INTRODUCTION

Anthracyclines are potent chemotherapeutic agents for treatment of multiple solid and haematologic malignancies. Despite their high efficacy in cancer treatment, anthracyclines can induce cardiotoxicity in both acute and chronic forms. Acute cardiotoxicity has been described as arrhythmia and transient left ventricular dysfunction, but these are rare as compared to chronic cardiotoxicity.1,2 High cumulative doses of anthracyclines can lead to left ventricular systolic dysfunction and heart failure. The highest incidence was observed in doxorubicin therapy in which a sharp rise in left ventricular systolic dysfunction occurred when the cumulative dose of doxorubicin reached 400 mg/m².3 The reported incidence of heart failure is 5%, 26% and 48% in patients receiving 400, 550 and 700 mg/m² of doxorubicin, respectively.4 From a recent prospective cohort study,5 the incidence of left ventricular systolic dysfunction was found to be
9% and the majority of the cases (98%) occurring within the first year of treatment. It has been shown that the risk of heart failure could be cumulative for up to 30 years after exposure to anthracyclines. Currently, there is no standard effective therapy for the prevention of anthracycline-induced cardiotoxicity.

There are several proposed mechanisms for doxorubicin-induced cardiotoxicity. Doxorubicin primarily targets topoisomerase IIβ (Top2β) and induces DNA double-strand breaks. It also disrupts cardiac pro-survival pathway, the neuregulin/ErbB signalling which results in mitochondrial dysfunction and apoptosis. Furthermore, the most pronounced mechanism responsible for doxorubicin-induced cardiotoxicity is the formation of reactive oxygen species (ROS), leading to oxidative stress. The generation of ROS occurs via multiple pathways. Mitochondria are subcellular organelle, which are the main source and target of ROS. Several studies indicated that doxorubicin-induced mitochondrial dysfunction, increased production of ROS which subsequently led to the development of cardiac muscle dysfunction and heart failure. Mitochondria occupy about 40% of each cardiomyocyte volume, and the majority of the energy produced in the cardiomyocyte is from mitochondrial respiration.

Doxorubicin can target to the mitochondria mainly through ROS production. ROS induces mitochondrial DNA damage and decreases mitochondrial transmembrane potential. In addition, doxorubicin also directly interferes with mitochondrial function by inhibiting electron transport chain proteins expression and promotes mPTP opening. Inhibition of mitochondrial function by doxorubicin contributes to cardiac energy starvation and cell death. All of these data suggest that mitochondria are one of the main regulators in the development of doxorubicin-induced cardiotoxicity.

Mitochondria are dynamic organelles whose primary function is ATP production. Mitochondria protect cells against cellular stress by several processes. Mitochondrial transcription factors such as peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) and mitochondrial transcription factor A (TFAM) mediate an increase in mitochondrial number in response to cellular damage. This process is called mitochondrial biogenesis which could regulate mitochondrial dynamics. Mitochondrial dynamics consists of mitochondrial fusion and fission. Maintaining balance of mitochondrial dynamics is a key for achieving normal mitochondrial function by controlling several aspects including mitochondrial respiration, mitochondrial metabolism and ROS production. Mitochondrial dynamics are regulated by guanosine triphosphatases (GTPases) in the dynamin family. Fusion is mediated by mitofusin-1 (MFN1) and mitofusin-2 (MFN2) proteins at the outer mitochondrial membrane and optic atrophy 1 (OPA1) at the inner mitochondrial membrane. Mitofusins initiate fusion by the process of mitochondrial tethering of two adjacent mitochondria and create homodimeric or heterodimeric complexes of MFN1 and MFN2 at the outer mitochondrial membrane. After fusion of the outer mitochondrial membrane, OPA1 mediates the inner mitochondrial membrane fusion resulting in a more interconnected mitochondrial network. With regards to mitochondrial fission, DRP1 is recruited from the cytosol and binds to DRP1 receptor proteins at the outer mitochondrial membrane including mitochondrial fission protein1 (MTPF1) and mitochondrial fission factor (MFF). Fission leads to mitochondrial fragmentation and enhances the generation of ROS. There is growing evidence that doxorubicin disrupts mitochondrial dynamics and mitochondrial function.

In this review, the effects of doxorubicin on mitochondrial dynamics and mitochondrial function have been comprehensively summarized. Consistent and controversial reports from in vitro and in vivo models have been presented. The proposed pharmacological interventions to ameliorate doxorubicin-induced cardiotoxicity have been presented and discussed.

### 2 | DOXORUBICIN-INDUCED CARDIOTOXICITY AND CARDIAC MITOCHONDRIAL DYNAMICS: EVIDENCE FROM IN VITRO AND IN VIVO STUDIES

Interference with mitochondrial dynamics is associated with the development of cardiovascular and metabolic diseases including ischaemia-reperfusion injury, metabolic syndrome and also in doxorubicin-induced cardiotoxicity. Doxorubicin increased mitochondrial fragmentation followed by ROS generation and apoptosis. These effects of doxorubicin could be explained by an imbalance of mitochondrial dynamics. In vitro studies in neonatal rat cardiomyocytes (NRCMs) treated with doxorubicin dosage between 0.86 and 1.72 µmol/L for 1-24 hours showed a significant decrease in mitochondrial fusion proteins, MFN1, MFN2, OPA1 and increase in phosphorylation of DRP1 at serine 616. Using H9c2 cell and postnatal rat cardiomyocytes also showed consistent result that phosphorylation of DRP1 at serine 616 was increased. These findings indicated that doxorubicin inhibited mitochondrial fusion and promoted mitochondrial fission. There are some conflicting results regarding mitochondrial fusion. Rats treated with doxorubicin dosage 2 mg/kg/wk for 7 weeks showed suppression of MFN1, MFN2 and OPA1 proteins expression. Another in vivo study in mice treated with single dose of doxorubicin (10 mg/kg) showed that doxorubicin increased RNA expression of Mfn2 and Opa1.
| Study model                     | Methods (Drug/Dose/Route/Duration) | Major Findings       | Interpretation                                           | Ref |
|--------------------------------|------------------------------------|----------------------|---------------------------------------------------------|-----|
| **in vitro reports**           |                                    |                      |                                                         |     |
| Isolated 1- to 4-d-old neonatal Sprague Dawley cardiomyocytes | Dox/0.86-1.72 µmol/L/8-24 h Transfected with Mfn2 • Dox/1.72 µmol/L/4-24 h | ↓MFN2 ↑MFN2 ↑Mitochondrial fragmentation ↓Mitochondrial fragmentation ↑ROS ↑Caspase-3 activity ↑TUNEL staining ↓ROS ↓Caspase3 activity ↓TUNEL staining | Dox decreased MFN2 expression which promoted mitochondrial fission, ROS production and apoptosis | 14  |
| 1-d-old neonatal Wistar rats cardiomyocytes | Dox/1.0 µmol/L/1-15 h Transfected with anti-miR-140 • Dox/1.0 µmol/L/1-12 h Transfected with Mfn1 • Dox/1.0 µmol/L/5-15 h | ↓MFN1 ↓MFN2 ↓OPA1 ↑MFN1 ↔MFN2 ↔OPA1 ↑Mitochondrial fragmentation ↓Mitochondrial fragmentation ↑TUNEL staining ↓TUNEL staining | MFN1 was negatively controlled by miR-140 and could regulate mitochondrial fission and apoptosis | 30  |
| Neonatal rat cardiomyocytes    | Dox/0.1-0.3 µmol/L/24 h Transfected with Ad-Sirt3 • Dox/0.1-0.3 µmol/L/24 h | ↑OPA1 acetylation ↓OPA1 acetylation ↑TUNEL-positive cells ↓TUNEL-positive cells | SIRT3 overexpression blocked Dox-mediated cell death by reducing OPA1 acetylation | 25  |
| Postnatal rat cardiomyocytes  | Dox/10 µmol/L/18 h Transfected with Bnip3-shRNA • Dox/10 µmol/L/18 h | ↑pSer616 DRP1 ↑Mitochondrial fragmentation ↓Mitochondrial fragmentation | BNIP3 was a critical mediator of mitochondrial fragmentation induced by Dox | 29  |
| H9c2 cell                      | Dox/5 µmol/L/24 h Treated with Mdivi-1/1 µmol/L/30 min • Dox/5 µmol/L/24 h | ↑pSer616 DRP1/DRP1 ↓pSer616 DRP1/DRP1 ↑Annexin V ↑Cleaved caspase3 ↓Annexin V ↓Cleaved caspase3 | Mdivi-1 blunted the increase in mitochondrial fission caused by Dox treatment | 31  |
| HL-1 cell                      | Dox/1-2 µmol/L/4-24 h Transfected with Mtfp1-shRNA • Dox/2 µmol/L/24 h Transfected with Mtfp1 cDNA • Dox/0.3 µmol/L/24 h | ↑MTFP1 ↑Mitochondria DRP1 ↓Cytosol DRP1 ↑Mitochondrial fission ↓Mitochondrial DRP1 ↑Cytosol DRP1 ↑Mitochondrial fission ↑Mitochondrial fission | MTFP1 was associated with DRP1 activation and mediated the signal required for Dox-induced mitochondrial fission and apoptosis | 28  |

*Continues*
Differences between dosage and analytical methods may explain the discrepancy in results found concerning the mitochondrial fusion process.

The precise mechanism of how doxorubicin regulates mitochondrial dynamics proteins is unclear. It has been shown that DRP1 translocation is controlled by MTFP1. Transfection of HL-1 cardiac cell with Mtfp1-shRNA inhibited mitochondrial fission and apoptosis. Interestingly, recent evidence showed that sirtuin-3 (SIRT3) could regulate mitochondrial dynamics. SIRT3 is a protein that possesses deacetylase activity and is responsible for deacetylating several mitochondrial proteins including OPA1. Acetylation of OPA1 represses OPA1 function. Transfection of the NRCMs with Ad-Sirt3 showed a decrease in OPA1 acetylation and apoptosis. This finding suggested that SIRT3 may have a protective role in a doxorubicin-induced cardiotoxicity model. Several studies have proposed the role of microRNA in the regulation of mitochondrial dynamics. Cardiomyocytes isolated from rat hearts showed that MFN1 was negatively controlled by microRNA-140. Another in vitro study using NRCMs indicated that microRNA-532-3p promoted mitochondrial fission by suppressing the expression of apoptosis repressor with caspase recruitment domain (ARC). Further studies are needed to investigate the role of different microRNA in this model. Overall, evidence from in vitro and in vivo models indicated that doxorubicin caused an imbalance in mitochondrial dynamics by inhibiting mitochondrial fusion and promoting mitochondrial fission. The findings from these reports are comprehensively summarized in Table 1.

### Table 1: DOXORUBICIN-INDUCED CARDIOTOXICITY AND CARDIAC MITOCHONDRIAL DYSFUNCTION: EVIDENCE FROM IN VITRO AND IN VIVO STUDIES

Molecular mechanisms associated with doxorubicin-induced cardiotoxicity are multifactorial and complex. After several decades of investigation, doxorubicin-induced cardiotoxicity is still a necessary focus in the field of cardio-oncology research as the precise mechanism associated with its cardiotoxicity remains unclear. Increased oxidative stress has been shown as a key feature of doxorubicin-induced heart failure. Cardiomyocytes are known to be more susceptible to oxidative damage than other cells due to their lower capacity for antioxidant defense and their high density of mitochondria, which are the main source of ROS generation. The chemical structure of doxorubicin is susceptible to redox cycling. As it is a quinone compound, doxorubicin is reduced by oxidoreductases within the cell including NADPH oxidase, xanthine oxidase and mitochondrial electron transport chain enzymes, especially complex I. Redox cycling leads to the formation of a semiquinone compound which interacts with oxygen to form the superoxide anion. The generation of ROS induces lipid peroxidation at the cellular membrane and has impact on other subcellular organelles including the nucleus and mitochondria.
| Study model                                      | Methods (Drug/Dose/Route/ Duration)                                                                 | Major Findings                                      | Oxidative phosphorylation | Autophagy Mitophagy |
|------------------------------------------------|-----------------------------------------------------------------------------------------------------|-----------------------------------------------------|---------------------------|----------------------|
| **in vitro reports**                            |                                                                                                     |                                                     |                           |                      |
| Neonatal rat cardiomyocyte                      | • Dox/0.1-0.3 µmol/L/24 h Transfected with Ad-Sirt3 • Dox/0.1-0.3 µmol/L/24 h                         | ↑Fragmented Mt                                      | ↑Swollen Mt               | ↓Fragmented Mt       |
|                                                |                                                                                                     |                                                     |                           | ↓Swollen Mt          |
| Postnatal rat cardiomyocyte                     | • Dox/10 µmol/L/18 h Bnip3-shRNA • Dox/10 µmol/L/18 h                                              | ↓OCR                                                | ↓RRC                     | ↑OCR                 |
| Isolated postnatal rat cardiomyocytes from 1- to 2-d-old Sprague Dawley rats | • Dox/5 or 10 µmol/L/18 h Ad-Bnip3 • Dox/5 or 10 µmol/L/18 h Bnip3-shRNA • Dox/5 or 10 µmol/L/18 h | ↓OCR                                                | ↓RRC                     | ↑OCR                 |
| Human right atrial trabeculae                   | • Dox/1 µmol/L/0-90 min                                                                             | ↓Developed force                                    | ↓State 2                 | ↓State 3             |
|                                                |                                                                                                     | ↓Maximal contraction velocity                        |                           |                      |
|                                                |                                                                                                     | ↓Maximal relaxation velocity                         |                           |                      |
| H9c2 cell                                       | • Dox/0.5-1 µmol/L/6-24 h                                                                            |                                                     |                           |                      |
| H9c2 cell                                       | • Dox/1 µmol/L/24 h                                                                                   |                                                     |                           |                      |
| H9c2 cell                                       | • Dox/1-10 µmol/L/24 h                                                                               | ↓Complex I,II,IV protein                             | ↑Complex I,II,IV protein  |                      |
| Human adult ventricular cardiomyocyte (AC16)    | • Dox/250 nmol/L/24 h                                                                               |                                                     | ↑LC3-II/LC3-I            |                      |
|                                                |                                                                                                     |                                                     | ↑Beclin1                 |                      |
|                                                |                                                                                                     |                                                     | ↓p62                     |                      |
|                                                |                                                                                                     |                                                     | ↑PINK1                   |                      |
|                                                |                                                                                                     |                                                     | ↑Parkin                  |                      |
| H9c2 cell                                       | • Dox/3 µmol/L/24 h                                                                                  |                                                     | ↑LC-3I                   |                      |
|                                                |                                                                                                     |                                                     | ↑LC-3II                  |                      |
|                                                |                                                                                                     |                                                     | ⇔LC-3I/LC-3I             |                      |
|                                                |                                                                                                     |                                                     | ↑p62                     |                      |
| Neonatal cardiomyocyte                          | • Dox/1 µmol/L/24 h + Medium APN/30 µg/mL/24 h AMPK inhibitor 1 µmol/L • Dox/1 µmol/L/24 h + High APN/100 µg/mL/24 h |                                                     |                           |                      |
| ROS/Apoptosis | MMP/mPTP | Mitochondrial protein | Interpretation | Ref |
|---------------|----------|-----------------------|----------------|----|
| SIRT3 expression preserved mitochondrial morphology after Dox treatment | 25 |
| ↑ROS ↑LDH ↑%Dead cells ↓%Dead cells | ↓MMP ↑mPTP ↑Mitochondria BNIP3 | Dox induced mitochondrial dysfunction and increased mitophagy activity through BNIP3 activation | 29 |
| ↑ROS ↑LDH ↑Cardiac troponin T ↓Cell viability ↓LDH ↓Cardiac troponin T ↑Cell viability | ↓MMP ↑mPTP ↑Mitochondria BNIP3 ↑MMP ↓mPTP | Dox-induced mitochondrial respiratory chain defect was linked to BNIP3 activation | 44 |
| ↓MMP ↑mPTP | Dox induced mitochondrial permeability transition pore opening and contractile dysfunction | 50 |
| ↑ROS ↑Nuclear p53 ↑Bax ↑Cytosolic cytochrome c ↑Caspase3,9 activity ↓DNA synthesis | ↓MMP | Dox induced mitochondrial dysfunction and activation of apoptotic pathway | 51 |
| ↑ROS ↑Bax ↑Cytosolic cytochrome c ↑Cleaved caspase3 ↓Cell viability | ↓MMP ↓ARE activity ↓Nuclear NRF2 ↔Cytosol Keap1 | Dox induced mitochondrial dysfunction, decreased HO1 protein expression and nuclear NRF2 translocation | 49 |
| ↓SOD2 ↑ROS | ↓SIRT3 ↓SIRT1 ↑PGC1α acetylation | | |
| ↓ROS ↓MMP ↓Cell viability | ↓SIRT3 ↓SIRT1 ↓PGC1α acetylation | Dox inhibited SIRT3, SIRT1 expression, mitochondrial respiration and increased ROS production | 18 |
| ↓ROS ↓MMP ↓Cell viability | ↓PGC-1α ↓NRF1 ↓TFAM | Dox-induced mitophagy and autophagosome formation which resulted in decreased mitochondrial biogenesis proteins expression and mitochondrial damage | 74 |
| ↑ROS ↑Caspase activity ↓Cell viability | ↑ROS ↓Cell viability | Dox reduced autophagic activity, increased ROS and decreased cell viability | 77 |
| ↓Bcl2 ↑Bax ↑Cytosol cytochrome c ↑Cleaved caspase3 ↑TUNEL-positive ↑Bcl2 ↓Bax ↓Cytosol cytochrome c ↓Cleaved caspase3 ↓TUNEL-positive ↓Bcl2 ↑Bax ↑Cytosol cytochrome c ↑Cleaved caspase3 ↑TUNEL-positive | ↓p-AMPKα ↑p-AMPKα | APN had cardioprotective effects against Dox-induced cardiomyopathy, and these effects could be involved in the regulation of AMPK signalling pathway | 56 |
| Study model | Methods (Drug/Dose/Route/Duration) | Major Findings | Oxidative phosphorylation | Autophagy Mitophagy |
|-------------|-----------------------------------|----------------|--------------------------|---------------------|
| Neonatal Sprague Dawley rats cardiomyocyte | Dox/1 µmol/L/2-24 h | **Heart function/Morphology** | **Oxidative phosphorylation** | **Autophagy Mitophagy** |
| H9c2 cell | Dox/10 nmol/L/1-72 h | | | |
| Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) | Dox/1 µmol/L/24 h | | | |
| Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) | Dox/3-10 µmol/L/16-24 h | | | |
| Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) | Acute model (Evaluated immediately after Dox) | | | |
| Human pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) | Chronic model (Evaluated at Day 14) | | | |
| in vivo reports | Male C57BL/6 mice | Dox/10 mg/kg/ip/single dose (Follow-up 1.5 wk) | ↓LVFS | ↓CR |
| in vivo reports | 8-week-old male Balb/c mice | Dox/15 mg/kg/ip/3 times/wk/2 wk | ↓LVEF ↑LVEDD ↑LVEDD ↑Mt width ↑Mt length ↓Mt Length/width ratio | ↓Complex I activity ↔Complex IV activity |
| in vivo reports | 6-week-old male Sprague Dawley rats | Dox/2 mg/kg/wk/ip/7 wk | | ↑Beclin1 ↑Beclin1/Bcl2 ↑LC3-II ↑PINK1 ↑p62 |
| in vivo reports | Male Sprague Dawley rats | Dox/4 mg/kg/dose/ip/every 5 d/3 doses (Total 12 mg/kg) | ↓LVEF ↑LVEF | ↑PINK1 ↓Parkin ↓p62 ↓LC3-II ↓PINK1 ↑Parkin ↑p62 ↑LC3-II |
| ROS/Apoptosis                        | MMP/mPTP/Mitochondrial protein | Interpretation                                      | Ref |
|--------------------------------------|--------------------------------|----------------------------------------------------|-----|
| ↑p-p53                               | ↓MMP                           | Dox-induced cardiomyocyte apoptosis by increased   | 59  |
| ↑Bax                                 | ↑p-AMPK_α                       |                                                     |     |
| ↓Bcl-2                               | ↑AMP/ATP                        |                                                     |     |
| ↑Caspase3                            | ↑p-ACC                          |                                                     |     |
| ↑TUNEL-positive cells                |                                |                                                     |     |
| ↑ROS                                 | ⇔p-AMPK                         | Dox induced ROS production but had no significant  | 83  |
| ↑LDH                                 | (72 h)                          | changes in the AMPK signalling pathway             |     |
| ↓Cell viability                      |                                |                                                     |     |
| ↑ROS                                 | ↓MMP                            |                                                    |     |
| ↓MMP                                 | ↑Apoptosis cell                 |                                                    |     |
| ↑ROS                                 | ↑ATP                            |                                                    |     |
| ↓MMP                                 | ↑Apoptosis cell                 |                                                    |     |
| ↑↑ROS                                | ↓↓ATP                           |                                                    |     |
| ↓↓MMP                                | ↑↑Apoptosis cell                |                                                    |     |
| ↑DNA double-strand breaks            | ↑Apoptosis cell                 |                                                    |     |
| ↑Intracellular calcium               |                                |                                                    |     |
| ⇔MMP                                 | ↓Cell number                    |                                                    |     |
| ⇔Mitochondrial calcium               | ↓Cell number                    |                                                    |     |
| ↓MMP                                 |                                |                                                    |     |
| ↑Mitochondrial calcium               |                                |                                                    |     |
| ↑mPTP                                |                                  | Dox decreased myocardial contractile function,     | 13  |
| ↑PGC1_α                              |                                  | mitochondrial function and increased mPTP opening   |     |
| ↑Mt DNA                              |                                  |                                                    |     |
| ↑Cleaved caspase3                    |                                  | Dox decreased LV function, mitochondrial respiration| 31  |
| ↑TUNEL staining                      |                                  | and increased apoptosis                            |     |
| ↑Caspase3,9                           | ↑mPTP                           | Dox increased autophagic activity and apoptosis     | 32  |
| ↑Bax/Bcl2 ratio                      |                                |                                                    |     |
| ↓Sestrins2                           |                                | Overexpression of SESN2 protected against Dox-     | 76  |
| ↑Sestrins2                           |                                | induced cardiotoxicity by alleviating Dox-induced  |     |
|                                       |                                | inhibition of Parkin-mediated mitophagy            |     |

(Continues)
| Study model | Methods (Drug/Dose/Route/Duration) | Major Findings |
|-------------|-----------------------------------|----------------|
| Mice aged 8-10 wk | • Dox/20 mg/kg/ip/single dose **Bnip3** -/- mice • Dox/20 mg/kg/ip/single dose | **Heart function/Morphology** | **Oxidative phosphorylation** | **Autophagy Mitophagy** |
| | | ↑Swollen Mt | ↓OCR | ▶**OCR** |
| | | ↑Loss of Mt cristae | ↑OCR | ↑RRC |
| | | ↑Mt vacuolization | ↓LVFS | ↑LVFS |
| | | ↓LVFS | Intact cristae |  |
| | | ↓Mt vacuolization | ↑LVFS |  |
| | | ↑LVFS |  |  |
| C57BL mice | • Dox/15 mg/kg/ip/single dose | ↓LVEF | ↓FS | ↑Myocardial swelling and vacuolization |
| 8-week-old female C57BL6 mice | • Dox/8 mg/kg/wk/ip/4 wk | ↓Complex I,II,IV protein |  | |
| LC3 transgenic mice inoculated with E0771 cells | • Dox/20 mg/kg/ip/split into 2 doses | ▼LC-3II/LC-3I |  | ↑p62 |
| C57BL/6 mice | WT mice • Dox/4 mg/kg/wk/6 wk **APN transgenic sense (APN-SE)** • Dox/4 mg/kg/wk/6 wk **APN transgenic antisense (APN-AS)** • Dox/4 mg/kg/wk/6 wk | ↓LVFS | ↑Myocardial fibrosis | ▼APN |
| | | ↑Myocardial fibrosis | ↑LVFS |  |
| | | ↓Myocardial fibrosis | ↓LVFS |  |
| | | ↑↑Myocardium fibrosis |  |  |
| Male Sprague Dawley rats | • Dox/20 mg/kg/ip/single dose | ↓LVEDV | ↓Stroke volume | ↓LVEF |
| Male Wistar Albino rats | • Dox/20 mg/kg/ip/divided 2 doses/day 2 and 4 | ↑Myocyte degeneration | ↑Myocardial degeneration | ▼Beclin-1 |
| | | ↑Interrupted muscle fibre | ↑Intersticial inflammation | ↓LC3B-II |
| | | ↑Wide interstitial spaces | ↑Intersticial haemorrhage | ↑p62 |
| Male Wistar rats | • Dox/15 mg/kg/ip/single dose | ↑Myocardial degeneration | ↑Myocardial degeneration | ▼Beclin-1 |
| | | ↑Intersticial inflammation | ↑Intersticial haemorrhage | ↓LC3B-II |
| | | ↑Intersticial inflammation | ↑Intersticial haemorrhage | ↑p62 |
| Sprague Dawley rats | • Dox/3 mg/kg/EOD/ip/6 doses | ▼Aortic flow | ▼Cardiac output | ▼Beclin-1 |
| | | ▼Stroke volume | ▼Myocardial thickness | ↓LC3B-II |

**Abbreviations:** ACC, Acetyl-CoA carboxylase; APN, Adiponectin; ARE, Antioxidant-responsive elements BNIP3, BCL2/adenovirus E1B 19 kD protein-interacting protein 3; CAT, Catalase; COX1, cytochrome c oxidase subunit1; Dox, Doxorubicin; FHC, Ferritin heavy chain; GSH, Reduced glutathione; GSSG, Oxidized glutathione; GSTα, Glutathione S-transferase-α; HO1, Haem oxgenase1; Keap1, Kelch-like ECH-associated protein 1; LVEDD, Left ventricular end-diastolic dimension; LVEDV, Left ventricular end-diastolic volume; LVEF, Left ventricular ejection fraction; LVESD, Left ventricular end-systolic dimension; LVFS, Left ventricular fractional shortening; MDA, Malondialdehyde; MMP, Mitochondrial membrane potential; mPTP, Mitochondrial permeability transition pore; Mt, Mitochondria; NRF, Nuclear respiratory factor; OCR, Oxygen consumption rate; P, phosphorylation; PDGFRβ, Platelet-derived growth factor receptor β; PKA, Protein kinase A; RCR, Respiratory control ratio; ROS, Reactive oxygen species; RRC, Reserve respiratory capacity; SIRT, Sirtuin; SOD2, Superoxide dismutase-2; TBA, Thiobarbituric acid; TFAM, Mitochondrial transcription factor A; UCP3, Mitochondrial uncoupling protein 3.
| ROS/Apoptosis | MMP/mPTP | Mitochondrial protein | Interpretation | Ref |
|--------------|----------|-----------------------|----------------|-----|
| ↑LDH        | ↑BNIP3   |                       | Dox activated BNIP3 and induced mitochondrial respiratory chain defects in mouse hearts | 44  |
| ↓Survival   |          |                       |                |     |
| ↑LDH        |          |                       |                |     |
| ↑Survival   |          |                       |                |     |
| ↑Lipid peroxidation | ↓PGC1α |                       | Dox induced cardiac contractile dysfunction and apoptosis through a decrease in mitochondrial biogenesis | 41  |
| ↑Protein carbonylation | ↓NRF-1 |                       |                |     |
| ↓GSH/GSSG   | ↓MtDNA copy number |                       |                |     |
| ↑Cytochrome cytochrome c | ↑TUNEL-positive |                       |                |     |
| ↓SOD2       | ↓SIRT3   |                       | Dox reduced SIRT3 expression, oxidative phosphorylation and SOD2 expression | 18  |
| ↑ Cleaved caspase3 | ↓Bcl2   |                       | Dox reduced autophagic activity, decreased survival and tumour growth | 77  |
| ↓Survival   |          |                       |                |     |
| ↓Tumour growth |        |                       |                |     |
| ↓Bcl2       | ↑p-AMPKα |                       | APN had cardioprotective effects in Dox-induced cardiomyopathy and was involved in the AMPK signalling pathway | 56  |
| ↑Bax        | ↑p-AMPKα |                       |                |     |
| ↑Caspase3   | ↑↓p-AMPKα|                       |                |     |
| ↓Survival   |          |                       |                |     |
| ↑Bcl2       |          |                       |                |     |
| ↓Bax        |          |                       |                |     |
| ↓Caspase3   |          |                       |                |     |
| ↑Survival   |          |                       |                |     |
| ↓↓Bcl2      | ↑↑Bax    | ↑↑Caspase3          |                |     |
| ↓Survival   |          |                       |                |     |
| ↑p-p53      | ↑p-AMPKα |                       | Dox increased p-AMPKα and apoptosis | 59  |
| ↓Bcl2       |          |                       |                |     |
| ↑ Cleaved caspase3 | ↑TNF-α |                       | Dox increased myocardial damage, ROS generation and apoptosis | 73  |
| ↑TUNEL-positive |        |                       |                |     |
| ↓Survival   |          |                       |                |     |
| ↓Bcl-2      |          |                       |                |     |
| ↑Caspase3   |          |                       |                |     |
| ↑CK-MB      |          |                       |                |     |
| ↑LDH        |          |                       |                |     |
| ↓GSH        |          |                       |                |     |
| ↑TBA        |          |                       |                |     |
| ↑LDH        |          |                       | Dox induced myocardial damage, oxidative stress and apoptosis | 107 |
| ↑CK-MB      |          |                       |                |     |
| ↑MDA        |          |                       |                |     |
| ↓SOD        |          |                       |                |     |
| ↑Caspase3   |          |                       |                |     |
| ↔LDH        | ↔p-AMPK  |                       | Dox impaired the autophagic process, increased oxidative damage and cardiac dysfunction | 78  |
| ↔CK-MB      |          |                       |                |     |
| ↑Troponin T |          |                       |                |     |
| ↔MDA        |          |                       |                |     |
production of ROS causes oxidative damage to mitochondrial DNA (mtDNA), increased mitochondrial depolarization and alteration in the morphology of mitochondria as indicated by mitochondrial swelling and fragmentation. The negative consequences of mitochondrial dysfunction result in cardiac contractile dysfunction indicated by both reduced left ventricular fractional shortening and ejection fraction. These could be the effect of ROS production and dysregulation of calcium channels or transporters, which are susceptible to redox cycling. Furthermore, doxorubicin interferes with mitochondrial respiration at electron transport chain (ETC) level by inhibiting complex I, II, IV proteins and complex I activity. These ETC complexes are necessary for the process of oxidative phosphorylation which is the major source of ATP production. Alteration in mitochondrial respiration was observed in doxorubicin models as shown by an increase in state 4, and a decrease in state 3 respiration and respiratory control ratio. These findings indicated that doxorubicin inhibited mitochondrial respiration and led to mitochondrial bioenergetics failure. There is evidence to suggest that inhibition of oxidative phosphorylation is secondary to dysregulation in mitochondrial calcium homeostasis regulated by an opening of mitochondrial permeability transition pores (mPTP). Oxidative stress induces mPTP opening and increases permeability of the inner mitochondrial membrane, thus allowing passage of small molecules into the membrane, leading to matrix swelling, mitochondrial transmembrane potential disruption and release of calcium from the matrix.

Recent evidence found that the opening of mPTP was induced by BCL2/adenovirus E1B 19 kD protein-interacting protein 3 (BNIP3). Knockdown of Bnip3 in mice treated with doxorubicin (20 mg/kg single dose) inhibited mPTP opening and restored mitochondrial function. These findings suggested that BNIIP3 was responsible for the cardiotoxic effects of doxorubicin and could be a molecular target for the attenuation of doxorubicin-induced cardiotoxicity. Furthermore, several studies also investigated the effects of doxorubicin on mitochondrial function using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs). Consistent with the results found in other cell types, doxorubicin decreased mitochondrial membrane potential, increased mitochondrial calcium loading and decreased cell viability in these hiPSC-CMs. Suppression of Sirt3 in hiPSC-CMs also increased the toxicity of doxorubicin which suggested the protective role of SIRT3, and this finding is also consistent with previous reports using NRCM and H9C2 cells. A summary of these reports is shown in Table 2.

In addition to increasing ROS production, doxorubicin can also suppress cardiac antioxidant defence system. This has been postulated to be partially mediated by alteration in the sirtuins family proteins particularly SIRT1 and SIRT3. Suppression of sirtuins expression inhibited various endogenous antioxidant enzyme. Doxorubicin reduced endogenous antioxidant enzyme activity including that of haem oxygenase 1 (HO-1), superoxide dismutase (SOD) and glutathione peroxidase (GPx), the levels being measured in both in vitro and in vivo models. Transcription and translation of mitochondrial antioxidant enzymes required the activation of nuclear respiratory factor-1 and 2 (NRF1, NRF2). Several studies indicated that doxorubicin decreased NRF1, nuclear NRF2, TFAM and HO1 expression. These findings suggested that doxorubicin altered the cardiac antioxidant defence system and could potentially disrupt mitochondrial biogenesis. Taken together, these underlies the importance of mitochondria as the mediator of doxorubicin-induced cardiotoxicity. A summary of reports on cardiac oxidative stress due to doxorubicin is shown in Table 2.

Although oxidative stress is the most notable of mechanisms involved in doxorubicin-induced cardiotoxicity, there is accumulating evidence to suggest that doxorubicin can induce apoptosis through mechanisms that are independent of the ROS production. Doxorubicin has been shown to directly interfere with the nucleus of cardiomyocytes causing DNA damage and secondary activation of p53, thus promoting transcription of pro-apoptotic proteins and the release of cytochrome c which induced apoptosis. The effects of doxorubicin on cardiac mitochondrial function and apoptosis are shown in Figure 1. Moreover, alteration in autophagy and mitophagy is another proposed mechanisms in doxorubicin-induced cardiotoxicity. Mitophagy is the cellular process for removal of damaged mitochondria. Recent in vitro study demonstrated that in human adult ventricular cardiomyocyte cell (AC16) treated with doxorubicin (250 nmol/L/24 h), the PINK1 and Parkin protein expressions were increased with subsequently resulted in mitochondrial biogenesis proteins (PGC-1α, NRF-1 and TFAM) suppression and mitochondrial damage. Consistently, in an in vivo study in rats treated with chronic doxorubicin (2 mg/kg/wk/ip/7 wk) showed a significant increase in PINK1. These findings suggested that doxorubicin-induced mitophagy and contributed to mitochondrial dysfunction. However, there is evidence to suggest that doxorubicin inhibited mitophagy. An in vivo study in rats received doxorubicin (4 mg/kg/dose/ip/every 5 d/3 doses) indicated that doxorubicin suppressed Parkin-mediated mitophagy. Inhibition of mitophagy resulted in accumulation of damaged mitochondria, mitochondrial dysfunction and impairment in heart function. With regard to the role of autophagy, the autophagic proteins were affected by doxorubicin. Doxorubicin altered LC3-II and increased p62 level. There is an emerging consensus that doxorubicin blocked the lysosomal degradation process and increased accumulation of autophagosome and autolysosome; thus, the autophagic process cannot be completed. These findings suggested that doxorubicin dysregulated autophagy and inhibited autophagic clearance which accompanied by ROS production, myocardial dysfunction and apoptosis. Reports on these findings are summarized in Table 2.

### 4 | THE ROLE OF AMPK ON DOXORUBICIN-INDUCED CARDIOTOXICITY

AMPK is a key energy sensor and is activated in response to cellular energy...
Iron signalling plays an important role in the mechanism of doxorubicin-induced cardiotoxicity. Specifically, doxorubicin is able to chelate free iron forming doxorubicin-iron complexes which promotes oxidative stress. In addition to the ROS pathway, evidence suggests that doxorubicin cardiotoxicity is also mediated through the oxidative-independent mechanism which is considered to be a key process. By interfering with iron regulatory proteins (IRPs), doxorubicin treatment could result in the accumulation of free iron within the cardiomyocytes. This occurs from doxorubicinol, a product of doxorubicin metabolism that removes the Fe-S cluster from the IRP-1 which is then converted into apo-IRP1 which allows IRP1 binding to the iron-responsive element (IRE). The apo-IRP1 could bind to IRE at the mRNA of several iron signalling proteins including ferritin and transferrin receptor1. This complex process contributes to the inhibition of ferritin synthesis and activation of transferrin expression, thus increasing iron overload. Accumulation of free iron enhances the cardiotoxic effects of doxorubicin. Optimal IRE/IRP interaction is an important factor for achieving balance in iron homeostasis. In addition, doxorubicin could directly interfere with IRE mRNA of ferritin heavy chains (FHC) and suppress ferritin expression. However, several studies indicated that FHC gene and protein expression are increased following doxorubicin exposure. This finding is considered to be a defensive mechanism against cytotoxicity of ROS production. Moreover, a recent in vitro study demonstrated that doxorubicin induced the accumulation of free iron, and this was aggravated by haem degradation process. Accumulation of iron within mitochondria caused lipid peroxidation on its membrane which triggered a specific type of programmed cell death called ‘ferroptosis’. The ferroptosis is a distinct form of regulated cell death which is an iron-dependent process. Blocking of ferroptosis showed a reduction of mortality in mice treated with doxorubicin (20 mg/kg). These reports indicated that doxorubicin can induce cell death through ferroptosis apart from the notable apoptosis. Due to this evidence, prevention of cardiac iron overload by targeting the iron signalling pathway could be an effective strategy in the amelioration of doxorubicin-induced cardiotoxicity. Moreover, inhibition of ferroptosis could be a potential intervention to prevent doxorubicin-induced cardiotoxicity. Future studies are needed to elucidate this hypothesis.

Since doxorubicin has been shown to alter the level of mitochondrial fusion and fission proteins, targeting the mitochondrial dynamic GTPase proteins could be promising in the prevention of doxorubicin cardiotoxicity. Currently, there is a limited number of studies which have investigated the roles of pharmacological interventions concerning doxorubicin-induced cardiotoxicity on mitochondrial dynamics in the heart.

Sacubitril/Valsartan (LCZ696) is an angiotensin receptor nephrilysin inhibitor. It is a standard treatment in patients with heart failure with reduced ejection fraction (HFrEF). Pretreated H9c2 cell with LCZ696 20 µmol/L for 30 minutes before given doxorubicin (5 µmol/L/24 h) demonstrated that LCZ696 inhibited mitochondrial fission and apoptosis by decreasing DRP1 phosphorylation. Overexpression of DRP1 abolished the protective effect of LCZ696. These findings indicated that the beneficial effect of LCZ696 is associated with the inhibition of mitochondrial fission. Since LCZ696 inhibited mitochondrial fission and apoptosis, it is possible that LCZ696 might promote mitochondrial fusion regulators (MFN1 MFN2 or OPA1 expression) in response to doxorubicin toxicity. However, this hypothesis needs to be validated in future studies. Another agent targeting DRP1 phosphorylation is polyphenolic elagic acid (EA). EA is a natural antioxidant compound found in numerous fruits and vegetables. Co-treatment of EA 10 µmol/L with doxorubicin 10 µmol/L for 18 hours showed that EA inhibited mitochondrial fission and fragmentation by the suppression of BNIP3. This information provides new insight into the modulation of DRP1 signalling and the inhibition of mitochondrial fission as a potential therapeutic option for alleviating doxorubicin-induced cardiotoxicity. These reports are summarized in Table 3.

Doxorubicin inhibits mitochondrial fusion and promotes mitochondrial fragmentation which is accompanied by an increased susceptibility to mPTP opening and ROS generation. Co-treatment of cyclosporin A, an mPTP inhibitor, with a dose of 1 mg/kg/alternate day in mice treated with doxorubicin (10 mg/
| Study model            | Methods (Drug/Dose/Route/Duration)                                                                 | Major Findings                                                                 | Interpretation                                                                                       | Ref |
|-----------------------|--------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|-----|
| **in vitro reports**  |                                                                                                 |                                                                                 |                                                                                                  |     |
| H9c2 cell             | • Pretreated with LCZ696/20 µmol/L/30 min + Dox/5 µmol/L/24 h                                     | ↓pSer616 DRP1 ↔ pSer616 DRP1                                                   | LCZ696 attenuated Dox-induced apoptosis by decreasing p-DRP1 and mitochondrial fission            | 31  |
|                       | • Pretreated with LCZ696/20 µmol/L/30 min + Drp1-expression lentivirus + Dox/5 µmol/L/24 h       | ↓Annexin V ↔ Cleaved caspase3                                                   |                                                                                                  |     |
|                       |                                                                                                 |                                                                                |                                                                                                  |     |
| Postnatal rat         | • Dox/10 µmol/L/18 h + Ellagic acid/10 µmol/L/18 h                                              | ↓pSer616 DRP1 ↔ pSer616 DRP1                                                   | Ellagic acid suppressed Dox-induced mitochondrial fission by decreasing p-DRP1                    | 29  |
| cardiomycyte          |                                                                                                 | ↓Mitochondrial fragmentation                                                    |                                                                                                  |     |
|                       |                                                                                                 |                                                                                |                                                                                                  |     |
| **in vivo reports**   |                                                                                                 |                                                                                 |                                                                                                  |     |
| 8-week-old male       | • Dox/15 mg/kg/ip/3 times/wk/2 wk + LCZ696/60 mg/kg/d/4 wk (Started 1 d after Dox)              | ↓pSer616 DRP1 ↔ pSer616 DRP1                                                   | LCZ696 attenuated Dox-induced apoptosis by decreasing p-DRP1 and mitochondrial fission         | 31  |
| Balb/c mice           |                                                                                                 | ↓Cleaved caspase3                                                              |                                                                                                  |     |
|                       |                                                                                                 |                                                                                |                                                                                                  |     |
| Male C57BL/6 mice     | • Dox/10 mg/kg/ip/single dose + Cyclosporin A/1 mg/kg/ip/alternate d (Follow up at 1.5 wk)     | ↓Mfn2 ← Opa1 ↔ Drp1 ← Mfpl1 ↔ Drp1 ↔ Mitochondrial fragmentation               | Cyclosporin A prevented mitochondrial fragmentation and alterations in mitochondrial fusion balance | 13  |
|                       |                                                                                                 |                                                                                |                                                                                                  |     |
| 6-week-old male       | • Dox/2 mg/kg/wk/ip/7 wk + Free wheel activity unlimited access 24 h/d (Start 5 wk before Dox) | ↑Mfn1 ↑Mfn2 ↑Opa1 ↑Mfn1 ↑Mfn2 ↑Opa1                                           | Both chronic exercise models attenuated the alteration in mitochondrial dynamics                  | 32  |
| Sprague Dawley rats   | • Dox/2 mg/kg/wk/ip/7 wk + Treadmill training 5 d/wk/12 wk (Start 5 wk before Dox)             | ↔DRP1 ↔DRP1                                                                    |                                                                                                  |     |
|                       |                                                                                                 |                                                                                |                                                                                                  |     |
| Female C57BL6 mice    | Dox/8 mg/kg/wk/ip/4 wk + Treadmill training 5 d/wk/8 wk                                         | ↑Mfn1 ↔ Mfn2 ↔ Mfn1 ↔ Mfn2                                                     | Both aerobic exercise training and RESV increased the expression of mitofusin proteins          | 91  |
|                       | • Dox/8 mg/kg/wk/ip/4 wk + RESV/320 mg/kg/d                                                   |                                                                                |                                                                                                  |     |

Abbreviations: Dox, Doxorubicin; DRP1, Dynamin-related protein1; LCZ696, Sacubitril/valsartan; Mfn1, Mitofusin1; Mfn2; Mitofusin2; Opa1, Optic atrophy1; pSer616, Phosphorylation serine616; RESV, Resveratrol; ROS, Reactive oxygen species.
FIGURE 1  The effects of doxorubicin on cardiac mitochondrial dynamics and mitochondrial function. Doxorubicin inhibits mitochondrial fusion proteins (MFN1, MFN2 and OPA1) and promotes mitochondrial fission by increasing DRP1 phosphorylation. Doxorubicin undergoes redox cycling and generation of ROS. ROS, in turn, induces lipid peroxidation at cellular membrane and targets subcellular organelle causing mitochondrial DNA damage and decreases mitochondrial transmembrane potential. Doxorubicin inhibits electron transport chain proteins subunit I, II, IV and induces mPTP opening which initiates apoptotic signalling pathway. In addition, doxorubicin binds to topoisomerase II in the nucleus causing DNA double-stranded breaks and induces apoptosis. Dox, Doxorubicin; MDA, Malondialdehyde; mPTP, mitochondrial permeability transition pore; mtDNA, Mitochondrial DNA; ROS, Reactive oxygen species; ΔΨm, Mitochondrial transmembrane potential.

FIGURE 2  The effects of pharmacological and non-pharmacological interventions on mitochondrial biogenesis, mitochondrial dynamics and mitochondrial function. Various pharmacological interventions have been shown to attenuate apoptosis by promoting mitochondrial function. The CVB-D promotes mitochondrial biogenesis by preservation of PGC1α and mitochondrial DNA copy number. Balancing the mitochondrial dynamics by increasing mitochondrial fusion proteins and inhibiting mitochondrial fission process also attenuates ROS production and apoptosis. BNIP3, BCL2/adenovirus E1B 19 kD protein-interacting protein 3; CsA, Cyclosporin A; CVB-D, Cycloverobuxine-D; EA, Ellagic acid; LCZ696, Sacubitril/valsartan; Mt, Mitochondria; mPTP, mitochondrial permeability transition pore; mtDNA, Mitochondrial DNA; RESV, Resveratrol; ROS, Reactive oxygen species; SIRT3, Sirtuin3; SOD2, Superoxide dismutase-2; ΔΨm, Mitochondrial transmembrane potential.
| Study model                        | Methods (Drug/Dose/Route/Duration)                                                                 | Major Findings                                                                 |
|-----------------------------------|-----------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|
| **In vitro reports**              |                                                                                               |                                                                              |
| Human right atrial trabeculae     | Dox/1 µmol/L/0-90 min + Cyclosporin A/1 µmol/L/10 min before dox                                | ↑Developed force                                                              |
|                                  |                                                                                               | ↑Maximal contraction velocity                                                 |
|                                  |                                                                                               | ↑Maximal relaxation velocity                                                  |
|                                  |                                                                                               | ↓State 2                                                                     |
|                                  |                                                                                               | ↑State 3                                                                     |
|                                  |                                                                                               | ↑RCR                                                                         |
|                                  |                                                                                               | Cyclosporin A inhibited mPTP opening improved mitochondrial respiration and cell contraction |
|                                  |                                                                                               |                                                                              |
| Postnatal rat cardiomyocyte       | Dox/10 µmol/L/18 h + Ellagic acid/10 µmol/L/18 h                                              |                                                                              |
|                                  |                                                                                               |                                                                              |
| H9c2 cell                         | Dox/1 µmol/L/24 h + RESV/50 µmol/L/24 h                                                         | ↑Mt size                                                                     |
|                                  | Sirt3-null MEFs                                                                               | ↔Mt size                                                                     |
|                                  | Dox/1 µmol/L/6 h                                                                               |                                                                              |
|                                  | Sirt3-null MEFs                                                                               |                                                                              |
|                                  | Dox/1 µmol/L/6 h + RESV/10 µmol/L/6 h                                                          |                                                                              |
|                                  |                                                                                               |                                                                              |
| H9c2 cell                         | Dox/5 µmol/L/24 h + Pretreated with LCZ696/20 µmol/L/30 min                                     | ↑Mt size                                                                     |
|                                  | Dox/5 µmol/L/24 h + Pretreated with LCZ696/20 µmol/L/30 min + Drp1-expression lentivirus (OE cell) | ↔Mt size                                                                     |
|                                  |                                                                                               |                                                                              |
| Neonatal Sprague Dawley rat cardiomyocyte | Pretreated with Berberine/1 µmol/L/20 min                                                            |                                                                              |
|                                  | Dox/1 µmol/L/2-24 h                                                                           |                                                                              |
|                                  |                                                                                               |                                                                              |
| HL-1 cell                         | Dox/5 µmol/L/15 or 24 h + Metformin/4 mmol/L/24 h                                              |                                                                              |
|                                  | Transfected with AdipoR1 or AdipoR2 siRNA                                                      |                                                                              |
|                                  | Dox/5 µmol/L/15 h + Metformin/4 mmol/L/24 h                                                    |                                                                              |
|                                  |                                                                                               |                                                                              |
| H9c2 cell                         | Dox/10 nmol/L/1-72 h                                                                          |                                                                              |
|                                  | + Metformin/0.1 mmol/L/1-72 h                                                                 |                                                                              |
|                                  | Dox/10 nmol/L/1-72 h                                                                          |                                                                              |
|                                  | + Metformin/1.0 mmol/L/1-72 h                                                                  |                                                                              |
|                                  | Treated with AMPK inhibitor 10 µmol/L                                                          |                                                                              |
|                                  | Dox/10 nmol/L/1-72 h                                                                          |                                                                              |
|                                  | + Metformin/0.1 mmol/L/1-72 h                                                                  |                                                                              |
|                                  |                                                                                               |                                                                              |
| In vivo reports                   |                                                                                               |                                                                              |
| Male C57BL/6 mice                 | Dox/10 mg/kg/ip/single dose + Cyclosporin A/1 mg/kg/ip/alternate day (Follow up at 1.5 wk)     | ↑LVFS                                                                        |
|                                  |                                                                                               | ↔Mt density                                                                   |
|                                  |                                                                                               | ↑Mt size                                                                     |
|                                  |                                                                                               | ↑Mt elongated                                                                 |
|                                  |                                                                                               | ↑RCR                                                                         |
| 8-week-old male Balb/c mice      | Dox/15 mg/kg/ip/3 times/wk/2 wk + LCZ696/60 mg/kg/d/4 wk (Start 1 d after Dox)                | ↑LVFS                                                                        |
|                                  |                                                                                               | ↓LVEDD                                                                       |
|                                  |                                                                                               | ↓LVESD                                                                       |
|                                  |                                                                                               | ↔Mt width                                                                    |
|                                  |                                                                                               | ↑Mt length                                                                    |
|                                  |                                                                                               | ↔Mt length/width                                                             |
|                                  |                                                                                               | ↑Complex I                                                                    |
|                                  |                                                                                               | ↔Complex IV                                                                   |
| ROS/poptosis                        | MMP/mPTP/ Mitochondrial protein | Interpretation                                                                 | Ref |
|-----------------------------------|---------------------------------|--------------------------------------------------------------------------------|-----|
|                                    | ↑MMP                            | Cyclosporin A inhibited mPTP opening improved mitochondrial respiration and cell contraction | 50  |
| ↓ROS ↓LDH ↓%Dead cells            | ↑MMP                            | EA suppressed mitochondrial injury and cell death by abrogating BNIP3 activity   | 29  |
| ↑SOD2                             | ↑SIRT3                          | SIRT3 expression was necessary for RESV to attenuate Dox-induced ROS production | 18  |
| ↓ROS                              |                                 |                                                                               |     |
| ↔SOD2                             |                                 |                                                                               |     |
| ↑ROS                              |                                 |                                                                               |     |
| ↓AnnexinV                         |                                 |                                                                               | 31  |
| ↓Cleaved caspase3                 |                                 |                                                                               |     |
| ↔Cleaved caspase3                 |                                 |                                                                               |     |
| ↓p-p53                            | ↑MMP                            | Berberine suppressed Dox-induced cardiomyocyte apoptosis through the inhibition of AMPK phosphorylation | 59  |
| ↓Bax                              | ↓p-AMPKα                         |                                                                               |     |
| ↑Bcl-2                            | ↓AMP/ATP                         |                                                                               |     |
| ↓TUNEL-positive                    | ↓p-ACC                           |                                                                               |     |
| ↓Caspase 3,9 activity             | ↑p-AMPK                          | The protective effects of metformin against Dox-induced cardiotoxicity were considered to be involved in the regulation of the adiponectin system | 70  |
| ↓TUNEL-positive cells             |                                 |                                                                               |     |
| ↑Cell viability ↑Catalase activity |                                 |                                                                               |     |
| ↑GPx activity                     |                                 |                                                                               |     |
| ↑SOD activity                     |                                 |                                                                               |     |
| ↔Cell viability                   |                                 |                                                                               |     |
| ↔Catalase activity                |                                 |                                                                               |     |
| ↔GPx activity                     |                                 |                                                                               |     |
| ↔SOD activity                     |                                 |                                                                               |     |
| ↓LDH                              | ↑p-AMPK                          | Low-dose metformin exerted cardioprotective effects against Dox by regulating AMPK pathway | 83  |
| ↓ROS                              | ↑p-ACC                           | High-dose metformin reverted the protective effects by suppressing PDGFR expression |     |
| ↑Cell viability                   | ↑PKA activity                    |                                                                               |     |
| ↔Cell viability                   | ↑p-PDGFRβ                       |                                                                               |     |
| ↔LDH                              | ↑p-AMPK                          |                                                                               |     |
| ↔ROS                              | ↑p-ACC                           |                                                                               |     |
| ↔Intracellular calcium            | ↑PKA activity                    |                                                                               |     |
| ↔Cell viability                   | ↓p-PDGFRβ                       |                                                                               |     |
|                                    | ↔PKA activity                    |                                                                               |     |
|                                    | ↓mPTP                            | Cyclosporin A inhibited mPTP opening, mitochondrial potential loss and contractile depression | 13  |
| ↓Cleaved caspase3                 | ↔PGC1α                           | LCZ696 improved cardiac function, mitochondrial respiration and decreased apoptosis | 31  |
| ↓TUNEL staining                   |                                 |                                                                               |     |
TABLE 4 (Continued)

| Study model                        | Methods (Drug/Dose/Route/Duration)                                                                 | Heart function/Morphology | Oxidative phosphorylation | Autophagy Mitophagy |
|------------------------------------|-----------------------------------------------------------------------------------------------|---------------------------|---------------------------|---------------------|
| C57BL mice                         | Pretreated with CVB-D 1 mg/kg/d/4 d  
- Dox/15 mg/kg/ip/single dose         | ↑LVEF                      |                           |                     |
|                                    |                                                                                               | ↑FS                       | ↓Myocardial swelling,   |                     |
|                                    |                                                                                               |                           | vacuolization           |                     |
| Male Sprague Dawley rats           | • Dox/20 mg/kg + Berberine/60 mg/kg/dose/4 d                                                   | ↑LVEDV                    |                           |                     |
|                                    |                                                                                               | ↑Stroke volume            |                           |                     |
|                                    |                                                                                               | ↑LVEF                     |                           |                     |
| Male Sprague Dawley rats           | • Dox/2 mg/kg/wk/sc/7 wk  
- Dox/2 mg/kg/wk/sc/7 wk + Carvedilol/1 mg/kg/wk/ip/7 wk | ↑Swollen mitochondria     | ↓Stage3                   |                     |
|                                    |                                                                                               | ↓Swollen mitochondria     | ↑Stage4                   |                     |
|                                    |                                                                                               |                           | ↓RCR                     |                     |
|                                    |                                                                                               |                           | ↑Complex I               |                     |
|                                    |                                                                                               |                           | ↑Stage3                   |                     |
|                                    |                                                                                               |                           | ↑RCR                     |                     |
|                                    |                                                                                               |                           | ↑Complex I               |                     |
| Adult male Wistar Albino rats      | • Dox/3 mg/kg/EOD/ip/6 doses + Metformin/50 mg/kg/d/oral/11 d  
- Dox/3 mg/kg/EOD/ip/6 doses + Metformin/500 mg/kg/d/oral/11 d | No Myocardial fibre injury |                           |                     |
|                                    |                                                                                               | ↓Inflammatory infiltration |                           |                     |
|                                    |                                                                                               | Normal myocardial fibres  |                           |                     |
| Male Wistar rats                   | • Dox/15 mg/kg/ip/single dose + Metformin/250 mg/kg/d/oral/7 d                                 | ↓Myocardial degeneration  |                           |                     |
|                                    |                                                                                               | ↓Interstitial inflammation|                           |                     |
|                                    |                                                                                               | ↓Interstitial haemorrhage |                           |                     |
| Male Wistar Albino rats            | • Dox/20 mg/kg/ip/divided into 2 doses + Metformin/500 mg/kg/d/oral/7 d                     | ↓Myocyte degeneration     |                           |                     |
|                                    |                                                                                               | ↓Interrupted muscle fibre |                           |                     |
|                                    |                                                                                               | ↓Wide interstitial spaces |                           |                     |
| Male Wistar Albino rats            | • Dox/4 mg/kg/dose/ip/twice a week/4 doses + Metformin/250 mg/kg/d/oral/14 d                | ↑LVEF                     |                           |                     |
|                                    |                                                                                               | ↓LVESD                    |                           |                     |
|                                    |                                                                                               | Almost normal histology   |                           |                     |
| ROS/poptosis | MMP/mPTP/ Mitochondrial protein | Interpretation | Ref |
|--------------|---------------------------------|----------------|-----|
| ↓Lipid peroxidation | ↑PGC1α | CVB-D protected against Dox-induced cardiomyopathy by suppression of oxidative damage and mitochondrial biogenesis impairment | 41 |
| ↓Protein carbonylation | ↑NRF-1 | | |
| ↑GSH/GSSG | ↑mtDNA copy number | | |
| ↓Cytosolic cytochrome c | | | |
| ↓TUNEL-positive | | | |
| ↓p-p53 | ↓p-AMPK | Berberine attenuated Dox-induced apoptosis by increased Bcl2 expression and decreased p53-AMPK pathway | 59 |
| ↑Bcl2 | | | |
| ↓Cleaved caspase3 | | | |
| ↓TUNEL-positive | | | |
| ↑Survival | | | |
| ↔CK-MB | ↔Acetyl-CoA | Metformin prevented Dox-induced cardiotoxicity by inhibiting Dox-induced oxidative stress and energy starvation | 72 |
| ↔LDH | ↔ATP | | |
| ↑GSH | ↓Acetyl-CoA | | |
| ↑GSTα | ↑ATP | | |
| ↔CAT | | | |
| ↔NQO1 | | | |
| ↓CK-MB | | | |
| ↓LDH | | | |
| ↑GSH | | | |
| ↓GSTα | | | |
| ↓HO-1β | | | |
| ↑CAT | | | |
| ↑NQO1 | | | |
| ↓LDH | Metformin attenuated Dox-induced cardiotoxicity in rats due to its antioxidant, anti-inflammatory and anti-apoptotic properties | 107 |
| ↓CK-MB | | | |
| ↓MDA | | | |
| ↑SOD | | | |
| ↓COX-2 | | | |
| ↓Caspase3 | | | |
| ↓CK-MB | Metformin exerted protective effects against Dox-induced cardiotoxicity by inhibition of apoptotic pathway | 73 |
| ↓LDH | | | |
| ↑GSH | | | |
| ↓TBA | | | |
| ↓Caspase3 | | | |
| ↑Bcl2 | | | |
| ↔Catalase | ↔TNF-α | Metformin preserved contractile function and attenuated histological damage | 53 |
| ↔SOD | ↔BNP | | |
| ↔GPx | | | |
| ↔Apoptotic cells | | | |

(Continues)
TABLE 4 (Continued)

| Study model          | Methods (Drug/Dose/Route/Duration) | Major Findings                                      | Heart function/ Morphology | Oxidative phosphorylation | Autophagy Mitophagy |
|----------------------|------------------------------------|-----------------------------------------------------|----------------------------|---------------------------|---------------------|
| Sprague Dawley rats | • Dox/3 mg/kg/EOD/ip/6 doses + Metformin/250 mg/kg/d/oral/14 d | ↑Aortic flow                                 | ↑Aortic flow                              | ↑Cardiac output                       | +Beclin-1           |
|                     |                                    | ↑Cardiac output                                   | ⇔Stroke volume                | ↑Myocardial thickness        | +p62                |
| 6-week-old male Sprague Dawley rats | • Dox/2 mg/kg/wk/ip/7 wk + Free wheel activity unlimited access 24 h/d (Start 5 wk before Dox) | +LVEF                                  | +LVEF                                   | +Complex I                        | +Beclin1/Bcl2 ratio |
|                     | • Dox/2 mg/kg/wk/ip/7 wk + Treadmill training 5 d/wk/12 wk (Start 5 wk before Dox) | ⇔LVEF                                   | ⇔LVEF                                   | ⇔Complex II                       | ⇔p62                |
| Female C57BL6 mice  | • Dox/8 mg/kg/wk/ip/4 wk + Treadmill training 5 d/wk/8 wk | | | | |
|                     | • Dox/8 mg/kg/wk/ip/4 wk + RESV/320 mg/kg/d | ↑Complex I                                  | ↑Complex I                        | ⇔Complex IV                      | ⇔Beclin1/Bcl2 ratio |
|                     |                                    | ⇔Complex II                                     | ⇔Complex II                       | +Complex IV                       | ⇔p62                |
|                     |                                    | ⇔Complex I                                      | ⇔Complex I                        | ⇔Complex IV                       | ⇔p62                |
|                     |                                    | ⇔Complex II                                     | ⇔Complex II                       | ⇔Complex IV                       | ⇔p62                |

Abbreviations: ACC, Acetyl-CoA carboxylase; AdipoR, Adiponectin receptor; BNIP3, BCL2/adenovirus E1B 19 kD protein-interacting protein 3; CAT, Catalase; COX1, Cytochrome c oxidase subunit1; CVB-D, Cyclovirobuxine; Dox, Doxorubicin; GPx, Glutathione peroxidase; GSH, Reduced glutathione; GSSG, Oxidized glutathione; GSTx, Glutathione S-transferase-α; HO-1β, Haem oxygenase-1β; LCZ696, Sacubitril/valsartan; LVEDD, Left ventricular end-diastolic dimension; LVEDV, Left ventricular end-diastolic volume; LVEF, Left ventricular ejection fraction; LVESD, Left ventricular end-systolic dimension; LVFS, Left ventricular fractional shortening; MDA, Malondialdehyde; MMP, Mitochondrial membrane potential; MnSOD, Manganese superoxide dismutase; mPTP, Mitochondrial permeability transition pore; Mr, Mitochondria; NQO1, NAD(P)H:quinone oxidoreductase 1; NRF, Nuclear respiratory factor; OCR, Oxygen consumption rate; P, Phosphorylation; PDGFβ, Platelet-derived growth factor receptor β; PKA, Protein kinase A; RCR, Respiratory control ratio; RESV, Resveratrol; ROS, Reactive oxygen species; SIRT, Sirtuin; SOD2, Superoxide dismutase-2; TBA, Thiobarbituric acid.

kg) indicated that cyclosporine A could normalize the mitochondrial fusion gene Mfn2 and Opa1, thus maintaining the mitochondrial fusion balance and preserving mitochondrial ultrastructural changes.13

Physical exercise is a non-pharmacological intervention used in the strategy to reduce cardiac toxicity from doxorubicin. However, the mechanisms responsible for the beneficial effects of exercise are not well characterized. Studies in rats showed that treadmill training and freewheel exercise preconditioning prior to doxorubicin treatment increased the levels of mitochondrial fusion proteins, MFN1, MFN2 and OPA1.32,91 It is suggested that the beneficial effects of physical exercise are at least through the regulation of mitochondrial dynamics. The potential therapeutic targets of doxorubicin on mitochondrial dynamics are demonstrated in Figure 2.

Currently, there are limited clinical studies that target mitochondrial dynamics modulation as an intervention in doxorubicin-induced cardiotoxicity. Future studies are needed to investigate this target and explore whether these interventions can provide cardioprotection in this model.

7 | EFFECTS OF PHARMACOLOGICAL AND NON-PHARMACOLOGICAL INTERVENTIONS ON CARDIAC MITOCHONDRIAL FUNCTION IN DOXORUBICIN-INDUCED CARDIOTOXICITY: EVIDENCE FROM IN VITRO AND IN VIVO STUDIES

The oxidative stress hypothesis is the most widely accepted mechanism for the cause of doxorubicin-induced cardiotoxicity. Several antioxidant agents have been studied in both in vitro and animal models.35,57,60,92 Antioxidants showed cardioprotective effects through reduced ROS generation and decreased apoptosis. However, these effects did not translate into a beneficial outcome in clinical study.93,94 Therefore, choosing other interventions that directly promote mitochondrial function could be a promising strategy. Pretreatment or co-treatment with cyclosporin A, an mPTP inhibitor, has shown beneficial effects by improving mitochondrial respiration and cardiac contractility in both in vitro and in vivo studies.13,50 Consistent with the use of
Oxidative phosphorylation; LCZ696, Sacubitril/valsartan; LVEDD, Left ventricular end-diastolic dimension; LVEF, Left ventricular ejection fraction; LVESD, Left ventricular end-systolic dimension; LVFS, Left ventricular fractional shortening; MDA, Malondialdehyde; MMP, Mitochondrial membrane potential; MnSOD, Manganese superoxide dismutase; mPTP, Mitochondrial permeability transition pore; Mt, Mitochondria; NQO1, NAD(P)H: quinone oxidoreductase 1; NRF, Nuclear respiratory factor; OCR, Oxygen consumption rate; P, Phosphorylation; PDGFR, Platelet-derived growth factor receptor-β; PKA, Protein kinase A; RCR, Respiratory control ratio; RESV, Resveratrol; ROS, Reactive oxygen species; SIRT, Sirtuin; SOD2, Superoxide dismutase-2; TBA, Thiobarbituric acid.

Female C57BL6 6-week-old Sprague Dawley mice

• Dox/8 mg/kg/wk/ip/4 wk + RESV/320 mg/kg/d Treadmill training

• Dox/2 mg/kg/wk/ip/7 wk + Treadmill training

• Dox/2 mg/kg/wk/ip/7 wk + RESV/250 mg/kg/d/oral/14 d Metformin doses +

5 d/5 d/5 d/; HO-1; α, Glutathione S-transferase-α; β, Haem oxygenase-1

↑Complex IV

↔Complex IV

↑Complex II

↔Mitophagy

↑Autophagy

↔Parkin

↓PINK1

↔p62

↓LC3B-II

↔Beclin-1

↓Beclin1/Bcl2 ratio

↔Beclin1

↓Parkin

↓PINK1

↓LC3-II

↓Beclin1/Bcl2 ratio

↔Beclin1

ROS/poptosis | MMP/mPTP/ Mitochondrial protein | Interpretation | Ref |
---|---|---|---|
↔LDH | ↔p-AMPK | Administration of metformin with Dox normalized the autophagic activity and conferred cardioprotection | 78 |
↓CK-MB | | | |
↓Trop T | | | |
↓MDA | | | |
↓Bax/Bcl2 ratio | ↓mPTP | Both chronic exercise models attenuated apoptotic signalling and alterations in autophagy | 32 |
↓Caspase3,9 | ↓mPTP | | |
↓Bax/Bcl2 ratio | | | |
↓Caspase3,9 | | | |
↓4-HNE | ↑MnSOD | Both aerobic exercise training and RESV reduced oxidative stress, promoted expression of mitochondrial electron transport chain proteins and improved heart function | 91 |
↑MnSOD | | | |
↓4-HNE | | | |
↔MnSOD | | | |
ellagic acid (EA), a natural antioxidant which suppressed BNIP3 and promoted mitochondrial function by inhibiting mPTP opening, increasing mitochondria transmembrane potential and reducing cell death in postnatal rat cardiomyocyte. Furthermore, resveratrol (RESV), a polyphenol found in grapes and berries, exerts cardioprotective effects by promoting SIRT3 expression. SIRT3 is involved in the deacetylation of several mitochondrial proteins and increased SIRT3 expression by RESV attenuated mitochondrial dysfunction and ROS generation in H9c2 cells. Impairment in mitochondrial biogenesis is considered to be an important process in doxorubicin-induced cardiotoxicity. PGC-1α is a key regulator in mitochondrial biogenesis which is inhibited by doxorubicin treatment. Pretreated with CVB-D 1 mg/kg/d for 4 days before given doxorubicin (15 mg/kg) in mice showed that CVB-D exerted cardioprotective effects by the preservation of PGC-1α, NRF1 and mitochondrial DNA copy number. The potential therapeutic targets of doxorubicin on mitochondrial biogenesis could be another effective strategy in the prevention of doxorubicin-induced cardiotoxicity.

Targeting of iron signalling by the iron chelator, dexrazoxane, has been demonstrated in animal models and translated into clinical trials in cancer patients treated with doxorubicin. Dexrazoxane significantly reduced the risk of heart failure but had no difference on the survival outcome. In addition to the iron chelator concept, the cardioprotective effects of dexrazoxane are considered to be involved in the inhibition of anthracyclines binding to Top2β. However, concern about the risk of the development of secondary malignancies limits dexrazoxane usage to patients receiving a cumulative dose of doxorubicin of more than 540 mg/m² according to the European Medicine Agency (EMA).

Choosing the interventions that have been widely used in clinical practice could be a favourable way due to the less concern of their adverse reactions. In this regard, beta blocker, statin and metformin have been studied in doxorubicin model. For statin reports, the proposed cardioprotective mechanism of lovastatin is associated with the inhibition of RAC1 signalling with subsequent reduction in apoptosis. A recent in vivo study showed that lovastatin...
attenuated mitochondrial dysfunction by reducing mitochondrial proliferation. Atorvastatin and rosuvastatin also have been shown to prevent doxorubicin-induced cardiotoxicity by reducing oxidative stress and inhibition of apoptosis. In addition to statin, beta blocker has also been investigated in doxorubicin model. For example, carvedilol possesses a distinct cardioprotective properties due to its antioxidant effects and the ability to inhibit lipid peroxidation within myocardial cells. The studies in animal models indicated that co-administration of carvedilol with doxorubicin prevented the inhibitory effects of doxorubicin on mitochondrial respiration. Carvedilol also prevented mitochondrial damage and the decrease in mitochondrial calcium loading capacity in rats treated with doxorubicin. These findings suggested that carvedilol could prevent cardiac mitochondrial dysfunction in doxorubicin model.

Several reports from both in vitro and in vivo have demonstrated that metformin had a cardioprotective role in doxorubicin model. The findings from in vitro studies indicated that adiponectin system and AMPK could both play a role in the preventive effects of metformin (Table 4). It has been shown that inhibition of adiponectin receptor1 (adipoR1) and adiponectin receptor2 (adipoR2) abrogated the protective effects of metformin in HL-1 cell. Since adiponectin could activate AMPK, this finding indicated the regulation of AMPK as the mechanism responsible for the attenuation of the doxorubicin-induced cardiotoxicity. Consistent with another report using H9c2 cell which demonstrated the efficacy of the cardioprotective effects of low-dose metformin through the increase in p-AMPK and its downstream regulators. A recent report showed that berberine, a natural alkaloid extracted from a variety of plants, improved mitochondrial function and decreased myocardial apoptosis by inhibiting AMPK phosphorylation in rats and NRCMs treated with doxorubicin.

Evidence from in vivo studies also supports the protective effect of metformin in doxorubicin models (Table 4). Co-treatment with metformin in rats demonstrated that metformin exerted cardioprotective effects by increasing cardiac antioxidant enzyme level including reduced glutathione (GSH) and SOD. The potential therapeutic targets of doxorubicin on mitochondrial function are demonstrated in Figure 2. Moreover, co-treatment with metformin in Wistar rats showed that metformin attenuated apoptosis afterdoxorubicin therapy. These effects contributed to the preservation of mitochondrial morphology and attenuated myocardial damage. Another proposed mechanism for the cardioprotective effects of metformin is its role in the regulation of autophagy. Doxorubicin impaired autophagy by altering the process of autophagosome formation and inhibition of autophagic clearance. Co-treatment with metformin normalized the expression of autophagic enzymes and mitigated the cardiotoxic effects of doxorubicin. A summary of the reports of all of these findings is shown in Table 4.

With regard to non-pharmacological intervention, various physical exercises have been shown to be the effective interventions against doxorubicin-induced cardiotoxicity. Treadmill training in mice treated with doxorubicin showed a reduction in oxidative stress, increased expression of mitochondrial electron transport chain proteins and an improved heart function. The beneficial effects of swim training was involved in an increase in heat shock proteins of the 60 kD family (HSP60). Furthermore, both treadmill training (TM) and free wheel activity (FW) could normalize the increase in the autophagic initiation protein, beclin-1/bcl2 ratio and reduced apoptosis. This finding suggested that lower intensity and longer duration (FW) may be as protective against doxorubicin toxicity as higher intensity and shorter duration exercise (TM). These data strengthen the role of physical exercise in attenuating doxorubicin-induced cardiotoxicity apart from the regulation in mitochondrial dynamics. The comprehensive summary of these findings is shown in Table 4.

Various pharmacological and non-pharmacological interventions have been studied in both in vitro and animal model as we have discussed. Whether these interventions contribute to beneficial outcome in clinical trials are inconsistent and controversial. Most of the clinical trials evaluated only heart function and did not demonstrate the possible involved cardioprotective mechanism. In addition, there is lack of clinical study which investigate the effects of the intervention drugs on mitochondrial dynamics and function. Further clinical studies in this field are needed to improve outcome in these patients.

8 | CONCLUSION

The mechanisms involved in doxorubicin-induced cardiotoxicity are complex. Oxidative stress, mitochondrial dysfunction and apoptosis play an important role in the development of cardiomyopathy. The balance of mitochondrial dynamics and normal mitochondrial function are disrupted by doxorubicin which results in myocardial damage. Successful identification of interventions that could attenuate doxorubicin-induced cardiotoxicity would be of tremendous clinical benefit for cancer patients treated with doxorubicin.

ACKNOWLEDGEMENTS

This work was supported by Thailand Research Fund grant RTA6080003 (SCC), the NSTDA Research Chair grant from the National Science and Technology Development Agency Thailand (NC) and the Chiang Mai University Center of Excellence Award (NC).

CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

NO performed the literature search, drafted the manuscript, made the figure and tables; AP, SCC, NC designed the concept and revised the manuscript; NC revised the manuscript and provided final approval of the version to publish.
REFERENCES

1. Hayek ER, Speakman E, Rehmus E. Acute doxorubicin cardiotoxicity. N Engl J Med. 2005;352:2456-2457.

2. Steinberg JS, Cohen AJ, Wasserman AG, et al. Acute arrhythmogenicity of doxorubicin administration. Cancer. 1987;60:1213-1218.

3. Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. Cancer. 2003;97:2869-2879.

4. Zamorano JL, Lancellotti P, Rodríguez Muñoz D, et al. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines: the Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J. 2016;37:2768-2801.

5. Cardinale D, Colombo A, Bacchiani G, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation. 2015;131:1981-1988.

6. Mulrooney DA, Yeazel MW, Kawashima T, et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. BMJ. 2009;339:b4606.

7. Lyu YL, Kerrigan JE, Lin CP, et al. Topoisomerase IIbeta mediated DNA double-strand breaks: implications in doxorubicin cardiotoxicity and prevention by dexrazoxane. Cancer Res. 2007;67:8839-8846.

8. Horie T, Ono K, Nishi H, et al. Acute doxorubicin cardiotoxicity is associated with miR-146a-induced inhibition of the neuregulin-ErbB pathway. Cardiovasc Res. 2010;87:656-664.

9. Rohrbach S, Muller-Werdan U, Werdan K, et al. Apoptosis-modulating interaction of the neuregulin/erbB pathway with anthracyclines in regulating Bcl-xS and Bcl-xL in cardiomyocytes. J Mol Cell Cardiol. 2005;38:485-493.

10. Hahn VS, Lenihan DJ, Ky B. Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. J Am Heart Assoc. 2014;3:e000665.

11. Dan Dunn J, Alvarez LAJ, Zhang X, et al. Reactive oxygen species and mitochondria: a nexus of cellular homeostasis. Redox Biol. 2015;6:472-485.

12. Kuznetsov AV, Margreiter R, Amberger A, et al. Changes in mitochondrial redox state, membrane potential and calcium precede mitochondrial dysfunction in doxorubicin-induced cell death. Biochim Biophys Acta. 2011;1813:1144-1152.

13. Marechal X, Montaigne D, Marciniak C, et al. Doxorubicin-induced cardiac dysfunction is attenuated by ciscoprin treatment in mice through improvements in mitochondrial bioenergetics. Clin Sci (Lond). 2011;121:405-413.

14. Tang H, Tao A, Song J, et al. Doxorubicin-induced cardiomyocyte apoptosis: role of mitofusin 2. Int J Biochem Cell Biol. 2017;88:55-59.

15. Ventura-Clapier R, Garnier A, Vekslar V. Energy metabolism in heart failure. J Physiol. 2004;555:1-13.

16. Moon SB, Kajiyama K, Hino Y, et al. Effect of adriamycin on lipid peroxide, glutathione peroxidase and respiratory responses of mitochondria from the heart, liver and kidney. Kurume Med J. 1983;30:1-4.

17. Guo J, Guo Q, Fang H, et al. Cardioprotection against doxorubicin by metallothionein is associated with preservation of mitochondrial biogenesis involving PGC-1alpha pathway. Eur J Pharmacol. 2014;737:117-124.

18. Cheung KG, Cole LK, Xiang BO, et al. Sirtuin-3 (SIRT3) protein attenuates doxorubicin-induced oxidative stress and improves mitochondrial respiration in H9c2 cardiomyocytes. J Biol Chem. 2015;290:10981-10993.

19. Archer SL. Mitochondrial dynamics — mitochondrial fission and fusion in human diseases. N Engl J Med. 2013;369:2236-2251.

20. Suliman HB, Carraway MS, Tatro LG, et al. A new activating role for CO in cardiac mitochondrial biogenesis. J Cell Sci. 2007;120:299-308.

21. Ryan JJ, Marsboom G, Fang YH, et al. PGC1alpha-mediated mitofusin-2 deficiency in female rats and humans with pulmonary arterial hypertension. Am J Respir Crit Care Med. 2013;187:865-878.

22. Sebastian D, Palacin M, Zorzano A. Mitochondrial dynamics: coupling mitochondrial fitness with healthy aging. Trends Mol Med. 2017;23:201-215.

23. Dorn GW. Evolving concepts of mitochondrial dynamics. Annu Rev Physiol. 2019;81(1):1-17.

24. Youle RJ, van der Bliek AM. Mitochondrial fission, fusion, and stress. Science. 2012;337:1062-1065.

25. Samant SA, Zhang HJ, Hong Z, et al. SIRT3 deacetylates and activates OPA1 to regulate mitochondrial dynamics during stress. Mol Cell Biol. 2014;34:807-819.

26. Ong S-B, Subrayan S, Lim SY, et al. Inhibiting mitochondrial fission protects the heart against ischemia/reperfusion injury. Circulation. 2010;121:2012-2022.

27. Zorzano A, Liesa M, Palacin M. Role of mitochondrial dynamics proteins in the pathophysiology of obesity and type 2 diabetes. Int J Biochem Cell Biol. 2009;41:1846-1854.

28. Aung LHH, Li R, Prabhakar BS, et al. Knockdown of Mtfp1 can minimize doxorubicin cardiotoxicity by inhibiting Dnm1-mediated mitochondrial fission. J Cell Mol Med. 2017;21:3394-3404.

29. Dhiranga A, Jayas R, Afshar P, et al. Ellagic acid antagonizes Bnip3-mediated mitochondrial injury and necrotic cell death of cardiac myocytes. Free Radic Biol Med. 2017;112:411-422.

30. Li J, Li Y, Jiao J, et al. Mitofusin 1s is negatively regulated by microRNA 140 in cardiomyocyte apoptosis. Mol Cell Biol. 2014;34:1788-1799.

31. Xia Y, Chen Z, Chen AO, et al. LC3Z696 improves cardiac function via alleviating Drp1-mediated mitochondrial dysfunction in mice with doxorubicin-induced dilated cardiomyopathy. J Mol Cell Cardiol. 2017;108:138-148.

32. Marques-Aleixo I, Santos-Alves E, Torrella JR, et al. Exercise and doxorubicin treatment modulate cardiac mitochondrial quality control signaling. Cardiovasc Toxicol. 2018;18:43-55.

33. Wang J-X, Zhang X-J, Feng C, et al. MicroRNA-532-3p regulates mitochondrial fission through targeting apoptosis repressor with caspase recruitment domain in doxorubicin cardiotoxicity. Cell Death Dis. 2015;6:e1677.

34. Ghigo A, Li M, Hirsch E. New signal transduction paradigms in anthracycline-induced cardiotoxicity. Biochim Biophys Acta. 2016;1863:1916-1925.

35. de Tassigny AD, Assaly R, Schaller S, et al. Mitochondrial translocator protein (TSPO) ligands prevent doxorubicin-induced mechanical dysfunction and cell death in isolated cardiomyocytes. Mitochondrion. 2013;13:688-697.

36. Dong Q, Chen L, Lu Q, et al. Quercetin attenuates doxorubicin cardiotoxicity and improves OPA1 to regulate mitochondrial dynamics during stress. Mol Cell Biol. 2016;36:3068-3074.

37. Zhang X, Azhar G, Nagano K, et al. Differential vulnerability to oxidative stress in rat cardiac myocytes versus fibroblasts. J Am Coll Cardiol. 2001;38:2055-2062.

38. Doroshov JH, Davies KJ. Redox cycling of anthracyclines by cardiac mitochondria. II. Formation of superoxide anion, hydrogen peroxide, and hydroxyl radical. J Biol Chem. 1986;261:3068-3074.

39. Ravi D, Das KC. Redox-cycling of anthracyclines by thiorodoxin system: increased superoxide generation and DNA damage. Cancer Chemother Pharmacol. 2004;54:449-458.
41. Guo Q, Guo J, Yang R, et al. Cyclovirobuxine D attenuates doxorubicin-induced cardiomyopathy by suppression of oxidative damage and mitochondrial biogenesis impairment. *Oxid Med Cell Longev.* 2015;2015:151972.

42. Suliman HB, Carraway MS, Ali AS, et al. The CO/HO system reverses inhibition of mitochondrial biogenesis and prevents murine doxorubicin cardiomyopathy. *J Clin Invest.* 2007;117:3730-3741.

43. Asensio-Lopez MC, Sanchez-Mas J, Pascual-Figal DA, et al. Ferritin heavy chain as main mediator of preventive effect of metformin against mitochondrial damage induced by doxorubicin in cardiomyocytes. *Free Radic Biol Med.* 2014;67:19-29.

44. Dinghra R, Margulets V, Chowdhury SR, et al. Binip3 mediates doxorubicin-induced cardiac myocyte necrosis and mortality through changes in mitochondrial signaling. *Proc Natl Acad Sci U S A.* 2014;111:E5537-E5544.

45. Gao S, Li H, Cai Y, et al. Mitochondrial binding of alpha-eno-lase stabilizes mitochondrial membrane: its role in doxorubicin-induced cardiomyocyte apoptosis. *Arch Biochem Biophys.* 2014;542:46-55.

46. Guo R, Lin J, Xu W, et al. Hydrogen sulfide attenuates doxorubicin-induced cardiotoxicity by inhibition of the p38 MAPK pathway in H9c2 cells. *Int J Mol Med.* 2013;31:644-650.

47. He H, Luo Y, Qiao Y, et al. Curcumin attenuates doxorubicin-induced cardiotoxicity via suppressing oxidative stress and preventing mitochondrial dysfunction mediated by 14-3-3 gamma. *Food Funct.* 2018;9:4404-4418.

48. Lai H-C, Liu T-J, Ting C-T, et al. Insulin-like growth factor-1 prevents loss of electrochemical gradient in cardiac muscle mitochondria via activation of PI 3 kinase/Akt pathway. *Mol Cell Endocinol.* 2003;205:99-106.

49. Li BO, Kim DS, Yadav RK, et al. Sulforaphane prevents doxorubicin-induced oxidative stress and cell death in rat H9c2 cells. *Int J Mol Med.* 2015;36:53-64.

50. Montaigne D, Marechal X, Preau S, et al. Doxorubicin induces mitochondrial permeability transition and contractile dysfunction in the human myocardium. *Mitochondrion.* 2011;11:22-26.

51. Sardão VA, Oliveira PI, Holy J, et al. Doxorubicin-induced mitochondrial dysfunction is secondary to nuclear p53 activation in H9c2 cardiomyoblasts. *Cancer Chemother Pharmacol.* 2009;64:811-827.

52. Plantadosi CA, Carraway MS, Babiker A, et al. Heme oxygenase-1 regulates cardiac mitochondrial biogenesis via Nrf2-mediated transcriptional control of nuclear respiratory factor-1. *Circ Res.* 2008;103:1232-1240.

53. Argum M, Uzum K, Sonmez MF, et al. Cardioprotective effect of metformin against doxorubicin cardiotoxicity in rats. *Anatol J Cardiol.* 2016;16:234-241.

54. Gao S, Li H, Feng XJ, et al. alpha-Enolase plays a catalytically independent role in doxorubicin-induced cardiomyocyte apoptosis and mitochondrial dysfunction. *J Mol Cell Cardiol.* 2015;79:92-103.

55. Huang L, Zhang K, Guo Y, et al. Honokiol protects against doxorubicin cardiotoxicity via improving mitochondrial function in mouse hearts. *Sci Rep.* 2017;7:11989.

56. Konishi M, Haraguchi GO, Ohigashi H, et al. Adiponectin protects against doxorubicin-induced cardiomyopathy by anti-apoptotic effects through AMPK up-regulation. *Cardiovasc Res.* 2011;89:309-319.

57. Lai H-C, Yeh Y-C, Ting C-T, et al. Doxycycline suppresses doxorubicin-induced oxidative stress and cellular apoptosis in mouse hearts. *Eur J Pharmacol.* 2010;644:176-187.

58. Liu G, Liu Y, Wang R, et al. Spironolactone attenuates doxorubicin-induced cardiotoxicity in rats. *Cardiovasc Ther.* 2016;34:216-224.

59. Lv X, Yu X, Wang Y, et al. Berberine inhibits doxorubicin-triggered cardiomyocyte apoptosis via attenuating mitochondrial dysfunction and increasing Bcl-2 expression. *PLoS ONE.* 2012;7:e47351.

60. Min K, Kwon O-S, Smuder AJ, et al. Increased mitochondrial emission of reactive oxygen species and calpain activation are required for doxorubicin-induced cardiac and skeletal muscle myopathy. *J Physiol.* 2015;593:2017-2036.

61. Zhang C, Feng Y, Qu S, et al. Resveratrol attenuates doxorubicin-induced cardiomyocyte apoptosis in mice through SIRT1-mediated deacetylation of p53. *Cardiovasc Res.* 2011;90:538-545.

62. Zhu C, Wang YL, Liu H, et al. Oral administration of Ginsenoside Rg1 prevents cardiac toxicity induced by doxorubicin in mice through anti-apoptosis. *OncoTarget.* 2017;8:83792-83801.

63. Gao WD, Liu Y, Marban E. Selective effects of oxygen free radicals on excitation-contraction coupling in ventricular muscle. *Circulation.* 1996;94:2597-2604.

64. Cadete VJJ, Deschênes S, Cuillerier A, et al. Formation of mitochondrial-derived vesicles is an active and physiologically relevant mitochondrial quality control process in the cardiac system. *J Physiol.* 2016;594:5343-5362.

65. Marques-Aleixo I, Santos-Alves E, Mariani D, et al. Physical exercise prior and during treatment reduces sub-chronic doxorubicin-induced mitochondrial toxicity and oxidative stress. *Mitochondrion.* 2015;20:22-33.

66. Solem LE, Henry TR, Wallace KB. Disruption of mitochondrial calcium homeostasis following chronic doxorubicin administration. *Toxicol Appl Pharmacol.* 1994;129:214-222.

67. Louisse J, Wüst RCJ, Pistollato F, et al. Assessment of acute and chronic toxicity of doxorubicin in human induced pluripotent stem cell-derived cardiomyocytes. *Toxicol In Vitro.* 2017;42:182-190.

68. Maillet A, Tan K, Chai X, et al. Modeling doxorubicin-induced cardiotoxicity in human pluripotent stem cell derived-cardiomyocytes. *Sci Rep.* 2016;6:25333.

69. Yang NA, Ma H, Jiang Z, et al. Dosing depending on SIRT3 activity attenuates doxorubicin-induced cardiotoxicity via elevated tolerance against mitochondrial dysfunction and oxidative stress. *Biochem Biophys Res Commun.* 2019;517(1):111-117.

70. Asensio-Lopez MC, Lax A, Pascual-Figal DA, et al. Metformin protects against doxorubicin-induced cardiotoxicity: involvement of the adiponectin cardiac system. *Free Radic Biol Med.* 2011;51:1861-1871.

71. Asensio-López MC, Sánchez-Más J, Pascual-Figal DA, et al. Involvement of ferritin heavy chain in the preventive effect of metformin against doxorubicin-induced cardiotoxicity. *Free Radic Biol Med.* 2013;57:188-200.

72. Ashour AE, Sayed-Ahmed MM, Abd-Allah AR, et al. Metformin rescues the myocardium from doxorubicin-induced energy starvation and mitochondrial damage in rats. *Oxid Med Cell Longev.* 2012;2012:434195.

73. Sheta A, Elsakkar M, Hamza M, et al. Effect of metformin and sirtuin-3 on doxorubicin-induced cardiotoxicity in adult male albino rats. *Hum Exp Toxicol.* 2016;35:1227-1239.

74. Yin J, Guo J, Zhang Q, et al. Doxorubicin-induced mitophagy and mitochondrial damage is associated with dysregulation of the PINK1/parkin pathway. *Toxicol In Vitro.* 2018;51:1-10.

75. Hoshino A, Mita Y, Okawa Y, et al. Cytosolic p53 inhibits Parkin-mediated deacetylation of p53. *Cardiovasc Res.* 2011;90:538-545.

76. Wang P, Wang L, Lu J, et al. SESN2 protects against doxorubicin-induced cardiomyopathy via rescuing mitophagy and improving mitochondrial function. *J Mol Cell Cardiol.* 2019;133:125-137.

77. Sishi BJN, Loos B, van Rooyen J, et al. Autophagy upregulation promotes survival and attenuates doxorubicin-induced cardiotoxicity. *Biochem Pharmacol.* 2013;85:124-134.
96. Herman EH, Ferrans VJ. Reduction of chronic doxorubicin cardiotoxicity in dogs by pretreatment with (±)-1,2-Bis[3,5-dioxopiperazinyl-1-yl)propane (ICRF-187). Cancer Res. 1981;41:3436-3440.

97. van Dalen EC, Caron HN, Dickinson HO, et al. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev. 2006;2:CD003917.

98. Tebbi CK, London WB, Friedman D, et al. Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J Clin Oncol. 2007;25:493-500.

99. Huelsenbeck J, Henninger C, Schad A, et al. Inhibition of Rac1 signaling by lovastatin protects against anthracycline-induced cardiac toxicity. Cell Death Dis. 2011;2:e190.

100. Ohlig J, Henninger C, Zander S, et al. Rac1-mediated cardiac damage causes diastolic dysfunction in a mouse model of subacute doxorubicin-induced cardiotoxicity. Arch Toxicol. 2018;92:441-453.

101. Henninger C, Huelsenbeck S, Wenzel P, et al. Chronic heart damage following doxorubicin treatment is alleviated by lovastatin. Pharmacol Res. 2015;91:47-56.

102. Sharma H, Pathan RA, Kumar V, et al. Anti-apoptotic potential of rosuvastatin pretreatment in murine model of cardiomyopathy. Int J Cardiol. 2011;150:193-200.

103. Svrs R, Trivedi PP, Kushwaha S, et al. Protective role of atorvastatin against doxorubicin-induced cardiotoxicity and testicular toxicity in mice. J Physiol Biochem. 2013;69:513-525.

104. Oliveira PJ, Bjork JA, Santos MS, et al. Carvedilol-mediated antioxidant protection against doxorubicin-induced cardiac mitochondrial toxicity. Toxicol Appl Pharmacol. 2004;200:159-168.

105. Dulin B, Abraham WT. Pharmacology of carvedilol. Am J Cardiol. 2004;93:3b-6b.

106. Santos DL, Moreno AJ, Leino RL, et al. Carvedilol protects against doxorubicin-induced myocardial cardiotoxicity. Toxicol Appl Pharmacol. 2002;185:218-227.

107. Kelleni MT, Amin EF, Abdelrahman AM. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in rats: impact of oxidative stress, inflammation, and apoptosis. J Toxicol. 2015;2015:424813.

108. Ascensão A, Magalhães J, Soares J, et al. Endurance training attenuates doxorubicin-induced cardiac oxidative damage in mice. Int J Cardiol. 2005;100:451-460.

109. Acar Z, Kale A, Turgut M, et al. Efficiency of atorvastatin in the protection of anthracycline-induced cardiomyopathy. J Am Coll Cardiol. 2011;58:988-989.

110. Avila MS, Ayub-Ferreira SM, de Barros Wanderley MR, et al. Carvedilol for prevention of chemotherapy-related cardiotoxicity: the CECCY trial. J Am Coll Cardiol. 2018;71:2281-2290.

111. Kalay N, Basar E, Ozdogru I, et al. Protective effects of carvedilol against anthracycline-induced cardiomyopathy. J Am Coll Cardiol. 2006;48:2258-2262.