Journal of Exploratory Research in Pharmacology **2024** vol. 000(000) | 000–000 Epub
DOI: 10.14218/JERP.2023.00025S

Review Article

Cytotoxic Properties of Cyclophosphamide: A Focus on Its Mechanistic Impacts on Male Gonadal Functions

Temitope G. Olowe[1,2], Mega O. Oyovwi[3*], Kingsley E. Nwangwa[2], Ejiro Peggy Ohwin[2] and Onome B. Oghenetega[4]

[1]Department of Physiology, University of Medical Sciences, Ondo, Ondo, Nigeria; [2]Department of Physiology, Faculty of Basic Medical Sciences, Delta State University, Abraka, Delta, Nigeria; [3]Department of Physiology, Faculty of Basic Medical Science, Adeleke University, Ede, Osun, Nigeria; [4]Department of Physiology, School of Basic Medical Science, Babcock University, Illisan, Ogun, Nigeria

Received: September 17, 2023 | **Revised:** February 21, 2024 | **Accepted:** March 14, 2024 | **Published online:** May 11, 2024

Abstract

Cyclophosphamide (CP) is a potent chemotherapeutic agent utilized in the treatment of various types of cancer. However, in addition to its efficacy in combating cancer, CP also has severe side effects, including damage to male gonadal functions. This paper aims to explore the cytotoxic properties of CP and its mechanistic impacts on male gonadal functions. The search strategy was conducted using several reviewed articles indexed in PubMed, Science Direct, EBSCO, Scopus, Cochrane Library, Sage Journals, and Google Scholar. CP is an alkylating agent that damages cancer cell DNA, inhibiting growth and division. It also affects healthy cells, leading to severe cytotoxicity. In male gonadal tissues, CP damages germ cells, Leydig cells, and Sertoli cells, causing decreased sperm count, testosterone levels, and disruption of the blood-testis barrier. The metabolism of CP in the liver generates reactive oxygen species, leading to oxidative damage and cell death. Moreover, CP also affects the hypothalamic-pituitary-gonadal axis, regulating male gonadal functions. CP disrupts the production and secretion of gonadotropin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone, resulting in a decrease in testosterone levels and impaired spermatogenesis. Additionally, CP exerts its cytotoxic effects by inhibiting the proliferation and differentiation of germ cells, leading to a decrease in sperm production. It also affects Leydig cells, which are responsible for the production of testosterone, thus decreasing testosterone levels. In conclusion, CP exhibits potent cytotoxic properties that not only affect cancer cells but also severely damage male gonadal functions. The mechanisms involved in CP-induced gonadal toxicity include oxidative stress and disruption of the hypothalamic-pituitary-gonadal axis. Therefore, it is crucial to consider the potential gonadal toxicity of CP when prescribing it for cancer treatment and to closely monitor the gonadal functions of male patients receiving CP therapy.

Keywords: Cyclophosphamide; Spermatotoxicity; Gonadotoxicity; Genotoxicity; Cardiotoxicity; Oxidative damage; Phosphoramide; Heat stress shock; Reprotoxicity.

Abbreviations: ATP, adenosine triphosphate; Bcl-2, B-cell lymphoma 2; CHD4, chromodomain-helicase-DNA-binding protein 4; CP, cyclophosphamide; FSH, follicle-stimulating hormone; HIST1H2BL, histone H2B type 1-L; HSP, heat shock proteins; IL-10, interleukin-10; IL-4, interleukin-4; LH, luteinizing hormone; NMOPP, nitrogen-mustard, oncovin (vincristine), procarbazine, and prednisone; PRKR, protein kinase R; PRKRIP1, protein kinase R Interacting Protein 1; PURA, purine-rich element binding protein A; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; CAT, catalase; SOD, superoxide dismutase; GPX, glutathione peroxidase.

***Correspondence to:** Mega O. Oyovwi, Department of Physiology, Faculty of Basic Medical Science, Adeleke University, Ede, Osun 232101, Nigeria. ORCID: https://orcid.org/0000-0002-8892-0770. Tel: +2348066096369, E-mail: megalect@gmail.com and oyovwi.obukohwo@adelekeuniversity.edu.ng

How to cite this article: Olowe TG, Oyovwi MO, Nwangwa KE, Ohwin EP, Oghenetega OB. Cytotoxic Properties of Cyclophosphamide: A Focus on Its Mechanistic Impacts on Male Gonadal Functions. *J Explor Res Pharmacol* 2024;000(000):000–000. doi: 10.14218/JERP.2023.00025S.

Introduction

Cyclophosphamide (CP) is one of the most commonly used alkylating agents in pediatric cancer treatment.[1] Studies have demonstrated a dose-dependent effect of CP on spermatogenesis; patients receiving total cumulative doses below 3.5 g/m^2 or exceeding 10 g/m^2 are at high risk of permanent gonadal damage.[2–4] Moreover, CP exerts its cytotoxic effects by cross-linking DNA strands, thereby inhibiting cell division and promoting apoptosis in rapidly proliferating cells.[5] While this mechanism is crucial for targeting cancer cells, it also affects healthy dividing cells, including those in the gonads.[6] The gonads, comprising the testes in males and ovaries in females, are primary sites of gametogenesis and hormone production.[7] Therefore, any damage inflicted upon these structures can lead to infertility and hormonal imbalances.[8] The continuous utilization of novel cytotoxic medications and various treatment protocols underscores the ongoing need for long-term

Table 1. The risk of impaired spermatogenesis following chemotherapy

High risk	Medium risk	Low risk
Cyclophosphamide	Ciplatin	Vincristine
Ifosfamide	Carboplatin	Methotrexate
Chlometine	Doxorubicin	Dactinomycin
Busulfan	BEP	Bleomycin
Melphalan	ABVD	Mercaptopurine
Procarbazine		Vinblastine
Decarbazine		
Chlorambucil		
MOPP		

The recovery of spermatogenesis depends on the drugs used and on the cumulative dose given. ABVD, adriamycin, bleomycin, vinblastine, and dacarbazine; BEP, bleomycin, etoposide, and cisplatin; MOPP, nitrogen-mustard, oncovin (vincristine), procarbazine and prednisone.

follow-up investigations. These studies are invaluable for assessing the potential enduring consequences of newly introduced treatment regimens.

Extensive research has explored the adverse effects of cytotoxic drugs on spermatogenesis, as evidenced by a recent comprehensive review conducted by Heidarizadi *et al*.[9] Most deleterious substances include nitrogen mustard derivatives, like busulphan and melphalan, as well as alkylating medicines such as CP and procarbazine. Table 1 provides a comprehensive overview of the potential risks associated with the disruption of spermatogenesis caused by various cytotoxic medications. The administration of combination chemotherapies, such as the NMOPP regimen consisting of nitrogen-mustard, oncovin (vincristine), procarbazine, and prednisone, is associated with a significant likelihood of inducing sterility in patients with Hodgkin's disease (Table 1). Additional multi-drug treatment protocols utilized in pediatric cancer patients, such as adriamycin, bleomycin, vinblastine, and dacarbazine for Hodgkin's disease and bleomycin, etoposide, and cisplatin for testicular germ cell tumors, have shown a diminished likelihood of causing irreversible infertility.

Thus, the primary aim of this review is to explore the mechanistic impacts of CP on male gonadal functions. Understanding these impacts is crucial due to their significant implications for a patient's overall health, quality of life, and fertility. It is imperative to take appropriate measures to mitigate these effects in patients undergoing treatment with this drug.

Search strategy

Several electronic databases, including SCOPUS, PubMed, Web of Science, and Google Scholar, were utilized to search for published material. The following medical subject headings (MeSH) keywords were used in the search, including "Pharmacology of Cyclophosphamide", "Cyclophosphamide and its properties", "Cyclophosphamide and its metabolism", "infertility and Cyclophosphamide", "Cyclophosphamide and birth defect", "Cyclophosphamide and spermatogenesis", "Cyclophosphamide and gonadotropin", and Cyclophosphamide and genotoxicity. Additionally, bibliographies of collected literature were examined for related in vivo and in vitro research investigating the mechanism of CP-induced testicular toxicity.

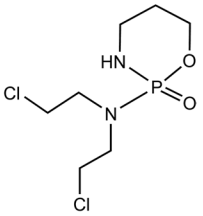

Fig. 1. Chemical structure of cyclophosphamide.

Pharmacology of CP

CP, a common anticancer medication and immunosuppressant, exerts its primary pharmacological effects by inhibiting normal mitosis and cell division in rapidly growing tissues. It affects cells at various stages of their life cycles and lacks specificity for particular cell types. The primary mechanism of action of CP as a chemotherapeutic drug involves the induction of DNA strand breaks and crosslinks.[10] This alkylating agent interferes with the growth of cancer cells, primarily by impeding DNA synthesis. By generating alkyl radicals that interact with DNA molecules, CP induces strand breaks and cell death. Furthermore, alkylating chemicals create active carbonium ion intermediates that covalently bind to target molecules, disrupting normal biological functions.[11]

Metabolism of CP

The hepatic cytochrome P450 enzyme system converts the nitrogen mustard CP into its active metabolite, 4-hydroxy-CP, which exists in equilibrium with the acyl tautomer aldophosphamide. Spontaneous-elimination of this metabolite leads to the production of toxic metabolites such as phosphoramide, mustard, and aerosol. These active metabolites facilitate CP's biological function.[12] Hepatic aldehyde oxidase may also detoxify CP, producing carboxyphosphamide and 4-ketocyclophosphamide.[13] According to Swystun *et al*.,[14] the cytotoxic effects of CP are dose-dependent and gradually eliminated from the body. CP is primarily metabolized through glucuronidation and is predominantly excreted in urine, with a half-life of approximately 30 minutes. It is available in tablet, capsule, and injection forms.

Formula and molecular weight

The chemical formula for CP is $C_7H_{15}Cl_2N_2O_2P$, with a relative molecular mass of 261.1 (Fig. 1).

Chemical and physical properties

CP is a fine white crystalline powder, characterized by odorlessness and slightly bitter taste, with a melting point ranging from 41–45°C (Table 2). A 2% solution of CP typically has a pH of 4 to 6.

Mechanism of CP action

CP, a nitrogen mustard medication, inhibits protein synthesis through DNA and RNA cross-linking.[15] The primary cause of its antineoplastic effects is the production of phosphoramide during the metabolism of liver enzymes. CP is metabolized by liver enzymes into hydroxyl CP and aldophosphamide, which further breaks down into phosphoramide mustard and acrolein. According to Verma *et al*.,[16] the phosphoramide metabolite forms long-lasting crosslinks that induce programmed cell death.

Table 2. Physiochemical properties of cyclophosphamide

Description	The powder form of cyclophosphamide is white and crystalline. Without smell and slightly bitter in flavor. 41–45°C melting point. The pH range of a 2% solution is 4–6. use as an anti-cancer agent in medicine.
pKa	9.5 (66% Dimethylformamide)
Solubility Profile	Soluble. 1–5 g/100 mL at 23°C
Melting Point	41–45°C
pH	0.8

pH, power of hydrogen; pKa, acid dissociation constant (Ka) of a solution °C, degree celcius; mL, milliliter; g, gram.

However, the exact mechanism of CP action is not fully understood. It is believed to act in two main ways: firstly, by interfering with the protein synthesis pathway of cancer cells, leading to the death of these cells, and secondly, by suppressing the activity of the immune system to prevent it from attacking normal healthy cells. Additionally, CP may interfere with cancer cell division and growth, as well as cause DNA damage.

Overall, CP possesses antimitotic, antineoplastic, and immunosuppressive properties. It is utilized for selective immunomodulation of regulatory T cells and the elimination therapy for malignant hematopoietic cells. While CP reduces the secretion of interferon-gamma and IL-12, it increases the production of Th2 cytokines including IL-4 and IL-10.[17] Therefore, it is beneficial for managing immune-mediated illnesses, post-transplant alloreactivity management, and tumor vaccination procedures. Although the precise mechanism underlying its immunomodulatory effects remains unclear, studies indicate that it reduces regulatory T cells, activates T cell growth factors, and primes host cells to accept donor T cells.[17]

Use of CP

CP is commonly used in the treatment of various malignancies, including Hodgkin and non-Hodgkin lymphomas, lymphocytic lymphomas, small lymphocytic lymphomas, Burkitt's lymphomas, and multiple myelomas, especially at Ann Arbor Stage III and IV.[18,19] Additionally, it is used to treat ovarian adenocarcinoma, disseminated neuroblastoma, retinoblastoma, nephrotic syndrome, breast cancer, leukemia, and retinoblastoma.[20,21] To prevent graft rejection and complications related to graft-versus-host disease, CP is also used as an immunosuppressant before transplantation.[22,23]

Doses of CP

A patient can receive CP intravenously, orally, or intramuscularly. Less frequently, the medication has been injected intramuscularly, delivered intraperitoneally, intrapleurally, or directly perfused.

When there are no hematological deficits, adults and children often initiate CP monotherapy. An intravenous loading dose of 40–50 mg/kg is given over two to five days in divided doses as part of induction therapy. Oral doses range from 1 to 5 mg/kg per day, depending on the patient's tolerance. Children with nephrotic syndrome may require a daily dose of 2–4 mg/kg for 60–90 days. Patients undergoing stem cell transplantation may receive high doses of CP as part of their conditioning regimen. Breast cancer is treated with CP-containing combination chemotherapy regimens, with a typical regimen for early breast cancer consisting of 100 mg/m^2 orally supplemented with intravenous methotrexate and fluorouracil.[24] Cycles are repeated every month for 6–12 months of therapy. Oral tablets and vials for parenteral administration are both options for CP.

Toxicity data of CP in human subjects

Follow-up studies in patients with diseases such as glomerulonephritis, bone marrow transplantation, and cancer are the primary sources of information regarding the adverse effects of clonidine (CP) medication in humans. According to Ghobadi *et al*.,[15] chronic CP treatment in prepubertal individuals leads to abnormalities such as decreased ejaculate volume, sperm density, and decreased testosterone levels. Various doses of CP used in chemotherapy have been shown to cause aberrations in sperm parameters, as well as elevated levels of LH and follicle-stimulating hormone (FSH).[25] Azoospermia and hormonal abnormalities are induced by exposure to CP at various dosages, with higher doses resulting in more significant harm.

Cardiotoxicity effects of CP

CP, according to Morandi *et al*.,[26] can induce myelosuppression, potentially culminating in sepsis and septic shock. In specific circumstances, the medical team may opt for additional monitoring and administration of antibiotic treatment. Cardiotoxicity, pulmonary toxicity, veno-occlusive liver disease, and secondary malignancies have been reported in certain cases involving the use of CP. Potential hazards include myocarditis, pericardial effusion leading to cardiac tamponade, pneumonitis, and respiratory insufficiency. Morandi *et al*.[26] reported a positive correlation between increased dosages of CP and elevated occurrences of adverse effects, as well as increased mortality rates.

Genotoxicity of CP

Protamines, which are crucial chromatin compartments, are primary targets of CP-induced DNA damage. Through alkylation, CP alters the interaction between protamines and DNA, thereby damaging DNA. Round spermatids sustain more damage because protamines play the role of histones during spermatogenesis. This results in elongating spermatids that are transcriptionally inactive. Direct alkylation of protamine thiol groups and decreased P1 gene expression, affecting chromatin condensation ability, are likely responsible for the decrease in protamine content.[15] Acute CP treatment increases gene expression in spermatogenic cells, while prolonged exposure suppresses it due to DNA damage and the formation of crosslinks in DNA and transcription machinery.[15] CP also affects gene expression, with acute treatment increasing gene expression in spermatogenic cells. Chromosome cross-linking can impede RNA polymerases and hinder gene expression. Pachytene spermatocytes and spherical, elongating spermatocytes exhibit variable expression of stress response genes, increasing their vulnerability to chromosomal cross-linking.[27] Due to chromatin modification during spermatogenesis, round spermatids are more susceptible than other types. Pachytene spermatocytes undergo apoptosis and DNA repair processes to compensate for DNA damage. Chronic CP exposure leads to increased transcript levels,

decreasing the vulnerability of elongating spermatids.[15] This results from altered interactions between mRNA-binding proteins and RNA-binding proteins after DNA strand breaks, as well as aberrant transcription.[28]

Acute exposure to CP can alter the expression of apoptosis-related genes in spermatocytes and spermatids. The pro-apoptotic Bcl-2-associated death promoter and the Bcl-2-associated x protein are two genes implicated in apoptosis. The expression of antiapoptotic Bcl-2 and Bcl-2-like (Bcl-xL) proteins decreases, while Fas expression increases.[15] Apoptosis results from the activation of the caspase cascade by apoptotic proteins. Both pro- and anti-apoptotic proteins are comparatively elevated in round spermatids, resulting in a low incidence of apoptosis. After CP therapy, the levels of chromatin- and DNA-binding proteins such as single-stranded DNA-binding protein alpha, structure-specific recognition protein 1, and HNRNPK also increase. Round spermatids exhibit an increase in extracellular signal-regulated kinases, which activate either intrinsic or extrinsic apoptotic pathways.[29]

According to the previous study, pachytene sperm exhibit decreased expression of heat shock proteins (HSPs), while round and elongated sperm exhibit increased expression in response to oxidative stress. HSPs and co-chaperones are essential for preventing misfolding, aggregation, and degradation of proteins, and their altered expression due to continuous therapy with CP causes harm. HSP70 likely aids in the construction of the flagellar axoneme. CP treatment affects flagella formation and sperm motility, with HSP70 playing a role in regulating apoptosis.[30] Additionally, CP affects the gene expression involved in DNA repair pathways, including nucleotide excision repair, base excision repair, homologous recombination repair, and mismatch repair pathways.[15]

Acute exposures to CP increase gene expression in pachytene spermatocytes and round spermatids, while chronic exposures increase gene expression in elongating spermatids. These dysregulations lead to genetic instability and the loss of sperm function and viability.[15] CP also enhances the expression of DNA-binding proteins like SSRP1, HNRNPK, and PURA, contributing to the cytotoxicity of CP. Abnormalities in ornithine decarboxylase expression are observed after CP treatment, with acute exposures increasing its expression in pachytenes and round spermatids, and chronic exposures increasing its expression in elongating spermatids. Overexpression of ornithine decarboxylase has been associated with infertility.[15] Sperm-stimulating substances called polyamines have been associated with male infertility. Sperm capability can be inhibited by abnormal concentrations of these compounds in sperm.[15] The sperm proteome is impacted by modifications in gene expression following CP therapy for HNRPA1, PRKRIP1, CHD4, PPAR-y, and Akt-1. Fatty Acid Binding Protein 9 is involved in sperm structure, while CHD4, HIST2H4, HIST1H2BL, and Zona pellucida binding protein are involved in chromatin organization and fertilization. The expression of PRKR Inhibitory Protein-1, a PRKR inhibitor, decreases following CP exposure. Levels of PPAR- and Akt-1 also decrease, affecting lipid metabolism. Testes lacking Akt-1 undergo premature apoptosis and mitochondrial malfunction,[15] highlighting the role of Akt-1 in protecting germ cells and inhibiting apoptosis.

Reproductive and impairment of fertility studies

Studies have demonstrated decreased testis weight,[31] transient oligozoospermia,[32] decreased DNA synthesis in spermatogonia, and reduced RNA and protein synthesis in spermatids.[32,33] CP has been associated with adverse effects on male reproduction. Both humans and animals have experienced malformations due to CP treatment, including a child born without a hand whose father underwent treatment for Behrets disease.[34] Male animals treated with CP are more likely to have offspring with abnormalities such as open eyes, omphalocele, generalized edema, syndactyly, gigantism, and dwarfism.[35] After treatment, chromosomal translocations in the F1 gene are also induced, potentially impacting the F2 generation.[36] CP treatment can lead to complex phenotypic changes in the second and third generations, resulting in mutations that can be inherited by subsequent generations. Chronic low doses of CP can induce early embryonic defects, malformations, and growth retardation in surviving offspring.[37] Retreatment with low doses of CP may exacerbate pre- and post-implantation losses and malformations, with post-implantation loss rates reaching up to 95% after six weeks.[38] These abnormalities persist into subsequent generations, but normal offspring recover rapidly after treatment discontinuation. Chronic exposure to low doses of CP directly affects sperm and can lead to birth defects, which are specific and heritable. CP can also affect oogenesis and spermatogenesis processes, leading to sterility in both sexes.[38] Patients should be informed about the risks of infertility before commencing CP therapy.

Various aspects of the mechanism of CP-induced testicular toxicity

Hormonal disturbances

Treatment with CP decreases the activity of testicular steroidogenic enzymes, such as 3-hydroxysteroid dehydrogenase and 17-hydroxysteroid dehydrogenase, as well as serum levels of testosterone, LH, and FSH.[39] This may be attributed to oxidative damage caused by CP and alterations in gene expression patterns in Leydig cells (Fig. 2), as suggested by Dong *et al*.[40] and Mahmoodi *et al*.[41]

Testicular weight and histopathological abnormalities

According to various investigations, individuals treated with CP exhibit a significant reduction in testicular weight and histological abnormalities, which are crucial signs of reproductive toxicity. Testicular weight influences both the ability to reproduce and spermatogenesis,[42] with decreased gonadotropin and testosterone release being linked to this decrease, thereby interfering with spermatogenesis.

Abnormal sperm morphology

After CP exposure, significant reductions in sperm concentration and motility are observed, along with various additional abnormalities.[43] DNA damage induction, peroxidation of crucial thiol groups in proteins, membrane lipid peroxidation, oxidative stress in mitochondria, and decreased tricarboxylic acid cycle enzyme activity result in decreased availability of adenosine triphosphate (ATP).[43] Exposure to reactive oxygen species (ROS) has been demonstrated to lower ATP levels and impede motility. Moreover, anomalies in flagellar function post-therapy have been attributed to a decrease in heat shock proteins.

Testicular Oxidative damage

The testis is particularly susceptible to oxidative damage due to the presence of numerous ROS-generating mechanisms and elevated levels of polyunsaturated fatty acids (PUFAs).[15] The antioxidant system plays a crucial role in protecting this tissue from the detrimental effects of oxidative damage, which is a significant contributor to testicular failure. ROS are generated as natural by-products during cellular metabolism, with oxygen (O2) being the most prev-

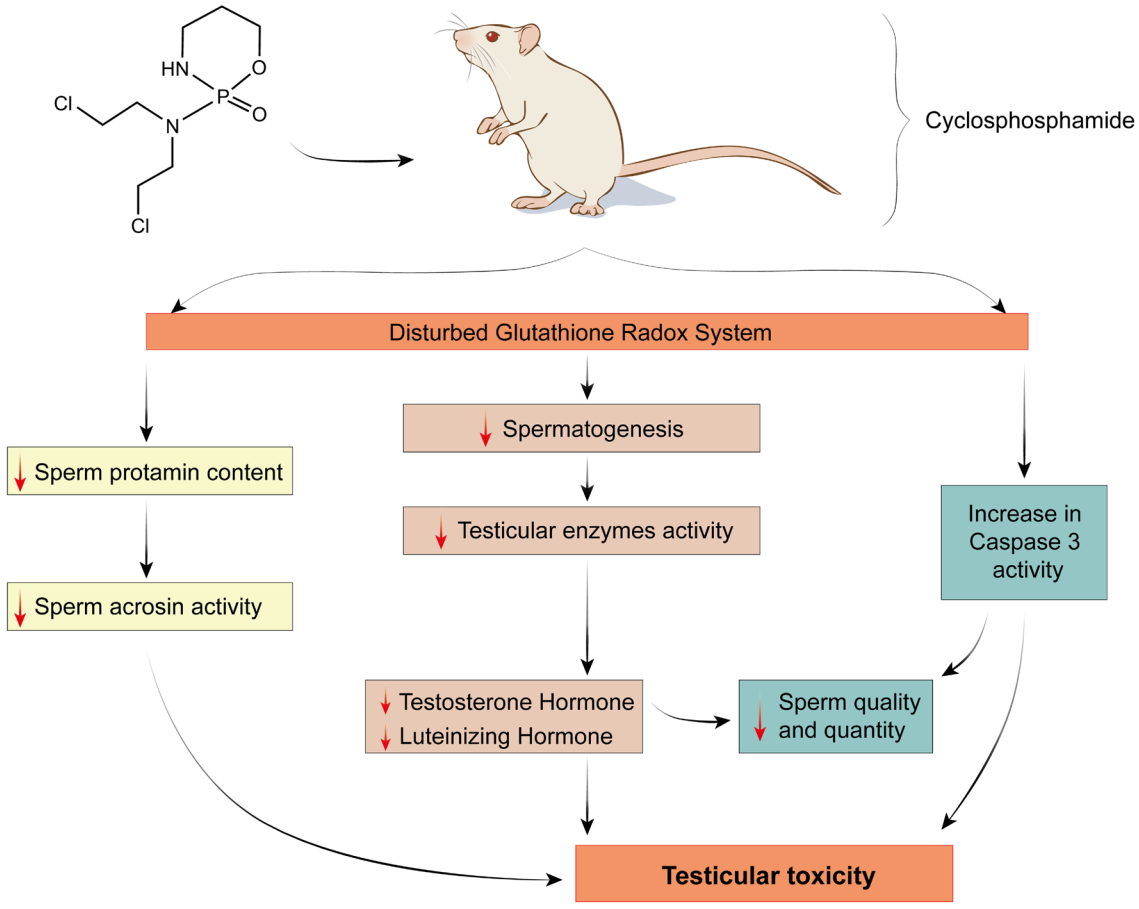

Fig. 2. The mechanism of cyclophosphamide associated with disturbed glutathione redox system and increased caspase 3 activity results in decreased male sex hormone levels, poor sperm quality, and testicular toxicity.

alent among them. Cells employ various mechanisms to mitigate the harmful effects of ROS, including the utilization of antioxidant molecules such as glutathione, vitamins E and C, as well as antioxidant proteins such as thioredoxin, glutaredoxin, metallothioneins, and albumin. Additionally, cells utilize enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPX) to counteract ROS-induced damage.[15] The process of ROS quenching involves the conversion of molecular oxygen (O2) to hydrogen peroxide (H2O2), which is then eliminated from the system through the action of GPX and CAT. According to Ghobadi *et al*.,[15] the administration of CP decreases the levels of key antioxidant enzymes such as SOD, CAT, and glutathione (GSH) in cells. Additionally, CP has been observed to decrease the activity of glucose-6-phosphate dehydrogenase, GPX, glutathione reductase, as well as the levels of vitamins E and C. This cumulative effect of CP on these cellular components renders the cell more susceptible to oxidative damage. According to Song *et al*.,[43] there is a notable decrease in sperm concentration and motility, as well as the presence of many other abnormalities following exposure to CP. The induction of DNA damage, peroxidation of essential thiol groups in proteins, membrane lipid peroxidation, oxidative stress in the mitochondria, and reduced activity of tricarboxylic acid cycle enzymes have been found to lead to a decrease in the availability of ATP.[43] Exposure to ROS has been shown to decrease ATP levels and impair motility. The observed deviations in flagellar function following therapy have also been attributed to a reduction in heat shock proteins.

Research has indicated that exposure to CP has the potential to disrupt the balance of redox in tissues, which may result in biochemical and physiological problems. Phosphoramide mustard, the primary active metabolite of CP, could potentially contribute to oxidative stress induced by CP. Nitrogen mustard, a byproduct of CP metabolism, has been found to induce oxidative and nitrosative stress by activating inducible nitric oxide synthase, leading to the production of nitric oxide and peroxynitrite.[44] Additionally, it has been observed to decrease the activity of GPX.[45] Previous studies have demonstrated that CP can increase the concentration of nitric oxide in the bloodstream. Acrolein, an aldehyde generated by CP metabolism, can enhance lipid peroxidation and form covalent connections with DNA and proteins.[46,47] According to Kishino *et al*.,[48] the presence of acrolein has been shown to decrease the levels of SOD, CAT, and glutathione peroxidase (GPx). Additionally, exposure to acrolein has been found to disturb the cytoskeletal structure and enhance the expression of P38mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinases, which regulate various cellular processes, such as proliferation, differentiation, growth, and apoptosis. According to Ghobadi *et al*.,[15] the presence of xanthine oxidase or aldehyde dehydrogenase leads to the generation of hydroxyl and superoxide radicals via acroleinyl radicals and GS-propionaldehyde.

Possible mechanisms of male-related birth defects

Various factors contribute to birth defects of paternal origins, including drug transfer during sexual intercourse, alteration of the reproductive tract environment, and direct impacts on sperm. Some drugs, such as methadone, morphine, and thalidomide, can enter the seminal fluid, potentially affecting early conception. Notably, CP has been observed to permeate all tissues within the male reproductive tract and increase pre-implantation loss in male rats. Alternatively, it has been suggested that birth defects may result from the inflammatory response of the epididymis, rather than a direct chemical reaction.[49] Drugs can also exert their effects directly on sperm by crossing barriers like the blood-testis or blood-epididymal barriers to reach germ cells. They can impact seminiferous tubule epithelial cells, hinder germ cell division, and gain direct access to the male genome without necessarily altering sperm morphology or fertilization capacity.[50] Research has demonstrated that chronic administration of low-dose CP can have direct effects on sperm, with the spermatid stage being particularly sensitive to its influence.[51,52] This finding implies that the effects of the drug on sperm may be indirect rather than a direct action on the sperm itself.

Cytotoxic properties of CP on female gonadal functions

CP is a cytotoxic drug with known potential side effects on female gonadal functions, including infertility and other reproductive complications.[53] This drug works by inhibiting normal immune function and interfering with the production of DNA in rapidly dividing cells. As a result, CP can severely affect the female reproductive system, leading to changes in hormone levels, ovarian failure, and other fertility problems.[54]

One of the main effects of CP on female gonadal function is the suppression of ovarian endocrine activity.[44] This can lead to a decrease in estradiol and progesterone production, resulting in amenorrhea (stoppage of menstruation), precocious puberty, and menopausal-like symptoms such as hot flashes, night sweats, and vaginal dryness. Long-term treatment with CP can also cause permanent infertility.[53] In some cases, ovarian tissue may be damaged, making it impossible for fertilization and implantation of an embryo. Additionally, exposure to CP can lead to a decrease in ovarian follicles, which can put women at increased risk for early menopause.[54] In addition, this drug is known to cause decreased ovarian reserve, resulting in a decreased response to ovulation-stimulating drugs and decreased chances of successful pregnancy in women undergoing assisted reproductive technologies. Furthermore, the ovarian suppression and low circulating estradiol levels caused by CP can lead to reduced bone mineral density, increased risk of fractures, and increased risk of osteoporosis.

Mechanism of CP on female gonadal functions

CP is also known to interfere with the hypothalamic-pituitary-gonadal axis in females, leading to the suppression of gonadal hormones and ovarian activity.[55] This inhibition is thought to primarily affect the function of the aromatase enzyme. Aromatase is responsible for synthesizing estrogen in females, so its suppression by CP results in decreased levels of circulating estrogen (estradiol), disrupting normal ovulatory cycles and suppressing estrogen-dependent reproductive processes. In addition to its effects on estrogen levels, CP has also been shown to suppress other female reproductive hormones, such as progesterone, FSH, and LH.[56] This disruption of the normal hormonal balance further contributes to the disruption of ovarian activity and reproductive function. CP is known to cause infertility in female patients, as the effects of the drug on the ovarian and reproductive functions of the body can persist for months or even years. Therefore, it is important for females undergoing treatment with CP to discuss any potential fertility issues with their healthcare providers and explore methods to preserve fertility before beginning treatment.

Potential biochemical and molecular pathways mediating the cytotoxic effects of CP on gonadal functions

CP is known to have cytotoxic effects on gonadal functions, and its impacts on the body are mediated through several biochemical and molecular pathways.

One of the primary pathways through which CP exerts its cytotoxic effects is by generating ROS and reactive nitrogen species (RNS).[57] These reactive species are generated either directly by the drug itself or as a result of its enzymatic metabolism by cytochrome P450 enzymes in the body. The presence of ROS and RNS leads to oxidative stress within cells, disrupting normal cellular functions and potentially causing cell death. This oxidative stress is mediated by the enzyme CYP2B, which produces ROS when CP is converted into an active metabolite.[58] In addition to ROS/RNS-mediated cytotoxicity, CP can also affect cells by binding to DNA and DNA repair proteins.[59] This can lead to the inhibition of DNA replication, transcription, and other processes that are essential for normal cellular functioning. In addition, CP has been found to induce DNA damage in gonadal cells, possibly by inhibiting topoisomerase II.[27,54] Topoisomerase II is an enzyme responsible for relaxing and unknotting DNA, and inhibition of this enzyme leads to breaks in DNA strands and cellular toxicity.

Finally, CP has been found to cause disruptions in the endocrine system,[15,60] Specifically, it has been linked to reduced secretion of FSH and LH in both men and women, which can lead to decreased fertility and reproductive disorders (Fig. 2). Additionally, CP may competitively inhibit the activity of gonadal enzymes, such as cytochrome P450 enzymes,[15] leading to decreased detoxification of gonadal toxins and potential reproductive toxicity. CP may also act on gonadal cells by directly interfering with the production of steroid hormones. This could lead to decreased levels of testosterone and other sex steroid hormones, which may lead to reproductive toxicity.

Moreover, CP decreases the number of mitochondria in gonadal cells,[61] primarily through the opening of the mitochondrial permeability transition pore in response to oxidative stress induced by the drug. This can lead to the destruction and collapse of the mitochondria, ultimately causing apoptosis. Additionally, CP may induce mitochondrial DNA damage, resulting in decreased energy production in gonadal cells,[62,63] and subsequent cell death due to the accumulation of ROS and calcium ions, as well as the release of pro-apoptotic proteins.

CP also increases apoptosis signaling in gonadal cells.[61] This process is mediated by the release of apoptosis-inducing factor from the mitochondria, triggering programmed cell death. Notably, the identified pathway is the caspase-mediated apoptotic pathway. Caspases are proteins that are crucial for apoptosis – the programmed death of cells. CP has been shown to activate caspases and induce apoptosis in gonadal cells, which may disrupt gonadal functions.[15,64,65] The activation of caspase-3 begins with the binding of CP to its target enzyme, cytochrome c, resulting in its release from the mitochondrial transmembrane. Once cytochrome c is released, several downstream proteins and pathways are activated,

ultimately leading to the activation of caspase-3 and subsequent apoptosis.

CP also induces the stress response in gonadal cells, leading to cytotoxic effects.[15] This process is mediated by increased levels of HSPs, which trigger apoptosis in response to changes in the cellular environment.

Finally, another pathway that may be affected by CP is the MAPK pathway.[66–68] This pathway plays a crucial role in regulating cell proliferation and differentiation, both of which are vital for gonadal functions. Studies have found that CP can activate MAPKs, leading to the disruption of these processes and consequent disruptions of gonadal functions.[66–68]

In summary, CP can exert its cytotoxic effects on gonadal functions through various biochemical and molecular pathways, including the generation of ROS/RNS, binding to DNA and DNA repair proteins, MAPK, increased levels of HSPs, caspase-mediated apoptosis, and disruption of the hypothalamic-pituitary-gonadal axis, leading to hormonal changes that can affect gonadal functions. In addition, CP has been suggested to interfere with estrogen- and androgen-mediated pathways, resulting in endocrine disruption.

Future directions

This review illustrates the pharmacological effects of CP by focusing on its mechanistic impacts on gonadal function. It is anticipated that future research on the reproductive anomalies induced by CP will reference the success rates of previous studies as a benchmark. To mitigate its cytotoxic effects, various antioxidant supplements, such as taurine,[69–72] quercetin,[71,73,74] D-ribose-L-cysteine,[75,76] Lutein,[77] Coenzyme Q10,[67,70,78,79] N-acetylcysteine, zinc sulfate,[80,81] Kolaviron,[82] Arjunolic acid,[83,84] Epigallocatechin-gallate,[85] Diosmin,[86] S-allylcysteine,[87] melatonin,[88] Rutin,[89] diallyl disulfide,[90] ascorbic acid,[91] vitamin E,[92] Rosmarinic,[93] and DL-alpha-lipoic acid[94] could serve as therapeutic adjuncts for preventing the reproductive anomalies caused by CP. Thus, combining drug delivery with a potent antioxidant agent may present a viable approach to mitigate the side effects of CP.

Significance statement

This review examines the novel cytotoxic effects of CP on gonadal functions. Previous studies have indicated that CP therapy results in gonadal dysfunction, including amenorrhea, infertility, and testicular damage. Our review revealed that long-term or cumulative CP therapy can cause decreased ovarian reserve, increased anti-mullerian hormone levels, and impaired ovulation. It has been suggested that CP-induced changes in ovarian reserve are caused by direct effects of the drug, its metabolites, and immunological stimulation. In addition, CP therapy may cause increased follicular atresia, leading to an altered ovarian reserve and a reduction in ovarian function. Regarding testicular damage, our review revealed that CP administration may cause germ cell apoptosis and Leydig cell damage, resulting in a decrease in the number of spermatozoa and a decrease in testosterone and LH levels. Furthermore, CP-induced inhibition of spermatogenesis may lead to oligospermia and azoospermia. In summary, our review revealed evidence of novel cytotoxic effects of CP on gonadal functions, including ovarian reserve, Leydig cell damage, and spermatogenesis impairments. The results of several studies indicate that CP-treated individuals exhibit a considerable loss of testicular and ovarian function, as well as histological abnormalities, which are critical indicators of reproductive impairment. Notably, spermatogenesis, oogenesis, and reproductive ability decrease as testis and ovarian weight decrease.[47,95] This decline may be attributed to decreased gonadotropin and steroidal hormone production, which interferes with spermatogenesis and oogenesis. The mechanisms underlying the cytotoxic effects of CP are also described in this work.

The potential biochemical and molecular pathways (DNA damage, endocrine disruption, oxidative stress, cellular toxicity, and immune modulation) that mediate the cytotoxic effects of CP on gonadal functions are also well discussed. Finally, we highlighted the need for further research to develop methods to prevent or reduce the cytotoxic effects of CP on gonadal functions.

Conclusions

In conclusion, the cytotoxic properties of CP significant impact male gonadal functions. Its ability to induce DNA damage, affect sperm production and maturation, damage Leydig cells, disrupt the hypothalamic-pituitary-gonadal axis, and cause birth defects can all contribute to male infertility, sexual dysfunction, and other complications. Therefore, it is crucial for healthcare professionals to carefully monitor male patients receiving CP treatment and provide appropriate interventions to minimize these adverse effects. Future research is needed to develop strategies to mitigate the cytotoxic effects of CP on male gonadal functions and improve the quality of life for male cancer patients.

Acknowledgments

None.

Funding

This research received no external funding.

Conflict of interest

The authors declare no competing interests related to this publication.

Author contributions

All authors have made important contributions to the manuscript. MOO, EPO, and KEN were involved in the conceptualization; the validation of resources, data curation, and writing were performed by all the authors; MOO, OBO, KEN, and TGO reviewed and edited the manuscript. All of the authors read and approved the final manuscript.

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