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Effect of captopril on the oxidative damage caused by pentylenetetrazole in the SHSY-5Y human neuroblastoma cell line

Kaptoprilin SHSY-5Y insan nöroblastom hücre hattında pentilentetrazolün neden olduğu oksidatif hasar üzerindeki etkisi

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SUMMARY

Objective: Epilepsy is a crucial brain disorder that seizures could cause a neuronal loss in the hippocampus. Oxidative stress has an important role in the pathology of this way. The aim of this study was to investigate the neuroprotective effect of captopril, on pentylenetetrazole (PTZ) induced epileptic seizures in SH-SY5Y cell line.
Method: In this XTT cell viability assay, captopril was performed *in vitro* SH-SY5Y cell culture to evaluate PTZ-induced neurotoxicity. Tissue TOS concentrations at the cell supernatants were quantified with the automated assay method. Hydrogen peroxide was used for the calibration of the assay.
Results: When XTT cell viability results were evaluated, captopril did not affect neuronal viability in SH-SY5Y cell line. Moreover, captopril did not have significant effect on TOS levels (***P>0. 001)
Conclusions: Results showed that, captopril did not have neuroprotective properties in SH-SY5Y cell line after PTZ-induced neurotoxicity.
Keywords: Neurotoxicity, captopril, PTZ.

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ÖZET

Amaç: Epilepsi, nöbetlerin hipokampusta nöron kaybına neden olduğu önemli bir beyin hastalığıdır. Oksidatif stres bu yolun patolojisinde önemli bir role sahiptir. Bu çalışmanın amacı, kaptoprilin, SH-SY5Y hücre hattında pentilentetrazol (PTZ) ile indüklenen epileptik nöbetler üzerindeki sinir koruyucu etkisini araştırmaktır.
Yöntem: XTT hücre canlılığı deneyinde PTZ' nin neden olduğu nörotoksisiteyi değerlendirmek için kaptopril *in vitro* SH-SY5Y hücre kültüründe uygulandı. Hücre süpernatanlarındaki doku TOS konsantrasyonları otomatik test yöntemi ile ölçüldü. Analizin kalibrasyonu için hidrojen peroksit kullanıldı.
Bulgular: XTT hücre canlılığı sonuçları değerlendirildiğinde, kaptopril, SH-SY5Y hücre hattında nöronal canlılığı etkilemedi. Ayrıca, kaptoprilin TOS seviyeleri üzerinde de önemli bir etkisi olmamıştır (P> 0. 001).
Sonuç: Sonuçlar, kaptoprilin, PTZ ile indüklenen SH-SY5Y hücre hattı nörotoksisitesinde sinir koruyucu etkiye sahip olmadığını gösterdi.
Anahtar sözcükler: Nörotoksisite, kaptopril, PTZ.

INTRODUCTION

Epilepsy is defined as a central nervous system disorder characterized by repetitive seizures that generally occur due to a genetic predisposition or chronic pathological situation[1,2]. Assessment the role of oxidative stress in the pathophysiology of seizures is curicial to describe suitable therapeutic strategies. Components with antioxidant and neuroprotective function may perform positive effects when associated with antiepileptic properties. The frequent event of epileptic seizures considerably decreases the memory and learning capacity in patients with epilepsy[3]. It has been shown that pentylenetetrazole (PTZ)-induced seizures can cause memory impairment in rodents[4]. The hippocampus and cortex are both effective on memory and learning. Epileptic seizures lead to neuronal death via aggravating calcium influx into cells. Thus, the brain neuro-degenerates, particularly in the hippocampus, and changes the function of variable synapses that store information, which may be a statement of the observed learning disorder following seizure[5,6]. Experimental epilepsy models have been performed to explain the basic mechanisms include in epileptic seizures. PTZ is a chemical agent used to create experimental seizures which is a selective antagonist of the GABA-A receptor. PTZ causes adverse effects on the neuronal membrane, affects calcium and potassium channels, releases intracellular calcium ion reserves, and decreases the neurotransmitter-induced chloride conductivity. This model is clinically considered as an answer to temporal lobe epilepsy and complex partial epilepsy [7,8]. Captopril is one of the most commonly prescribed antihypertensive drugs that inhibits Angiotensin II (Ang II) converting enzyme (ACE). Captopril is commonly used for secondary prevention of cardiovascular events in patients with diabetes. It can selectively lower the Ang II, endothelin, and oxidative stress, which may have a potential role in its blood pressure-lowering effect[5,9]. It can pass the blood-brain barrier readily. Current literature show that decreased angiotensin II activity caused by ACE inhibitors can be associated with antidepressant and anxiolytic effects [10,11].

The objective of the present study was to examine the neuroprotective effects of the ACE inhibitor captopril in SHSY-5Y cell lines.

MATERIAL AND METHODS

In vitro studies

Cell culture

SHSY-5Y (CRL-2266) cell lines were obtained from American Type Culture Collection and cultured in DMEM (Thermo Fisher Scientific, Altrincham, UK) containing 10 % Fetal Bovine Serum (FBS) (Sigma-Aldrich Co., St Louis, MO, USA), 1% penicillin/streptomycin (Sigma Aldrich Co., St Louis, MO, USA), and 1% L-glutamine (Sigma-Aldrich Co., St Louis, MO, USA). The cells were maintained in an incubator (at 37 °C and 5% CO_2 humidified atmosphere). Captopril and pentylenetetrazole (PTZ) (Sigma-Aldrich Co., St Louis, MO, USA) were dissolved in DMEM and stock solutions were prepared before treatment.

Cell viability assay

Cell viability was evaluated using the XTT assay (Roche Diagnostic, MA, USA). Initially, SHSY-5Y cells were seeded in 96-well plates at a density of 1×10^4 cells in 100- µL DMEM per well and incubated overnight before treatment. The following day, four-cell groups were prepared to assess the neuroprotective effect of captopril. The control group did not perform any treatment. Cells in the PTZ group were treated with 30 mM PTZ for 24 h. Cells in the captopril group were treated with various concentrations (12.5, 25, 50, 100, and 200 µM) of captopril for 24 h. Cells in the PTZ+captopril group were treated with various concentrations (12.5, 25, 50, 100, and 200 µM) of captopril for 1 h and then implemented to 30 mM PTZ for 24 h. After incubation, the medium was removed and wells were washed with phosphate-buffered saline (PBS). Then, 100 µL DMEM without phenol red, and 50 µL XTT labeling solution was added to all the wells, and then the plates were maintained at 37 °C for 4 h. The absorbance of samples was detected using an ELISA micro-plate reader (Thermo Fisher Scientific, Altrincham, UK) at 450 nm. All the experiments were performed three times and the cell viability was measured as viable cell percentages compared to the control group (untreated cells).

Preparation of cell homogenates

The cells were collected in sterile tubes and were centrifuged at 2000 rpm for 10 min. Then, the supernatants were removed and the components of the cells were suspended using PBS (pH 7.4) to dilute cell suspension of the cell concentration (approximately 1 million/mL). The cells were damaged through repeated freeze–thaw cycles to

let out the internal components of the cells and were centrifuged at 4000 rpm for 10 min at a temperature of 4 °C. Then, the supernatants were collected for biochemical analysis of total oxidant status (TOS) by using TOS commercial kit (Real Assay Kit Diagnostics, Antep, Turkey). The Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) was used to determine the total protein levels in the samples.

Measurement of TOS

Tissue TOS concentrations at the cell supernatants were quantified with the automated assay method of Erel [2]. Because ferrous ions are oxidized into ferric ions when sufficient quantities of oxidants are available in the medium, the method allows for quantifying TOS levels by measuring tissue levels of ferric ions with the utilize of xylenol orange. Hydrogen peroxide was used for the calibration of the assay[3]. The results of the assay were expressed in micromolar hydrogen peroxide equivalents per milligram tissue protein (μmol H_2O_2 Eq/mg protein).

Statistical analysis

The results were expressed as a mean ± standard deviation of mean (SEM). The data analyses were evaluated using a one-way analysis of variance (ANOVA). Newman Tukey test was utilized to determine the differences between the experimental groups.

RESULTS

Effects of captopril on cell viability after PTZ-induced neurotoxicity in SH-SY5Y cells

An XTT cell viability assay was performed to assess the neuroprotective effects of captopril on PTZ-induced SH-SY5Y cell toxicity. In the previous studies, the IC50 value of PTZ in the SH-SY5Y cell was found as 30 mM and this value was used in this study[8]. As presented in Fig. 1, constant concentration of PTZ (30 mM) treatment considerably reduced cell viability compared to the control ($P<0.001$). According to the results, it can be said that PTZ has a neurotoxic effect in SH-SY5Y cell line. However, when applied together, captopril did not exhibit neuroprotective effects at 12.5–200 mg/mL concentrations (***$P>0.001$). In addition, treatment of captopril at the dose of 12.5 mg/mL showed the least neurotoxicity compared to other applied doses of captopril in SH-SY5Y cells (Figure 1). There were not significant differences to the cell viability of PTZ-induced SH-SY5Y cells when the treatment of captopril at different doses. It was expected to be highest SH-SY5Y cell viability at the treatment of captopril with 200 mg/ml dose. However, at this dose, SH-SY5Y cells showed the least cell viability. In addition, there was no significant difference in viability of control group and SH-SY5Y cells treated with different concentrations of captopril in cells without the treatment of PTZ.

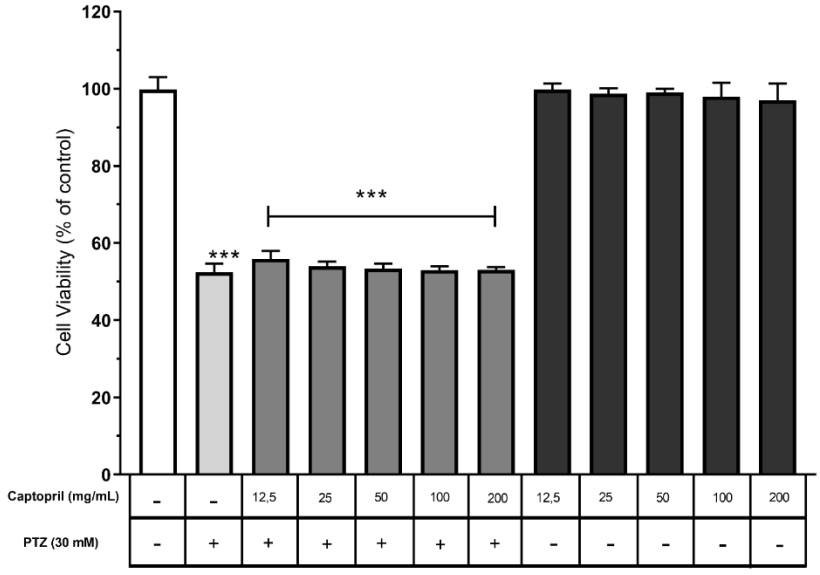

Figure 1: Effects of captopril on cell viability after PTZ induced neurotoxicity in SH-SY5Y cells. Values are presented as mean±SD (***$P>0.001$).

Assessment of captopril on TOS levels after PTZ-induced neurotoxicity in SH-SY5Y cells

The TOS levels in SH-SY5Y cells were measured using commercial kits. TOS levels in SH-SY5Y cells were significantly increased in only PTZ-treated cells compared to the control ($P<0.001$; Figure 2). Captopril alone had no notable effect on the TOS level in SH-SY5Y cells (***$P>0.001$). Captopril treatment at a dose of 100 mg/mL did not crucial effect on TOS levels in SH-SY5Y cells after PTZ-induced neurotoxicity (***$P>0.001$).

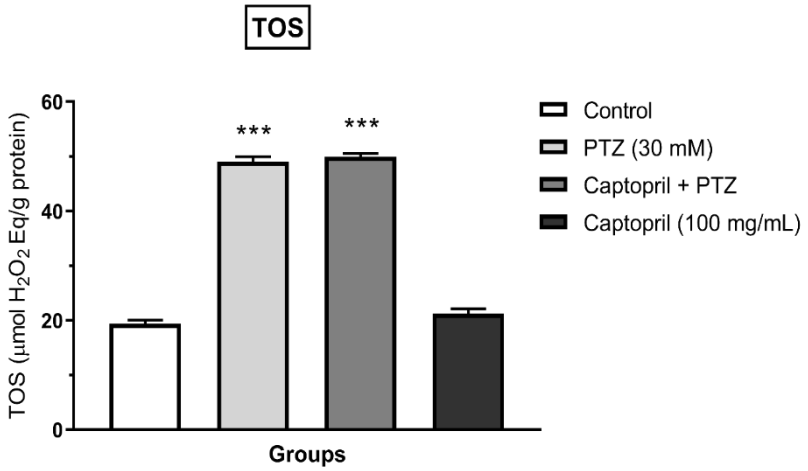

Figure 2: Effects of captopril on TOS levels after PTZ-induced neurotoxicity in SH-SY5Y cells. Values are presented as mean±SD (***$P>0.001$).

DISCUSSION

Captopril is commonly utilized ACE inhibitory, selectively and potently inhibiting ACE. Several recent studies have shown that oxidative stress and inflammation consisted of crucial part in the pathogenesis of epileptogenesis. It has been reported that captopril reduces oxidative stress and inflammation levels in tissues and they significantly increased the epileptic threshold[12]. Several experimental studies show that captopril has neuroprotective and antioxidant effects under different conditions in the rodent brain. A study reported that captopril improved the memory function by reducing oxidative stress in the hippocampus [13]. Our present study results showed that there were not significant differences to the cell viability of PTZ-induced SH-SY5Y cells when the treatment of captopril. Captopril treatment at a determined dose did not significant effect on TOS levels in SH-SY5Y cells after PTZ-induced neurotoxicity.

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Disclosure Statement

The authors have no conflict of interest to disclose.

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