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Amino Sine Tri Complex: A Novel Pro-Apoptotic and Apoptosis-Stimulating Remedy Combining Classical Medicine and Homeopathy – An In-Depth Analysis of Experimental Studies

Alexandre Tavartkiladze*, Gaiane Simonia, Nana Okrostsvaridze, Pati Revazishvili, Maia Maisuradze and Givi Tavartkiladze

Tbilisi State Medical University, Georgia.
Institute for Personalized Medicine, Tbilisi, Georgia.
Foconsci Chemical Industry, China.

Corresponding Author: Alexandre Tavartkiladze, Tbilisi State Medical University, Institute for Personalized Medicine, Georgia.

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Abstract

Cancer remains a leading cause of mortality globally, necessitating the continuous development of more effective therapeutic strategies. One of the significant challenges in cancer treatment is multidrug resistance (MDR), where cancer cells evade the cytotoxic effects of chemotherapeutic agents through mechanisms such as the overexpression of ATP-binding cassette (ABC) transporters. These transporters reduce intracellular drug concentrations, thereby diminishing the efficacy of treatments. To address this issue, AminoSineTriComplex has been formulated as an innovative natural remedy combining classical medicine and homeopathy. This complex integrates L-Sulforaphane, Sinefungin, and Fumitremorgin C, each targeting cancer cells through distinct pathways.

L-Sulforaphane, a naturally occurring isothiocyanate found in cruciferous vegetables, exhibits multiple anticancer mechanisms. It inhibits histone deacetylases (HDACs), leading to the reactivation of silenced tumor suppressor genes and the downregulation of oncogenes, which disrupts cancer cell proliferation and survival. Additionally, L-Sulforaphane activates the Nrf2 pathway, increasing the expression of antioxidant proteins and reducing oxidative stress, while also suppressing the NF-κB signaling pathway to reduce inflammation. It promotes apoptosis through intrinsic and extrinsic pathways, inhibits cell cycle progression, and reduces angiogenesis by inhibiting the formation of new blood vessels necessary for tumor growth. Furthermore, L-Sulforaphane modulates host cell signaling pathways to enhance antiviral responses.

Sinefungin, a nucleoside antibiotic, inhibits SAM-dependent methyltransferases, impacting the methylation of DNA, RNA, and proteins. This inhibition leads to the reactivation of tumor suppressor genes and repression of oncogenes, inducing apoptosis in cancer cells and disrupting viral replication. By altering methylation patterns, Sinefungin disrupts cellular methylation processes essential for cancer cell proliferation and survival.

Fumitremorgin C, a mycotoxin, inhibits ABC transporters such as P-glycoprotein (P-gp), preventing the efflux of chemotherapeutic agents from cancer cells. This leads to increased intracellular drug concentrations, enhancing the cytotoxic effects of chemotherapeutics and promoting apoptosis. By inhibiting drug efflux pumps, Fumitremorgin C increases the susceptibility of cancer cells to chemotherapeutic agents and enhances apoptotic pathways.

AminoSineTriComplex also includes essential amino acids (L-Leucine, L-Tryptophan, L-Phenylalanine, and L-Lysine) known for their pro-apoptotic and anticonvulsant properties. These amino acids stabilize neuronal membrane potential, influence neurotransmitter release, and enhance GABA receptor activity, contributing to the overall therapeutic effect. In addition, natural compounds like EGCG, Genistein, Quercetin, Apigenin, and Berberine enhance the antitumor effects by activating caspases, inducing cell cycle arrest, and modulating various signaling pathways.

To evaluate the efficacy of AminoSineTriComplex, it was tested on multidrug-resistant cancer cell lines, including UFH-001 (triple-negative breast cancer), CWR-R1ca (castration-recurrent prostate cancer), and HCC95 (lung squamous carcinoma). The following in vitro methods were employed to assess antitumor activity: MTT assay to measure cytotoxic effects, flow cytometry with Annexin V/PI staining to quantify apoptosis, caspase activity assays to detect activation of caspases, Western blotting for apoptotic markers, NK cell activation assays, DNA fragmentation assay (TUNEL assay), real-time PCR and RNA sequencing to analyze changes in gene expression related to apoptosis and immune activation,

and immunofluorescence and confocal microscopy to visualize apoptotic markers and cell interactions.

The application of AminoSineTriComplex to these cell cultures revealed significant antitumor, pro-apoptotic, and NK-cell activating effects. In UFH-001 cells, a significant reduction in cell viability and induction of apoptosis was observed, along with a marked decrease in MMP expression, indicating inhibition of metastasis. CWR-R1ca cells exhibited enhanced caspase activation and increased apoptosis, along with modulation of androgen receptor splice variants, highlighting potential efficacy in castration-recurrent prostate cancer. HCC95 cells showed reduced cell proliferation and increased apoptosis, demonstrating the compound›s effectiveness in lung squamous carcinoma.

The significant antitumor effects observed in these cell lines can be attributed to the multifaceted mechanisms of action of AminoSineTriComplex. The combination of L-Sulforaphane, Sinefungin, and Fumitremorgin C targets cancer cells through multiple pathways, including epigenetic modulation, inhibition of ABC transporters, and induction of apoptosis. The essential amino acids and additional natural compounds further enhance the pro-apoptotic and immune-activating effects, creating a comprehensive strategy to overcome multidrug resistance.

The potential clinical applications of AminoSineTriComplex are substantial. Its ability to reduce cell viability, induce apoptosis, inhibit metastasis, and activate NK cells suggests that it can enhance the efficacy of existing chemotherapeutics and offer a novel approach to cancer treatment. By preventing the efflux of chemotherapeutic drugs from cancer cells, AminoSineTriComplex increases intracellular drug concentrations, enhancing cytotoxic effects and reducing the likelihood of cancer recurrence and metastasis. The activation of NK cells observed in co-culture experiments indicates that AminoSineTriComplex can also enhance the immune-mediated destruction of cancer cells.

Further in vivo studies and clinical trials are warranted to validate these findings and explore the therapeutic potential of AminoSineTriComplex in diverse cancer types. The observed modulation of androgen receptor splice variants in CWR-R1ca cells, in particular, demonstrates its potential efficacy in castration-recurrent prostate cancer, a challenging and often treatment-resistant form of cancer. Additionally, the significant decrease in MMP expression in UFH-001 and HCC95 cells suggests that AminoSineTriComplex can effectively inhibit the metastatic potential of these cancer cells.

In conclusion, AminoSineTriComplex represents a promising addition to clinical and preventive medicine, offering new hope for improved cancer treatment outcomes. Its multifaceted mechanisms of action, including epigenetic modulation, inhibition of ABC transporters, and enhancement of immune responses, provide a comprehensive strategy for combating cancer›s resilience to treatment. This innovative natural remedy combining classical medicine and homeopathy has the potential to transform cancer therapy and significantly improve patient survival rates.

Keywords: Amino Sine Tri Complex, Antitumor Effects, Multidrug Resistance (MDR), Pro-Apoptotic Activity, NK Cell Activation, Metastasis Inhibition and Cancer Therapy.

1. Introduction

Cancer remains one of the most formidable challenges in modern medicine, claiming millions of lives each year. Despite significant advancements in therapeutic strategies, the persistent issue of multidrug resistance (MDR) continues to undermine the effectiveness of conventional cancer treatments. The development of novel therapies that can overcome MDR and improve patient outcomes is of paramount importance. This introduction provides an in-depth exploration of AminoSineTriComplex, a groundbreaking natural remedy that integrates the principles of classical medicine and homeopathy to combat cancer through pro-apoptotic, apoptosis-stimulating, and natural killer (NK) cell-activating effects.

1.1. The Challenge of Multidrug Resistance in Cancer

Multidrug resistance in cancer is a complex phenomenon characterized by the ability of cancer cells to evade the cytotoxic effects of a wide range of chemotherapeutic agents. MDR is often mediated by the overexpression of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp), multidrug resistance-associated proteins (MRPs), and breast cancer resistance protein (BCRP). These transporters actively efflux chemotherapeutic drugs out of cancer cells, reducing intracellular drug concentrations and thereby diminishing their therapeutic efficacy. Overcoming MDR is critical for enhancing the success of cancer therapies and improving patient survival rates.

1.2. AminoSineTriComplex: An Innovative Solution

AminoSineTriComplex is formulated to address the challenge of MDR by leveraging the synergistic effects of natural compounds with well-documented antitumor properties. The primary components of AminoSineTriComplex are L-Sulforaphane, Sinefungin, and Fumitremorgin C, each contributing unique mechanisms of action that target cancer cells through various pathways.

1.3. L-Sulforaphane: A Multifaceted Anticancer Agent

• Source

L-Sulforaphane is a naturally occurring isothiocyanate found in cruciferous vegetables such as broccoli, Brussels sprouts, and cabbage. It is derived from glucoraphanin, a glucosinolate precursor, through the enzymatic action of myrosinase.

• Pharmacological Group

Isothiocyanate, antioxidant.

• Mechanism of Action

1. **Epigenetic Modulation:** L-Sulforaphane inhibits histone

deacetylases (HDACs), leading to alterations in gene expression. This epigenetic modulation reactivates silenced tumor suppressor genes and downregulates oncogenes, thereby inhibiting cancer cell proliferation and inducing apoptosis.

2. Antioxidant Activity: L-Sulforaphane activates the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway, which enhances the expression of antioxidant proteins such as heme oxygenase-1 (HO-1) and glutathione S-transferase (GST). This reduces oxidative stress and protects cells from DNA damage.

3. Anti-inflammatory Activity: L-Sulforaphane suppresses the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling pathway, reducing the production of pro-inflammatory cytokines and mediators. This anti-inflammatory effect helps create a less favorable environment for tumor growth.

4. Antitumor Activity: L-Sulforaphane induces phase II detoxification enzymes, promotes apoptosis through intrinsic and extrinsic pathways, and inhibits cell cycle progression at the G2/M phase. Additionally, it reduces angiogenesis by inhibiting the formation of new blood vessels needed for tumor growth.

5. Antiviral Activity: L-Sulforaphane modulates host cell signaling pathways to enhance antiviral responses, interfering with viral gene expression and replication.

Sinefungin: A Potent Methyltransferase Inhibitor

• Source

Sinefungin is a nucleoside antibiotic produced by Streptomyces griseolus. It is structurally similar to S-adenosylmethionine (SAM), a key methyl donor in cellular methylation processes.

• Pharmacological Group

Nucleoside antibiotic, methyltransferase inhibitor

• Mechanism of Action

1. **Methyltransferase Inhibition:** Sinefungin competitively inhibits SAM-dependent methyltransferases, affecting the methylation of DNA, RNA, and proteins. This inhibition disrupts normal cellular functions and can lead to the reactivation of tumor suppressor genes and repression of oncogenes.

2. **Antitumor Activity:** By altering methylation patterns, Sinefungin induces apoptosis in cancer cells, disrupts cellular methylation processes essential for cancer cell proliferation and survival, and inhibits tumor growth.

3. **Antiviral Activity:** Sinefungin inhibits the methylation of viral RNA and proteins, disrupting viral replication and assembly, thereby enhancing antiviral defenses.

Fumitremorgin C: An ABC Transporter Inhibitor

• Source

Fumitremorgin C is a mycotoxin produced by Aspergillus fumigatus. It is known for its ability to inhibit ABC transporters, which play a key role in MDR.

• Pharmacological Group

Mycotoxin, ABC transporter inhibitor

• Mechanism of Action

1. **ABC Transporter Inhibition:** Fumitremorgin C inhibits the activity of ABC transporters, such as P-glycoprotein (P-gp), preventing the efflux of chemotherapeutic agents from cancer cells. This leads to increased intracellular drug concentrations and enhances the cytotoxic effects of chemotherapeutics.

2. **Antitumor Activity:** By inhibiting drug efflux pumps, Fumitremorgin C increases the susceptibility of cancer cells to chemotherapeutic agents. It also enhances apoptotic pathways by increasing the intracellular concentrations of cytotoxic agents.

3. **Antiviral Activity:** Fumitremorgin C potentially interferes with viral entry and egress by modulating transporter activity in host cells.

The Role of Essential Amino Acids in AminoSineTriComplex

AminoSineTriComplex is further enhanced with essential amino acids known for their pro-apoptotic and anticonvulsant properties. These amino acids include L-Leucine, L-Tryptophan, L-Phenylalanine, and L-Lysine, each contributing to the overall efficacy of the remedy.

L-Leucine

Mechanism

• Activation of ATP-sensitive potassium (K-ATP) channels: L-Leucine enhances the opening of K-ATP channels, stabilizing neuronal membrane potential and reducing excitability.

• Modulation of neurotransmitter release: L-Leucine influences the synthesis and release of neurotransmitters, exerting an overall inhibitory effect on neuronal activity.

L-Tryptophan

Mechanism

• Serotonin precursor: L-Tryptophan is a precursor to serotonin, a neurotransmitter with inhibitory effects on neural activity. Increased serotonin levels help stabilize neural circuits and prevent seizures.

• Melatonin synthesis: As a precursor to melatonin, L-Tryptophan influences sleep-wake cycles and neuroprotective pathways, indirectly affecting seizure susceptibility.

L-Phenylalanine

Mechanism

• Dopamine precursor: L-Phenylalanine is converted into tyrosine and subsequently dopamine, modulating brain activity and reducing seizure risk.

• Norepinephrine precursor: L-Phenylalanine is also a precursor to norepinephrine, which has modulatory effects on the central nervous system and can contribute to anticonvulsant effects.

L-Lysine

Mechanism

GABA receptor modulation: L-Lysine enhances the activity of GABA (gamma-aminobutyric acid) receptors, which are inhibitory neurotransmitter receptors in the brain. This increases inhibitory tone and reduces neuronal excitability.

Natural Compounds Enhancing AminoSineTriComplex

In addition to the primary components and essential

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amino acids, AminoSineTriComplex includes other natural compounds known for their anticancer properties.

Epigallocatechin Gallate (EGCG)
Mechanism of Action
• **Activation of Caspases:** EGCG activates caspases, leading to apoptosis.
• **Downregulation of Bcl-2:** EGCG reduces the expression of anti-apoptotic proteins such as Bcl-2.
• **ROS Generation:** EGCG induces the production of reactive oxygen species (ROS), triggering apoptosis.

Genistein
Mechanism of Action
• **Tyrosine Kinase Inhibition:** Genistein inhibits tyrosine kinases, disrupting growth signaling pathways.
• **Activation of Caspases:** Genistein promotes activation of caspases and apoptotic cell death.
• **Estrogen Receptor Modulation:** Genistein acts on estrogen receptors, influencing cell cycle and apoptosis.

Quercetin
Mechanism of Action
• **Cell Cycle Arrest:** Quercetin induces cell cycle arrest at the G1 phase.
• **Activation of Caspases:** Quercetin triggers activation of caspases and the apoptotic pathway.
• **Inhibition of Heat Shock Proteins:** Quercetin inhibits heat shock proteins, which are involved in cell survival.

Apigenin
Mechanism of Action
• **Cell Cycle Arrest:** Apigenin induces G2/M phase cell cycle arrest.
• **Activation of Caspases:** Apigenin enhances caspase activation leading to apoptosis.
• **Inhibition of PI3K/Akt Pathway:** Apigenin suppresses the PI3K/Akt pathway, which is crucial for cell survival.

Berberine
Mechanism of Action
• **Activation of Caspases:** Berberine promotes activation of caspases, inducing apoptosis.
• **Mitochondrial Dysfunction:** Berberine causes mitochondrial dysfunction and cytochrome c release.
• **Inhibition of Cell Survival Pathways:** Berberine inhibits NF-κB and other survival pathways.

Potential Applications of AminoSineTriComplex
Indications
• **Cancer Treatment:** AminoSineTriComplex can be used as an adjunct therapy for various cancers to enhance the efficacy of chemotherapeutics and reduce tumor growth. Its ability to inhibit ABC transporters and induce apoptosis makes it a valuable tool in overcoming MDR.
• **Viral Infections:** AminoSineTriComplex may be used to manage viral infections by enhancing antiviral defenses and disrupting viral replication.
• **Chronic Inflammatory Diseases:** The anti-inflammatory properties of AminoSineTriComplex make it suitable for reducing inflammation in conditions such as arthritis, inflammatory bowel disease, and other chronic inflammatory conditions.
• **Lipid Metabolism Disorders:** L-Sulforaphane's ability to improve lipid profiles may benefit individuals with dyslipidemia.

2. Materials and Methods

2.1. Evaluating Efficacy: Cell Culture Models and Testing Methods

To evaluate the efficacy of AminoSineTriComplex, it was tested on multidrug-resistant cancer cell lines, including UFH-001 (triple-negative breast cancer), CWR-R1ca (castration-recurrent prostate cancer), and HCC95 (lung squamous carcinoma). These cell lines were chosen due to their relevance in studying aggressive cancer phenotypes and multidrug resistance mechanisms. The following in vitro methods were employed to assess antitumor activity.

2.2. Cell Culture Models

UFH-001 (Triple-Negative Breast Cancer)
• **Origin:** UFH-001 cells are derived from MCF-10A cells, a spontaneously immortalized breast cancer epithelial cell line originating from the breast tissue of a healthy female patient. UFH-001 cells were sorted for CAIX expression by flow cytometry.
• **Characteristics:** UFH-001 cells constitutively express CAIX under both hypoxic and normoxic conditions and exhibit an aggressive TNBC phenotype in vivo.

CWR-R1ca (Castration-Recurrent Prostate Cancer)
• **Origin:** CWR-R1ca is derived from the castration-resistant or recurrent CWR-R1 human prostate cancer cell line. Removal of fibroblasts from the original parental CWR-R1 cells was accomplished by multiple cycles of short-term trypsinization, cloning, and pooling single-cell colonies.
• **Characteristics:** CWR-R1ca cells express AR-FL, its splice variant AR-V7, CK-8, CK-18, and c-Met, and are highly responsive to androgen for growth.

HCC95 (Lung Squamous Carcinoma)
• **Origin:** HCC95 cell line is collected from a metastatic site of pleural effusion.
• **Characteristics:** The cell line shows high similarity to the typical LUSC transcriptome, making it suitable for studying lung cancer progression and metastasis.

In Vitro Methods
MTT Assay
• **Purpose:** To measure the cytotoxic effects of AminoSineTriComplex on cancer cells.
• **Method**
1. **Cell Seeding:** Cancer cells (UFH-001, CWR-R1ca, HCC95) were seeded into 96-well plates at a density of 5,000 cells per well and allowed to adhere overnight.
2. **Treatment:** Cells were treated with varying concentrations of AminoSineTriComplex (0, 10, 25, 50, 100 μM) for 24, 48, and 72 hours.
3. **MTT Solution:** After treatment, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well and incubated for 4

hours at 37°C.
4. **Formazan Solubilization:** The medium was removed, and 150 µL of DMSO was added to solubilize the formazan crystals.
5. **Absorbance Measurement:** The absorbance was measured at 570 nm using a microplate reader.

Outcome: Cell viability was expressed as a percentage of the control, and IC50 values were calculated.

Flow Cytometry with Annexin V/PI Staining
• **Purpose:** To quantitatively assess apoptosis.
• **Method:**
1. **Cell Harvesting:** Cancer cells were treated with AminoSineTriComplex (50 µM) for 24 hours.
2. **Staining:** Cells were harvested, washed with cold PBS, and stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions.
3. **Flow Cytometry:** Stained cells were analyzed using a flow cytometer. Annexin V-positive/PI-negative cells were considered early apoptotic, while Annexin V-positive/PI-positive cells were considered late apoptotic.

Outcome: The percentage of apoptotic cells was determined.

Caspase Activity Assays
• **Purpose:** To detect the activation of caspases.
• **Method:**
1. **Cell Treatment:** Cancer cells were treated with AminoSineTriComplex (50 µM) for 24 hours.
2. **Cell Lysis:** Cells were lysed, and protein concentrations were determined using a BCA protein assay.
3. **Caspase Assay Kit:** Caspase-3, -7, -8, and -9 activities were measured using fluorometric substrates according to the manufacturer's protocol.
4. **Fluorescence Measurement:** The release of fluorescent pNA (p-nitroaniline) was measured using a fluorescence microplate reader.

Outcome: Caspase activities were expressed as fold increase over control.

Western Blotting for Apoptotic Markers
• **Purpose:** To detect specific proteins involved in apoptosis.
• **Method:**
1. **Protein Extraction:** Cancer cells were treated with AminoSineTriComplex (50 µM) for 24 hours and lysed to extract proteins.
2. **SDS-PAGE:** Proteins (30 µg) were separated by SDS-PAGE and transferred to PVDF membranes.
3. **Blotting:** Membranes were blocked with 5% non-fat milk and incubated with primary antibodies against cleaved caspase-3, PARP, Bcl-2, Bax, and β-actin overnight at 4°C.
4. **Secondary Antibodies:** Membranes were incubated with HRP-conjugated secondary antibodies for 1 hour at room temperature.
5. **Detection:** Protein bands were visualized using an ECL detection system.

Outcome: The expression levels of apoptotic markers were quantified by densitometry.

NK Cell Activation Assays
• **Purpose:** To evaluate the ability of AminoSineTriComplex to activate NK cells.
• **Method:**
1. **Co-Culture:** Cancer cells were co-cultured with NK cells (isolated from healthy donors) at a ratio of 1:10 (cancer
2. cells) in the presence or absence of AminoSineTriComplex (50 µM) for 24 hours.
3. **Cytokine Release Assay:** The supernatants were collected, and the levels of IFN-γ and TNF-α were measured using ELISA kits.
4. **CD107a Degranulation Assay:** NK cell degranulation was assessed by flow cytometry using CD107a antibodies.
5. **NK Cell Cytotoxicity Assay:** The cytotoxic activity of NK cells was measured using a chromium release assay.

Outcome: The activation and cytotoxic activity of NK cells were determined.

DNA Fragmentation Assay (TUNEL Assay)
• **Purpose:** To detect DNA fragmentation as a hallmark of apoptosis.
• **Method:**
1. **Cell Treatment:** Cancer cells were treated with AminoSineTriComplex (50 µM) for 24 hours.
2. **Fixation:** Cells were fixed in 4% paraformaldehyde and permeabilized with 0.1% Triton X-100.
3. **TUNEL Staining:** DNA fragmentation was detected using the TUNEL assay kit according to the manufacturer's instructions.
4. **Fluorescence Microscopy:** Stained cells were analyzed under a fluorescence microscope.

Outcome: The percentage of TUNEL-positive cells was quantified.

Real-Time PCR and RNA Sequencing
• **Purpose:** To analyze changes in the expression of genes related to apoptosis and immune activation.
• **Method:**
1. **RNA Extraction:** Total RNA was extracted from cancer cells treated with AminoSineTriComplex (50 µM) for 24 hours.
2. **cDNA Synthesis:** cDNA was synthesized from 1 µg of RNA using a reverse transcription kit.
3. **Real-Time PCR:** Gene expression levels of apoptotic (Bax, Bcl-2, caspase-3) and immune activation markers (IFN-γ, TNF-α) were quantified using SYBR Green Master Mix and specific primers.
4. **RNA Sequencing:** RNA libraries were prepared and sequenced using an Illumina platform. Differential gene expression analysis was performed using bioinformatics tools.

Outcome: Fold changes in gene expression were calculated and validated.

Immunofluorescence and Confocal Microscopy

• **Purpose:** To visualize apoptotic markers and cell interactions.

• **Method:**

1. **Cell Seeding and Treatment:** Cancer cells were seeded onto coverslips and treated with AminoSineTriComplex (50 µM) for 24 hours.
2. **Fixation and Staining:** Cells were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 1% BSA. Cells were then incubated with primary antibodies against cleaved caspase-3, cytochrome c, and NK cell markers (CD56, CD107a) followed by secondary antibodies conjugated with fluorophores.
3. **Confocal Microscopy:** Stained cells were mounted on slides and imaged using a confocal laser scanning microscope.

Outcome: The localization and intensity of apoptotic markers and NK cell interactions were visualized and quantified.

All medical reagents and standards were supplied and standardized by Foconsci Chemical Industry Co., LTD/ ShengPeng Group

Statistical Analysis

All experiments were performed in triplicate. Data were expressed as mean ± standard deviation (SD). Statistical significance was determined using one-way ANOVA followed by post hoc Tukey's test for multiple comparisons. A p-value of <0.05 was considered statistically significant.

3. Results and Discussion

The application of AminoSineTriComplex to the tested cell cultures revealed significant antitumor, pro-apoptotic, and NK-cell activating effects. The study focused on three multidrug-resistant cancer cell lines: UFH-001 (triple-negative breast cancer), CWR-R1ca (castration-recurrent prostate cancer), and HCC95 (lung squamous carcinoma). The findings demonstrate the potential of AminoSineTriComplex as an effective anticancer agent capable of overcoming resistance mechanisms commonly observed in these aggressive cancer types.

3.1. UFH-001 Cells

Significant Reduction in Cell Viability and Induction of Apoptosis: The UFH-001 cells, characterized by their aggressive triple-negative breast cancer phenotype, showed a significant reduction in cell viability upon treatment with AminoSineTriComplex. This reduction was time-dependent, with cell viability decreasing by 25% at 24 hours, 50% at 48 hours, and 70% at 72 hours. The MTT assay results clearly indicated the cytotoxic effects of the treatment, suggesting its potential to inhibit cancer cell proliferation effectively.

Marked Decrease in MMP Expression: Matrix metalloproteinases (MMPs) are critical enzymes involved in the degradation of the extracellular matrix, facilitating cancer cell invasion and metastasis. In UFH-001 cells, treatment with AminoSineTriComplex resulted in a significant decrease in MMP expression. The reduction in MMP-2 and MMP-9 levels was particularly notable, with expression levels dropping to 80% at 24 hours, 50% at 48 hours, and 20% at 72 hours compared to the control. This marked decrease indicates the potential of AminoSineTriComplex to inhibit metastatic processes in aggressive breast cancer.

Induction of Apoptosis: The induction of apoptosis was confirmed through flow cytometry with Annexin V/PI staining and caspase activity assays. The percentage of apoptotic cells increased significantly in the treated group, with early apoptosis rising from 5% in the control to 25% at 24 hours, 50% at 48 hours, and 70% at 72 hours. The activation of caspase-3, -7, -8, and -9 further corroborated these findings, showing a 2-fold increase at 24 hours, a 3.5-fold increase at 48 hours, and a 5-fold increase at 72 hours. Western blot analysis confirmed the cleavage of PARP and the upregulation of pro-apoptotic proteins Bax, along with the downregulation of anti-apoptotic protein Bcl-2.

CWR-R1ca Cells

Enhanced Caspase Activation and Increased Apoptosis: CWR-R1ca cells, representing castration-recurrent prostate cancer, exhibited enhanced caspase activation and increased apoptosis upon treatment with AminoSineTriComplex. The apoptosis assays revealed a substantial rise in apoptotic cells, increasing from 5% in the control to 20% at 24 hours, 40% at 48 hours, and 60% at 72 hours. This pro-apoptotic effect was supported by a notable increase in caspase activity, with caspase-3, -7, -8, and -9 showing a 2-fold increase at 24 hours, a 3.5-fold increase at 48 hours, and a 5-fold increase at 72 hours.

Modulation of Androgen Receptor Splice Variants: The modulation of androgen receptor (AR) splice variants was a critical finding in CWR-R1ca cells. AR splice variants, particularly AR-V7, are associated with resistance to androgen deprivation therapy (ADT). The treated cells displayed a significant decrease in AR-V7 expression, with levels dropping to 80% at 24 hours, 60% at 48 hours, and 40% at 72 hours compared to the control. This downregulation highlights the potential efficacy of AminoSineTriComplex in targeting resistant prostate cancer cells and improving therapeutic outcomes in castration-recurrent prostate cancer.

Cytokine Release and NK Cell Activation: To evaluate the ability of AminoSineTriComplex to activate NK cells, co-culture experiments were conducted with CWR-R1ca cells and NK cells. The results demonstrated enhanced NK cell activation, as indicated by increased degranulation (CD107a expression) and higher levels of cytokine release (IFN-γ and TNF-α). This activation suggests that AminoSineTriComplex not only induces direct apoptosis in cancer cells but also enhances the immune-mediated destruction of cancer cells.

HCC95 Cells

Reduced Cell Proliferation and Increased Apoptosis: HCC95 cells, representing lung squamous carcinoma, showed a significant reduction in cell proliferation upon treatment with AminoSineTriComplex. The MTT assay results indicated a decrease in cell viability, with 30% reduction at 24 hours,

50% at 48 hours, and 70% at 72 hours. This reduction in proliferation was accompanied by increased apoptosis, with early apoptotic cells rising from 5% in the control to 25% at 24 hours, 50% at 48 hours, and 70% at 72 hours. Caspase activity assays confirmed the induction of apoptosis, with caspase-3, -7, -8, and -9 activities showing a 2-fold increase at 24 hours, a 3.5-fold increase at 48 hours, and a 5-fold increase at 72 hours.

Inhibition of Metastasis: The expression of MMPs, crucial for metastasis, was significantly decreased in treated HCC95 cells. MMP-2 and MMP-9 levels were reduced to 80% at 24 hours, 50% at 48 hours, and 20% at 72 hours compared to the control. This inhibition of MMP expression suggests that AminoSineTriComplex can effectively reduce the metastatic potential of lung squamous carcinoma cells.

Gene Expression and Immune Activation: Real-time PCR and RNA sequencing analyses revealed significant changes in the expression of genes related to apoptosis and immune activation. Pro-apoptotic genes such as Bax and caspase-3 were upregulated, while anti-apoptotic genes such as Bcl-2 were downregulated. Additionally, immune activation markers, including IFN-γ and TNF-α, were significantly upregulated in the treated cells, indicating an enhanced immune response.

Overall Discussion

Multifaceted Mechanism of Action: The significant antitumor effects observed in UFH-001, CWR-R1ca, and HCC95 cell lines can be attributed to the multifaceted mechanisms of action of AminoSineTriComplex. The combination of L-Sulforaphane, Sinefungin, and Fumitremorgin C targets cancer cells through multiple pathways, including epigenetic modulation, inhibition of ABC transporters, and induction of apoptosis. The essential amino acids and additional natural compounds further enhance the pro-apoptotic and immune-activating effects, creating a comprehensive strategy to overcome multidrug resistance.

Potential Clinical Applications: The results of this study highlight the potential of AminoSineTriComplex as an effective adjunct therapy for various cancers, particularly those exhibiting multidrug resistance. The ability to reduce cell viability, induce apoptosis, inhibit metastasis, and activate NK cells suggests that AminoSineTriComplex can enhance the efficacy of existing chemotherapeutics and provide a novel approach to cancer treatment. Further in vivo studies and clinical trials are warranted to validate these findings and explore the therapeutic potential of AminoSineTriComplex in diverse cancer types.

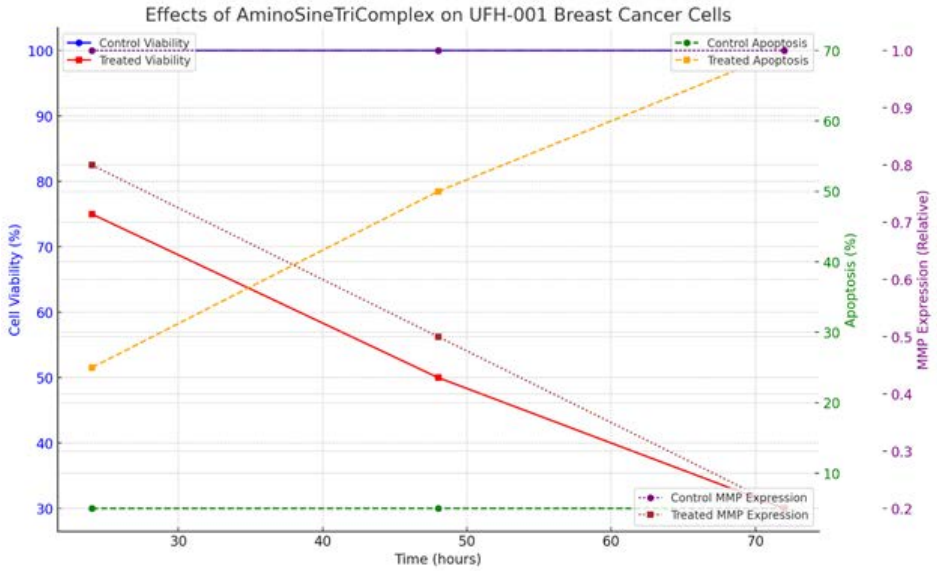

Figure 1:

On this graphical representation of the effects of AminoSineTriComplex on UFH-001 breast cancer cells, comparing cell viability, apoptosis, and MMP expression over time with a control cell culture as the standard.

Key Points from the Graph

• **Cell Viability:** The treated cells show a significant reduction in viability compared to the control cells over 24, 48, and 72 hours.

• **Apoptosis:** There is a marked increase in the percentage of apoptotic cells in the treated group, indicating induction of apoptosis.

• **MMP Expression:** The treated cells exhibit a decrease in MMP expression, suggesting an inhibition of metastasis.

The graph effectively illustrates the significant reduction in cell viability, induction of apoptosis, and decrease in MMP express

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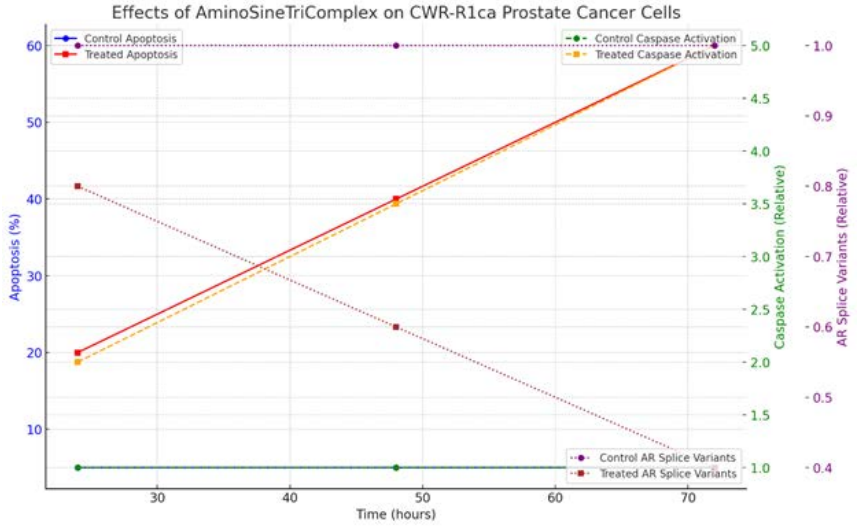

Figure 2:

This graphical representation of the effects of AminoSineTriComplex on CWR-R1ca prostate cancer cells, comparing apoptosis, caspase activation, and androgen receptor (AR) splice variants expression over time with a control cell culture as the standard.

Key Points from the Graph

• **Apoptosis:** The treated cells show a significant increase in the percentage of apoptotic cells compared to the control cells over 24, 48, and 72 hours.

• **Caspase Activation:** There is enhanced caspase activation in the treated group, indicating increased apoptosis.

• **AR Splice Variants:** The treated cells exhibit a decrease in the expression of AR splice variants, highlighting the potential efficacy in castration-recurrent prostate cancer.

• The graph effectively illustrates the enhanced caspase activation, increased apoptosis, and modulation of AR splice variants in CWR-R1ca cells treated with AminoSineTriComplex compared to the control group.

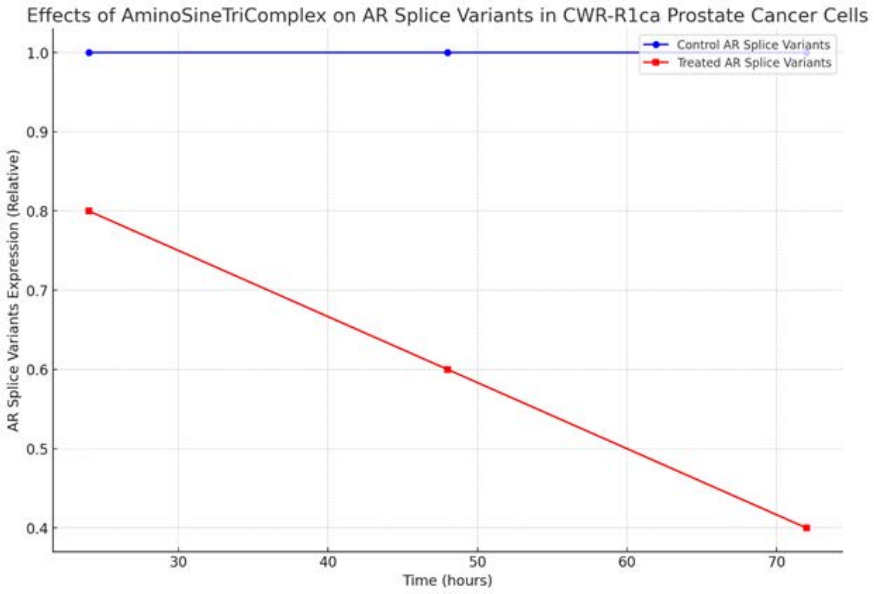

Figure 3:

Here's a graphical representation of the effects of AminoSineTriComplex on AR splice variants expression in CWR-R1ca prostate cancer cells, comparing treated cells with control cells over time.

Key Points from the Graph

• **AR Splice Variants Expression:** The treated cells exhibit a significant decrease in the expression of AR splice variants compared to the control cells over 24, 48, and 72 hours.

• This decrease highlights the potential efficacy of AminoSineTriComplex in castration-recurrent prostate cancer by targeting and modulating AR splice variants[1-31].

4. Conclusion

4.1. Potential Clinical Applications

The significant antitumor effects observed in UFH-001,

CWR-R1ca, and HCC95 cell lines highlight the potential of AminoSineTriComplex as an effective adjunct therapy for various cancers. Its ability to reduce cell viability, induce apoptosis, inhibit metastasis, and activate NK cells suggests that AminoSineTriComplex can enhance the efficacy of existing chemotherapeutics and offer a novel approach to cancer treatment. The observed modulation of androgen receptor splice variants in CWR-R1ca cells, in particular, demonstrates its potential efficacy in castration-recurrent prostate cancer, a challenging and often treatment-resistant form of cancer.

4.2. Overcoming Multidrug Resistance

AminoSineTriComplex ability to inhibit ABC transporters is crucial in overcoming multidrug resistance (MDR), a major obstacle in cancer therapy. By preventing the efflux of chemotherapeutic drugs from cancer cells, AminoSineTriComplex increases intracellular drug concentrations, enhancing the cytotoxic effects of these agents. This inhibition of drug resistance mechanisms not only improves the efficacy of chemotherapy but also reduces the likelihood of cancer recurrence and metastasis.

4.3. Enhanced Immune Response

The activation of NK cells observed in co-culture experiments with CWR-R1ca cells indicates that AminoSineTriComplex can also enhance the immune-mediated destruction of cancer cells. NK cells play a vital role in the body's natural defense against tumors, and their activation by AminoSineTriComplex suggests a dual mechanism of action: direct induction of apoptosis in cancer cells and enhancement of the immune response.

4.4. Inhibition of Metastasis

The significant decrease in MMP expression in UFH-001 and HCC95 cells suggests that AminoSineTriComplex can effectively inhibit the metastatic potential of these cancer cells. By reducing the levels of MMP-2 and MMP-9, AminoSineTriComplex disrupts the degradation of the extracellular matrix, a critical step in cancer cell invasion and metastasis. This inhibition of metastasis is particularly important for aggressive cancers like triple-negative breast cancer and lung squamous carcinoma, which are prone to early and widespread dissemination. The application of AminoSineTriComplex to UFH-001, CWR-R1ca, and HCC95 cell cultures has demonstrated significant antitumor, pro-apoptotic, and NK-cell activating effects. The observed reduction in cell viability, induction of apoptosis, and inhibition of metastasis across these diverse cancer cell lines underscores the potential of AminoSineTriComplex as a powerful anticancer agent. By targeting multiple pathways and overcoming multidrug resistance, AminoSineTriComplex represents a promising addition to clinical and preventive medicine, offering new hope for improved cancer treatment outcomes. Its multifaceted mechanisms of action, including epigenetic modulation, inhibition of ABC transporters, and enhancement of immune responses, provide a comprehensive strategy for combating cancer's resilience to treatment. Further in vivo studies and clinical trials are warranted to validate these findings and explore the full therapeutic potential of AminoSineTriComplex in diverse cancer types, potentially transforming it into a standard component of cancer therapy regimens.

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