Original Article

Improvement of Antiproliferative Activity of Recombinant Truncated Form of *Pseudomonas aeruginosa* Exotoxin (PE38) by Vitamin E in MCF-7 Cells

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Abstract

Background: In addition to beneficial roles of vitamin E in many metabolic processes and its antitumor activities, vitamin E derivatives have extensively been considered as permeability enhancers. Using these enhancers, permeability of a wide spectrum of drugs was reported to be significantly increased. PE38, a toxic substance with a potential application in cancer therapy, is a truncated form of Pseudomonas exotoxin A (PE), which lacks Ia and a portion of domain Ib. **Objective:** Here, the antiproliferative potential of PE38 and alpha-tocopherol (αT) form of vitamin E were assessed in MCF-7 cells. The role of vitamin E in PE38 cytotoxicity level was also evaluated. **Materials and Methods:** The antiproliferative potential of PE38, vitamin E, and a combination of them were colorimetrically evaluated in a cellular breast cancer model (MCF-7), using the MTT assay. P values of <0.05 were considered significant. **Results:** Compared to control cells, the PE38 inhibited the proliferation of MCF-7 cells (80% ± 1.37% cell viability) only at the highest concentration used (500 μg/mL) ($P < 0.05$). MTT assay also showed that 0.1, 1, and 10mg/mL of vitamin E could significantly ($P < 0.001$) decrease the cell viability of MCF-7 cells to 57% ± 1.37%, 26.8% ± 1.37%, and 14.7% ± 1.37% at 24 h, respectively. Moreover, the coadministration of vitamin E (0.1 mg/mL) with 31.25, 62.5, 125, 250, and 500 μg/mL concentrations of PE38 decreases in cell viability from 100% in control cells to 35.61% ± 4.29%, 37.8% ± 6.45%, 36.42% ± 5.79%, 32.33% ± 4.62%, and 29.97% ± 5.07% at 24 h, respectively ($P < 0.001$). **Conclusion:** The results of this study suggest that vitamin E can enhance the antiproliferative activity of PE38 toward MCF-7 cells.

Keywords: *Antiproliferative activity, MCF-7 cells, MTT, PE38, vitamin E*

Key Messages: The results of this study suggest that vitamin E can enhance the antiproliferative activity of PE38 toward MCF-7 cells.

Introduction

Pseudomonas exotoxin A (PE) is the most toxic substance in *P. aeruginosa*, which is able to inhibit the protein synthesis of the host cell. On the basis of X-ray crystallographic studies, PE is composed of three major structural domains: domain I (residues 1–252 [Ia] and 365–404 [Ib]), which is responsible for cell binding; the target site for this toxin is α2-macroglobulin receptor that is present in many types of normal and cancerous cells; domain II (residues 253–364), which is able to translocate the carboxyl terminus of the protein into the cytoplasm; and the enzymatic domain III (residues 405–613), which is able to arrest protein synthesis via ADP-ribosylation of elongation factor-2.[1] Investigations on the structure–function relationship of PE led to the generation of genetically modified truncated forms, which could be further used to develop the recombinant immunotoxins. Modified PEs with deletions in domain 1a were widely studied.[2] PE38 is such a truncated form of PE, which lacks Ia (residues 1–252) and a portion of domain Ib (residues 365–380). Due to deletion in domain Ia, PE38 molecule is not able to bind to cells. Moreover, in PE38KDEL, amino acids 609–613 (REDLK) at the carboxyl terminus of PE38 are also replaced by KDEL.[3] On the basis of data extracted from the published reports, various modified forms of PE in which the cell binding site was deleted show low toxicity to human or mouse cells.[2] For example, using MTT assay, cytotoxicity of the recombinant protein PE38KDEL was tested on ch-hep-3, ch-hep-1, and Hut102 cells and results showed slightly toxicity in the three cell lines.[1] However, translocation properties as well as enzymatic activities of these truncated proteins have been remained.

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The vitamin E family consists of eight lipophilic molecules (four tocopherols and four tocotrienols) with distinct antioxidant activities.[4] In addition, vitamin E has an important role in immune function, cell signaling, modulation of gene expression, and other metabolic processes.[5] On the basis of previous studies, various forms of vitamin E have been reported to cause death in cancerous cell lines. For instance, growth inhibition and morphological changes were reported in mouse melanoma (B-16) cells treated with D-αT (vitamin E) acid succinate.[6]

In addition to the aforementioned properties, due to physico-chemical properties and bio-efficacies of vitamin E derivatives, they have been extensively used to develop a wide range of drug delivery systems. Solvent capacity, biocompatibility, and the biological properties make vitamin E derivatives attractive in drug delivery.[7] They were shown to be able to enhance *in vitro* permeability of a broad spectrum of drugs as well as their oral bioavailability in animal models. For instance, vitamin E-TPGS (D-αT polyethylene glycol 1000 succinate) is able to increase *in vitro* permeability of celiprolol and paclitaxel. E- D-αT polyethylene glycol 1000 succinate is a water-soluble derivative of natural source vitamin E.[8,9] Moreover, the effects of dietary vitamin E on the permeability of rat organs including brain, heart, kidney, eye, and liver were assessed and an increased permeability was shown in heart and eye organs.[10]

This study aimed to investigate the ability of vitamin E to deliver PE38 through MCF-7 cell line. So, the effect of vitamin E on the cytotoxicity level of PE38 was assessed *in vitro* for the first time. Moreover, antiproliferative potential of PE38 and alpha-tocopherol (αT) form of vitamin E was also evaluated in MCF-7 cell line.

Subject and Methods

Materials

Human breast cancer cell lines (MCF-7) were obtained from Pasture Institute of Iran in Tehran. Cell culture reagents were prepared from Gibco /BRL (Paisley, UK). αT was purchased from Sigma (Hamburg, Germany) Dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and all other chemicals were purchased from Sigma (Hamburg, Germany).

Methods

Cell culture

MCF-7 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Sigma, Hamburg, Germany) consisting 10% fetal bovine serum (FBS) (Gibco Laboratories, North Andover, MA, USA) and 1% penicillin/streptomycin (50 IU/mL and 50 µg/mL, respectively) and incubated at 37°C in a humidified atmosphere with 5% CO_2. At 80%–90% confluence, cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) (Sigma, Hamburg, Germany) solution.[11]

Tocopherol preparation

αT was dissolved in DMSO at 50 mM and Tween 80 was added to it. Samples were kept cold during preparation and were protected from exposure to light. In controls, the corresponding amounts of DMSO and Tween 80 were added.[12]

PE38 preparation

The purified recombinant PE38 protein[3] was provided as a gift by Javid Biotechnology Institute.

Assessment of cell proliferation and cytotoxicity

5×10^3 MCF-7 cells/well were seeded into 96-well plates. After cells had been incubated for 24 h[11] with increasing concentrations of PE38 (31.25, 62.5, 125, 250, and 500 µg/mL) or 0.01, 0.1, 1 and 10 mg/mL of vitamin E as well as 0.1 mg/mL of vitamin E in combination with increasing concentrations of PE38 (31.25, 62.5, 125, 250, and 500 µg/mL), the proliferative response was evaluated using the MTT assay (Sigma-Aldrich, St. Louis, MO, USA). After the incubation period, cells were treated with 100 µL of MTT solution (0.5 mg/well) (Sigma, Hamburg, Germany) and incubated further for 4 h at 37°C in humidified CO_2. The formazan crystals were then solubilized with 100-µL DMSO. Absorbance was measured at 570 nm using a MultiSkan plate reader (LabSystems, Helsinki, Finland). The percentage of viable cells was calculated as follows: cell proliferation (%) = (OD of experimental group/OD of control group) × 100. Phosphate-buffered saline was used as a negative control. The half maximal growth inhibitory concentration (IC_{50}) values in this study were obtained by using a linear regression equation that expresses the relationship between the sample concentrations with the average radical catch activity of the replication series of measurements.

Statistical analysis

To compare the mean of the two and more groups, differences were determined using Student's t-test and analysis of variance (ANOVA) test, respectively. All experiments were performed in eight replicates, and error bars in figures represent SD (standard deviation) values. Statistical analyses were performed with GraphPad Prism (version 5.01) software (GraphPad Software, San Diego, CA, USA). *P* values of <0.05 were considered significant.

Results

Effect of PE38 on the cell viability of MCF-7 cells

The antiproliferative effect of PE38 on MCF-7 cells was determined under five different concentrations (31.25–500 µg/mL). As shown in Figure 1A, exposure of MCF-7 cell line to increasing concentrations of PE38 up to 250 µg/mL caused no significant cytotoxic effects. At the highest concentration (500 µg/mL), the PE38 significantly inhibited the proliferation in MCF-7 cells ($P = 0.0418$) and compared to control cells, 80% ± 1.37% cell viability was observed.

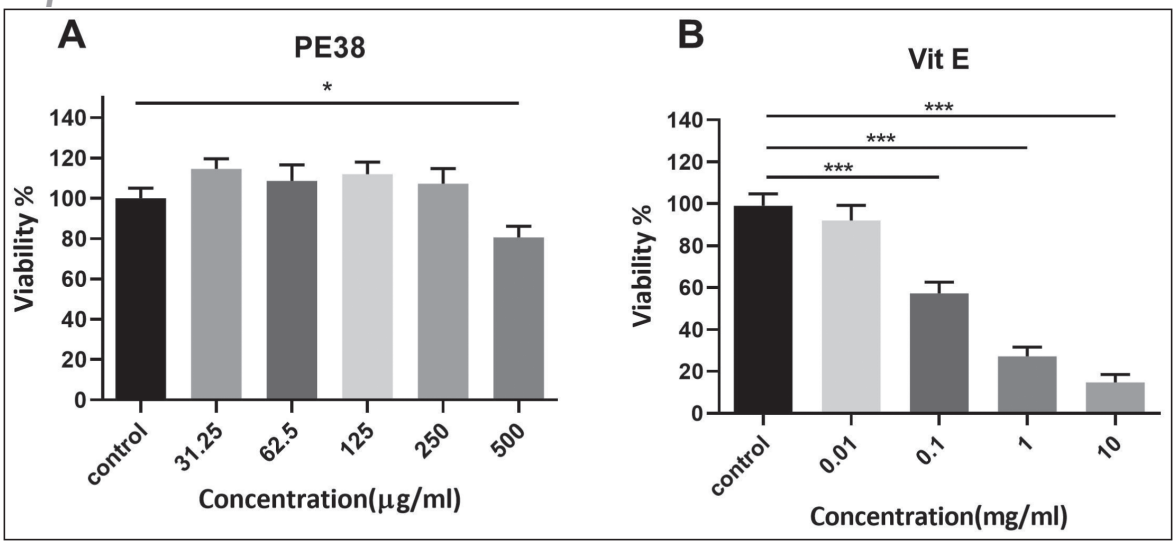

Figure 1: Effects of PE38 and αT form of vitamin E on the viability of MCF-7 cell line. The cells were treated with increasing concentrations of (A) PE38 (31.25, 62.5, 125, 250, and 500 µg/mL) or (B) 0.01, 0.1, 1, and 10 mg/mL of vitamin E and the proliferative response was evaluated using the MTT assay. Data are presented as mean ± standard deviation (SD) (n = 8). *P < 0.05, and ***P < 0.001

In vitro antiproliferative effect of vitamin E against MCF-7 cells

Using MTT assay, the potential antiproliferative activity of vitamin E was evaluated in breast carcinoma MCF-7 cells. The MTT results at different concentrations of vitamin E (0.01–10 mg/mL) are summarized in Figure 1. As shown in Figure 1B, when MCF-7 cells were treated with vitamin E, no cytotoxic effects were observed at 0.01 concentration. Compared to control cells, cell viability of 57% ± 1.37% ($P < 0.001$), 26.8% ± 1.37% ($P < 0.001$), and 14.7% ± 1.37% ($P < 0.001$) was observed for 0.1, 1, and 10 mg/mL of vitamin E, respectively. These results showed the dose-dependent inhibition effects of vitamin E on MCF-7 cells. Using these data, subsequent experiments were conducted with the minimum effective dose (0.1 mg/mL) of vitamin E on MCF-7 cells. Also, pairwise comparison analysis showed that all concentrations of vitamin E were significantly different in cytotoxic effects ($P < 0.05$). In addition, the IC_{50} value measured for MCF-7 cells was 147.3 µg/mL for vitamin E after 24 h.

Antiproliferative activity of the combinatorial treatment of PE38 with vitamin E

The antiproliferative effect of combinatorial treatments of PE38 with vitamin E was also investigated in this study. As presented in Figure 2, the coadministration of 0.1 mg/mL of vitamin E with increasing concentrations of PE38 (31.25–500 µg/mL) led to a significant enhancement of the antiproliferative effect of PE38 in MCF-7 cells. Compared to control, the viability of cells was significantly ($P < 0.001$) reduced under the combinatorial treatment. cell viability of 35.61% ± 4.29%, 37.8% ± 6.45%, 36.42% ± 5.79%, 32.33% ± 4.62%, and 29.97% ± 5.07% was detected after 24 h of exposure to vitamin E (0.1 mg/mL) coadministered with 31.25, 62.5, 125, 250, and 500 µg/mL concentrations of PE38, respectively [Figure 2].

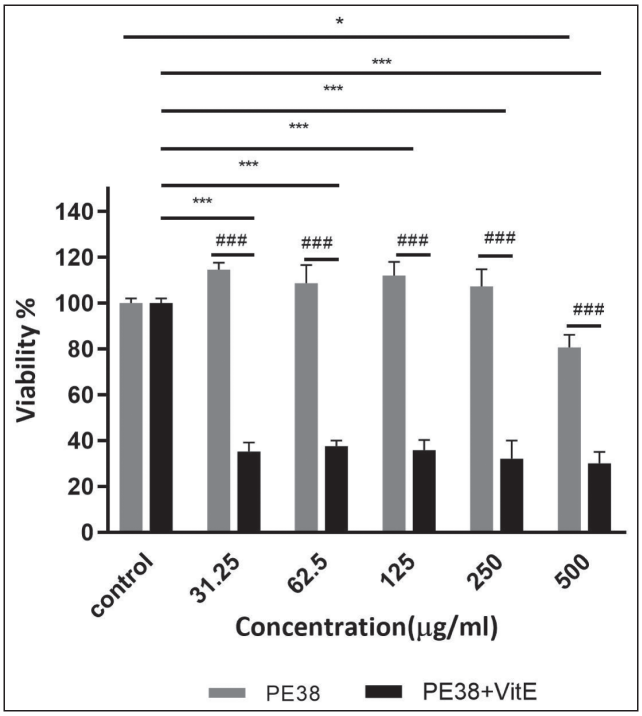

Figure 2: Antiproliferative activity of the combinatorial treatment of PE38 with vitamin E toward MCF-7 cells. Using MTT assay, the viability of cells was evaluated after 24 h of exposure to vitamin E (0.1 mg/mL) coadministered with 31.25, 62.5, 125, 250, and 500 µg/mL concentrations of PE38. Data are presented as mean ± standard deviation (SD) (n = 8). *P < 0.05, ***P < 0.001, and ###P < 0.001

Moreover, compared with the single administration of PE38 (in all concentrations), a markedly increase in the inhibitory effect was observed on the proliferation of MCF-7 cells treated with PE38 combined with vitamin E. The relevant IC_{50} value of the MCF-7cells was 15.67 µg/mL for combinatorial treatments of PE38 with vitamin E.

Discussion

Various recombinant forms of truncated PE have been previously expressed including PE38 that lacks Ia and a portion of domain Ib.[2] In this study, for the first time, the antiproliferative potential of PE38, vitamin E, and a combination of them were colorimetrically evaluated in a cellular breast cancer model (MCF-7). By cytotoxicity assay, increasing concentrations of PE38 up to 125 μg/mL had no cytotoxic effects on MCF-7 cells. Results indicated that only at the highest concentration (500 μg/mL), PE38 was slightly toxic [Figure 1A]. This result is consistent with a previous finding, in which cytotoxicity level of PE38 was studied.[13] No significant cytotoxicity for this recombinant form was shown toward human umbilical vein endothelial cell (HUVEC), MCF-7, and human fibroblast cells at concentration of 10 μg/mL.[13] But its translocation ability and inhibitory effect were retained when delivered as a conjugated molecule with a targeting agent (anti-VEGFR2/PE38). Cell viability was significantly decreased in the VEGFR2 expressing cell lines (HUVEC, MCF-7) by anti-VEGFR2/PE38 showing that the bioactivities of both anti-VEGFR2 antibody and PE38 toxin were maintained.[13] This low cytotoxic activity was also reported by other truncated forms. PE38KDEL is another form, which has shown slight level of toxicity on Hut102, ch-hep-1, and ch-hep-3 cells in MTS assay.[1]

Here, cytotoxicity potential of αT form of vitamin E was also evaluated using MTT assay. Compared to control cells, a significant reduction of viability was observed in MCF-7 cells treated with vitamin E in a dose-dependent manner [Figure 1B]. Results obtained here are in good agreement with Schwartz and Shklar report that indicated a selective cytotoxicity of αT on seven malignant cells including two oral carcinoma cell lines (SCC-25 and SQ-38), two lung carcinoma cell lines (CALV3 and SK-MES), two breast cancer cell lines (ZR75 and MCF-7), and one malignant melanoma cell line, A375. In Schwartz and Shklar's[14] study, compared with the untreated tumor cells, a consistent morphologic change as well as a decrease in proliferation was observed in tumor cells treated with vitamin E regardless of their original origin. This inhibition of proliferation was not reported in αT-treated normal human keratinocytes (NHK). Moreover, our results are in accordance with a previous study that showed the antiproliferative activity of αT in an *in vitro* assay at concentrations of 70 or 300 μM. αT was shown to give 50%–75% inhibition of cell density of squamous cell carcinoma (SK-MES) cells as well as 19%–36% inhibition of cell density of SCC-25 cells (oral carcinoma). The cell density of normal keratinocytes treated with vitamin E did not differ significantly from those of controls.[15]

The role of vitamin E in PE38 cytotoxicity level was also evaluated in this investigation. On the basis of obtained results, the coadministration of vitamin E at its minimum effective dose was shown to significantly enhance the antiproliferative effect of PE38 in MCF-7 cells [Figure 2]. However, the mechanisms involved in this synergistic effect remain to be elucidated. This effect may be attributed to permeability enhancement ability of vitamin E leading to a more effective inhibition of MCF-7 cells proliferation by the PE38. This effect was also supported by Giasuddin and Diplock,[16] who found that vitamin E could be able to act as a permeability enhancer. They simulated conditions of selenium, vitamin E, and essential fatty acid deficiency in a tissue culture model and determined the effect of the addition of vitamin E on permeability of the plasma membrane to 2-deoxyglucose. They found that the presence of linoleic acid, vitamin E, and cholesterol in the medium significantly could affect optimal growth and played a determinative role in the ability of the cell membrane to take up 2-deoxyglucose. Moreover, the maximum transport of 2-deoxyglucose was achieved when αT together with arachidonic acid or linoleic acid and cholesterol were present in the medium.[16] Similar to these reports, the Yu *et al*.'s[17] study showed that vitamin E-TPGS, D-αT polyethylene glycol 1000 succinate, could increase both solubility and Caco-2 cells permeability of amprenavir, a potent HIV protease inhibitor. *In vitro* permeability of celiprolol and paclitaxel was also reported to be enhanced with vitamin E-TPGS.[8,9]

In this study for the first time, vitamin E was used for delivery of PE38 to MCF-7 cell line. The obtained results showed that vitamin E could be used as a carrier agent for transferring PE38 or even other protein toxins through the cells. However, more studies are needed to confirm the role of vitamin E as a delivery agent.

Conclusion

In summary, here we showed that recombinant PE38 was slightly toxic to MCF-7 cells only in the highest concentration (500 μg/mL) used. In addition, compared to control cells, a significant decrease in viability was observed in MCF-7 cells treated with vitamin E in a dose-dependent manner. Also, our results for the first time showed that αT is able to enhance the antiproliferative activity of PE38 toward MCF-7 cells. However, the mechanisms involved in this synergistic effect remain to be elucidated.

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Conflicts of interest

There are no conflicts of interest.

References

1. Song S, Xue J, Fan K, Kou G, Zhou Q, Wang H, *et al*. Preparation and characterization of fusion protein truncated *Pseudomonas* exotoxin A (PE38KDEL) in *Escherichia coli*. Protein Expr Purif 2005;44:52-7.
2. Langari J, Karimipoor M, Golkar M, Khanahmad H, Zeinali S, Omidinia S, *et al*. *In vitro* evaluation of Vegf-*Pseudomonas* exotoxin: A conjugated on tumor cells. Adv Biomed Res 2017;6:144.

3. Pastan IH, Onda M, Liu W. *Pseudomonas* exotoxin A with less immunogenic B cell epitopes. Google Patents; 2018.
4. Kamal-Eldin A. Antioxidative Activity of Vitamin E. In: Weber P., Birringer M., Blumberg J., Eggersdorfer M., Frank J. (eds) Vitamin E in Human Health. Nutrition and Health. Cham. Totowa, NJ, USA: Humana Press, 2019.
5. Jiang Q. Natural forms of vitamin E: Metabolism, antioxidant, and anti-inflammatory activities and their role in disease prevention and therapy. Free Radic Biol Med 2014;72:76-90.
6. Prasad KN, Edwards-Prasad J. Effects of tocopherol (vitamin E) acid succinate on morphological alterations and growth inhibition in melanoma cells in culture. Cancer Res 1982;42:550-5.
7. Parsa A, Saadati R, Abbasian Z, Azad Aramaki S, Dadashzadeh S. Enhanced permeability of etoposide across everted sacs of rat small intestine by vitamin E-TPGS. Iran J Pharm Res 2013;12: 37-46.
8. Varma MV, Panchagnula R. Enhanced oral paclitaxel absorption with vitamin E-TPGS: Effect on solubility and permeability *in vitro*, *in situ* and *in vivo*. Eur J Pharm Sci 2005;25:445-53.
9. Cornaire G, Woodley J, Hermann P, Cloarec A, Arellano C, Houin G. Impact of excipients on the absorption of P-glycoprotein substrates *in vitro* and *in vivo*. Int J Pharm 2004;278:119-31.
10. Demirel-Yilmaz E, Dinçer D, Yilmaz G, Turan B. The effect of selenium and vitamin E on microvascular permeability of rat organs. Biol Trace Elem Res 1998;64:161-8.
11. Pourani Z, Hashemi A. Stability assessment of reference genes for reliable analysis of silver nanoparticles cytotoxicity in HepG2 cell line. J Clust Sci 2018;28:2623-34.
12. Jiang Q, Wong J, Fyrst H, Saba JD, Ames BN. γ-Tocopherol or combinations of vitamin E forms induce cell death in human prostate cancer cells by interrupting sphingolipid synthesis. PNAS 2004;101:17825-30.
13. Safari E, Zavaran Hosseini A, Hassan Z, Khajeh K, Ardestani MS, Baradaran B. Cytotoxic effect of immunotoxin containing the truncated form of *Pseudomonas* exotoxin A and anti-VEGFR2 on HUVEC and MCF-7 cell lines. Cell J 2014;16:203-10.
14. Schwartz J, Shklar G. The selective cytotoxic effect of carotenoids and alpha-tocopherol on human cancer cell lines *in vitro*. J Oral Maxillofac Surg 1992;50:367-73; discussion 373-4.
15. Schwartz JL, Singh RP, Teicher B, Wright JE, Trites DH, Shklar G. Induction of a 70 kD protein associated with the selective cytotoxicity of beta-carotene in human epidermal carcinoma. Biochem Biophys Res Commun 1990;169:941-6.
16. Giasuddin AS, Diplock AT. The influence of vitamin E and selenium on the growth and plasma membrane permeability of mouse fibroblasts in culture. Arch Biochem Biophys 1979;196:270-80.
17. Yu L, Bridgers A, Polli J, Vickers A, Long S, Roy A, *et al*. Vitamin E-TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability. Pharm Res 1999;16:1812-7.