Original Article Open Access

Archives Of Breast Cancer

DOI: 10.32768/abc.2023102148-158

Synergistic Effects of the Association of BR2 Peptide with 2-Aminoethyl Dihydrogen Phosphate on Triple-Negative Breast Cancer Cells

Laertty Garcia de Sousa Cabral[a,b], Cyntia Silva de Oliveira[c], Rosely Cabette Barbosa Alves[b], Vani Xavier Oliveira Jr.[c,d], Jean-Luc Poyet[e,f], Durvanei Augusto Maria*[a,b]

[a]*Faculty of Medicine, University of Sao Paulo, FMUSP; Sao Paulo, Brazil*

[b]*Laboratory of Development and Innovation, Butantan Institute, Sao Paulo, Brazil*

[c]*Federal University of Sao Paulo, UNIFESP, Sao Paulo, Brazil*

[d]*Federal University of ABC, Center for Natural and HumanSciences, Santo Andre, Brazil*

[e]*Université Paris Diderot et Institut De Recherche Saint-Louis, Hôpital Saint-Louis, Paris, France*

ARTICLE INFO

Received:
2 December 2022
Revised:
20 March 2023
Accepted:
23 March 2023

Keywords:
triple-negative breast cancer, peptide, monophosphoester, synergism

ABSTRACT

Background: Breast cancer is one of the most common diseases among women worldwide. The triple negative subtype is the most aggressive, with low tumor-free survival and the worst clinical evolution, requiring the development of more effective and targeted therapies. The present study investigated the in vitro pharmacological effects of the association of BR2 peptide with 2-aminoethyl dihydrogen phosphate (2-AEH_2P) on MDA-MB-231 and 4T1 triple-negative breast cancer cells.

Methods: The physical-chemical analysis of the peptide was performed using the Heliquest software, the cell viability was assessed using the MTT colorimeter method and the predictive pharmacological effect was evaluated using the Synergy Finder software.

Results: The results showed the BR2 tumor penetration peptide and the 2-AEH_2P+BR2 association significantly increased cytotoxicity in the MDA MB-231 and 4T1 tumor lines, without compromising the viability of the normal fibroblastic cells. The results also showed that depending on the time and concentration, a synergistic effect was observed for the association with tumor cells, with a therapeutic window between 0.8 and 50µm for MDA-MB-231 tumor cells in 48h.

Conclusion: The results demonstrated in vivo antitumor and antiproliferative efficiency for MDA-MB-231 and 4T1 tumor cells with low toxicity for normal fibroblast cells, with MDA MB-231 cells being more sensitive to treatments.

INTRODUCTION

Cancer is one of the most prevalent health problems in the world, and its incidence has been increasing due in part to the aging population and changes in the distribution and prevalence of risk factors, especially those associated with socioeconomic development.[1-3] Among the types of cancer, breast cancer is the most common among women worldwide, with an estimated 2 million cases

***Address for correspondence:**
Durvanei Augusto Maria, M.D.,
Laboratory of Development and Innovation, Butantan Institute, Av Vital Brasil, Sao Paulo-SP, Brazil.
Tel: +551126279300
Email: durvanei@usp.br

diagnosed, corresponding to 25.2% of all female cancers.[1]

The triple-negative subtype, so named because it does not express estrogen receptors (ER), progesterone receptors (PR) and HER-2, also known as basal-type breast cancer with increased incidence in patients with a BRCA1 and 2 mutations germinal, has a worse prognosis, with a low tumor-free survival rate and a high rate of recurrence followed by metastasis within 5 years, often treated with mastectomy, followed by systemic chemotherapy.[4,5]

Efficient intracellular delivery is essential to overcome the current drawbacks of cancer therapeutics. Tumor-penetrating peptides offer the possibility of efficient intracellular transport and therefore the development of drug delivery systems using tumor-penetrating peptides as molecular carriers is an interesting strategy to minimize the current disadvantages. The possibility of incorporating peptides that direct drugs to the tumor to increase the specificity of these therapeutic agents, is an efficient method to improve the intracellular mechanisms of drug release.[6]

Peptide delivery is targeted and has been shown to increase specifically the accumulation of drugs and antibodies in the tumor microenvironment. Studies in mice have showed improved antitumor efficacy, with less damage to normal tissues, both isolated and associated with traditional chemotherapy, antibodies and nanoparticulate drugs and in a xenograft model.[7,8]

The use of cell penetration peptides as drug drivers in drug delivery systems has great clinical potential for the treatment of numerous pathologies, and in cancer, the reduction of systemic toxicity generated by traditional chemotherapy would be reduced, improving the outcome of the treatment and the prognosis.

The 2-aminoethyl dihydrogen phosphate (2-AEH_2P) monophosphoester is a molecule involved in phospholipid turnover, acting as a precursor in the synthesis of membrane phospholipids. It shows anti-proliferative and pro-apoptotic effects on melanoma cells (B16-F10 and SK-MEL)[9,10], human breast adenocarcinoma tumor cells MCF-7[11], human and murine breast adenocarcinoma triple-negative (MDA-MB-231 and 4T1)[12,13] and chronic myelogenous leukemia (K562 and K562 MDR+).[14,15]

Given the great need to develop new therapies for the treatment of triple-negative breast cancer, and since the prognosis is currently poor and the treatment consists of total breast removal, emptying the axillary lymph nodes, followed by systemic chemotherapy, and with high rates of recurrence in 5 years, and metastases mainly in the skin and bones, the present work seeks to evaluate the pharmacological effects of the association of the tumor peptide BR2 with 2-aminoethyl dihydrogen phosphate in a model of triple-negative breast cancer.

METHODS

Sequence analysis and database screening

A helical sequence submitted by the user was analyzed by a sliding window (14–54 aa, i.e., up to three repeats of a complete helical wheel of 18 aa). The analysis module presents a table for each segment reporting its net charge z (at pH = 7.4), mean hydrophobicity <H>, and hydrophobic moment <μH>, calculated on a standard hydrophobicity scale, and statistics on its composition (percentage or enumeration of specific residues). A helical wheel representation of each segment with its <μH> vector is downloadable.

Cell culture

The cell line cultures were triple-negative MDA-MB-231 human breast cancer (ATCC® CRM-HTB-26), murine breast cancer 4T1 (ATCC® CRL-2539), and normal human fibroblast FN1. The cell lines were grown in RPMI-1640 medium (LGC Biotecnologia, Cotia, SP, Brazil), except the MDA-MB-231 line that was grown in Leibovitz medium (LGC Biotecnologia, Cotia, SP, Brazil). The medium was supplemented with 2 mM L-glutamine (Cultilab, Campinas, SP, Brazil), 10 mM HEPES (Cultilab, Campinas, SP, Brazil), 24 mM sodium bicarbonate, 0.01% antibiotics, and 10% fetal bovine serum (Cultilab, Campinas, SP, Brazil). The cells were cultivated in a 5% CO2 atmosphere at 37 °C. A viability assessment was conducted using the Trypan Blue exclusion test when cell viability was over 94%.

Cytotoxicity assay by the colorimetric method (MTT)

Tumor and normal cells were incubated in 96-well plates at 1×10^5 cells/mL for 24h and 48h and treated with the BR2 peptide, 2-AEH_2P, and the combination of 2-AEH_2P+BR2 at different concentrations. The treatment was based on the fixed concentration of 2-AEH_2P and varied concentrations of the BR2 peptide. The IC50 values for each cell were fixed to half of the IC50 value of 2-AEH_2P per well and the BR2 peptide at different concentrations. After 24h and 48h of treatment, the supernatant was aspirated and 100μL of 5mg/mL MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2h-tetrazolium·bromide) was added, and finally the cells were incubated for 3h in an atmosphere containing 5% CO_2 at 37°C. Then, the content was removed and 100μL of methanol was added to dissolve the formazan crystals. The absorbance at 540nm was then assessed using a microplate reader.

Evaluation of the therapeutic index of in vitro treatments

The therapeutic index is a measure similar to the therapeutic window and is used for measuring the relative safety of a drug, and comparing the dose required for therapeutic effects and the dose causing toxicity, and is calculated by dividing the toxic dose for 50% of the population by the minimum effective dose for 50% of the population, as observed in the formula below:

TD_{50} = toxic dose for 50% of the population
ED_{50} = effective dose for50% of the population

$$\text{Therapeutic index} = \frac{TD_{50}}{ED_{50}}$$

SynergyFinder 2.0 analysis of multiple drug combinations

To check the potential synergy of the drug, a matrix study was conducted with the BR2 peptide and the 2-AEH_2P. The combination matrix was tested on two cell lines: MDA-MB-231 and 4T1. SynergyFinder 2.0 software determined the degree of synergy as the excess over the multiplicative effects of single drugs as if they acted independently (Bliss). The following higher-order formulations were used to quantify drug combination (S) synergy for multiple drug combination effects measured between 2 drugs:

Statistical analysis

All values attained from the various cell lines were expressed as mean ± standard deviation. After obtaining the individual values of both treated and controlled cell lines, the results were analyzed using Graphpad, Version 5.0, and Version 8.0. Data analysis was done by comparing two or more groups with a nonparametric distribution using analysis of variance (ANOVA), followed by the TUKEY-KRAMER multiple comparison tests, with $P \leq 0.05$ considered as the critical level for significance.

RESULTS

Physicochemical properties of the BR2 peptide using the Helquest Freeware program

Data on physicochemical properties were calculated using the Helquest Freeware program.[16] Studies have shown that both antimicrobial and antitumor activity can be attributed to the peptide load (z). The BR2-NH2 peptide has a net positive charge of +6, hydrophobicity (H) of 0.48 and hydrophobic moment (μH) of 0.4, as shown in Figure 1.

A total of 13 (2.2%) patients had no stated age at the time of data collection.

Cytotoxicity of isolated and associated BR2 peptide and 2-AEH_2P

The results of the evaluation of cell viability in 4T1 tumor cells demonstrated that 2-AEH_2P induced cytotoxicity with an IC50 value of 17.4mM and 2.6mM at 24h and 48h, respectively (Figure 2A-B). The BR2 peptide promoted cytotoxicity at all concentrations tested, resulting in the IC50 for BR2 at 24h and 48h at values of 28μM and 18μM (Figure 2A-B). The 2-AEH_2P+BR2 association was also cytotoxic at all concentrations evaluated, with IC50 of 17.5μM and 10μM of BR2 and at the fixed concentration of 9mM of 2-AEH_2P at 24h and 48h (Figure 2A-B).

MDA-MB-231 triple-negative human breast cancer tumor cells treated with 2-AEH_2P (0.7-200mM), with the BR2 peptide (10-100μM) and 2-AEH_2P+BR2 association showing effects similar to those observed in the 4T1 tumor cells, described above (Figure 2A-C). Treatment of these cells with 2-AEH_2P (0.7-200mM) resulted in significant cytotoxicity, with IC50 values of 12mM and 6.5mM at 24h and 48h (Figure 2A-C). The BR2 peptide significantly reduced cell viability, showing IC50 values of 14μM and 9.5μM at 24h and 48h, respectively (Figure 2A-C). The association 2-AEH_2P+BR2 induced cytotoxicity, with an 89±2.3% reduction in cell viability at the lowest concentration, proving to be more efficient than the drugs combination tested at 24h (Figure 2A-C).

$$S_{BLISS} = E_{A,B} - (E_A + E_B)$$

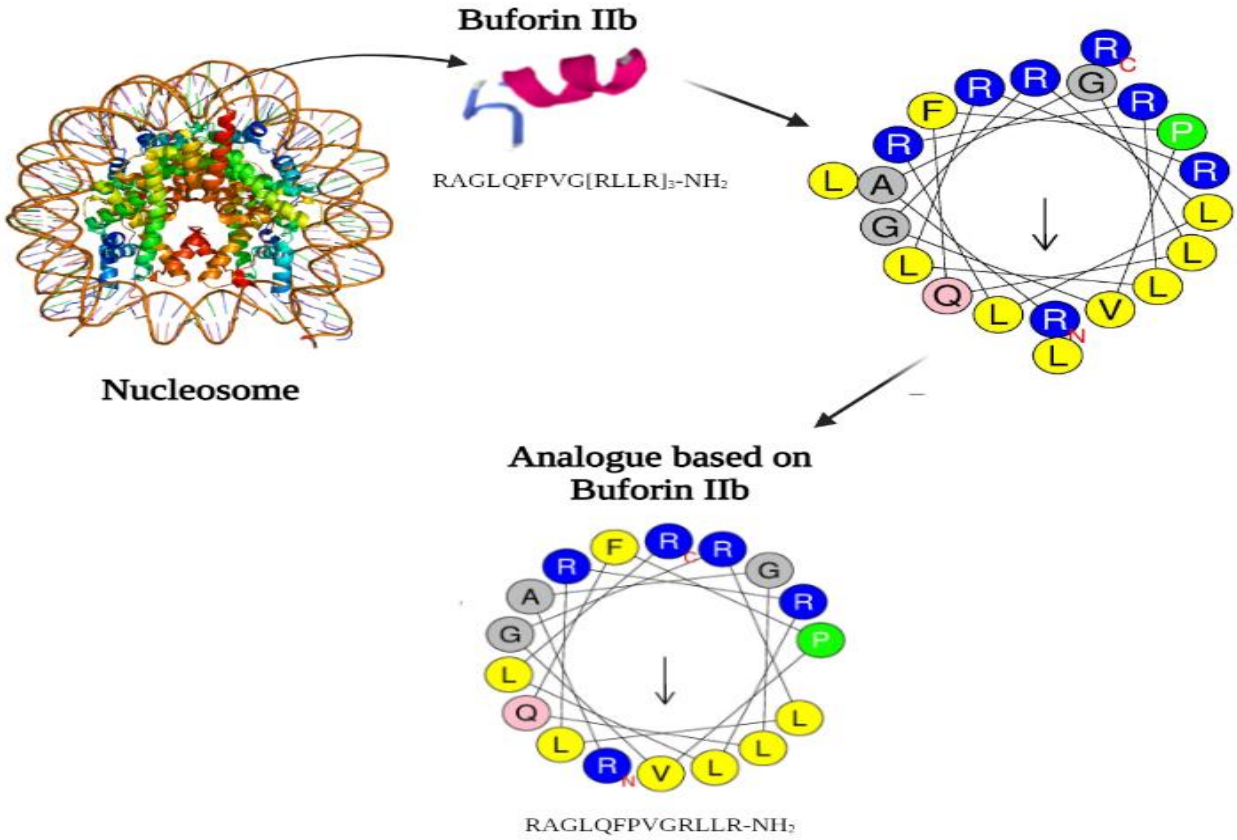
$$SynergyScore = \frac{-10g(p)}{\log(0.05)} \times \frac{t}{|t|}$$

In normal human fibroblast FN1, 2-AEH_2P did not induce significant cytotoxicity, obtaining IC50 of 56mM and 41mM at 24h and 48h (Figure 2A-D). The BR2 peptide produced significant cytotoxicity at the highest concentrations tested, being less significant when compared to tumor cells, with IC50 of 35μM and 36μM at 24h and 48h (Figure 2A-D). The association 2-AEH_2P+BR2 (2-AEH_2P 28mM - BR2 peptide 10-100μM) showed more pronounced cytotoxicity at 24h and 48h with IC50 of 29.5μM and 27μM, respectively (Figure 2A-D).

When analyzing the cytotoxicity of the treatments for the normal L929 cell line fibroblast, the results were similar to those observed for the FN1 cell line, where 2-AEH_2P did not induce significant cytotoxicity, obtaining IC50 of 57.3mM and 43.5mM at 24h and 48h (Figure 2A-E). The BR2 peptide produced significant cytotoxicity at the highest concentrations tested, being less significant when compared to tumor cells, with IC50 of 65.4μM and 66.8μM at 24h and 48h (Figure 2A-E). The 2-AEH_2P+BR2 association (29mM 2-AEH_2P - 10-100μM BR2 peptide) showed more pronounced cytotoxicity at 48h and with an IC50 of 53.6μM, the

IC50 value for the 24h treatment was 77.2µM (Figure 2A-E).

The 2-AEH$_2$P+BR2 association showed a greater cytotoxic effect but did not induce significant cytotoxicity at the inhibitory concentrations of 50% used in tumor cell lines; therefore, it did not significantly compromise the viability of the normal FN1 human fibroblast cell.



Peptide	Sequence	Molecular weight (Da)		H	*µH*	*z*
		Calculated	Observed			
BR2	CRAGLQFPVGRLLRRLLR	2122,3	2127	0,48	0,4	+6

Figure 1. Helical wheel projections of synthetic Buforin IIb peptide derivative. The inner color of each circle indicates the amino acid group: yellow for aromatic and aliphatic hydrophobic residues; gray for residues with hydrophobicity close to zero; blue for positively charged basic residues; purple for uncharged polar residues; red for negatively charged residues; green for the pseudo amino acid proline. The black arrows indicate the direction and intensity of the hydrophobic moment, calculated using the online server Heliquest. The arrows and red rings indicate the positions along the peptide sequence where substitutions were made. The table with the values of the physical-chemical analysis of the BR2 peptide was generated by Heliquest

Therapeutic index of in vitro drug combination

The combination of drugs (peptide BR2+2-AEH$_2$P) for the 4T1 murine triple-negative breast cancer tumor cell within 24h showed a sensitivity response ~2 times greater than the FN1 and ~3 times larger than the fibroblast cell lineage L929 (Figure 3A), with an in vitro therapeutic index of 1.75 and a therapeutic window of 10-30µM (Figure 3E). In the 48h period of treatment, the response to treatment was ~5 times greater when compared to the FN1 cell and ~8 times greater when compared to the L929 cell (Figure 3B). The in vitro therapeutic index was 1.25 with a therapeutic window of 10-50µM (Figure 3E).

The drug combination for the triple-negative human breast cancer cell MDA MB-231 showed better results compared to the 4T1 cell. The sensitivity of the MDA MB-231 cell was ~6-fold when compared to the FN1 cell and ~10.9-fold when compared to the L929 cell over the 24h period (Figure 3C). The therapeutic index was 4.1 and the therapeutic window was 1.1-30µM (Figure 3E). Within 48h, the sensitivity of the MDA-MB-231 tumor cell was ~12 times greater when compared to the FN1 fibroblast cell and ~37 times greater than that for the L929 fibroblast cell (Figure 3D). The therapeutic index was 2.6 with a therapeutic window of 0.8-50µM (Table 1).

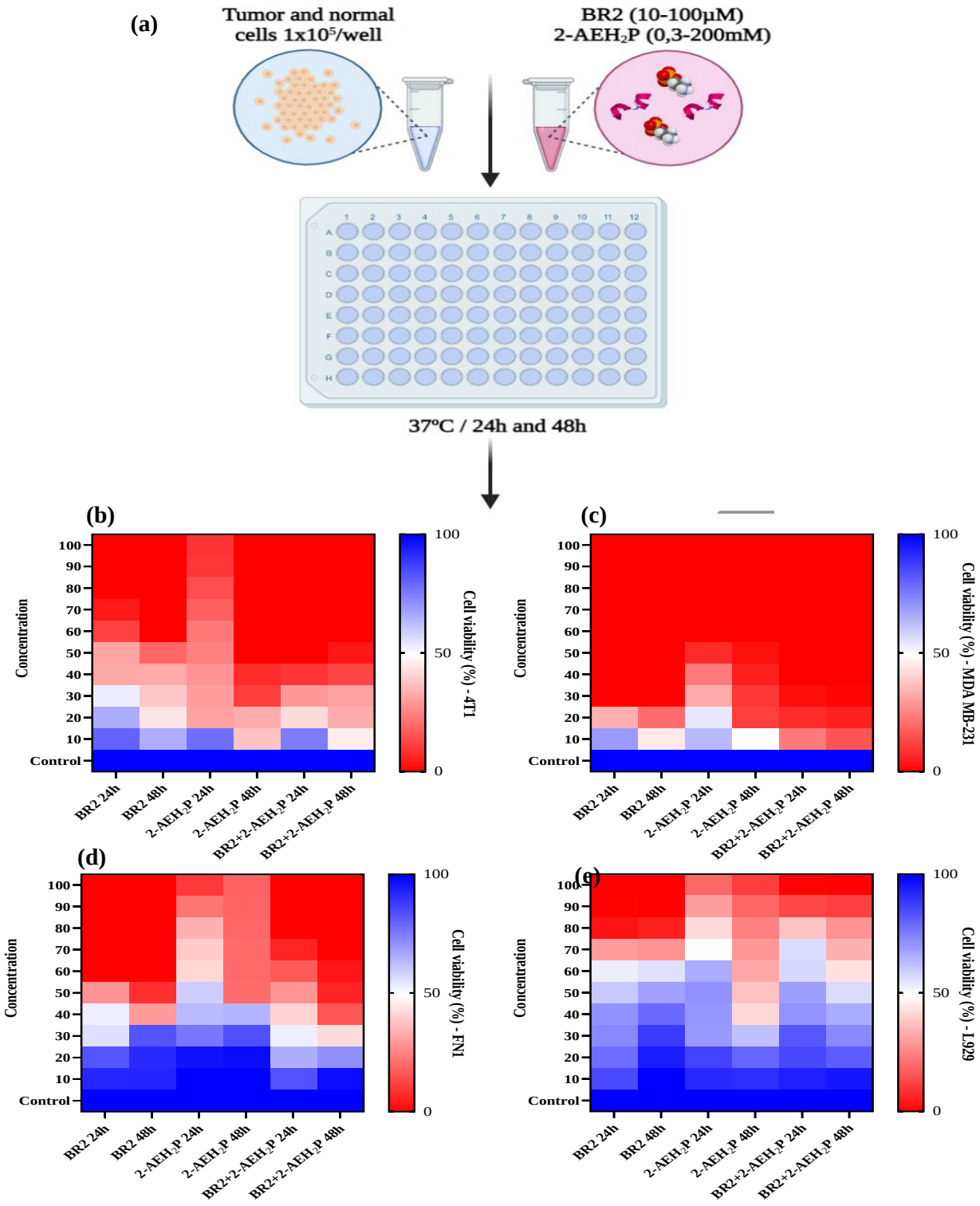


Figure 2. Determination of cytotoxicity in human triple-negative breast cancer MDA MB-231, murine 4T1, and normal human FN1 fibroblast tumor cells by the MTT colorimetric method. The cells were treated with different concentrations of the BR2 peptide, the 2-AEH$_2$P monophosphoester and the 2-AEH$_2$P+BR2 association, for a period of 24h and 48h. **(a)** Cells in RPMI medium were seeded in 96-well plates followed by the addition of each compound (BR2 peptide, 2-AEH$_2$P and association); **(b)** Heatmap showing the correlation of the cytotoxic effect expressed as mean±SD of three independent experiments for 4T1 murine triple-negative breast cancer tumor cell; **(c)** Heatmap showing the correlation of cytotoxic effect expressed as mean±SD of three independent experiments for human triple-negative breast cancer tumor cell MDA MB-231; **(d)** Heatmap showing the correlation of cytotoxic effect expressed as mean±SD of three independent experiments for FN1 standard human fibroblast cell; **(e)** Heatmap showing the correlation of cytotoxic effect expressed as mean±SD of three independent experiments for L929 standard human fibroblast cell.

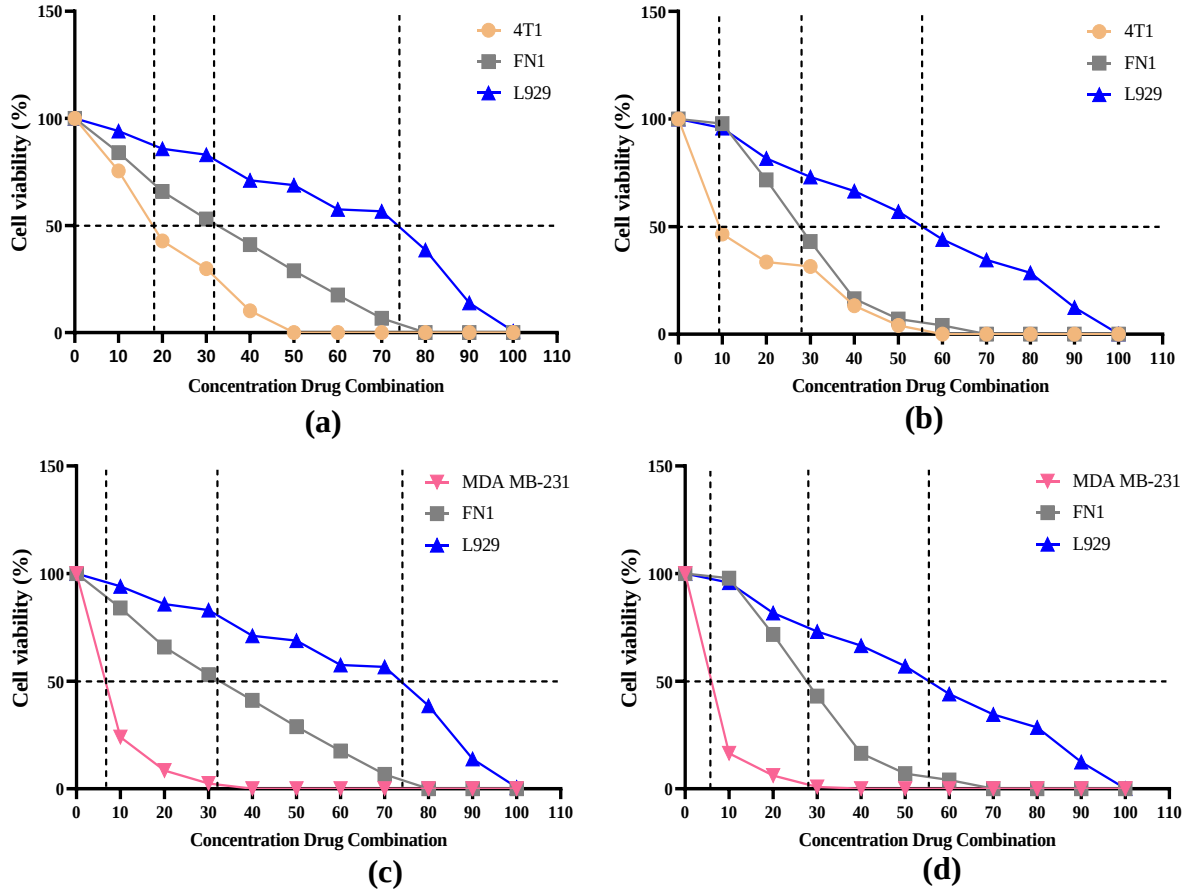


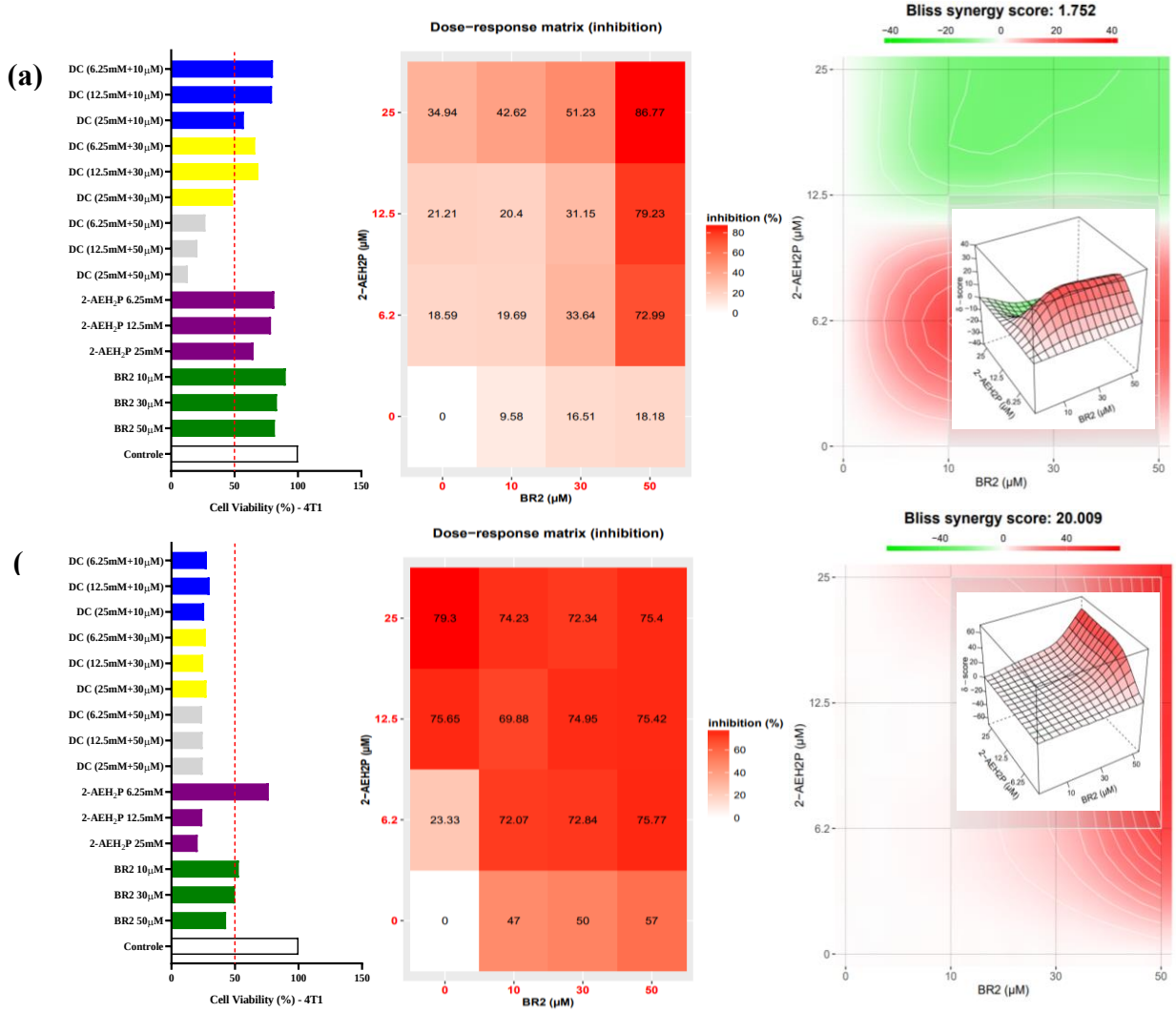
Figure 3. Evaluation of the therapeutic index of in vitro treatments for MDA MB-231 and 4T1 triple-negative breast cancer tumor cells. The cells were treated with a fixed concentration of 2-AEH$_2$P and varied concentrations of the BR2 peptide (10-100µM) at 24h and 48h. **(a)** Bar graph showing the correlation between the 4T1 tumor cell and normal FN1 and L929 expressed by the mean of three independent experiments over the 24h period; **(b)** Bar graph showing the correlation between the 4T1 tumor cell and normal FN1 and L929 expressed by the mean of three independent experiments over a 48h period; **(c)** Bar graph showing the correlation between MDA MB-231 tumor cell and normal FN1 and L929 expressed by the mean of three independent experiments over a 24h period; **(d)** Bar graph showing the correlation between the MDA MB-231 tumor cell and normal FN1 and L929 expressed by the mean of three independent experiments over a 48h period

Table 1. The therapeutic index of in vitro treatments for MDA MB-231 and 4T1 triple-negative breast cancer tumor cells.

Cell	Drug Combination	Period	Therapeutic index	Therapeutic window
4T1	BR2+2-AEH$_2$P	24h	1.75	10-30 µM
		48h	1.25	10-50 µM
MDA		24h	4.1	1.1-30 µM
MB-231		48h	2.6	0.8-50 µM

Analysis of the pharmacological effects of treatments with BR2 peptide and 2-AEH$_2$P

The antagonistic effect is observed in the color space between white and green (≤0), and the additive effect and synergism are observed in the color space between white and red (>0 and <10 additives; >10 synergistic). The combinatorial activity of BR2 peptide and 2-AEH$_2$P was investigated in MDA-MB-231 and 4T1 tumor cells over 24h and 48h using SynergyFinder 2.0 with the Bliss analysis model. The pharmacological effect observed for the association in the 4T1 cell in the 24h period was additive, with an analysis value of 1.75 (Figure 3A), when treated in the 48h period, the reduction in cell viability was accentuated, increasing the value obtained by the software, being 20.0, thus presenting a synergistic effect (Figure 3B). A more significant result was observed for MDA-MB-231 tumor cells when treated with the association 2-AEH$_2$P + BR2 within 24h, showing a synergistic effect with a synergy score of 12.1 (Figure 4A). The synergistic effect was also observed for the 48h treatment with the association, with a synergy value of 14.3 (Calculation was done by Bliss).



(c)

Cell	Period	Drug Combination	Synergy score	Effect	Method
4T1	24h	BR2+2-AEH$_2$P	1.75	Additive	Bliss
	48h	BR2+2-AEH$_2$P	20.0	Synergistic	Bliss

DC= Drug Combination

Figure 3. Determination of the pharmacological effect of the BR2 peptide association with 2-AEH$_2$P monophosphoester in triple-negative breast cancer tumor cells 4T1. **(a)** Bar graphs show additive or synergistic activity for concentrations of treatments in tumor cells over the 24h period; **(b)** Bar graphs show additive or synergistic activity for concentrations of treatments in tumor cells over the 48h period; The antagonistic effect is observed in the color space between white and green (≤0), the additive effect and synergism are observed in the color space between white and red (>0 and <10 additive;>10 synergistic). Color saturation is proportional to the magnitude of the difference between these values; **(c)** The table shows drug combination and synergy score for that combination.

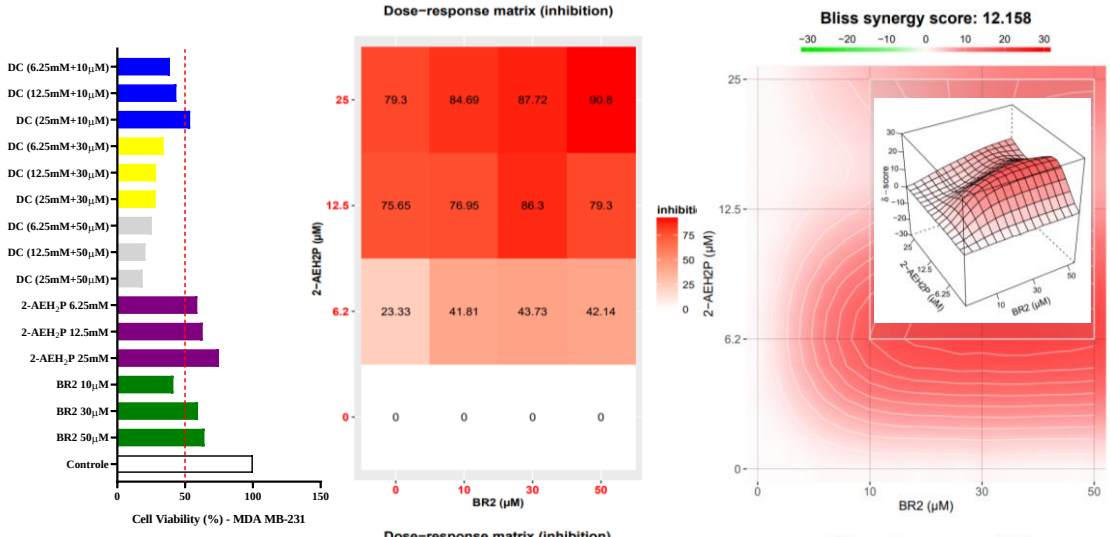
DISCUSSION

In this study, we analyzed the ability of the BR2 tumor-penetrating peptide to potentiate the effects of in vitro therapy when associated with 2-aminoethyl dihydrogen phosphate (2-AEH$_2$P) in human triple-negative breast cancer tumor cells (MDA-MB-231) and murine (4T1). After the association (2-AEH$_2$P+BR2), with half of the IC50 values obtained for both treatments, more significant results were observed, with better responses at lower concentrations.

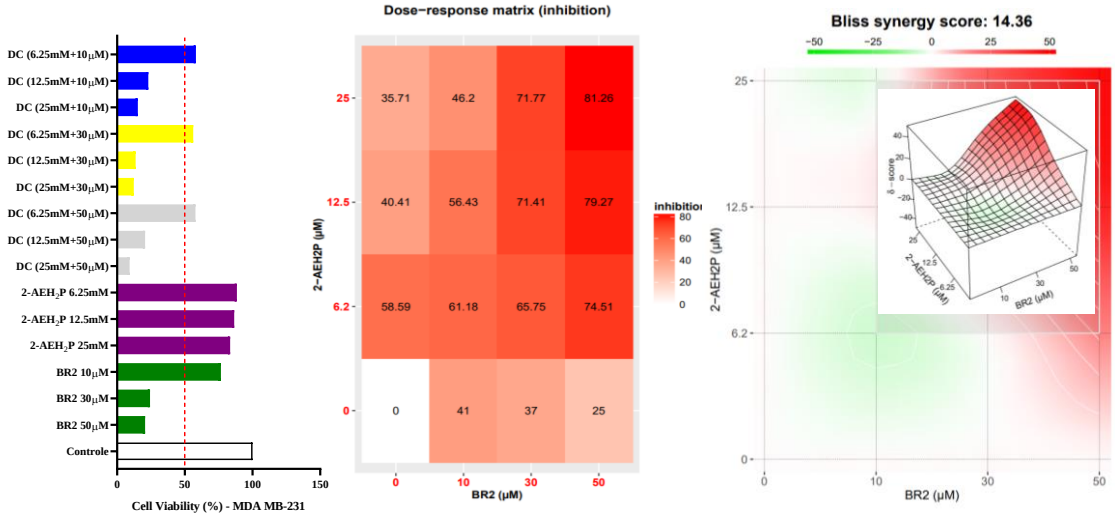
There was a pharmacological effect of the association as a result of treatment time and concentration, mostly presenting a synergistic effect.

The activity of antitumor drugs is often limited due to a lack of satisfactory tumor specificity and bioavailability. Conjugating antitumor drugs to specific delivery systems can increase tumor selectivity, reaching the tumor microenvironment and potentiating its activity. It is still a challenge to develop compounds that have selectivity and penetration of tumor cells.[17,18]

(a)



(b)



(c)

Cell	Period	Drug Combination	Synergy score	Effect	Method
MDA MB-231	24h	BR2+2-AEH$_2$P	12.1	Synergistic	Bliss
	48h	BR2+2-AEH$_2$P	14.3	Synergistic	Bliss

DC= Drug Combination

Figure 4. Determination of the pharmacological effect of the BR2 peptide association with 2-AEH$_2$P monophosphoester in triple-negative breast cancer tumor cells MDA MB-231. **(a)** Bar graphs show additive or synergistic activity for concentrations of treatments in tumor cells over the 24h period; **(b)** Bar graphs show additive or synergistic activity for concentrations of treatments in tumor cells over the 48h period; The antagonistic effect is observed in the color space between white and green (≤0), the additive effect and synergism are observed in the color space between white and red (>0 and <10 additive;>10 synergistic). Color saturation is proportional to the magnitude of the difference between these values; **(c)** Table showing drug combination and synergy score for that combination.

The BR2 peptide is a non-specific tumor penetration analog of the buforin IIb peptide, derived from histone H2A, which crosses the cell membrane of tumor cells without damaging them through interaction with gangliosides 54,70. Studies have shown that the BR2 peptide has antitumor potential, inducing cytotoxicity in several tumor cell lines in vitro, such as HepG2 (Hepatocarcinoma); HeLa (human cervical cancer); MCF-7 (human breast adenocarcinoma); HCT116 (human colon cancer); B16-F10 (murine melanoma).[19-21]

One of the main hypotheses for the selective cytotoxicity of cationic peptides in tumor cells is that plasma membranes of tumor cells present greater electronegativity compared to normal cell membranes, due to an increased expression of anionic elements, such as sodium sulfate heparan, phosphatidylserine, and sialic acid, making tumor cells more susceptible to the uptake of cationic peptides. Another characteristic of tumor cell plasma membranes is the increased presence of microvilli, increasing the contact surface with this type of peptide.[22,23]

The monophosphoester 2-aminoethyl dihydrogen phosphate (2-AEH$_2$P) is a molecule involved in the turnover of phospholipids, acting as a precursor in the synthesis of membrane phospholipids. This compound has broad antitumor potential, inducing cytotoxicity in different tumor cell lines, such as EAT (Ehrlich's ascitic tumor); B16F10 cells (murine melanoma); MCF-7 cells (human breast adenocarcinoma); MDA MB-231 cells (human triple-negative breast cancer); H292 cells (human lung carcinoma); Skmel-28 cells, Mewo (human melanoma); Hepa1c1c7 cells (hepatocarcinoma); K562 and K562-Lucena (Chronic Myeloid Leukemia - MDR+).[10,11,24-28]

For treatment with the BR2 peptide, the tested concentrations showed cytotoxicity for tumor cells, with a smaller effect on normal cells, corroborating the data already described in the literature.[21] The pharmaceutical association of these two molecules at lower concentrations also had positive results, showing cytotoxicity for tumor cells. The data corroborate a possible additive and synergistic effect of the combination, thus enhancing its effects. Numerous works show that tumor penetration peptides potentiate the action of other molecules, in immunotherapy, facilitating the permeability of these cells to the tumor microenvironment and as a drug director in target-specific therapies.[29,30]

Most cationic antitumor peptides have antitumor properties, including the ability to rapidly kill target cells, with a broad spectrum of activity and some specificity for cancer cells.[31] Some factors of cationic antitumor peptides have been identified to be important for their antitumor activity, including hydrophobicity, net charge, amphipathicity, secondary structure in the membrane and their ability to oligomerize. Among these factors, hydrophobicity plays an important role in anticancer activity due to the hydrophobic environment of the cell membrane.[32]

The cytotoxic effect of the BR2 peptide was greater than that of the 2-AEH$_2$P monophosphoester, considering the IC50 values obtained and the concentrations necessary to cause a reduction in cell proliferation and death. The results observed for the BR2 peptide are similar to those found in the literature for cationic peptides, inhibiting proliferation and migration.[31,33]

Numerous studies address therapeutic strategies using combination therapies, which are in clinical practice and have been widely used for many diseases, including cancer.[33,34] The co-administration of different drugs with different mechanisms and targets and actions, aims to improve the therapeutic efficacy and/or reduce the systemic side effects of the administration.[35] The combined action of two peptides, one from collagen IV and the other from a protein containing somatotropin domain in a peptide-peptide combination, has been observed to synergize the antitumor activity.[36] Zhao *et al*. showed that the antitumor activity of the HPRP-A1 peptide and doxorubicin (DOX) and epirubicin (EPI) act synergistically against different cancer cell lines both in in vitro and in vivo studies.[37]

We found important data corroboratng the synergistic effect of the action of the BR2 peptide, as other works have focused on this pharmacological effect for tumor cells, increasing the therapeutic efficiency for human and murine breast cancer cells, and colon cancer HCT116 cells both in vitro and in vivo.[8,38] Given that in vitro studies in cells in monolayers are limited and in vivo results can be very different, other studies should evaluate the systemic effects of this association, and its biodistribution and bioavailability in the body.

CONCLUSION

The results obtained in the present work demonstrated that the BR2 peptide and the association 2-AEH$_2$P+BR2 produced greater cytotoxicity in the human triple-negative breast cancer tumor cell lines MDA MB-231 and murine 4T1 compared to the monophosphoester 2-AEH$_2$P, which, even with cytotoxicity, was significantly lower. MDA MB-231 tumor cells were the most sensitive to all treatments with lower IC50 values. The results showed important pharmacological effects, with a synergistic effect for the treatments at 24h and 48h for the MDA MB-231 tumor cell and for the 4T1 tumor cell only at 48h. The in vitro results demonstrated that the treatments presented more specific cytotoxicity for tumor cells, obtaining IC50 two to three times lower than for the normal fibroblast cell FN1.

ACKNOWLEDGMENTS

We thank the Coordination for the Improvement of Higher Education Personnel – CAPES – Cabral, LGS – 88882.328116/2019-01 and theNational Council for Scientific and Technological Development – CNPq – Maria, DA - 305056/2019-0.

CONFLICT OF INTERESTS

There are no conflicts of interest to declare.

FUNDING

This research has not benefited from any funding sources from public and private sectors.

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7-33. doi:10.3322/caac.21708
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424. doi:10.3322/caac.21492
3. INCA. Estimativa 2020 : Incidência de Câncer No Brasil.; 2019.
4. Sørlie T, Tibshirani R, Parker J, et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. PNAS. 2003;100(14):8418-8423. www.stanford.edubreastcancer
5. Carey LA, Perou CM, Livasy CA, et al. Race, Breast Cancer Subtypes, and Survival in the Carolina Breast Cancer Study. American Medical Association. 2006;295(21):2492-2502. https://jamanetwork.com/
6. Torres MDT, Pedron CN, Araújo I, Silva PI, Silva FD, Oliveira VX. Decoralin Analogs with Increased Resistance to Degradation and Lower Hemolytic Activity. ChemistrySelect. 2017;2(1):18-23. doi:10.1002/slct.201601590
7. Alberici L, Roth L, Sugahara KN, et al. De Novo design of a tumor-penetrating peptide. Cancer Res. 2013;73(2):804-812. doi:10.1158/0008-5472.CAN-12-1668
8. de Sousa Cabral LG, Hesse H, Freire KA, et al. The BR2 peptide associated with 2-aminoethyl dihydrogen phosphate is a formulation with antiproliferative potential for a triple-negative breast cancer model. Biomedicine & Pharmacotherapy. 2022;153:113398. doi:10.1016/j.biopha.2022.113398
9. Luna ACDL, Santos Filho JRDA, Hesse H, Neto SC, Chierice GO, Maria DA. Modulation of pro-apoptotic effects and mitochondrial potential on B16F10 cells by DODAC/PHO-S liposomes. BMC Res Notes. 2018;11(1). doi:10.1186/s13104-018-3170-7
10. Ferreira AK, Meneguelo R, Neto SC, Chierice GO, Maria DA. Synthetic Phosphoethanolamine Induces Apoptosis Through Caspase-3Pathway by Decreasing Expression of Bax/Bad Protein and Changes CellCycle in Melanoma. Journal of Cancer Science & Therapy. 2011;3(3):53-59. Accessed April 23, 2022. https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1064212
11. Ferreira AK, Meneguelo R, Pereira A, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine induces cell cycle arrest and apoptosis in human breast cancer MCF-7 cells through the mitochondrial pathway. Biomedicine and Pharmacotherapy. 2013;67(6):481-487. doi:10.1016/j.biopha.2013.01.012
12. Manuela Garcia Laveli da Silva, Luciana Bastianelli Knop, Durvanei Augusto Maria. Meclizine Chloridrate and Methyl-β-Cyclodextrin Associated with Monophosphoester Synthetic Phosphoethanolamine Modulating Proliferative Potential in Triple-Negative Breast Cancer Cells. Journal of Pharmacy and Pharmacology. 2019;7(7). doi:10.17265/2328-2150/2019.07.006
13. Manuela Garcia Laveli da Silva, Laertty Garcia de Sousa Cabral, Monique Gonçalves Alves, et al. 2-aminoethyl Dihydrogen Phosphate as a Modulator of Proliferative and Apoptotic Effects in Breast Cancer Cell Lines. Journal of Pharmacy and Pharmacology. 2021;9(3). doi:10.17265/2328-2150/2021.03.001
14. Conceição TO, Cabral LGS, Laveli-Silva MG, et al. New potential antiproliferative monophosphoester 2-aminoethyl dihydrogen phosphate in K-562 and K-562 MDR+ leukemia cells. Biomedicine and Pharmacotherapy. 2021;142. doi:10.1016/j.biopha.2021.112054
15. Conceição T de O, Laveli-Silva MG, Maria DA. Phosphomonoester Phosphoethanolamine Induces Apoptosis in Human Chronic Myeloid Leukemia Cells. Journal of Pharmacy and Pharmacology. 2019;7(7). doi:10.17265/2328-2150/2019.07.009
16. Gautier R, Douguet D, Antonny B, Drin G. HELIQUEST: A web server to screen sequences with specific α-helical properties. Bioinformatics. 2008;24(18):2101-2102. doi:10.1093/bioinformatics/btn392
17. Kamaly N, Xiao Z, Valencia PM, Radovic-Moreno AF, Farokhzad OC. Targeted polymeric therapeutic nanoparticles: Design, development and clinical translation. Chem Soc Rev. 2012;41(7):2971-3010. doi:10.1039/c2cs15344k
18. Blanco E, Shen H, Ferrari M. Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol. 2015;33(9):941-951. doi:10.1038/nbt.3330
19. Zhang X, Lin C, Lu A, et al. Liposomes equipped with cell penetrating peptide BR2 enhances chemotherapeutic effects of cantharidin against hepatocellular carcinoma. Drug Deliv. 2017;24(1):986-998. doi:10.1080/10717544.2017.1340361
20. Lim KJ, Sung BH, Shin JR, et al. A Cancer Specific Cell-Penetrating Peptide, BR2, for the Efficient Delivery of an scFv into Cancer Cells. PLoS One. 2013;8(6). doi:10.1371/journal.pone.0066084
21. Marqus S, Pirogova E, Piva TJ. Evaluation of the use of therapeutic peptides for cancer treatment. J Biomed Sci. 2017;24(1). doi:10.1186/s12929-017-0328-x
22. Huang W, Seo J, Willingham SB, et al. Learning from host-defense peptides: Cationic, amphipathic peptoids with potent anticancer activity. PLoS One. 2014;9(2). doi:10.1371/journal.pone.0090397
23. Mulder KCL, Lima LA, Miranda VJ, Dias SC, Franco OL. Current scenario of peptide-based drugs: the key roles of cationic antitumor and antiviral peptides. Front Microbiol. 2013;4. doi:10.3389/fmicb.2013.00321
24. Ferreira AK. Alquil Fosfatado Sintético Precursor Dos Fosfolipídios de Membrana Celular Com Potencial Efeito Antitumoral e Apoptótico Em Modelos de Tumores Experimentais.; 2013.
25. Ferreira AK, Meneguelo R, Pereira A, Filho OMR, Chierice GO, Maria DA. Anticancer Effects of

Synthetic Phosphoethanolamine onEhrlich Ascites Tumor: An Experimental Study. Anticancer Res. 2012;32:95-104.
26. Ferreira AK, Meneguelo R, Pereira A, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine induces cell cycle arrest and apoptosis in human breast cancer MCF-7 cells through the mitochondrial pathway. Biomedicine and Pharmacotherapy. 2013;67(6):481-487. doi:10.1016/j.biopha.2013.01.012
27. Ferreira AK;, Meneguelo R;, Neto SC;, Chierice GO;, Maria DA. Synthetic Phosphoethanolamine Induces Apoptosis Through Caspase-3Pathway by Decreasing Expression OfBax/Bad Protein and Changes CellCycle in Melanoma. Vol 3.; 2011. https://pesquisa.bvsalud.org/portal/resource/pt/biblio-1064212
28. Ferreira AK, Santana-Lemos BAA, Rego EM, Filho OMR, Chierice GO, Maria DA. Synthetic phosphoethanolamine has in vitro and in vivo anti-leukemia effects. Br J Cancer. 2013;109(11):2819-2828. doi:10.1038/bjc.2013.510
29. Jabir MS, Taha AA, Sahib UI, Taqi ZJ, Al-Shammari AM, Salman AS. Novel of nano delivery system for Linalool loaded on gold nanoparticles conjugated with CALNN peptide for application in drug uptake and induction of cell death on breast cancer cell line. Materials Science and Engineering C. 2019;94:949-964. doi:10.1016/j.msec.2018.10.014
30. Lee YW, Hwang YE, Lee JY, Sohn JH, Sung BH, Kim SC. VEGF siRNA delivery by a cancer-specific cell-penetrating peptide. J Microbiol Biotechnol. 2018;28(3):367-374. doi:10.4014/jmb.1711.11025
31. Hoskin DW, Ramamoorthy A. Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta Biomembr. 2008;1778(2):357-375. doi:10.1016/j.bbamem.2007.11.008
32. Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by K-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochimica et Biophysica Acta . 1999;1462:55-70. www.elsevier.com/locate/bba
33. Zhao J, Huang Y, Liu D, Chen Y. Two Hits Are Better than One: Synergistic Anticancer Activity of α-Helical Peptides and Doxorubicin/Epirubicin. Vol 6.; 2014. www.impactjournals.com/oncotarget/
34. Ning Zhang, Jia-Ning Fu, Ting-Chao Chou. Synergistic combination of microtubule targeting anticancer fludelone with cytoprotective panaxytriol derived from panax ginseng against MX-1 cells in vitro: experimental design and data analysis using the combination index method. Am J Cancer Res. 2016;6(1):97-104. www.combosyn.com
35. Bijnsdorp I v., Giovannetti E, Peters GJ. Analysis of Drug Interactions. In: ; 2011:421-434. doi:10.1007/978-1-61779-080-5_34
36. Koskimaki JE, Lee E, Chen W, et al. Synergy between a collagen IV mimetic peptide and a somatotropin-domain derived peptide as angiogenesis and lymphangiogenesis inhibitors. Angiogenesis. 2013;16(1):159-170. doi:10.1007/s10456-012-9308-7
37. Hu C, Chen X, Huang Y, Chen Y. Synergistic effect of the pro-apoptosis peptide kla-TAT and the cationic anticancer peptide HPRP-A1. Apoptosis. 2018;23(2):132-142. doi:10.1007/s10495-018-1443-1
38. Bolhassani A, Jafarzade BS, Mardani G. In vitro and in vivo delivery of therapeutic proteins using cell penetrating peptides. Peptides (NY). 2017;87:50-63. doi:10.1016/j.peptides.2016.11.011

How to Cite This Article

Cabral LGS, Oliveira CS, Alves RCB, Oliveira VX, Poyet JL, Maria DA. Synergistic Effects of the Association of BR2 Peptide with 2-Aminoethyl Dihydrogen Phosphate on Triple-Negative Breast Cancer Cells. Arch Breast Cancer. 2023; 10(2): 148-58.
Available from: https://www.archbreastcancer.com/index.php/abc/article/view/650