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Article

Targeting Voltage-Gated Potassium Channels in Breast Cancer: Mechanistic Insights into 4-Aminopyridine-Induced Cell Death

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Abstract

Cancer has recently been proposed as a type of channelopathy due to the aberrant expression of various ion channels. Voltage-gated potassium (K^+) channels are notably upregulated during tumor proliferation, while voltage-gated sodium (Na^+) channels are predominantly associated with the invasive stage of cancer progression. Among these, the Kv10.1 channel has been found to be overexpressed in breast cancer, making it a promising therapeutic target. 4-Aminopyridine, a non-selective voltage-gated potassium channel (VGKC) blocker, has emerged as a potential novel agent for breast cancer treatment. In this study, we aimed to elucidate the mechanism of action of 4-aminopyridine in breast cancer cells. To investigate the involvement of various cell death pathways, cycloheximide (a paraptosis inhibitor), Z-VAD-FMK (a pan-caspase inhibitor), and 2-APB (a phosphoinositide 3-kinase [PI3K] inhibitor) were employed. Experiments were conducted using the MCF-7 human breast cancer cell line and the L929 mouse fibroblast cell line as a healthy control. Assessments included cell viability assays, intracellular calcium (Ca^{2+}) and potassium (K^+) concentration measurements, and plasma membrane potential analysis. Our findings aim to contribute to the understanding of the therapeutic potential and cellular effects of VGKC blockers, particularly 4-aminopyridine, in breast cancer treatment strategies.

Keywords: voltage gated K channels; membrane potential; intracellular Ca concentration; ion channel blockers; breast cancer cell line

1. Introduction

Ion channels play crucial roles in tumor pathophysiology, contributing to the regulation of membrane potential, cell cycle progression, cellular osmolarity, motility, invasion, migration, and proliferation [1,2]. Among these, potassium (K^+) channels—encoded by approximately 77 genes—represent the most diverse and abundant group in excitable cells. These channels are essential for maintaining ionic homeostasis, selectively permitting potassium ion flux across membranes in response to various environmental stimuli, while restricting the movement of other ions.

Based on their activation mechanisms, ion channels are generally classified into voltage-gated, ligand-gated, and mechanically gated types. The term “ion channel” often refers specifically to voltage-gated channels, whose activity is predominantly regulated by membrane potential. These channels are named according to the ions that most readily permeate them.

Recently, cancer has increasingly been considered a channelopathy, and there is growing interest in targeting ion channel modulation as a novel therapeutic approach, particularly in breast cancer [1].

Potassium channel proteins are involved in a wide range of physiological and pathological processes [2]. Notably, dysregulation and abnormal expression of potassium channels have been observed in several types of cancer, including breast [3], colorectal [4], prostate [5], lung [6], liver [7], and glioma [8]. Several subtypes, such as KCNN4 [9], KCNA1 [10], Kv11.1[11], KCNK9 [11,12], KCNE1 [13], and GIRK1 [14], have been implicated in the malignant transformation and progression

of breast cancer. Additionally, KCNK6 has been reported to be overexpressed in both breast cancer [15] and thyroid carcinoma [16].

Hou et al. demonstrated that KCNK6 expression is elevated in breast cancer cells, leading to weakened cell adhesion [17]. Pharmacological inhibition of this channel enhanced cellular adhesion and biophysical function, suggesting that upregulation of KCNK6 may facilitate cancer cell proliferation, migration, and invasion. Similarly, Sun et al. reported that KCNK1 is overexpressed in breast cancer and associated with poor prognosis [18]. Silencing of KCNK1 reduced cell proliferation and migration while enhancing sensitivity to paclitaxel chemotherapy.

In MCF-7 breast cancer cells, the “membrane potential” model proposed by Wonderlin et al. suggests that both proliferation and cell cycle progression are tightly linked to K^+ channel activity [19]. Inhibition of these channels causes membrane depolarization, which in turn suppresses cell proliferation. Electrophysiological studies have revealed that at least six distinct K^+ currents—differing in their dependence on voltage, intracellular Ca^{2+} , and ATP—are present in MCF-7 cells [20–23].

However, the precise mechanisms by which non-selective K^+ channel blockers such as 4-aminopyridine (4-AP) induce cell death in breast cancer cells remain unclear. 4-Aminopyridine (4-AP) blocks potassium channels, leading to cell depolarization and calcium influx. Due to this property, it enhances neurotransmitter release by increasing Ca^{2+} influx in the presynaptic region and accelerates axonal conduction, making it useful in the treatment of central and peripheral nervous system disorders such as Multiple Sclerosis (MS), spinal cord injury (SCI), botulism, and Lambert-Eaton syndrome [24].

Given 4-AP's action as a potassium channel blocker, the altered expression of potassium channels in cancer cells, and the effects of channel blockade on cell proliferation [13,25,26], studies have shown that 4-AP inhibits cancer cell proliferation and induces apoptosis [27].

In this study, we hypothesized that blocking K^+ channels with 4-AP alters membrane potential and calcium signaling, leading to regulated cell death. To investigate this, we employed pharmacological inhibitors—including cycloheximide (CHX), Z-VAD-FMK, and 2-APB—to dissect the involvement of apoptotic and calcium-dependent cell death pathways. The effects of 4-AP on cell viability, membrane potential, and intracellular calcium concentration were systematically evaluated.

2. Results

2.1. Determination of IC_{50} Values and the Investigation of Cell Death Mechanisms Induced by 4-AP in L929 and MCF-7 Cell Lines

The IC_{50} values of 4-aminopyridine (4-AP) for L929 (healthy fibroblast) and MCF-7 (breast cancer) cell lines were determined using the trypan blue exclusion method with a hemocytometer. A concentration range of 1–5 mM was used to determine the IC_{50} values. The IC_{50} value of 4-AP was found to be 4 mM for MCF-7 cells and 5 mM for L929 cells. The higher IC_{50} observed in L929 cells indicates lower sensitivity to 4-AP compared to MCF-7 cells. L929 cells, which do not exhibit overexpression of voltage-gated potassium channels, were selected as the healthy control line. The considerable difference in IC_{50} values between the two cell lines suggests that 4-AP exhibits selective cytotoxicity, potentially related to differential ion channel expression.

In subsequent experiments, the effects of 4-AP on cell death mechanisms were investigated using cycloheximide (CHX), z-VAD-FMK, and 2-APB based on concentrations previously reported in the literature.

For CHX experiments, a 20 μ M concentration was chosen. At this dose, cell viability was $61.6\% \pm 2.4$ for L929 and $77\% \pm 1.5$ for MCF-7. Cells were pretreated with CHX for 1 hour, and then treated with 4-AP for 24 hours. After the combined treatment, cell viability increased to $78\% \pm 2.2$ in L929 and $88\% \pm 1.2$ in MCF-7 cells (Figure 1A,B). If 4-AP-induced cytotoxicity were solely due to paraptosis, these viability levels would be expected to be even higher, suggesting the involvement of other cell death mechanisms.

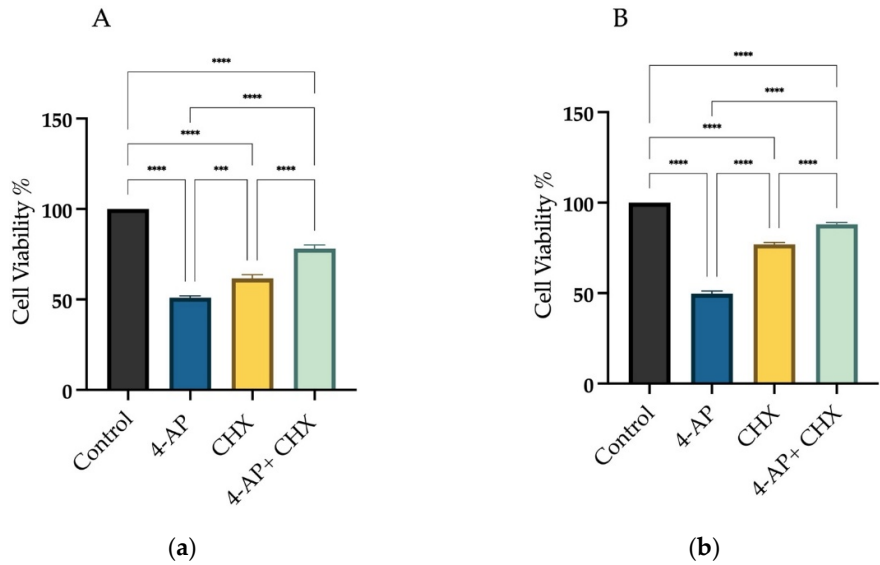


Figure 1. Assessment of cell viability following CHX and 4-AP treatments. Cells were treated with the IC₅₀ concentration of 4-aminopyridine (4-AP), and cell viability was assessed using the trypan blue exclusion method with a hemocytometer. For cycloheximide (CHX) treatment, 20 μ M dose was selected. Cells were pre-incubated with CHX for 1 hour prior to 4-AP exposure. CHX pre-treatment resulted in increased cell viability in both the L929 (A) and MCF-7 (B) cell lines. ***p < 0.001, and ****p < 0.0001.

To explore the involvement of apoptosis, Z-VAD-FMK, a pan-caspase inhibitor, was used at a concentration of 50 μ M. Treatment with Z-VAD-FMK alone resulted in cell viabilities of 83.6% \pm 1.9 for L929 and 91% \pm 0.9 for MCF-7. Following 1-hour pretreatment with Z-VAD-FMK, cells were incubated with 4-AP for 24 hours. Under these conditions, cell viability decreased to 72.3% \pm 2.4 in L929 and 85% \pm 1.2 in MCF-7 (Figure 2A,B). These findings indicate that other cell death pathways may also be involved.

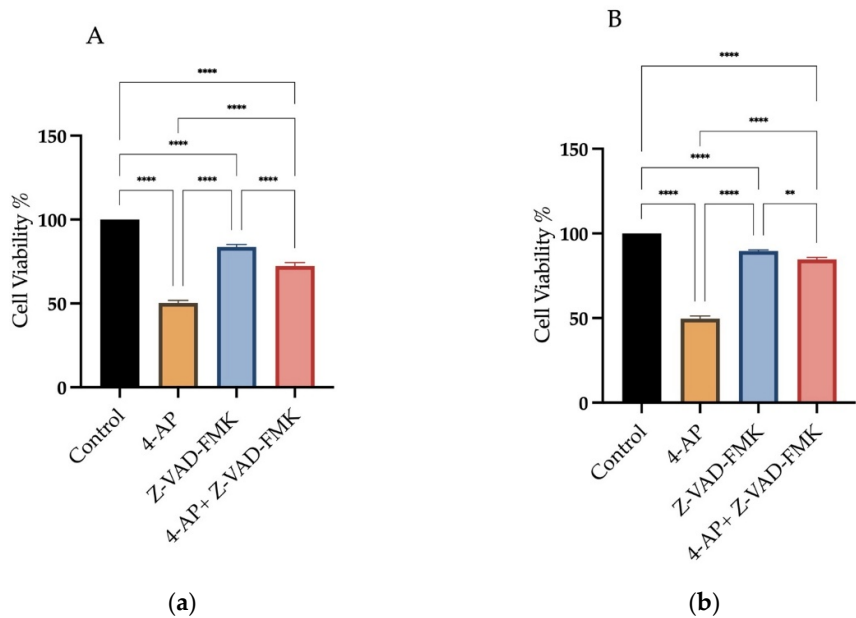


Figure 2. Assessment of cell viability following Z-VAD-FMK and 4-AP treatments. Cells were treated with 50 μ M Z-VAD-FMK, either alone or in combination with 4-aminopyridine (4-AP). Cell viability was assessed using the trypan blue exclusion method with a hemocytometer. Treatment with Z-VAD-FMK alone increased cell

viability, whereas co-treatment with 4-AP reduced viability in both L929 (A) and MCF-7 (B) cell lines. $**p < 0.01$, $****p < 0.0001$.

To assess the role of calcium signaling in 4-AP-induced cell death, 2-APB, an IP₃ receptor inhibitor, was employed at a concentration of 10 μ M. Treatment with 2-APB alone resulted in cell viabilities of $73.3\% \pm 1.9$ for L929 and $73\% \pm 2.1$ for MCF-7. Following 1-hour pretreatment with 2-APB and subsequent incubation with 4-AP for 24 hours, cell viability increased to $82\% \pm 2.5$ for L929 and $92\% \pm 2.0$ for MCF-7 (Figure 3A,B). These findings suggest that IP₃-mediated calcium signaling plays a significant role in 4-AP-induced cytotoxicity. The high cell viability observed after combined 2-APB and 4-AP treatment implies that inhibition of calcium signaling may prevent both apoptosis and paraptosis.

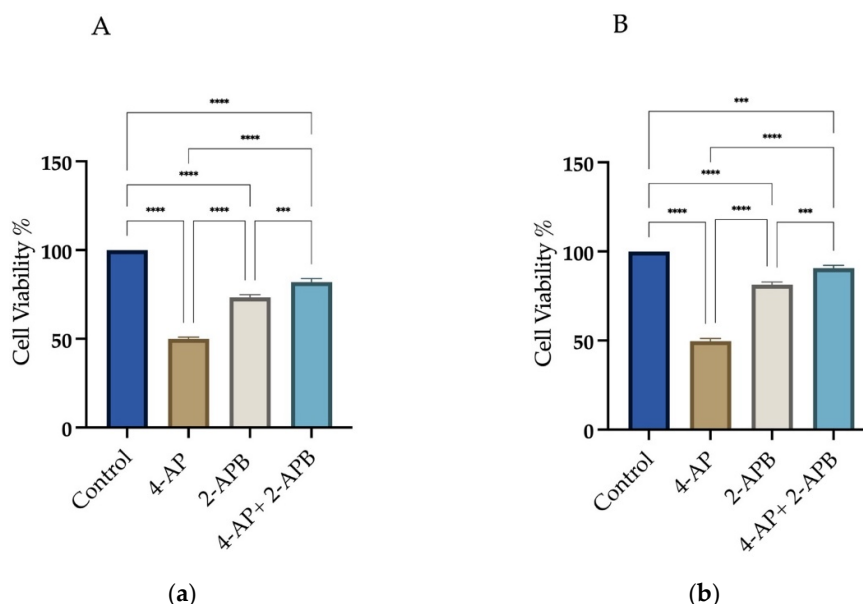


Figure 3. Assessment of cell viability following 2-APB and 4-AP treatments. Cells were treated with 10 μ M 2-APB, either alone or in combination with 4-aminopyridine (4-AP). Cell viability was assessed using the trypan blue exclusion method with a hemocytometer. Co-treatment with 4-AP increased cell viability in both L929 (A) and MCF-7 (B) cell lines. $***p < 0.001$, and $****p < 0.0001$.

2.2. Assessment of Intracellular Calcium Changes Following Drug Treatments

After drug treatments, changes in intracellular calcium levels were measured using the Screen Quest Fura-2 AM no-wash calcium assay kit. Fura-2 crosses the cell membrane and enters the cytoplasm. When Fura-2 binds to free calcium ions, its fluorescence emission wavelength shifts towards the blue region. When agonists or other signals stimulate the cell, receptor activation triggers the release of calcium, leading to an increase in the fluorescent intensity of Fura-2. For analysis of intracellular Ca^{2+} changes, Fura-2 measurements were taken immediately after drug addition for 20 hours. The results at 20th hour are presented as a histogram (Figure 4A,B).

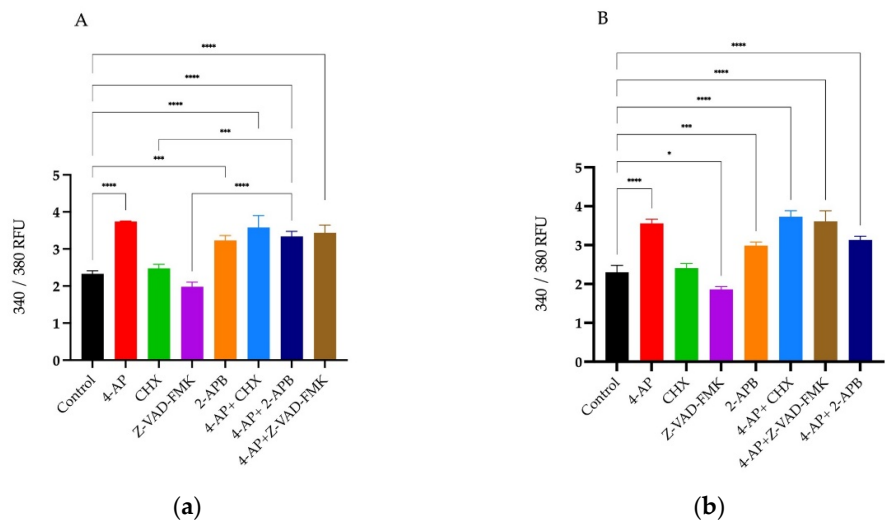


Figure 4. Percentage changes in intracellular Ca^{2+} levels following treatments. Intracellular calcium concentrations were measured using the Fura-2 fluorescent dye. Percentage changes in Ca^{2+} levels were calculated relative to the untreated control group. Treatment with 4-AP and its combinations increased intracellular Ca^{2+} levels. CHX and Z-VAD-FMK alone did not induce significant increases, whereas 2-APB alone elevated intracellular Ca^{2+} concentrations in both L929 (A) and MCF-7 (B) cell lines. *p < 0.05, ***p < 0.001, and ****p < 0.0001. Non-significant results are not indicated in the figure.

The percentages of changes in intracellular Ca^{2+} concentration and cell viabilities after treatments are presented in the table relative to the control group (Table 1). Briefly, 4-AP and its combinations caused an increase in intracellular Ca^{2+} concentration. CHX induced a slight increase, whereas Z-VAD-FMK led to a decrease in intracellular Ca^{2+} levels. Although 2-APB is an IP_3 receptor blocker, its treatment resulted in an unexpected increase in intracellular Ca^{2+} concentration. Both 2-APB alone and in combination with 4-AP increased intracellular Ca^{2+} levels. The combination effect of 4-AP and 2-APB can be explained by the blockade of voltage-gated potassium channels (VGKC) by 4-AP, which causes membrane depolarization. This depolarization likely opens voltage-gated calcium channels (VGCC), leading to an increase in intracellular Ca^{2+} concentration.

Table 1. The percentages of changes in intracellular Ca^{2+} concentration and cell viabilities.

Treatments	MCF-7		L929	
	Cell Viability	The changes in Intracellular Ca^{+2}	Cell Viability	The changes in Intracellular Ca^{+2}
4-AP	49.6% ±2.9	55% ±0.13	50.3% ± 2.3	60% ±0.19
CHX	77% ±1.5	4% ±0.11	61.6% ± 2.4	6% ±0.20
Z-VAD-FMK	91% ±0.9	-19% ±0.10	83.6% ±1.9	-16% ±0.30
2-APB	73% ±2.1	30% ±0.11	73.3% ±1.9	38% ±0.10
4-AP+ CHX	88% ±1.2	62% ±0.17	78% ± 2.2	53% ±0.37
4-AP+ Z-VAD-FMK	85% ±1.2	57% ±0.26	72.3% ±2.4	47% ±0.10
4-AP+ 2-APB	92% ±2.0	36% ±0.12	82% ±2.5	42% ±0.10

2.3. Membrane Potential Measurements: Effect of Drug Treatments on Membrane Polarization

Changes in membrane potential induced by drug treatments were measured using the DiBAC4(3) fluorescent dye. DiBAC4(3) is a hydrophobic dye that easily passes through the cell membrane, enabling detection of membrane potential alterations. In the absence of stimulation, the

negative charge on the cytoplasmic side of the membrane prevents the ionized dye from entering the cell. Upon membrane depolarization triggered by stimulation, the dye binds to intracellular proteins and lipids, leading to an increase in fluorescence intensity. Conversely, during hyperpolarization, the fluorescence intensity of the dye decreases.

To determine changes in membrane potential following the inhibition of 4-AP-induced cell death, drugs and their combinations with 4-AP were administered, and DiBAC₄(3) fluorescence measurements were performed using a microplate reader 15 minutes after treatments for 24 hours.

Measurements were taken in a time-dependent manner. In the L929 cell line, 4-AP treatment induced membrane depolarization. Since 4-AP blocks voltage-gated potassium channels (VGKCs), this blockade results in membrane depolarization. The combination of 4-AP and 2-APB also led to depolarization (Figure 5A). The depolarization caused by 4-AP likely activated voltage-gated calcium channels (VGCCs), enhancing calcium influx and resulting in a greater degree of depolarization than 4-AP treatment alone. Combinations of 4-AP with CHX or Z-VAD-FMK also induced membrane depolarization; however, the depolarization levels were lower compared to 4-AP alone or the 4-AP + 2-APB combination. This difference may be attributed to intracellular accumulation of potassium ions without additional VGCC activation. Treatment with 2-APB alone did not induce membrane depolarization.

In the MCF-7 cell line, a similar pattern was observed. 4-AP treatment alone induced membrane depolarization, and its combination with 2-APB enhanced this effect. However, 4-AP combined with CHX or Z-VAD-FMK caused only a slight depolarization of the membrane (Figure 5B).

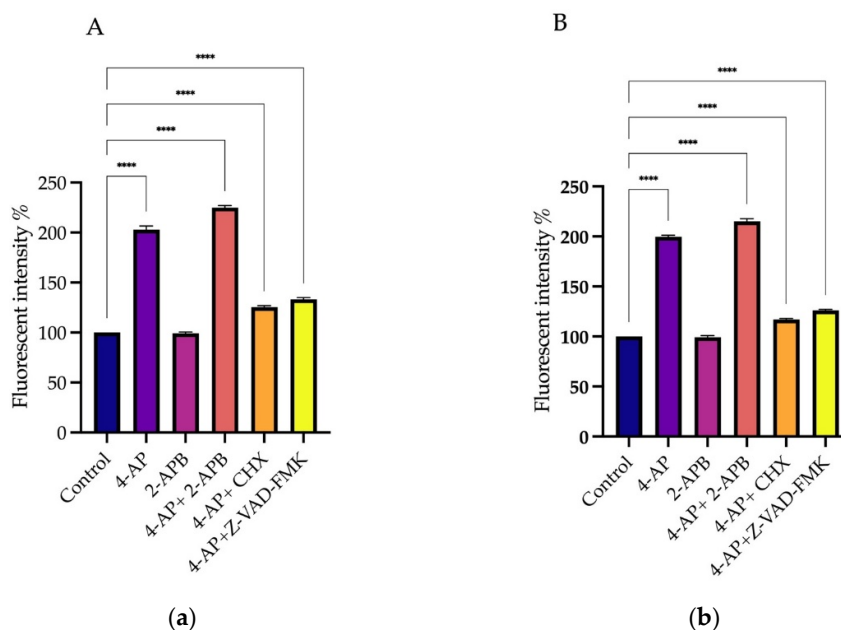


Figure 5. Effect of drug treatments on membrane polarization. To assess the effects of elevated intracellular Ca²⁺ levels on membrane potential, DiBAC₄(3) fluorescent dye was used. Since CHX and Z-VAD-FMK did not induce significant increases in intracellular Ca²⁺ levels, they were excluded from DiBAC₄(3) measurements. Treatment with 4-AP and its combinations increased membrane depolarization, with the most pronounced effect observed following co-treatment with 2-APB. In contrast, 2-APB alone did not cause substantial depolarization in either L929 (A) or MCF-7 (B) cell lines. ****p < 0.0001. Non-significant results are not indicated in the figure.

3. Discussion

In this study, we demonstrated that 4-aminopyridine (4-AP), a known potassium channel blocker used in neurological disorders, induces cell death in MCF-7 breast cancer cells through both apoptotic and paraptotic pathways. Despite the development of chemotherapeutic agents, resistance

to apoptosis remains a major limitation in effective cancer treatment [28]. Therefore, triggering alternative cell death mechanisms, such as paraptosis, has become increasingly relevant in overcoming therapeutic resistance.

Our results showed that treatment with 4-AP caused vacuolization and cell swelling in MCF-7 cells, indicative of paraptotic or paraptosis-like cell death. These findings are in line with previous studies reporting that paraptosis is characterized by cytoplasmic vacuole formation, endoplasmic reticulum (ER) and mitochondrial swelling, and a dependence on protein synthesis [29,30]. The protective effect of the protein synthesis inhibitor cycloheximide (CHX) and therefore paraptotic cell death [31], which increased MCF-7 cell survival from 53% to 88%, supports the involvement of a paraptotic mechanism.

Furthermore, we assessed whether apoptotic pathways were simultaneously involved in the 4-AP-induced cell death process. The application of the pan-caspase inhibitor z-vad-fmk significantly increased survival rates in both MCF-7 and L929 cells, suggesting that apoptosis also contributes to the observed cytotoxicity. These findings align with other studies reporting simultaneous activation of multiple cell death pathways in cancer cells exposed to various compounds, including celastrol, iturin A, and honokiol [32–34].

Interestingly, although caspase-9 activity is reported to be required for paraptosis [35], Sperandio et al. demonstrated that paraptosis is not inhibited by z-vad-fmk, indicating mechanistic complexity [31]. Our findings that z-vad-fmk increases survival suggest partial overlap or co-activation of apoptotic and paraptotic pathways. To delineate the sequence and dominance of these pathways, future experiments using selective caspase-9 inhibitors and paraptosis-specific blockers such as AIP1/Alix are needed.

Another key observation in our study was the intracellular calcium (Ca^{2+}) influx following 4-AP treatment. Using Fura-2-based analyses, we confirmed that 4-AP induced significant increases in intracellular Ca^{2+} levels. Pre-incubation with the IP3 receptor antagonist 2-APB improved survival rates (92% in MCF-7 cells), indicating that ER-derived Ca^{2+} release contributes to 4-AP-induced cell death. These findings are consistent with the notion that disruption of Ca^{2+} homeostasis is central to both apoptosis and paraptosis [36].

However, we also observed that 2-APB alone induced Ca^{2+} elevation greater than 4-AP. This may be explained by its known activation of Orai3 channels in estrogen receptor-positive breast cancer cells, as previously reported [37,38]. Therefore, the context-dependent action of 2-APB and its concentration-specific effects on calcium channels must be carefully considered in interpreting these data [39].

The observed ER and mitochondrial changes, combined with altered calcium signaling, support the idea that 4-AP simultaneously triggers both mitochondrial-mediated apoptosis and ER-related paraptosis. Nevertheless, temporal dynamics remain unclear. Confocal microscopy-based kinetic analyses of intracellular Ca^{2+} distribution, in combination with selective pathway inhibitors, will be crucial to clarify the sequence of events.

In conclusion, our findings suggest that 4-AP induces cancer cell death via dual activation of paraptotic and apoptotic pathways. This dual action may overcome apoptosis resistance in certain cancer types. Although 4-AP is unlikely to serve as a standalone anticancer agent, its ability to modulate ion channel activity and disrupt Ca^{2+} homeostasis makes it a promising candidate for combination therapy with conventional chemotherapeutics. As demonstrated by our previous findings that 4-AP in combination with paclitaxel increased cell death of both MCF-7 and MDA-MB-231 breast cancer cell lines [40].

4. Materials and Methods

4.1. Cell Lines and the Growth Condition

The breast cancer cell line MCF-7 (ATCC HTB 22) and healthy mouse fibroblast cell line L929 (ATCC CCL-1) were used in this study. Cells were grown in DMEM medium containing L-glutamine (Capricorn Scientific GmbH, Germany) supplemented with 10% Fetal Bovine Serum (FBS; Capricorn

Scientific GmbH, Germany), 1% penicillin/streptomycin (Capricorn Scientific GmbH, Germany) in a humidified atmosphere containing 5% CO₂ at 37° C.

4.2. Cell Viability and Cytotoxicity of 4-AP, Z-VAD-FMK, CHX, 2-APB; Determination of IC₅₀ Values

4-AP, Z-VAD-FMK, CHX, and 2-APB (Sigma Aldrich St. Louis MO, USA) stock solutions were prepared by dissolving in DMSO (Sigma Aldrich St. Louis MO, USA) and stored at -20° C. Growth medium was used for preparing the desired concentration from the stock solution. The final concentration of DMSO was 0.01% and the control groups were with and without DMSO. From cell survival point of view no difference was observed between the control without DMSO and the control with DMSO.

Determinations of viability and IC₅₀ values were performed by the trypan blue exclusion method on hemocytometer. MCF-7 and L929 cells were seeded on 6 wells plates, cell numbers were 1.8x10⁵ cells/well in 3 ml growth medium, and incubated overnight in a humidified atmosphere containing 5% CO₂ at 37° C. After incubation, cells were treated with different concentrations of 4-AP, Z-VAD-FMK, CHX, and 2-APB and incubated for 24 h and then counted with a hemocytometer. Results were compared with control groups.

4.3. Determination of Intracellular Ca²⁺ Concentration

Activation of calcium channels or G protein-coupled receptors leads to changes in intracellular calcium concentrations, which can be homogeneously detected using the Fura-2 calcium indicator. Once inside the cell, esterases cleave the lipophilic and blocking groups of Fura-2, trapping the negatively charged fluorescent dye within the cell.

Changes in intracellular Ca²⁺ concentration were measured using the Screen Quest™ Fura-2 No-Wash Calcium Assay Kit (AAT Bioquest Inc., Pleasanton, CA, USA) with a microplate reader (Synergy H1, BioTek Instruments, VT, USA). Fura-2 AM was dissolved in DMSO and used according to the manufacturer's protocol.

Cells were seeded in black 96-well plates at a density of 1 × 10⁴ cells per well in 100 µL of growth medium and incubated overnight. Intracellular calcium levels were detected by measuring fluorescence at dual excitation wavelengths of 340 nm and 380 nm, and an emission wavelength of 510 nm.

Fluorescence measurements were performed for 90 minutes immediately following drug addition to monitor dynamic calcium responses.

4.4. Measurement of Transmembrane Potential Using DiBAC₄(3)

The transmembrane potential was measured using the fluorescent dye Bis-(1,3-dibutyl barbituric acid) trimethine oxonol (DiBAC₄(3); AAT Bioquest Inc., Pleasanton, CA, USA) with a microplate reader (Synergy H1, BioTek Instruments, VT, USA). DiBAC₄(3) is a hydrophobic, slow-response dye that passively diffuses across the cell membrane. Upon incubation, the dye migrates from the extracellular aqueous medium into the lipid bilayer due to its hydrophobic nature. Unlike cationic carbocyanine dyes, DiBAC₄(3) is not absorbed by mitochondria and is primarily selective for changes in membrane potential.

The dye was prepared at a stock concentration of 20 mM in spectroscopic-grade DMSO. Cells were seeded in black 96-well plates at a density of 5 × 10⁴ cells per well in 100 µL of growth medium and incubated overnight. After incubation, the medium was removed and replaced with 100 µL of HBSS. Drug treatments were applied and cells were incubated for 2 hours. Following drug treatment, DiBAC₄(3) was added to each well to reach a final concentration of 2 µM in 100 µL HBSS, and the cells were incubated for 30 minutes at room temperature.

To remove excess dye, cells were washed with HBSS, and drug treatments were repeated. Fluorescence measurements were performed in HBSS medium with an excitation wavelength of 540

nm and an emission wavelength of 590 nm. Fluorescence intensity was recorded every 30 minutes over a 24-hour period to monitor dynamic changes in membrane potential.

4.5. Statistical Analyzes

Data in this study were obtained from at least three independent experiments. Results are presented as mean ± standard deviation (SD) in the figures. Statistical analyses were conducted using a two-tailed unpaired Student’s t-test for comparisons between two groups, and one-way analysis of variance (ANOVA) for comparisons among multiple groups, post-hoc analysis was performed using Tukey’s test. For all analyses, n ≥ 3. Differences were considered statistically significant at p < 0.05 and are indicated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

Author Contributions: Conceptualization, E.M.C.A and P.I.; methodology, E.M.C.A.; software, E.M.C.A.; validation, E.M.C.A, P.I, and A.I.G.; formal analysis, E.M.C.A; investigation, E.M.C.A., P.I.; data curation, E.M.C.A.,P.I.; writing—original draft preparation, E.M.C.A.; writing—review and editing, E.M.C.A., A.I.G.; visualization, E.M.C.A.; supervision, A.I.G.; project administration, A.I.G.; funding acquisition, A.I.G. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

4-AP	4-Aminopyridine
2-APB	2-Aminoethoxydiphenyl borate
CHX	Cycloheximide
DiBAC4(3)	Bis-(1,3-dibutylbarbituric acid) trimethine oxonol
ER	Endoplasmic reticulum
VGKC	Voltage gated potassium channel
VGCC	Voltage gated Calcium channel
IP3	Inositol 1,4,5-trisphosphate
MS	Multiple Sclerosis
SCI	spinal cord injury
DMEM	Dulbecco’s Modified Eagle’s Medium
FBS	Fetal bovine serum
DMSO	Dimethyl sulfoxide
Orai3	Orai Calcium Release-Activated Calcium Modulator 3

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