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RESEARCH ARTICLE

The Effect of Nimodipine towards Calcium Ion (Ca^{2+}) Expression in SH-SY5Y Neuron Cell Culture Exposed By Chronic Hyperglycaemia

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Abstract

Diabetic neuropathy is a complication of diabetes mellitus which forms clinical or subclinical that affecting the peripheral nervous system. In diabetes condition, changes in calcium ion signalling contribute to the development of distal symmetrical sensorimotor polyneuropathy (DSN) and pain. Increasing of cytosolic calcium involved in neuron degenerative and cell apoptotic process. This study discusses the effect of nimodipine, an L-type calcium channel blocker, on calcium ions (Ca^{2+}) in SH-SY5Y culture cell line exposed to chronic hyperglycemia. This study, using in vitro research model. Before measuring calcium ions (Ca^{2+}), the study sample was divided into three groups with 6 days of glucose administration; 5 mM noromoglycemia, 25 mM hyperglycemia, and 50 mM hyperglycemia. Each group was given different treatment; without nimodipine, nimodipine 10 nM and nimodipine 100 nM for 30 minutes, then proceed to observe of Ca^{2+} concentration parameters by fluo-3 dye using confocal laser scanning microscopy (CLSM). The results showed that higher dose of glucose could increase the calcium ion significantly. The administration of nimodipine at 10 nM and 100 nM doses significantly reduced calcium ions in the 25 mM and 50 mM glucose groups. In the 5 mM glucose group with administration of 10 nM and 100 nM nimodipine, Ca^{2+} has shown increased. The conclusion of this study is the administration of nimodipine could decrease calcium ions in SH-SY5Y cells exposed to chronic hyperglycemia.

Keyword: *Nimodipine, Calcium Ion (Ca^{2+}), Diabetic Neuropathy.*

Introduction

Diabetes Mellitus is a chronic metabolic disease, occurs because of a disturbance in the pancreas, either the pancreas cannot produce enough insulin or when the body cannot use insulin produced by the body properly [1]. Long-term complications of diabetes, including retinopathy, which has the potential to lose vision; nephropathy which can lead to kidney failure; peripheral neuropathy, which can be a risk of foot ulcers, amputations, and Charcot joints [2].

It is estimated that in developed countries, there are about 87% - 91% of patients with type 2 diabetes, 7% - 12% of patients with type 1diabetes, and 1% - 3% of other types of diabetics. Of all the world's population, 425 million, or about 8.8% of adults aged 20-79 years, estimated to have diabetes [3]. In Indonesia, it is estimated that the number of people with diabetes is around 12 million, disturbed glucose tolerance is around 52 million and disturbed Fasting Plasma Glucose around 64 million [4].

Diabetic neuropathy is a complication of diabetes mellitus in the form of clinical or subclinical symptoms that affect the peripheral nervous system [5]. The progressive development of the sensation of feeling lost, pain and autonomic dysfunction are a common symptom that occurs in diabetes neuropathy [6]. Diabetes can damage peripheral nerves in various ways,

but the most common is distal symmetric polyneuropathy (DSN). Patients with severe DSN have a great risk for ulceration and amputation in the lower extremity [7].

The mechanism of diabetes neuropathy can be caused by oxidative stress, AGE pathway, poylyol pathway, hexosamine pathway, protein kinase C pathway, polyol (ADP-Ribose) polymerase and decreased neurotopic factors [8]. Calcium ion (Ca^{2+}) is a second messenger that is useful for controlling biological cellular responses that are brief such as muscle contraction and neurotransmission, to those that are long-lasting such as cell proliferation and organ development.

Cytosol is the main compartment in calcium ion signaling. When calcium ions enter the cytosol, calcium ions will interact with calcium binding protein, which will cause the activation or inhibition of cellular processes [9, 10].

Increased local concentrations of calcium ions at the injured site or in the spinal cord contribute to the development of neuropathic pain. In diabetes, changes in calcium signaling contribute to the development of distal symmetrical sensorimotor polyneuropathy and pain [11]. Nimodipine, which is a calcium channel blocker (CCB), works by penetrating well into the blood brain barrier.

Nimodipine itself is often used in studies to determine the effect of CCB on neuronal function. In dorsal roots ganglion neurons (DRG) and substantial gelatinous neurons (SG), nimodipine treatment can improve the abnormalities of calcium homeostasis caused by diabetes.

In the study, long-term use of nimodipine was found to be effective not only in normalizing disruption of calcium homeostasis in DRG and SG neurons in experimental animals with diabetes, but also in reducing hypoalgesia induced by diabetes as well as noxious stimuli responses [12].

Therefore, this research is needed to see the effect of nimodipine administration on calcium reduction in diabetics so that it can be an alternative medicine to overcome the symptoms of diabetes neuropathy complications.

Materials and Methods

This study uses True Experimental Design in a laboratory in vitro. This study aims to determine the effect of calcium channel blocker (CCB) on Ca^{2+} expression in neuron cells exposed to chronic hyperglycemia.

Cell line SH-SY5Y Culture

SH-SY5Y cells are used and grown in culture media containing high glucose DMEM and low glucose DMEM, added with FBS 10%, L-glutamine 200 mM 1% and penicillin-Streptomisiyn 1%. The cell is then put in in the C02 incubator. The media are replaced every two days and the cells are observed until 80% confluent. Sub culture was carried out into 2 flasks, Then continue with cell treatment in well 24. Glucose induction is given with various concentrations; 5 mM, 25 mM and 50 mM for 6 days, then on day 6 were given nimodipine treatment of 10 nM and 100 nM for 30 minutes.

Calcium Dye

Cells that have been given nimodipine for 30 minutes, then removed from the incubator. Cells were washed with PBS 3x and then given 250 µL fluo-3 coloring for each well plate. The cells were then placed in an incubator for an hour and then washed with PBS 1x. Cells were immediately observed with Confocal Laser Scanning Microscope (CLSM).

Data and Statistical Analysis

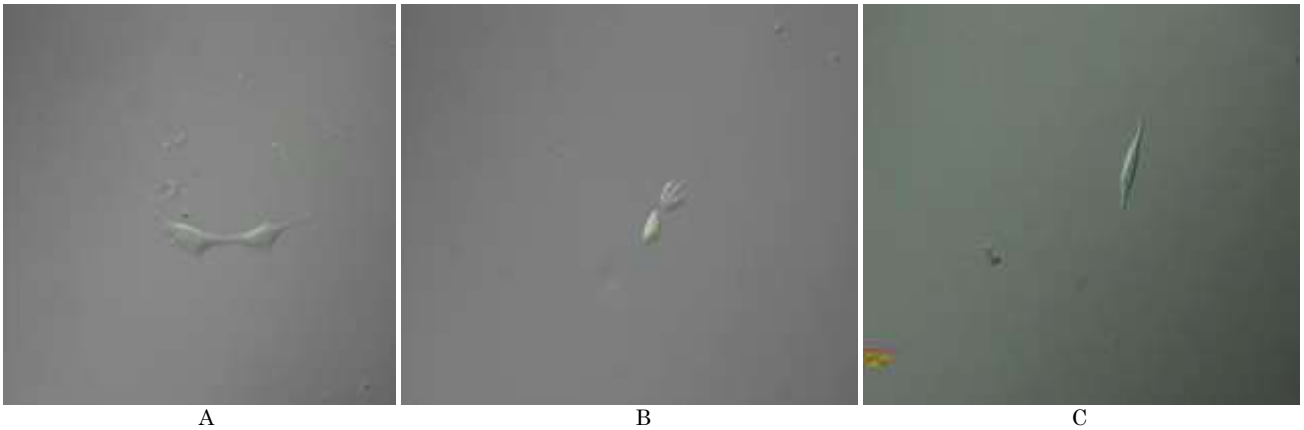
Calcium expression then observed with OIympus fIuoview Ver4.2a and obtained a green fluorescent as a parameter of calcium expression. The data obtained were then analyzed using the calculation of normality test, homogeneity test, one-ways ANOVA test, Post-hoc Tuckey test and Pearson correlation test using an SPSS software application for Windows 25.0. It was said to be significant if $p < 0.05$.

Result and Discussion

Morphologically the results of observations of the effect of nimodipine on the expression of calcium ions (Ca^{2+}) in the culture of SH-SY5Y cell line neurons exposed to hyperglycemia at various concentrations are shown in Figures 1, 2, 3 and 4.

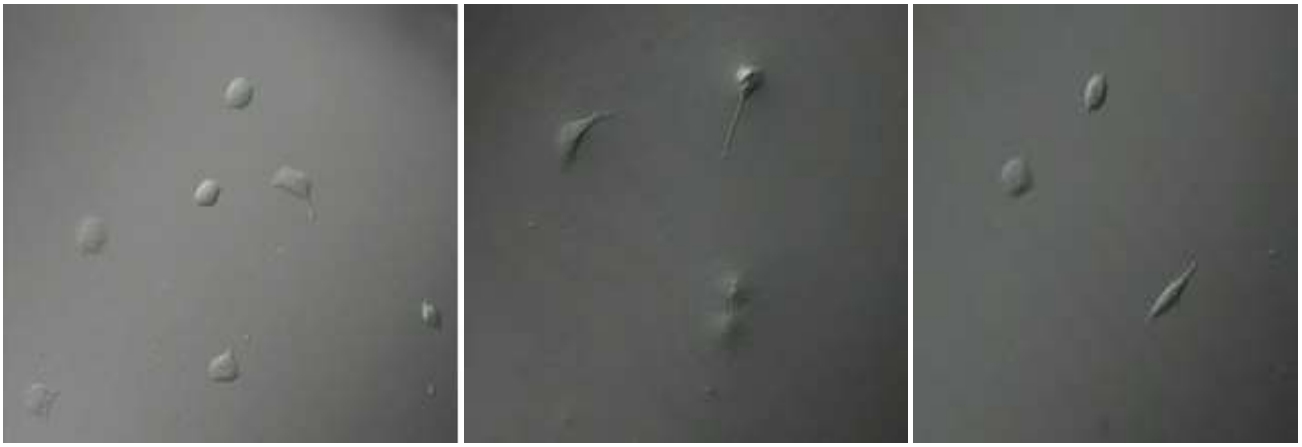
A B C

Fig. 1: Ca^{2+} expression on SH-SY5Y cells with exposure of hyperglycemia.(A) 5 mM normoglycemia (B) 25 mM hyperglycemia (C) 50 mM hyperglycemia



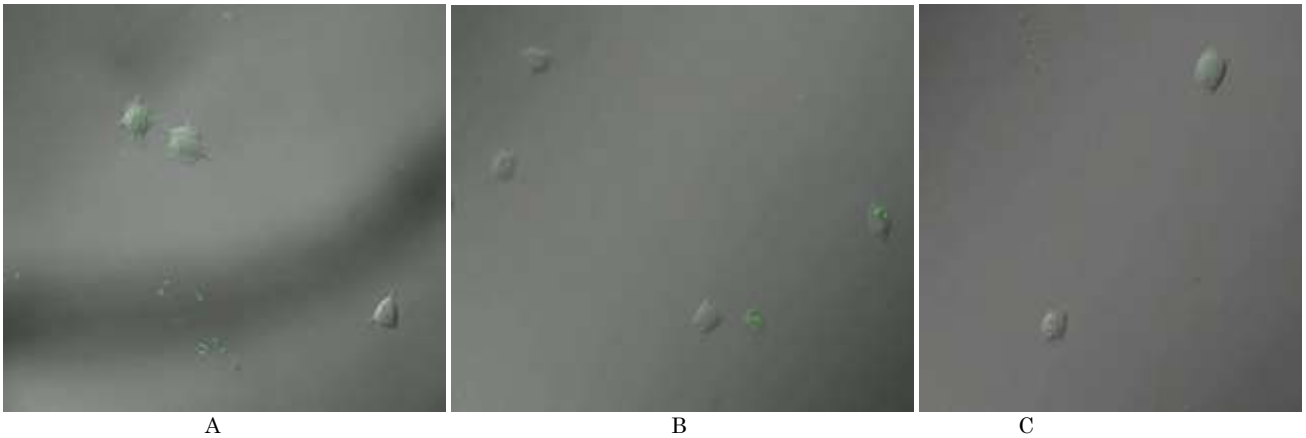
A B C

Fig. 2: Ca^{2+} expression in SH-SY5Y cells with 5 mM normoglycemia. (A) Treatment without nimodipine (B) Treatment with nimodipine 10 nM (C) Treatment with nimodipine 100 nM



A B C

Fig. 3: Ca^{2+} expression in SH-SY5Y cells with 25 mM hyperglycemia. (A) Treatment without nimodipine (B) Treatment with nimodipine 10 nM (C) Treatment with nimodipine 100 nM



A B C

Fig. 4: Ca^{2+} expression in SH-SY5Y cells with 50 mM hyperglycemia. (A) Treatment without nimodipine (B) Treatment with nimodipine 10 nM (C) Treatment with nimodipine 100 nM

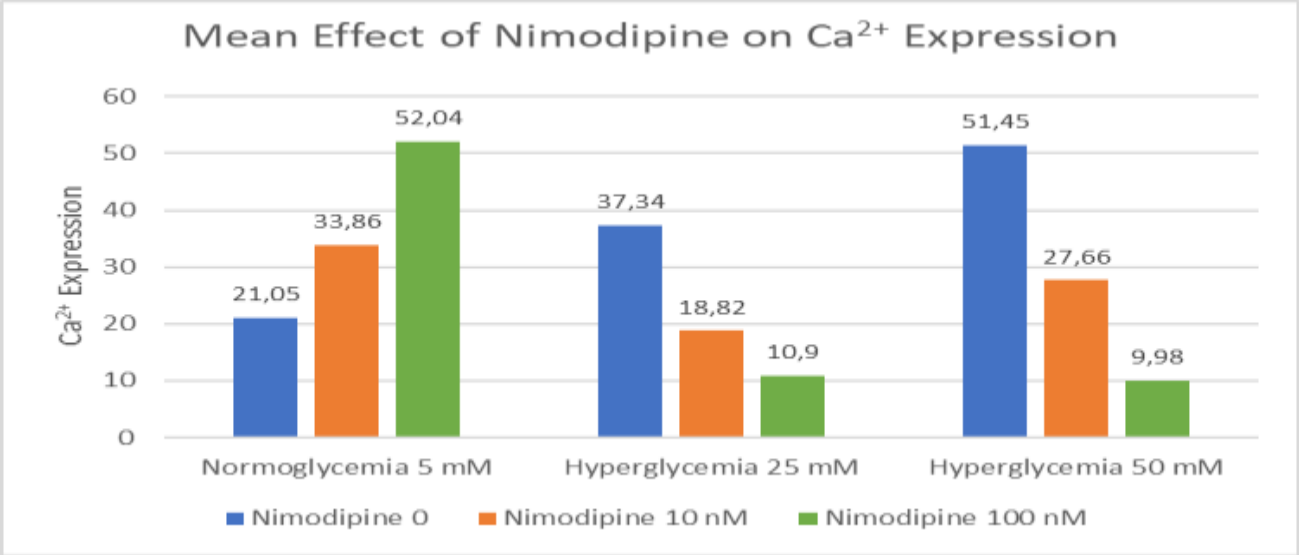


Fig. 5: Mean Effect of Nimodipine on Ca^{2+} Expression

Besides morphologically, the mean of Ca^{2+} expression is shown in Fig. 5 and the results of data analysis are shown in Table 1.

Table 1: Results of the One-Way ANOVA Test and Correlation Test

	One-Way ANOVA	Correlation	
		Pearson	Sig. (2-ailed)
Hyperglycemia	0.000*	0.904	0.000*
Gluc 5 mM + Nimodipine	0.000*	0.952	0.000*
Gluc 25 mM + Nimodipine	0.000*	-0.947	0.000*
Gluc 50 mM + Nimodipine	0.000*	-0.950	0.000*

*= signifikan p<0.05

Effect of Hyperglycaemia on the Expression of Ca^{2+} on SH-SY5Y Neuron Cells

In this study it was found that Ca^{2+} concentrations in the 5 mM glucose group had significantly different values from the 25 mM and 50 mM glucose groups. Visually, the comparison of luminescence of the 50 mM glucose group was brighter compared to the 5 mM and 25 mM glucose groups. Brighter luminescence indicates increased Ca^{2+} expression.

In Koshimura study 2012, evaluating the effect of high glucose concentrations on PC12 cell survival, Seven-day culture with D-glucose (13.5 mg / dl) increases nitric oxide (NOx) metabolites in culture media and increases intracellular Ca^{2+} concentration in PC12 cells. This study shows that high concentrations of glucose induce cell death through NO production [13].

In STZ induced mice, intramitochondrial calcium levels increase and stimulate ROS production in the mitochondria. This is because an increase in TCA and oxidative phosphorylation thus makes mitochondria work faster [14]. Increased calcium in the mitochondria can induce the opening of PTP, which causes mitochondrial swelling, cytochrome C release and cell death due to apoptosis [15].

The Effect of Hyperglycaemia on the Expression of Ca^{2+}on SH-SY5Y Neuron Cells

In the administration of nimodipine 10 nM and 100 nM, it was able to significantly reduce Ca^{2+} levels in the 25 mM and 50 mM glucose groups compared to those without nimodipine. There were high correlation with a negative correlation direction indicates the higher the dose of nimodipine, the lower the concentration of Ca^{2+}.

This is supported by Singh study 2016, which aims to understand the neuroprotective mechanism of nimodipine at 1-methyl-4-phenyl pyridinium (MPP) which induces neuroblastoma SH-SY5Y cell death, given nimodipine (100 nM, 10 µM, 30 µM) for 15 minutes then the media is replaced with media containing MPP (1 mM) for 24-48 hours.

It was found that nimodipine was able to decrease the increase in intracellular calcium

and restore the integrity of mitochondria in SH-SY5Y cells [16]. Nimodipine, besides being an L-type CCB, is also an antioxidant and has inhibitory activity in phosphodiesterase 1 (PDE1) [17].

PDE1 inhibitors work by increasing the levels of cAMP / cGMP which triggers phosphorylation and activation of transcription cAMP responsive element binding protein (CREB) transcription factors causing neuroprotective molecular expression such as BDNF, FGF, and TGF as well as PCG-1a expression which acts as ROS scavenger [18].

Yoon's 2015 study using MN9D cells incubated with medium containing different glucose concentrations (5-35 mM) and 200 µM MPP showed that low glucose concentrations (5-10 mM) caused ROS to form and induce cell death [19]. Research by Masone et al. in 1999 stated that nimodipine did not achieve a significant antioxidant effect even with a dose of 10 µM [20]. Giving 5 mM glucose may induce ROS formation and cell death resulting in an increase in intracellular calcium, and administration of nimodipine does not affect the correction of calcium increase because of its lack of antioxidant effects.

Conclusions

From the results of the study it can be concluded that the induction of hyperglycemia (glucose 25 mM and 50 mM) has an influence in increasing the expression of calcium Ca^{2+}. ions. It was also found that administration of nimodipine at 10 nM and 100 nM in the 25 mM and 50 mM glucose groups reduced the expression of calcium ions.

Suggestion

The need for further research to determine the mechanism of increasing the expression of calcium Ca^{2+} ions in vitro or in vivo. It is also necessary to do repeat research regarding the effective dose of nimodipine to reduce calcium Ca^{2+} ions.

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