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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

The Difference Between The Response To Glutamate Excitotoxicity and The Role Of Ca^{2+} Channel Blockers in Cortical Neuron and SH-SY5Y Cells Cultures

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Abstract: Cortical neuron and SH-SY5Y cells are widely used in glutamate excitotoxicity studies, but it is unclear which one better reflects this model. Generally, glutamate induces toxicity conditions by leading to L and L/N-Ca^{2+}channels activation and cell death via lethal Ca^{2+} influx. To evaluate this hypothesis, the effects of L and L/N-Ca^{2+} channel blockers, lacidipine, and amlodipine under excitotoxic conditions were evaluated. At the same time, in this study, we aimed to determine that these two cell lines better reflect this model. To induce excitotoxicity, cortical neuron and SH-SY5Y cells were incubated with glutamate 10^{-5} mM. After 30 min incubation with glutamate, agents of different concentrations (1, 2, and 4 µg lacidipine and 20, 50, and 100 µM amlodipine) were applied to these cells. Possible neuroprotective roles of lacidipine and amlodipine were investigated through cell viability, oxidative stress, and apoptotic alterations. Our results showed that SH-SY5Y cells are the more ideal cell line for oxidative stress-mediated glutamate toxicity. Although 4 µg lacidipine and 100 µM amlodipine have important neuroprotective roles in these cells, the most protective effect was also detected in SH-SY5Y cells at 100 µM amlodipine concentration. The highest viability rate on cell lines was found at 88.8 % in SH-SY5Y cells treated with 100 µM amlodipine. Results from the TAC, TOS, LDH assays, and flow cytometry analysis were correlated to our MTT results. Taken together, our results indicate that SH-SY5Y cells are more effective at reflecting glutamate-induced excitotoxicity and 100µM amlodipine has a more protective effect in treating this toxicity.

Keywords: Amlodipine, calcium, lacidipine, neurotoxicity.

Kortikal Nöron ve SH-SY5Y Hücre Kültürlerinde Glutamat Eksitotoksisitesine Yanıt ile Ca^{2+} Kanal Blokerlerinin Rolü Arasındaki Fark

Öz: Kortikal nöron ve SH-SY5Y hücreleri glutamat eksitotoksisite çalışmalarında yaygın olarak kullanılmaktadır, ancak hangisinin bu modeli daha iyi yansıttığı belirsizdir. Genel olarak glutamat, L ve L/N-Ca^{2+} kanallarının aktivasyonuna ve öldürücü Ca2+ akışı yoluyla hücre ölümüne yol açarak toksisite koşullarını indükler. Bu hipotezi değerlendirmek için eksitotoksik koşullar altında L ve L/N-Ca^{2+} kanal blokerleri, lasidipin ve amlodipinin etkileri değerlendirildi. Aynı zamanda bu çalışmada bu iki hücre hattının bu modeli daha iyi yansıttığını belirlemeyi amaçladık. Eksitotoksisiteyi indüklemek için kortikal nöron ve SH-SY5Y hücreleri 10^{-5} mM glutamat ile inkübe edildi. Glutamat ile 30 dakikalık inkübasyondan sonra, bu hücrelere farklı konsantrasyonlarda (1, 2 ve 4 µg lasidipin ve 20, 50 ve 100 uM amlodipin) ajanlar uygulandı. Lasidipin ve amlodipinin olası nöroprotektif rolleri, hücre canlılığı, oksidatif stres ve apoptotik değişiklikler yoluyla araştırıldı. Sonuçlarımız SH-SY5Y hücrelerinin oksidatif stres aracılı glutamat toksisitesini daha iyi yansıttığını gösterdi. 4 µg lasidipin ve 100 µM amlodipin bu hücrelerde önemli nöroprotektif rollere sahip olmasına rağmen en yüksek koruyucu etki SH-

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SY5Y hücrelerinde 100 µM amlodipin konsantrasyonunda de belirlendi. En yüksek canlılık oranı, 100 µM amlodipin ile tedavi edilen SH-SY5Y hücrelerinde %88,8 olarak bulundu. TAC, TOS, LDH ve flow sitometrik analizinden elde edilen sonuçlar MTT sonuçlarımızla benzerdi. Tüm sonuçlar birlikte değerlendirildiğinde SH-SY5Y hücrelerinin glutamat kaynaklı eksitotoksisiteyi yansıtmada daha etkili hücre hattı olduğunu ve 100 µM amlodipinin bu toksisitenin tedavisinde daha koruyucu bir etkiye sahip olduğunu göstermektedir.

Anahtar kelimeler: Amlodipin, kalsiyum, lasidipin, nörotoksisite.

INTRODUCTION

Glutamate excitotoxicity is one of the most extensively researched processes of neuronal cell death, and it is involved in the pathophysiological mechanism of many brain related diseases such as Alzheimer's, Huntington's, and Parkinson's disease (Binvignat & Olloquequi, 2020). In physiological conditions, glutamate has a function in neuronal communication such as synaptic plasticity and learning (Valtcheva & Venance, 2019). However, excessive glutamate give rise to neuronal injury and death through overstimulation of glutamate receptors, particularly the N-methyl-D-aspartate (NMDA) subtype which can trigger influx of Ca^{2+} ions (Carvajal et al., 2016). Also, excessive Ca^{2+} flux through voltage-gated calcium channels (VGCC) such as L-, N- and P/Q-type amplify and propagate the phenomenon of glutamate excitotoxicity (Higley & Sabatini, 2012).

Ca^{2+} homeostasis is essential for the survival of neurons, on the other hand increased intracellular Ca^{2+} concentration disrupts calcium homeostasis, and free oxygen radicals formation which could degenerate neurons and synaptic loss (Godoy et al., 2021) (5). For this reason, blocking excitotoxicity through inhibition of VGCC activity in neurons are an especially appealing point for neuroprotection(Godoy et al., 2021; Higley & Sabatini, 2012; Kim et al., 2016). By contrast to neuroprotective effects of P/Q-type and N-type calcium channel blockers, there is a gap for L-type calcium channels blockers in relation to glutamate excitotoxicity (Vallazza-Deschamps et al., 2005; Wheeler et al., 1996). Although there are studies demonstrating the neuroprotective effects of lacidipine and amlodipine which are dihydropyridine calcium channel blockers, evidence supporting their roles as a neuroprotectant factor in glutamate excitotoxicity is almost not available (Choi et al., 2014; Khurana et al., 2021). Both lacidipine and amlodipine block L-channels, but their selectivity is different. Lacidipine is the more selective for L-channels, however, amlodipine blocks similar levels both N-type and L- type VGCC (Godfraind, 2017). Therefore, more research is needed to evaluate and define the mechanism by which types of Ca^{2+} channel blockers act more protectively against glutamate excitoxicity.

Various cell lines are widely used to research in vitro excitotoxicity. However, the methodological employed for signaling pathways activated in cell lines generally differ (Kritis et al., 2015). The rat cortical neuronal and human undifferentiated SH-SY5Y cells line is widely prefered in vitro toxicity researches. These cell lines are similar in their responses when exposed to glutamate, but it is questionable which one better reflects the this model (Barbosa et al., 2015; Krasil'Nikova et al., 2019; Kritis et al., 2015). L-type and N-type VGCC from these cells have been functionally characterized and identified

(Choi et al., 2014; Kritis et al., 2015; Wheeler et al., 1996). On the other hand, while cortical neuronal cells express NMDA receptors whether native/undifferentiated SHSY5Y cells express NMDA receptors is controversial (Sun et al., 2010; Yang et al., 2012).

Hence, cortical neuron and SH-SY57 (Human neuroblastoma) cells constitute an adequate model to research the problem posed here (i) to know whether L-type (lacidipine) and/or dual L/N-type (amlodipine) calcium channel blockers have neuroprotective effects in glutamate excitotoxicity; and (ii) to define which of the cortical neuron and SH-SY57 cells more efficacious to reflects the glutamate induced injury.

MATERIAL AND METHOD

Cell culture: Cortical neurons and human neuroblastoma SH-SY5Y cells were prepared and cultured as previously reported (Hu et al., 2012; Taghizadehghalehjoughi & Naldan, 2018). Frozen rat cortical neuronal cells obtained from the Pharmacology and Toxicology Department of Veterinary Medicine Faculty of Ataturk University (Erzurum, Turkey). Neuronal cells were resuspended in neurobasal medium contain B27, 10% fetal bovine serum (FBS) and 0.1% antibiotic (penicillin–streptomycin-amphotericin B) (Taghizadehghalehjoughi & Naldan, 2018). The human neuroblastoma SH-SY5Y cell line was purchased from the American Type Culture Collection (ATCC® CRL-2266™). SH-SYSY cells were grown DMEM media containing 10% FBS, 100 U/ml penicillin, and 100 g/ml streptomycin (Hu et al., 2012). The cells were maintained at 37 °C and 5% CO2. The experiment was started when the cells reached 70-80% confluency.

Glutamate excitotoxicity model: Glutamate (Sigma®, USA) at 10^{-5} mM concentration was applied to the cell culture induce excitotoxic injury (Taghizadehghalehjoughi & Naldan, 2018). 30 minutes after glutamate administration, commercially obtained amlodipine and lacidipine (Sigma®, USA) were applied at different concentration (1, 2 and 4 µg lacidipine and 20, 50 and 100 µM amlodipine) to anayze ameliorating effects against glutamate excitotoxicity after 24h incubation. Each sample was prepared as triplicates and 10^{-5} mM glutamate was used as a positive control.

Lacidipine and amlodipine treatment: Cells were divided into sixteen groups at randomly: For cortical neuron and SHSY5Y cells; Control group, positive control group (only 10^{-5} mM glutamate) and different concentrations of lacidipine+glutamate groups (final concentration; 1, 2 and 4 µg) and different concentrations of amlodipine + glutamate groups (final concentration; 20, 50 and 100 µM). Cells were cultured in the plates for 24 hours and treated with lacidipine and amlodipine for another 24 hours. Glutamate was added 30 min prior to different concentration of lacidipine and amlodipine exposure to induce excitotoxicity.

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) analysis: The susceptibility of the cells to amlodipine and lacidipine was determined in vitro by MTT (Sigma®, USA)-based colorimetric assay (Taghizadehghalehjoughi & Naldan, 2018). The MTT assay depends on evaluating the reduction, by dehydrogenases of metabolically active cells, of the reduction of yellow-colored MTT tetrazolium to purple-colored formazan. The intracellular formazan is soluble and can be measured by spectrophotometry. Briefly, neuronal and SH SY5Y cells were seeded in triplicate wells in a flat-bottomed 96-well plate at a density of 2×10^5 and 5×10^3 cells/cm^2 respectively and then administered with agents as previously described. MTT reagent (0.5 mg/ml final concentration) was added to the cells and the plates were incubated to a humidified chamber kept in a 37°C-5% CO_2 incubator for 4 h. Then the medium removed, and the 100 µl DMSO (Sigma®, USA) was added to each well for dissolving crystals. Absorbance at 570 nm was determined using Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, Canada, USA). The cell viability (%) was calculated by the formula below;

Viability % ratio= (Sample absorbance value/ Control group absorbance value)X100 (18).

Determination of membrane integrity using an LDH Release assay: Lactate dehydrogenase (LDH) is a decisive enzyme found in all cell types that are speedily spread into the cell culture medium upon injury to the plasma membrane. LDH is the most commonly used marker for conducting a cytotoxicity test. The LDH assay protocol depends on an enzymatic coupling reaction: LDH released from the cell oxidizes lactate to produce NADH, which then reacts with WST to form a yellow color. The intensity of the generated color associated directly with the number of lysed cells. The cells were seeded to 96-well plates and then the agents were applied as previously described. After that 100 µL supernatant was transferred to a fresh 24-well plate and 100 µL of the reaction mixture was added to the samples and incubated for 30 min at room temperature. Finally, a microplate reader (Thermo Scientific, Canada, USA) was used to measure the wavelength of 490 nm.

Determination of oxidative stress markers: TAC (Total antioxidant capacity) and TOS (Total Oxidant Status) kits were obtained commercially (manufactured by Rel Assay Diagnostics® Company, Gaziantep, Turkey). TAC TOS were determined using the the Erel method that is a novel automated colorimetric measurement (Erel, 2004; Erel, 2005). The measurement of TAC levels was depends on the ability of antioxidants to inhibit the formation of 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS)+ from the oxidation of ABTS at wavelength 660 nm by spectrophotometrically. TOS levels are based on the measurement of color intensity at 530 nm by oxidation of ferrous ioneo-dianisidine complex to ferric ion in the presence of various oxidative species under acidic condition. TAC results was shown as mM Trolox equivalent per liter (Trolox Equiv/mmol L^{-1}) whereas TOS results was shown in terms of micromolar hydrogen peroxide equivalent per liter (mmol H_2O_2 Equiv/mmol L^{-1}). Spectrophotometric measurements were done using Multiskan™ GO Microplate Spectrophotometer reader (Thermo Scientific, Canada, USA).

Annexin V-FITC (Fluorescein Isothiocyanate) and propidium iodide (PI) staining assay: The cortical neuron and SH-SY5Y cells were centrifuged to remove the medium, washed with PBS, and stained with 5 µ l Annexin V-FITC and 10 µ l of 20 µ g/ml PI in the binding buffer after treatment with different concentrations of lacidipine and amlodipine after 24 hours. Stained cells were determined using a flow cytometer (Beckman, S. Kraemer Boulevard Brea, CA,USA). Viable cells were negative for both Annexin V and PI ; apoptotic cells were negative for PI and positive for Annexin V, and late-apoptotic dead cells demonstrated both Annexin V and PI positivity Non-viable cells, which suffer from necrosis, were positive for PI and negative for Annexin V.

Statistical analysis: The significance of the mean difference between groups was investigated by the one-way ANOVA with the Tukey HSD multiple comparison. Descriptive statistics for continuous variables were expressed as mean ± standard deviation. *P*-value <0.05 was considered statistically.

RESULTS

MTT assay results: The cytotoxic effect of lacidipine and amlodipine was determined glutamate-induced excitoxicity in neuron and neuroblastoma cells using MTT assay. As seen in Figure 1. Glutamate (10^{-5} mM) treatment at 24 hours reduced the number of neuron and SH-SY5Y neuroblastoma cells by 37% and 41% compared to controls, respectively. When the neuron cells were treated with lacidipine after applying with glutamate, cell viability statistically remarkably increased in dose-dependently. 2 µg and 4 µg lacidipine increased cell viability to 71% and 79%, respectively, relative to glutamate control cells ($P < 0.05$). However, only the highest dose of lacidipine (4 µg) had a statistically protective effect in glutamate-induced excitotoxicity of SH-SY5Y cells, with 24 % increases in cell viability. 50 µM and 100 µM amlodipine showed 9% ($P<0.05$) and 19% ($P<0.05$) rate increased cell viability on neuronal cells compared to glutamate control group, respectively. On the other hand, 100 µM amlodipine exhibited approximately a 30% increasing in cell viability as opposed to the glutamate control group in SH-SY5Y cells ($P< 0.001$). But the 20 µM and 50 µM amlodipine did not show a significant effect on cell viability in SH-SY5Y cells (Figure 1). The highest viability rate on cell lines was found 88.8 % in SH-SY5Y cells applied with 100 µM amlodipine.

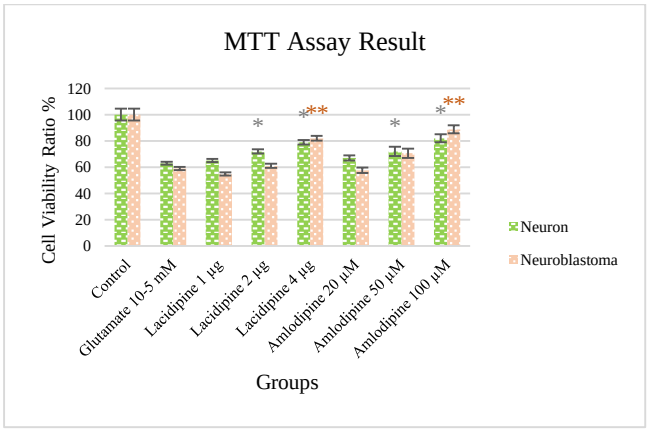

Figure 1. Effects of lacidipine and amlodipine on cell viability on glutamate- excitotoxicity in cortical neuron and SH-SY5Y cells. Indicated cells were treated with the 10^{-5} mM concentration of glutamate for 24 h. * and ** indicate $P<0.05$, and $P<0.001$, respectively, compared to glutamate control cells.

LDH assay results: We compared the sensitivity of our MTT assay to the LDH release assay (Figure 2). 10^{-5} mM glutamate increased LDH release in both cortical neuron and SH-SY5Y cells compared to untreated controls. As shown in Figure 2, lacidipine treatment of 1 µg and 2 µg different doses did not protect cells from glutamate-induced excitotoxicity, but 4 µg lacidipine exhibit protective effect in neuronal cells with glutamate excitotoxicity. Also, different concentrations of lacidipine did not demonstrated a remarkable protective effect on LDH release except for 4 µg lacidipine in SH-SY5Y cells. Both the neuron and neuroblastoma cells were detected 1 µg and 2 µg lacidipine had no protective effects compared to control, while 4 µg lacidipine showed significantly a protective effect ($P < 0.05$). The highest dose of amlodipine only was determined to significantly decrease LDH release in neuron ($P< 0.05$) and SH-SY57 ($P < 0.001$) cells, however at 20 µM and 50 µM amlodipine concentration did not observe a significant change in both cell lines compared to glutamate control. The lowest LDH release on cell lines was found in SH-SY57 cells applied with 100 µM amlodipine. The MTT test was much more sensitive than the LDH test. Significant differences in cell death were measured at lower concentrations of amlodipine and lacidipine by MTT assay.

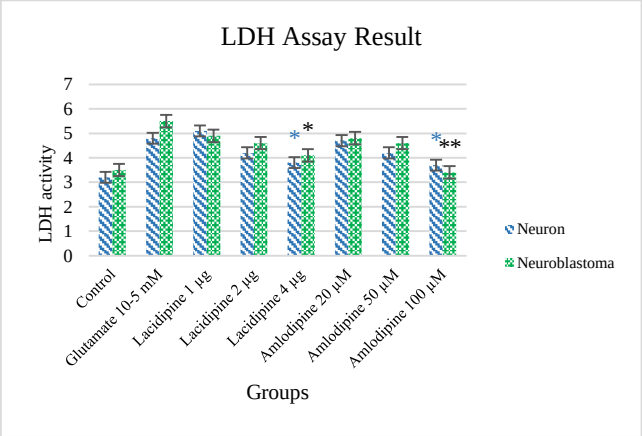

Figure 2. Effects of lacidipine and amlodipine on LDH release on glutamate excitotoxicity in cortical neuron and SH-SY5Y cells. Indicated cells were treated with the 10^{-5} concentration of glutamate for 24 h. * and ** indicate $P<0.05$, and $P<0.001$, respectively, compared to glutamate control cells.

TAC and TOS results: In this study, the effects of lacidipine and amlodipine on oxidative changes caused by toxicity in neuronal and SH-SY5Y cultures were determined by TAC-TOS measurement (Figure 3a and 3b). In the control groups, TAC levels were determined 6.5 and 6.3 Trolox Equiv/mmol L^{-1} in neuron and SH-SY5Y cells, respectively. Applying of 10^{-5} mM glutamate reduced TAC levels in neuron and neuroblastoma cells to 2.8 and 2.4 Equiv/mmol L^{-1}respectively. 1 µg and 2 µg lacidipine did not show significant effect of TAC levels these cell cultures (2.9, 3.4; neuron, 2.6, 3.1; SH-SY5Y; respectively), however 4 µg lacidipine lead to significantlly increases TAC levels in neuron ($P < 0.05$) and SH-SY5Y ($P < 0.001$) cell 4,8 and 5,4 Trolox Equiv/mmol L^{-1}, respectively. In both cell cultures applying of 50 µM and 100 µM amlodipine significantly increased TAC levels (neuron 4.8 ($P < 0.05$) and 5.1 ($P < 0.001$); SH-SY5Y 4.5 ($P < 0.001$) and 5.8 ($P < 0.001$), respectively). 20 µM amlodipine increased TAC level compared to the glutamate control in both cell culture, however this increasing was not statistically significant. The

findings showed that TOS levels decreased in parallel with the increase in TAC. And, applications with glutamate led to increasing of TOS levels in neuronal and SH-SY5Y cells (4.8 and 5.8 H_2O_2 Equiv/mmol L^{-1}, respectively). Exposure of 1 and 2 µg lacidipine, and 20 µM amlodipine in both cell cultures did not reduce a statistically significant in TOS level. On the contrary, at 4 µg lacidipine, and 50 and 100 µM amlodipine concentrations were determined TOS levels decreased compared to control. This decrease was statistically $P < 0.001$ at 4 µg lacidipine and 100 µM amlodipine, but at 50 µM amlodipine $P < 0.05$.

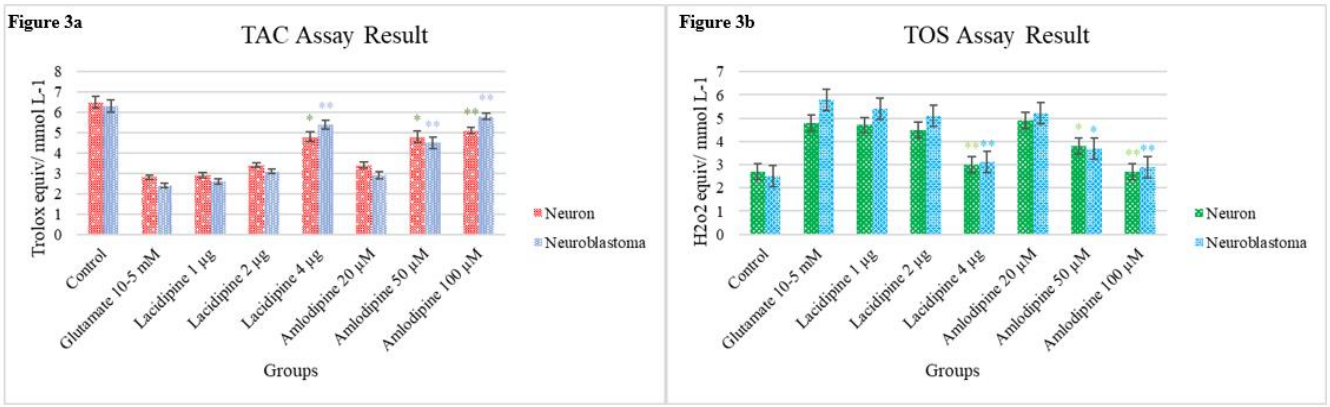

Figure 3a-b. Effects of lacidipine and amlodipine on TAC-TOS capacity on glutamate- excitotoxicity in cortical neuron and SH-SY5Y cells. Indicated cells were treated with the 10^{-5} concentration of glutamate for 24 h. * and ** indicate P<0.05, and P<0.001, respectively, compared to glutamate control cells.

Annexin V-FITC (Fluorescein Isothiocyanate) and propidium iodide (PI) staining assay results: Annexin v-FITC and PI were used for evaluation apoptosis. We examined the development of apoptosis at the early apoptosis level or late apoptosis stage. The early apoptosis stage is a reversible process and cells can come back to normal. However, late apoptosis is an irreversible process for cells and is referred to by some researchers as the early stage of necrosis. Results are shown in Figure 4a and 4b.

According the neuron culture results (Figure 4a); the cell viability of control group was %96.43 and glutamate control group 50.49%. 10^{-5}mM glutamate increased the necrosis, early and late apoptosis (respectively 29.6%8, 9.39% and 10.44%) more than negative group (respectively 3.57%, 0.00% and 0.00%). In lacidipine treatment groups; The highest cell viability and the lowest necrosis, early and late apoptosis ratio were found 4 µg lacidipine group (respectively 67.06 %, 0.24 %, 32.15% and 0.55%). Among the amlodipine groups, the maximum viability (79.07%) was found in 100 µM amlodipine group. Minimum early (2.43%) and late apoptosis (0.56%) rate were seen in the 20 µM amlodipine group, however the necrosis rate (0.26%) in 50 µM amlodipine group.

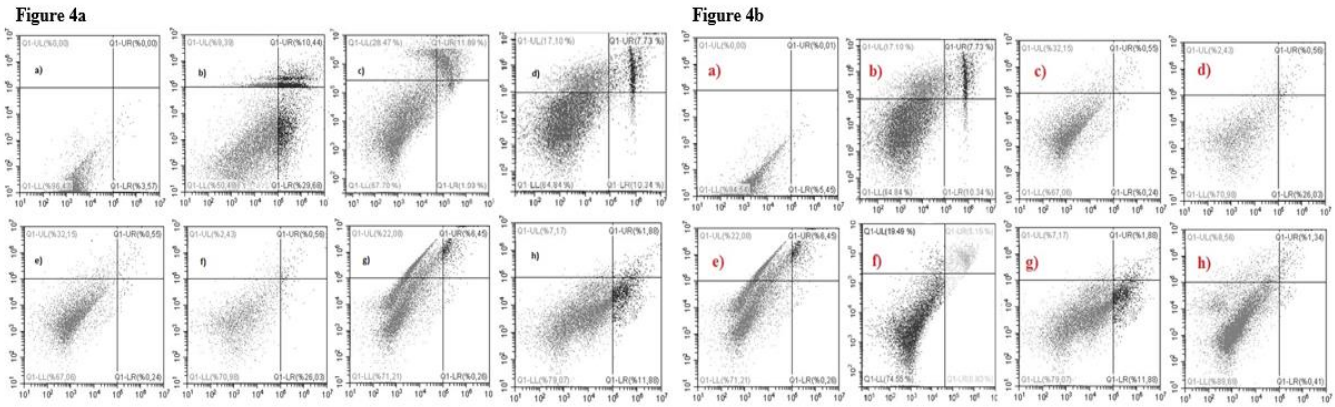

Figure 4a-b. Flow cytometry results for cortical neuron and SH-SY5Y cells, respectively. a) control group; b) glutamate control 10^{-5} mM; c) 1 µg lacidipine; d) 2 µg lacidipine; e) 4 µg lacidipine; f) 20 µM amlodipine; g) 50 µM amlodipine; h) 100 µM amlodipine.

According the SH-SY57 neuroblastoma cell culture results (Figure 4b); the cell viability of control group was 94.54% and glutamate control group was 64.84%. 10^{-5}mM glutamate increased the necrosis, early and late apoptosis (respectively 10.24%, 17.10% and 7.73%) more than negative group (respectively 5.45%, 0.00% and 0.01%). In lacidipine treatment groups; the highest viability rate and the lowest necrosis rate were

found in the 4 µg lacidipine group (respectively 74.55% and 0.26%). The highest early apoptosis rate (32.15%), and the lowest late apoptosis (0.55%) were seen in 2 µg lacidipine group. Among the amlodipine groups, the highest viability rate and the lowest necrosis and late apoptosis rate were found in the 100µM amlodipine group (respectively 89.69%, 0.41%, and 1.34%). However the highest early apoptosis rate was seen in 20µM amlodipine group (19.49%).

According to our findings, the highest viability rate among the treatment groups was found in the at 100 µM amlodipine in SH-SY5Y cell line. Our findings demonstrate a correlation with the MTT and LDH assay results.

DISCUSSION

The main finding of this study was to evaluate the protective roles of L-type (lacidipine) and/or dual L/N-type (amlodipine) calcium channel blockers in glutamate-induced excitotoxicity and to determine which of the cortical neuron and SH-SY57 cells reflects glutamate injury. MTT, LDH, TAC-TOS analysis and flow cytometry were performed sequentiall. Herein, firstly we found that SH-SY5Y cells were more sensitive to oxidative stress damage and cell viability reduction caused by glutamate than cortical neuron cell culture. Secondly, amlodipine, a dual inhibitor L/N VGCCs blocker, had more protective effects against glutamate toxicity than lacidipine, which is only L-type VGCCs blocker and this effects were dose dependent manner.

The molecular pathways of excitotoxicity following glutamate receptor activation are still unclear. Increased intracellular concentrations of Ca^{2+} are classically thought to be a supreme event in inducing cell death (3, 5). Even though Ca2+ can pass into via NMDA glutamate receptors, glutamate can alternatively depolarize neurons, giving a start to the opening of VGCC (Chávez-Castillo et al., 2017; Higley & Sabatini, 2012). This hypothesis is supported by the neuroprotective effect of Ca^{2+} channel blockers against glutamate induced excitotoxic injury. Although it is well established that N-type VGCCs participate in glutamate release, recently. It demonstrated that increased expression of L-type voltage-gated Ca^{2+} channels at presynaptic terminals irregularly induced glutamate release (Giansante et al., 2020). Therefore, we used cortical neuron cells and SH-SY5Y cell lines, which are two dissimilar cell lines expressing L and N-type calcium channels in our study. Applying glutamate decreased cell viability more in SH-SY57 cells than in neuron cells (589 % vs. 63%). Also, such results from the MTT analysis have been supported by the evaluation of LDH findings. Although in two cultures treated glutamate, the activity of LDH was remarkably higher in SH-SY5Y cells when compared to cortical neuron cells. Additionally, our TAC-TOS and flow cytometric results were also in parallel with MTT and LDH assay. As a result of our literature search, we could not find any study showing which one better reflects glutamate damage in neuronal cells and SH-SY5Y cell lines. Therefore, we did not compare to our results with other studies. However, it is known that alteration of intracellular Ca2+ concentrations induces numerous intracellular situations, inclusive of oxidative stress, necrosis, and apoptosis. Ca^{2+} influx via L- and N- type channels is an crucial event during the initial phases of cell death (Dong et al., 2009). Therefore, we think that Ca^{2+} loading is variable in neurons and SH-SY5Y cells due to depolarization caused by toxicity. SH-SY5Y cells appear to be more susceptible to Ca^{2+} overload and subsequent oxidative stress and apoptotic cell death due to L and N-type Ca^{2+} channels.

Numerous studies highlight the important role of VGCC blockers to treat glutamate excitotoxicity (Godoy et al., 2021; Higley & Sabatini, 2012; Kim et al., 2016; Zyśk et al., 2018). In the light of the results summarized in the literature, N-type blockers are known to have protective effects toxicity of NMDAR-mediated Ca^{2+} entry (Kimura et al., 1999; Vallazza-Deschamps et al., 2005). However, there are inconsistent results regarding the role of L-type calcium channel blockers in glutamate excitotoxicity. Some findings reported that L-type calcium channels are not liable for glutamate excitotoxicity so antagonists of this channel have no protection from glutamate-induced neuronal damage (Stuiver et al., 1996; Kimura et al., 1999). On the other hand, Giansante et al., (2020) demonstrated that L-type VCGC channels abnormally contribute to evoked glutamate release and Vallazza-Deschamps et al., (2005) found that diltiazem, a blocker of L-type Ca^{2+} channels, limit the cell death triggered by glutamate. Sanchez et al., (2016) demonstrated that inhibition of L-type Ca^{2+} channels with nimodipine protect cerebrocortical cells under presence of neurotoxicity. In light of the results just summarized, it is time to reconsider the relationship between L and dual L/N VCGC antagonists and glutamate excitoxicity. Although there are no finding the effects of lacidipine on glutamate toxicity, it is avaiable data showing lacidipine its role as a neuroprotectant in neurodegenerative diseases by reducing oxidative stress (Khurana & Bansal, 2019; Manev et al., 1997). At the same time, Manev et al., (1997) found that amlodipine ameliorated both mitochondrial injury and cell death in zinc neurotoxicity in rat cerebellar granule cells. It is declared that Zn^{2+} induce neuronal death by enhancing glutamate excitotoxicity (Kim et al., 2020). Specifically, our results show that similar to previous reports lacidipine and amlodipine alleviated glutamate-induced

excitotoxicity in neuron and SH-SY5Y cells in a concentration-dependent. However, the most protective impact was determined 100 µM amlodipine concentration on SH-SY5Y cells. Compared with glutamate-damaged cultures, we found that the percentage of cell viability was significantly the highest (58.9 % vs. 88.8 %), and LDH release into the culture medium was statistically the lowest in 100 µM amlodipine-treated SH-SY5Y cells. On the other hand TAC-TOS results, oxidative stress parametres, parallel with the MTT and LDH analysis, respectively. Therefore, amlodipine that decreases oxidative stress parameters may also be neuroprotective. Consistent with our findings, Mahajan et al., (2007) reported that amlodipine improved vascular damage by reducing oxidative stress and improving antioxidant status, thanks to its antioxidant properties.

Oxidative stress often involves induction of apoptotic cell death (Lee et al., 2021). In our study, the used concentration of glutamate lead to the apoptotic pathway of celluar death in mostly the cortical neurons neurons but early apoptosis in SH-SY5Y cells. The highest viability and lowest necrosis rate among all treatment groups were determined in the 100 µM amlodipine in SH-SY5Y cells group. In 100 µM amlodipine- applied cultures, the number of viable cells was remarkably higher than in the cultures applied with glutamate alone (89.69% vs. 64.84 %) although the percentage of necrotic cells was remarkably lower (10.24% vs. 0.41%).

CONCLUSION

These results show that SH-SY5Y cells are more effective at reflecting glutamate-induced excitotoxicity, including oxidative stress and apoptotic cell death more than cortical neuron cells. Although lacidipine, an L-type calcium channel blocker, had protective effects in the treatment of glutamate-induced injury, these effects were more effective than 100 µM amlodipine, an L/N-type calcium channel blocker. However, this hypothetical explanation requires further investigation.

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