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CYCLOPHOSPHAMIDE-INDUCED IMMUNOLOGIC AND MORPHO-FUNCTIONAL ALTERATIONS OF THE SPLEEN IN EXPERIMENTAL MODELS WITH EMPHASIS IN CHINCHILLA RABBITS

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Abstract

Cyclophosphamide is an alkylating agent widely used in oncology, autoimmune diseases, and experimental immunosuppression models. Despite its therapeutic efficacy, cyclophosphamide induces profound immunotoxic and morpho-functional alterations in lymphoid organs, particularly the spleen. This literature review critically analyzes experimental data from the last decade, with special emphasis on splenic injury induced by cyclophosphamide in Chinchilla rabbits and other animal models.

Experimentally, cyclophosphamide undergoes hepatic bioactivation, leading to oxidative stress, mitochondrial dysfunction, and activation of intrinsic apoptotic pathways. These molecular processes result in selective lymphocyte depletion, alterations in cytokine profiles, and disruption of splenic microarchitecture.

From an immunological perspective, cyclophosphamide suppresses T- and B-cell proliferation, reduces the $CD4^+/CD8^+$ ratio, weakens the humoral immune response, and alters macrophage and dendritic cell function. Morphologically and functionally, white pulp atrophy, regression of germinal centers, reduction of the marginal zone, sinusoidal dilation, vascular congestion, and increased apoptotic index are observed.

Oxidative stress and mitochondria-mediated apoptosis represent key mechanisms linking cellular injury to organ-level dysfunction. In rabbit models, including the Chinchilla breed, dose- and duration-dependent changes are generally similar to those observed in rodents; however, species-specific differences exist in lymphoid organization and regenerative capacity. This review integrates pharmacological, immunological, and histopathological findings, identifies methodological limitations, and outlines directions for future research.

1. Introduction

Cyclophosphamide is one of the most widely used cytotoxic and immunosuppressive agents in clinical practice. Initially developed as an antineoplastic drug, it later gained broad application in autoimmune diseases, organ transplantation, and experimental immunosuppression models (Emadi et al., 2009; Sistigu et al., 2014). Its ability to suppress lymphocyte proliferation has made it an essential tool in immunological research.

The spleen is the largest secondary lymphoid organ and plays a central role in systemic immune surveillance, antigen presentation, and humoral immunity. It continuously produces, activates, and renews large populations of lymphoid cells, many of which are in an active proliferative state. Rapidly dividing cells are particularly sensitive to DNA-damaging agents or substances that disrupt cell replication. Moreover, the spleen possesses an extensive and well-perfused vascular system, allowing high concentrations of circulating drugs and reactive metabolites to reach the tissue. Consequently, splenic tissue is especially vulnerable to cytotoxic compounds and oxidative stress–inducing factors. Experimental administration of cyclophosphamide frequently results in splenic atrophy and functional immunosuppression (Zhang et al., 2018).

Chinchilla rabbits are increasingly used in experimental immunology due to their well-developed splenic architecture and clinical–experimental relevance. However, compared to murine and rat models, the mechanisms of cyclophosphamide-induced alterations in rabbits remain insufficiently investigated.

2. Pharmacological and Immunological Mechanisms of Cyclophosphamide

Cyclophosphamide is metabolized in the liver by cytochrome P450 enzymes (primarily CYP2B6 and CYP3A4) into 4-hydroxycyclophosphamide. This metabolite subsequently decomposes into two major cytotoxic products: phosphoramide mustard and acrolein (Emadi et al., 2009).

Phosphoramide mustard forms inter- and intra-strand crosslinks at the N7 position of guanine in DNA. As a result, DNA replication and transcription are impaired, leading to cell cycle arrest and apoptosis. Rapidly proliferating T and B lymphocytes are particularly sensitive.

Acrolein primarily induces toxicity through oxidative stress and lipid peroxidation. It depletes glutathione and promotes the accumulation of reactive oxygen species (ROS) (Zhang et al., 2018).

Immunologically, cyclophosphamide suppresses clonal expansion of T cells, reduces IL-2 production, and decreases Th1 cytokines such as IFN-γ and TNF-α. B-cell proliferation and antibody synthesis decline significantly. At low doses, a transient reduction in regulatory T cells (Tregs) may occur, whereas higher doses result in generalized immunosuppression (Sistigu et al., 2014).

3. Normal Structure and Immunological Function of the Spleen

The spleen consists of white pulp and red pulp, separated by the marginal zone.

White pulp contains periarteriolar lymphoid sheaths (PALS) and lymphoid follicles. PALS are rich in $CD3^+$ T cells, while follicles contain $CD20^+$ B cells. Germinal centers form during antigenic stimulation.

The marginal zone contains specialized macrophages and B cells responsible for trapping blood-borne antigens.

The red pulp consists of Billroth cords and venous sinusoids and plays a role in erythrocyte filtration and iron metabolism.

This microarchitecture ensures coordination between innate and adaptive immune responses.

4. Immunological Changes Induced by Cyclophosphamide

Cyclophosphamide reduces proliferating T and B lymphocytes, causing profound lymphopenia. $CD4^+$ T cells decrease, leading to a reduced $CD4^+/CD8^+$ ratio (Li et al., 2019). IL-2 expression declines.

B cells, particularly in germinal centers, are markedly reduced, resulting in decreased IgM and IgG production.

Macrophage phagocytic activity and cytokine secretion are altered. Consequently, both humoral and cellular immunity are weakened.

5. Morpho-Functional and Histopathological Changes in the Spleen

Cyclophosphamide induces white pulp atrophy, regression of germinal centers, and thinning of PALS. The marginal zone becomes narrowed.

In the red pulp, sinusoidal dilation, vascular congestion, and increased erythrophagocytosis are observed. The number of apoptotic cells progressively increases (Zhang et al., 2018).

High doses or chronic exposure may lead to stromal remodeling and fibrosis.

6. Molecular and Oxidative Stress Mechanisms

DNA crosslinking activates the p53 pathway, increasing pro-apoptotic proteins such as Bax. Mitochondrial membrane permeability increases, cytochrome c is released, and the caspase cascade is initiated.

Elevated ROS levels damage lipids, proteins, and DNA. Antioxidant defenses such as glutathione, superoxide dismutase, and catalase decrease. Lipid peroxidation intensifies.

Caspase-3 activation represents the final stage of lymphocyte apoptosis.

7. Experimental Evidence in Rabbits and Other Animal Models

Recent studies in rabbits (10–50 mg/kg) demonstrate reduced splenic mass and regression of lymphoid tissue. In Chinchilla rabbits, germinal centers are significantly diminished, and oxidative stress markers increase.

Rodent models show similar dose-dependent immunosuppression (Li et al., 2019; Zhang et al., 2018).

However, data on rabbits remain limited, highlighting the need for standardized experimental investigations.

9. Discussion

Cyclophosphamide induces combined immunological and morphological alterations through DNA damage and oxidative stress. Lymphocyte depletion explains white pulp atrophy and germinal center regression, while reactive oxygen species amplify apoptotic signaling.

Although rabbit models have high translational relevance (bridging experimental research and clinical application), available data remain insufficient.

11. Conclusion

Cyclophosphamide induces dose-dependent profound immunological and morpho-functional alterations in the spleen. DNA alkylation, oxidative stress, and caspase-mediated apoptosis constitute the principal mechanisms. Chinchilla rabbits represent a promising but insufficiently studied experimental model.

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