

THE INVESTIGATION OF THE MPTP TREATMENT AND THE MONOAMINE OXIDASE B EXPRESSION LEVELS IN GLIOBLASTOMA CELL LINES

GLİOBLASTOMA HÜCRE HATLARINDA MPTP MUAMELESİNİN VE MONOAMİN OKSİDAZ B'NİN İFADE SEVİYELERİNİN ARAŞTIRILMASI

Gizem DÖNMEZ YALÇIN

Adnan Menderes Üniversitesi, Tıp Fakültesi Tıbbi Biyoloji Anabilim Dalı, Aydın

Keywords: Glioblastoma, glia, MPTP, MAO-B

Anahtar Sözcükler: Glioblastom, glia, MPTP, MAO-B

Yazının alınma tarihi: 02.10.2019

Kabul tarihi: 24.04.2020

Online basım: 11.05.2020

SUMMARY

Introduction: Glioblastomas are the most common and aggressive brain tumors derived from glia cells. Despite intense studies, unfortunately a satisfactory cure regimen is not available. Parkinson's Disease (PD) is the second most common neurodegenerative disorder without a major cure. Existing therapies are helpful only to a certain point. The association between brain tumors and PD remains controversial. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) is a reliable and reproducible chemical model for PD. Monoamine Oxidase B (MAO-B) enzymes, expressed by glia cells, convert MPTP to MPP⁺ (1-Methyl-4-phenylpyridinium) which inhibits Complex I and induces cell death. It is important to investigate the effect of MPTP and also analyze the protein expression levels of MAO-B in glioblastoma cell lines and compare with control glia cells.

Materials and Methods: The effect of MPTP on the viability of glioblastoma cell lines (A172 and U87) was investigated using MTT assay and compared with control glia cells (IHA-immortalized human astrocytes). The level of protein expression of Monoamine Oxidase B (MAO-B) was also analyzed in glioblastoma cell lines and compared with control glia cells using ELISA assay.

Results: The cell viability was significantly increased in glioblastoma cells when compared to glia cells after MPTP administration. The protein expression levels of MAO-B are significantly lower in glioblastoma cells compared to control glia cells.

Conclusion: Glioblastoma cells might be resistant against MPTP treatment due to having expressed lower amount of MAO-B enzyme so that it will be interesting in the future to investigate the MAO-B-related pathways and design therapeutics accordingly for brain tumors.

ÖZ

Giriş: Glioblastomalar, glia hücrelerinden farklılaşan en agresif ve yaygın tümörlerdir. Yoğun çalışmalara rağmen tatmin edici bir tedavi yöntemi henüz yoktur. Parkinson Hastalığı (PH) ikinci en yaygın nörodejeneratif hastalıktır ve tedavisi yoktur. Var olan tedaviler ancak belli bir noktaya kadar yardımcıdır. Beyin tümörleri ve PH arasındaki ilişki tartışmalıdır. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) güvenilir ve tekrar edilebilir bir PH kimyasal modelidir. Glia hücrelerinde ifade edilen Monoamine Oksidase B (MAO-B) enzimleri, MPTP'yi MPP⁺ (1-Methyl-4-phenylpyridinium)'ye dönüştürür. MPP⁺, Complex 1'i inhibe eder ve hücre ölümünü tetikler. MPTP'nin etkisinin ve MAO-B enziminin protein ifade seviyelerini glioblastoma hücrelerinde araştırmak önemli olacaktır.

Gereç ve Yöntem: MPTP'nin glioblastoma hücrelerinin (A172 ve U87) canlılığı üzerine etkisi MTT assay ile analiz edilmiş ve kontrol glia hücreleri (IHA-immortalize insan astrositleri) ile karşılaştırılmıştır. MAO-B'nin protein ifade seviyeleri de ELISA assay kullanarak glioblastoma hücrelerinde ölçülmüş ve kontrol glia hücreleri ile karşılaştırılmıştır.

Bulgular: MPTP muamelesinden sonra, hücre sağkalımı, glioblastoma hücrelerinde kontrol glia hücrelerine göre istatistiksel olarak anlamlı bir şekilde daha fazladır. MAO-B protein ifade seviyeleri de glioblastoma hücrelerinde kontrole göre istatistiksel olarak anlamlı bir şekilde düşük bulunmuştur.

Sonuç: Glioblastoma hücrelerinin, MPTP muamelesine, kontrol glia hücrelerine göre daha dirençli olmalarının nedeninin MAO-B enzimini daha az seviyede ifade etmeleri olduğu düşünülmüştür. Bu neden MAO-B ile ilgili yolların çalışılması beyin tümörlerine tedavi geliştirilmesi için önemli olacaktır.

INTRODUCTION

Glioblastoma (GBM) is the most common malignant primary brain tumor type (1) with an average survival of 5 years after being detected. Patients having high grade tumors have a survival of 6-12 months (1). The molecular biology of gliomas still needs intensive research to develop therapies. Glioblastomas are malignant Grade IV type of tumors, which are infiltrative and invasive to other tissues. They are nourished by blood supply from vessels. The tumors mainly contain glia cells. GBMs are classified according to their histopathological and molecular features into four grades (I-IV) by WHO (World Health Organization). The survival of patients is inversely correlated with the tumor grade (1). GBM patients have a median survival of just 4.6 months, which can rise to 14 months with an optimal treatment.

An intense basic research has been conducted with cell and animal models at a molecular level in order to understand the underlying pathways of glioblastoma with the aim of developing therapies. Among the cells used, A172 and T98G cell lines were found to maintain the main features of glioblastomas and therefore can be used for the research of this type of neoplasms (2).

Parkinson's disease (PD) is second common neurodegenerative disease in the brain (3). The disease biology and the physiology are both progressive. Tremor, rigidity and bradykinesia are the most common motor deficits observed in this disease. Molecular studies showed that the loss of dopaminergic neurons and the accumulation of alpha-synuclein aggregates are observed in the patient brains. The familial mutations associated with Parkinson's Disease include alpha-synuclein and enzymes expressed in mitochondria such as PARK, UCHL-1, DJ-1, PINK (4).

Monoamine oxidases (MAO) catalyze the

oxidation of monoamines, using oxygen to cleave their amine group. They are mostly bound to the outer membrane of mitochondria (5).

MPTP (1 - methyl - 4 - phenyl - 1, 2, 3, 6-tetrahydropyridine) is a reliable and reproducible chemical model for PD (6). It leads to the destruction of nigrostriatal dopaminergic pathway after systemic administration. MPTP is converted to 1-methyl 4-phenylpyridinium (MPP⁺) by MAO-B enzyme in glia cells. MPP⁺, which is a toxic metabolite, is taken up by the dopamine transporter (DAT) into dopaminergic neurons, MPP⁺ inhibits the Complex I of mitochondria leading to the generation of extensive ROS and oxidative stress (7). The presence of DATs on dopaminergic neurons are thought to be the reason for the selective effect of MPP⁺ on these cells (8).

The association between Parkinson's Disease and glioblastomas remain controversial (9). Parkinsonism is observed among patients with brain tumors. One of the reasons is thought to be the distortion and stress to the midbrain or neuronal loss in the substantia nigra due to the tumor invasion. These cases were thought to respond to dopamine replacement. However, recent reports showed that the dopamine replacement exerted almost no or weak therapeutic effects. In a recent study, researchers linked a gene related to Parkinson's Disease called PARK2 to many types of cancer including glioblastoma. PARK2 was found to act as a tumor suppressor (10).

In this study, the effect of MPTP was investigated on glioblastoma cell lines and compared with the control glia cells. Additionally, the levels of protein expression of Monoamine Oxidase B (MAO-B) in glia cells (IHA-immortalized human astrocytes) and glioblastoma cell lines (A172 and U87) were analyzed. The cell viability is increased in glioblastoma cells when compared to glia cells after MPTP administration. The

protein expression level of MAO-B enzyme was shown to be lower in glioblastoma cells compared to the control glia cells. Therefore, it will be interesting in the future to investigate the MAO-B related pathways and design therapeutics accordingly for brain tumors.

MATERIALS AND METHODS

Cell Culture

A172 and U87 cell lines were obtained from ATCC. IHA (Immortalized Human Astrocytes) (11) cells were a gift from Prof. Yavuz Oktay (Izmir Biomedicine and Genome Center). IHA, A172 and U87 cells were grown in DMEM with %10 FBS, 100 µg/ml penisilin/streptomisin and 2 mM L-glutamin.

MPTPTreatment

MPTP was dissolved in H₂O. Control represents vehicle-treated cells. MPTP treatment was carried out at 1 mMfor 24 hours.

MTT Cell Viability assay

MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) Cell Viability Assay

(Biotum) is a standard test to determine the number of living cells. MTT is a tetazlium salt metabolized by living cells and converted to purple formazan crystal. It is dissolved in DMSO and measured at 570 nm. The assay was performed according to the manufacturer's guidelines in the protocol.

Monoamine Oxidase B (MAO-B) ELISA Assay

Human Monoamine Oxidase B (MAO-B) ELISA Kit was obtained from Aviva Systems Biology, the experiment was carried out according to the manufacturer's guidelines and the assay was measured using Microplate Reader from Molecular Devices.

Statistics

All the statistical analyses were performed using statistical software (GraphPad Software, Inc., San Diego, USA). Namely, "unpaired, one-tail, equal variance and two-sample t-test" was performed using Prism 5 software. Significant differences are shown with different symbols indicating $p < 0.05$. Error bars in figures represent standard error of the mean (SEM).

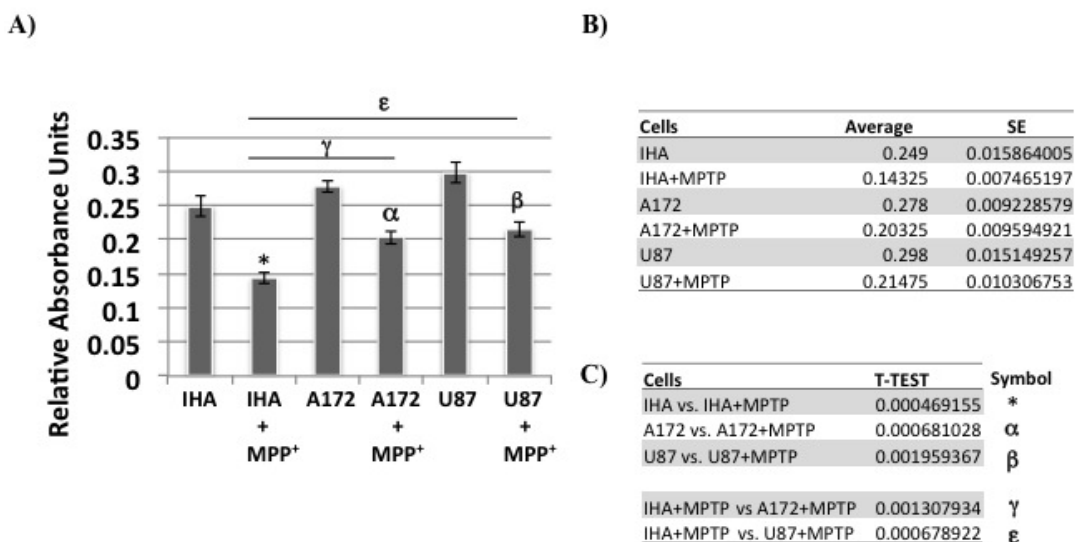


Figure 1. The effect of MPTP on cell viability. Cell viability was measured using an MTT assay. The absorbance at 570 nm is expressed as relative absorbance units. The values significantly different from the relative controls are indicated with symbols. $p < 0.05$. **A)** IHA, A172 and U87 cells were treated 1 mMof MPTP for 24 h. Vehicle-treated control cells and MPTP treated cells were then assayed with MTT. The measurements were carried out with four repeats. **B)** The table shows the average values and SEMs (Standard Error of the Mean). **C)** The table shows the comparisons and the Student's t-test results. Significance was shown with symbols. * $p < 0.05$.

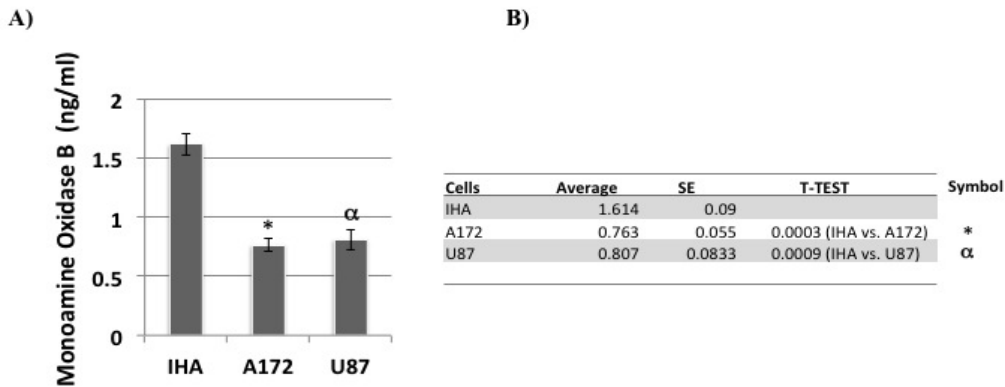


Figure 2. The Monoamine Oxidase B (MAO-B) ELISA Assay. **A)** The protein expression level of MAO-B was assayed with ELISA in IHA, A172 and U87 cells. The measurements were carried out with three repeats. **B)** The table shows the average values, SEMs (Standard Error of the Mean), the comparisons and the Student's t-test results. Significance was shown with symbols. * $p < 0.05$.

RESULTS

Cell viability is higher in glioblastoma cell lines compared to control glia cells

Glia (IHA), A172 and U87 cells were treated with 1 mM MPTP for 24 hours and assayed to determine the cell viability with MTT ((3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide)) assay. A significant decrease in cell viability was observed in IHA cells when compared to vehicle-treated control cells. Similarly, the cell viability was significantly decreased in MPTP-treated A172 or U87 cells when compared to vehicle-treated controls. However, when MPTP-treated A172 was compared with the MPTP-treated IHA cells, it was observed that the cell viability was significantly higher in MPTP-treated A172 cells indicating that this glioblastoma cell line was more resistant to MPTP treatment compared to control glia cells. Similarly, MPTP-treated U87 cells also showed a higher cell viability after 24-hour treatment of 1 mM MPTP compared to MPTP-treated glia cells. This shows that glioblastoma cells were more resistant to MPTP-induced cell death compared to glia cells.

Glioblastoma cell lines display reduced expression of Monoamine Oxidase B (MAO-B) compared to control glia cells

Monoamine Oxidase B (MAO-B) is normally expressed on glia cells and thought to play a role in metabolizing MPTP to MPP⁺. Therefore, it is important to find out the level of MAO-B

expression on glioblastoma cell lines and control glia cells. Using Human MAO-B ELISA Kit, the levels of protein expression of MAO-B in IHA, U87 and A172 were measured. The level of MAO-B protein expression is significantly lower in both U87 and A172 cells compared to control IHA cells.

DISCUSSION

MPTP is a Parkinsonism inducing toxin that is metabolized in glia cells. After systemic administration, MPTP is able to cross blood-brain barrier as is and taken up by glial cells. MAO-B enzyme in glial cells converts MPTP to MPDP⁺. Afterwards, MPDP is oxidized to MPP⁺ in glial cells. Then, MPP⁺ is released to extracellular space and enters dopaminergic neurons via dopamine transporters (DATs). The affinity between MPP⁺ and dopamine transporters are thought to be the reason for the destruction of majorly dopaminergic cells by MPP⁺ and therefore MPTP being a reliable and reproducible chemical model for Parkinson's Disease.

The association between glioblastomas and Parkinson's Disease has long been known and remains controversial. The association arised from glioblastoma patients having PD syndrome (9) and also a PD gene being observed in different cancers including glioblastoma (10). Therefore, in this study, two glioblastoma cell lines U87 and A172 and a control glial cell line IHA were treated with 1 mM MPTP for 24 hours and then assayed for cell viability. U87 and A172 cells displayed a significantly higher cell viability

compared to control glia cells indicating a higher resistance to MPTP and its effects. There can be various reasons for this, one of which might be the tolerance shown to MPTP by the cells. The major defining factor for this is how much MPTP is converted by glial cells. Therefore, the protein expression levels of MAO-B enzyme in the cells were analyzed using ELISA Assay. It was shown that glioblastoma cell lines both express significantly lower levels of MAO-B enzyme compared to control glial cells. It is thought that since the MAO-B enzyme level is lower in glioblastoma cells, the toxic MPP⁺ metabolite generated is also lower and therefore the cell viability is higher compared to glia cells.

It is clearly not known in literature how MPTP enters glial cells. The entrance of MPTP into glial cells might be another tolerance or resistance mechanism and might cause a difference between the glial cells and glioblastoma cells. Monoamine oxidases A and B (MAO-A and MAO-B) were found to be highly expressed in cancers. Via immunohistochemical staining, MAO-B was found to display higher expression in gliomas and correlated with tumor grade and hypoxia-inducible factor 1-alpha (HiF-1α) expression (12).

Additionally, MAO-B has recently been shown as a potential therapeutic target for Alzheimer's disease because of its relation to the γ-aminobutyric acid (GABA) in reactive astrocytes (13). Although short-term treatment with irreversible MAO-B inhibitors improves cognitive deficits in patients, long-term treatments showed disappointing and discouraging results. The association between PD and cancer is complicated since epidemiological studies support an inverse correlation, however, increasing evidence points to a positive association (14). Therefore, molecular studies such as the current study will enlighten the overlapping molecular pathways.

Finally, the results of the current study show for the first time the resistance of A172 and U87 glioblastoma cell lines against MPTP treatment when compared to control glial cells and again significantly lower MAO-B protein expression. This feature of glioblastoma cell lines could be harvested for studies to develop therapeutics against Parkinson's Disease. MAO-B is an important candidate in many brain diseases including glioblastomas to develop therapies. Therefore, the addition of information regarding its expression in different cell lines is critical.

REFERENCES

- Ohgaki H, Kleihues P. The definition of primary and secondary glioblastoma. *Clin Cancer Res* 2013; 19(4):764-72.
- Kiseleva LN, Kartashev AV, Vartanyan NL, Pinevich AA, Samoilovich MP. A172 and T98G Cell Lines Characteristics. *Cell and Tissue Biology* 2016;10 (5): 341–8.
- Dauer W, Przedborski S. Parkinson's Disease: Mechanisms and Models. *Neuron* 2003; 39: 889–909
- Klein C, Westenberger A. Genetics of Parkinson's Disease. *Cold Spring Harb Perspect Med.* 2012; 2(1): a008888.
- Gaweska H, Fitzpatrick PF. Structures and mechanism of the monoamine oxidase family. *Biomol Concepts* 2011; 2(5): 365–77.
- Smeyne RJ, Jackson-Lewis V. The MPTP model of Parkinson's disease. *Mol Brain Res.* 2005; 134(1): 57-66.
- Jankovic J. Parkinson's Disease: clinical features and diagnosis. *J Neurol Neurosurg Psychiatry* 2008; 79: 368-76.
- Kalia LV, Lang AE. Parkinson's Disease. *Lancet* 2015; 386(9996): 896-912.
- Ye R, Shen T, Jiang Y, Xu L, Si X, Zhang B. The relationship between Parkinson Disease and brain tumor: a meta-analysis. *PLoS One.* 2016; 11(10): e0164388.
- Veeriah S, Taylor BS, Meng S, Fang F, Yilmaz E, Vivanco I et al. Somatic mutations of the Parkinson's disease-associated gene PARK2 in glioblastoma and other human malignancies. *Nat Genet* 2010 ; 42(1):77-82.
- Turcan S, Rohle D, Goenka A, Walsh LA, Fang F, Yilmaz E et al. IDH1 mutation is sufficient to establish the glioma hypermethylator phenotype. *Nature* 2012; 483(7390): 479-83.
- Sharpe MA, Baskin DS. Monoamine oxidase B levels are highly expressed in human gliomas and are correlated with the expression of HiF-1α and with transcription factors Sp1 and Sp3. *Oncotarget* 2016; 7(3): 3379–93.

13. Park JH, Ju YH, Choi JW, Song HJ, Jang BK, Woo J et al. Newly developed reversible MAO-B inhibitor circumvents the shortcomings of irreversible inhibitors in Alzheimer's disease. *Sci Adv* 2019; 5(3): eaav0316
14. Feng, DD, Cai W, Chen X. The associations between Parkinson's disease and cancer: the plot thickens. *Transl Neurodegener* 2015; 4: 20.

Corresponding author

Gizem DÖNMEZ YALÇIN (Doç. Dr.)
Adnan Menderes Üniversitesi Tıp Fakültesi,
Tıbbi Biyoloji Ana Bilim Dalı, Aydın
E-mail: gizem.yalcin@adu.edu.tr
ORCID : 0000-0002-5121-8232