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T-type Calcium Channel Blocker, NNC 55-0396, Suppress Cell Proliferation and Promote Apoptosis in SNU-1 Gastric Cancer Cells

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ABSTRACT

Accumulating evidence reports that T-type calcium channels play crucial roles in tumor formation and development. However, the roles of inhibiting calcium channels in tumor cells with various inhibitors in tumor progression remain unclear. This study aimed to investigate the cytotoxic and apoptotic effects of NNC 55-0396, a T-type calcium channel inhibitor, against SNU-1 gastric cancer cells. Cytotoxic and apoptotic effects of NNC 55-0396 were evaluated by the XTT assay and flow cytometry. The results showed that NNC 55-0396 had concentration-dependent cytotoxicity in SNU-1 cells and its the half-maximal inhibitory concentration (IC50) value was calculated as 4.17 µM. The results of the Annexin V experiments also showed that this inhibitor significantly increased apoptosis in SNU-1 cells. In conclusion, these results demonstrated that NNC 55-0396 induces cytotoxic effects by increasing apoptosis in gastric cancer cells. However, further research is required for its use as a possible therapeutic agent in the treatment of gastric cancer.

Keywords: Gastric cancer, NNC 55-0396, Cytotoxicity,Apoptosis.

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Introduction

Gastric cancer, the fifth most diagnosed malignancy worldwide, is still considered a major health problem. Because of its often advanced stage at diagnosis, gastric cancer has a high mortality rate and is the third leading cause of cancer-related deaths, with 784,000 deaths worldwide in 2018 [1]. Many factors such as age, gender, genetic factors, smoking, race, unhealthy diet, and being infected with Helicobacter pylori bacteria are known as important risk factors for gastric cancer. Traditionally, chemotherapy, radiotherapy, surgery, and immunotherapy have been used success in its treatment [2, 3]. However, unfortunately, most of the patients cannot overcome the side effects associated with these treatments [4]. Therefore, new cellular targets urgently need to be discovered to increase the therapeutic efficacy and minimize the side effects of existing treatment modalities.

Collective reports have revealed that ion channels, the special membrane proteins that allow ion flow, play a role in the development of many diseases, including cancer [5]. The regulation of calcium signaling is used for various therapeutic purposes such as hypertension, coronary artery disease, and pain control [6]. Moreover, as a secondary messenger, calcium plays important roles in cell proliferation, cell cycle progression, differentiation, migration, and apoptosis. It is also known that abnormal activation of calcium channels is related to tumor formation and development [7, 8]. Therefore, calcium channel blockers (CCBs) appear to be a notable anticancer target, as both decreased and increased intracellular calcium levels induce apoptotic cell death [9]. A large number of reports have also shown that the expression of T-type calcium channels is significantly increased in many cancer types such as glioma, hepatoma, colon, breast, esophageal, and ovarian cancers. Besides, high calcium channel levels can be detected in tumor tissue samples collected from various cancer patients [10].

In light of CCB data obtained in the last decades, blocking T-type calcium channels is seen as a promising strategy in cancer treatment. However, the use of NNC 55-0396 in SNU-1 gastric cancer cells has not yet been disclosed. This study aimed to investigate the cytotoxic and apoptotic effects of NNC 55-0396, which is used as T-type CCB, on gastric cancer SNU-1 cells.

Materials and Methods

Cell Culture

Human stomach cancer cells SNU-1 were purchased from ATCC (CRL-5971) and incubated at 37°C under a 5% CO_2 humidified atmosphere. The cells were cultured in an RPMI-1640 (Sigma-Aldrich) mixture supplemented with 10% fetal bovine serum (FBS, Gibco, Thermo Fisher Scientific) and 50 U/mL penicillin/streptomycin (Gibco, Thermo Fisher Scientific). A 10 mM stock solution of NNC 55-0396 (Tocris) in DMSO was prepared and the concentrations to be applied to the cells were prepared from this stock by diluting with RPMI-1640 mixture.

Cell Viability Assay

After 24 h incubation, cell viability was evaluated by the XTT assay (Roche Diagnostic) as described previously [11]. In short, SNU-1 cells were exposed to NNC 55-0396 at increasing concentrations (1, 5, 10, 20, and 40 µM) for 24 h, and XTT test was carried out. The half-maximal inhibitory concentration (IC50) value of NNC 55-0396 in SNU-1 cells was calculated using Graph Prism 7 software.

Annexin V Binding Assay

Initially, SNU-1 cells were treated with NNC 55-0396 at the previously determined IC_{50} concentration and incubated for 24 h. After the incubation period, the cells were collected by centrifugation and incubated in 100 µL of Annexin V reagent (Millipore) for 20 min in the dark [11]. Then, the apoptotic effect of NNC 55-0396 was determined using the muse cell analyzer (Merck, Millipore).

Results

Cytotoxic Effect of NNC 55-0396 in SNU-1 Cells

Cell viability findings are shown in Figure 1 and it has been demonstrated that when compared to the untreated cells NNC 55-0396 has a dose-dependent inhibitory effect at 24 h ($P < 0.01$). The IC_{50} value of NNC 55-0396 was determined as 4.17 µM for 24 h in SNU-1 cells and this value was utilized for the subsequent apoptosis assessment experiments.

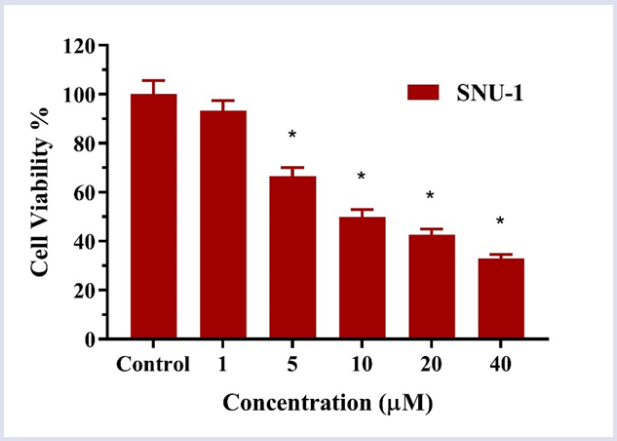

Figure 1. Antiproliferative effect of NNC 55-0396 treatment in SNU-1 cells. Results are exhibited as mean with a standard deviation of the independent three replicates. *Significantly different when compared to cell viability of untreated cells ($P < 0.01$).

Apoptotic Effect of NNC 55-0396 in SNU-1 Cells

According to the findings, when compared to the untreated cells, NNC 55-0396 treatment markedly promoted the early and late apoptotic cell population ($P < 0.01$). As presented in Figure 2, the early, late apoptotic cell population % and dead cell population % in untreated cells (4.27 ± 1.30%, 5.77 ± 1.10 %, and 0.76 ± 0.90%, respectively) significantly increased to 11.03 ± 1.79% and 49.42 ± 3.68%, and 7.38 ± 1.07%, respectively, in NNC 55-0396 administered group ($P < 0.01$).

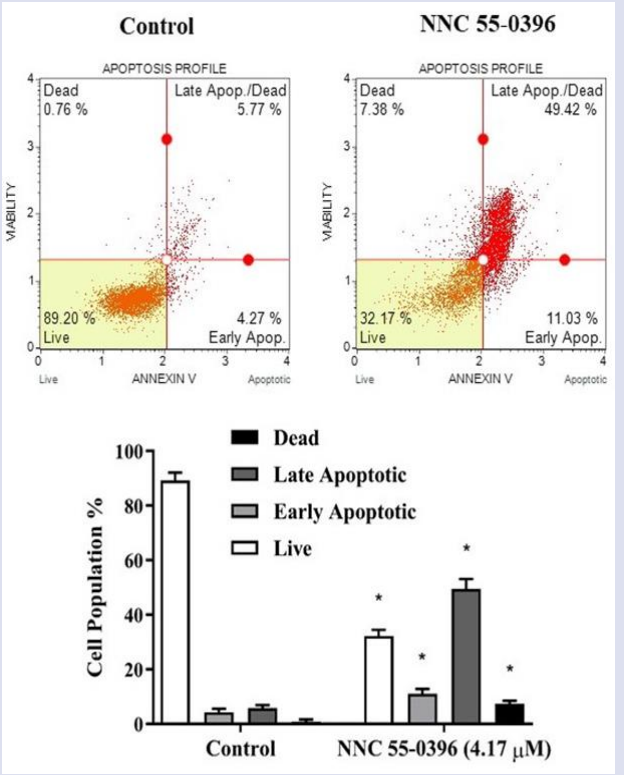

Figure 2. Apoptotic effect of NNC 55-0396 treatment in SNU-1 cells. All experiments were carried out in triplicate. *Significantly different when compared to the cell population rate of control cells ($P < 0.01$).

Discussion

Although several researchers reported the anticancer potential of NNC 55-0396 in various cancer cells, as far as we know, there is a gap in the literature evaluating the cytotoxic and apoptotic effects of NNC 55-0396 in SNU-1 cells. In this context, the current investigation demonstrated that gastric cancer cells showed reduced cell proliferation when treated with selective T-type CCB, NNC 55-0396, in a concentration-dependent manner. Mechanistically, this inhibitor was found to cause significant apoptotic cell death in SNU-1 gastric cancer cells. These data revealed that calcium channel inhibition by NNC 55-0396 may cause meaningful anticancer effects through the promotion of apoptosis in SNU-1 gastric cancer cells.

It has long been known that significant changes in intracellular calcium levels are closely associated with apoptotic cell death. In connection with this, in cells, calcium channels play several roles in physiological and pathological processes, including cancer. Especially, T-type calcium channels are also frequently abnormally expressed in various cancer cells and are involved in the proliferation, cell cycle progression, and survival [12]. Moreover, abnormal activation of T-type calcium channels is associated with various pathological conditions and has made these channels fascinating drug

targets [13]. In this context, many studies have shown that pharmacological or RNAi-mediated suppression of calcium channels diminishes cell proliferation and increases cell death in various cancer cells [14, 7]. In a previous study, Huang et al. reported that NNC 55-0396 application showed a concentration-dependent cytotoxic effect on different leukemia cells by increasing calcium transfer from the endoplasmic reticulum to the cytosol. In the same study, the NNC 55-0396 application showed a significant apoptotic effect, especially late apoptosis, in Jurkat and MOLT-4 cells [10]. In a similar fashion, Nam et al. showed that newly synthesized CCBs were significantly cytotoxic in two human cancer cells (colon cancer HCT-116 and lung cancer A549) [13]. In another study, it was shown that the treatment of specific T-type calcium channel blockers mibefradil or pimozide had significant cytotoxic effects on melanoma cells M16 and JG cells. Inhibitors have also been shown to induce apoptotic cell death in the cells in a caspase-dependent manner [15]. In different studies, the T-type calcium channel inhibitor mibefradil has also been shown to have significant apoptotic effects on breast and brain cancer cells [16, 17]. Many previous studies have also reported that calcium channel antagonists such as mibefradil or NNC 55-0396 have cytotoxic and apoptotic effects on various cancer cells such as colon, pancreas (MiaPaCa2), glioma (U87MG) and lung (A549), in line with our data [18].

All the studies mentioned in the previous paragraph exhibit that T-type calcium channel blockers play a crucial role in cancer development and progression, and inhibition of these channels with specific inhibitors suppresses the proliferation of cancer cells. Consistent with these studies, it has been shown in this study that NNC 55-0396, a specific calcium channel blocker, has significant cytotoxic and apoptotic effects on SNU-1 gastric cancer cells. At the same time, this study has some limitations. The use of different gastric cancer cell lines and the evaluation of the apoptotic effect of NNC 55-0396 with different methods may support the obtained data. Moreover, further in vitro and in vivo studies are needed to elucidate the potential use of NNC 55-0396 for gastric cancer therapy.

Conflicts of interest

The author declared no conflict of interest.

References

[1] Smyth E.C., Nilsson M., Grabsch H.I., van Grieken N.C., & Lordick F., Gastric cancer, Lancet (London, England), 396 (10251) (2020) 635–648.

[2] Karimi P., Islami F., Anandasabapathy S., Freedman N.D., & Kamangar F., Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention, *Cancer Epidemiol Biomarkers Prev*., 23 (5) (2014) 700–713.

[3] Joshi S.S., Badgwell B.D., Current treatment and recent progress in gastric cancer, *CA Cancer J Clin.*, 71 (3) (2021) 264-279.

[4] Harada K., Sakamoto N., Ukai S., Yamamoto Y., Pham Q.T., Taniyama D., Honma R., Maruyama R., Takashima T., Ota H., Takemoto Y., Tanabe K., Ohdan H., & Yasui, W., Establishment of oxaliplatin-resistant gastric cancer organoids: importance of myoferlin in the acquisition of oxaliplatin resistance, *Gastric Cancer*, 24(6) (2021) 1264–1277.

[5] Gonçalves J.C.R., Coulidiati T.H., Monteiro A.L., Carvalho-Gonçalves L.C.T., Valença W.O., Oliveira R.N., Câmara C.A., Araújo D.A.M., Antitumoral activity of novel 1,4-naphthoquinonederivative involves L-type calcium channel activation in human colorectal cancer cell line, *Journal of Applied Biomedicine*, 14(3) (2016) 229-234.

[6] Monteith G.R., Davis F.M., Roberts-Thomson S.J., Calcium channels and pumps in cancer: changes and consequences, *J. Biol. Chem.*, 287(38) (2012) 31666-31673.

[7] Wu L., Lin W., Liao Q., Wang H., Lin C., Tang L., Lian W., Chen Z., Li K., Xu L., Zhou R., Ding Y., & Zhao, L., Calcium Channel Blocker Nifedipine Suppresses Colorectal Cancer Progression and Immune Escape by Preventing NFAT2 Nuclear Translocation, *Cell Reports*, 33(4) (2020) 108327.

[8] Phan N.N., Wang C.Y., Chen C.F., Sun Z., Lai M.D., & Lin Y.C., Voltage-gated calcium channels: Novel targets for cancer therapy, *Oncology Letters*, 14(2) (2017) 2059–2074.

[9] Mason R.P., Calcium channel blockers, apoptosis and cancer: is there a biologic relationship?, *Journal of the American College of Cardiology*, 34(7) (1999) 1857–1866.

[10] Huang W., Lu C., Wu Y., Ouyang S., & Chen Y., T-type calcium channel antagonists, mibefradil and NNC-55-0396 inhibit cell proliferation and induce cell apoptosis in leukemia cell lines, *J. Exp. Clin. Cancer Res*., 34(1) (2015) 54.

[11] Ergul M., Bakar-Ates F., A specific inhibitor of polo-like kinase 1, GSK461364A, suppresses proliferation of Raji Burkitt's lymphoma cells through mediating cell cycle arrest, DNA damage, and apoptosis, *Chem Biol Interact*., 332 (2020) 109288.

[12] Dziegielewska B., Gray L.S., Dziegielewski J., T-type calcium channels blockers as new tools in cancer therapies, *Pflugers Arch*., 466(4) (2014) 801-810.

[13] Nam Y., Ryu K.D., Jang C., Moon Y.H., Kim M., Ko D., Chung K.S., Gandini M.A., Lee K. T., Zamponi G.W., & Lee J.Y., Synthesis and cytotoxic effects of 2-thio-3,4-dihydroquinazoline derivatives as novel T-type calcium channel blockers, *Bioorganic & Medicinal Chemistry*, 28(11) (2020) 115491.

[14] Panner A., & Wurster R.D., T-type calcium channels and tumor proliferation, *Cell Calcium*, 40(2) (2006) 253–259.

[15] Das A., Pushparaj C., Herreros J., Nager M., Vilella R., Portero M., Pamplona R., Matias-Guiu X., Martí R.M., & Cantí C., T-type calcium channel blockers inhibit autophagy and promote apoptosis of malignant melanoma cells, *Pigment cell & Melanoma Research*, 26(6) (2013) 874–885.

[16] Valerie N.C., Dziegielewska B., Hosing A.S., Augustin E., Gray L.S., Brautigan D.L., Larner J.M., & Dziegielewski J., Inhibition of T-type calcium channels disrupts Akt signaling and promotes apoptosis in glioblastoma cells, *Biochemical Pharmacology*, 85(7) (2013) 888–897.

[17] Ohkubo T., & Yamazaki J., T-type voltage-activated calcium channel Cav3.1, but not Cav3.2, is involved in the inhibition of proliferation and apoptosis in MCF-7 human breast cancer cells, *International Journal of Oncology*, 41(1) (2012) 267–275.

[18] Granados K., Hüser L., Federico A., Sachindra S., Wolff G., Hielscher T., Novak D., Madrigal-Gambo, V., Sun Q., Vierthaler M., Larribère L., Umansky V., & Utikal J., T-type calcium channel inhibition restores sensitivity to MAPK inhibitors in de-differentiated and adaptive melanoma cells, *British Journal of Cancer*, 122(7) (2020) 1023–1036.