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In vitro antitumor efficacy of atorvastatin against acute monocytic leukemia cells

Karim S. El-Said [1,*], Bassant Ezzat Abdelmoaty[1], Elsayed I. Salim[2]

[1] Biochemistry Division, Chemistry Department, Faculty of Science, Tanta University, Tanta 31527, Egypt.
[2] Research Lab. of Molecular Carcinogenesis, Zoology Department, Faculty of Science, Tanta University, Tanta 31527, Egypt.

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Corresponding author:
Karim Samy El-Said, Ph. D.
Biochemistry Division, Chemistry Department، Faculty of Science, Tanta University, Egypt
E-mail: kreem.ali@science.tanta.edu.eg
Mobile: (+2)01002977062

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ABSTRACT

Chemotherapy is a potential setting for the treatment of acute monocytic leukaemia (AML). It has recently been demonstrated that statins (hydroxymethyl glutaryl CoA reductase inhibitors) are involved in some antitumor pathways against tumour cells to overcome side effects and increase the efficacy of chemotherapy. The present study was designed to address the molecular and biochemical effects of atorvastatin (ATOR) therapy against the human leukaemia monocytic THP-1 cell line. By *in vitro* studies, MTT assay was performed to determine the IC_{50} levels of ATOR against the THP-1 cell line. Also, apoptosis and cell cycle were determined after exposure of THP-1 cells to an IC_{50} dose of ATOR versus the non-treated THP-1 cells. Flow cytometry analysis showed apoptotic-inducing capacity and arrested the cell cycle in the G2/M phase after the treatment with ATOR. In conclusion, ATOR increases the percentages of apoptotic leukemic THP-1 cells and arrested their cell cycle.

Keywords: Atorvastatin, Antitumor effect; Acute monocytic leukemia.

1. Introduction

Cancer is the primary cause of death and a significant obstacle to raising life expectancy (Sung et al., 2020). Leukemia is a group of cancers that affect bone marrow leading to the production of aberrant blood cells (Varotto et al., 2022). The most prevalent form of leukemia linked to genetic abnormalities that are evident in morphological and immunophenotypic features is acute myeloid leukemia (AML) (Swerdlow et al., 2017). Myeloid cells' malignant growth, inhibition of differentiation, and disorganized apoptosis were the hallmarks of AML, which destroyed the healthy hematopoietic function. (Vetrie et al., 2020).

Chemotherapy and radiotherapy have been utilized to treat leukemia patients, also novel approaches such as stem cell therapy were used (Moreno et al., 2019). When used alone or in conjunction with other therapies, chemotherapy is thought to be the most effective and popular modality for treating various cancer types; however, it is toxic to important organs and has several side effects (EL-Hussein et al., 2021). Conventional chemotherapy has several drawbacks, such as difficult dosage selection, poor specificity, fast drug metabolism, and extremely dangerous side effects (Mondal et al., 2014). AML is a heterogeneous hematological malignant tumor originating from hematopoietic cells that frequently relapse after standard chemotherapy. Therefore, there is a need for the development of novel chemotherapeutic agents and regimens that could treat AML more effectively (Heo et al., 2020).

Statins are useful for the treatment of hypercholesterolemia and are among the most widely prescribed drugs. Beyond simply lowering cholesterol, statins have shown antitumor activity in various forms of cancer and could potentially decrease the risk of developing certain malignancies (Dale et al., 2006; Altwairgi et al., 2015). In terms of leukemias, some statin compounds have shown pre-clinical activity against acute lymphoblastic leukemia (ALL) and chronic lymphocytic leukemia (CLL) (Sheen et al., 2011; Żołnierczyk et al., 2013). Atorvastatin was reported to induce apoptosis in AML cell lines (Wong et al., 2002). The current investigation addressed the molecular and biochemical underpinnings of ATOR therapy against the THP-1 cell line.

2. Materials and Methods

Chemicals and reagents

Pharma Check Company (Cairo, Egypt) provided the 80 mg atorvastatin (ATOR) tablets. following supplies were acquired from Sigma-Aldrich (St Quentin Fallavier, France): fetal bovine serum, RPMI-1640, HEPES buffer solution, L-glutamine, phenol red, and 0.25% Trypsin-EDTA. Trypan blue dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were acquired from Sigma-Aldrich (St. Louis, Mo., USA). For flow cytometry, an apoptosis kit containing Annexin-V FITC and PI was obtained from Invitrogen (Catalog no: V13242). The dye cycle violet stain was purchased from Invitrogen, (Catalog no: V35003). Colorimetric assay kits for determination of the total antioxidants capacity (TAC) (Catalog no: E-BCK136-M), The USA-based Elabscience Biotechnology company was the source of the glutathione peroxidase (GSH-Px) activity (Catalog no: E-BC-K096-S), glutathione reductase (GR) activity (Catalog no: E-BC-K099-S), and malonaldehyde (MDA) level (Catalog no: E-BC-K025-S).

Cancer cell line

A human leukemia monocytic cell line (THP-1) was acquired from the VACSERA Co., Giza, Egypt's Tissue Culture Unit. Cells were propagated in culture media (RPMI-1640) containing 20 mM HEPES, L-glutamine, and phenol red. Cells were sub-cultured twice a week and kept at 37 °C in a humidified environment with 5% CO_2.

Cytotoxicity assessment by MTT assay

THP-1 cells were grown in RPMI-1640 medium and centrifuged for five minutes at 37 °C with 5% CO_2 and 95% air. At 70–80% confluence, passage was completed. ATOR was evaluated for its cytotoxic effects on THP-1 cell lines using the MTT colorimetric assay protocol. The yellow tetrazolium MTT is converted into the insoluble (E, Z)-5-(4,5-dimethylthiazol-2-yl)1,3-diphenylformazann (formazan) by the enzyme NAD(P)H-dependent cellular oxidoreductase. DMSO was used to dissolve the produced formazan, which produces a purple color with a 540 nm absorption peak. Indicating cell viability, the intensity of the purple color is inversely proportional to the number of cells.

Assessment of apoptosis using Annexin/PI staining by flow cytometry in harvested THP-1 cells

FITC-Annexin V and propidium iodide (PI) stains were used to stain THP-1 cells both in the absence of treatment and in the presence of ATOR. The monoclonal antibodies use recombinant annexin-V, which is conjugated to a fluorescent dye, to detect the externalization of phosphatidylserine in apoptotic cells, and PI, which stains necrotic cells red fluorescently, to identify dead cells. Apoptotic cells exhibit green fluorescence following treatment with both probes, whereas dead cells exhibit red and green fluorescence and living cells exhibit little to no fluorescence. After adding 400 µl of 1X binding buffer, the BD FACSC anto™ II flow cytometer was used for analysis. The data from the flow cytometer was analyzed using Beckman Coulter's Navios software.

Assessment of cell cycle assay by flow cytometry analysis in harvested THP-1 cells.

To display the distribution of DNA content at various stages of the cell cycle, THP-1 cells are fixed with cold methanol, stained with dye cycle violet stain, and then subjected to flow cytometry analysis. One mL of cell suspension in complete media at a cell concentration of 1×10^6 cells/mL was added to 1 µL of Vybrant® Dye Cycle™ Violet stain and mixed well. The

final stain concentration becomes 5 µM. The Stained cells are incubated at 37 ˚C for 30 minutes, protected from light, and kept cells at 37 ˚C until acquisition. Using an excitation wavelength of approximately 405 nm and an emission wavelength of approximately 440 nm, the samples are analyzed on a flow cytometer without being washed (Beckman Coulter, Navios software). The distribution of apoptotic sub-G1 cells, G0/G1 phase, S phase, and G2/M phase is shown by the population analysis. The CELLQUEST program (Becton Dickinson Immuno-cytometry Systems, San Jose, CA) was used to calculate the cell cycle.

Determination of antioxidants/oxidants biomarkers

Cell number (10%) divided by homogenization medium was the ratio at which the medium was added. In an ice water bath, cells were sonicated or manually ground. Spin for ten minutes at 1500 g., then take the supernatant and preserved it on ice for detection. Total antioxidant capacity was determined according to Bartosz (2003), glutathione peroxidase (Lubo et al., 2011), glutathione reductase activities (Gill et al., 2013), and malonaldehyde levels (Del et al., 2005) were determined.

Statistical analysis

The one-way ANOVA results were analyzed using Graph Pad Prism software (San Diego, CA) and Tukey was used for multiple comparisons to assess the significance of the differences between the treatment groups and the control. A p-value of less than 0.05 was considered acceptable.

3. Results

Effect of ATOR treatment on THP-1 cell viability.

The results show that ATOR has a potent anti-proliferative activity against THP-1 cells in a concentration-dependent manner. the IC_{50} value of ATOR was 0.6967 ± 0.0839 µg/mL after 24 hrs. and 0.6779 ± 0.2085 µg/mL after 48 hrs. (Fig. 1).

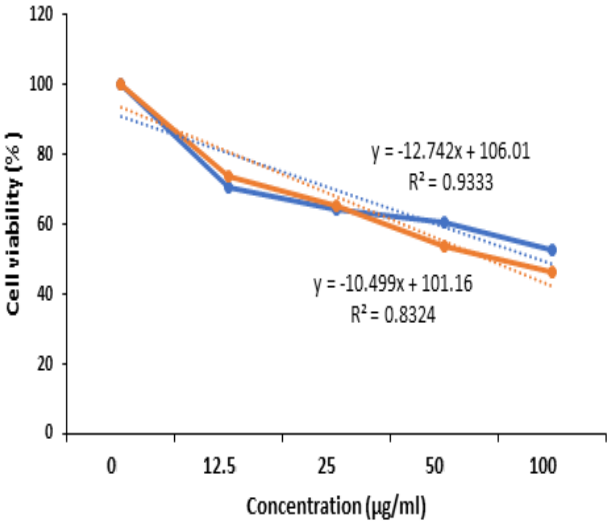

Fig. 1. Percentage of cell viability of THP-1 cells treated with ATOR after 24 and 48 hours.

Effect ATOR on the percentages of apoptotic and necrotic THP-1 cells

Using flow cytometry, the percentages of early, late, and necrotic THP-1 cells were ascertained. When compared to THP-1 cells that were not treated, the results demonstrate that the distribution of THP-1 cells based on their staining with Annexin/PI was altered following various treatments using ATOR. When compared to the untreated THP-1 cells, the treatment with ATOR resulted in a significant decrease in the percentage of live THP-1 cells and a significant increase in the percentage of apoptotic and necrotic THP-1 cells (Fig. 2).

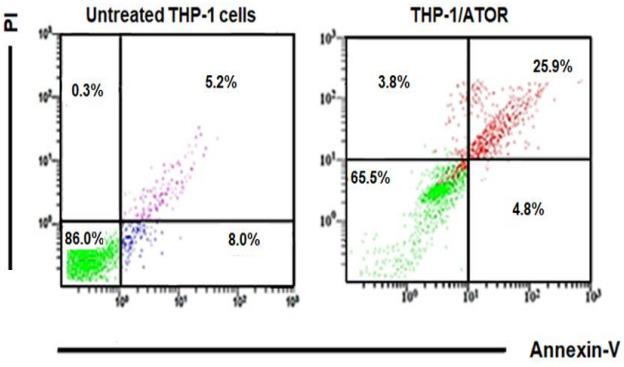

Fig. 2. Apoptosis in THP-1 cells treated for 48 hours is shown in representative flow cytometry plots using Annexin V-FITC/PI staining.

Cell cycle analysis

Using flow cytometry, the impact of ATOR on the THP-1 cell cycle was calculated. Compared with the untreated THP-1, the results revealed a cell cycle arrest in the G2/M phase (Fig. 3).

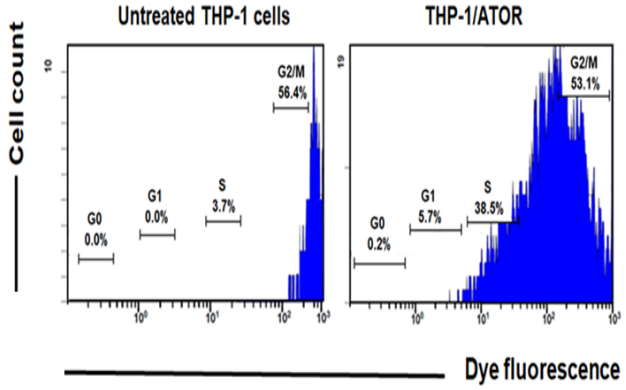

Fig. 3. Cell cycle analysis data in THP-1 cells with different treatments showing average percentage numbers of cells at G0/1, S, and G2/M stages of cell cycle. Flow cytometry histograms for cell cycle.

Effect of ATOR treatment on the antioxidant/oxidants' homeostasis in acute monocytic leukemia cells

The results show that the treatment with ATOR led to a significant increase in the total antioxidant level. The level of antioxidant was recorded in the THP-1 cells treated with ATOR (4.86 ± 0.54 U/mg protein), When compared to no-treated THP-1 cells, the activities of glutathione peroxidase (GPx) and glutathione reductase (GR) in THP-1 cells were significantly reduced ($p < 0.05$). Malondialdehyde (MDA) level was determined in the harvested THP-1 cells after the treatment with ATOR. The results showed that there was a significant decrease in the MDA level of THP-1 cells that were treated with ATOR (Table 1).

Table 1. Effect of the treatment with atorvastatin on the antioxidant/oxidant status of THP-1 cells.

Parameter/Condition	Untreated cells	ATOR
TAO (U/mg protein)	2.78 ± 0.13	4.86 ± 0.5
GSH-Px (nmol/mg protein)	51.34 ± 3.28	78.8 ± 4.9
GR (U/mg protein)	342.7 ± 35.6	650.5 ± 43.8
MDA (nmol/mg protein)	45.18 ± 3.26	29.8 ± 2.5

The values represented as means ± S.D.; TAO: Total antioxidants; GSH-Px: Glutathione peroxidase; GR: Glutathione reductase; MDA: Malondialdehyde.

4. Discussion

Acute leukemia is a malignant disorder with different rates in various areas of the world (Tebbi et al., 2021). Although there is a high death rate linked to AML, new treatments are improving results (Stubbins et al., 2022). ATOR treatment led to a decrease in cholesterol (Egom et al., 2016). Novel therapies are needed to improve the outcome of leukemia patients. Therefore, this study was designed to investigate the biochemical and molecular mechanisms of the treatment with ATOR against THP-1 cells to overcome the chemotherapy side effects and increase its efficacy. This study reported that ATOR was shown to exert a potent anti-proliferative activity on the cells. These findings agreed with previous studies that identified the potential predictive markers for clinical response to chemotherapeutics (Macanas-Pirard et al., 2017). Statins potentiate the cytotoxic effect in lymphoma by suppressing protein prenylation, and ATOR exerts antileukemia activity in K562 and HL60 cells (Zhang et al., 2019). Combining medications is a common method in the treatment of cancer. Natural anti-cancer products are widely accessible, safe, and they may be used with anti-cancer medications to have a synergistic therapeutic impact and minimize chemotherapy dosage, toxicity, and drug resistance (Chou et al., 2010). Furthermore, it has been demonstrated that the combination of statin with chemotherapy inhibits proliferation, and tumor-induced cytotoxicity in leukemic cells (Henslee et al., 2018).

In this study, the distribution of THP-1 cells according to their staining with Annexin/PI was changed after different treatments. These findings were in accordance with previous studies indicating the effects of the treatment with ATOR on apoptosis of AML cells (Qi et al., 2020; Tomic et al., 2022). It has been reported that the co-treatment with cytarabine and radotinib inhibited AML cell viability and induced apoptosis (Heo et al., 2020). A previous study reported that ATOR-induced apoptosis in AML cell lines involved ROS-related mitochondrial apoptotic signaling and activation of Bax/Caspase-9/Caspase-3/PARP pathway

(Zhan et al., 2019; Jansen et al., 2023) .

It has been reported that chemotherapy-induced survival regulation in AML cells increases the expression levels of apoptosis-related proteins and of cell-cycle inhibitors (Otevřelová et al., 2021). It has been reported that statins cause cells to become more sensitive to radiation by stopping them in the late G1 phase of the cell cycle (Altwairgi et al., 2015). ATOR induced G0/G1 arrest in HL60 cells by up-regulating p27 and down-regulating cyclin D1 and p-pRb, but it inhibited K562 and HL60 cell proliferation and induced G2/M cell cycle arrest in K562 cells by down-regulating cyclin B1 and cdc2 (Huang et al., 2019). AML cells are frequently characterized by altered cellular redox status and elevated reactive oxygen species (ROS) levels (Mattes et al., 2019). The present study showed that the ATOR led to significant enhancement of the total antioxidant levels. Previous study revealed that ATOR restored the changes of MDA, ROS, GPx and superoxide dismutase (SOD) (Wu et al., 2023). Because it encourages the activation of oncogenes and the inactivation of tumor suppressor genes, an increased redox state is correlated with mutational events (Zhang et al., 2015). These mutated pathways up-regulate pathways that support cell survival, proliferation, invasion, and migration, which leads to genomic instability and accelerates the onset and spread of leukemia (Liou et al., 2010) Interestingly, strong antioxidant systems in AML cells compensate for high ROS levels to prevent excessive ROS production and shield leukemic cells from oxidative stress-induced cell death (Snezhkina et al., 2019).

5. Conclusions

Treatment with ATOR drug showed potential antitumor efficacy in acute monocytic leukemia cells.

Conflicts of interest

There are no conflicts of interest to declare.

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