WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Pharmaceutical and Pharmacological Insights into Cyclophosphamide

Anjana Lekshmi S S[a] , Nashwa R P[b], Nabeesath Sahada[b],Nafeesath Ashifa N H[b]

[a] Lecturer, Malik Deenar College of Pharmacy, Seethangoli, Kasaragod, Kerala-671321
Affiliated to Kerala University of Health Sciences, Thrissur
[b]B.Pharm Student, Malik Deenar College of Pharmacy, Seethangoli, Kasaragod, Kerala 671321
Affiliated to Kerala University of Health Sciences, Thrissur.

ABSTRACT :

Cancer represents a complex group of diseases characterized by uncontrolled cell growth and spread, often driven by genetic mutations and environmental factors. Among these, malignant lymphomas- specifically Hodgkin and non-Hodgkin lymphoma- are cancers of the lymphatic system marked by disruptions in apoptosis, immune regulation, and epigenetic alterations. Cyclophosphamide, a nitrogen mustard-derived alkylating agent, is widely employed in chemotherapy, especially for lymphomas. It acts by cross-linking DNA strands, ultimately inhibiting replication and triggering cell death. This study details the pharmacological profile, mechanism of action, pharmacokinetics, and side effects of cyclophosphamide, along with its physicochemical and micromeritic preformulation properties, including solubility, melting point, partition coefficient, pKa, and flow characteristics. These parameters are crucial for optimizing its formulation and therapeutic efficacy in clinical oncology.

Keywords: Cancer, Cyclophosphamide, Alkylating agents, Preformulation studies

INTRODUCTION

A class of disorders known as cancer is defined by the unchecked development and dissemination of aberrant cells. Death may ensue if the spread is not stopped. Any region of the body may be affected, and it is brought on by genetic abnormalities that are either acquired or inherited and cause the normal cellular functions of growth, division, and death to be dysregulated. Prostate, colorectal, lung, and breast cancers are common varieties. The development of cancer is influenced by a number of factors, including genetics, lifestyle, infections, and environmental exposures[(1)].

1.1 MALIGNANT LYMPHOMA

A class of tumors known as malignant lymphomas develops in the lymphatic system and mostly affects lymphocytes, which are white blood cells essential to the immune response. Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) are the two main types, and each has unique clinical and pathological characteristics. The two major categories are Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL), each with distinct pathological and clinical features.

1.1.1 PATHOPHYSIOLOGY

1.1.1.1 Chromosomal translocations and genetic mutations

• The clonal growth of lymphocytes is caused by the activation of proto-oncogenes or the inactivation of tumor suppressor genes.
• Typical translocations include t(14;18) in mantle cell lymphoma (overexpression of cyclin D1) and follicular lymphoma (overexpression of BCL2).

1.1.1.2 Dysregulation of the Immune System

Chronic antigen stimulation, as seen in extranodal marginal zone lymphoma, can lead to genetic mutations and lymphoma development.

1.1.1.3 Disruption of the apoptotic pathway

By blocking planned cell death, mutations in genes that regulate apoptosis, such as those encoding the BCL-2 family of proteins, contribute to the pathophysiology of lymphomas**.**

1.1.1.4 Changes in Epigenetics

The course of lymphoma can be accelerated by alterations in DNA methylation and histone modification patterns, which can either activate oncogenes or mute tumor suppressor genes[(2)].

1.2 Alkylating agents

These agents generate extremely reactive carbonium ion intermediates that, through the formation of covalent bonds, transfer alkyl groups to biological macromolecules. Alkylation causes DNA strands to scissor, cross-link, or pair abnormally. It is also possible for nucleic acids and proteins to crosslink.

Cytotoxic and radiomimetic (similar to ionizing radiation) effects are exhibited by alkylating agents. Many are cell cycle non-specific, i.e. at on dividing as well as resulting cells. Some have CNS stimulant and cholinergic properties.

1.2.1 Nitrogen mustards

Nitrogen mustards are a class of alkylating agents that exert their cytotoxic effects by forming highly reactive aziridinium ion intermediates, which alkylate DNA and other cellular macromolecules. Cell replication and function are eventually disrupted as a result of DNA cross-linking, aberrant base pairing, and strand breaking. Originally developed as chemical warfare agents, nitrogen mustards like mechlorethamine, cyclophosphamide, and ifosfamide are now widely used in chemotherapy for cancers such as lymphomas and leukemias. They are generally cell cycle non-specific and may also exhibit radiomimetic and immunosuppressive properties[3].

1.3 Cyclophosphamide

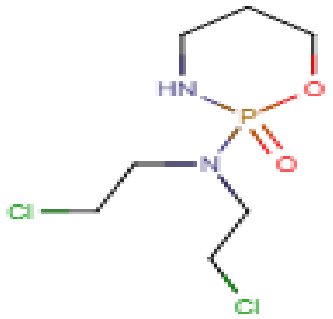

Fig 1: Structure of cyclophosphamide[4].

Molecular formula : $C_7H_{15}C_{12}N_2O_2P$

Molecular mass: 279.10 g/mol

Table 1: Different formulations of cyclophosphamide injection

BRAND NAME	MANUFACTURER	STRENGTHS AVAILABLE	USES
Cycloxan	Zydus lifesciences	200 mg, 500 mg, 1000 mg	Widely used in oncology; available in multiple strengths.
Endoxan-N	Baxter	1000 mg	Used in cancer treatment.

1.3.1 Mechanism of action

A nitrogen mustard derivative, cyclophosphamide is a multipurpose alkylating agent.

The active metabolite of cyclophosphamide, phosphoramide mustard, exhibits the alkylating action. Phosphoramide mustard, a cyclophosphamide metabolite, demonstrates the alkylating activity. Following the biological change caused by oxidation by hepatic microsomal enzymes under the spontaneous ß-elimination of acrolein from aldophosphamide, phosphoramide mustard is produced. The primary causes of the active metabolite's cytotoxic activity are suppression of DNA synthesis and crosslinking of DNA and RNA strands. Cyclophosphamide is a potent immunosuppressive agent that also causes marked and persistent inhibition of cholinesterase activity[5].

1.3.2 Side effects

Fever or chills, Lower back or side pain, Missing menstrual periods, Painful or difficult urination, Dizziness, Joint pain[6].

1.3.3 Dose

2-3 mg/kg/day oral, 10-15 mg/kg IV every 7-10 days, IM also possible.

ENDOXAN, CYCLOXAN: 50 mg tab; 200, 500,1000 mg injection[7].

1.3.4 Pharmacokinetics

- **Absorption:** Cyclophosphamide is well absorbed orally. The bioavailability of oral formulation ranges from 87% to 96% compared to IV administration.
- **Distribution:** The distribution into the total body water is indicated by the volume of distribution (V_d), which is around 30 to 50 L. While its metabolites show stronger protein binding, cyclophosphamide has little protein binding (-20%).
- **Metabolism:** Cytochrome P450 (CYP50) enzymes, mainly CYP2B6, activate the prodrug cyclophosphamide in the liver to produce active metabolites such aldophosphamide and 4-hydroxycyclophosphamide.
- **Excretion:** The main way that cyclophosphamide is removed is through metabolites. 10-20% is excreted unchanged in the urine and 4% excreted in the bile following IV administration[8].

Half life- 3-12 hours

1.3.5 Toxicity

Neutropenia, febrile neutropenia, fever, alopecia, nausea, vomiting, and diarrhea are the most frequently reported adverse effects[9].

PREFORMULATION STUDIES OF CYCLOPHOSPHAMIDE

Preformulation study is defined as an investigation of physical and chemical properties of a drug substance alone and when combined with excipients[10].

2.1 Organoleptic properties

Cyclophosphamide is a fine, white, crystalline powder form. Tastes little bitter and has no smell[11].

2.2 Physical properties

2.2.1 Melting point

Thiele's melting point device used the capillary method to determine the cyclophosphamide's melting point. In this method, the temperature was noted at which point the sample started melting to finish. For this, a capillary tube containing cyclophosphamide was sealed at one end and knotted so that it would stay submerged in a liquid paraffin bath. The melting point was then recorded. Cyclophosphamide has a melting point of approximately 41 to 45°C[11].

2.2.2 Solubility studies

The solubility is the maximum quantity of a solute that can be dissolved in certain quantity of a solute that can be dissolved in certain quantity of solution at a specified temperature. Cyclophosphamide (10mg) was dissolved in a 10ml of different solvents (i.e. ethanol (95%), water and ether) at room temperature and kept for 3 days for equilibrium in a separating funnel. The funnel was shaken often. The U.V. spectrophotometer was used to analyze the supernatant in order to estimate equilibrium solubility[11].

Table 2: Solubility analysis of cyclophosphamide in various solvents.

SOLVENT	SOLUBILITY
Water	Soluble
Ethanol	Freely soluble
Methanol	Rapidly soluble
Glacial acetic acid	Slighlty soluble

2.3 Chemical properties

2.3.1 Partition coefficient

The ratio of unionized medication dispersed between the organic and aqueous phases at equilibrium is known as the "partition coefficient." To determine the drug's solubility and lipophilic behavior, the partition coefficient was examined in both the aqueous and oil phases. It was determined by shaking 10 mg cyclophosphamide in a separating funnels containing 10 ml of n-octanol and 10ml water. The separating funnels were shaken for 2hrs using wrist action shaker equilibration. Two phases were separated and the amount of drug in aqueous phases was analyzed spectrophotometrically at 300nm after appropriate dilution.

The partition coefficient of the drug was calculated by the formula;

$$\text{Partition coefficient} = \frac{\text{Concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}} \qquad (1)$$

2.3.2 Dissociation constant

The dissociation constant (pK_a) of Cyclophosphamide can be determined by potentiometric titration. Since cyclophosphamide is a weak base (or acid, depending on the functional group of interest), the pK_a can be calculated by monitoring the pH change as a function of titrant volume using potentiometric titration. To find the pK_a of cyclophosphamide, dissolve about 0.1 g of the drug in deionized water. If it doesn't dissolve fully, add a small amount of acid or base. Calibrate the pH meter with standard buffers, then stir the solution and record its initial pH. Slowly titrate with 0.1 M NaOH or HCl, adding small volumes and recording the pH after each step. Plot a pH vs. volume graph, and determine the pK_a from the pH at the half-equivalence point of the titration curve[13].

2.4 Micromeritic properties

2.4.1 Angle of repose

The angle of repose, representing the steepest angle between a powder pile and a horizontal surface was measured using the funnel method. This involved pouring the mixture through a vertically adjustable funnel until a predetermined cone height was reached. The angle of repose (θ) was computed using the following formula once the heap's radius was measured;

$$Tan\ \theta = \frac{h}{r} \qquad (2)$$

Where r = pile's base radius and h = pile's height [25].

Table 3 : Angle of repose

S/N	Angle of repose	Flow property
1	<25	Excellent
2	25-30	Good
3	30-40	Passable
4	>40	Poor

2.4.2 Compressibility index

Compressibility, a measure of how easily a material can be made to flow, was the most straight forward method for determining the powder's flow.

$$Compressibility\ index = \frac{Tapped\ density - bulk\ density}{Tapped\ density} \times 100 \qquad (3)$$

Table 4 : Compressibility index

S/N	Compressibility index	Flow property
1	5-11	Excellent
2	12-17	Good
3	18-21	Fair to passable
4	22-34	Poor
5	>35	Very poor

2.4.3 Hausner ratio

It is the ratio of bulk density to tapped density. Cohesion reflecting particle friction is measured using it. Hausner ratio value that are less than 1.25 exhibit good flow property while more than 1.25 exhibit poor flow property.

$$Hausner's\ ratio = \frac{Tapped\ density}{bulk\ density} \qquad (4)$$

2.4.4 Bulk density

The blend was poured into a graduated cylinder to determine the apparent bulk density. The bulk density was determined by dividing the mass of the powder by its bulk volume.

$$Apparent\ bulk\ density = \frac{weight\ of\ powder}{bulk\ volume} \qquad (5)$$

2.4.5 Tapped density

Using a density device, the measuring cylinder containing a known mass of blend was tapped 100 times. After tapping, the blend's weight and the constant minimum volume occupied in the cylinder were measured [14].

$$Tapped\ density = \frac{weight\ of\ powder}{Tapped\ volume} \qquad (6)$$

Acknowledgement

The authors would like to express their sincere gratitude to the faculty and staff of the Department of Pharmaceutics, Malik Deenar College of Pharmacy, Kasaragod for their valuable guidance, constant support, and encouragement throughout the preparation of this review.

REFERENCES

1. 1. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer (Review). Mol Clin Oncol. 2021 Dec 1;15(6):1–8.
2. Kumar, V., Abbas, A.K., & Aster, J.C. (2020). Robbins and Cotran Pathologic Basis of Disease (10th ed.). Elsevier.
3. Ahmed AR, Hombal ShM. Cyclophosphamide - A review on relevant pharmacology and clinical uses. J Am Acad Dermatol 1984, 11(6): 1115-1126.
4. Wishart DS, Feunang YD, Guo AC, Lo EJ, Marcu A, Grant JR, et al. Drug Bank 5.0: a major update to the Drug Bank database for 2018.

Nucleic Acids Res. 2018 Jan 4;46(D1):D1074–82.

5. Tripathy KD. Essentials of Medical Pharmacology**.** 8th ed. New Delhi: Jaypee Brothers Medical Publishers; 2018. p. 859–60.

6. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. *Nat Rev Clin Oncol*. 2009 Nov;6(11):638–47.

7. Tchekmedyian NS, Legha SS, Wiernik PH, Collins JM. A phase I clinical and pharmacokinetic study of cyclophosphamide administered by five-day continuous intravenous infusion. Cancer Chemother Pharmacology. 1986;18(1):33–8.

8. Grochow LB, Colvin M. Clinical pharmacokinetics of cyclophosphamide. Clinical Pharmacokinetics 4: 380–394, 1978.

9. Skeel RT. *Handbook of Cancer Chemotherapy*. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2002.

10. Kumar D, Dixit G, Dixit K. An overview on preformulation studies. Indo American journal of pharmaceutical science. 2015; 2 (10) : 1399-1407.

11. Lewis ,R.J., Sr (Ed).Hawley's Condensed Chemical Dictionary. 13th edition. New York, John wiley and sons, Inc. 1997., page no. 722.

12. Chakradhar DM, Sunitha A, Sofia B, Srikar G. Formulation and characterization of cyclophosphamide injections using lyophilization technique. *Int J Pharm Chem Anal*. 2019;6(3):69-75.

13. *Shah SM, Jain AS, Kaushik R, Nagarsenker MS, Nerurkar MJ.* Preclinical formulations: insight, strategies, and practical considerations. *AAPS PharmSciTech*. 2014;15(5):1307–23.

14. S*axena V, Panicucci R, Joshi Y, Garad S.* Impact of preformulation on drug development. *J Pharm Science*. 2013;102(5):1677–85.