Cyclophosphamide-induced cardiotoxicity

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Abstract: One of the commonest antitumor drugs is cyclophosphamide (CP). CP that is used in management of many tumors and autoimmune disease as well as in polychemotherapy regimens and immunosuppressive protocols. It doesn't only have a specific effect on cancer cells, but also it produces many toxic effects. One of the most important side effects of CP that limits its use in clinical practice is its dose dependent toxicity on the heart. The incidence of cardiotoxicity at doses over 120-150 mg/kg is of 8-20% in adults, and 5% in children. The incidence of heart failure after high doses of CP varies widely from less than 5% and up to 10-29%. This review article discusses the molecular mechanism of CP cardiotoxicity with some of different signaling pathways that contribute in the development of such toxicity. Our main concern from discussing these molecular mechanisms of CP induced cardiotoxicity is to help in development of new therapies.

Keywords: Cyclophosphamide; Anti-neoplastic; Cardiotoxicity; Signaling pathways; Molecular mechanism.

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1. INTRODUCTION

Malignancy represents an aggressive disorder that causes mortality equal nearly 10,000,000 deaths worldwide 1. In 2020, about 19.3 million new patients with malignancy and almost 10.0 million mortality due to malignant diseases were recognized worldwide. The number is expected to increase in 2040 to be 28.4 million patients with 47% rise from nowadays 2. Due to its higher incidence, several pharmacological therapies against tumors were developed. However, some of these drugs lead to cardiotoxicity, representing an important challenge for the healthcare professionals. Therefore, it is important to solve this problem as it affects the survival of patients treated with chemotherapeutic agents. CP is a nitrogen mustard drug that causes alkylation of DNA. The drug is not cell-cycle phase-specific and is converted to an active form that inhibits protein synthesis by DNA and RNA crosslinking 3,4. Its main function is achieved by its metabolite phosphoramid mustard. Phosphoramid mustard is only found in cells that possess minimal concentrations of aldehyde dehydrogenase. It results in DNA crosslinking both between and within DNA strands at guanine N-7 positions. This linking is permanent and causes cell apoptosis 5,6. CP has also immunosuppressive action mainly on T cells.

2. CYCLOPHOSPHAMIDE-INDUCED CARDIOTOXICITY

It is difficult to establish the true rate of CP cardiotoxicity due to its difficult assessment; as the literature depends on case reports with multiple concentrations of CP. Also, CP is usually used with other cardiotoxic drugs 7-10. The incidence of cardiotoxicity that occurs at concentrations more than 120-150 mg/kg occur at a rate of 8-20% in old age and 5% in paediatrics. CP causes heart failure (HF) at rates that vary from below 5% and up to 10-29% 11-16.

Cyclophosphamide and its metabolites aldophosphamide, 4-hydroxycyclophosphamide and acrolein are identified as cardiotoxic agents. Indeed, acrolein is identified as its most toxic metabolite 17. Cardiomyocytes are extremely sensitive to acrolein as it is an unsaturated highly reactive aldehyde 18,19. CP or its metabolites interact with proteases and causes release of oxygen free radicals in addition to massive inflammatory effect in cardiomyocytes 20.
Furthermore, the production of endothelial nitric oxide synthase (NOS) phosphorylation and eNOS formation is also decreased.

Several research studies documented that CP decreases the eNOS dimer and elevates eNOS monomer, this leads to eNOS uncoupling and release of nitrative stress by synthesis of peroxynitrite. Remarkably, acrolein, the main toxic metabolite of CP, has surprising chemical nature as it produces cytoplasmic and nuclear protein adducts in heart cells, causing damage of the heart. Acrolein combines with lysine and interacts with reduced glutathione (GSH) leading to oxidative stress. Also, it forms compounds with cysteine in cardiac cells, leading to enhancement of caspases and NF-kB-p65. Stimulation of caspases lead to apoptosis, while the stimulated NF-kB moves to the nucleus in which it increases the transcription of cytokines such as interleukin-6 (IL-6) and tumour necrosis factor alpha (TNFα). The formed compounds as well as the metabolites of cyclophosphamide react with Fas ligand and TNF-α receptors leading to apoptosis of cardiac cells, via activation of the extrinsic pathways. Cardiac mitochondria are also destructed by protein adducts, which decreases ATP synthesis and furthers cardiotoxicity. CP intake can also cause blood vessels damage, vasoconstriction, and endothelial damage.

Another mechanism by which CP also causes cardiotoxicity is through stimulation of p53 and p38 mitogen-stimulating protein kinase pathways that leads to cardiac hypertrophy, inflammation, and apoptosis. Furthermore, the concentration of E3 ligase and MuRF1 are increased by the stimulated p38 in cardiomyocytes, leading to myosin heavy chain destruction. Previous studies found that CP causes changes in Ca²⁺ homeostasis in the heart leading to Ca²⁺ overload, increased activity of the heart muscle, increased blood pressure, endoplasmic reticulum (ER) stress, decrease ATP synthesis and increased sympathetic activity causing cardiomyopathy and HF.

### 3. MOLECULAR MECHANISMS OF CP-INDUCED CARDIOTOXICITY

#### 3.1. Alteration in energy production

The cardiomyocytes depend on fatty acids (FA) and glucose to produce energy. Normally, long chain fatty acids (LCFA) oxidation represents more than 60–90 percent of ATP synthesis. Glucose and lactate are responsible for synthesis of the remaining ATP.

FA metabolism is controlled by two main factors (i) heart fatty acid binding protein [H-FABP] and (ii) carnitine palmitoyl transferase-I [CPT-I]. H-FABP is present in the cytoplasm of cardiac cells and has a critical function as it transfers FA to the mitochondria. This transfer releases acetyl-CoA in mitochondria to produce energy through kreb's cycle and to eliminate the free FA and their toxic intermediates from the cytoplasm. CPT-I, however, is responsible for the transfer of LCFA through the membrane of mitochondria in cardiomyocytes. Several research studies showed that any change in the concentration of these metabolic intermediates lead to massive cardiac dysfunction and cardiomyopathy. It is well defined that CP, doxorubicin and other anti-tumour therapy associated with cardiomyopathy were reported to be secondary to the inhibition of the expression of H-FABP and CPT-I in cardiac tissues. CP decreases H-FABP leading to decrease in the transfer of FFA from the cytoplasm to mitochondria. Furthermore, it causes damage to the heart by enhancing reduction of CPT-1, promoting the action of Acetyl CoA carboxylase that leads to acetyl CoA carboxylation, and synthesis of Malonyl-CoA. Malonyl-CoA is known for decreasing CPT-I and malonyl CoA decarboxylase (MCD) that decarboxylate malonyl-CoA to acetyl CoA. Therefore, reduction in MCD concentration leads to increased malonyl-CoA action and decreased CPT-I activity, causing reduction in β-oxidation. The end result of the inactivation of LCFA oxidation decreases ATP synthesis. When cardiac tissue is depleted from ATP, Ca²⁺ level increases in mitochondria, and ER stress occurs. Decreasing ATP also causes insufficiency of Na⁺-Ca²⁺ pump, Ca²⁺-ATPase, and Na⁺-ATPase activities that causes increased intracellular Ca²⁺ level in cytosol and the sarcoplasm. Increased concentration of intracellular Ca²⁺ mediates different pathological actions as it changes the contraction of the cardiac muscle, promotes inflammation of cardiac cells, and increases oxidative stress leading to many myocardial disorders such as cardiomyopathy, myocardial hypertrophy, and myocardial failure.
myocardial antioxidants. Previous research indicated that CP (150–200 mg/kg i.p) as a single dose causes oxidative and nitrative stress within 7 days. Additionally, it was demonstrated that CP treatment (50 mg/kg, i.p) for 3 successive days lead to increased oxidative stress. Oxidative stress and nitrative stress mediate apoptosis via accumulation of calcium intracellularly, which ultimately activates caspases. Oxidative stress also triggers inflammation via different signaling pathways like p-38 MAPK and TLR-4 that finally causes myocardial hypertrophy and HF.

3.3. Endoplasmic reticulum stress

Acrolein the main toxic metabolite of CP increases the generation of ROS creating oxidative stress environment as well as damage the mitochondria, thus increase the probability of endoplasmic reticulum stress and apoptosis. Ca²⁺ concentration in the cytoplasm is also altered due to sarcoplasmic reticulum Ca²⁺-ATPase (SERCA2a) inactivation and increased activity of RyR receptors. The excess Ca²⁺ is then taken up by mitochondria and causes liberation of cytochrome c, which plays a major role in apoptosis. Also, there is evidence for abnormal calcium homeostasis that is caused by changes in SERCA2a, phospholamban (PLB) and calsequestrin. These changes lead to systolic and diastolic abnormalities.

3.4. Nitric oxide abnormalities

Cyclophosphamide intake was related to increase in nitric oxide that may due to its effect on iNOS or eNOS. ER stress leads to elevation of Ca²⁺ concentration that increases synthesis of NO. It is well documented that NO is highly reactive with superoxide anion (O₂⁻) to form peroxynitrite radical (ONOO⁻) that causes inflammation, cell death and cardiac toxicity. NO has cardioprotective effects under normal conditions by elevating the concentration of cGMP and PKG through the effect of guanine cyclase enzyme. Elevated level of PKG is essential for protection of the cardiac muscle by transforming Ca²⁺ to ER via enhancing SERCA²⁻ and Na⁺/Ca²⁺ pump. iNOS/eNOS has protective effect on cardiac muscle that is mediated by its NO production capacity, but its toxic effect is mediated by the production of peroxynitrite (ONOO⁻). Cardiomyopathy may be induced by iNOS and eNOS, but eNOS has dual function (depending upon O₂⁻ level); however, iNOS is generally toxic to the heart. CP induced cardiotoxicity causes elevation in the concentration of iNOS and NO causing nitrative stress that is associated with the release of reactive oxygen species. Besides, activation of the apoptotic pathway is controlled by the nitrative stress through p38/JNK cascades.

3.5. Inflammation

Myocardial inflammation is documented after exposure to a cardiotoxic drugs or agents like CP. NF-κB signal transduction pathway is important for the synthesis of pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β. Exposure of heart cells to CP leads to activation of NF-κB signalling. It is demonstrated that when transforming growth factor-beta (TGF-β) is phosphorylated, it stimulates NF-κB under the effect of interleukin-12 and TGF-β-activated kinase. A study by Jiang et al., 2017, demonstrated that treatment with CP (80 mg/kg i.p) for 3 days massively increased TNF-α, NF-κB, IL-6 and IL-1β levels. Other researchers showed that when CP is injected (200 mg/kg, i.p), it causes stimulation of CK, IL-6, IL-1β, TNF-α, and NF-κB signalling pathways.

3.6. Cyclophosphamide and calcium dysregulation

The proper concentration of Ca²⁺ in the cell membrane is preserved by SERCA2a, RyR, and IP3R. Any abnormality in the control of Ca²⁺ lead to different degree of cardiotoxicity. Under physiological conditions, Ca²⁺ is stored in the ER, via L type Ca²⁺ channels. CP causes elevation of intracellular Ca²⁺. During systolic stimulation, Ca²⁺ is liberated from the ER into the cytosol through RyR and IP3R. Ca²⁺ should be transported back into the ER to cause muscle relaxation. However, after CP intake, abnormal Ca²⁺ transport is caused by decreased/suppressed SERCA2a and PLB. Increase level of Ca²⁺ in the cytoplasm leads to peroxidation of lipids, mitochondrial stress, stimulated TGF-β and NF-κB, and elevation of ROS levels, which further causes inactivation of SERCA-2a and phosphorylation of PLB. The net result of abnormal regulation of Ca²⁺ is cardiac hypertrophy.
Concisely, the following figure summarizes the most studied molecular mechanisms of CP cardiotoxicity.

![Figure 1](https://aijpms.journals.ekb.eg/)

**Figure 1.** Schematic diagram for the most studied molecular mechanisms of CP cardiotoxicity.

### 5. CONCLUSIONS

Depending on the proposed mechanistic pathways derived from previous research, we summarize that the cardiotoxic effects of CP result from its induction of pathological changes of blood vessels of the myocardium, necrosis of myocytes, and gastro-intestinal bleeding. CP causes toxicity to the heart not only by increasing oxidative stress, but also by other pathways that contribute to myocardial inflammation, apoptosis, damage of the endothelium, and changes in H-FABP and ATP synthesis. Administration of multiple drugs that improves oxidative stress, nitrative stress, uphold H-FABP and maintain integrity of endothelium may be important in reduction of CP-induced cardiotoxicity.

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**List of Abbreviations:**
- ACC: Acetyl-CoA Carboxylase
- ALDH2: Aldehyde Dehydrogenase-2
- ATP: Adenosine Triphosphate
- cTnT: Cardiac Troponin T
- CPT I: Carnitine palmitoyltransferase I
- CK-MB: Creatine Kinase-Mb
- CP: Cyclophosphamide
- DNA: Deoxyribonucleic Acid
- ELISA: Enzyme-Linked Immunosorbent Assay
- eNOS: Endothelial Nitric Oxide Synthase
- ER: Endoplasmic Reticulum
- FFA: Free Fatty Acid
- H-FABP: Heart-Fatty Acid Binding Protein
- HIF: Hypoxia Inducible Factor
- iNOS: Inducible Nitric Oxide Synthase
- IP3R: Inositol Triphosphate Receptor
- LCFA: Long Chain Fatty Acid
- LDH: Lactate Dehydrogenase
- MCD: Malonyl-CoA Decarboxylase
- MuRF1: Muscle RING-Finger Protein-1
- NADPH: Nicotinamide Adenine Dinucleotide Phosphate
- NF-κB: Nuclear Factor kappa-light-chain-enhancer of activated B cells
- PLB: Phospholamban
- RNS: Reactive Nitrogen Species
- ROS: Reactive Oxygen Species
- RyR: Ryanodine Receptor
- SERCA2a: Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ ATPase 2a
- TGF-β: Transforming Growth Factor-beta
- TNF-α: Tumor Necrosis Factor-alpha

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