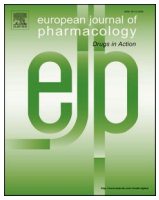
European Journal of Pharmacology 928 (2022) 175083

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar



Investigation of drugs for the prevention of doxorubicin-induced cardiac events using big data analysis

Shiori Nishiuchi [a,b,1], Kenta Yagi [c,1], Hiroumi Saito [a], Yoshito Zamami [a,d,**], Takahiro Niimura [c], Koji Miyata [a], Yoshika Sakamoto [a], Kimiko Fukunaga [a], Shunsuke Ishida [b], Hirofumi Hamano [a,d], Fuka Aizawa [b], Mitsuhiro Goda [a,b,*], Masayuki Chuma [e], Yuki Izawa-Ishizawa [f], Hideki Nawa [g], Hiroaki Yanagawa [c], Yasunari Kanda [h], Keisuke Ishizawa [a,b,c]

[a] Department of Clinical Pharmacology and Therapeutics, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan
[b] Department of Pharmacy, Tokushima University Hospital, Tokushima, Japan
[c] Clinical Research Centre for Developmental Therapeutics, Tokushima University Hospital, Tokushima, Japan
[d] Department of Pharmacy, Okayama University Hospital, Okayama, Japan
[e] Department of Hospital Pharmacy and Pharmacology, Asahikawa Medical University, Asahikawa, Japan
[f] Department of Pharmacology, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan
[g] Department of Pharmacy, Faculty of Pharmacy, Shujitsu University, Okayama, Japan
[h] Division of Pharmacology, National Institute of Health Sciences, Kanagawa, Japan

ARTICLE INFO

Keywords:
Drug–drug interaction
Cardiology
Data analysis
Chemotherapy
Doxorubicin

ABSTRACT

Aim: Doxorubicin, an anthracycline anti-tumour agent, is an essential chemotherapeutic drug; however, the adverse events associated with doxorubicin usage, including cardiotoxicity, prevent patients from continuing treatment. Here, we used databases to explore existing approved drugs with potential preventative effects against doxorubicin-induced cardiac events and examined their efficacy and mechanisms.

Methods: The Gene Expression Omnibus (GEO), Library of Integrated Network-based Cellular Signatures (LINCS), and Food and Drug Administration Adverse Events Reporting System (FAERS) databases were used to extract candidate prophylactic drugs. Mouse models of doxorubicin-induced cardiac events were generated by intraperitoneal administration of 20 mg/kg of doxorubicin on Day 1 and oral administration of prophylactic candidate drugs for 6 consecutive days beginning the day before doxorubicin administration. On Day 6, mouse hearts were extracted and examined for mRNA expression of apoptosis-related genes.

Results: GEO analysis showed that doxorubicin administration upregulated 490 genes and downregulated 862 genes, and LINCS data identified sirolimus, verapamil, minoxidil, prednisolone, guanabenz, and mosapride as drugs capable of counteracting these genetic alterations. Examination of the effects of these drugs on cardiac toxicity using FAERS identified sirolimus and mosapride as new prophylactic drug candidates. In model mice, mosapride and sirolimus suppressed the *Bax/Bcl*-2 mRNA ratio, which is elevated in doxorubicin-induced cardiotoxicity. These drugs also suppressed the expression of inflammatory cytokines Il1b and Il6 and markers associated with myocardial fibrosis, including Lgal3 and Timp1.

Conclusion: These findings suggest that doxorubicin-induced cardiac events are suppressed by the administration of mosapride and sirolimus.

* Corresponding author. Department of Clinical Pharmacology and Therapeutics, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan.

** Corresponding author. Department of Clinical Pharmacology and Therapeutics, Graduate School of Biomedical Sciences, Tokushima University, Tokushima, Japan.

E-mail addresses: zamami@tokushima-u.ac.jp (Y. Zamami), mgoda@tokushima-u.ac.jp (M. Goda).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.ejphar.2022.175083
Received 8 November 2021; Received in revised form 29 May 2022; Accepted 30 May 2022
Available online 31 May 2022

1. Introduction

Doxorubicin, an anthracycline anti-tumour drug, exerts its effect by inserting into the DNA base pairs of tumour cells and inhibiting DNA polymerase, RNA polymerase, and topoisomerase II reactions, thereby suppressing DNA and RNA biosynthesis. The efficacy of doxorubicin has been demonstrated in several cancer types, including lung, gastrointestinal, breast, and bladder cancers, as well as malignant lymphoma and osteosarcoma (Damiani et al., 2016), resulting in its consideration as an essential chemotherapeutic agent. Although doxorubicin exerts a strong anti-tumour effect, it is also associated with the development of cardiotoxicity, which prevents patients from continuing treatment (Koleini and Kardami, 2017).

Doxorubicin cardiotoxicity includes acute and chronic disorders. Chronic myocardial injury is known to occur in a cumulative, dose-dependent manner (McGowan et al., 2017) and can lead to heart failure, with a 3-year survival rate of approximately 50% in the absence of treatment (Felker, 2000). Doxorubicin-induced chronic myocardial injury is addressed in real-world clinical practice by dose restriction. However, acute myocardial injury does not correlate with dosage and is reported to occur within a few days of doxorubicin administration, resulting in transient ventricular hypofunction and other symptoms. No prophylaxis for doxorubicin-induced acute myocardial injury has been developed to date. The underlying mechanism of doxorubicin-induced myocardial injury is probably associated with oxidative stress due to free radical production, ferotosis, apoptosis owing to mitochondrial damage in cardiomyocytes, and DNA damage due to topoisomerase II inhibition (Mustafa et al., 2017; Songbo et al., 2019; Sheibani et al., 2020, 2021; Tadokoro et al., 2020). The effects of dexrazoxane on doxorubicin-induced cardiotoxicity and doxorubicin-specific anti--tumour effects are controversial, according to various studies(Reichardt et al., 2018; Lipshultz et al., 2014; Ghasemi et al., 2021; Chow et al., 2022). In clinical trials conducted in the United States, an increased risk of acute myeloid leukemia and myelodysplastic syndrome was reported in paediatric patients and adolescents treated with dexrazoxane to prevent cardiomyopathy caused by long-term administration of anthracyclines (Tebbi et al., 2007; Salzer et al., 2010; Schwartz et al., 2009; Swain et al., 1997). The Committee for Medicinal Products for Human Use (CHMP) reviewed this report and concluded in 2011 that the use of dexrazoxane to prevent cardiomyopathy in patients younger than 18 years of age was not warranted. It was recommended that the use of dexrazoxane be contraindicated (EMA/491205/2011).

Recently, a new strategy was proposed for drug discovery called drug repositioning, where existing approved drugs are investigated for alternate pharmacological effects, thereby exploiting their potential to treat other diseases (Ashburn and Thor, 2004). The construction of large-scale medical information databases providing information on altered gene expression and side effects has made it possible to successfully repurpose drugs.

The Food and Drug Administration (FDA) Adverse Events Reporting System (FAERS) is the largest database containing reports of spontaneous adverse events from medical professionals and companies in the United States and abroad (Nagashima et al., 2016). The Gene Expression Omnibus (GEO), maintained by the National Centre for Biotechnology Information (NCBI), contains gene expression information that includes microarray data of >3 million samples (Edgar et al., 2002). The Library of Integrated Network-based Cellular Signatures (LINCS) program is a drug-discovery tool that simulates changes in the expression of ~20,000 genes in response to chemical compounds in human cell lines and has been used to study the development of novel therapeutic agents (Keenan et al., 2018; Duan et al., 2016). These databases have been employed in many studies, including those performed by our research team, to identify drugs capable of preventing adverse events associated with high-risk drugs (Zamami et al., 2019a; Izawa-Ishizawa et al., 2019; Okada et al., 2019).

In this study, we used these databases to explore existing approved drugs with potential preventative effects against doxorubicin-induced cardiac events and examine their efficacies and associated mechanisms.

2. Material and methods

2.1. Doxorubicin-induced changes in gene expression

The microarray dataset associated with doxorubicin-induced cardiotoxicity was obtained from the NCBI GEO database (http://www.ncbi.nlm.nih.gov/geo/; accession no. GSE23598). The dataset comprised mouse heart mRNA samples collected 4 days after administration of 15 mg/kg doxorubicin or an equivalent amount of saline. Two pre-processing methods were used to process the microarray data. The robust multi-array average (RMA) method is a quantile normalisation method that sorts the data of each sample by signal intensity and corrects it to the same value if it is of the same rank in each sample. The MAS5 method is a global normalisation protocol that corrects each sample to ensure that all signal averages are the same.

The statistical analysis programming language R (https://www.r-project.org/) along with its packages “affy” and “RobLoxBioC” was used for each process. We identified a group of genes with significantly altered expression ratios in the doxorubicin-treated group relative to the saline-treated group.

2.2. Extraction of drugs to counteract changes in gene expression

For the identified gene groups, we extracted drugs that counteract the doxorubicin-induced changes in gene expression using the LINCS search engine L1000CDS2. A group of genes upregulated by doxorubicin was entered into L1000CDS2 as “up genes”, and a downregulated group was entered as “down genes”. We then searched for compounds that countered the altered gene expression and selected the top 50 FDA-approved drugs as prophylactic candidate drugs.

2.3. Targeting prophylactic candidate drugs

The FAERS data for 7,738,415 spontaneous adverse events from Q1 2007 through Q1 2017 were downloaded from the FDA website (https://www.fda.gov/Drugs/GuidanceCompliance -RegulatoryInformation/Surveillance/AdverseDrugEffects/ucm082193.htm). Duplicate data were excluded according to the methodology recommended by the FDA, and the remaining 6,994,117 events were used for analysis. In the FAERS database, the names of drugs were standardised to generic names, as this database allows drugs to be registered under any name, including trade names and abbreviations.

Adverse events were designated according to the 93 extracted terms from the “10019280/Heart failures/Heart failure” group, which is based on the Medical Dictionary for Regulatory Activities/J (v.21.0; International Glossary of Pharmaceutical Terms of International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use) (Table S1).

The risk of the reported adverse events was evaluated by calculating the 95% confidence interval (CI) and reporting odds ratios (RORs) according to previously reported methods (Nagashima et al., 2016; Li et al., 2008). Doxorubicin-treated patients from the FAERS database were then classified into four groups: patients who (A) received the candidate drug and reported heart failure as an adverse effect, (B) received the candidate drug and did not report heart failure as an adverse effect, (C) did not receive the candidate drug and reported heart failure as an adverse effect, and (D) did not receive the candidate drug and did not report heart failure as an adverse effect. The number of reports for each group was calculated based on these classifications, after which ROR and 95% CIs were calculated according to Eq. (1):

$$\mathrm{ROR} = (A/B)/(C/D);\ 95\%\ \mathrm{CI} = \exp\left[\log(\mathrm{ROR}) \pm 1.96\ \mathrm{square}\ 1/A + 1/B + 1/C + 1/D\right] \quad (1)$$

where A, B, C, and D represent the number of reports in each group, and log represents the natural logarithm. We defined drugs with a ROR > 1 as those that increased the risk of adverse effects, whereas drugs with a ROR < 1 were considered to decrease the risk of adverse effects and defined as prophylactic candidate drugs.

2.4. Animals

Twenty-four male C57BL/6J mice (aged 8 weeks) were purchased from Nippon CLEA (Tokyo, Japan). The mice were housed and acclimatised for 1 week before being placed at a constant temperature (23–24 °C) with a 12-h light/dark cycle (light: 8 A.M. to 8 P.M.). Food and water were provided ad libitum. All experimental procedures performed on the mice were in accordance with the guidelines of the Animal Research Committee of Tokushima University Graduate School, and the protocol was approved by the Institutional Review Board of Tokushima University Graduate School for animal protection (permit no. T30-85).

2.5. Doxorubicin-induced myocardial flame model mice and drug administration

Mouse models of doxorubicin-induced cardiac disease were prepared as previously described (Zhao et al., 2018a, 2018b; Gupta et al., 2018; Yuan et al., 2018). Male C57BL/6J mice (8-weeks old) were randomised into the following groups: vehicle (n = 8), doxorubicin-treated (n = 5), mosapride (3 mg/kg) or sirolimus (2 mg/kg) only (n = 6/group), and doxorubicin treatment plus mosapride (doxorubicin + mosapride) or sirolimus (doxorubicin + sirolimus) (n = 5/group). The mice were intraperitoneally administered a single dose of doxorubicin (20 mg/kg; Funakoshi Co., Ltd., Tokyo, Japan) dissolved in saline. Vehicle-treated mice were administered only saline. Mosapride (3 mg/kg; Wako, Osaka, Japan) and sirolimus (2 mg/kg; Funakoshi Co., Ltd.) were dissolved in 0.5% carboxymethyl cellulose, and each drug was orally administered to mice once daily for 6 consecutive days beginning 1 day before doxorubicin treatment. The method of administration and dosage concentrations of mosapride and sirolimus were determined based on previously reported methods used in animal experiments. After 5 days of doxorubicin administration, experimental mice were anaesthetised, and their hearts were collected. Anaesthesia was induced by inhalation of isoflurane under a 4% diluted vaporiser setting (vaporised in oxygen, 1 L/min) and maintained with isoflurane under a 2% vaporiser setting. The isoflurane was delivered via a small face mask.

2.6. Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

The collected cardiac apex tissue was placed in ISOGEN II (Nippon Gene, Tokyo, Japan) and homogenised on ice, followed by extraction of total RNA according to the manufacturer's instructions. The extracted RNA concentration was measured using a Nanodrop 1000 (Thermo Fisher Scientific, Waltham, MA, USA), and cDNA solutions were prepared using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan).

Each reaction was performed using a TaKaRa PCR thermal cycler (Dice; Takara Bio). qRT-PCR was performed using Thunderbird SYBR qPCR mix (Toyobo, Osaka, Japan) and a StepOnePlus system (Applied Biosystems, Waltham, MA, USA) with the following cycling conditions: one cycle at 95 °C, followed by 40 cycles of 15 s at 95 °C and 35 s at 60 °C. The expression level of each gene was determined using mouse β-actin gene as the endogenous standard gene. All data were analysed using CFX Manager software (Bio-Rad, Hercules, CA, USA).

2.7. Statistical analysis

All experiments were independently repeated a minimum of three times, and the resulting data were expressed as the mean ± standard error of the mean (SEM). Data were analysed using a two-way analysis of variance, followed by the Tukey test. Categorical variables were compared using Fisher's exact test. A $p < 0.05$ was considered significant.

3. Results

3.1. Identification of agents capable of preventing doxorubicin-induced cardiotoxicity

First, we extracted genes showing altered expression following doxorubicin administration. Following normalisation of data from the GEO database (using the RMA method), doxorubicin treatment was found to upregulate the expression of 1259 genes and downregulate that of 1158 genes. Similarly, data normalised by the MAS5 method showed that doxorubicin treatment upregulated 490 genes and downregulated 862 genes.

Next, the L1000CDS2 search engine identified six approved drugs that counteracted doxorubicin-altered gene expression. Specifically, sirolimus was identified in the RMA-normalised gene groups, whereas verapamil, minoxidil, prednisolone, guanabenz, and mosapride were detected in the MAS5-normalised gene groups.

FAERS was subsequently used to examine the effects of these six drugs on the prevention of doxorubicin-induced cardiotoxicity. From the 6,994,117 reports obtained following the exclusion of duplicate data, 36,329 patients using doxorubicin were included in the analysis. No reports on the concomitant use of guanabenz with doxorubicin were available. The RORs for sirolimus, mosapride, prednisolone, verapamil, and minoxidil were 0.32, 0.36, 0.65, 1.36, and 6.95, respectively (Table 1). However, the preventative effect exerted by prednisolone is moderate (Aguilar et al., 2014). Therefore, sirolimus and mosapride were identified as novel preventive drug candidates.

3.2. Evaluation of cardiac effects elicited by doxorubicin and candidate drug treatment

The contribution of candidate drugs to the protection of cardiac function was examined in a mouse model of doxorubicin-induced cardiac events. The body weight of each group on Day 6 was 23.06 ± 0.49 g for the vehicle group, 24.42 ± 0.79 g for the mosapride only group, 23.42 ± 0.22 g for the sirolimus only group, 17.60 ± 0.26 g for the doxorubicin only group, 18.30 ± 0.50 g for the doxorubicin + mosapride group, and 18.70 ± 0.39 g for the doxorubicin + sirolimus group. Body weights in the vehicle, mosapride only, and sirolimus only groups were unchanged compared with those on Day 1. From the day

Table 1
Effect of concomitant medications on the rate of reported doxorubicin-induced heart failure.

Concomitant with Drug B	Without Drug B (%)	With Drug B (%)	ROR[a]	95% CI[b]	p
Sirolimus	2092/36221 (5.78)	2/108 (1.85)	0.32	0.04–1.19	0.13
Mosapride	2093/36281 (5.77)	1/48 (2.08)	0.36	0.01–2.11	0.52
Prednisolone	1912/31666 (6.04)	182/4663 (3.90)	0.65	0.55–0.76	<0.0001
Verapamil	2090/36278 (5.76)	4/51 (7.84)	1.36	0.36–3.71	0.54
Minoxidil	2092/36324 (5.76)	2/5 (40)	6.95	0.66–42.46	0.05

[a] ROR: reporting odds ratio.
[b] CI: confidence interval.

after doxorubicin treatment (Day 2), body weights were reduced in the doxorubicin only, doxorubicin + mosapride, and doxorubicin + sirolimus groups, with a significant reduction observed after 5 days of doxorubicin treatment. Although combining doxorubicin with these prophylactic agents did not significantly inhibit weight loss, reduced weight loss was observed compared with that in the doxorubicin only group (Fig. 1).

3.3. Changes in apoptosis-related gene expression by doxorubicin and candidate drug treatment

We then evaluated the effects of doxorubicin and candidate drug administration on B-cell lymphoma-2 (*Bcl-2*) and *Bcl-2*-associated X-protein (*Bax*) expression in the mouse heart. The *Bax/Bcl-2* mRNA expression ratio was 1.00 ± 0.12 for the vehicle group, 0.75 ± 0.07 for the mosapride only group, 0.96 ± 0.08 for the sirolimus only group, 2.87 ± 0.71 for the doxorubicin only group, 1.37 ± 0.22 for the doxorubicin + mosapride group, and 1.53 ± 0.17 for the doxorubicin + sirolimus group. Although doxorubicin treatment significantly increased the *Bax/Bcl-2* mRNA ratio, a trend towards the reduction of the ratio was observed in the doxorubicin + mosapride and doxorubicin + sirolimus groups (Fig. 2).

We then evaluated changes in the expression of inflammatory markers associated with cardiac fibrosis following doxorubicin and candidate drug treatment. Quantification of the expression of inflammatory cytokines in cardiomyocytes revealed no increases in interleukin-1b (*Il1b*) mRNA expression in the mosapride only and sirolimus only groups compared with that in the vehicle group, although a decreasing trend in *Il6* mRNA expression was observed in these groups compared with that in the vehicle group. Additionally, we found that doxorubicin treatment significantly increased *Il1b* and *Il6* expression; however, combined treatment with mosapride or sirolimus resulted in a decreasing trend of *Il1b* expression (Fig. 3A) and significant suppression of *Il6* expression (Fig. 3B).

Furthermore, we evaluated mRNA levels of *Lgal3* (encoding galectin-3) and tissue inhibitor of metalloproteinase (*Timp*)*1* as biomarkers of cardiac remodelling. We found no increases in *Lgal3* or *Timp1* mRNA levels in the mosapride and sirolimus groups compared with those in the vehicle group, but the expression of both was significantly increased by doxorubicin treatment. Moreover, their expression was significantly suppressed by combined treatment with either mosapride or sirolimus compared with that observed in the doxorubicin alone group (Fig. 4A and B).

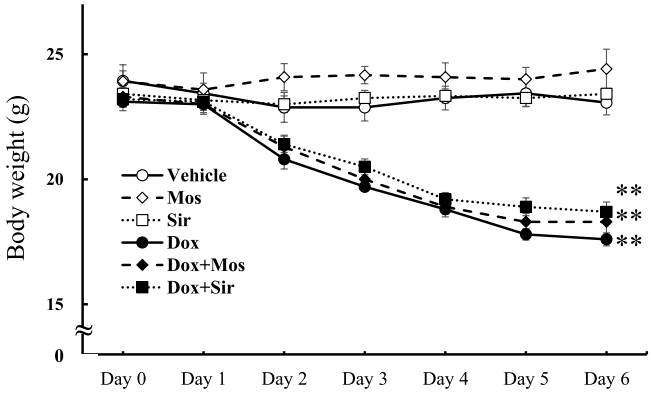


Fig. 1. Changes in body weight in the mouse model of doxorubicin-induced cardiotoxicity. Changes in body weight of 8-week-old male C57BL/6J wild-type mice treated with doxorubicin alone or in combination with mosapride or sirolimus for 6 days. Error bars represent the mean ± standard error of the mean (SEM; n = 5–8). **p < 0.01 vs. vehicle.

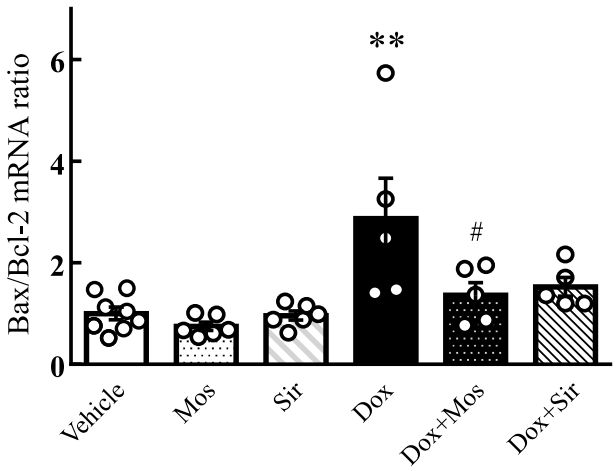


Fig. 2. Expression of apoptosis-related genes in the heart tissue of mouse models of doxorubicin-induced cardiotoxicity. Ratio of *Bax/Bcl-2* mRNA levels. mRNA levels were adjusted to those in the vehicle group, which were set to 1. Error bars represent the mean ± standard error of the mean (SEM; n = 5–8). *p < 0.05 vs. vehicle; #p < 0.05 vs. the doxorubicin-treated group.

4. Discussion

In this study, we searched for candidate drugs that reduced doxorubicin-induced cardiotoxicity by analysing three medical information databases (GEO, LINCS, and FAERS) and determining the efficacy and mechanism of action of these candidates.

Doxorubicin-specific adverse effects, including cardiotoxicity, causes significant clinical challenges by interfering with the continuation of treatment (Koleini and Kardami, 2017). Using GEO, we found that doxorubicin alters the expression of genes associated with inflammatory responses and apoptosis, which are involved in the mechanism of cardiotoxicity (Yu et al., 2020; Tacar et al., 2013). Therefore, we used LINCS to search for drugs capable of suppressing changes in the gene expression induced by doxorubicin, which resulted in the identification of six drugs (verapamil, minoxidil, prednisolone, guanabenz, mosapride, and sirolimus).

FAERS was then used to compare the frequency of doxorubicin-induced cardiac events in patients administered with doxorubicin, with and without the use of candidate drugs. Prednisolone, mosapride, and sirolimus achieved an ROR < 1, suggesting that they reduced the development of doxorubicin-induced heart disease (Table 1). Prednisolone, a steroid, should not be administered indiscriminately for an extended period, although a study has shown its efficacy against cardiotoxicity (Aguilar et al., 2014). Therefore, we chose mosapride and sirolimus as candidate drugs for further analysis. Although the multiple databases helped identify drugs capable of preventing the development of adverse reactions and predict mechanisms associated with drug combinations, this strategy does not allow the elucidation of the true effects of drug combinations. Therefore, we investigated the associated mechanisms using a mouse model of doxorubicin-induced cardiac events. Since epidemiological studies and clinical reports suggest that myocardial damage is generally more common in males than in females (Kytö et al., 2013; Zamami et al., 2019b), this study was conducted on male mice. Since FAERS does not provide information on the number of doses, to evaluate the effects of cumulative toxicity is challenging. Therefore, when trying to obtain results linked to FAERS in animal experiments, it is appropriate to consider a single-dose acute toxicity model. Specifically, using a model of acute cardiotoxicity induced by a single 15 mg/kg dose of doxorubicin (Yuan et al., 2018; Jiao et al., 2022), we examined the effects of doxorubicin and prophylactic drug

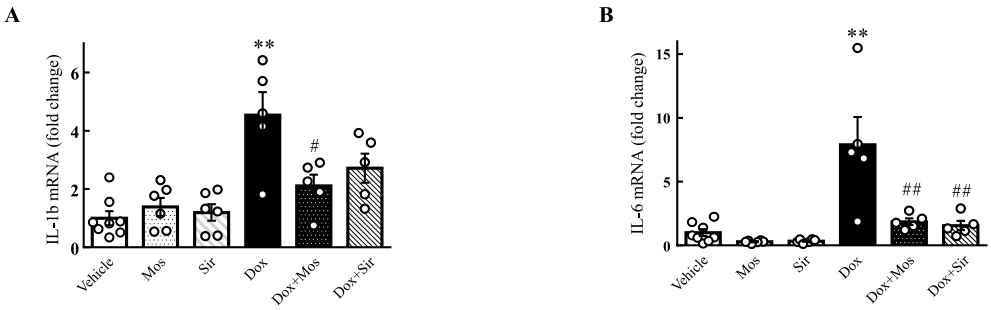


Fig. 3. Inflammatory cytokine gene expression in the heart tissue of mouse models of doxorubicin-induced cardiotoxicity. (A) *Il1b* and **(B)** *Il6* mRNA levels. mRNA levels were adjusted to those of the vehicle group, which were set to 1. Error bars represent the mean ± standard error of the mean (SEM; n = 5–8). *p < 0.05; **p < 0.01 vs. vehicle; #p < 0.05; ##p < 0.01 vs. the doxorubicin-treated group.

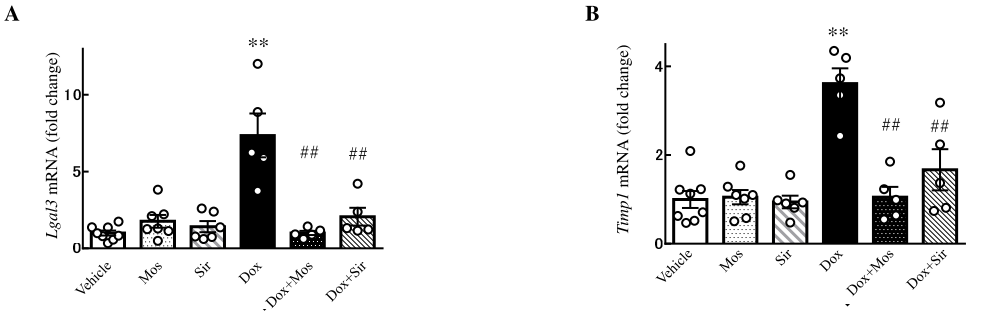


Fig. 4. mRNA levels of cardiac fibrosis-related markers in the heart tissue of moue models of doxorubicin-induced cardiotoxicity. (A) *Lgal3* and **(B)** *Timp1* levels. mRNA levels were adjusted to those of the vehicle group, which were set to 1. Error bars represent the mean ± standard error of the mean (SEM; n = 5–8). *p < 0.05; **p < 0.01 vs. vehicle; #p < 0.05; ##p < 0.01 vs. the doxorubicin-treated group.

candidates, focusing on body weight changes, apoptosis and ferotosis in doxorubicin-treated mice. Previous reports have shown that apoptosis involving *Bax/Bcl* and ferotosis involving Gpx4 (glutathione peroxidase 4) are involved in doxorubicin-induced cardiotoxicity as a pathway of cell death. The combination of prophylactic drug candidates with doxorubicin treatment tended to reduce weight loss compared with doxorubicin treatment alone (Fig. 1). In addition, the expression of apoptosis- and ferotosis-related genes was investigated: *Bax* promotes apoptosis in the mitochondrial apoptosis pathway by increasing the permeability of the mitochondrial outer membrane, whereas *Bcl-2* inhibits apoptosis by preventing the influx of apoptosis-inducing proteins into the cytoplasm (Aguilar et al., 2014; Adams and Cory, 1998; Oltvai et al., 1993). Gpx4 is also known to be involved in ferotosis (Tadokoro et al., 2020). We found that doxorubicin treatment significantly increased the *Bax/Bcl*-2 mRNA expression ratio in cardiomyocytes of doxorubicin-induced heart failure mice, but this effect was suppressed by the co-administration of mosapride or sirolimus (Fig. 2). On the other hand, no difference in *Gpx4* mRNA was observed with prophylactic treatment (data not shown). Thus, we suggest that mosapride and sirolimus may inhibit doxorubicin-induced myocardial apoptosis and reduce doxorubicin-induced cardiotoxicity.

Next, we evaluated inflammatory cytokines and markers associated with cardiac fibrosis. Inflammatory cytokines are implicated in the development of myocardial infarction, with high serum levels of IL-1b and IL-6 that are associated with poor prognosis(Tsutamoto et al., 1998; Zhu et al., 2010, 2011). Additionally, other studies have shown the involvement of the inflammatory response in doxorubicin-induced cardiotoxicity (Sheibani et al., 2020, 2021). In the present study, we found that doxorubicin-induced elevations in *Il6* expression were significantly suppressed by combination treatment of doxorubicin with mosapride or sirolimus; a trend towards suppression of *Il1b*, a proinflammatory cytokine that promotes cardiac remodelling (Bujak et al., 2008), expression was also observed by combination treatment (Fig. 3). Similarly, we identified significant doxorubicin-induced upregulation of *Lgal3* and *Timp1* expression as biomarkers of cardiac remodelling, with subsequent significant suppression in their levels observed by combination treatment with mosapride or sirolimus (Fig. 4). These results suggest that treatment with mosapride or sirolimus improves prognosis after the onset of cardiac disease. In doxorubicin-induced cardiomyopathy, the inflammatory response causes fibrosis, which leads to cardiac dysfunction (Tanaka et al., 2020), and it is suggested that the inflammatory response and suppression of fibrosis attenuate doxorubicin-induced cardiomyopathy.

This study has certain limitations. First, the gene-expression data recorded in LINCS is based on data from cell lines. However, we evaluated the efficacy of LINCS data against the reported rate of doxorubicin-induced cardiac events using FAERS rather than determining the candidate drugs by LINCS alone. In fact, the results of FAERS analysis showed that verapamil and minoxidil (extracted from LINCS) were ineffective against doxorubicin-induced cardiac events, resulting in their exclusion as candidate drugs. Second, FAERS data report spontaneous adverse events; however, all patients who have used doxorubicin or concomitant medications are not included, because those who have not experienced adverse events are not registered. Moreover, some patients who experienced adverse events are not reported. Therefore, the rate of doxorubicin-induced heart failure calculated by FAERS analysis might differ from that observed in the clinic. Third, the effect of any confounding factors was not applied to FAERS analysis. Although the ROR was calculated based on the number of reports on the use of concomitant medications, we did not adjust for age, gender, or medical history. A high percentage of patients with heart failure has high blood pressure (Adams et al., 2005), with the average age of these patients at 70 years (Nieminen et al., 2006). We were unable to evaluate the medical history of patients, as this information is not provided in FAERS. Owing to these limitations, the results of the FAERS review do not reflect real-world patients. Nevertheless, considering the difficulty in collecting

case reports of inconsistently occurring adverse effects, such as doxorubicin-induced cardiac events, the results of FAERS analysis, which incorporates a large amount of actual patient data, are effective in investigating the real-world clinical practice. Therefore, further evidence, including multicentre and randomised clinical trials, is needed to determine the possible effects of mosapride or sirolimus in preventing cardiac events in patients receiving doxorubicin.

In the present study, a model of doxorubicin-induced acute cardiotoxicity was generated in male mice with a single dose of 15 mg/kg doxorubicin (Yuan et al., 2018; Jiao et al., 2022). The current study examined the prophylactic effects of mosapride and sirolimus on doxorubicin-induced acute myocardial injury, but further studies are needed to examine their effects under other conditions. Since sex differences in doxorubicin-induced cardiotoxicity have been reported, it may be useful to perform studies using female mice. It may also be worthwhile to examine the prophylactic effects of mosapride and sirolimus using a model of chronic cardiotoxicity induced by multiple doses of doxorubicin.

In conclusion, this study demonstrates that doxorubicin-induced adverse cardiac events may be suppressed by the administration of the candidate drugs mosapride and sirolimus.

Funding

This work was supported by a grant from the Japan Society for the Promotion of Science Grants-in-Aid for Scientific Research [grant no. 18K06785], a Grant-in-Aid for Transformative Research Areas (B) [grant no. 20H05798], and the Japan Research Foundation for Clinical Pharmacology [grant no. 2018A10].

The funding sources had no involvement in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the article for publication.

CRediT authorship contribution statement

Shiori Nishiuchi: Methodology, Investigation, Writing – original draft. **Kenta Yagi:** Writing – original draft. **Hiroumi Saito:** Methodology, Investigation, Software, Validation. **Yoshito Zamami:** Conceptualization, Project administration. **Takahiro Niimura:** Methodology, Software, Validation, Formal analysis. **Koji Miyata:** Software, Validation. **Yoshika Sakamoto:** Methodology, Software, Validation. **Kimiko Fukunaga:** Software, Validation. **Shunsuke Ishida:** Writing – review & editing. **Hirofumi Hamano:** Methodology, Software, Validation. **Fuka Aizawa:** Methodology, Software, Validation. **Mitsuhiro Goda:** Conceptualization, Formal analysis, Visualization, Project administration. **Masayuki Chuma:** Writing – review & editing. **Yuki Izawa-Ishizawa:** Writing – review & editing. **Hideki Nawa:** Writing – review & editing. **Hiroaki Yanagawa:** Writing – review & editing. **Yasunari Kanda:** Writing – review & editing. **Keisuke Ishizawa:** Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We thank Editage (www.editage.com) for English language editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejphar.2022.175083.

References

Adams, J.M., Cory, S., 1998. The Bcl-2 protein family: arbiters of cell survival. Science 281, 1322–1326. https://doi.org/10.1126/science.281.5381.1322.

Adams Jr., K.F., Fonarow, G.C., Emerman, C.L., LeJemtel, T.H., Costanzo, M.R., Abraham, W.T., Berkowitz, R.L., Galvao, M., Horton, D.P., ADHERE Scientific Advisory Committee and Investigators, 2005. Characteristics and outcomes of patients hospitalized for heart failure in the United States: rationale, design, and preliminary observations from the first 100,000 cases in the Acute Decompensated Heart Failure National Registry (ADHERE). Am. Heart J. 149, 209–216. https://doi.org/10.1016/j.ahj.2004.08.005.

Aguilar, D., Strom, J., Chen, Q.M., 2014. Glucocorticoid induced leucine zipper inhibits apoptosis of cardiomyocytes by doxorubicin. Toxicol. Appl. Pharmacol. 276, 55–62. https://doi.org/10.1016/j.taap.2014.01.013.

Ashburn, T.T., Thor, K.B., 2004. Drug repositioning: identifying and developing new uses for existing drugs. Nat. Rev. Drug Discov. 3, 673–683. https://doi.org/10.1038/nrd1468.

Bujak, M., Dobaczewski, M., Chatila, K., Mendoza, L.H., Li, N., Reddy, A., Frangogiannis, N.G., 2008. Interleukin-1 receptor type I signaling critically regulates infarct healing and cardiac remodeling. Am. J. Pathol. 173, 57–67. https://doi.org/10.2353/ajpath.2008.070974.

Chow, E.J., Aplenc, R., Vrooman, L.M., Doody, D.R., Huang, Y.-S.V., Aggarwal, S., Armenian, S.H., Baker, K.S., Bhatia, S., Constine, L.S., Freyer, D.R., Kopp, L.M., Leisenring, W.M., Asselin, B.L., Schwartz, C.L., Lipshultz, S.E., 2022. Late health outcomes after dexrazoxane treatment: a report from the Children's Oncology Group. Cancer 128, 788–796. https://doi.org/10.1002/cncr.33974.

Damiani, R.M., Moura, D.J., Viau, C.M., Caceres, R.A., Henriques, J.A., Saffi, J., 2016. Pathways of cardiac toxicity: comparison between chemotherapeutic drugs doxorubicin and mitoxantrone. Arch. Toxicol. 90, 2063–2076. https://doi.org/10.1007/s00204-016-1759-y.

Duan, Q., Reid, S.P., Clark, N.R., Wang, Z., Fernandez, N.F., Rouillard, A.D., Readhead, B., Tritsch, S.R., Hodos, R., Hafner, M., Niepel, M., 2016. L1000CDS2: LINCS L1000 characteristic direction signatures search engine. NPJ Syst. Biol. Appl. 2, 16015 https://doi.org/10.1038/npjsba.2016.15.

Edgar, R., Domrachev, M., Lash, A.E., 2002. Gene Expression Omnibus: NCBI gene expression and hybridization array data repository. Nucleic Acids Res. 30, 207–210. https://doi.org/10.1093/nar/30.1.207.

Felker, G.M., Thompson, R.E., Hare, J.M., Hruban, R.H., Clemetson, D.E., Howard, D.L., Baughman, K.L., Kasper, E.K., 2000. Underlying causes and long-term survival in patients with initially unexplained cardiomyopathy. N. Engl. J. Med. 342, 1077–1084. https://doi.org/10.1056/nejm200004133421502.

Ghasemi, K., Vaseghi, G., Mansourian, M., 2021. Pharmacological interventions for preventing anthracycline-induced clinical and subclinical cardiotoxicity: a network meta-analysis of metastatic breast cancer. J. Oncol. Pharm. Pract. 27, 414–427. https://doi.org/10.1177/1078155220965674.

Gupta, S.K., Garg, A., Bär, C., Chatterjee, S., Foinquinos, A., Milting, H., Streckfuß-Bömeke, K., Fiedler, J., Thum, T., 2018. Quaking inhibits doxorubicin-mediated cardiotoxicity through regulation of cardiac circular RNA expression. Circ. Res. 122, 246–254. https://doi.org/10.1161/circresaha.117.311335.

Izawa-Ishizawa, Y., Imanishi, M., Zamami, Y., Toya, H., Nagao, T., Morishita, M., Tsuneyama, K., Horinouchi, Y., Kihira, Y., Takechi, K., Ikeda, Y., Tsuchiya, K., Yoshizumi, M., Tamaki, T., Ishizawa, K., 2019. Development of a novel aortic dissection mouse model and evaluation of drug efficacy using in-vivo assays and database analyses. J. Hypertens. 37, 73–83. https://doi.org/10.1097/hjh.0000000000001898.

Jiao, Y., Li, Y., Zhang, J., Zhang, S., Zha, Y., Wang, J., 2022. RRM2 alleviates doxorubicin-induced cardiotoxicity through the AKT/mTOR signaling pathway. Biomolecules 12, 299. https://doi.org/10.3390/biom12020299.

Keenan, A.B., Jenkins, S.L., Jagodnik, K.M., Koplev, S., He, E., Torre, D., Wang, Z., Dohlman, A.B., Silverstein, M.C., Lachmann, A., Kuleshov, M.V., Ma'ayan, A., Stathias, V., Terryn, R., Cooper, D., Forlin, M., Koleti, A., Vidovic, D., Chung, C., Schürer, S.C., Vasiliauskas, J., Pilarczyk, M., Shamsaei, B., Fazel, M., Ren, Y., Niu, W., Clark, N.A., White, S., Mahi, N., Zhang, L., Kouril, M., Reichard, J.F., Sivaganesan, S., Medvedovic, M., Meller, J., Koch, R.J., Birtwistle, M.R., Iyengar, R., Sobie, E.A., Azeloglu, E.U., Kaye, J., Osterloh, J., Haston, K., Kalra, J., Finkbiener, S., Li, J., Milani, P., Adam, M., Escalante-Chong, R., Sachs, K., Lenail, A., Ramamoorthy, D., Fraenkel, E., Daigle, G., Hussain, U., Coye, A., Rothstein, J., Sareen, D., Ornelas, L., Banuelos, M., Mandefro, B., Ho, R., Svendsen, C.N., Lim, R. G., Stocksdale, J., Casale, M.S., Thompson, T.G., Wu, J., Thompson, L.M., Dardov, V., Venkatraman, V., Matlock, A., Van Eyk, J.E., Jaffe, J.D., Papanastasiou, M., Subramanian, A., Golub, T.R., Erickson, S.D., Fallahi-Sichani, M., Hafner, M., Gray, N.S., Lin, J.R., Mills, C.E., Muhlich, J.L., Niepel, M., Shamu, C.E., Williams, E. H., Wrobel, D., Sorger, P.K., Heiser, L.M., Gray, J.W., Korkola, J.E., Mills, G.B., LaBarge, M., Feiler, H.S., Dane, M.A., Bucher, E., Nederlof, M., Sudar, D., Gross, S., Kilburn, D.F., Smith, R., Devlin, K., Margolis, R., Derr, L., Lee, A., Pillai, A., 2018. The library of integrated network-based cellular signatures NIH program: system-level cataloging of human cells response to perturbations. Cell Syst 6, 13–24. https://doi.org/10.1016/j.cels.2017.11.001.

Koleini, N., Kardami, E., 2017. Autophagy and mitophagy in the context of doxorubicin-induced cardiotoxicity. Oncotarget 8, 6663–6680. https://doi.org/10.18632/oncotarget.16944.

Kytö, V., Sipilä, J., Rautava, P., 2013. The effects of gender and age on occurrence of clinically suspected myocarditis in adulthood. Heart 99, 1681–1684. https://doi.org/10.1136/heartjnl-2013-304449.

Li, C., Xia, J., Deng, J., Jiang, J., 2008. A comparison of measures of disproportionality for signal detection on adverse drug reaction spontaneous reporting database of

Guangdong province in China. Pharmacoepidemiol. Drug Saf. 17, 593–600. https://doi.org/10.1002/pds.1601.
Lipshultz, S.E., Franco, V.I., Sallan, S.E., Adamson, P.C., Steiner, R.K., Swain, S.M., Gligirov, J., Minotti, G., 2014. Dexrazoxane for reducing anthracycline-related cardiotoxicity in children with cancer: an update of the evidence. Prog. Pediatr. Cardiol. 36, 39–49. https://doi.org/10.1016/j.ppedcard.2014.09.007.
McGowan, J.V., Chung, R., Maulik, A., Piotrowska, I., Walker, J.M., Yellon, D.M., 2017. Anthracycline chemotherapy and cardiotoxicity. Cardiovasc. Drugs Ther. 31, 63–75. https://doi.org/10.1007/s10557-016-6711-0.
Mustafa, H.N., Hegazy, G.A., El-Awdan, S.A., AbdelBaset, M., 2017. Protective role of CoQ10 or L-carnitine on the integrity of the myocardium in doxorubicin induced toxicity. Tissue Cell 49, 410–426. https://doi.org/10.1016/j.tice.2017.03.007.
Nagashima, T., Shirakawa, H., Nakagawa, T., Kaneko, S., 2016. Prevention of antipsychotic-induced hyperglycaemia by vitamin D: a data mining prediction followed by experimental exploration of the molecular mechanism. Sci. Rep. 6, 26375 https://doi.org/10.1038/srep26375.
Nieminen, M.S., Brutsaert, D., Dickstein, K., Drexler, H., Follath, F., Harjola, V.-P., Hochadel, M., Komajda, M., Lassus, J., Lopez-Sendon, J.L., Ponikowski, P., 2006. EuroHeart Failure Survey II (EHFS II): a survey on hospitalized acute heart failure patients: description of population. Eur. Heart J. 27, 2725–2736. https://doi.org/10.1093/eurheartj/ehl193.
Okada, N., Niimura, T., Zamami, Y., Hamano, H., Ishida, S., Goda, M., Takechi, K., Chuma, M., Imanishi, M., Ishizawa, K., 2019. Pharmacovigilance evaluation of the relationship between impaired glucose metabolism and BCR-ABL inhibitor use by using an adverse drug event reporting database. Cancer Med. 8, 174–181. https://doi.org/10.1002/cam4.1920.
Oltvai, Z.N., Milliman, C.L., Korsmeyer, S.J., 1993. Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programmed cell death. Cell 74, 609–619. https://doi.org/10.1016/0092-8674(93)90509-o.
Reichardt, P., Tabone, M.D., Mora, J., Morland, B., Jones, R.L., 2018. Risk-benefit of dexrazoxane for preventing anthracycline-related cardiotoxicity: re-evaluating the European labeling. Future Oncol. 14, 2663–2676. https://doi.org/10.2217/fon-2018-0210.
Salzer, W.L., Devidas, M., Carroll, W.L., Winick, N., Pullen, J., Hunger, S.P., Camitta, B. A., 2010. Long-term results of the pediatric oncology group studies for childhood acute lymphoblastic leukemia 1984–2001: a report from the children's oncology group. Leukemia 24, 355–370. https://doi.org/10.1038/leu.2009.261.
Schwartz, C.L., Constine, L.S., Villaluna, D., London, W.B., Hutchison, R.E., Sposto, R., Lipshultz, S.E., Turner, C.S., deAlarcon, P.A., Chauvenet, A., 2009. A risk-adapted, response-based approach using ABVE-PC for children and adolescents with intermediate-and high-risk Hodgkin lymphoma: the results of P9425. Blood 114, 2051–2059. https://doi.org/10.1182/blood-2008-10-184143.
Sheibani, M., Faghir-Ghanesefat, H., Azizi, Y., Mokhtari, T., Yousefi-Manesh, H., Badkoubeh, R.S., Emami, A.H., Dehpour, A.R., 2021. Anti-inflammatory and antioxidative effects of sumatriptan against doxorubicin-induced cardiotoxicity in rat. Acta Med. Iran. 59, 406–415. https://doi.org/10.18502/acta.v59i7.7020.
Sheibani, M., Nezamoleslami, S., Faghir-Ghanesefat, H., Emami, A.H., Dehpour, A.R., 2020. Cardioprotective effects of dapsone against doxorubicin-induced cardiotoxicity in rats. Cancer Chemother. Pharmacol. 85, 563–571. https://doi.org/10.1007/s00280-019-04019-6.
Songbo, M., Lang, H., Xinyong, C., Bin, X., Ping, Z., Liang, S., 2019. Oxidative stress injury in doxorubicin-induced cardiotoxicity. Toxicol. Lett. 307, 41–48. https://doi.org/10.1016/j.toxlet.2019.02.013.
Swain, S.M., Whaley, F.S., Gerber, M.C., Weisberg, S., York, M., Spicer, D., Jones, S.E., Wadler, S., Desai, A., Vogel, C., Speyer, J., Mittelman, A., Reddy, S., Pendergrass, K., Velez-Garcia, E., Ewer, M.S., Bianchine, J.R., Gams, R.A., 1997. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J. Clin. Oncol. 15, 1318–1332. https://doi.org/10.1200/jco.1997.15.4.1318.
Tacar, O., Sriamornsak, P., Dass, C.R., 2013. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J. Pharm. Pharmacol. 65, 157–170. https://doi.org/10.1111/j.2042-7158.2012.01567.x.
Tadokoro, T., Ikeda, M., Ide, T., Deguchi, H., Ikeda, S., Okabe, K., Ishikita, A., Matsushima, S., Koumura, T., Yamada, K.-i., Imai, H., Tsutsui, H., 2020. Mitochondria-dependent ferroptosis plays a pivotal role in doxorubicin cardiotoxicity. JCI Insight 5, e132747. https://doi.org/10.1172/jci.insight.132747.
Tanaka, R., Umemura, M., Narikawa, M., Hikichi, M., Osaw, K., Fujita, T., Yokoyama, U., Ishigami, T., Tamura, K., Ishikawa, Y., 2020. Reactive fibrosis precedes doxorubicin-induced heart failure through sterile inflammation. ESC Heart Fail 7, 588–603. https://doi.org/10.1002/ehf2.12616.
Tebbi, C.K., London, W.B., Friedman, D., Villaluna, D., De Alarcon, P.A., Constine, L.S., Mendenhall, N.P., Sposto, R., Chauvenet, A., Schwartz, C.L., 2007. Dexrazoxane-associated risk for acute myeloid leukemia/myelodysplastic syndrome and other secondary malignancies in pediatric Hodgkin's disease. J. Clin. Oncol. 25, 493–500. https://doi.org/10.1200/JCO.2005.02.3879.
Tsutamoto, T., Hisanaga, T., Wada, A., Maeda, K., Ohnishi, M., Fukai, D., Mabuchi, N., Sawaki, M., Kinoshita, M., 1998. Interleukin-6 spillover in the peripheral circulation increases with the severity of heart failure, and the high plasma level of interleukin-6 is an important prognostic predictor in patients with congestive heart failure. J. Am. Coll. Cardiol. 31, 391–398. https://doi.org/10.1016/s0735-1097(97)00494-4.
Yu, X., Ruan, Y., Huang, X., Dou, L., Lan, M., Cui, J., Chen, B., Gong, H., Wang, Q., Yan, M., Sun, S., Qiu, Q., Zhang, X., Man, Y., Tang, W., Li, J., Shen, T., 2020. Dexrazoxane ameliorates doxorubicin-induced cardiotoxicity by inhibiting both apoptosis and necroptosis in cardiomyocytes. Biochem. Biophys. Res. Commun. 523, 140–146. https://doi.org/10.1016/j.bbrc.2019.12.027.
Yuan, Y.P., Ma, Z.G., Zhang, X., Xu, S.-C., Zeng, X.-F., Yang, Z., Deng, W., Tang, Q.-Z., 2018. CTRP3 protected against doxorubicin-induced cardiac dysfunction, inflammation and cell death via activation of Sirt1. J. Mol. Cell. Cardiol. 114, 38–47. https://doi.org/10.1016/j.yjmcc.2017.10.008.
Zamami, Y., Niimura, T., Koyama, T., Shigemi, Y., Izawa-Ishizawa, Y., Morita, M., Ohshima, A., Harada, K., Imai, T., Hagiwara, H., Okada, N., Goda, M., Takechi, K., Chuma, M., Kondo, Y., Tsuchiya, K., Hinotsu, S., Kano, M.R., Ishizawa, K., 2019a. Search for therapeutic agents for cardiac arrest using a drug discovery tool and large-scale medical information database. Front. Pharmacol. 10, 1257. https://doi.org/10.3389/fphar.2019.01257.
Zamami, Y., Niimura, T., Okada, N., Koyama, T., Fukushima, K., Izawa-Ishizawa, Y., Ishizawa, K., 2019b. Factors associated with immune checkpoint inhibitor-related myocarditis. JAMA Oncol. 5, 1635–1637. https://doi.org/10.1001/jamaoncol.2019.3113.
Zhao, L., Qi, Y., Xu, L., Tao, X., Han, X., Yin, L., Peng, J., 2018a. MicroRNA-140-5p aggravates doxorubicin-induced cardiotoxicity by promoting myocardial oxidative stress via targeting Nrf2 and Sirt2. Redox Biol. 15, 284–296. https://doi.org/10.1016/j.redox.2017.12.013.
Zhao, L., Tao, X., Qi, Y., Xu, L., Yin, L., Peng, J., 2018b. Protective effect of dioscin against doxorubicin-induced cardiotoxicity via adjusting microRNA-140-5p-mediated myocardial oxidative stress. Redox Biol. 16, 189–198. https://doi.org/10.1016/j.redox.2018.02.026.
Zhu, J., Zhang, J., Xiang, D., Zhang, Z., Wu, M., Zhu, S., Zhang, R., Han, W., 2010. Recombinant human interleukin-1 receptor antagonist protects mice against acute doxorubicin-induced cardiotoxicity. Eur. J. Pharmacol. 643, 247–253. https://doi.org/10.1016/j.ejphar.2010.06.024.
Zhu, J., Zhang, J., Zhang, L., Du, R., Xiang, D., Wu, M., Zhang, R., Han, W., 2011. Interleukin-1 signaling mediates acute doxorubicin-induced cardiotoxicity. Biomed. Pharmacother. 65, 481–485. https://doi.org/10.1016/j.biopha.2011.06.005.