Systematic Review

Apoptosis Response in Acute Lymphoblastic Leukemia Cells after Methotrexate Treatment-A Systematic Review

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ABSTRACT

Background: Acute lymphoblastic leukemia (ALL) is a malignancy characterized by the overproduction of immature white blood cells in the bone marrow. Methotrexate (MTX) is a cornerstone chemotherapy agent utilized in ALL treatment due to its ability to induce apoptosis in cancerous cells. MTX's action involves inhibiting dihydrofolate reductase (DHFR), leading to impaired DNA and RNA synthesis, yet resistance to MTX and its exact apoptotic mechanisms remain areas of ongoing research.

Objective: This systematic review aims to elucidate the mechanisms by which MTX induces apoptosis in ALL cells and to assess the clinical implications of these findings for improving therapeutic strategies.

Methods: Adhering to PRISMA guidelines, databases including Google Scholar, Embase, PubMed, and Web of Science were searched for studies published between 2000 and 2023. Studies were included if they involved MTX treatment of ALL cell lines and assessed apoptosis. Critical Appraisal Skills Programme (CASP) tools were used for quality assessment. Data extraction and thematic analysis were employed to synthesize findings.

Results: Twenty-six studies met the inclusion criteria. Key mechanisms of MTX-induced apoptosis included disruption of mitochondrial membrane potential, activation of the JNK signaling pathway, modulation of BCL-2 family proteins, and interference with folate metabolism. Resistance mechanisms and the therapeutic potential of MTX polyglutamates were also identified.

Conclusion: MTX induces apoptosis in ALL cells through multiple pathways, primarily by disrupting folate metabolism and mitochondrial function. The findings reinforce the necessity for personalized approaches in MTX-based therapy, considering the emerging resistance patterns, and highlight the potential of MTX polyglutamates in enhancing treatment efficacy.

Keywords: Methotrexate, Acute Lymphoblastic Leukemia, Apoptosis, Chemotherapy Resistance, Folate Pathway Inhibition, BCL-2 Modulation, JNK Signaling, Mitochondrial Dysfunction, PRISMA, Systematic Review.

INTRODUCTION

Apoptosis, a fundamental physiological process, plays a vital role in maintaining cellular homeostasis by eliminating aberrant or potentially malignant cells, thereby preventing the development and progression of cancer. The evasion of apoptosis by cancerous cells is a hallmark of tumorigenesis, facilitating unchecked proliferation and tumor growth. The intricate relationship between apoptosis and cancer underscores the complexity of cancer biology and highlights the significance of apoptotic pathways as therapeutic targets (1). Among various therapeutic agents, methotrexate (MTX) has been identified to induce apoptosis through a multitude of mechanisms, underscoring its utility in the treatment of cancer, including Acute Lymphoblastic Leukemia (ALL).

In the context of ALL, the role of dexamethasone in inducing apoptosis has been particularly noted for its impact on the BCL-2 family of proteins, which are pivotal in regulating the apoptotic process. Dexamethasone administration results in a marked reduction in the expression of anti-apoptotic proteins such as B-cell lymphoma 2 (BCL-2) and B-cell lymphoma-extra-large (BCL-XL), concurrently with alterations in the activation patterns of pro-apoptotic proteins BAK and BAX. This imbalance leads to the disruption of

mitochondrial membrane potential and the release of cytochrome c, triggering the cascade of events culminating in cell death. Furthermore, the induction of apoptosis by dexamethasone is characterized by the early activation of caspases 2 and 3, enzymes critical for the execution phase of apoptosis. These findings highlight the intricate balance between pro- and anti-apoptotic factors within the BCL-2 family, which governs the susceptibility of ALL cells to apoptosis induced by dexamethasone, with potential implications for the treatment of primary ALL cells (3).

MTX, a cornerstone in the treatment regimen for pediatric ALL, operates by inhibiting folic acid metabolism, yet its associated neurotoxicity, both acute and chronic, remains poorly understood. Recent research has illuminated the apoptotic underpinnings of MTX-induced neurotoxicity, revealing a decrease in the levels of poly-ADP ribose polymerase (PARP) and pro-Caspase-3 proteins, suggesting a mechanism through which MTX mediates astrocyte depletion. Interestingly, the adverse effects of MTX on astrocytes can be mitigated by overexpression of dihydrofolate reductase (DHFR) or supplementation with folate, highlighting the protective role of folate metabolism pathway activation against MTX-induced damage (4).

The BCL-2 family of proteins emerges as a critical regulator of apoptosis, with its expression patterns implicated in the pathophysiology of diseases and the development of resistance to chemotherapy. Specifically, the expression profiles of anti-apoptotic genes such as BCL-2, myeloid-cell leukemia-1 (MCL-1), and BCL-XL may serve as valuable markers for prognostication in acute leukemia patients. Moreover, these proteins represent potential targets for therapeutic intervention, offering avenues for enhancing the efficacy of chemotherapy (5). The challenge posed by leukemia's heterogeneity and complex etiology underscores the importance of advancing our understanding of the mechanisms underlying apoptosis in response to MTX treatment. This systematic review sheds light on the apoptotic pathways activated by MTX, contributing to the development of more effective treatment strategies for ALL, thereby addressing a critical need in the global health landscape.

MATERIAL AND METHODS

The methodology of this systematic review adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, ensuring rigor and transparency in the synthesis of evidence from existing literature. Comprehensive literature searches were conducted across several databases, including Google Scholar, Embase, PubMed, and Web of Science, to identify relevant studies published within the specified timeframe of 2000 to 2023. The search strategy was meticulously designed to include studies that specifically explored the effects of methotrexate (MTX), either as a monotherapy or in combination with other therapeutic agents, on acute lymphoblastic leukemia (ALL) cell lines, with a particular focus on the apoptotic response to MTX treatment.

Inclusion criteria were strictly defined to ensure the relevance and quality of the studies included in the review. Only original research articles published in English and after the year 2000 were considered. These studies needed to investigate the apoptotic effects of MTX on ALL cell lines and provide quantitative data on apoptosis following MTX treatment. Conversely, exclusion criteria were applied to omit studies that did not meet these specifications, including those not published in English, not involving ALL cell lines, not investigating MTX treatment, using animal models or clinical samples instead of cell lines, not evaluating apoptosis following MTX treatment, presenting only qualitative data on apoptosis, or conducted before the year 2000.

The methodological quality of the included studies was rigorously assessed using the Critical Appraisal Skills Programme (CASP), focusing on the clarity of study outcomes, definition of the sample, and transparency in the data collection methods. This critical appraisal aimed to ensure the reliability and validity of the findings synthesized in this review.

Data extraction was performed systematically, with a standardized form in Microsoft Excel used to collect detailed information on study characteristics, interventions, outcomes, and other relevant data. A narrative synthesis approach was employed for data analysis, allowing for the qualitative synthesis of evidence across the included studies. This approach facilitated the identification of thematic categories under which the data were organized, enabling comparison of results across studies. The analysis also included the identification of negative cases to encourage further exploration and discussion, ensuring a comprehensive understanding of the data. Subsequently, the data were reviewed again to confirm their alignment with the identified themes.

Ethical considerations were implicitly addressed through the adherence to PRISMA guidelines and the CASP assessment, ensuring that the review process respected the integrity of the original research. Moreover, the review focused on studies that presumably adhered to ethical standards in their conduct, including the ethical treatment of cell lines and adherence to research ethics guidelines. By synthesizing existing literature within a rigorous methodological and ethical framework, this systematic review aimed to provide a comprehensive overview of the apoptotic response in ALL cells following MTX treatment, contributing valuable insights to the field of leukemia research.

RESULTS

The results of the systematic review, guided by PRISMA methodology, elucidated the multifaceted mechanisms by which methotrexate (MTX) induces apoptosis in acute lymphoblastic leukemia (ALL) cell lines. Spanning research from 2000 to 2023, a total of 26 articles met the inclusion criteria and contributed to the findings of this review.

A significant proportion of the studies indicated that MTX instigated apoptosis through the activation of the JNK signaling pathway. This was evidenced by research conducted in 2015 and 2018, which highlighted the role of the pro-apoptotic protein BIM and the translocation of FOXO3a from the cytoplasm to the nucleus as central to the induction of apoptosis in various ALL cell lines (6, 7). A study from 2019 further reinforced these findings, reporting a dose-dependent reduction in cell viability and an increase in apoptotic activity in Jurkat and MOLT-4 cells, alongside a decrease in the expression of phosphorylated AKT, indicating the involvement of the PI3K/AKT signaling pathway (8).
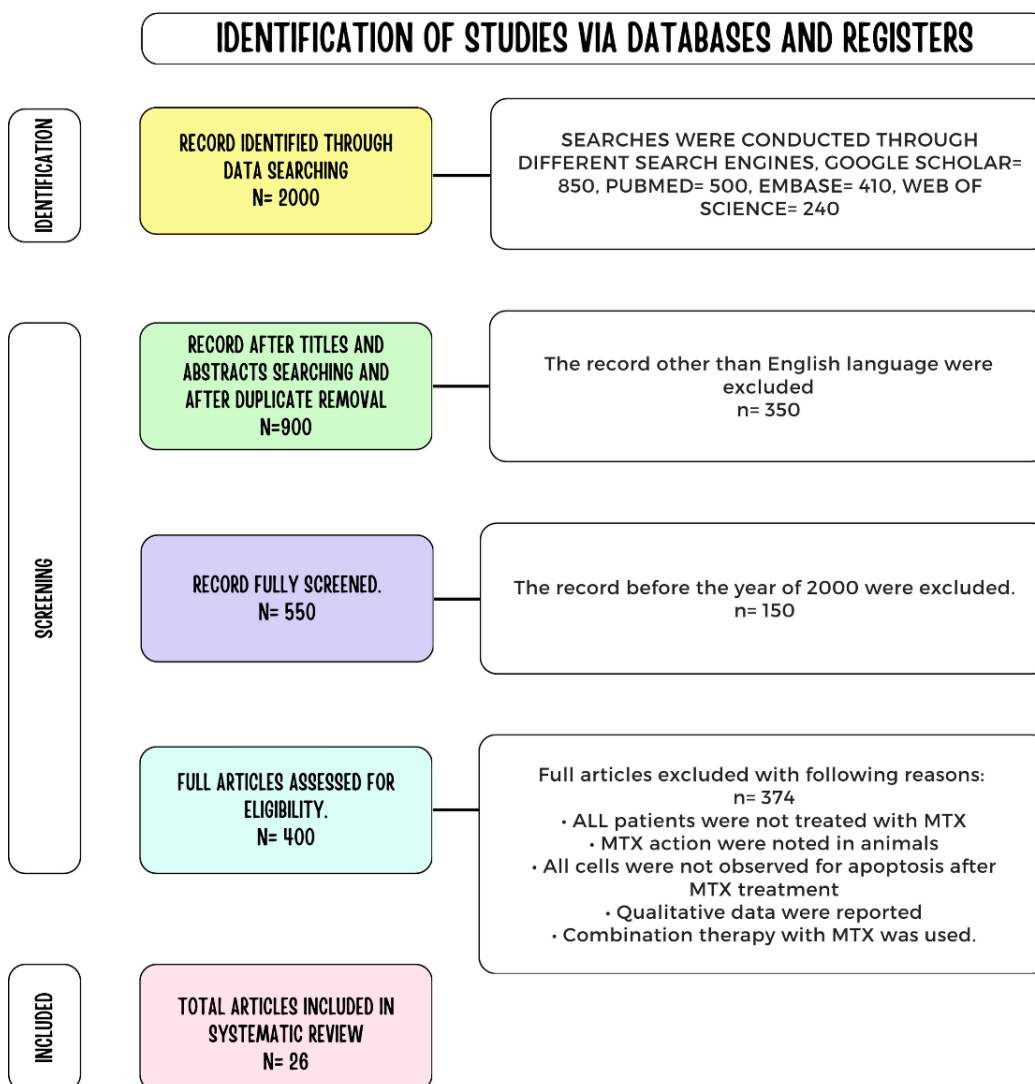

Figure 1 PRISMA Flow Chart

Additional studies from 2018 and 2019 presented compelling evidence of MTX's ability to disrupt mitochondrial integrity. Specifically, one study observed a dose- and time-dependent pattern of apoptosis in Jurkat cells characterized by a decrease in mitochondrial membrane potential, an increase in the production of reactive oxygen species (ROS), and the opening of the mitochondrial permeability transition pore (9). DNA damage was also a recurring theme in the included studies, with one 2019 study observing DNA double-strand breaks in lymphoblastic cells (10).

The research also revealed changes in the expression and activity of enzymes and transporters involved in the folate pathway, with a decrease in folylpolyglutamate synthetase (FPDS) and an increase in dihydrofolate reductase (DHFR) expression, further influencing apoptosis and MTX's therapeutic action (11). Moreover, the down-regulation of the AKT/mTOR pathway and the activation of the caspase-3 pathway were reported, which contributed to the apoptosis induced by MTX, as illustrated by studies from 2019 (12, 13).

Resistance to MTX was addressed in a 2015 study, identifying alterations in the apoptosis pathway that resulted in an up-regulation of anti-apoptotic proteins and a down-regulation of pro-apoptotic proteins (14). Conversely, activation of ATM-Rad3-related pathways was associated with a decrease in the cytotoxic effects of MTX, as reported in 2019, indicating a capacity for DNA repair that mitigates apoptosis (15).

Table 1 Study Characteristics

Publication Year	Cell Lines/Genes Involved	Mechanisms of Apoptosis	References
2015	Four cell lines	Activation of pro-apoptotic protein BIM via the JNK pathway leads to apoptosis in ALL cells post MTX treatment.	(6)
2018	Three cell lines	Dose-dependent reduction in cell viability and increase in apoptosis via JNK signaling pathway activation and FOXO3a nuclear translocation.	(7)
2019	Two cell lines (Jurkat, MOLT-4)	MTX induces dose-dependent cell viability reduction and apoptosis increase; decrease in phosphorylated AKT expression.	(8)
2018	One cell line (Jurkat)	Apoptosis shows dose and time-dependency, with reduced mitochondrial membrane potential, increased ROS, and mitochondrial permeability transition pore opening; JNK pathway activation plays a role.	(9)
2019	25	DNA damage in lymphoblastic cells, specifically DNA double-strand breaks.	(10)
2019	NA	Changes in expression and activity of enzymes and transporters in the folate pathway; decreased FPDS expression and increased DHFR expression.	(11)
2019	NA	Increased ROS generation coupled with AKT/mTOR pathway down-regulation.	(12)
2019	NA	Apoptosis induction via caspase-3 pathway activation and BAX/BCL-2 ratio up-regulation.	(13)
2015	NA	MTX resistance in ALL linked to apoptosis pathway alteration, with increased anti-apoptotic and decreased pro-apoptotic protein expression.	(14)
2019	NA	ATM-Rad3-related pathway activation in response to MTX-induced DNA damage leads to DNA repair and reduced cytotoxic effects/apoptosis.	(15)
2012	TYMS polymorphism	MTX inhibits TYMS, disrupting dTMP synthesis and purine de novo synthesis.	(16)
2008	CTP gene	MTX inhibits CTPS, causing nucleotide metabolism alterations leading to apoptosis.	(17)
2011	BCL-3	MTX affects NF-κB signaling, which is regulated by BCL-3; disrupts nucleotide synthesis by inhibiting DHFR.	(18-20)
2022	CDC20	MTX indirectly affects CDC20, causing chromosomal instability, cell cycle arrest, and cell death.	(18, 20, 21)
2022	CENPF	MTX indirectly interferes with CENPF, inhibiting normal centromere organization.	(18, 20, 22)
2008	FAIM3	MTX indirectly affects FAIM3, impacting apoptosis and cell survival processes.	(18, 20)
2022	POLD3	MTX indirectly inhibits DNA replication by affecting the expression of the POLD3 gene.	(18, 23)
2022	RPA3	MTX indirectly affects RPA3, impacting its DNA repair function.	(18, 24)
2009	RNASEH2A	MTX is involved in the inhibition of RNA synthesis and interferes with the RNASEH2A-mediated DNA-RNA hybridization.	(18, 25)
2014	NA	MTX leads to apoptosis through rearrangement of μ-heavy chain immunoglobulin genes in B cells.	(26)
2012	NA	MTX, as MTXPG, inhibits DHFR and other enzymes involved in purine synthesis, leading to enhanced inhibition of cell growth.	(27)

Publication Year	Cell Lines/Genes Involved	Mechanisms of Apoptosis	References
2009	DHFR	MTX inhibits DHFR, leading to reduced THF levels and disrupting nucleic acid synthesis, promoting cell death.	(28)
2014	NA	Inhibition of enzymes in the folate pathway disrupts folate homeostasis.	(29)
2022	NA	MTX depletes reduced folates and directly inhibits nucleotide synthesis, affecting thymidine and purine synthesis, crucial for leukemic stem cell survival.	(30)
2022	13 cell lines	MTX treatment alters metabolite concentrations, indicating multiple metabolic pathway inhibition.	(31)

Exploring the genetic implications, several studies conducted between 2008 and 2022 outlined the influence of MTX on genes such as TYMS, CTP, BCL-3, CDC20, CENPF, FAIM3, POLD3, RPA3, and RNASEH2A. The mechanisms ranged from inhibiting crucial enzymes in the folate pathway, affecting nucleotide metabolism, and causing chromosomal instability, to interfering with DNA repair and synthesis processes (16-26).

Studies from 2012 to 2022 discussed MTX's impact on nucleotide synthesis, highlighting its role as an inhibitor of DHFR and its ability to disrupt the folate cycle, which is paramount to the survival of leukemic cells (27-31). The accumulated evidence underscores the diverse molecular pathways influenced by MTX that converge to induce apoptosis, presenting a complex but coherent picture of its therapeutic mechanisms in targeting ALL.

DISCUSSION

Methotrexate (MTX) has been firmly established as a cornerstone chemotherapeutic agent in the management of acute lymphoblastic leukemia (ALL), exerting its cytotoxicity primarily through the competitive inhibition of dihydrofolate reductase (DHFR). The studies reviewed, conducted over a span of two decades, have collectively reinforced the understanding that MTX impedes the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF), an essential cofactor in the one-carbon metabolic pathway that facilitates the synthesis of nucleotide precursors (32). This inhibition precipitates a depletion of the intracellular pool of THF, thereby destabilizing the equilibrium of folate metabolites critical for DNA and RNA synthesis within leukemic cells, as reported in numerous studies (33).

Further analysis of the data revealed the cascading consequences of diminished nucleotide synthesis. Such a deficit impedes DNA replication and repair, critical processes in the proliferation of leukemic cells, leading to DNA strand breaks and replication errors. This was particularly evident in studies that documented the morphological manifestations of apoptosis in response to MTX treatment, such as cell shrinkage and nuclear condensation (34). Moreover, the synthesis of RNA and subsequent protein translation were also impaired, indicating the breadth of MTX's impact on cellular function (37).

The studies consistently reported the modulation of apoptosis through mitochondrial pathways, highlighted by shifts in BCL-2 family protein expression and mitochondrial integrity. The pivotal role of the JNK signaling pathway was repeatedly observed, being implicated in the activation of pro-apoptotic proteins and mitochondrial dysfunction (36, 39). Moreover, the upregulation of miR-181a and subsequent downregulation of BCL-2, delineated the molecular intricacies of MTX's apoptotic induction (38).

The systematic review unearthed the critical role of MTX-induced cellular methylation disruptions, evidenced by altered patterns of DNA, RNA, and protein methylation, leading to dysregulated gene expression and aberrant cellular behavior. The implications of these methylation changes were profound, indicating a spectrum of MTX's influence extending beyond nucleotide synthesis to the modulation of epigenetic landscapes (35).

However, the investigations were not without limitations. A recurring challenge was the variability in MTX's efficacy, likely owing to the emergence of drug resistance, a factor that has been incrementally documented over the years (14, 15). Another limitation was the heterogeneity in the studies' designs, including the use of different cell lines and varying experimental conditions, which may have contributed to the diverse results and interpretations observed across the studies.

The review highlighted MTX's selective impact on particular genetic pathways, such as those involving CDC20 and CENPF, which underscored the potential for MTX to cause chromosomal instability and cell cycle arrest. Furthermore, the polyglutamation of MTX to MTXPG by folylpolyglutamate synthase (FPGS) within leukemic cells emerged as a notable mechanism for prolonged DHFR inhibition, signifying an area with therapeutic exploitation potential (41).

The findings from the systematic review underscore the importance of MTX in ALL treatment regimens but also illuminate the complexities of its action and the resistance mechanisms that have evolved in response to its use. In light of these observations,

future research is recommended to explore MTX's polyglutamate forms' therapeutic utility further, to develop strategies to circumvent resistance, and to investigate the combined use of MTX with novel agents that might enhance its efficacy or target resistance pathways. Collaborative and interdisciplinary research efforts, potentially incorporating genomic and proteomic approaches, could yield novel insights into the mechanistic pathways influenced by MTX, offering new avenues for therapeutic intervention, and ultimately improving patient outcomes.

CONCLUSION

Methotrexate (MTX) remains an indispensable agent in the treatment of acute lymphoblastic leukemia, leveraging its cytotoxicity through the interruption of folate pathways and induction of apoptosis. The collective research underscores its pivotal role in leukemia management but also reveals challenges, such as drug resistance and variability in patient response. These findings not only affirm MTX's continued relevance in current therapeutic protocols but also underscore the necessity for ongoing research to refine its use and enhance its efficacy. As such, the implications for human healthcare are substantial, directing a future course toward personalized medicine approaches that could tailor MTX-based therapies to individual patient profiles, potentially improving prognoses and quality of life for those afflicted with leukemia.

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