



Short communication

Antiproliferative activity of parthenolide against three human cancer cell lines and human umbilical vein endothelial cells

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Abstract:

Parthenolide is a major sesquiterpene lactone derived from feverfew (*Tanacetum parthenium*) with known anti-inflammatory activity. Moreover, the anticancer potential of this compound was suggested. In this study, we determined the effect of parthenolide on proliferation of three human cancer cell lines: human lung carcinoma (A549), human medulloblastoma (TE671), human colon adenocarcinoma (HT-29) and human umbilical vein endothelial cells (HUVEC) *in vitro*. Cell proliferation was assessed by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IC₅₀ value (the concentration of drug necessary to induce 50% inhibition) together with confidence limits was calculated.

Parthenolide inhibited proliferation of all three types of cancer cells (A549, TE671, HT-29) and HUVEC with the following IC₅₀ values (in μM): 4.3, 6.5, 7.0 and 2.8, respectively. Thus, the antiproliferative potential of parthenolide was confirmed.

Key words:

parthenolide, human lung carcinoma (A549), human medulloblastoma (TE671), human colon adenocarcinoma (HT-29), human umbilical vein endothelial cells (HUVEC), proliferation, *in vitro*

Abbreviations: A549 – human lung carcinoma, HT-29 – human colon adenocarcinoma, HUVEC – human umbilical vein endothelial cells, JNK – Jun N-terminal kinase, MAP – mitogen-activated protein, MTT – 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, NF-κB – nuclear factor kappa B, TE671 – human rhabdomyosarcoma/medulloblastoma

Introduction

Parthenolide is the principal sesquiterpene lactone isolated from the herb feverfew (*Tanacetum parthenium*). Many studies have shown that parthenolide

has anti-inflammatory properties and is well known as an anti-inflammatory drug [6]. It has been historically used as a folk remedy to treat migraine, stomachache, toothache, menstrual irregularities, fever and rheumatoid arthritis [6, 15].

Parthenolide is a potent nuclear factor kappa B (NF- κ B) inhibitor which inhibits a common step in its activation by preventing the degradation of I κ B- α and I κ B- β . Specifically, it inhibits subunits β -I κ B of the I κ B kinase complex [4, 7]. NF- κ B is important for the expression of multiple cytokine genes involved in the cellular inflammatory, immune responses and has been also implicated in the regulation of cell proliferation, transformation, and tumor development. It regulates genes important for tumor invasion, metastasis and chemoresistance [12]. NF- κ B migrates into the nucleus and activates the expression of numerous target genes that are important for the cell proliferation [5, 11] and regulation of apoptosis [1]. It has been shown that NF- κ B is constitutively active in some cancers [12]. Besides the inhibition of DNA binding of transcription factors NF- κ B and STATs, the antitumor activity of the parthenolide is believed to be due to the activation of Jun N-terminal kinase (JNK), reduction in mitogen-activated protein (MAP) kinase activity and generation of reactive oxygen species [10]. The ability of parthenolide to alter the function of three transcription factors makes it an ideal antitumor agent that can sensitize cancer cells to chemotherapy by reducing the activity of antiapoptotic genes [12, 16].

In this study, the effect of parthenolide on viability of three cancer cell lines and human umbilical vein endothelial cells was investigated.

Materials and Methods

Drugs

Parthenolide was purchased from Tocris Bioscience, USA and suspended in DMSO.

Cancer cell lines

The human rhabdomyosarcoma/medulloblastoma cell line (TE671) was obtained from the European Collection of Cell Cultures (Centre for Applied Microbiol-

ogy and Research, Salisbury, U.K.). Human Caucasian lung carcinoma cell line (A549) and human colon adenocarcinoma cell line (HT-29) were obtained from the Institute of Immunology and Experimental Therapy Polish Academy of Sciences, Wrocław, Poland.

TE671 was grown in 1:1 mixture of DMEM and Nutrient mixture F-12 Ham (Ham's F-12) (Sigma). The culture medium for A549 cell line consisted of 2:1 mixture of DMEM/Ham's F-12. HT29 cell line was maintained in DMEM culture medium (Sigma). All media were supplemented with 10% fetal bovine serum (FBS, Life Technologies, Karlsruhe, Germany), penicillin (100 U/ml) (Sigma) and streptomycin (100 μ g/ml) (Sigma). Cultures were kept at 37°C in a humidified atmosphere of 95% air and 5% CO₂.

Human umbilical vein endothelial cells (HUVEC)

Human umbilical vein endothelial cells (HUVEC) were isolated from the umbilical cord according to the method described by Morgan [9]. The umbilical vein was washed with warm PBS supplemented with penicillin (200 U/ml), streptomycin (200 μ g/ml) and amphotericin B (0.5 μ g/ml). Next the lumen of the vein was digested by 0.2% collagenase type I (Sigma) solution for 10 min. Dislodged cells were suspended in culture medium and centrifuged at 1,000 rpm for 10 min. The cell pellet was resuspended in culture medium and transferred into 25 cm² tissue culture flasks (Nunc). The culture medium consisted of 1:1 mixture of DMEM and Nutrient mixture F-12 Ham (Ham's F-12) (Sigma), 10% FBS (Life Technologies), penicillin (100 U/ml), streptomycin (100 μ g/ml) (Sigma) and with 75 μ g/ml of endothelial cell growth factor (ECGF) (Sigma). Cells were grown at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Endothelial cell morphology was confirmed by typical cobble-stone morphology as well as by staining for von Willebrand factor (Dakopatts, Denmark). For experiments, HUVEC at passage 1–2 were plated on 96-well microplates at a density of 3×10^4 cells/ml.

Cell proliferation assessment

Cells were plated on 96-well microplates (Nunc) at a density of 1×10^4 (TE671, A549) and 3×10^4 (HT29). On the following day, the culture medium was removed and cells were exposed to serial dilutions of parthenolide prepared in the fresh medium.

Cell proliferation was assessed after 96 h by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Proliferation Kit I, Roche Diagnostics, Germany) in which the yellow tetrazolium salt is metabolized by viable cells to purple formazan crystals. Cells were incubated for 3 h with MTT solution (5 mg/ml). Formazan crystals were solubilized overnight in sodium dodecyl sulfate (SDS) buffer (10% SDS in 0.01N HCl) and the product was quantified spectrophotometrically by measuring absorbance at 570 nm wavelength using E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA).

Statistics

Data were presented as the mean value and standard error of the mean (SEM). Statistical analysis was performed using the ANOVA with Tukey *post-hoc* test. Significance was accepted at $p < 0.05$. The IC_{50} value (the concentration of drug necessary to induce 50% inhibition), together with confidence limits, was calculated using computerized linear regression analysis of quantal log dose-probit functions, according to the method of Litchfield and Wilcoxon [8].

Results

Parthenolide decreases viability of cancer cells

Parthenolide reduced viability of all three cancer cell lines in a concentration-dependent manner. The IC_{50} value of parthenolide against A549, TE671 and HT-29 was 4.3 (3.4–5.6) μ M, 6.5 (4.7–8.9) μ M and 7.0 (5.2–9.6) μ M, respectively (Fig. 1 A, B, C).

Parthenolide decreases viability of human umbilical vein endothelial cells (HUVEC)

Parthenolide decreased viability of HUVEC in a concentration-dependent manner. The IC_{50} value was 2.8 (2.3–3.3) μ M (Fig. 2).

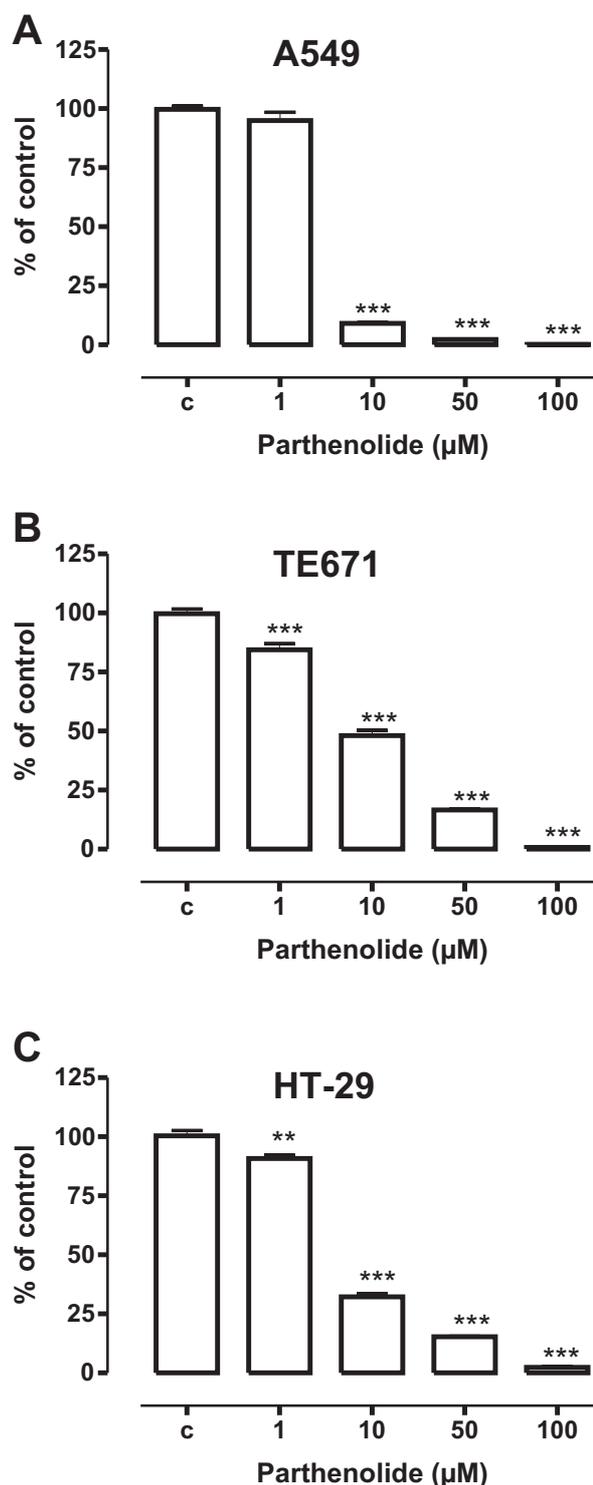


Fig. 1. Effect of parthenolide on viability of (A) human lung carcinoma cells (A549), (B) human rhabdomyosarcoma/medulloblastoma cells (TE671) and (C) human colon adenocarcinoma cells (HT-29) measured by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay *in vitro*. Results are expressed as the mean percentage of control \pm SEM of $n = 6$ independent experiments; ** $p < 0.01$; *** $p < 0.001$ vs. control. c – control

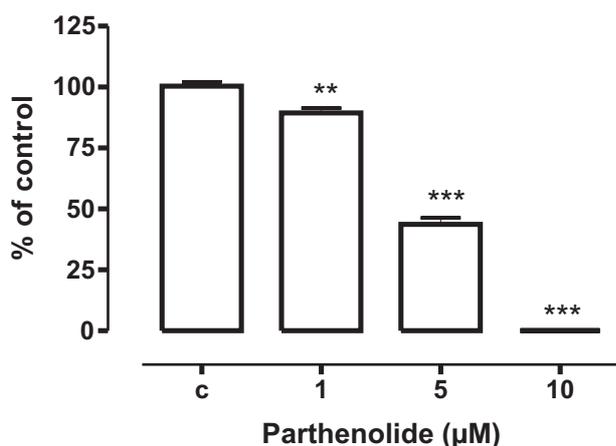


Fig. 2. Effect of parthenolide on proliferation of human umbilical vein endothelial cells (HUVEC) measured by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay *in vitro*. Results are expressed as the mean percentage of control \pm SEM of $n = 6$ independent experiments; ** $p < 0.01$; *** $p < 0.001$ vs. control. c – control

Discussion

It was found that parthenolide decreased proliferation of three human tumor cell lines A549, TE671 and HT-29 *in vitro*. Previously, it has been reported that parthenolide and golden feverfew extract exerted anti-proliferative and anticancer activity against different human cancer cell lines. They inhibited growth of human breast cancer cells, cervical cancer cells [19], pancreatic cancer cells [20], human lymphoma cell line (TK6), lymphocytic leukemia [3] and mouse fibrosarcoma [14].

Despite numerous studies with parthenolide showing its antitumor properties *in vitro*, there are only a few studies in which parthenolide was used in animal models of cancer *in vivo*. It was found that parthenolide possessed a strong anticancer activity in ultraviolet B-induced skin cancer in mice [18] and in metastases xenograft model of breast cancer *in vivo* [16].

The antitumor effects of parthenolide and the molecular mechanisms involved are poorly understood. It is believed to be due to the inhibition of DNA binding of transcription factors NF- κ B and STAT-3, reduction in MAP kinase activity and the generation of

reactive oxygen [10]. NF- κ B plays an essential role in preventing apoptotic cell death. A recent report demonstrated that parthenolide induced apoptosis in human colorectal cancer cells [21] and invasive sarcomatoid hepatocellular carcinoma cells, mainly through the induction of oxidative stress [17]. Wen and his colleagues demonstrated that parthenolide-induced apoptosis involved caspase activation and mitochondrial dysfunction in hepatoma cells [17]. Besides oxidative stress, multiple pathways might be involved in parthenolide-induced apoptotic cell death, including endoplasmic reticulum stress, intracellular thiol depletion, caspase activation, and mitochondrial dysfunction [17, 21]. Evidence has been provided that the inhibitory effects on activator protein-1 (AP-1) and MAP kinases serve as one of the underlying mechanisms for the cancer chemopreventive property of parthenolide [18].

Patel et al. demonstrated that parthenolide enhanced the sensitivity of human breast cancer cells to the chemotherapeutic drug paclitaxel via its inhibitory effect on NF- κ B [12]. Importantly, other authors have found, that co-treatment with the parthenolide markedly enhances sensitivity of resistant breast cancer tumor cells to antiestrogen tamoxifen [2] and fulvestrant [13]. NF- κ B is constitutively active in a subset of breast cancers, and parthenolide, a potent inhibitor of this transcription factor, may be useful in increasing the sensitivity of cancers with constitutively active NF- κ B [12].

Moreover, as shown by Yip-Schneider et al., treatment with the combination of parthenolide and sulindac inhibited cell growth synergistically or additively in pancreatic carcinoma cells [20]. In addition, this combination lowered the threshold for induction of apoptosis and these data provide preclinical support for a combined chemotherapeutic approach with NF- κ B inhibitors and non-steroidal anti-inflammatory drugs for the treatment of pancreatic adenocarcinoma [20].

We have also shown that parthenolide significantly inhibited the proliferation of HUVEC at concentrations comparable to those effective in the human cancer cell lines studied. It is well known that the transcription factor NF- κ B plays a critical role in resistance to apoptosis and the induction of angiogenesis and invasion of cancers [2]. It is possible that parthenolide, an inhibitor of NF- κ B, inhibits development of cancers also through inhibition of angiogenesis.

Based on our results and experiments performed by others, it may be suggested that parthenolide pos-

sesses antiproliferative properties and its use as an anticancer drug should be considered and carefully evaluated in clinical studies.

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