

Cytotoxic activity of Galantamine hydrobromide against HeLa cell line

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Abstract

Cytotoxic activity of Galantamine HBr against human cervical adenocarcinoma cell line HeLa in different concentrations (1.875 μM \div 30 μM) was evaluated. Inhibition of HeLa cell growth after treatment with Galantamine HBr and index of cell viability were determined. From the experimental results was proven that the drug exerts cytotoxic activity towards HeLa cell line, with antiproliferative activity presented as the value of $\text{IC}_{50} = 30 \mu\text{M} \pm 0.22$.

Keywords

Cytotoxic activity, Galantamine HBr, Adenocarcinoma cell line HeLa

Introduction

Galantamine HBr is a specific long-acting centrally active competitive (reversible) inhibitor of enzyme acetylcholinesterase (Danchev and Nikolova 2006; Ago et al. 2011), possessing positive allosteric modulatory effect on $\alpha 7$ -subtype of nicotinic acetylcholine receptors (Arias et al. 2004; Wang et al. 2007) and antioxidant activity (Traykova et al. 2003; Tsvetkova et al. 2013a).

In accordance with these data, the aim of current study is the investigation of cytotoxic effect on human cervical adenocarcinoma cell line HeLa of Galantamine HBr, which possess acetylcholinesterase and γ -secretase inhibitory activity (Vezenkov et al. 2012) and antioxidant properties in ferric reducing/antioxidant power (FRAP) method (Tsvetkova et al. 2013b).

MTT-test of Mosmann has been widely applied for the investigation of cytotoxic activity of different compounds due to the advantages: it is rapid, versatile, quantitative, highly reproducible and sensitive. The MTT assay determines the ability of viable cells to convert a soluble yellow tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) into an insoluble violet formazan crystal, that can be dissolved in an organic solvent and can be determined spectrophotometrically at $\lambda = 570 \text{ nm}$. Tetrazolium salt accepts electrons from oxidized substrates or appropriate enzymes. MTT is reduced at the ubiquinone and cytochrome B and cytochrome C sites of the mitochondrial electron transport system and is the result of succinate dehydrogenase activity. The reduction takes place only when mitochondrial reductase enzymes are active and therefor conversion can be directly related

to the number of viable cells. An increase in cell number results in an increase in the amount and absorbance of formazan. When the amount of formazan produced by cells treated with an agent is compared with the amount of formazan produced by untreated control cells, the effectiveness of the agent in causing death of cells can be deduced, through the production of a dose-response curve (Mosmann 1983).

Human epithelia cervical carcinoma cell line (HeLa) is the first type of human cancer cell lines, which has been cultured continuously. HeLa is isolated from the aggressive glandular cervical cancer of a young woman in 1951. Till now the knowledge of many processes occurring in human cells has been obtained using HeLa cells (Masters 2002).

Materials and methods

In vitro cancer test systems – cell lines

In experiments for the assessment of cytotoxic activity of Galantamine HBr, HeLa cervical adenocarcinoma cell line cultured in medium Minimum Essential Medium Eagle was used.

Tested drug: Galantamine HBr

The structure of Galantamine is shown in Fig. 1.

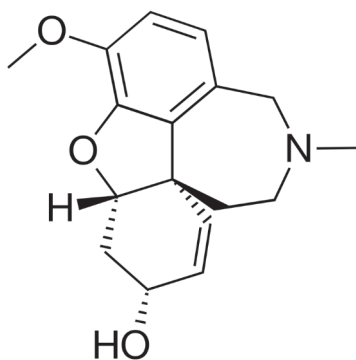


Figure 1. Structure of Galantamine: (1S,12S,14R)-9-methoxy-4-methyl-11-oxa-4-azatetracyclo [8.6.1.0^{1,12}.0^{6,17}] heptadeca-6(17),7,9,15-tetraen-14-ol).

Reagents with analytical grade quality

Fetal bovine serum (FBS), 100 IU/ml of Penicillin, 100 µg/ml of Streptomycin, standard MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyl-tetrazolium bromide), dimethylsulfoxide, 0.25% Trypsin EDTA 1X.

Preparation of solutions of Galantamine HBr

The examined drug Galantamine HBr was dissolved in DMSO to obtain solutions with concentrations 1.875 µM ÷ 30 µM.

Preparation of solution of MTT

Solution of MTT was prepared by dissolving of MTT in phosphate buffer solution (pH 7,4) to obtaining solution with concentration of MTT 5 mg/ml. This solution is stable 1 month at storage at 4 °C.

Cell seeding

Cytotoxic activity of *Galantamine HBr* was evaluated in 96-well microplates. HeLa cervical cancer cells were cultured in 75 cm² flasks in Minimum Essential Medium Eagle, supplemented with 5% of fetal bovine serum, 100 IU/ml Penicillin and 100 µg/ml Streptomycin at 37 °C in a fully humidified atmosphere of 5% CO₂. Exponentially growing cells were trypsinized, centrifuged and counted with the help of haemocytometer. Cell suspension was diluted with culture medium to obtain concentration: 6 × 10⁴ HeLa cells/ml. 100 µl of cell suspension were introduced into all wells and were incubated for 24 h at 37 °C in a fully humidified atmosphere of 5% CO₂. The culture medium was removed after incubation.

MTT-test assay

MTT-test of Mosmann was applied (Mosmann 1983). 200 µl of solution of esters with certain amino acids that are the subject of published studies ester in media were added separately in different concentrations (1.875 µM ÷ 30 µM) into each well. Experiments were performed in triplicates for each ester in each concentration and the average values were calculated. After 48 hours, 200 µL of MTT (0.5 mg/ml) was added to each well. The plates were incubated further for 4 h at 37 °C in 5% CO₂ incubator. The supernatant was removed and to each well were added 100 µl of dimethylsulfoxide to solubilize the formed formazan, which absorbance was measured at λ = 570 nm.

The cytotoxicity was recorded as concentration causing 50% growth inhibition (IC₅₀) of HeLa cells. Index cell viability V (%) and cell growth inhibition I (%) by the following equations were calculated:

$$V(\%) = \frac{A(t) - A(-)}{A(+)} \cdot 100$$

$$I(\%) = 100 - V(\%) = 100 - \frac{A(t) - A(-)}{A(+)} \cdot 100$$

V (%) – index cell viability

I (%) – cell growth inhibition

A(t) – mean absorbance value of formazan, obtained after treatment of HeLa cells with test compound in presence of solution of MTT

A(+) – mean absorbance of formazan, obtained with positive control (HeLa cell line treated with solution of MTT without addition of the examined compounds)

A(-) – mean absorbance value of formazan, obtained with negative control (solution of MTT, without addition of the examined compounds).

Results

Absorbances of positive: A(+) and negative: A(-) controls are presented on Table 1.

Table 1. Absorbances of positive A(+) and negative A(-) controls.

N:	A(+)	A(-)
1	1.575	0.082
2	1.634	0.083
3	1.645	0.084
4	1.668	0.084
5	1.791	0.085
6	1.828	0.086
\bar{X}	1.690	0.084
SD	0.098	0.001

As a standard was used Doxorubicin. Absorbances of formazan obtained after treatment of HeLa cells with Galantamine HBr are summarized on Table 2.

Table 2. Absorbances of formazan produced from HeLa cell line treated with Galantamine HBr.

C_{GAL} [μ M]	Absorbance of formazan [AU]				
	1	2	3	\bar{X}	SD
1.875	1.635	1.645	1.650	1.643	0.008
3.75	1.584	1.590	1.596	1.590	0.006
7.5	1.490	1.505	1.510	1.502	0.010
15	1.289	1.295	1.315	1.300	0.014
30	0.887	0.890	0.894	0.890	0.004

The accordance between the decreased values of absorbances of formazan and concentrations of Galantamine HBr is illustrated on Fig. 2.

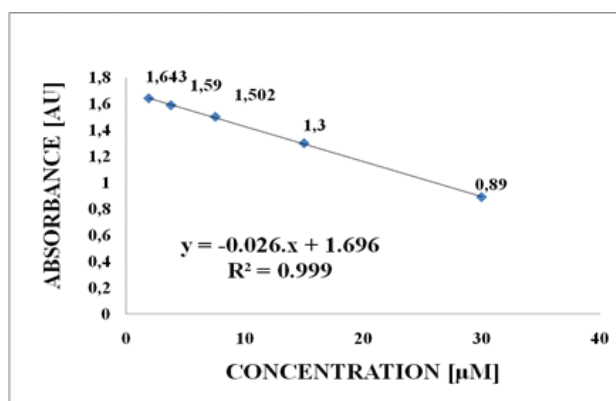


Figure 2. Effect of Galantamine HBr on the absorbances of formazan produced from HeLa cell line.

Effect of different concentrations (1.875 μ M ÷ 30 μ M) Galantamine HBr on the survival of HeLa cells is shown on Table 3.

The decreased data of index of HeLa cell viability after treatment with Galantamine HBr are presented on Fig. 3.

The experimental results from the investigation of cytotoxic activity of Galantamine HBr against HeLa cells are summarized on Table 4.

Table 3. Index of HeLa cell viability after treatment with Galantamine HBr.

C_{GAL} [μ M]	Index of HeLa cell viability [%]				
	1	2	3	\bar{X}	SD
1.875	96.58	97.20	97.51	97.10	0.47
3.75	93.40	93.77	94.15	93.77	0.38
7.5	87.55	88.48	88.79	88.27	0.65
15	75.03	75.40	76.65	75.69	0.85
30	50.0	50.19	50.44	50.21	0.22

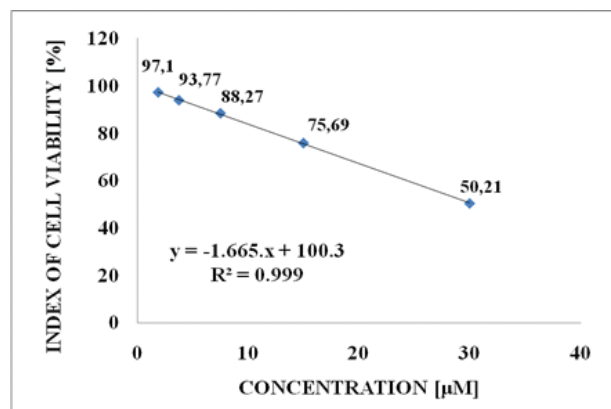


Figure 3. Effect of Galantamine HBr on HeLa cell viability.

Table 4. Inhibitory effect of Galantamine HBr on proliferation of HeLa cell line.

C_{GAL} [μ M]	Inhibition of HeLa cell growth [%] treated with GAL				
	1	2	3	\bar{X}	SD
1.875	3.42	2.80	2.49	2.90	0.47
3.75	6.60	6.23	5.85	6.23	0.38
7.5	12.45	11.52	11.21	11.73	0.65
15	24.97	24.60	23.35	24.31	0.85
30	50.0	49.81	49.56	49.79	0.22

The obtained data for inhibition concentration 50% (IC_{50}) are: Galantamine HBr (30 μ M \pm 0.22), standard Doxorubicin (3.1 μ M).

The decreased growth of HeLa cells treated with Galantamine HBr is illustrated on Fig. 4.

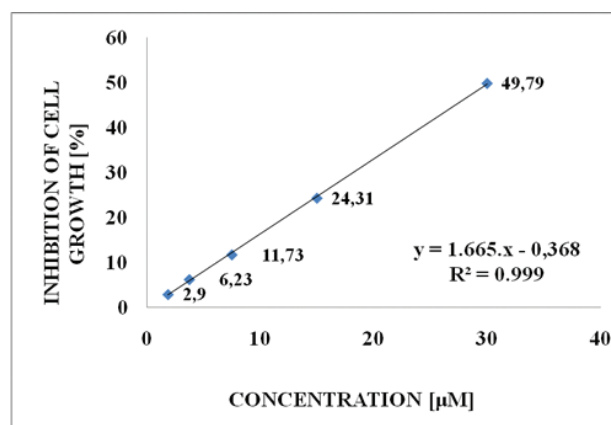


Figure 4. Cytotoxic activity of Galantamine HBr on HeLa cell line.

Discussion

HeLa cell line was treated separately with each of the examined peptide esters in different concentrations (1.875 μM \div 30 μM) and MTT assay was evaluated. Experimental data show that the treatment of HeLa cells, resulted in reduced concentration of formazan, which indicates a growth inhibitory property of Galantamine HBr.

Inhibition of HeLa cell growth obtained after treatment with GAL is proved by the fact that in concentration 30 μM it inhibits 30.59% of cell growth with index of cell survival 69.41%. The cytotoxicity is recorded as concentration causing 50% growth inhibition (IC_{50}) of cells. It was reported that 0.1 μM Doxorubicin reduced survival of HeLa cells to 40% (Sadeghi-Aliabadi et al. 2010).

Experimental results for cell growth inhibition are plotted on regression analysis using Microsoft Excel Program.

For Galantamine HBr $\text{IC}_{50} = 30 \mu\text{M} \pm 0.22$ is higher than experimental obtained data with standard Doxorubicin ($\text{IC}_{50} = 3.1 \mu\text{M}$). This result prove that Galantamine HBr possesses lower antiproliferative activity than standard Doxorubicin.

It is reported that antitumor drug Ledakrin and other 1-nitro-9-aminoacridines produce a potent dose – dependent inhibition of DNA synthesis and replication in HeLa S3 cells by inhibition of thymidine incorporation (Wojnarowski and Bartoszek 1985).

Inhibitor of phosphatidylinositol 3-kinases promotes mitotic cell death in HeLa cells (Hou et al. 2012). Anticancer activity on HeLa cell lines of coumarime derivative Calanone, isolated from genus *Calophyllum* ($\text{IC}_{50} = 22.887 \mu\text{g/ml}$) (Ekowati et al. 2010) and of ethanolic extract of *Canthium parviflorum* Lam [$\text{IC}_{50} = 43.15 \mu\text{g/ml}$] (Purushoth et al. 2011) is proved.

It is demonstrated that the inhibitory effect on HeLa cell line of an antitumour triterpenoid: cyano enone of methyl boswellates at 1 μM is 78.5% and $\text{IC}_{50} = 0.27 \mu\text{M}$. (Ravanan et al. 2011).

It is reported that DNA methyltransferase inhibitor Zebularine (You and Park 2012) and synthetic antioxidants butylated hydroxyanisole (Moon and Park 2011) and propylgallate (Han et al. 2010) inhibit the growth of HeLa cells via caspase-dependent apoptosis, as a result of activation caspase 3 and caspase 8. The observed data for IC_{50} are respectively: Zebularine ($\text{IC}_{50} = 130 \mu\text{M}$) (You and Park 2012), butylated hydroxyanisole ($\text{IC}_{50} = 150 \mu\text{M}$) (Moon and Park 2011), propylgallate ($\text{IC}_{50} = 800 \mu\text{M}$) (Han et al. 2010). The low value of IC_{50} correspond to the high cytotoxic activity. From the presented results it is obvious that triterpenoid is more effective in suppression of cell proliferation, followed by Zebularine, butylated hydroxyanisole and propylgallate.

(DIPP-Trp)2-Lys-OCH₃ induced a dose-dependent inhibitory effect on the proliferation of HeLa cells. 100 μM (DIPP-Trp)2-Lys-OCH₃ inhibits 85% of HeLa cells. The inhibitory concentration 50% (IC_{50}) is 42.23 μM (Liu et al. 2008).

Conclusion

In comparison with the data from our experimental results it is obvious that Galantamine HBr $\text{IC}_{50} = 30 \mu\text{M}$ is more active on HeLa cells in comparison with Zebularine, butylated hydroxyanisole and propylgallate.

Galantamine HBr treatment resulted in reduced concentration and absorbance of formazan, which indicates its growth inhibitory activity. The experimental results for inhibitory concentration IC_{50} are 30 $\mu\text{M} \pm 0.22$ and proved that Galantamine HBr exert cytotoxic activity against HeLa cell line.

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