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Research Article | Araştırma Makalesi

CAPSAICIN ATTENUATES BUPIVACAINE ANESTHESIA-INDUCED NEUROTOXICITY IN SH-SY5Y CELLS BY REGULATING APOPTOSIS

KAPSAİSİN APOPTOZU DÜZENLEYEREK SH-SY5Y HÜCRELERİNDE BUPİVAKAİN ANESTEZİSİNİN NEDEN OLDUĞU NÖROTOKSİSİTEYİ HAFİFLETMEKTEDİR

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ABSTRACT

Objective: Bupivacaine administered for local anesthesia can cause critical neurotoxicity and neurological dysfunctions. Any substance that can reduce bupivacaine-mediated toxic effects will be of great interest during surgical procedures and in the pain management process. In this context, we evaluated capsaicin, an alkaloid of *Capsicum annuum* (cayenne pepper), which has been intensively researched for its neuroprotective effect due to its various biological effects such as cardioprotective, anti-inflammatory, analgesic, thermogenic, and benefits on the gastrointestinal tract.

Methods: In this study, we researched the *in vitro* effect of capsaicin in SH-SY5Y cells with a model of bupivacaine-mediated neurotoxicity. Cell proliferation assay was handled by XTT, and apoptosis was determined by flow cytometry analysis.

Results: We observed a notable increase in apoptosis induction with a significant decrease in the viability of cells exposed to bupivacaine at 1 mM. We found that bupivacaine-mediated cytotoxicity was reduced when increasing concentrations of capsaicin were applied to bupivacaine-treated cells. At the same time, capsaicin also reduced apoptosis in SH-SY5Y cells exposed to bupivacaine.

Conclusion: According to our results, it is thought that the administration of capsaicin against bupivacaine-mediated neurotoxicity may be an alternative neuroprotective agent by suppressing the apoptosis response in neurons.

Keywords: Apoptosis, capsaicin, neurotoxicity, SH-SY5Y

ÖZ

Amaç: Lokal anestezi için uygulanan bupivakain, nöronlarda ciddi nörotoksisite ve nörolojik komplikasyonlara neden olabilmektedir. Bupivakain aracılı toksik etkileri azaltabilen olası bir maddenin, cerrahi işlemler sırasında ve ağrı yönetimi sürecinde büyük ilgi görecektir. Bu kapsamda, kardiyoprotektif, anti-inflamatuar, analjezik, termojenik gibi çeşitli biyolojik etkileri ve gastrointestinal sistem üzerinde faydaları nedeniyle yoğun olarak araştırılan kırmızı biberin (*Capsicum annuum*) bir alkaloidi olan kapsaisinin nöroprotektif etkisini değerlendirdik.

Yöntem: Bu çalışmada, kapsaisinin SH-SY5Y hücrelerinde bupivakain kaynaklı nörotoksisite üzerindeki *in vitro* etkisini araştırdık. Hücre canlılığı, XTT testi ile ölçüldü ve apoptoz, akış sitometri analizi ile ölçüldü.

Bulgular: 1 mM'de bupivakaine maruz kalan hücrelerin canlılığında önemli bir azalma ile apoptoz indüksiyonunda kayda değer bir artış gözlemledik. Bupivakain ile tedavi edilen hücrelere artan konsantrasyonlarda kapsaisin uygulandığında bupivakain aracılı sitotoksisitenin azaldığını bulduk. Aynı zamanda kapsaisin, bupivakaine maruz kalan SH-SY5Y hücrelerinde apoptozu da azaltmıştır.

Sonuç: Elde ettiğimiz sonuçlara göre, bupivakain aracılı nörotoksisiteye karşı kapsaisin uygulanmasının, nöronlardaki apoptoz yanıtının baskılanması yoluyla, alternatif bir nöroprotektif ajan olabileceği düşünülmektedir.

Anahtar Kelimeler: Apoptoz, kapsaisin, nörotoksisite, SH-SY5Y

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Introduction

Local anesthetics have a good efficacy profile and a wide range of applications, from surgical pain relief to postoperative pain management. However, the neuron damage and neurotoxicity they cause remain a problem.[1] Clinical studies have shown that prolonged or high-dose exposure to local anesthetics can cause spinal neurotoxicity or cause irreversible neurological dysfunctions. A growing body of evidence shows that bupivacaine, which is frequently preferred due to its effect of blocking the impulse transmission in the nerves by inhibiting the permeability of sodium ions, causes severe neurotoxicity in both animals and humans through various neuronal signaling pathways.[2] Since local anesthetic drugs are necessary for clinical applications in medicine, developing a safe-effective agent that can prevent neurodysfunction is of critical importance in protecting against bupivacaine-mediated neurotoxicity. To date, there is still no effective method to reduce local anesthetic-induced neurotoxicity. For this reason, it is very important to reveal the mechanisms that lead to local anesthesia-induced neurotoxicity and to add potent and effective adjuvants to clinical local anesthetic administration protocols.[3]

Capsaicin, which constitutes 80-90% of capsaicinoids, is responsible for the bitterness of red pepper and is the alkaloid found naturally and mainly in Capsicum species.[4] Capsaicin and capsaicinoids, which are stated to have beneficial effects on many metabolic and dermatological diseases, have beneficial activities such as antioxidant, analgesic, anti-inflammatory, and neuroprotective effects.[5]

Despite the fact indicating that capsaicin exhibited an important effect against neurotoxicity, the effect of capsaicin on bupivacaine-induced neurotoxicity in SH-SY5Y cells remains unclear. Thus, the present study attempted to investigate whether capsaicin may protect bupivacaine-induced SH-SY5Y cells from death and explore the underlying mechanisms under such effects.

Methods

Cell Culture

SH-SY5Y human neuroblastoma cells were purchased from American Type Culture Collection (Manassas, VA, USA) and maintained in DMEM (Lonza, Walkersville, MD, USA) supplemented with 10% FBS (Sigma-Aldrich St. Louis, MO, USA), 2 mM L-glutamine, 100 mg/ml streptomycin, and 100 units/ml penicillin (Gibco Thermo Fisher Scientific) at 37°C in a humidified incubator containing 5% CO_2.

XTT Assay

To determine the neuroprotective effect of capsaicin, cell viability and XTT assay were applied. For experimental conditions, SH-SY5Y cells were maintained in a humidified incubator with 5% CO_2 at 37°C. Four different groups were assigned for the experimental protocol: 1. control group, 2. capsaicin group (5, 10, 20, 40, 80, and 100 µM), 3. bupivacaine group (0.1, 0.5, 1, 2, 2.5, and 5 mM), 4. capsaicin + bupivacaine group (Cells exposed to capsaicin at concentrations of 2.5, 5, and 10 µM for 24 hours were then treated with bupivacaine (1 mM) for 2 hours. XTT test reagent was added to the groups whose incubation times were completed, and absorbance measurements were made by means of a spectrophotometer (Thermo, Germany) at 450 nm. Test results obtained with three independent replicates were expressed as % viability compared to control.

Determination of Cell Apoptosis by Flow Cytometry

Apoptotic and late-apoptotic cells were determined in accordance with the Muse® Annexin V & Dead Cell kit manufacturer's instructions by flow cytometry analysis. SH-SY5Y cells treated with bupivacaine (1 mM) and/or capsaicin (60 µM) for 24 hours were then incubated with Annexin V and Dead Cell Reagent (Luminex, Austin, USA) for 20 minutes at 20-22°C. After that, early and late apoptotic cells were determined by the Guava® Muse® Cell Analyzer (Luminex, Europe).

Statistical Analysis

All data offered as mean ± SD were analyzed with GraphPad Prism 7.0 software. ANOVA or Student's t-test was used for statistical comparisons. Statistical significance was accepted as $p<0.05$.

Results

Bupivacaine-induced Neurotoxicity in SH-SY5Y Cells was Relieved by Capsaicin

The XTT assay was applied to appraise the effects of capsaicin on the SH-SY5Y cell proliferation. There was no significant change in the viability of the cells with the alone administration of capsaicin (5, 10, 20, or 40 µM) compared to the control (Figure 1A). The IC_{50} for capsaicin was 63 µM. In contrast, increasing concentrations of bupivacaine suppressed SH-SY5Y cell viability, while the concentration that induced 50% cell growth inhibition was 1 mM for bupivacaine (Figure 1B). Therefore, 1 mM bupivacaine was maintained in the subsequent experiment steps. Besides, 10 µM capsaicin administration strikingly reversed the inhibition of bupivacaine on SH-SY5Y cell viability (Figure 1C). These findings proposed that capsaicin could decrease bupivacaine-induced neurotoxicity in SH-SY5Y cells.

Capsaicin Suppressed Bupivacaine-induced Apoptosis of SH-SY5Y Cells

To understand the mechanism mediating the contribution of capsaicin to the reversal of bupivacaine-induced neurotoxicity, its effect on apoptosis was investigated by flow cytometry. The induction of apoptosis with bupivacaine alone was markedly suppressed by capsaicin (Figure 2). As a result, it has been suggested that capsaicin's suppression of SH-SY5Y cell

apoptosis is the mechanism mediating the alleviation of bupivacaine-induced neurotoxicity.

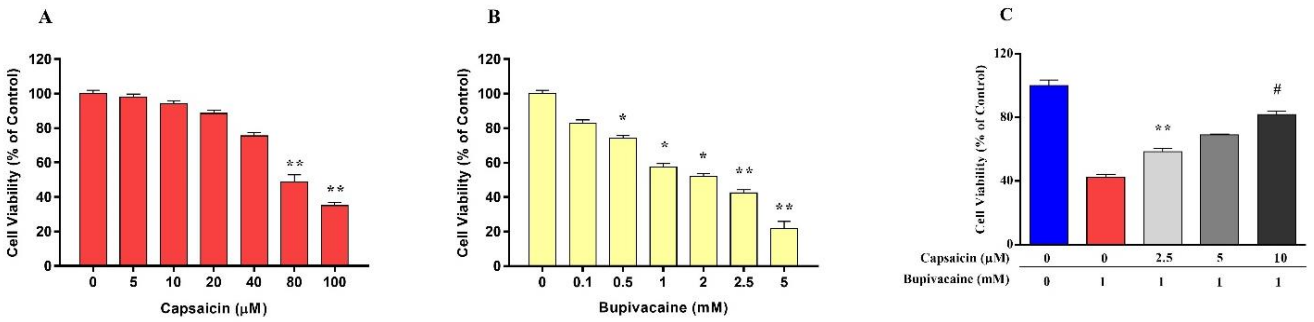
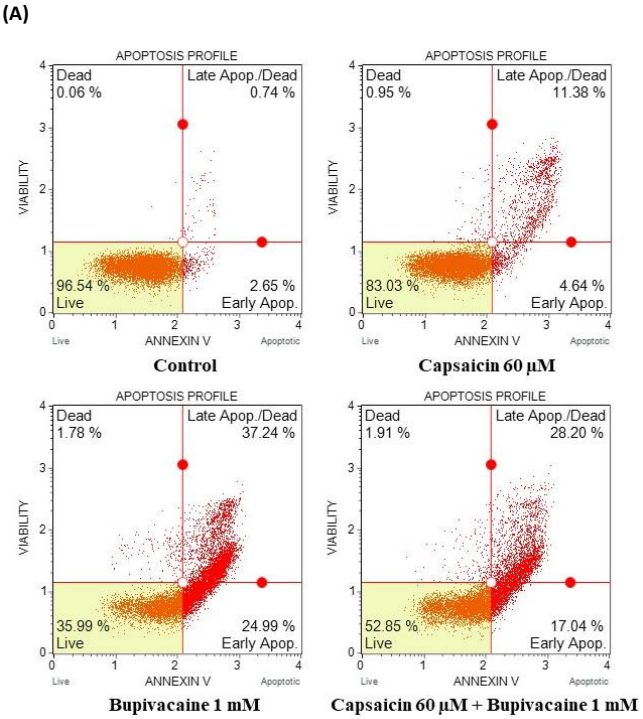


Figure 1. Capsaicin attenuated bupivacaine-induced cytotoxicity in SH-SY5Y cells. **(A)** Cell proliferation of SH-SY5Y cells treated with capsaicin (0, 5, 10, 20, 40, 80, and 100 μM) for 24 h was determined using XTT assay. **(B)** Cell proliferation of SH-SY5Y cells exposed with bupivacaine (0.1, 0.5, 1, 2, 2.5, and 5 mM) for 24 h was detected using XTT assay. **(C)** Cell viability of SH-SY5Y cells treated with capsaicin (0, 2.5, 5, and 10 μM) and/or bupivacaine (1 mM) was detected using XTT assay. *p<0.05, **p<0.01 compared with 0 μM group; ##p<0.01 compared with 1 mM bupivacaine group.



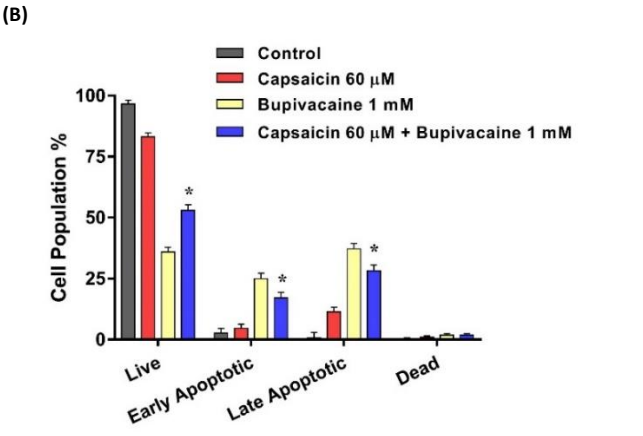


Figure 2. Capsaicin decreased bupivacaine-induced cytotoxicity via inhibition of apoptosis in SH-SY5Y cells. SH-SY5Y cells were treated with 60 μM capsaicin with or without 1 mM bupivacaine. (A) The quadrant graph representation of apoptotic cells detected by Annexin V and PI double staining. (B) The bar graph representation of calculated apoptosis cell rates.

Discussion

Capsaicin, the irritant cause of cayenne peppers, is an inhibitor of TRPV1 ion channels. According to the exhaustively describing the effects of capsaicin on sensory receptors, it was found that it initiates the painful burning sensation in peripheral sensory receptors by TRPV1 channel activation mechanism.[6]

There is evidence in the literature that TRPV1 is related to neurodegeneration: (I). Marinelli et al. suggested that TRPV1 has an essential role in brain ischemia by regulation of glutamate release and activating excitatory amino-acid receptors. (II). It has been shown that TRPV1 receptors are present at the brain sites where the cells are more susceptible to neurodegeneration.[7,8] (III). Capsaicin and vanilloid antagonist capsazepine (CZP) showed a neuroprotective effect against the ouabain-induced neurotoxicity in rats when administrated peripherally.[9] It has been reported that capsaicin has protective effects in terms of neurotoxicity and oxidative stress; however, no studies to date have focused on the role of capsaicin in bupivacaine-induced neurotoxicity.

Although there are many studies focusing on protection against local anesthetic-mediated nerve injury, it remains a clinical challenge.[10] New methods and deeper understandings are still needed. SH-SY5Y cells are extensively used to study the neurotoxicity of bupivacaine as they can simulate the biological properties of neurons.[11,12] Increasing evidence has suggested that a number of biochemical processes and molecular mechanisms, such as excessive oxidative stress, mitochondrial dysfunction, and activation of the apoptotic cascade, are recognized as mediators of bupivacaine-induced neurotoxicity.[13] Oxidative stress occurs when ROS is overproduced, and this is considered an important reason for promoting neuronal cellular dysfunction. Oxidative stress-mediated DNA damage is one of the most important threats to the genome stability of neurons.[14] Neuroblastoma SH-SY5Y cell, a type of tumor cell with low differentiation in the human nervous system, is frequently used in studies designed to

investigate the pathogenesis, prevention, and treatment of central nervous system diseases. Hence, scientists are using this model to study the mechanisms that mediate the peripheral neurotoxicity of local anesthetics.[15,16]

The concentrations used in *in vitro* experiments investigating the neurotoxicity of Local Anesthetics varied widely. In the previous study of Zhao et al., the IC_{50} (half-maximum inhibitory concentration) of bupivacaine in the SH-SY5Y cell line model was approximately 1.5 mMol/L.[10] Wang et al. used 500 µM bupivacaine applied model in their study designed to investigate the neuroprotective effect of TET in SH-SY5Y cells.[16] In our study, considering the concentrations presented in the literature to determine the IC_{50} of bupivacaine, when we applied increasing concentrations of bupivacaine, we determined the IC_{50} of bupivacaine as 1 mM, for *in vitro* neurotoxicity modeling. In this study, 1 mM bupivacaine remarkably suppressed SH-SY5Y cell proliferation, as demonstrated by the XTT assay results. In addition, it was observed that capsaicin had no significant effect on SH-SY5Y cell proliferation in the concentration range of 5-100 µM, but the toxicity of bupivacaine-induced SH-SY5Y cells was able to prevent it.

Apoptosis is an essential process that accompanies a variety of physiological conditions. Increased oxidative stress and DNA damage caused by it have been accepted as basic mechanisms for the induction of cytotoxicity and apoptosis in neurons treated with bupivacaine.[2] Based on this observation; we performed apoptosis assay by flow cytometry to further demonstrate the protective function of capsaicin against bupivacaine-induced neurotoxicity. The results confirmed that capsaicin administration attenuated bupivacaine-induced neuronal apoptosis. After promising studies back in the literature, Pezzoli et al. showed that capsaicin crosses the blood-brain barrier and affects the central nervous system.[17] Lee et al. demonstrated that capsaicin reduces kainic acid-mediated epileptogenesis.[18] Huang et al. reported that capsaicin maintains cortical neurons against ischemia/reperfusion injury. They also show that this neuroprotective effect is strictly related to the dose.[19] In the present study, we also found that the apoptosis induced by bupivacaine in SH-SY5Y cells was reduced by the administration of capsaicin.

The findings of the present study are in accordance with the literature. In the current study, we managed to show that bupivacaine has a concentration-dependent strong cytotoxic effect on neuron cells. This finding verified that we could establish our neurotoxicity model. The single administration of capsaicin has shown another cornerstone of our hypothesis. Capsaicin did not have any cytotoxic effect on neuron cells which is relatively new data for literature since single administration of capsaicin is usually missed in the study setups.

Consequently, this study showed for the first time that capsaicin can alleviate bupivacaine-mediated neurotoxicity in SH-SY5Y cells by suppressing apoptosis. As a result, it has been suggested that capsaicin may be an attractive candidate for reducing the neurotoxicity resulting from the use of bupivacaine and other similar local anesthetics.

Compliance with Ethical Standards

Ethical approval for this study was granted by the Cumhuriyet University Clinical Research Ethics Committee with the approval number of 2021-06/18.

Conflict of Interest

The author declares no conflicts of interest.

Author Contribution

AA: Study idea, hypothesis, study design, material preparation, data collection and analysis

Financial Disclosure

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