Research Article

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Effect of various doses of metformin on map-kinase and apoptosis in penicillin-induced secondary rat cortical astrocytes-can metformin be antiepileptic?

Penisilinle indüklenen sekonder sıçan kortikal astrositlerinde çeşitli metformin dozlarının map-kinaz ve apoptoz üzerine etkisi- metformin antiepileptik olabilir mi?

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Abstract

Purpose: Astrocytes in the brain and spinal cord perform many functions and it express plasma membrane carriers for various neurotransmitters. Often sclerotic areas in the hippocampus are known as the cause of epilepsy. In reality, astrocytes initiate glutamate release in sclerotic tissue. In experimental epilepsy, penicillin is widely used to initiate this neuronal stress and establish the epilepsy model. The aim of this study is to demonstrate the protective effect of metformin, which has been proven to be effective in neurodegenerative diseases, on penicillin-induced astrocyte degeneration on the primary astrocyte cell line. This study is thought of as the first study about the effect of metformin on penicillin-induced astrocyte death.

Materials and methods: For this purpose, secondary astrocyte cell culture which was previously obtained from the newborn rat brain was used.

Results: The viability of penicillin-induced astrocyte cells was statistically significantly decreased (p=0.01), While cell viability increased statistically significantly in the metformin group at all metformin doses (p=0.01).

Conclusions: We conclude that metformin has protective effects on damage caused by the penicillin secondary astrocyte cell line. These results suggest that penicillin reduces astrocyte cell viability, but metformin protects astrocytes against the lethal effect of penicillin.

Keywords: Astrocyte, penicillin, insulin, rat, cell culture.

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Öz

Amaç: Beyin ve omurilikteki astrositler birçok işlevi yerine getirir ve çeşitli nörotransmitterler için plazma membran taşıyıcıları eksprese ederler. Hipokampustaki sklerotik alanlar sıklıkla epilepsinin nedeni olarak bilinir. Gerçekte, astrositler sklerotik dokuda glutamat salınımını başlatır. Deneysel epilepside, bu nöronal stresi başlatmak ve epilepsi modelini oluşturmak için penisilin yaygın olarak kullanılır. Bu çalışmanın amacı, nörodejeneratif hastalıklarda etkili olduğu kanıtlanmış olan metforminin, birincil astrosit hücre hattında penisilin kaynaklı astrosit dejenerasyonuna karşı koruyucu etkisini göstermektir. Bu çalışma, metforminin penisilin kaynaklı astrosit ölümüne etkisi hakkında yapılan ilk çalışma olarak kabul edilmektedir.

Gereç ve yöntem: Bu amaçla, daha önce yeni doğmuş sıçan beyninden elde edilen ikincil astrosit hücre kültürü kullanılmıştır.

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Bulgular: Penisilin kaynaklı astrosit hücrelerinin canlılığı istatistiksel olarak anlamlı derecede azalmıştır (p=0,01). Metformin grubunda ise tüm metformin dozlarında hücre canlılığı istatistiksel olarak anlamlı derecede artmıştır (p=0,01).
Sonuç: Metforminin, penisilin kaynaklı ikincil astrosit hücre hattı hasarına karşı koruyucu etkileri olduğu sonucuna vardık. Bu sonuçlar, penisilinin astrosit hücre canlılığını azalttığını, ancak metforminin astrositleri penisilinin öldürücü etkisine karşı koruduğunu göstermektedir.

Anahtar kelimeler: Astrosit, penisilin, insülin, sıçan, hücre kültürü.

Özdemir H, Özdemir MB, Tokgün O, Akça H, Kurbetli N, Aygün D, Dedeakayoğulları H, Elmas L, Demirci M. Penisilinle indüklenen sekonder sıçan kortikal astrositlerinde çeşitli metformin dozlarının map-kinaz ve apoptoz üzerine etkisi- metformin antiepileptik olabilir mi? Pam Tıp Derg 2026;19:531-538.

Introduction

Astrocytes, also collectively known as astroglia, are characteristic star-shaped glial cells in the brain and spinal cord. They perform many functions such as biochemical support of endothelial cells forming the blood-brain barrier, supply of nutrients to the nervous tissue, maintenance of extracellular ion balance, and the repair and scarring process of the brain and spinal cord after traumatic injuries. Also, astrocytes express plasma membrane transporters such as glutamate transporters for various neurotransmitters, including glutamate, ATP, and gamma aminobutyric acid (GABA). It has been shown recently that astrocytes secrete glutamate or ATP in a vesicular Ca2+ dependent manner [1-3]. On the other hand, the common antibiotic penicillin is a chemical convulsant [4, 5]. It causes neuronal degeneration and neuronal loss [6, 7] by reducing the GABAergic effect [8], increasing excitatory glutamatergic neurotransmission [6] or activating Ca2+ and Na+ transmission in ion channels. Glutamate excitotoxicity is believed to be associated with neuronal stress [9-12].

The GABAergic effect is important in astrocyte cell death [8]. Increased excitatory glutamatergic neurotransmission and activated Ca2+ and Na+ transmission in ion channels may also be the mechanism of astrocyte cell death [7-11].

Metformin is an antidiabetic drug that is effective in the treatment of non-insulin-dependent diabetes mellitus [type 2 diabetes] and is increasingly used all over the world [1]. In addition, it has been studied in many areas from cardiovascular diseases to its protective effect on cancer and neurodegeneration, and its effect has been demonstrated. Metformin has been one of the most studied drugs in recent years. In a study, a relatively low dosage of metformin was shown to reduce insulin resistance and related cardiovascular risk parameters in patients with HIV-infected lipodystrophy. It has been suggested that metformin keeps glucose levels at an optimal level, and its immune-modulating property may have a beneficial effect on the outcomes of patients [12-17]. There is increasing evidence of its benefits as well. There are studies showing that certain neurodegenerative diseases and diabetes are clearly linked, and that metformin in combination with other diabetes medications can reduce neurological symptoms in some patients and reduce disease phenotypes in animal and cell models.

In our study, we wanted to show the protective effect of metformin in penicillin-induced astroglial cell culture. Because astrocytes actually initiate the release of glutamate in the sclerotic tissue. Glutamate excites surrounding neurons and seizure activity occurs. It explained several moleculer-level signaling pathways related to astrocytes. One of these is down-regulated MAP kinases. Fellin et al. [18] determined that astrocytic glutamate has a role in the actual ictal period, not in the onset of epileptiform seizures [14, 19-22]. This may have been the case in epileptiform seizures seen as a result of high fever in children. Viruses such as meningitis, herpes [6], and herpes simplex [7] may also have caused seizures by the same mechanism. The high incidence of astrocytes in epileptic seizures suggests the possibility of using them as an effective antiepileptic.

Materials and methods

The study PAUHADYEK-2019/35, titled "The Effect of Various Doses of Metformin on Map Kinases and Apoptosis in Penicillin-Induced Secondary Rat Cortical Astrocytes," was discussed at our meeting held on October 9, 2019 (no: 2019/07). Following the discussions, approval from the Animal Experiments Ethics Committee is not required, in accordance with Article 1 of the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, dated February 15, 2014, and numbered 28914.

The astroglial cell line obtained as a primer in our own laboratory and stored in a suitable environment for later use was used in our study (Pamukkale University, Faculty of Medicine). Experimental groups were divided into a control group, a PBS group, a penicillin group (300, 500, 1000, 1500 and 2000 IU), a metformin group (0.25, 0.5, 1 and 2 micrograms) and, finally, a penicillin-metformin group. Cell viability was found to be at least 1000 IU in the penicillin group (Table 1, Graph 1). Astrocytes affected with 1000 IU of penicillin were given 0.25, 0.5, 1, and 2 micrograms of metformin, respectively.

Statistical Analysis

Values are expressed as the mean of percentages from 18 independent experiments. Statistical significance was determined by One Way Analysis of Variance (ANOVA) followed by a post-hoc test (Fisher's Protected Least Significant Difference (Fisher's PLSD) using StatView software (Abacus concepts, Berkeley, CA, USA). $p<0.05$ was considered statistically significant.

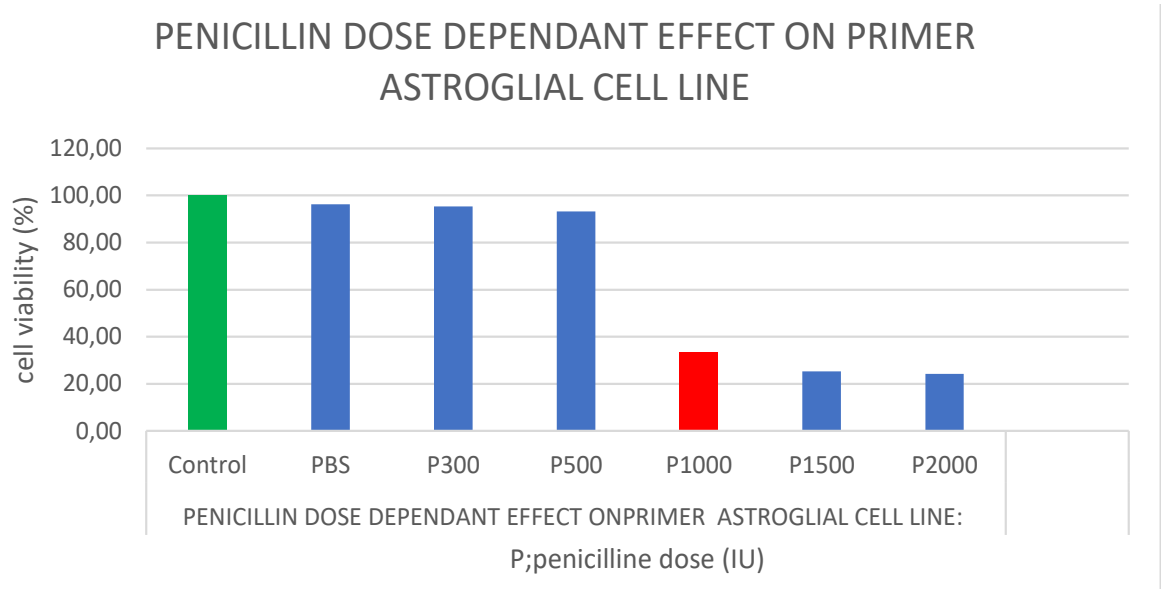
Results

The viability of penicillin-induced astrocyte cells was statistically significantly decreased (p=0.01; F=4.46). The group with the highest cell viability decreased was the penicillin dose of 1000 IU (Table 1, Graph 1).

Table 1. Penicillin dose dependant effect on primer astroglial cell line

	Control	PBS	P300	P500	P1000	P1500	P2000
Cell Viability [%]	100.00	96.30	95.36	93.19	33.36	25.22	24.15

P: Penicilline, dose [IU], (p=0.01; F=4.46); F: One Way Analysis of Variance



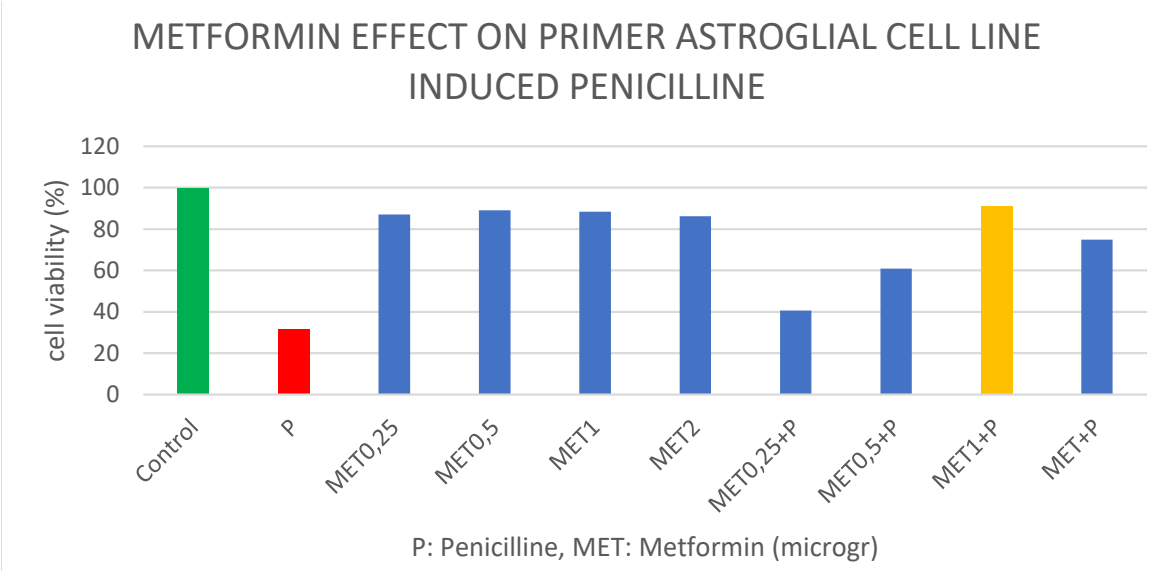
Graph 1. Penicillin dose dependant effect on primer astroglial cell line

While cell viability decreased up to 31 percent in the penicillin group, it increased statistically significantly in the metformin group at all metformin doses (p=0.01; F=3.46) Especially in the group given 1 microgram, cell viability increased the most (Table 2, Graph 2).

Table 2. Metformin effect on primer astroglial cell line induced penicilline

Control	P	MET0,25	MET0,5	MET1	MET2	MET0,25+P	MET0,5+P	MET1+P	MET+P
100	31.87	87.1	89.11	88.33	86.38	40.58	60.89	91.072	27.89

P: Penicilline, dose [IU], MET; Metformin, (p=0.01; F=3.46); F: One Way Analysis of Variance



Graph 2. Metformin effect on primer astroglial cell line induced penicilline

Discussion

These results suggest that penicillin reduces astrocyte cell viability, but metformin protects astrocytes against the lethal effect of penicillin. In fact, this study aimed to create a cellular-level epilepsy model. Furthermore, the protective effect of metformin was demonstrated. Experiments were conducted by applying different metformin concentrations to cell lines in penicillin-free and penicillin-containing culture media. Finally, while penicillin-induced astrocyte viability decreased, a significant increase in viability was observed in the metformin group at all metformin doses.

However, some limitations should be noted. The first limitation is that the greatest decrease in cell viability decreased was observed in the 1000 IU penicilin dose group. The other finding was that the treated group with 1 microgram metformin showed the greatest increase in cell viability.

Metformin is an antidiabetic drug that is effective in the treatment of non-insulin-dependent diabetes mellitus [type 2 diabetes] and is increasingly used all over the world [13]. Metformin is a cheap and safe drug with few side effects. Therefore, it is used in in vitro and in vivo experiments [22]. Besides diabetes, positive effects of metformin in cardiovascular diseases, obesity, cancer, polycystic ovary and neurodegenerative diseases have been studied and shown.

Until 2017, metformin was used in a controlled manner in pregnant women since it was a pregnancy Category C. As a result of many years of research, it has been evaluated

as Category B by the US Food and Drug Administration (FDA) since 2017 and has been used safely in pregnant women [23]. It has been suggested that metformin exerts its effect on the virus by increasing insulin sensitivity [24]. In a US study of patients older than 24 years and patients hospitalized for pneumonia with a previous history of diabetes, pre-administration of metformin was associated with significantly lower mortality [25]. By and large, older people are more prone to COVID-19 than younger people. The mortality rate of elderly patients with COVID-19 is higher than that of young and middle-aged patients. Therefore, it is thought that it may be possible to reduce the mortality rate of COVID-19 by reducing obesity and pneumonia in elderly, obese and diabetic patients by using metformin [14].

Metformin has the ability to cross the blood-brain barrier and distribute to various parts of the brain. The neuroprotective effects of metformin have been demonstrated in a variety of brain diseases; however, the mechanisms involved are not fully understood. The most accepted molecular mechanism of metformin is the activation of AMPK, the cellular energy sensor activated under metabolic stress. The data also showed that AMPK is the primary target of metformin for its protective function against cognitive impairment, but downstream signaling pathways have been shown to differ in various disease models. Studies have shown that metformin-induced AMPK activation leads to the activation of the Brain-derived neurotrophic factor/P26S6 kinase (BDNF/P26S6K) pathway in hippocampal neurons to increase memory formation in passive avoidance tasks in a global cerebral ischemia/reperfusion rat model [26].

The link between diabetes and neurodegenerative diseases is widely accepted, although the data are inconclusive and the mechanisms involved are unclear. There is a large pool of data on the use of metformin in humans and animals with neurodegenerative diseases, but the therapeutic use of metformin has not yet been accepted, as results are often contradictory. There are studies showing that certain neurodegenerative diseases and diabetes are clearly linked, and that metformin in combination with other diabetes drugs can reduce neurological symptoms in some patients and disease phenotypes in animal and cell models [27].

Recent epidemiological studies have shown that metformin treatment prevents cognitive decline in diabetic patients at high risk of developing dementia. Preclinical studies have revealed that metformin reduces Alzheimer's-like pathology in mouse models of Alzheimer's Disease (AD). Metformin improved learning and memory in a mouse model of spontaneous onset AD. In addition, biochemical analyzes have shown that metformin improves memory by reducing APPc99 and pTau [28]. It was thought that metformin could be used in addition to treatment in Parkinson's disease (PD), which is also a neurodegenerative disease. Parkinson's disease is characterized by severe movement defects and is commonly treated with the dopamine (DA) precursor 3.4-dihydroxyphenyl-1-alanine (L-DOPA). However, long-term use of L-DOPA can lead to the development of L-DOPA-induced dyskinesia (LID). The results of the studies suggest that metformin may have therapeutic potential for the suppression or treatment of L-DOPA-induced motor complications in PD patients [29]. It is even argued that metformin may have the potential to improve the basic neurological symptoms of specific neurodegenerative diseases such as Fragile X [30].

In particular, other molecular targets of metformin, including p38 mitogen-activated protein kinase [p38 MAPK] and c-Jun N-terminal kinase (JNK), have been identified as responsible for the drug's effects in different experimental contexts. These results showed that the protective effect of metformin against oxidative stress-induced apoptosis is in part through inhibition of JNK (p19) activation [31, 32].

It has been understood that JNK is an important mediator in insulin resistance and is a critical element in the pathogenesis of fatty liver disease and type 2 diabetes [33]. However, the pro-apoptotic mechanisms are not fully understood. Scientists have been doing a lot of work on the effects of metformin on JNK and p38, especially in recent years. In another study, it was shown that metformin treatment

can activate AMP-activated protein kinase, JNK/p38 MAPK signaling pathway and caspases, and also increase the expression of gene 118 (GADD118) inducible by growth arrest and DNA damage. In the same study, it was shown that metformin induced apoptosis by activating the JNK/p38 MAPK pathway and GADD118.

Common causes of organic epilepsy are divided into sclerotic and non-sclerotic. The effects of different genes expressed in the sclerotic hippocampus on several signal pathways related to astrocytes at the molecular level have been described. In fact, astrocytes initiate the release of glutamate in the sclerotic tissue. Glutamate excites surrounding neurons and seizure activity occurs.

Hippocampal atrophy, neuronal cell loss and astrocyte cell proliferation were first demonstrated in the sclerotic hippocampus in 1880. In a surgical series, 116 patients with middle temporal lobe epilepsy (MTLE) had sclerosis. However, mass temporal lobe epilepsy [MaTLE] 28% and paradoxical temporal lobe epilepsy (PTLE) 12% are non-sclerotic cases. It has been seen here that hippocampal atrophy is not correlated with the severity and duration of the seizure [17].

It has also been observed that seizure remissions occur 2 years after the resection of the sclerotic area. The conclusion drawn from these findings is that hippocampal seizures in the sclerotic region contribute to astrocytes hyperexcitability. The role of astrocytes in absence seizures has been debated until recently. In summary, astrocytes may contribute to seizures as follows: Na+ channels increase in the astrocyte membrane, and the mRNA ionotropic glutamate receptor-1 (GluR1) becomes more sensitive to glutamate. Glutamate synthetase is down-regulated. Nuclear factor kappa B (NFkB) [which takes part in the inflammatory reaction] increases. As a result, calcium-dependent glutamate release occurs from astrocytes to the extracellular space in the sclerotic hippocampus. This triggers the seizure. EEG also showed hyperexcitability in the gyrus dentatus in patients with MTLE. However, this was not seen in the EEG of patients with PTLE and MaTLE. In patients with MaTLe, the seizure completely disappeared when the tumor was removed. In this study, when hippocampal sclerotic and non-sclerotic epilepsy were compared, 4-fold neuronal loss, 3-fold gliosis in the Cornu Ammonis area 1 [CA1] region, and hippocampal atrophy were also observed in patients. Astrocytes and microglial cells may play a central role in the brain's immune processes. Extracellular glutamate is very high in seizures. It may be a misconception that the cause or source of this is the sclerotic hippocampus. Because glutamergic neurons are already displaced here. Therefore, their source is thought to be astrocytes [16]. Astrocytic glutamate can stimulate the paroxysmal depolarization pathway in seizures [17]. And they can play a very important role in the ictal period in seizures [18]. Fellin et al. [18] determined that astrocytic glutamate has a role in the actual ictal period, not in the onset of the epileptiform seizure. [18]. It is the main source of outputs from the hippocampus to the brain. Viruses such as epileptiform seizures, meningitis, and herpes simplex seen in children as a result of high fever may also have caused seizures by the same mechanism. The occurrence of astrocytes in epileptic seizures suggests the possibility of playing an active role in antiepileptics [14, 19-21, 28].

In conclusion, astrocyte and microglial cells play a central role in the immune processes of the brain. They are rapidly activated in situations of neuronal stress in the brain. It has been shown in this study that penicillin causes neuronal stress as well as astrocyte cell stress. It is now known that neuron-astrocyte cell stress causes epileptic seizures due to astrocytic glutamate. Metformin is known primarily for its oral antidiabetic effects, as well as for its anticarcinogenic effects and neuroprotective effects. In our study, its protective effect against penicillin-induced astrocyte degeneration was demonstrated. With this result, it may be possible to use metformin as an antiepileptic.

Ethics committee approval: Approval from the Animal Experiments Ethics Committee is not required, in accordance with Article 1 of the Regulation on the Working Procedures and Principles of Animal Experiments Ethics Committees, dated February 15, 2014, and numbered 28914.

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Authors' contributions: O.H., O.M.B. constructed the main idea and hypothesis of the study. O.H., O.M.B., T.O. and A.H. developed the theory and arranged the Materials and Methods section. O.H., O.M.B., A.D., E.L., D.M., K.N. and D.H. evaluated the data in the Results section. The Discussion section was written by O.H., O.M.B. who also reviewed, corrected and approved the manuscript. In addition, all authors discussed the entire study and approved the final version.

Conflict of interest: No conflict of interest was declared by the authors.

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