Research in Molecular Medicine

Cytotoxic Effects of Dimethylamino Parthenolide on the Rheumatoid Arthritis Fibroblast-like Synoviocytes Cell Line

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Citation Sadeqi S, Yazdani Z, Zafari P, Ghafari S, RafieiA. Cytotoxic Effects of Dimethylamino Parthenolide on the Rheumatoid Arthritis Fibroblast-like Synoviocytes Cell Line. Research in Molecular Medicine. 2025; 13(4):255-258. https://doi.org/10.32598/rmm.13.4.1465.1

https://doi.org/10.32598/rmm.13.4.1465.1

Article Type:
Research Paper

Article info:
Received: 13 May 2025
Revised: 17 Oct 2025
Accepted: 27 Oct 2025

Keywords:
Dimethylamino parthenolide (DMAPT), Rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS)

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease in which fibroblast-like synoviocytes (FLS) play a critical role in its pathogenesis. Due to the significant side effects associated with conventional treatments and the lack of response to the therapy in some patients, researchers are investigating novel therapeutic approaches that offer improved efficacy and a more favorable safety profile. This study aimed to investigate the cytotoxic activity of dimethylamino parthenolide (DMAPT) on the RA fibroblast-like synoviocytes (RA-FLS) cell line.

Materials and Methods: For this purpose, the RA-FLS cell line, previously generated in our unpublished studies, was treated with varying concentrations of DMAPT (0-160 µM) for 24 and 48 hours. Cell viability was assessed using the MTT assay. The half-maximal inhibitory concentration (IC_{50}) values were derived from dose-response curves fitted using GraphPad Prism software, version 8.

Results: Our results demonstrated that DMAPT exerts cytotoxic effects on the RA-FLS cell line, leading to a significant reduction in cell viability. This cytotoxic effect is dose-dependent, such that cell viability decreased with increasing dose. The half-maximal IC_{50} values of DMAPT for RA-FLS were 51.02 µM at 24 hours and 45.8 µM at 48 hours.

Conclusion: Our results demonstrated that DMAPT exerts cytotoxic effects on RA-FLS.

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Introduction

Rheumatoid arthritis (RA) is a chronic systemic autoimmune inflammatory disorder resulting from the interplay between genetics and environmental risk factors, particularly smoking. It predominantly affects synovial joints, causing persistent synovitis that leads to progressive articular damage [1]. The global prevalence of RA is estimated at approximately 0.5-1% in the general adult population. The disease exhibits a clear female predominance, affecting women two or three times more frequently than men [2, 3]. Multiple factors contribute to the pathogenesis of RA, among which fibroblast-like synoviocytes (FLS) play a pivotal role. These cells undergo aggressive transformation, exhibiting invasive behavior, hyperplasia of the synovial tissue, and direct destruction of cartilage and bone, thereby significantly contributing to disease initiation and progression [4, 5]. Conventional treatment strategies for RA are typically stepwise. Initial symptomatic management focuses on nonsteroidal anti-inflammatory drugs and glucocorticoids to alleviate pain and control acute-phase inflammation. Subsequent disease-modifying anti rheumatic drugs, including methotrexate, hydroxychloroquine, and sulfasalazine, represent the cornerstone of therapy aimed at achieving disease remission or low disease activity [6, 7]. However, these agents in some cases are associated with significant adverse effects, such as gastrointestinal bleeding and ulceration, renal impairment, cardiovascular complications, dermatologic reactions, and others [8]. Furthermore, a substantial proportion of patients exhibit inadequate response or intolerance to existing therapies. Consequently, current research efforts are increasingly directed toward the development of novel therapeutic agents that offer improved efficacy and more favorable safety profiles. Dimethylamino parthenolide (DMAPT) is a synthetic analogue of parthenolide characterized by markedly improved aqueous solubility and bioavailability compared to parthenolide. Parthenolide, the principal bioactive sesquiterpene lactone isolated from the medicinal herb feverfew (*Tanacetum parthenium*), has been traditionally employed for centuries in the management of migraine and RA. Parthenolide exerts potent anticancer effects by inhibiting proliferation and inducing apoptosis in various cancer cell lines, including colorectal carcinoma, hepatocellular carcinoma, and cholangiocarcinoma. It also sensitizes tumor cells to conventional chemotherapeutic agents. These effects are largely mediated through inhibition of nuclear factor kappa B (NF-κB) signaling, disruption of mitochondrial integrity, generation of reactive oxygen species (ROS), and subsequent activation of apoptotic pathways [9]. This study aimed to investigate the cytotoxic effects of DMAPT on the FLS cell line, a key effector cell type implicated in the pathogenesis of RA.

Materials and Methods

Cell culture

FLS cells previously isolated and established from primary synovial tissue samples of RA patients were thawed and cultured in dulbecco's modified eagle medium/nutrient mixture f12 (DMEM/F-12) medium supplemented with 10% fetal bovine serum (FBS). Cells were maintained in a humidified incubator at 37 ˚C with 5% carbon dioxide (CO_2). Upon reaching appropriate confluence, the cells in the culture flasks were used for subsequent experiments.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell viability assay

Upon reaching 80–90% confluence, rheumatoid arthritis fibroblast-like synoviocytes (RA-FLS) were detached using 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA), counted using a neubauer hemocytometer, and seeded into 96-well plates at a density of 7,000 cells per well in DMEM/F12 supplemented with 10% FBS. After allowing attachment (typically overnight), the cells were treated with varying concentrations of DMAPT (0, 1.25, 2.5, 5, 10, 20, 40, 80, and 160 µM) for 24 h and 48 h. Following treatment, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT solution was added to each well, and plates were incubated for 4 h at 37 ˚C in a humidified 5% CO_2 incubator in the dark. The supernatant was carefully aspirated, and 100 µL DMSO was added to each well to solubilize the formed formazan crystals. Absorbance was measured at 570 nm with a reference wavelength of 630 nm using a microplate reader. Cell viability was expressed as a percentage relative to the untreated control.

Results

DMAPt exerts a cytotoxic effect on RA-FLS

As shown in Figure 1, cell viability of RA-FLS decreased in a dose-dependent manner with increasing concentrations of DMAPT, indicating that the cytotoxic effect of DMAPT on RA-FLS is dose-dependent. Furthermore, the half-maximal inhibitory concentration (IC_{50}) of DMAPT in RA-FLS was determined following 24 h and 48 h treatment. As presented in Figure 2, the

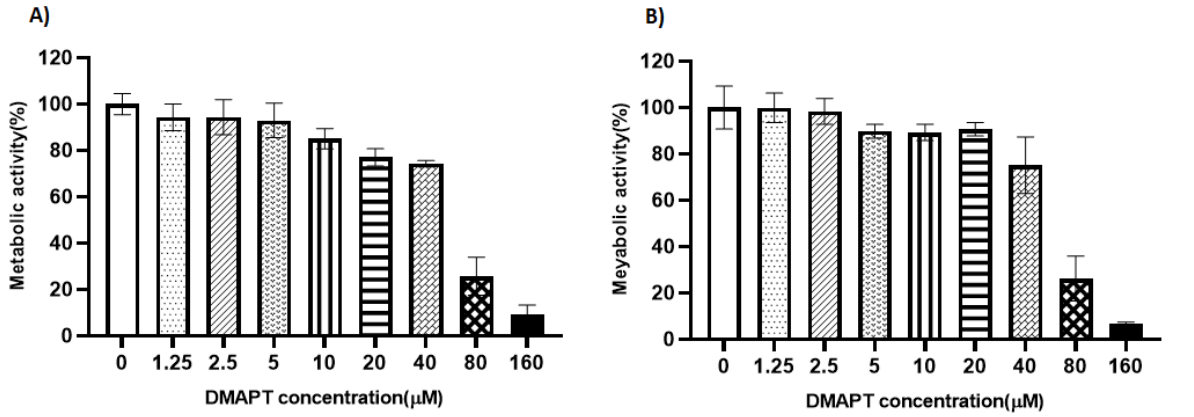

Figure 1. Cell viability of RA-FLS under treatment with various concentrations of DMAPT for 24 and 48 h

IC_{50} values were 45.8 μM for the 24-hour treatment and 51.02 μM for the 48-hour treatment.

Discussion

Previous studies have demonstrated that the IC_{50} value of DMAPT for primary chronic myeloid leukemia (CML) cells is approximately 7.5 μM at 24 hours and 5 μM at 48 hours for viability reduction. Similarly, the IC_{50} for pancreatic cancer cell lines (e.g. PANC-1) has been reported as 22.8 μM in MTT assays [10, 11].

Although no prior studies have investigated the cytotoxic effects of DMAPT on RA-FLS, the present study revealed that the IC_{50} values of DMAPT for RA-FLS were 51.02 μM at 24 hours and 45.8 μM at 48 h. These findings indicate that RA-FLS display markedly higher resistance to DMAPT-induced cytotoxicity compared to highly sensitive hematologic malignancy cells, such as primary CML progenitors (typically responsive in the 1–10 μM range) or certain solid tumor lines (e.g. pancreatic cancer). The slightly lower IC_{50} at 48 hours compared to 24 hours aligns with the expectation that prolonged exposure generally enhances potency, although cell-type-specific factors (e.g. adaptation, efflux mechanisms, or differential pathway engagement) may influence the time-dependent response.

DMAPT primarily exerts its effects through NF-κB inhibition and modulation of associated pathways, including ROS generation, inflammasome regulation, and signal transducer and activator of transcription (STAT) signaling in various models. Given the pivotal role of constitutive NF-κB hyperactivation in promoting the aggressive, invasive, and pro-inflammatory phenotype of RA-FLS—including enhanced cytokine secretion,

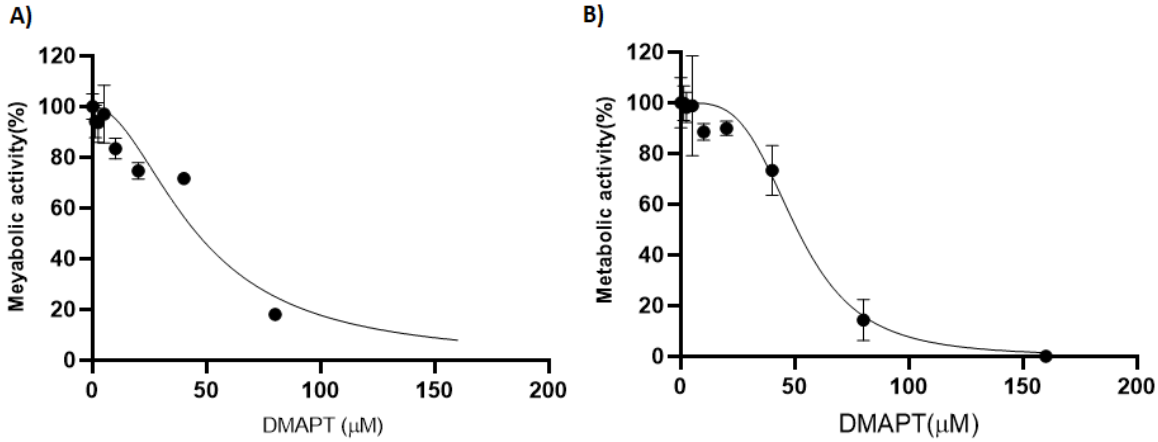

Figure 2. Half-maximal IC_{50} of DMAPT in RA-FLS following A) 24 and B) 48 h treatment

matrix metalloproteinase production, and synovial hyperplasia—the observed high IC_{50} values suggest that DMAPT has limited direct cytotoxic potential against RA-FLS at therapeutically achievable concentrations. Nevertheless, it may confer benefits in RA through sub-cytotoxic suppression of NF-κB-driven inflammatory and destructive processes, potentially supporting its evaluation as an adjunctive agent in combination therapies to augment sensitivity or target synergistic pathways in RA.

Conclusion

These findings demonstrate that DMAPT exerts cytotoxic effects on RA-FLS.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

Funding

This study was extracted from master's thesis of Sara Sadeghi, approved by the Department of Immunology and Microbiology, School of Sari Medical, Mazandaran University of Medical Sciences, Sari, Iran (Code: 21848), and financially supported by Mazandaran University of Medical Sciences, Sari, Iran (Code Patent Cell Line: 23909).

Authors contribution's

Investigation, data curation, formal analysis, and writing the original draft: Sara Sadeqi; Conceptualization, and project administration: Alireza Rafiei; Supervision, review and editing: Alireza Rafiei, Salman Ghafari, Parisa Zafari, and Zahra Yazdani.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgements

The authors thank Mazandaran University of Medical Sciences for its support and encouraging this work.

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