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 author to author: less than 5% and 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.

1. INTRODUCTION

Malignancy represents an aggressive disorder that causes mortality equal nearly 10,000,000 deaths worldwide [1]. Globally, about 19.3 million new patients with malignancy and almost 10.0 million mortalities due to malignant diseases were recognized worldwide in 2020. This 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 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 phosphoramide mustard. Phosphoramide 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 [21]. 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 [22]. 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-κB-p65 [23]. 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 [21]. 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 [19].

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 [24, 25]. Furthermore, the concentration of E3 ligase and MuRF1 are increased by the stimulated p38 in cardiomyocytes, leading to myosin heavy chain destruction [22, 26]. Previous studies found that CP causes changes in Ca^{2+} homeostasis in the heart leading to Ca^{2+} overload, increased activity of the heart muscle, increase blood pressure, endoplasmic reticulum (ER) stress, decrease ATP synthesis and increased sympathetic activity causing cardiomyopathy and HF [19, 27].

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 [28]. 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 [29].

FA metabolism is controlled by two main factors (i) heart fatty acid binding protein [H-FABP] and (ii) carnitine palmitoyl transferase-I [CPT-I] [30]. H-FABP is present in the cytoplasm of cardiac cells and has a critical function as it transfers FA to the mitochondria [31]. 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 [31]. CPT-1, however, is responsible for the transfer of LCFA through the membrane of mitochondria in cardiomyocytes [32]. 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-1 in cardiac tissues [33-35]. 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 [6]. The end result of the inactivation of LCFA oxidation leads to decreased ATP synthesis. When cardiac tissue is depleted from ATP, Ca^{2+} level increases in mitochondria, and ER stress occurs [36, 37]. Decreasing ATP also causes insufficiency of Na^+-Ca^{2+} pump, Ca^{2+}-ATPase, and Na^+-ATPase activities that causes increased intracellular Ca^{2+} level in cytosol and the sarcoplasm [37]. Increased concentration of intracellular Ca^{2+} 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 [38].

3.2. Oxidative stress

Cyclophosphamide causes oxidative stress in the cardiac cells by reducing the concentration of 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 [39]. Additionally, it was demonstrated that CP treatment (50 mg/kg, i.p) for 3 successive days lead to increased oxidative stress [40]. 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. Ca^{2+} concentration in the cytoplasm is also altered due to sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) inactivation and increased activity of RyR receptors. The excess Ca^{2+} 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 [41-44]. These changes lead to systolic and diastolic abnormalities.

3.4. Nitric oxide

Cyclophosphamide intake was related to increase in nitric oxide that may be due to its effect on iNOS or eNOS [45-50]. 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 (O^{2-}) 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 Ca^{2+} to ER via enhancing $SERCA^{-2}$ and Na^+/Ca^{2+} pump [6]. 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 ($ONOO^-$) [51]. 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 [52]. Besides, activation of the apoptotic pathway is controlled by the nitrative stress through p38/JNK cascades [53].

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 [54-57]. 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 [58]. 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 [57]. 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-kB signalling pathways [54, 59].

3.6. Cyclophosphamide and calcium dysregulation

The proper concentration of Ca^{2+} in the cell membrane is preserved by SERCA2a, RyR, and IP3R [60]. Any abnormality in the control of Ca2+ lead to different degree of cardiotoxicity. Under physiological conditions, Ca^{2+} is stored in the ER, via L type Ca^{2+} channels. CP causes elevation of intracellular Ca2+. During systolic stimulation, Ca^{2+} is liberated from the ER into the cytosol through RyR and IP3R. Ca^{2+} should be transported back into the ER to cause muscle relaxation. However, after CP intake, abnormal Ca^{2+} transport is caused by decreased/suppressed SERCA2a and PLB. Increase level of Ca^{2+} 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 [6].

Concisely, the following figure summarizes the most studied molecular mechanisms of CP cardiotoxicity.
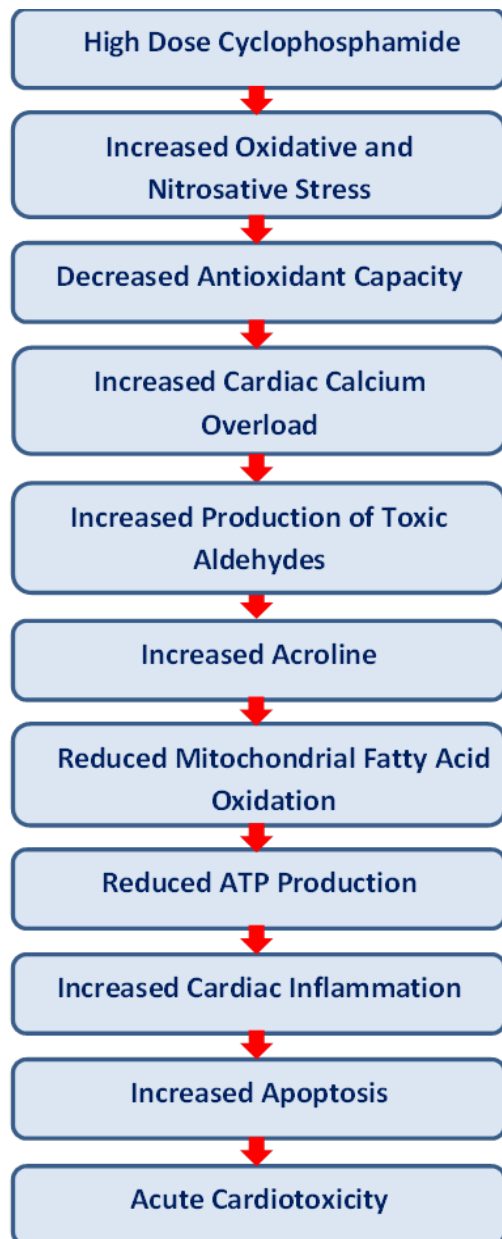
Figure 1. Schematic diagram for the most studied molecular mechanisms of CP cardiotoxicity.

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.

Conflicts of Interest: The authors have declared no conflict of interest.

Author Contribution: All listed authors have contributed significantly to the manuscript.

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-Myoglobin Binding, 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, 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 Ca^{2+}ATPase 2a, TGF-β; Transforming Growth Factor-beta, TNF-α; Tumor Necrosis Factor-alpha.

REFERENCES

1. Piñeros M, Mery L, Soerjomataram I, Bray F, Steliarova-Foucher E. Scaling up the surveillance of childhood cancer: a global roadmap. JNCI: Journal of the National Cancer Institute. 2021 Jan;113(1):9-15.

2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021 May;71(3):209-49.

3. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of

reactive oxygen and nitrogen species as well as PARP activation. Cell Biol Toxicol. 2007 Sep;23(5):303-12.

4. Mills KA, Chess-Williams R, McDermott C. Novel insights into the mechanism of cyclophosphamide-induced bladder toxicity: chloroacetaldehyde's contribution to urothelial dysfunction in vitro. Arch Toxicol. 2019;93(11):3291-3303. 10.1007/s00204-019- 02589-1

5. Hall AG, Tilby MJ (September 1992). "Mechanisms of action of, and modes of resistance to, alkylating agents used in the treatment of haematological malignancies". Blood Reviews. 6 (3): 163–73.

6. Iqubal A, Iqubal MK, Sharma S, Ansari MA, Najmi AK, Ali SM, Ali J, Haque SE. Molecular mechanism involved in cyclophosphamide-induced cardiotoxicity: old drug with a new vision. Life sciences. 2019 Feb 1;218: 112-31.

7. Appelbaum FR, Strauchen JA, Graw RG, Savage DD, Kent KM, Ferrans VJ, Herzig GP. Acute lethal carditis caused by high-dose combination chemotherapy. A unique clinical and pathological entity. Lancet. 1976;1: 58-62.

8. Goldberg MA, Antin JH, Guinan EC, Rappeport JM. Cyclophosphamide cardiotoxicity: an analysis of dosing as a risk factor. Blood. 1986; 68: 1114-1118.

9. Brockstein BE, Smiley C, Al-Sadir J, Williams SF. Cardiac and pulmonary toxicity in patients undergoing high-dose chemotherapy for lymphoma and breast cancer: prognostic factors. Bone Marrow Transplant. 2000; 25:8 85-894.

10. Kamezaki K, Fukuda T, Makino S, Harada M. Cyclophosphamide-induced cardiomyopathy in a patient with seminoma and a history of mediastinal irradiation. Intern Med. 2005; 44: 120-123.

11. Gottdiener JS, Appelbaum FR, Ferrans VJ, Deisseroth A, Ziegler J (1981). Cardiotoxicity associated with high-dose cyclophosphamide therapy. Arch Intern Med 141: 758-763.

12. Baello EB, Ensberg ME, Ferguson DW, Kugler JW, Gingrich RD, Armitage JO, Klassen LW, Kirchner PT, Kerber RE, Marcus ML. (1986). Effect of high-dose cyclophosphamide and total-body irradiation on left ventricular function in adult patients with leukemia undergoing allogeneic bone marrow transplantation. Cancer Treat Rep 70: 1187-1193.

13. Braverman AC, Antin JH, Plappert MT, Cook EF, Lee RT (1991). Cyclophosphamide cardiotoxicity in bone marrow transplantation: a prospective evaluation of new dosing regimens. J Clin Oncol 9: 1215-1223.

14. Ayash LJ, Wright JE, Tretyakov O, Gonin R, Elias A, Wheeler C, Eder JP, Rosowsky A, Antman K, Frei E. (1992). Cyclophosphamide pharmacokinetics: correlation with cardiac toxicity and tumor response. J Clin Oncol 10: 995-1000.

15. Auner HW, Tinchon C, Brezinschek RI, Eibl M, Sormann S, Maizen C, Linkesch W, Schmon-Kampel R, Quehenberger F, Tiran A, Sill H (2002). Monitoring of cardiac function by serum cardiac troponin T levels, ventricular repolarisation indices, and echocardiography after conditioning with fractionated total body irradiation and high-dose cyclophosphamide. Eur J Haematol 69: 1-6.

16. Zver S, Zadnik V, Cernelc P, Kozelj M (2008). Cardiac toxicity of high-dose cyclophosphamide and melphalan in patients with multiple myeloma treated with tandem autologous hematopoietic stem cell transplantation. Int J Hematol 88: 227-236.

17. Kurauchi K, Nishikawa T, Miyahara E, Okamoto Y, Kawano Y. Role of metabolites of cyclophosphamide in cardiotoxicity. BMC Res Notes. 2017;10(1):406. 10.1186/s13104-017-2726-2

18. Wang C, Armstrong DW, Chang CD. Rapid baseline separation of enantiomersand a mesoform of all-trans-astaxanthin, 13-cis-astaxanthin, adonirubin, andadonixanthin in standards and commercial supplements, J. Chromatogr. A 1194(2) (2008) 172–177.

19. Henning RJ, Johnson GT, Coyle JP, Harbison RD. Acrolein can cause cardi-ovascular disease: a review, Cardiovasc. Toxicol. 17 (2017) 227–236.

20. Maeshima T, Honda M, Chikazawa M, Shibata T, Kawai Y, Akagawa M, Uchida K. Quantitative analysis of acrolein-specific adducts generated duringlipid peroxidation–modification of proteins in vitro: identification of Nτ-(3-propanal) histidine as the major adduct, Chem. Res. Toxicol. 25 (2012)1384–1392.

21. Ismahil MA, Hamid T, Haberzettl P, Gu Y, Chandrasekar B, Srivastava S, Bhatnagar A, Prabhu SD. Chronic oral exposure to the aldehyde pollutant ac-rolein induces dilated cardiomyopathy, Am. J. Phys. Heart Circ. Phys. 301 (2011) H2050–H2060.

22. Moghe A, Ghare S, Lamoreau B, Mohammad M, Barve S, McClain C, Joshi-Barve S. Molecular mechanisms of acrolein toxicity: relevance to human disease,Toxicol. Sci. 143 (2015) 242–255.

23. Burcham PC, Thompson CA, Henry PJ. Invited review: acrolein and the lung:chemical, molecular, and pathological aspects, Advances in Molecular Toxicology,Elsevier, 2010, pp. 1–36.

24. Rom O, Kaisari S, Aizenbud D, Reznick AZ. The effects of acetaldehyde andacrolein on muscle catabolism in C2 myotubes, Free Radic. Biol. Med. 65 (2013)190–200.

25. DeJarnett N,Conklin DJ, Riggs DW, Myers JA, O'Toole TE, Hamzeh I, Wagner S, Chugh A, Ramos KS, Srivastava S. Acrolein exposure is associatedwith increased cardiovascular disease risk, J. Am. Heart Assoc. 3 (2014) e000934.

26. Willis S, Schisler JC, Li L, Rodríguez JE, Hilliard EG, Charles C. Patterson, Cardiac muscle ringfinger-1 increases susceptibility to heart failurein vivo, Circ. Res 105 (2009) 80–88.

27. Hyvelin JM, Roux E, Prévost MC, Savineau JP, Marthan R. Cellular me-chanisms of acrolein-induced alteration in calcium signaling in airway smoothmuscle, Toxicol. Appl. Pharmacol. 164 (2000) 176–183.

28. Khan V, Sharma S, Bhandari U, Sharma N, Rishi V, Haque SE. Suppression ofisoproterenol-induced cardiotoxicity in rats by raspberry ketone via activation ofperoxisome proliferator activated receptor-α, Eur. J. Pharmacol. 842 (2018) 157–166.

29. Lopaschuk GD, Stanley WC. Malonyl-CoA decarboxylase inhibition as a novelapproach to treat ischemic heart disease, Cardiovasc. Drugs Ther. 20 (2006)433–439.

30. Szewczak K, Niespialowska M, Jakubowska-Solarska B, Chalabis M, Gawlowicz J. Prospective of ischemic stroke biomarkers, Curr. Issues Pharm. Med.Sci. 30 (2017) 69–74.

31. Furuhashi M, Hotamisligil GS. Fatty acid-binding proteins: role in metabolic diseases and potential as drug targets, Nat. Rev. Drug Discov. 7 (2008) 489.

32. Bremer J. Carnitine–metabolism and functions, Physiol. Rev. 63 (1983)1420–1480.

33. Sayed-Ahmed MM, Rishk AM, Solmon S, Abdel-Aleem S. Protection by L-carnitine against the inhibition of gene expression of heart fatty acid binding protein bychronic administration of doxorubicin, J. Egypt. Natl. Canc. Inst. 12 (2000)275–281.

34. ElGhandour AH, El Sorady M, Azab S, ElRahman M. Human heart-type fattyacid-binding protein as an early diagnostic marker of doxorubicin cardiac toxicity,Hematol. Rev. 1 (2009).

35. 35) Cao Y, Wang X, Li S, Wang H, Yu L, Wang P. The effects of l-carnitine againstcyclophosphamide-induced injuries in mouse testis, Basic Clin. Pharmacol.Toxicol. 120 (2017) 152–158.

36. Santulli G, Xie W, Reiken SR, Marks AR. Mitochondrial calcium overload is akey determinant in heart failure, Proc. Natl. Acad. Sci. 112 (2015) 11389–11394.

37. Kohlhaas M, Nickel AG, Maack C. Mitochondrial energetics and calcium coupling in the heart, J. Physiol. 595 (2017) 3753–3763.

38. Dietl A, Maack C. Targeting mitochondrial calcium handling and reactive oxygenspecies in heart failure, Curr. Heart Fail. Rep. 14 (2017) 338–349.

39. Omole JG, Ayoka OA, Alabi QK, Adefisayo MA, Asafa MA, Olubunmi BO, Fadeyi BA. Protective effect of kolaviron on cyclophosphamide-induced cardiactoxicity in rats, J. Evid. Based Integr. Med. 23 (2018) (2156587218757649).

40. Swamy AV, Patel U, Koti B, Gadad P, Patel N, Thippeswamy A. Cardioprotective effect of saraca indica against cyclophosphamide induced car-diotoxicity in rats: a biochemical, electrocardiographic and histopathologicalstudy, Indian J. Pharm. 45 (2013) 44.

41. Sato Y, Kiriazis H, Yatani A, Schmidt AG, Hahn H, Ferguson DG, Sako H, Mitarai S, Honda R, Mesnard-Rouiller L. Rescue of contractile parameters andmyocyte hypertrophy in calsequestrin overexpressing myocardium by phospho-lamban ablation, J. Biol. Chem. 276 (2001) 9392–9399.

42. Gramolini AO, Kislinger T, Alikhani-Koopaei R, Fong V, Thompson NJ, Isserlin R, Sharma P, Oudit GY, Trivieri MG, Fagan A. Comparative pro-teomics profiling of a phospholamban mutant mouse model of dilated cardio-myopathy reveals progressive intracellular stress responses, Mol. Cell. Proteomics7 (2008) 519–533.

43. Younce CW, Burmeister MA, Ayala JE. Exendin-4 attenuates high glucose-in-duced cardiomyocyte apoptosis via inhibition of endoplasmic reticulum stress and activation of SERCA2a, Am. J. Phys. Cell Phys. 304 (2013) C508–C518.

44. Hamm NC, Stammers AN, Susser SE, Hlynsky MW, Kimber DE, Kehler DS, Duhamel TA. Regulation of cardiac sarco (endo) plasmic reticulum calcium-ATPases (SERCA2a) in response to exercise, Regulation of Ca2+-ATPases, V-ATPases and F-ATPases, Springer, 2016, pp. 187–206.

45. Fogli S, Nieri P, Breschi MC. The role of nitric oxide in anthracycline toxicity and prospects for pharmacologic prevention of cardiac damage, FASEB J. 18(2004) 664–675.

46. Rothe H, Faust A, Schade U, Kleemann R, Bosse G, Hibino T, Martin S, Kolb H. Cyclophosphamide treatment of female non-obese diabetic mice causesenhanced expression of inducible nitric oxide synthase and interferon-gamma, butnot of interleukin-4, Diabetologia 37 (1994) 1154–1158.

47. Souza-Fiho M, Lima M, Pompeu M, Ballejo G, Cunha F, Ribeiro RDA. Involvement of nitric oxide in the pathogenesis of cyclophosphamide-inducedhemorrhagic cystitis, Am. J. Pathol. 150 (1997) 247.

48. Nafees S, Rashid S, Ali N, Hasan SK, Sultana S. Rutin ameliorates cyclopho-sphamide induced oxidative stress and inflammation in Wistar rats: role of NFκB/MAPK pathway, Chem. Biol. Interact. 231 (2015) 98–107.

49. Aronsson P, Vesela R, Johnsson M, Tayem Y, Wsol V, Winder M, Tobin G. Inhibition of nitric oxide synthase prevents muscarinic and purinergic functionalchanges and development of cyclophosphamide-induced cystitis in the rat,Biomed. Res. Int. 2014 (2014).

50. Andersson M, Tobin G, Giglio D. Cholinergic nitric oxide release from the ur-inary bladder mucosa in cyclophosphamide-induced cystitis of the anaesthetizedrat, Br. J. Pharmacol. 153 (2008) 1438–1444.

51. Griendling KK, Touyz RM, Zweier JL, Dikalov S, Chilian W, Chen YR, Harrison DG, Bhatnagar A. Measurement of reactive oxygen species, reactivenitrogen species, and redox-dependent signaling in the cardiovascular system: ascientific statement from the American Heart Association, Circ. Res. 119 (2016) e39–e75.

52. Jo H, Otani H, Jo F, Shimazu T, Okazaki T, Yoshioka K, Fujita M, Kosaki A, Iwasaka T. Inhibition of nitric oxide synthase uncoupling by sepiapterin improves left ventricular function in streptozotocin-induced diabetic mice. Clinical and Experimental Pharmacology and Physiology. 2011 Aug;38(8):485-93.

53. Chong E, Chang SL, Hsiao YW, Singhal R, Liu SH, Leha T, Lin WY, Hsu CP, ChenYC, Chen YJ. Resveratrol, a red wine antioxidant, reduces atrial fibrillation susceptibility in the failing heart by PI3K/AKT/eNOS signaling pathway

activation, Heart Rhythm. 12 (2015) 1046–1056.

54. Song Y, Zhang C, Wang C, Zhao L, Wang Z, Dai Z, Lin S, Kang H, Ma X. Ferulic acid against cyclophosphamide-induced heart toxicity in mice by in-hibiting NF-κB pathway, Evid. Based Complement. Alternat. Med. 2016 (2016).

55. Hamada K, Nagai S, Kitaichi M, Jin G, Shigematsu M, Nagao T, Sato A, Mishima M. Cyclophosphamide-induced late-onset lung disease, Intern. Med. 42(2003) 82–87.

56. Asiri YA, Probucol attenuates cyclophosphamide-induced oxidative apoptosis, p53 and Bax signal expression in rat cardiac tissues, Oxidative Med. Cell. Longev.3 (2010) 308–316.

57. Jiang W, Liu J, Li P, Lu Q, Pei X, Sun Y, Wang G, Hao K. Magnesium iso-glycyrrhizinate shows hepatoprotective effects in a cyclophosphamide-inducedmodel of hepatic injury, Oncotarget 8 (2017) 33252.

58. Gingery A, Bradley EW, Pederson L, Ruan M, Horwood NJ, Oursler MJ. TGF-βcoordinately activates TAK1/MEK/AKT/NFkB and SMAD pathways to promote osteoclast survival, Exp. Cell Res. 314 (2008) 2725–2738.

59. Alhumaidha KA, Saleh DO, Abd El Fattah MA, El-Eraky WI, Moawad H. Cardiorenal protective effect of taurine against cyclophosphamide-induced toxicity in albino rats, Can. J. Physiol. Pharmacol. 94 (2015) 131–139.

60. Li L, Shang H, Fang P, Liao Y, Tao H. Hypericin protects against doxorubicin-induced dilated cardiomyopathy in rats via down-regulating expression of Cx43and inhibiting of TNF-α, ET-1, BNP, Int. J. Clin. Exp. Med. 9 (2016) 7453–7461.