Rosuvastatin and simvastatin attenuate cisplatin-induced cardiotoxicity via disruption of endoplasmic reticulum stress-mediated apoptotic death in rats: targeting ER-Chaperone GRP78 and Calpain-1 pathways

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ABSTRACT

Cisplatin (CP) is a powerful antineoplastic chemotherapeutic agent with broad-spectrum properties. Acute and cumulative cardiotoxicity are major limiting factors for CP therapy. Various pathogenic pathways have been suggested to CP-induced cardiotoxicity; oxidative damage, ER stress, and programmed cell death/apoptosis. The present study aimed to assess the signaling mechanisms related to the advantageous effects of rosuvastatin (RSV) and simvastatin (SMV) against CP-related cardiac ER stress dependent apoptotic death in rats. Acute cardiotoxicity was induced by a single dose of CP (10 mg/kg, i.p.) on the 10th day of the experiment. RSV (10 mg/kg/day) and SMV (10 mg/kg/day) were orally administered for 15 days. CP-treated rats showed significant alterations in electrocardiographic recordings and elevation in serum cardiac function biomarkers; troponin T content, lactate dehydrogenase and creatine kinase-MB levels as well as boost in the cardiac oxidative stress biomarkers. In addition, CP exposure resulted in GRP78 induction; an ER stress and elevation marker at calpain-1 content as well as activation of activated caspase-3 (ACASP3) and caspase-12 were reflected on CP-triggered apoptosis evidenced by elevation in the Bax/Bcl-2 ratio. However, RSV and SMV administration mitigate those adverse CP effects. Statins administration prominently alleviated CP-induced cardiac abnormalities exerting improvement in the ECG pattern and cardiac enzyme biomarkers. Interestingly, statins; RSV and SMV, disrupted CP-induced ER stress and the consequent apoptotic cell death evidenced by downregulation of ER-chaperone GRP78, calpain-1, ACASP3 and caspase-12 as well as decline in the Bax/Bcl-2 ratio. From all the previous findings, it can be suggested that statins namely; RSV and SMV, play protective role against CP-induced cardiac injury by regulating ER stress-mediated apoptotic pathways.

1. Introduction

Cisplatin (CP) is an essential chemotherapeutic agent commonly used for the management of many types of cancers [1]. However; despite its efficacy, major adverse effects immensely limit its use. These include nephrotoxicity, neurotoxicity, bone marrow suppression, ototoxicity, and cardiotoxicity [2]. Severe cardiotoxic events usually occur post CP administration which manifest as cardiotoxicity with a fraction of the midrange ejection, and arrhythmias [3] as well as electrocardiographic alterations, myocarditis, and cardiomyopathy [4]. In contrast to, anthracyclines, which are considered as well-established cardiotoxic compounds causing myocardial contractility suppression in a considerable number of patients [5]. Therefore, it is crucial to abate CP-induced cardiotoxicity through understanding the molecular mechanisms underlying it [6].

It has been postulated that oxidative stress, endoplasmic reticulum (ER) stress and apoptosis is primarily concerned with the pathogenesis of cardiotoxicity induced by CP. It exerts its cytotoxic action via formation of covalent adducts with DNA bases. This leads to massive reactive oxygen species formation (ROS); eventually causing redox stress [7,8]. Oxidative stress initiates myocardial cell damage via endoplasmic reticulum (ER) stress activation resulting in severe cardiotoxicity [9]. The well-organized functioning of the ER is vital for different cellular activities; ER is an intracellular Ca²⁺ storage organelle and among the most notable protein-folding compartments. The ER contains some molecular chaperones, which is responsible for ER stress cellular sensitivity and apoptosis. Disturbing ER function results in initiation and unfolded protein accumulation in attempt to restore...
2. Materials and methods

2.1. Animals

In the present study, adult male Wistar rats (weighing 180–200 g) obtained from the National Research Centre’s animal house colony (NRC, Egypt) were used. This study was carried out in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and the National Research Center–Medical Research Ethics Committee (NRC-MREC) for the use of animals.

2.2. Drugs and chemicals

This study consumed rosuvastatin (Crestor®, AstraZeneca, USA), simvastatin (Zocor®, MSD, USA) and cisplatin vials (Cisplatin®, Bristol Meyers Co., USA). All other chemicals that participated in this study were of the highest commercial grade available.

2.3. Experimental protocol

Forty rats were randomly divided into four equal groups. In group I, rats only received saline and served as a normal control group. Acute cardiotoxicity was induced by a single dose of cisplatin (CP; 10 mg/kg) injected on the 10th day of the experiment to the rats in the other three groups [4]. Group II served as CP-control group, while groups III and IV received oral doses of rosuvastatin (RSV; 10 mg / kg) and simvastatin (SMV; 10 mg / kg) for 15 consecutive days, respectively, each day as illustrated below.

2.4. Electrocardiogram recording

Electrocardiogram (ECG) was recorded using the ECG Powerlab module on the 16th day of the experiment and data was analyzed using ECG analyzer’s Lab Chart 7 software. RR Interval, heart rate, QRS Interval, QTc duration, P duration, ST Height and T amplitude were measured.

2.5. Serum and cardiac biochemical parameters analysis

Blood samples were collected from the retro-orbital venous plexus of rats, immediately after ECG recording. Blood samples collected were centrifuged at 4 °C and sera was separated. Commercially available kits were used to measure the serum troponin T content as well as lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) activities.

The rats were then sacrificed and the heart tissues were carefully separated and homogenized; the homogenous was centrifuged and the supernatant was then used to determine cardiac levels of the reduced glutathione (GSH) content, superoxide dismutase (SOD) activity, 78-kDa glucose-regulated protein (GRP78) and calpain-1 activity as well as the active caspase-3 activity (ACASP3) using commercially available ELISA kits.

2.6. Cardiac expression analysis of apoptosis related genes

Relative Cardiac caspase-12, Bax and Bcl-2 Quantitative Real Time-PCR gene expression was determined as follows:

2.6.1. RNA isolation and reverse transcription reaction

TRizol® extraction Chemical (Invitrogen) was used to isolate the total genomic RNA of the cardiac tissues of all treated animals. RNA pellet was stored in DEPC treated water after completion of the isolation procedures. The pellet of isolated RNA was treated with RNase-free DNase kit (Invitrogen, Germany) to digest the potential DNA residues. RNA aliquots were placed at −20 °C or utilized immediately for reverse transcription [20].

First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was utilized for the synthesis of the cDNA copy from the cardiac tissues via normal ER functionality [10]. This condition is referred to as ER stress. Protein accumulation is cytotoxic; therefore, many molecular events are associated with ER stress [11]. In order to stabilize protein folding and accumulation, the cell dissociates the ER chaperone viz. the 78-kDa glucose-regulated protein (GRP78) from its membrane receptors. GRP78 prompts the unfolded protein response in an attempt restore regular ER function [12,13]. When the ER stress is continued, or if the adaptive unfolded protein response fails in re-establishing ER homeostasis, ER stress-induced apoptotic death ensues. Apoptosis is induced through caspase-dependent pathways via activation and processing of calpain-1 which activates caspase 3 (ACASP3) among other caspasases including caspasases 6, 7, 8, 9 and 12 [14,15]. ER stress-induced apoptotic death interferes in the pathophysiology of numerous diseases as Alzheimer disease, Huntington disease as well as many cardiovascular disorders [11]. CP-cardiotoxicity is linked to mitochondrial abnormalities, boosted ER stress and apoptosis [16]. Many drug moieties have been tested to abate CP-induced cardiotoxicity; however, little achievement has been reached [17]. Statins; 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase in-hibitors, used clinically to enhance biosynthesis of cholesterol [8]. Statins possess many multiple effects, namely anti-oxidant, anti-inflammatory, and anti-thrombotic as well as reno-, vasculo- and cardio-protective actions. Statins’ use has been linked to decreased mortality and morbidity in cardiovascular patients, primarily via their anti-apoptotic actions on the cardiomyocytes [18,19]. Nevertheless, the molecular mechanisms underlying statins’ anti-apoptotic effects in hindering the CP-induced cardiotoxicity have not been yet revealed.

Based on the assumption that ER stress may play a pivotal role in the apoptotic CP cardiomyopathy demonstrated by myocardial contractile function and the proposed mechanisms involved. Therefore, the current study aims at testing the anti-apoptotic effects of RSV and SMV and outlining the status of key cardiac signaling pathways involved in cardiotoxicity to recognize prospective novel targets for intervention.
reverse transcription reaction. A reverse transcription reaction program of 25 °C for 10 min, then one hour at 42 °C then 5 min at 95 °C was employed to get the cDNA copy of the genome. Eventually, tubes of reaction containing cDNA copy were collected on ice until utilization for cDNA amplification [21].

2.6.2. Quantitative real Time-PCR
SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was consumed to attain the qRT-PCR analyses using the synthesized cDNA copies from the cardiac tissues. For each reaction a melting curve profile was conducted. The quantitative values of the target genes were normalized on the expression of the gene (Table 1). To determine the quantitative values of the specific genes for the GAPDH gene, the 2^{-ΔΔCT} method was used.

2.7. Statistical analysis
Values are shown as means ± Standard Median Error (SE). Data analysis was conducted using one-way variance analysis (ANOVA) followed by multiple post-hoc comparison testing at p 0.05 by Tukey. These statistical tests were done using GraphPad prism® software (version 6, San Diego, California, USA).

3. Results

3.1. Effects of RSV and SMV on ECG pattern in CP-induced cardiotoxicity
Injection of CP (10 mg/kg, i.p) was associated with marked changes in the ECG pattern as compared to normal ECG evidenced by elongation of QTc duration with increased ST height and T wave amplitude as compared to normal ECG. It showed significant elevation in heart rate, QTc duration, P amplitude, ST height and T amplitude to 113 %, 145 %, 191 %, 293 % and 238 %, respectively, as well as significant reduction in RR and QRS intervals to 77 % and 67 %, respectively relative to the normal control group.

Rosuvastatin led to an improvement in the ECG pattern whereas QTc duration and ST height have been restored with amelioration of T wave amplitude. It showed marked reduction in heart rate, QTc duration, P amplitude, ST height and T amplitude to 84 %, 77 %, 61 %, 49 % and 65 %, respectively as well as significant elevation in RR and QRS intervals to 125 % and 104 %, respectively. Similarly, SMV administration resulted in an improvement in QTc duration and ST height with amelioration of T wave amplitude with prominent reduction in heart rate, QTc duration, P amplitude, ST height and T amplitude to 86 %, 78 %, 75 %, 56 % and 67 %, respectively as well as significant elevation in RR and QRS intervals to 119 % and 140 %, in comparison with CP-control group (Figs. 1 & 2).

Table 1

| Gene      | Forward Reference (Accession number) | Reference |
|-----------|--------------------------------------|-----------|
| Caspase-12| F: CTG GAA GGA ATC TGT GGG GT NM_130422.1 R: GGC TAT CCC TTT GCT TGT GG |
| Bax       | F: GGT GCT TTC AGG GCT TTT CA AB046392.1 R: TGT GAA GTA GCA GCA GGT CA |
| Bcl2      | F: CTT CAG GGA TGG GGT GAA CT NM_016993.1 R: ATC AAA CAG AGG TCG CAT GC |
| GAPDH     | F: AGG TGT TCT CCT GTG ACT TC NM_017008 R: CTG TTG CTG TAG CCA TAT TC |

GAPDH: Glyceraldehydes-3 phosphate dehydrogenase.

![Fig. 1. Effects of RSV and SMV on ECG pattern in CP-induced cardiotoxicity in rats.](image-url)
Fig. 2. Effects of RSV and SMV on ECG parameters in CP-induced cardiotoxicity in rats. Values are shown as mean ± S.E. (n = 10). * significantly different at $p < 0.05$ vs normal control group and $\# p < 0.05$ significantly different vs CP-control group.
LDH and CK-MB activities to 82%, 69% and 73% respectively, while RSV resulted in marked reduction in serum troponin T content as well as LDH and CK-MB activities to 70%, 78% respectively, in comparison with the normal control group. Similarly, SMV administration caused a significant reduction in serum troponin T content as well as LDH and CK-MB activities to 218%, 159% and 176% respectively, relative to CP-control group (Table 2).

Table 2
Effects of RSV and SMV on serum cardiac troponin t content (a) as well as lactate dehydrogenase (b) and creatine kinase-MB (c) activities in CP-induced cardiotoxicity in rats.

| Groups              | Troponin T (pg/mL) | Lactate dehydrogenase (U/L) | Creatine kinase-MB (U/L) |
|---------------------|--------------------|-----------------------------|--------------------------|
| Normal control      | 46.80 ± 1.86       | 41.73 ± 1.95                | 22.75 ± 1.25             |
| CP-control (10 mg/kg, i.p.) | 102.2 ± 2.44@     | 66.26 ± 2.57@               | 39.95 ± 0.94@            |
| CP + RSV (10 mg/kg, p.o.) | 84.11 ± 1.50##    | 45.90 ± 1.67##              | 29.35 ± 1.43##           |
| CP + SMV (10 mg/kg, p.o.) | 71.64 ± 1.82##    | 51.70 ± 2.70##              | 31.00 ± 1.40##           |

Values are shown as mean ± S.E. (n = 10). @ significantly different at p < 0.05 vs normal control group and # significantly different at p < 0.05 vs cisplatin-control group.

3.2. Effects of RSV and SMV on serum cardiac function biomarkers in CP-induced cardiotoxicity

Cisplatin injection caused a prominent elevation in serum troponin T content as well as LDH and CK-MB activities to 218%, 159% and 176% respectively, in comparison with the normal control group. Similarly, RSV resulted in marked reduction in serum troponin T content as well as LDH and CK-MB activities to 82%, 69% and 73% respectively, while SMV oral administration caused a significant reduction in serum troponin T content as well as LDH and CK-MB activities to 70%, 78% and 77%, respectively relative to CP-control group (Table 2).

3.3. Effects of RSV and SMV on cardiac tissue oxidative stress markers in CP-induced cardiotoxicity

Cardiac tissue GSH content and SOD activity were reduced in CP treated rats to 47% and 57% respectively when compared to normal control group. RSV administration showed significant elevation in GSH content and SOD activity to 131% and 118% while SMV administration showed significant elevation in GSH content and SOD activity to 156% and 141% respectively; when compared to CP-control group (Fig. 3).

3.4. Effects of RSV and SMV on cardiac ER stress-mediated apoptotic death markers in CP-induced cardiotoxicity

Cardiac tissue contents of the GRP78 and calpain-1 as well as ACASP3 activity were augmented in CP-treated rats to 146%, 180% and 190% respectively, relative to the normal control group. However, RSV and SMV administration showed prominent reduction in GRP78 and calpain-1 as well as ACASP3 activities to 57%, 71% and 56% as well as 74%, 68% and 69%, respectively; relative to CP-control group (Fig. 4).

3.5. Effects of RSV and SMV on cardiac tissue relative caspase-12
Quantitative Real Time-PCR gene expression in CP-induced cardiotoxicity in rats

Cardiac caspase-12 gene expression was expressively elevated in CP treated rats as relative to normal control group. Treatment of CP-induced cardiotoxicity with either RSV or SMV significantly decreased cardiac caspase-12 gene expression when compared to CP-control group (Fig. 5).

3.6. Effects of RSV and SMV on cardiac tissue Bax and Bcl-2
Quantitative Real Time-PCR gene expression in CP-induced cardiotoxicity

Cisplatin was associated with overexpression of pro-apoptotic Bax gene and low expression of anti-apoptotic Bcl-2 gene. Furthermore, cardiac Bax/Bcl-2 ratio was significantly elevated in CP treated rats to reach 8.5 relative to normal control group. On the other hand, treatment of CP-induced cardiotoxicity with SMV and RSV showed a shift of the pro-apoptotic Bax gene and to the anti-apoptotic Bcl-2 gene towards anti-apoptosis. RSV administration decreased Bax/Bcl-2 ratio to 4.54 as well as SMV administration decreased Bax/Bcl-2 ratio to 3.31 relative to CP-control group (Fig. 6).

4. Discussion

Cisplatin; a broadly active cytotoxic anticancer and antitumor drug, whose role on DNA damage and cell apoptosis is totally unclear. However, involvement of various signaling pathways including ER stress as well as non-nucleus-dependent apoptotic signal stimulation have been recently demonstrated [13]. Besides, some experimental studies suggested a direct damaging effect of CP on the cardiomyocyte [22–24]. Therefore, the current study aims on clarifying the underlying mechanism of CP-induced cardiotoxicity through ER-stress mediated apoptotic death in rats and the role of statins namely; RSV and SMV, in counter-acting the CP detrimental effects on the cardiac tissue.

The current study in agreement with the case study of Hu et al. has shown that CP was associated with marked impairment in the cardiac functions evidenced by alterations in the ECG pattern as compared to normal ECG evidenced by; elongation of QTc duration with increased heart rate, P amplitude, ST height and T wave amplitude as compared to normal control group.
normal ECG [3]. It also showed marked elevation serum cardiac function biomarkers; troponin T content, LDH and CK-MB activities thus indicating cardiac injury [25]. On the other hand, the results of this study demonstrated that pretreatment with RSV and SMV resulted in an improvement of the ECG pattern while a marked decrease in heart rate, QTc duration, P amplitude, ST height and T amplitude as well as significant elevation in RR and QRS intervals were observed relative to CP-control group. Statins also attenuated the serum measured cardiac function biomarkers viz, troponin T content, LDH and CK-MB activities. The restoration of abnormal levels of these biomarkers indicates the cardioprotective potential of statins [25, 26].

It has been previously observed that the imbalance in the redox homeostasis, ER stress, as well as apoptosis are involved in CP-induced cardiotoxicity pathogenesis [27]. This contributes to the generation of massive ROS; which ultimately causes oxidative stress evidenced in the current study by reduction in the cardiac GSH content and SOD activity in CP treated rats. There has been always a close relation between cardiotoxicity and elevated levels of oxidative stress biomarkers [28–30]. Oxidative stress initiates myocardial cell damage by stress activation of the ER associated with impaired mitochondrial function, resulting in severe cardiotoxicity [31].

In accordance with previous studies, our findings showed that CP-induced ER stress, indicated by increased protein expression of GRP-78 and calpain-1, which eventually resulted in ER stress-mediated apoptosis [32]. Moreover, the current study showed that CP is linked with overexpression of pro-apoptotic Bax gene and low expression of anti-apoptotic Bcl-2 gene, thus high Bax/Bcl-2 ratio; as a proapoptotic
Fig. 6. Effects of RSV and SMV on cardiac tissue (a) relative Bax gene expression, (b) relative Bcl-2 gene expression and (c) Bax/Bcl-2 ratio in CP-induced cardiotoxicity in rats. Values are shown as mean ± S.E. (n = 10). @ significantly different at p < 0.05 vs normal control group and # p < 0.05 significantly different vs CP-control group.

Fig. 7. Involvement of ER stress signaling pathways in CP-induced cardiotoxicity and the regulatory role of statins via modulation of ER-Chaperone GRP78 and Calpain-1 Pathways. CP activation of ER stress initiates the unfolded protein response (UPR) by triggering the master chaperone protein ER-resident, 78 kDa glucose-regulated protein (GRP78). GRP78 interacts preferentially with hydrophobic patches on misfolded ER-resident proteins, releasing the UPR’s three signaling pathways. In addition, CP-induced ER stress activates Ca\(^{2+}\)-dependent-calpain-1 pathway which further induces caspase-12 and activated caspase-3 (ACASP3) apoptotic mechanism. Statins disrupt ER stress-mediated apoptotic cell death through regulation of GRP78, calpain-1 and ACASP3 in CP-induced cardiotoxicity.
index [33], which act as a regulator that determines the susceptibility of the cells to apoptosis [34,35]. In various pathological processes such as myocardial infarction, dilated cardiomyopathy, and ischemic cardiac disease, it has been found that the ratio of the pro-apoptotic Bax gene and to the anti-apoptotic Bcl-2 gene is shifted towards pro-apoptotic [36].

ER stress-induced protein response (UPR) stimulation is an imperative protein quality control mechanism in ER that stimulates cell survival and restores ER homeostasis [37]. A master ER-resident protein chaperone, GRP78 [38] regulates the three transmembrane signaling cascades of UPR: the ER stress controller protein PKR-like ER kinase (PERK), the inositol-requiring enzyme 1 (IRE-1) and the stimulating transcription factor 6 (ATF6) and their down-regulation of the signaling pathways [39]. GRP78 preferentially interrelates with the ER-resident misfolded proteins when it accumulates, thereby liberating the three signaling pathways of the UPR. Initiation of the UPR causes transient decline in protein translation, incrimination of ER protein-folding chaperones, and thus up-regulation of ER-associated protein degradation. However, apoptotic cell death is triggered when the protein-folding stress is intense or persistent [40]. Upon activation, PERK phosphorylates the eukaryotic initiation factor eIF2α, conducting apoptosis induction by activating ATF4 and CHOP via stimulation of a cascade reaction [41].

Endoplasmic reticulum stress showed a vital shift in the determination of the cardiomyocyte fate during CP treatment. Whereas, CP phosphorylates eIF2α, thus activates GRP78 in addition to its ability to activate calpain-1 which induce ACASP3 which promotes the formation of apoptosome. These two pathways induce apoptosis of the cardiomyocytes thus lead to CP-induced cardiotoxicity [16,42] as illustrated in Fig. 7. Initiating of the ER stress-related apoptotic signaling triggers death of cells in myocardial I/R, playing detrimental role in cardiotoxicity. ER stress progresses an ischemic cardiomyopathy in a variety of models ranging from mice to humans were UPR stimulation was observed [43,44]. The increase in unfolded protein response stimulation by ischemia is shown by activation of GRP78 in cardiomyocytes to regulate efficient protein folding and ER homeostasis [43].

Previous studies showed that prolonged ischemia as well as ischemia–reperfusion injury, persistent URP activation causes apoptosis by promoting the ACASP3 upregulation which contributes to the cardiac insufficiency [45] which was also found in the ischemic human heart [46]. Furthermore, apoptotic cardiac injury has been shown to be improved by mitigating the outbreak of ER stress via GRP78 down-regulation [47].

The stimulation of calpain-1 pathway was accompanied by ER stress, which indicates that CP targeted ER. Moreover, CP has shown to upregulate of ER stress marker expression, GRP78 and promote calpain-dependent enhancement of the ER-specific caspase-12 gene expression which plays a pivotal role in CP-induced apoptosis [48]. Calpain enhancement has been involved in heart failure and myocardial remodeling [49] while genetic reduction of calpain decreases myocardial dysfunction and cardiac remodeling in hypertrophic animal model [50]. Furthermore, lipotoxicity-induced apoptosis is prevented by the disturbed calpain in cardiomyocytes and cardiac dysfunction in mice fed high fat diet [51].

Statins namely; SMV and RSV, in the current study, have shown to interfere with this cascade as illustrated in Fig. 7. It has been hypothesized that RSV and SMV may inhibit the CP-induced ER stress by inhibiting GRP78 and calpain-1, which suppresses apoptosis and subsequently reduce the cardiotoxicity. This result was aligned with Yuan-nyan et al. [52] that revealed that SMV inhibits the ER stress-associated apoptosis in-vitro via attenuating GRP78 protein expression [52]. Moreover, statins have shown a prominent decrease in caspase-12 expression, thus reduce cardiomyocyte apoptosis. It has been previously presented that atorvastatin declined caspase-12 expression in a post-myocardial infarction-induced heart failure model [53].

Noteworthy, the current study showed that treatment of CP-induced cardiotoxicity with SMV and RSV showed a shift of the pro-apoptotic Bax gene and to the anti-apoptotic Bcl-2 gene towards anti-apoptotic evidenced by low Bax/Bcl-2 ratio indicating cardiomyocyte resistance towards apoptosis [52]. Hence, this study presents a novel target for avoiding cardiotoxicity mediated by CP.

Of note, deregulated UPR signaling sensitzes chemotherapy-resistance to agents that induce ER stress. Significant upregulation of GRP78 indicates that CP resistance was accompanied with ER stress [42]. Furthermore, GRP78/Bip has been overexpressed in several types of cancers both at the gene and at the protein levels. GRP78 induces chemoresistance acquisition properly via PI3K/Akt proliferative pathway [54]. Mechanistically, hyperactivation of ER-stress pathways is important for UPR signaling in chemotherapy resistance, providing a novel rationale through altering ER stress to cure patients with chemotherapy-resistance [55]. Functional studies of GRP78 silencing suggested that GRP78 knockdown plays a significant role in sensitizing cancer cells to CP. As a consequence, it has been speculated that GRP78 may be a potential future cancer therapeutic target [56]. Given limited data about the role of ER stress signaling in the crosstalk between cell survival, apoptosis, and cell death along with chemo-resistance, further investigations and testing new pharmacological intervention are warranted to improve the clinical outcome of CP, diminish the adverse effects and overcome resistance to chemotherapy.

5. Conclusion

In summary, it has been clarified the relationships between ER stress-induced apoptosis and CP-induced cardiotoxicity through upregulating GRP78 and calpain-1 pathways. The role of statins in attenuating the detrimental effects of CP on the ER stress-mediated cardiac apoptotic death has been extensively studies via targeting those two pathways evidenced by shifting the Bax/Bcl-2 expression towards anti-apoptosis. Thus, statins are recommended for further studies on CP-resistant in-vitro and in-vivo cancer models via GRP78 pathway regulation based on the current findings.

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Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process.

Declaration of Competing Interest

The authors are not reporting any conflicts of interest.

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