AL-KUFA JOURNAL FOR BIOLOGY
ISSN (Print): 2073-8854 **ISSN (online):** 2311-6544

Original Research Paper

Embryonic histological changes in rats exposed to different doses of cyclophosphoamide

Hydar M Khalfa[1], haider L Al-msaid, hawara muhsin[2]

[1]*Department of biology, Faculty of Science, University of kufa, Kufa, Iraq.*
[2] *university of al-Tousi, najaf, iraq*

Article history
Received: 1/5/2024
Revised: 15/6/2024
Accepted: 17/8/2024

*Corresponding Author: *Hydar Khalfa, Department of biology, Faculty of s*cience, University of kufa, Kufa, Iraq;
Doi: 10.36320/ajb/v16.i2.17565

Email:
hydarm.jmaiwai@uokufa.edu.iq

Abstract: Cyclophosphamide is a common anticancer drug that belongs to the nitrogen mustard group of alkylating agents, their acts by adding an alkyl group to DNA. Due to this cytotoxic property it is used extensively in a variety of carcinomas singly or in combination with other drugs to treat a variety of cancers. Therefore, the present study was conducted to compare and correlate histomorphological alterations in different anatomical sites of embryonic wistar albino rats induced by a gradual increase in cyclophosphoamide dose administered intraperitoneally through maternal delivery. Laboratory wistar albino rats were injected with different bolus doses of cyclophosphoamide intraperitoneally and rat pups were dissected after delivery . Liver and skin tissues were examined histologically and compared to control animals. Results: histological examination of all tissues in both groups shows gradual cytotoxic effect of cyclophosphoamide reaching extreme histological damage with increased bolus dose. Tissue damage included hemorrhaging and lymphocytic collection. There is a significant epidermal thickness between the control group and the groups treated with different cyclophosphoamide concentrations

Keywords: cyclophosphoamide, epidermis , embryonic, histology.

1.Introduction

Cyclophosphamide is a synthetic anticancer drug that belongs to the nitrogen mustard group of alkylating agents that acts by adding an alkyl group (CnH2n+1) to DNA. It acts by attaching an alkyl group to the Guanine base of DNA, at number 7 nitrogen atom of the imidazole ring. Thus, causing irreversible cross-linkages in the DNA strands that leads to cell death in G2- and S-phases of the cell-cycle [1]. Due to this cytotoxic property it is used extensively in a variety of carcinomas singly or in combination with other drugs to treat a variety of leukemias, Hodgkin's lymphoma, Multiple myeloma, Mycosis fungigoides, Non-Hodgkin's lymphoma, breast cancer, ovarian cancer and Retinoblastoma. Cyclophosphamide is also used at lower doses for some autoimmune diseases such as systemic lupus erythematosus, severe rheumatoid arthritis, Wegener's granulomatosis, multiple sclerosis, some connective tissue disorders, minimal lesion glomerulonephritis, several forms of vasculitis, Nephrotic Syndrome and post organ transplantation for prevention of rejection of the organ.[2] Cyclophosphamide is a prodrug that gets activated to alkylating phosphoramide mustard in the liver and excreted primarily (70%) in urine in forms of metabolites [3].

Adverse effects of cyclophosphamide include alopecia, nausea, vomiting, thrombocytopenia, mucosal ulcerations, brief spells of dizziness, transverse striations in the nails, increased skin pigmentation, pulmonary fibrosis, facial abrasions, leukopenia, hematuria, diarrhoea, hemorrhagic cystitis and petechial

DOI: 10.36320/ajb/v16.i2.17565

haemorrhage in lungs and small bowel.[1,4] General endocrinological imbalances in rats, ovarian failure, abnormal sperm production reduced fertility and outcome, decreased implantation, malformed and growth retarded fetuses have been observed in Sprague-Dawley rats.[1-2,5-8] Cyclophosphamide is also documented to cause hepatotoxicity in Fischer rats and cardiopulmonary toxicity in Mongrel dogs.[9-11] In an attempt to reduce this high level of toxicity and check increase in the resistance to this drug, various studies have been conducted in the past to test the efficacy of low doses of cyclophosphamide in treatment of carcinomas with positive results. Chronic administration in a low dose through drinking water was safe and an effective form of low dose therapy in severe combined immunodeficient (SCID) mice, injected with human prostate, colon, melanoma and breast cancer cell lines subcutaneously [12].

2.Methodology

Experimental Animals: 15 adult female Wistar albino rats weighing between (200-250) g were obtained from the Animal house at the department of biology, university of kufa between the period of December 2021- April 2021. The animals were kept in adjusted laboratory conditions (temperature= 25 ℃, humidity = 60 ± 10%). Animals had free access to standard rat chow and water. Guidelines of the Ethical Committee of the animal Research were followed, which conform to the recommendations on Health Guide for Care and Use of Laboratory Animals. Female rats were allowed to Animals were divided into three groups (control = N5, maternal intraperitoneal injection group (150 mg/kg): N 5 intraperitoneal injection group (300 mg/kg) : N5). Each group was injected daily with allocated dose of cyclophosphoamide intraperitoneally until delivery.

Bolus dose: Each pregnant animal in each group received 1 ml of specific dose of cyclophosphamide (baxter Germany) either intraperitoneally while control group received 1mil of PBS (phosphate buffered saline) intraperitoneally. Dose formulation of cyclophosphoamide was achieved following standard protocols and specific doses used in this experiment were 150 mg/kg, 300 mg/kg.

Sacrifice and processing: To carry out a histological analysis, skin, and liver were excied from all liter after birth using the surgical blade and were fixed in 4 % formaldehyde for three days. Tissues were processed and embedded in paraffin wax using TP1020 tissue processor (Leica Microsystem, UK). Five-micron sections were cut using a microtome (Leica Microsystem, UK), which were then placed on slides (Leica Microsystem, UK). The sections were deparaffinised in xylol and then rehydrated by using descending concentrations of alcohol before rehydration in distilled water. Haematoxylin and Eosin were used to stain the sections (Leica Microsystem, UK). Histological investigation of the prepared tissues was carried out by light microscopy with a magnification of 10x. Image J software (NIH, USA) was used to analyse microscopical images to measure the epidermal thickness. The epidermal thickness was measured from the base of the stratum germinativum to the surface of the keratin layer.

Statistical analysis: The mean of the epidermal thickness are measured using image j software. The statistical analysis of the treated groups were compared to the control group. The data were analysed using GraphPad Prism V9.0 software.

3.Results

Histomorphological changes: The results of the present study shows histological changes in animals injected with cyclophosphamide. Figure 1 shows histological appearance of rat pup tissues examined from different anatomical sites after gestational delivery of clophosphamide intraperitoneally. The figure shows normal histological appearance in skin and kidney in control pups. The figure shows abnormal histological appearance with more abundant hemorrhaging and cellular vaculation present in the kidney of both groups who received 150 and 300 mg/ml during gestational period. Skin histology shows normal skin architecture under normal conditions while histological disturbance is seen in animals who received 150 and 300 mg/ml of cyclophosphoamide (figure 1).

The figure shows a profound histological damage with more abundant hemorrhaging and lymphocytic infiltration in the liver and skin sites along with sinusoid enlargement in the liver and profound dermal and epidermal cytoarchitectural changes present. The liver shows vaculated cells present along with condensed nuclear chromosomes as well as signs of tissue necrosis. The skin also showed morphological and histological changes including excessive hemorrhaging and sloughing of corneum layer. Both the testes and the kidneys showed a minor histological damage including a slight hemorrhaging present in both tissues (figure 1).

DOI: 10.36320/ajb/v16.i2.17565

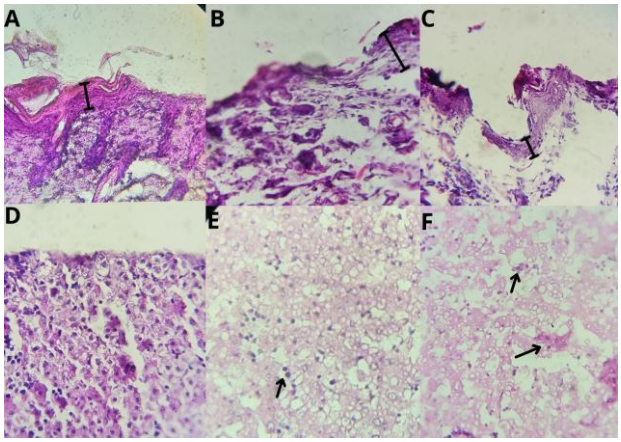

Fig.1. : histological appearance of tissues examined from both liver and skin of rats pups injected with 150 and 300 mg/kg of cyclophosphamide through maternal delivery intraperitoneally. The figure shows normal histological appearance with a slight damage present in liver and skin of control animals. Skin which shows normal histological architecture and some vaculated liver cells present. Figure 1 shows histological appearance of tissues examined from anatomical sited of rats injected with a bolus dose of 150 mg/kg of cyclophosphamide subcutaneously. The figure shows abnormal histological appearance with more abundant hemorrhaging present in all examined tissues including hemorrhaging in the epidermis of the skin. Figure 1 also shows histological appearance of tissues examined from anatomical sited of rats injected with a bolus dose of 250 mg/kg of cyclophosphamide subcutaneously. The figure shows a profound histological damage with more abundant hemorrhaging and lymphocytic infiltration in the liver and skin sites along with sinusoid enlargement in the liver and profound dermal and epidermal cytoarchitectural changes present. The liver shows vaculated cells present along with condensed nuclear chromosomes as well as signs of tissue necrosis. The skin also showed morphological and histological changes including excessive hemorrhaging and sloughing of corneum layer. Both the testes and the kidneys showed a minor histological damage including a slight hemorrhaging present in both tissues (figure 1) H&E X400.

General decrease in epidermal thickness is seen in both groups of rat pups injected with cyclophosphoamide intraperitoneally figure 2. This is also seen in the histological appearance of tissue architecture in figure 1.

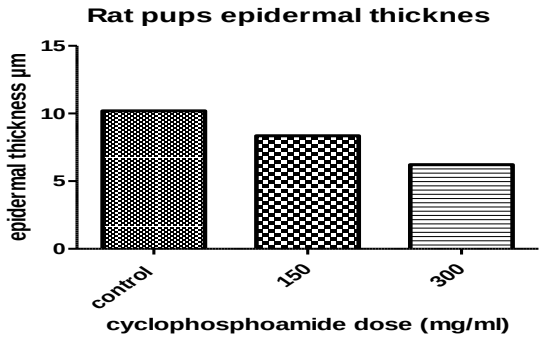

Fig.2. Histomorphometric analysis of the epidermal thickness between the control group and the groups treated with different cyclophosphoamide concentrations (mg/ ml).

The results of this study shows that there is a significant histological changes that takes place during exposure to cyclophosphoamide during embryogenesis. A study by al howail and colleagues [1] shows significant histological differences in rat liver injected with a weight specific dose of cyclophosphoamide. In their study Hepatdestructive changes were gradually increased from slight hemorrhaging to lymphocytic infiltration indication of cytotoxic effect of cyclophosphoamide injected intraperitoneally. The current study shows similar results to that documented by alhowail and team [1] who demonstrated the profound effect on histomorphological changes in various rat organs. Histological damage present in the current study is concurrent with many scientific evidence which demonstrated the cytotoxic and histological damage caused by cyclophosphoamide injection. However there was lacking scientific evidence present that compares histological changes induced by cyclophosphoamide injected using two different methods intraperitoneally and subcutaneously.

conclusion

This study concludes that cyclophosphoamide has a strong cytotoxic effect on certain organs if injected at a high dose intraperitoneally of subcutaneously. However histological damage is more prominent in intraperitoneal injected animals compared to subcutaneously injected animals. Cytotoxic effect and histological damage is confirmed by the gradual decrease in epidermal thickness of rat pups as well the profound histological changes that is seen in various organs of rat pups.

Funding Information

This project was privately funded by all authors.

Author's Contributions

All authors had an equall contribution in the preparation and of the manuscript.

References

1. Alhowail, A., et al. (2019). Effect of Cyclophosphamide and Its Combination with Metformin on the Survival Rate in Mice. Journal of Pharmaceutical Research International, 1-6.

2. Ataya, K. M., Pydyn, E. F., & Ramahi-Ataya, A. J. (1990). The effect of "activated" cyclophosphamide on human and rat ovarian granulosa cells in vitro. Reproductive Toxicology, 4(2), 121-125.

3. Sharbaugh, R. J., & Grogan, J. B. (1969). Suppression of Reticuloendothelial Function in the Rat with Cyclophosphamide. Journal of Bacteriology, 100(1), 117-122.

4. Saoji, V. (2008). Premature ovarian failure due to cyclophosphamide: A report of four cases in dermatology practice. Indian Journal of Dermatology, Venereology and Leprology, 74(2), 128.

5. Qiu, J., Hales, B. F., & Robaire, B. (1995). Effects of Chronic Low-Dose Cyclophosphamide Exposure on the Nuclei of Rat Spermatozoa1. Biology of Reproduction, 52(1), 33-40.

6. Burnstock, G., Vaughn, B., & Robson, S. C. (2013). Purinergic signalling in the liver in health and disease. Purinergic Signalling, 10(1), 51-70.

7. Trasler, J. M., Hales, B. F., & Robaire, B. (1986). Chronic Low Dose Cyclophosphamide Treatment of Adult Male Rats: Effect on Fertility, Pregnancy Outcome and Progeny1. Biology of Reproduction, 34(2), 275-283.

8. Sulkowska, M., et al. (1998). Cyclophosphamide-induced generation of reactive oxygen species. Comparison with morphological changes in type II alveolar epithelial cells and lung capillaries. Experimental and Toxicologic Pathology, 50(3), 209-220.

9. Corsi, A., Calabresi, F., & Greco, C. (1978). Comparative effects of cyclophosphamide and isophosphamide on Lewis lung carcinoma. British Journal of Cancer, 38(5), 631-633.

10. Emmenegger, U., et al. (2007). Pharmacodynamic and pharmacokinetic study of chronic low-dose metronomic cyclophosphamide therapy in mice. Molecular Cancer Therapeutics, 6(8), 2280-2289.

11. Pearson, R., et al. (2002). Purinergic and Muscarinic Modulation of the Cell Cycle and Calcium Signaling in the Chick Retinal Ventricular Zone. The Journal of Neuroscience, 22(17), 7569-7579.

12. Allcorn, S., Catsicas, M., & Mobbs, P. (1996). Developmental Expression and Self-regulation of Ca2+Entry via AMPA/KA Receptors in the Embryonic Chick Retina. European Journal of Neuroscience, 8(12), 2499-2510.

13. Mishra, S. K. (2006). Extracellular nucleotide signaling in adult neural stem cells: synergism with growth

factor-mediated cellular proliferation. Development, 133(4), 675-684.

14. Peng, H., et al. (2016). Purinergic and Store-Operated Ca2+ Signaling Mechanisms in Mesenchymal Stem Cells and Their Roles in ATP-Induced Stimulation of Cell Migration. STEM CELLS, 34(8), 2102-2114.

15. Greig, A. V. H., Linge, C., & Burnstock, G. (2008). Purinergic receptors are part of a signalling system for proliferation and differentiation in distinct cell lineages in human anagen hair follicles. Purinergic Signalling, 4(4), 331-338.

16. Liu, S., Zhang, H., & Duan, E. (2013). Epidermal Development in Mammals: Key Regulators, Signals from Beneath, and Stem Cells. International Journal of Molecular Sciences, 14(6), 10869-10895.

17. Burnstock, G. (2006). Historical review: ATP as a neurotransmitter. Trends in Pharmacological Sciences, 27(3), 166-176.

18. Langer, D., et al. (2007). The ectonucleotidases alkal ine phosphatase and nucleoside triphosphate diphosphohydrolase 2 are associated with subsets of progenitor cell populations in the mouse embryonic, postnatal and adult neurogenic zones. Neuroscience, 150(4), 863-879.

19. Neary, J. T., et al. (1999). Mitogenic Signaling by ATP/P2Y Purinergic Receptors in Astrocytes: Involvement of a Calcium-Independent Protein Kinase C, Extracellular Signal-Regulated Protein Kinase Pathway Distinct from the Phosphatidylinositol-Specific Phospholipase C/Calcium Pathway. The Journal of Neuroscience, 19(11), 4211-4220.

20. Khalfa, H. M., Albideri, A., & Jaffat, H. S. (2018). Cytological and Histological Study of Adult and Neonate Epidermis in Thick and Thin Skin of Various Anatomical Sites. International Journal of Pharmaceutical Quality Assurance, 9(2).

21. Khalfa, H. M., Albideri, A., & Jaffat, H. S. (2018). Histological and cytoarchitectural measurements of human epidermis in different anatomical sites of embryonic, fetal and neonatal Iraqi subjects in Al- Hilla/Iraq Maternity Hospital. Journal of pharmaceutical science & research, 10(4), 812-818.

22. Li, D., et al. (2012). Roles of Purinergic Receptor P2Y, G Protein–Coupled 12 in the Development of Atherosclerosis in Apolipoprotein E–Deficient Mice. Arteriosclerosis, Thrombosis, and Vascular Biology, 32(8).

23. Alarcón-Vila, C., Pizzuto, M., & Pelegrín, P. (2019). Purinergic receptors and the inflammatory response mediated by lipids. Current Opinion in Pharmacology, 47, 90-96.