each well for 3 hours before the end of the incubation time. Finally, the absorbance at 440 nm with a reference wavelength of 650 nm was measured by a microplate reader BioTek ELX 800 (BioTek Instruments Inc., USA), and the results were expressed as the percentage of the control.

Statistical analysis

In the viability assay, mean values of at least three separate experiments ($n = 3$) performed in seven repetitions \pm standard error of the mean (SEM) were calculated. Statistical analysis was performed employing 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. The significance level was established at the value of $p < 0.05$ (*) or $p < 0.01$ (**).

RESULTS

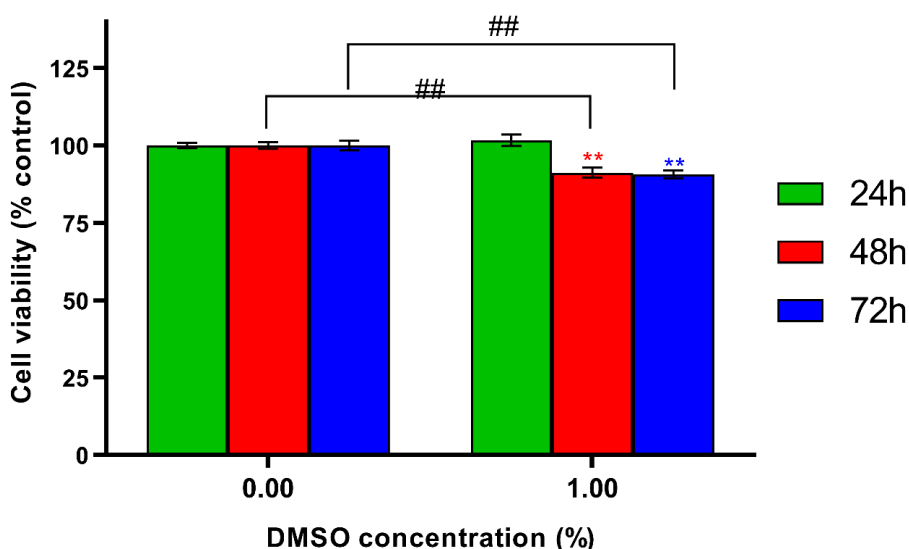
The effect of perphenazine, prochlorperazine dimaleate, or DMSO on the viability of human astrocytes

At the beginning, we analyzed the effect of DMSO (1%) on the viability of the cells after 24, 48, and 72 hours of incubation (Fig. 1). In this case, as the control, a growth medium without DMSO was used.

We observed a statistically significant decrease in human astrocyte viability by 10.46 and 11.03%, respectively after 48 and 72 hours of incubation with DMSO (1%) in comparison to the 24 hours of incubation with DMSO (1%). The study also showed a significant decrease in cellular viability by 8.78 and 9.34%, respectively after 48 and 72 hours of incubation with DMSO (1%) in comparison to the control after 48 and 72 hours of incubation.

Perphenazine does not significantly decrease the viability of human astrocytes (Fig. 2A). In the case of perphenazine and prochlorperazine as the control, we used cells incubated in a growth medium with DMSO (1%). After the 72-hour incubation of cells with perphenazine in the concentrations 0.5, 1.0, 5.0 μM , we observed a significant increase in viability by 16.5, 13.1, and 11.2%, respectively in comparison to the control. Moreover, after 72 hours of incubation with perphenazine 10.0 μM , there was a significant decrease by 12.9% in comparison to perphenazine 0.5 μM .

In the case of prochlorperazine dimaleate (Fig. 2B), we found a statistically significant decrease in human astrocyte viability by 13.2% after the 24-hour incubation of cells with prochlorperazine in the concentration of 10.0 μM in comparison to the control. After 72 hours of incubation of the cells with prochlorperazine in the concentrations 1.0 and 10.0 μM , we also observed a concentration-dependent decrease in the viability of human astrocytes by 11.1 and 20.9%, respectively in comparison to the control.



** comparison 48h vs. control, one-way Anova, $p < 0.01$

** comparison 72h vs. control, one-way Anova, $p < 0.01$

comparison time and concentration, two-way Anova, $p < 0.01$

Fig. 1. Impact of DMSO (1%). Mean values \pm SEM from three independent experiments ($n = 3$) performed in seven repetitions are presented. Values $p < 0.05$ and $p < 0.01$ were established as statistically significant.

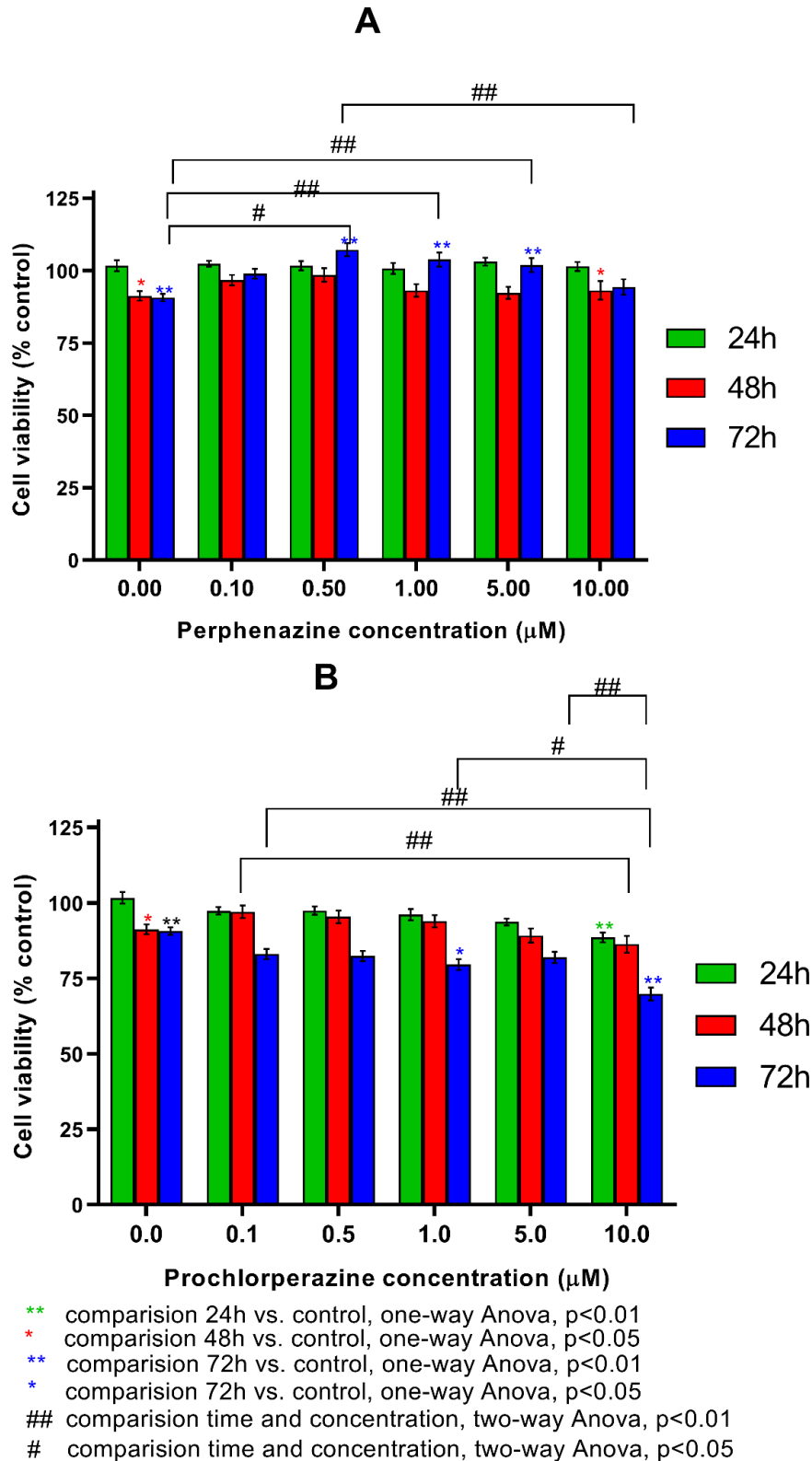


Fig. 2. Impact of perphenazine (A) and prochlorperazine dimaleate (B) on viability of human astrocytes using WST-1 assay. Cells were seeded 5000 cells/well in poly-L-lysine covered wells and incubated for 24, 48, and 72 hours. Viability is expressed as percentage of control. Mean values \pm SEM from three independent experiments ($n = 3$) performed in seven repetitions are presented. Values $p < 0.05$ and $p < 0.01$ were established as statistically significant.



Finally, we also compared the obtained results between perphenazine and prochlorperazine to see if the differences were statistically significant. The obtained results showed significant differences ($p < 0.01$) after 24-hour incubation between both drugs in concentrations of 0.1, 5.0, and 10.0 μM . Moreover, after 72 hours of incubation, significant differences ($p < 0.01$) were observed in all of the analyzed concentrations. After 48-hour incubation, significant changes were not observed.

DISCUSSION

In this study, we evaluated the impact of perphenazine and prochlorperazine dimaleate on the viability of human astrocytes. We also observed a time-dependent significant decrease in the viability of human astrocytes in comparison to the growth medium without DMSO.

In 2014, Yuan et al. [8] noticed a 16% decrease in the viability of mouse cortical astrocytes after 24-hour incubation with DMSO (1%) as well as a 32% decrease in viability after incubation with DMSO (5%). It is worth noting that the authors did not observe an impact on cellular survival and apoptosis after incubation with DMSO (1%) but there was a 42% decrease in cell density and an increase in apoptosis with DMSO (5%). The authors also suggested that DMSO in the concentration range from 0.5 to 1.5% is the most used solvent of different agents in *in vitro* and *in vivo* studies. In 2017, Zhang et al. [9] showed dose- and time-dependent changes in the neurons of neonatal Sprague-Dawley rats caused by DMSO treatment. Low concentrations of DMSO (0.25 and 0.50%) do not affect the viability of rat cortical neurons after 12-, 24-, or 48-hour incubation, while in higher concentrations of DMSO (1.00, 5.00, and 10.00%) a significant decrease in viability was observed. A 12-hour incubation of cells with DMSO (1.00, 5.00, and 10.00%) caused a decrease in the cell number from about 30 to 20, 18, and 12, respectively. A 24-hour incubation of cells with DMSO caused a reduction in the cell number from about 30 to 18, 15, and 7, respectively. A 48-hour incubation of cells with DMSO caused a decline in the cell number from about 30 to 15, 12, and 5, respectively. Moreover, the authors also observed a significant increase in the cell number from 18 to 24 and 25, respectively after the 24 and 48-hour incubation of rat astrocytes with DMSO (1%). The above-mentioned publications confirm our results since we also observed a time-dependent decrease in viability after DMSO treatment. What is more, it also suggests that the effect of viability after DMSO treatment depends on the cell type – in mouse astrocytes 24-hour incubation with DMSO (1%)

decreases viability, while in rat astrocytes it increases viability.

In case of the perphenazine, we did not observe a significant drop in viability after 24-, 48- or 72-hour incubation in the concentration range from 0.1 to 10.0 μM . Only after 72-hour incubation with perphenazine (0.5, 1.0, and 5.0 μM) did we observe a significant increase in human astrocytes in comparison to the control.

Unfortunately, there are not many published papers regarding perphenazine and the viability of normal astrocytes or normal neurons. Gil-ad et al. [10] observed that the incubation of neurons and neurons with glia with perphenazine (10 μM) lead to viability of 85.6 ± 3.6 and $94.9 \pm 4.6\%$, respectively. At higher concentrations (50 and 100 μM), a significant decrease in viability was observed. In contrast, in neuroblastoma cells (SK-N-SH), perphenazine (10 μM) reduces viability by about 90%. The authors used a primary mouse embryo-selected neuronal culture. The study showed that a low concentration of perphenazine did not induce significant neurotoxic activity. In addition, the authors suggest that “the glial cells play an important role in protecting the brain from neuroleptic-induced toxicity”. It is worth noting the opposite situation in comparison to neuroblastoma (NB cells) where as low as 1 μM affects the cell cycle. Jackson [11] observed that perphenazine (10 μM) is lethal for neurons.

It suggests that our results are in line with other researchers' results since we did not observe a significant decrease in human astrocyte viability either.

In the case of prochlorperazine dimaleate, we observed a statistically significant decrease in human astrocyte viability after 24-hour incubation with perphenazine (10 μM) as well as after 72-hour incubation with the same drug (1 and 10 μM). Unfortunately, to date no *in vitro* studies using brain cell lines have been conducted to analyze viability after prochlorperazine treatment. Moreover, based on the comparison of our results for prochlorperazine and perphenazine, we conclude that perphenazine is much safer for human astrocytes than prochlorperazine dimaleate. It is very important since we demonstrated in 2018 that perphenazine and prochlorperazine have a very similar effect on the viability of U-87 MG glioblastoma [7].

CONCLUSIONS

In conclusion, we noticed that the incubation of human astrocytes with perphenazine in the concentration range from 0.5 to 10 μM does not significantly impact cellular viability. In the case of



prochlorperazine dimaleate, we observed a statistically significant impact of the drug on the viability of the cells after 24 and 72 hours of incubation. Furthermore, statistically significant differences between perphenazine and prochlorperazine dimaleate were also observed after 24- and 72-hour incubation. These findings suggest perphenazine is safer for the analyzed cells in comparison to prochlorperazine. It is worth noting that we did not observe a decrease in the viability of human astrocytes in the concentration of 1.0 μM after 24-hour treatment either, which is similar to the EC_{50} for human glioblastoma U-87 MG. It suggests that phenothiazine derivatives may be potentially used in human glioblastoma treatment since both drugs reduce the viability of human glioblastoma with no impact on the viability of human astrocytes. However, further *in vitro* research is

important to find the mechanism of action of phenothiazines on human astrocytes and glioblastoma cells to explain the mechanism of protective action.

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Studies involving human and/or animals

Not applicable

Conflicts of interest

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

Author's contribution

Study design – M. Otręba

Data collection – M. Otręba

Data interpretation – M. Otręba, A. Rzepecka-Stojko

Statistical analysis – M. Otręba

Manuscript preparation – M. Otręba, A. Rzepecka-Stojko, J. Stojko, A. Kabała-Dzik, A. Kleczka

Literature research – M. Otręba, A. Kleczka

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