Cumhuriyet Sci. J., 45(4) (2024) 683-687
DOI: https://doi.org/10.17776/csj.1535242

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Oxidative Stress Mediates Anti-proliferative Effects of Nifedipine on AGS Cells

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Research Article

History
Received: 18/08/2024
Accepted: 16/12/2024

ABSTRACT

Previous studies have demonstrated the anticancer properties of certain calcium channel blockers when administered as sole agents. This exploration aimed to explore the potential anti-proliferative activity of nifedipine on AGS gastric cancer cells and to determine the contribution of oxidative stress in mediating this response. To assess the anti-proliferative impact of nifedipine, AGS cell viability was calculated employing the XTT assay across a concentration range of 25, 50, 100, 200, and 500 µg/mL. Concurrently, TAS and TOS kits were employed to evaluate the drug's influence on oxidative stress levels. Nifedipine exhibited a concentration-dependent cytotoxic effect on AGS cells, with a statistically significant reduction in cell viability. The IC50 value for AGS cells after 24 hours was determined to be 98.49 µg/mL. At this concentration, a substantial augmentation in TOS and a concurrent diminution in TAS levels were observed relative to the control group. These observations imply that nifedipine-induced oxidative stress is one of the mechanisms underlying its cytotoxic action against gastric cancer cells. Our results highlight the potential therapeutic utility of nifedipine in this cancer type.

Keywords: Proliferative Activity, Nifedipine, Oxidative Stress, AGS Cells.

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Introduction

Stomach cancer persists as a substantial worldwide health crisis, occupying the fifth position among the most frequently identified cancers. Due to its often advanced detection, this disease carries a high mortality rate, claiming 784,000 lives worldwide in 2018, consequently positioning it as the third leading cause of cancer-related deaths [1]. A multitude of factors contribute to gastric cancer risk, including age, sex, genetics, smoking habits, ethnicity, poor diet, and infection with the Helicobacter pylori bacterium. Traditionally, treatment options for gastric cancer have encompassed chemotherapy, radiation therapy, surgical intervention, and immunotherapy [2]. Unfortunately, a significant number of patients struggle to tolerate the adverse effects of these conventional treatments [3]. Consequently, there is a critical need to identify novel cellular targets to enhance treatment effectiveness and mitigate the adverse consequences of current therapies. Accumulating evidence indicates that ion channels, specialized membrane proteins regulating ion flow, are implicated in the pathogenesis of various diseases, including cancer [4]. Calcium signaling modulation has shown promise in treating a diverse range of diseases, such as high blood pressure, coronary heart disease, and pain [5]. Additionally, calcium acts as a crucial intracellular messenger, governing cellular functions including cell growth, cell cycle progression, specialization, movement, and programmed cell death. Importantly, aberrant calcium channel activity has been implicated in tumorigenesis and cancer progression [6]. Calcium signaling activation has been highlighted as a key driver of cancer progression. This is attributed to its role in fostering malignant cell behavior, interacting with multiple oncogenes and oncogenic signaling cascades, and suppressing immune responses [7]. Comparative analyses have revealed significantly elevated expression levels of CACNA1C and CACNA1D—genes encoding L-type calcium channels—in gastric, colorectal, breast, and prostate cancer tissues compared to their normal counterparts [8]. Research suggests that suppressing calcium signaling could be a promising approach for cancer treatment [7]. Furthermore, any ion channels or pumps that allow calcium ion passage represent potential therapeutic targets [9]. The calcium channel blocker nifedipine has demonstrated the ability to hinder the progression and immune evasion of colorectal cancer by inhibiting the translocation of NFATC2 into the cell nucleus [6]. Nifedipine has been shown to potentiate the antitumor efficacy of cisplatin against a range of human glioblastoma cells, including those exhibiting sensitivity, resistance, or even multidrug resistance to cisplatin [10]. Diltiazem has been shown to reverse the development of resistance to docetaxel and vincristine in human lung cancer cell populations [11]. Diltiazem has been demonstrated to suppress the growth and movement of hepatocellular cells in laboratory conditions [12]. The calcium channel inhibitors lercanidipine and amlodipine have exhibited the ability to suppress the transcription regulated by YY1, ERK, and TGF-β, thereby enhancing the responsiveness of gastric cancer cells to doxorubicin [13].

To date, the antiproliferative properties of nifedipine in gastric cancer have remained unexplored. Accordingly, this research sought to explore the inhibitory impact of nifedipine on the proliferation of AGS gastric cancer cells.

Materials and Methods

Cell culture

The CRL-1739 cell line was selected for this study due to its well-characterized nature as a human gastric adenocarcinoma cell line (AGS). AGS cells, obtained from the ATCC, were cultured in a 25 cm² flask under sterile conditions at 37°C and 5% CO2 in DMEM (1:1) cell culture medium supplemented with 1% L-glutamine, 1% penicillin-streptomycin, and 10% fetal bovine serum (FBS) (Gibco, Thermo Fisher Scientific). Cells were passaged when they reached 80% confluency, and experiments were initiated after the third passage. Nifedipine (Tocris) was applied at different concentrations (25, 50, 100, 200, and 500 µg/mL). Stock solution of nifedipine was created by dissolving the compound in saline. Working solutions of various concentrations were subsequently generated by diluting appropriate volumes of the stock solution with DMEM [14].

Cell Viability Assay

To assess nifedipine's impact on AGS cell survival, an XTT assay was conducted. AGS cells were cultured in 96-well plates at a seeding density of 10,000 cells per well. Cells were exposed to various nifedipine concentrations for 24 hours. The XTT assay operates on the principle that metabolically active cells convert the tetrazolium salt XTT into a soluble orange formazan product. The formazan concentration, measured spectrophotometrically at 450 nm, correlates with the number of viable cells. Following the treatment period, XTT reagent was added to each well, and the plate was incubated for four hours under controlled conditions. The untreated cell group served as the viability control, set at 100%, against which treatment group results were compared [15,16].

Cell Homogenate Preparation Reparation

Cells from each experimental group were collected aseptically and subjected to centrifugation at 2000 rpm for 10 minutes. Following the removal of the supernatant, the cell pellet was resuspended in phosphate-buffered saline (PBS, pH 7.4) to achieve a cell density of approximately 1 million cells per milliliter. To release intracellular components, the cell suspension underwent multiple freeze-thaw cycles. Subsequently, the lysate was centrifuged at 4000 rpm for 10 minutes at 4°C, and the resulting supernatant was collected for biochemical analyses [17,18]. Total protein concentration within the samples was determined using a Bradford protein assay kit (Merck Millipore, Darmstadt, Germany) [17].

Measurement of Total Antioxidant Status (TAS) and Total Oxidant Status (TOS)

TAS levels in cell culture supernatants were quantified using an automated assay kit (Rel Assay Kit Diagnostics, Antep, Turkey) established by Erel (2004). This method is based on the principle of colorimetric detection. Hydroxyl radicals, generated through the Fenton reaction, initiate a chain reaction producing colored dianisidyl radicals. Antioxidants in the sample inhibit this color development in proportion to their concentration [19,20] Results were expressed as micromolar Trolox equivalents per milligram of tissue protein (µmol Trolox Eq/mg protein). TOS levels in cell culture supernatants were determined using an automated assay (Rel Assay Kit Diagnostics, Antep, Turkey) developed by Erel (2005). The assay measures TOS indirectly by quantifying ferric ions, formed from the oxidation of ferrous ions by oxidants in the sample. Xylenol orange is employed to detect ferric ions, and hydrogen peroxide serves as the assay standard [21,22]. Results were expressed as micromolar hydrogen peroxide equivalents per milligram tissue protein (µmol H_2O_2 Eq/mg protein).

Statistical Analysis

The statistical analysis of the obtained data was performed using the SPSS Version 25.0 program. Since the data showed a normal distribution, one-way ANOVA (Analysis of Variance) was applied. Values with $p<0.05$ were considered statistically significant.

Results and Discussion

Nifedipine Inhibits AGS Cell Proliferation

Nifedipine was tested for its ability to inhibit AGS cell growth. Cells were exposed to concentrations ranging from 25 to 500 µg/mL for 24 hours. Cell viability was assessed using the XTT assay. No significant antiproliferative effect was observed at doses of 25 or 50 µg/mL. In contrast, a dose-dependent reduction in cell viability was evident at 100, 200, and 500 µg/mL ($p < 0.001$; Fig. 1). The half-maximalinhibitory concentration (IC50) of nifedipine, calculated using GraphPad Prism, was 98.49 µg/mL.

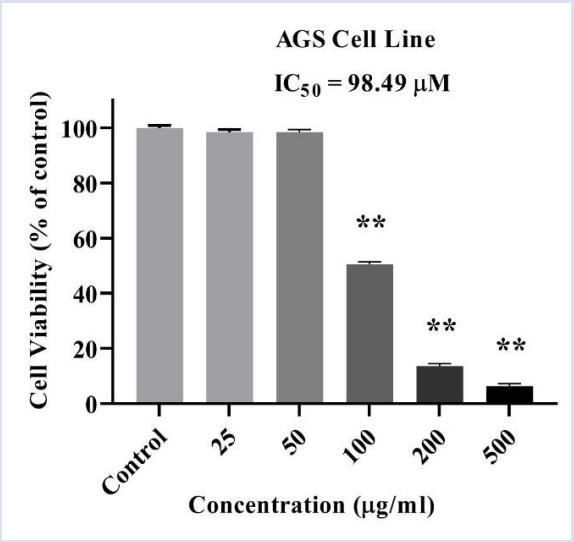

Figure 1. Cytotoxicity of nifedipine in AGS cells. Data represent mean ± SEM (n=6). **$p<0.001$ vs. control.

Nifedipine Modulates TAS and TOS in AGS Cells

Using commercial assay kits, Nifedipine's influence on TAS and TOS was evaluated in AGS cells. Treatment with 98.49 µg/mL nifedipine resulted in a significant decrease in TAS and a concomitant increase in TOS levels compared to the control group ($p < 0.001$; Figure 2.a,b).

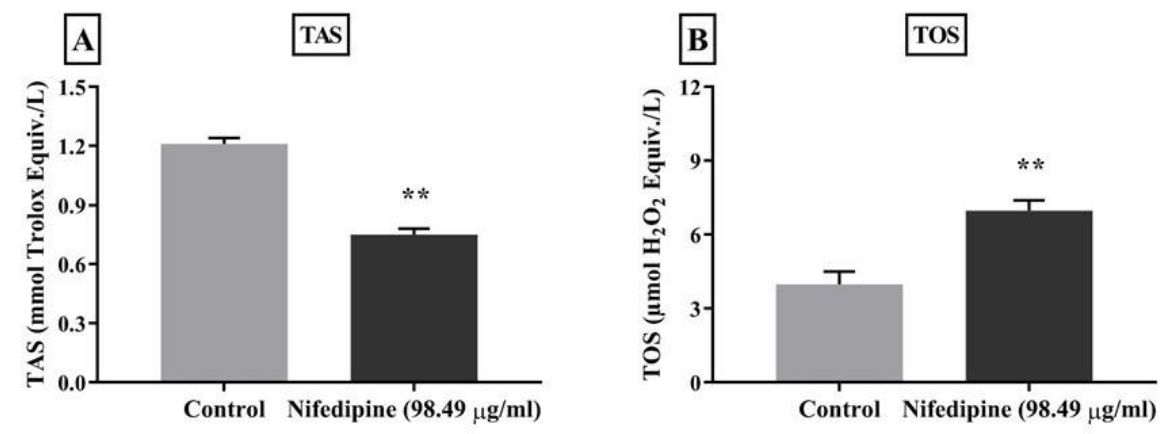

Figure 2. TAS (a) and TOS (b) levels in AGS cells treated with nifedipine. Data represent mean ± SEM. *$p < 0.001$ vs. control.

While multiple studies have documented Nifedipine's anticancer properties across various cancer cell types, to our knowledge, the literature lacks research specifically investigating nifedipine's cytotoxic impact on AGS cells. The present study revealed that the proliferation of gastric cancer cells was notably inhibited upon exposure to Nifedipine, a selective L-type calcium channel blocker, in a dose-dependent fashion. Mechanistic analysis indicated that this compound induced a marked elevation in TOS levels while concurrently diminishing TAS levels within AGS gastric cancer cells. These findings suggest that Nifedipine's inhibition of calcium channels may exert substantial anticancer effects by disrupting the oxidant-antioxidant balance in favor of oxidation. This oxidative shift is associated with the generation of free radicals, ultimately leading to cancer cell demise. Calcium ions (Ca2+) and their associated intracellular calcium channels are primarily recognized for their roles in regulating cardiovascular and neural function. However, emerging evidence increasingly implicates these channels as key contributors to the initiation and progression of tumor development [6]. In connection with this, in cells, calcium channels play several roles in physiological and pathological processes, including cancer. Calcium channels are also frequently abnormally expressed in various cancer cells and are involved in the proliferation, cell cycle progression, and survival [23]. A growing body of research underscores the pivotal role of calcium homeostasis in established oncogenic pathways [7]. Numerous studies have demonstrated the capacity of calcium signaling to drive the progression of various cancers, including glioma [24], prostate cancer [25], and breast cancer [26], through the activation of STAT3, a key transcriptional regulator in cancer. Mechanistically, calcium signals stimulate the production of reactive oxygen species (ROS) within mitochondria, which in turn triggers the phosphorylation and nuclear translocation of STAT3 [7]. Interfering with calcium signaling presents a potential strategy for enhancing antitumor immune responses. The MAPK pathway, another critical cellular signaling cascade, is interconnected with calcium signaling. A recent study demonstrated that downregulating KCNN4, a calcium-activated potassium channel, led to decreased expression of both MAPK and MMP-9 within cancer cells [7,27]. A significant finding is that numerous calcium channel blockers (CCBs), encompassing phenylalkylamine, dihydropyridine, and benzothiazepine subtypes, as well as other calcium antagonists, can effectively hinder drug efflux mediated by P-glycoprotein, thereby functioning as modulators of multidrug resistance (MDR) [28]. For instance, verapamil, a calcium channel blocker of the L-type, has been demonstrated to reduce P-gp expression in A704 (human kidney adenocarcinoma) and Caki-1 (human renal cancer) cell lines, consequently reversing multidrug resistance in renal cell carcinoma [29]. Furthermore, pretreatment with verapamil has demonstrated the ability to effectively counteract multidrug resistance to doxorubicin in ovarian cancer cells [30]. The calcium channel blocker nifedipine has been shown to inhibit the progression and immune evasion tactics of colorectal cancer by obstructing the nuclear translocation of NFAT2 [6]. Nifedipine has demonstrated the ability to amplify the antitumor efficacy of cisplatin across various human glioblastoma cell lines, including those exhibiting sensitivity, resistance, and even multidrug resistance to cisplatin [10]. Diltiazem has been shown to counteract the development of multidrug resistance induced by docetaxel and vincristine in human lung cancer cell lines [11]. Calcium channel blockers (CCBs) have also shown promise as standalone treatments. For

instance, diltiazem has been observed to suppress the proliferation and migratory behavior of hepatocellular cells in laboratory conditions [12,31]. Collectively, these findings suggest that disrupting calcium signaling could be a promising anticancer therapeutic approach. Carboxyamidotriazole (CAI), a compound that inhibits non-voltage-gated calcium channels and their associated signaling pathways, exemplifies this strategy [6]. Consistent with previous findings, our research demonstrated that nifedipine exhibits anti-proliferative properties against AGS gastric cancer cells. Oxidative stress, a common factor in numerous diseases, is significantly implicated in cancer progression. The body's reactive oxygen species (ROS) levels are pivotal in cancer development and metastasis. While moderate ROS can stimulate angiogenesis, metastasis, and cell survival by activating specific signaling pathways within the tumor microenvironment, excessive ROS can induce cancer cell apoptosis. This underscores the critical role of ROS concentration in determining cancer initiation or cell death [18]. The mechanism of action for many anticancer drugs involves inducing oxidative stress, believed to be the primary cause of extensive cellular macromolecular damage. These drugs target critical cellular components, including proteins, lipids within membranes, and DNA [32]. Total Oxidative Status (TOS) is a commonly used biomarker to evaluate the body's overall oxidative state [33]. Conversely, Total Antioxidant Status (TAS) is employed to assess the body's overall antioxidant capacity [19,34]. This study aimed to determine if nifedipine exerts a cytotoxic effect by altering TAS and TOS levels. Our results demonstrated that a 24-hour nifedipine treatment significantly increased TOS levels while simultaneously reducing TAS levels compared to control cells. These findings suggest that nifedipine induces oxidative stress in AGS cells, as evidenced by the elevated TOS and decreased TAS values. In conclusion, our study provides evidence that nifedipine, a commonly used calcium channel blocker, exhibits significant anti-proliferative effects on AGS gastric cancer cells. This effect is likely mediated through the induction of oxidative stress, as evidenced by the significant increase in TOS and decrease in TAS levels. These findings suggest that targeting calcium signaling pathways may represent a promising strategy for the development of novel anticancer therapies. While our study primarily focused on the antiproliferative effects of nifedipine and its association with oxidative stress in AGS cells, a more comprehensive understanding of the underlying mechanisms would benefit from further investigation. A cell cycle analysis, for instance, could provide additional valuable insights into the precise mode of action of nifedipine. Although such an analysis was beyond the scope of the current study due to resource limitations, our findings strongly suggest that nifedipine exerts its anti-cancer effects by inducing oxidative stress and inhibiting cell proliferation. Future studies could delve deeper into these molecular mechanisms by incorporating cell cycle analysis and exploring the involvement of specific cell cycle regulatory proteins. Such investigations could provide further evidence supporting the therapeutic potential of nifedipine in the management of gastric cancer and guide the development of more targeted and effective treatment strategies.

Conflicts of interest

There are no conflicts of interest in this work.

Acknowledgments

The authors would like to acknowledge the indispensable support and infrastructure provided by the CUTFAM Research Center at the School of Medicine, Sivas Cumhuriyet University, Sivas, Turkey, which were crucial to the successful completion of this research. This study was funded by Türkiye Bilimsel ve Teknolojik Araştırma Kurumu (2209-A Student Project, TÜBİTAK, NO. 1919B012221382, Turkey).

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