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The Role of Cardiac MRI in Animal Models of Cardiotoxicity: Hopes and Challenges

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Abstract

Animal models of chemotherapy-induced cardiotoxicity have been instrumental in understanding the underlying mechanisms of the disease. The use of cardiac magnetic resonance imaging (CMR) and nuclear magnetic resonance imaging (NMR) in preclinical models allow the non-invasive study of subclinical pathophysiological processes that influence cardiac function and establish imaging parameters that can be adopted into clinical practice to predict cardiovascular outcomes. Given the rising population of cancer survivors and the current lack of effective therapies for the management of cardiotoxicity, research combining clinically relevant animal models and non-invasive cardiac imaging remains essential to improve methods to monitor, predict, and treat cardiovascular adverse events. This comprehensive review summarizes the lessons learned from animal models of cardiotoxicity employing CMR and tissue characterization techniques and discusses the ongoing challenges and hopes for the future.

Keywords

Cardiotoxicity; Chemotherapy; Cardiac MRI; Nuclear magnetic resonance imaging Animal models

INTRODUCTION

The remarkable growth of the cardio-oncology field reflects the increased cardiovascular disease burden of patients undergoing cancer treatment and the rising population of cancer survivors. In this field, cardiologists, oncologists, and investigators in cancer and cardiovascular biology have joined efforts to uncover novel ways to detect, prevent, and treat the cardiovascular off-target effects of cancer therapies.

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Anthracycline agents are the classic cancer therapy associated with dose-dependent myocellular injury leading to left ventricular (LV) dysfunction and heart failure. Investigators have made significant progress in the understanding of the underlying cellular and molecular mechanisms of cardiotoxicity. Two fundamental investigative tools have made such progress possible: 1) cardiovascular imaging, especially cardiac magnetic resonance (CMR), due to its ability to accurately assess LV function and subclinical tissue injury; and 2) preclinical animal models of cardiotoxicity which can study the subcellular and molecular mechanisms driving heart failure. During the past decade, investigators have used animal model studies that employ CMR imaging to identify and describe CMR-derived tissue characteristics that correlate with histopathological findings and their prognostic utility. The combined strength of employing animal models with CMR better allows for not only understanding cardiotoxic processes, but also testing new therapeutic strategies that can prevent, delay, or even reverse the effects of cardiotoxicity.

In this review, we will focus on the lessons learned from animal models that employ CMR techniques and discuss the role of state-of-the-art imaging techniques such as nuclear magnetic resonance imaging (NMR) for the study of cardiotoxicity. It is important to recognize that the vast majority of these studies are anthracycline-based. In the last few years, a plethora of novel targeted anti-cancer therapies have emerged and subsequent cardiovascular complications have been observed in patients. While there is currently a paucity of non-anthracycline animal models, we will illustrate several examples of how CMR could inform the progression of cardiovascular disease induced by these new agents. Furthermore, this comprehensive review also includes practical recommendations specific to CMR techniques and animal modeling that would be beneficial for both the clinician and bench researcher. It is our hope that this report bolsters the collaborative multidisciplinary efforts integral to identifying cardiotoxicity and lead to successful treatment of cardiotoxicity in the near future.

CMR IN THE CARDIOVASCULAR EVALUATION OF THE CANCER PATIENT

CMR has become a critical imaging tool to detect cardiac abnormalities among patients receiving cardiotoxic cancer therapies, especially anthracyclines[1, 2]. While echocardiography is the primary non-invasive imaging modality recommended to monitor cardiotoxicity [3], CMR provides increased accuracy and reproducibility for LVEF quantification due to its high spatial and temporal resolution [4]; such precision is crucial for the safe administration of cancer therapies to prevent and diagnose cardiotoxicity [3]. Importantly, global longitudinal LV strain can also detect early functional deterioration after initiation of cancer therapies and CMR-derived feature-tracking analysis is an accurate tool to directly quantify the myocardial fiber deformation[5]. Abnormalities of the RV may also occur concomitantly with LV dysfunction after cardiotoxic chemotherapy [1]; however, the frequency and prognostic value of RV dysfunction remains unclear. Nonetheless, clinically, it is recommended to obtain a quantitative assessment of the RV structure and function [3]. Beyond the evaluation of LV structure and function, a unique feature of CMR imaging is its ability to identify subclinical pathological changes of the myocardial tissue and the interstitial space by mapping techniques. Prolongation of T1 and T2 mapping relaxation times reflect myocardial tissue injury and edema, respectively, and extracellular volume

(ECV) — a metric derived from T1 maps acquired before and after gadolinium contrast — measures the expansion of the interstitial space and denotes interstitial fibrosis and/or edema. Recently, a novel CMR technique has emerged that is capable of measuring the size of cardiomyocytes by water lifetime measurements (τ_{ic}) [6], which can discriminate whether cardiomyocyte atrophy contributes to the expansion of the ECV [7–9]. These imaging biomarkers have provided important insights about complex myocellular and interstitial remodeling processes of anthracycline-mediated cardiotoxicity. However, challenges remain regarding the prognostic implications of non-invasive phenotyping methods due to the heterogeneity of risk factors in patients, lack of longitudinal follow-up, threshold of abnormal values and limited access to human myocardial samples. Table 2 summarizes the potential evolution of the changes observed in CMR-derived tissue characteristics. The combination of all four techniques provide insights about the specific cardiac remodeling process. After the receipt of anthracyclines, ubiquitous increases of T1 and ECV measurements have been described [10, 11], denoting unspecific myocardial injury (myocellular injury, edema, fibrosis) and expansion of the interstitial space respectively; therefore, τ_{ic} and T2 values can facilitate the identification of which process is responsible for such increase of the interstitial space: is it due to edema (increase T2), cardiomyocyte atrophy (decreased τ_{ic}), or interstitial fibrosis (normal or decrease T2 and normal or elevated τ_{ic})? (Table 2). For these reasons, for anthracycline animal models employing CMR, it is recommended to adopt imaging sequences to evaluated cardiac anatomy (typically includes dark-blood T1- and T2 -weighted imaging, steady-state free precession (SSFP) imaging in both long and short axial views), function (cine white-blood SSFP imaging in short-axis view for volumetrics and strain imaging with tags or feature-tracking quantification methods) and tissue characterization techniques (Native T1, T2 mapping, post-contrast T1-maps for ECV calculation, τ_{ic} measurements) if available.

MODELING CARDIOTOXICITY IN ANIMALS

Preclinical animal research is the cornerstone of the development of preventive and treatment strategies for cancer therapy-related cardiovascular disease. Unlike *in vitro* or *ex vivo* studies, *in vivo* animal studies can investigate the effects of cancer therapies on cardiac structure and function with the advantage of histopathological and biochemical analysis. In addition, there is an abundance of genetically modified rodent models that can isolate the contributions of specific molecules to the pathophysiology of cardiotoxicity. The translatability of basic science discoveries relies on the careful selection of the animal model that a) resembles clinical doses and treatment intervals of anti-cancer regimens; b) allows the use of cardiac imaging tools similar to those used in clinical practice; and c) allows for long-term study of cardiotoxicity, with specific features characteristic of the human condition. Importantly, animal models can assess the independent influence of risk factors and comorbidities, although these factors are often neglected in these models since animals typically initiate experimental trials with a relatively healthy myocardium. It is now recognized that cardiovascular risk factors such as age, obesity, tobacco use, sedentary life style, diet, reproductive status, and pre-existing comorbidities such as diabetes and hypertension may influence the establishment and progression of cardiovascular toxicities [12]. Moreover, it has been previously described that both patients and experimental animals

with cancer may experience cardiac wasting or atrophy [7, 13], suggesting that cancer itself may play a role in cardiotoxicity.

Special Considerations for CMR Imaging in Animal Models

Chemotherapy Doses and Route of Administration—An important challenge is the inconsistent use of acute vs. chronic dosing schemes (i.e. high one-time doses vs. multiple small doses of chemotherapy) that frequently does not resemble clinical dosing regimens and the failure to account for the potential interaction of other concurrent cardiotoxic anti-cancer therapies. In order to increase translatability, preclinical studies should use smaller repeated doses, administered at intervals that maintain a reasonable and practical overall experimental time (e.g. once every 1 or 2 weeks) and that results in a progressive development of myocardial injury. The extrapolation of doses for experimental purposes between species remains a pharmacological controversy. Estimating doses based only on body weight differences is not accurate. One practical method of extrapolating human doses to animals and vice versa is through an allometric scaling approach that incorporates body surface area to account for differences in metabolic rates between species [14]. A key point for dose scaling is that large animals tend to have lower metabolic rates and therefore require smaller doses based on body weight. Table 1 provides examples of human equivalent doses (HED) corresponding to Dox doses frequently used in murine models, which were calculated based on the dose conversion guide by Nair and Jacob [14]. The administration route of chemotherapies adds another layer of complexity. Intravenous administration is encouraged because subcutaneous, intramuscular, and intraperitoneal drug delivery can induce local inflammatory reactions and tissue damage that may further alter the drug distribution, confound results that are difficult to interpret in the clinical context. However, intravenous delivery in small animals may be technically difficult, especially for serial tail injections in mice due to extravasation and necrosis of the surrounding tissues. In recent studies, a longstanding methodology of intracoronary administration of Dox to induce heart failure has been adopted to study cardiotoxicity in sheep [15] and pig models [8]. While this technique may avoid systemic adverse effects and improve animal survival rates, the resulting high myocardial concentration of Dox limits the ability to extrapolate findings to a clinical setting.

Anesthesia—An important consideration for CMR imaging is the choice of anesthesia for accurate, reliable, and reproducible LV function assessments. Factors that will determine the type of anesthetic include impact on physiological parameters (heart rate, blood pressures, body temperature, and cardiac function), duration of effect, recovery time, and availability of MRI compatible equipment. The most commonly used anesthetic for cardiac imaging in experimental animals is inhaled isoflurane because of its minimal cardiosuppressant effects [16]. A critical advantage of inhaled isoflurane over injectable anesthetics is the ability to monitor and control the depth of anesthesia and heart rate during the procedure, as well as a rapid recovery time. Large animals, like non-human primates, often require initial anesthesia with ketamine (an N-Methyl-D-Aspartate receptor agonist) to avoid stress from handling while inhaled anesthesia reaches effect and is a widely used anesthetic in veterinary medicine due to its safety profile. Despite the fact that ketamine increases catecholamine levels, it remains a superior injectable anesthetic for imaging due to relatively mild

cardiosuppressant effects [17]. Of note, ketamine is often used in conjunction with xylazine to avoid muscle rigidity [18]. However, this strategy may result in hypotension, bradycardia, and can cause significant cardiodepression. For these reasons, ketamine-xylazine in combination should be avoided in cardiovascular imaging [17]. Finally, to ensure reproducibility and reliability of study results, investigators should consistently report the following: type and dose of anesthesia, duration of the imaging procedure, frequency/timing of imaging acquisition, and monitored vital signs, especially heart rate, during image acquisition.

MRI Hardware—MRI scanners have a range of magnetic field strengths that determine the signal-to-noise ratio and the image resolution. Field strengths between 4.7 and 9.4T are commonly used for imaging small animals, while clinical fields strengths from 1.5 to 3T are often used for large animals [17, 19]. Because high-field systems are not readily available at many institutions, there are established methodologies to conduct small animal CMR imaging on clinical 1.5 and 3.0T scanners (please refer to an excellent methods paper by Gilson and Kraitichamn ([20])).

PROGNOSTIC VALUE OF CARDIAC MRI: LESSONS LEARNED FROM ANIMAL MODELS OF CARDIOTOXICITY

Large Animal Models of Cardiotoxicity

Rabbit—The rabbit heart shares several phenotypic and functional similarities with human myocardium, including the prevalence of β -myosin heavy-chains isoform and a similar pattern of excitation-contraction coupling [21] and therefore, offer an ideal intermediate step between murine models and larger animals like dogs or monkeys at a reduced cost of acquisition and housing. Importantly, the rabbit myocardium can be imaged utilizing 3 Tesla MRIs, with similar protocols to those used clinically. Pioneering studies of cardiotoxicity using ex-vivo preparations of rabbit myocardial tissue and ^{31}P NMR demonstrated that exposure of isolated cells to Dox increased the calcium current, inhibited sarcoplasmic reticulum function, and reduced myocyte adenosine triphosphate levels (ATP) [22]. More recently, in a landmark study in New Zealand rabbits by Hong et al., cardiotoxicity was modeled by administration of relatively low doses of Dox (1 mg/kg IV, twice a week) for up to 16 weeks [23]. Serial CMR imaging demonstrated subclinical early increases in native T1 and ECV values which were correlated with histologic fibrosis and preceded LVEF declines. These significant early increases in ECV also coincided with the loss of normal myofibrils and an increase in intracellular vacuolization. This study demonstrated that CMR-derived tissue characterization techniques may be useful to detect early changes in the interstitial space that may be related to diffuse myocardial fibrosis and may predict the development of LV dysfunction.

Pig—A pig model of cardiotoxicity recently identified early CMR tissue characterization changes with histopathological correlates [8]. In addition to T1 mapping and ECV, this study employed T2 mapping to detect myocardial edema. Three to five doses of Dox (0.45mg/kg/dose) were administered via angiography-guided intra-coronary bi-weekly injections, and the animals were followed for up to 16 weeks with CMR acquired pre- and post- every week

after Dox initiation. A group of animals were sacrificed after receiving only 3 doses and another after the initial CMR that served as controls. Several important findings were derived from this model: first, the earliest parameter to exhibit changes after Dox administration was the prolongation of T2 relaxation times (6 weeks after initiation of chemotherapy). Interestingly at this time point, T1 maps, ECV, or LVEF were not changed, which suggests the absence of interstitial edema. Nonetheless, histopathological examinations revealed an association with cardiomyocyte vacuolization denoting intra-cardiac water. In addition, the T2 changes which preceded increases in T1 mapping relaxation times and ECV were associated with histopathological myocardial fibrosis and LVEF declines. Notably, the cessation of Dox treatment upon detection of T2 mapping elevations prevented the progression of LV dysfunction and reversed cardiomyocyte vacuolizations. Although this study highlights the key role of inflammation as a potential therapeutic target to prevent the progression of LV dysfunction, some methodological features of this particular pig model may limit the extrapolation to clinical practice. Specifically, intracoronary administration of Dox can be challenging even in large animal models, it may accelerate cardiotoxic effects and it does not take into account the systemic contribution of other organs to cardiotoxicity. Moreover, the narrow window for intervention given the 2–3 week interval between T2 elevation and the development of LVEF declines and the need for serial frequent CMR imaging required to detect these changes limits the translatability of this model.

Sheep—One preclinical sheep model of cardiotoxicity revealed an association between delayed gadolinium uptake was correlated with decreases in LVEF in response to Dox exposure [24]. This model employed intracoronary injections of high doses of Dox (1 mg/kg bi-weekly), with CMR acquired before and 6 weeks after the final Dox dose. Histopathology revealed replacement fibrosis of patchy distribution, inflammation, and cardiomyocyte apoptosis. No significant changes in LV end-diastolic volume (EDV) were found in this model. More recently, other studies using similar sheep model of Dox-induced heart failure and CMR also confirmed a relationship between late gadolinium enhancement (LGE) and myocardial fibrosis [15]. While LGE and ECV can both measure myocardial fibrosis, it is important to recognize that LGE is useful only for the visualization and measurement of the area of focal dense replacement deposition of collagen. ECV, on the other hand, allows the quantification of diffuse interstitial myocardial fibrosis [25].

Non-human Primate—The pre-clinical African Green monkey model of cardiotoxicity [26] has elucidated important mechanistic information about CMR-derived ECV expansion changes after cancer therapy [8, 25, 27]. While ECV has been traditionally associated with diffuse myocardial fibrosis, it is important to recognize that ECV represents the ratio of the volume of the interstitial space and the total volume of myocardial tissue [7]; therefore, ECV increases may be attributed to one or more of the following causes: 1) an expansion of the extracellular space due to increased interstitial fibrosis [27] or edema [8] as described in mouse and pig models, respectively; or 2) a reduction of the total myocardial tissue volume mainly composed of cardiomyocytes [28]. Decrease in total myocardial tissue volume can occur either due to a decrease in cardiomyocyte size (atrophy) [7] or a reduction in the number of cardiomyocytes due to apoptosis or another cell death mechanism (Figure 1).

Female premenopausal monkeys aged 12–14 years old (equivalent to a ~40 year old woman) received a total cumulative dose of 240 mg/m² Dox via a vascular port and fractionated over five doses every 17 ± 3.5 days similar to adjuvant therapy for breast cancer [29]. LVEF, ECV, and LV total myocardial tissue volume were acquired with CMR before initiation of therapy and 15 weeks after the final Dox dose. Animals averaged an LVEF declined by 25% absolute percentage points consistent with CTRCD [3], LV myocardial mass increased by ~40%, and ECV increased by ~9%. Histopathologic analyses revealed that the increase in ECV was mainly due to diffuse interstitial fibrosis (interstitial factor) concomitant with cardiomyocyte loss (myocardial factors). The remaining cardiomyocytes exhibited hypertrophy which corresponded with the increases in the total cell volume and LV mass by CMR. These results suggest ECV expansion in this model is associated with interstitial factors, rather than a decrease in the LV myocardial tissue volume. These findings conflict with a recent landmark human study in which a unique non-invasive CMR technique known as intracellular water lifetime (τ_{ic}) measurement was used to assess cardiomyocyte size in breast cancer patients undergoing anthracycline therapy. The study demonstrated a decrease in LV myocardial mass that was observed approximately 45 weeks after Dox initiation (equivalent to 15 weeks in the monkey study) and was also associated with a reduction in cardiomyocyte size, suggesting that cardiomyocyte atrophy also contributes to the expansion of ECV in humans [7]. The reasons for such discrepancies remain unclear. One plausible explanation is the fact that these monkeys did not have cancer; prior studies have shown that both patients and experimental animals with cancer experience cardiac wasting or atrophy [13]. Therefore, the effect of cancer on the heart, even before initiation of chemotherapy, may define the cardiac remodeling process and long-term compensatory mechanisms. Alternatively, the monkeys in this model may have experienced cardiomyocyte atrophy at earlier time points.

Small Animal Models of Cardiotoxicity

Rat—Sprague-Dawley rats have been used to study early increases in CMR measures of gadolinium signal intensity within the LV and its capacity to forecast the development of LV dysfunction [30]. Dox was administered IV (1.5 to 2.5 mg/kg/week for up to 10 weeks) and bi-weekly CMR evaluations using a 1.5 Tesla scanner with a small phase-array surface coil. Changes in gadolinium signal intensity were associated with histopathological cardiomyocyte vacuolization. These findings were corroborated a few years later by another rat study (using Wistar rats), which concluded that gadolinium enhancement predicted LVEF reduction [31]. In fact, myocardial injury occurred several weeks prior to increases in gadolinium enhancement signal, as demonstrated by histological cardiomyocyte degeneration, inflammatory cell infiltration, followed by edema and diffuse myocardial fibrosis.

Mouse—Mice were used in a landmark study to assess the prognostic utility of CMR-derived tissue characteristics [27]. In an effort to mimic chemotherapy dosing schemes used to treat breast cancer and lymphoma, mice received 5 mg/kg/week by subcutaneous continuous infusion for 5 weeks and then were followed with CMR for 5, 10, and 20 weeks after completion of Dox therapy. At each imaging time point, a subset of the animals underwent necropsy to obtain histopathological correlates. Using tissue characterization

endpoints, this study found a 34% increase in ECV after 10 weeks post-Dox which corresponded with LVEF declines. T2 prolongation (detected at 5 weeks) preceded the ECV expansion. Both elevations of ECV and T2 correlated with histological measurements of interstitial myocardial fibrosis and cardiac water. Notably, this study demonstrated that myocardial fibrosis and edema (ECV and T2 elevations, respectively) and not LVEF declines predicted mortality due to cardiotoxicity. These observations underscore the contribution of inflammation and myocardial fibrosis to the development of LV dysfunction and heart failure induced by anthracyclines. A synopsis of the animal model studies that are discussed in this review are listed in Table 3.

Nuclear Magnetic Resonance and Mass Spectrometry Techniques in Cardiotoxicity Models

The molecular and cellular mechanisms of anthracycline cardiotoxicity have been investigated since the 1980s in small animal models. Seminal studies using Langendorff-perfused rat and rabbit hearts of chronically treated animals using ^{31}P NMR showed that adenosine triphosphate (ATP) consumption was significantly altered after Dox administration [32, 33]. Keller et al. demonstrated important metabolic differences between acute and chronic cardiomyopathy in rabbits treated with Dox. After 5 days of Dox treatment (5 mg/kg/day), the ATP-to-phosphocreatine (PCr) ratio increased compared to those treated for 7–10 weeks at lower doses (1.2 or 1.5 mg/kg twice a week) without significant differences in cardiac function, suggesting that mechanisms for chronic cardiomyopathy may be independent from metabolism pathways [34]. Subsequent studies using NMR have similarly showed decreased creatine kinase metabolism and impaired mitochondrial oxidative phosphorylation [34] supporting the hypothesis that creatine kinase impairment in ATP production contributes to cardiotoxicity during active treatment. Decreased creatinine kinase energetics measured with ^{31}P NMR have been shown to precede LVEF decreases and abnormal LV diastolic function in mice [35]. Furthermore, murine *in vitro* and *in vivo* models have shown that supplementation of normal creatinine kinase may be protective towards preserving LVEF after anthracycline-based chemotherapy [36].

^1H NMR is another promising modality used to study non-invasive markers in Dox-induced cardiotoxicity and is capable of measuring cardiac metabolites that include acetate, succinate, lactate, glutamine, and alanine [37]. One murine Dox model using quadrupole time-of-flight mass spectrometry demonstrated that these metabolite levels are increased as early as 1 day after initiation of chemotherapy and may be predictive of cardiotoxicity [38]. A summary of animal models of Dox-mediated cardiometabolic changes measured by NMR is shown in Table 4.

UTILITY OF CMR IMAGING BEYOND ANTHRACYCLINES

To date, the vast majority of preclinical studies have been conducted to study cardiotoxicity induced by anthracyclines, and of those, only a few studies have employed CMR techniques. There are virtually no reports of CMR imaging in non-anthracycline animal models. However, many clinical studies have used CMR to assess the cardiovascular effects of newer anti-cancer agents such as human epidermal growth factor receptor-2 inhibitors (HER-2) and checkpoint inhibitors.

Trastuzumab is a humanized monoclonal antibody that inhibits HER-2 and activates cytotoxic signaling in cancer cells, but in cardiomyocytes, it may interfere with fundamental contraction-relaxation mechanisms [39]. Importantly, administration of trastuzumab alone may be partially reversible [40], and early detection and treatment with cardioprotective medications (e.g., beta-blockers and angiotensin-converting enzyme inhibitors) have proven to be beneficial [41]. Using CMR, Fallah-Rad et al. [42] observed that the earliest changes after trastuzumab treatment were detected in peak global longitudinal strain (at 3 months) followed by LVEF decrease at 6 months. Recently, Gong et al. showed that feature tracking CMR-derived systolic strain and LVEF may be able to identify early signs of myocardial injury after trastuzumab injury [43].

Multiple case studies have reported fulminant myocarditis in patients receiving immune checkpoint inhibitors, especially those treated with combination therapies of ipilimumab (CTLA-4 inhibitor) and nivolumab (program death [PD]-1 inhibitor) [44, 45]. CMR is the recommended imaging modality for the diagnosis of myocarditis in these patients [1, 44]. Because inflammation and tissue injury facilitates the uptake of contrast agents, myocarditis presents with a rim-like pattern of LGE in the septal wall or patchy subepicardial LGE in the LV lateral wall [1]. While these techniques are useful for the diagnosis of myocarditis, their prognostic values remain to be determined.

In animal models, there are a few reports that have used murine models of non-anthracyclines cardiotoxicities. One study in rats showed that chronic administration with erlotinib (an epidermal growth factor receptor [EGFR] inhibitor) is associated with substance-P-mediated hypomagnesemia and LV diastolic dysfunction with moderate decreases in LVEF and LV posterior wall thinning [46]. A recent study in mice demonstrated that pembrolizumab (PD-1 inhibitor) when used in combination with trastuzumab increases cardiac production of interleukin-1 β and leukotrienes [47]. Other studies have also showed that sunitinib (a multitargeted anticancer agent) is associated with mitochondrial dysfunction and cardiomyocyte apoptosis and LV dysfunction [48]. These observations echoed clinical findings of the high incidence of LV dysfunction in patients treated with sunitinib, especially among those previously diagnosed with hypertension [49]. Lastly, myocarditis and heart failure have been described in PD-1-deficient BALB/c and MRL mice that exhibit fatal myocarditis and multi-organ inflammation; notably, myocarditis development is progressive in these mice and represent a suitable model for checkpoint inhibitor cardiotoxicities [50].

CHALLENGES, TRANSLATABILITY, AND FUTURE DIRECTIONS OF PRECLINICAL MODELS

While CMR-tissue characterization techniques have great potential in the risk stratification and early diagnosis of cardiotoxicity, several challenges remain. First, from both the clinical and animal research perspective, the interpretation of these studies is limited due to the lack of standardized protocols, magnetic field strengths, and variability across different vendors. It is yet to be determined whether the identification of these imaging biomarkers in small animals, typically acquired using 7 and 9.4 Tesla scanners, is feasible in clinical 1.5 and 3

Tesla scanners used in large animals and humans. There is a pressing need for standardized acquisition sequences and setting thresholds of abnormal values in humans.

Currently, anthracyclines therapy remains the most investigated cardiotoxic chemotherapy regimen. However, the range of cardiovascular abnormalities seen with other cancer therapies beyond anthracyclines remain understudied, and therefore, their underlying molecular causes remain poorly understood. Moreover, combination of therapies (for example, anthracyclines and trastuzumab, an effective anti-cancer regimen) can exacerbate cardiac toxicities [51, 52]. In fact, it is well documented that trastuzumab alone causes cardiac dysfunction [53]. Although the use of healthy animals fails to simulate a clinical scenario where human subjects often exhibit pre-existing cardiovascular comorbidities such as hypertension, coronary artery disease, and diabetes, there is a wide variety of small and large animal models of pressure and volume overload, metabolic dysfunction, and transgenic animals that allow the individual modeling of these critical features. Importantly, CMR mapping techniques are useful to distinguish such baseline myocardial abnormalities. Beyond anthracyclines, it is also critical to develop scientific models to thoroughly evaluate adverse cardiovascular effects of newer effective cancer therapies, such as vascular endothelial growth factor (VEGF) inhibitors, tyrosine kinase inhibitors (TKIs), and immune checkpoint inhibitors [40, 54, 55]. Thus, for successful clinical translation, the pathophysiologic changes of animal models should resemble those that occur in humans. In summary, further work is needed to demonstrate how early detection of subclinical CMR markers may lead to therapeutic interventions for the prevention of heart failure due to cardiotoxicity.

SUMMARY

In this review, we critically discuss valuable mechanistic information provided by animal models of cardiotoxicity that have employed CMR imaging to improve the understanding of cardiac tissue remodeling induced by cardiotoxic cancer therapies. Although each animal model described here has advantages and limitations, collectively, the lessons learned from these scientific communications offer new windows of opportunity for additional research to address cardiotoxicities in the growing cardio-oncology population. Importantly, this review offers fundamental knowledge to incorporate CMR imaging strategies in cardio-oncology clinical trials.

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Abbreviations

ATP	adenosine triphosphate
CMR	cardiac magnetic resonance
CTRCD	cancer therapy-related cardiac dysfunction
Dox	doxorubicin

ECV	extracellular volume
EDV	end-diastolic volume
LGE	late gadolinium enhancement
LV	left ventricular
LVEF	left ventricular ejection fraction
RVEF	right ventricular ejection fraction
NMR	nuclear magnetic resonance imaging
τ_{ic}	intracellular water lifetime
TKI	tyrosine kinase inhibitor
VEGF	vascular endothelial growth factor

REFERENCES

1. Jordan JH, et al., Cardiovascular Magnetic Resonance in the Oncology Patient. *JACC Cardiovasc Imaging*, 2018 11(8): p. 1150–1172. [PubMed: 30092971]
2. Vasu S and Hundley WG, Understanding cardiovascular injury after treatment for cancer: an overview of current uses and future directions of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*, 2013 15: p. 66. [PubMed: 23902649]
3. Plana JC, et al., Expert consensus for multimodality imaging evaluation of adult patients during and after cancer therapy: a report from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*, 2014 15(10): p. 1063–93. [PubMed: 25239940]
4. Bellenger NG, et al., Reduction in sample size for studies of remodeling in heart failure by the use of cardiovascular magnetic resonance. *J Cardiovasc Magn Reson*, 2000 2(4): p. 271–8. [PubMed: 11545126]
5. Muser D, et al., Clinical applications of feature-tracking cardiac magnetic resonance imaging. *World J Cardiol*, 2018 10(11): p. 210–221. [PubMed: 30510638]
6. Coelho-Filho OR, et al., Quantification of cardiomyocyte hypertrophy by cardiac magnetic resonance: implications for early cardiac remodeling. *Circulation*, 2013 128(11): p. 1225–33. [PubMed: 23912910]
7. Ferreira de Souza T, et al., Anthracycline Therapy Is Associated With Cardiomyocyte Atrophy and Preclinical Manifestations of Heart Disease. *JACC Cardiovasc Imaging*, 2018 11(8): p. 1045–1055. [PubMed: 30092965]
8. Galan-Arriola C, et al., Serial Magnetic Resonance Imaging to Identify Early Stages of Anthracycline-Induced Cardiotoxicity. *J Am Coll Cardiol*, 2019 73(7): p. 779–791. [PubMed: 30784671]
9. Hundley WG and Jordan JH, When Left Ventricular Extracellular Volume Fraction Changes After Anthracyclines: Is it Due to a Change in the Numerator, Denominator, or Both? *JACC Cardiovasc Imaging*, 2018 11(8): p. 1056–1058. [PubMed: 30092966]
10. Melendez GC, et al., Progressive 3-Month Increase in LV Myocardial ECV After Anthracycline-Based Chemotherapy. *JACC Cardiovasc Imaging*, 2016.
11. Jordan JH, et al., Anthracycline-Associated T1 Mapping Characteristics Are Elevated Independent of the Presence of Cardiovascular Comorbidities in Cancer Survivors. *Circ Cardiovasc Imaging*, 2016 9(8).

12. Mehta LS, et al., Cardiovascular Disease and Breast Cancer: Where These Entities Intersect: A Scientific Statement From the American Heart Association. *Circulation*, 2018 137(8): p. e30–e66. [PubMed: 29437116]
13. Willis MS, et al., Doxorubicin Exposure Causes Subacute Cardiac Atrophy Dependent on the Striated Muscle-Specific Ubiquitin Ligase MuRF1. *Circ Heart Fail*, 2019 12(3): p. e005234. [PubMed: 30871347]
14. Nair AB and Jacob S, A simple practice guide for dose conversion between animals and human. *J Basic Clin Pharm*, 2016 7(2): p. 27–31. [PubMed: 27057123]
15. Carbone A, et al., Dietary omega-3 supplementation exacerbates left ventricular dysfunction in an ovine model of anthracycline-induced cardiotoxicity. *J Card Fail*, 2012 18(6): p. 502–11. [PubMed: 22633309]
16. Janssen BJ, et al., Effects of anesthetics on systemic hemodynamics in mice. *Am J Physiol Heart Circ Physiol*, 2004 287(4): p. H1618–24. [PubMed: 15155266]
17. Lindsey ML, et al., Guidelines for measuring cardiac physiology in mice. *Am J Physiol Heart Circ Physiol*, 2018 314(4): p. H733–h752. [PubMed: 29351456]
18. Hildebrandt IJ, Su H, and Weber WA, Anesthesia and other considerations for in vivo imaging of small animals. *Ilar j*, 2008 49(1): p. 17–26. [PubMed: 18172330]
19. Price AN, et al., Cardiovascular magnetic resonance imaging in experimental models. *Open Cardiovasc Med J*, 2010 4: p. 278–92. [PubMed: 21331311]
20. Gilson WD and Kraitchman DL, Cardiac magnetic resonance imaging in small rodents using clinical 1.5 T and 3.0 T scanners. *Methods*, 2007 43(1): p. 35–45. [PubMed: 17720562]
21. Hasenfuss G, Animal models of human cardiovascular disease, heart failure and hypertrophy. *Cardiovasc Res*, 1998 39(1): p. 60–76. [PubMed: 9764190]
22. Kawabata H, Ryamoto T, and Ishikawa K, Effect of beta-blocker on metabolism and contraction of doxorubicin-induced cardiotoxicity in the isolated perfused rabbit heart. *Angiology*, 2000 51(5): p. 405–13. [PubMed: 10826857]
23. Hong YJ, et al., Early Detection and Serial Monitoring of Anthracycline-Induced Cardiotoxicity Using T1-mapping Cardiac Magnetic Resonance Imaging: An Animal Study. *Sci Rep*, 2017 7(1): p. 2663. [PubMed: 28572614]
24. Psaltis PJ, et al., An ovine model of toxic, nonischemic cardiomyopathy--assessment by cardiac magnetic resonance imaging. *J Card Fail*, 2008 14(9): p. 785–95. [PubMed: 18995184]
25. Ambale-Venkatesh B and Lima JA, Cardiac MRI: a central prognostic tool in myocardial fibrosis. *Nat Rev Cardiol*, 2015 12(1): p. 18–29. [PubMed: 25348690]
26. Melendez GC, et al., Myocardial Extracellular and Cardiomyocyte Volume Expand After Doxorubicin Treatment Similar to Adjuvant Breast Cancer Therapy. *JACC Cardiovasc Imaging*, 2019.
27. Farhad H, et al., Characterization of the Changes in Cardiac Structure and Function in Mice Treated With Anthracyclines Using Serial Cardiac Magnetic Resonance Imaging. *Circ Cardiovasc Imaging*, 2016 9(12).
28. Zhou P and Pu WT, Recounting Cardiac Cellular Composition. *Circ Res*, 2016 118(3): p. 368–70. [PubMed: 26846633]
29. Peto R, et al., Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet*, 2012 379(9814): p. 432–44. [PubMed: 22152853]
30. Lightfoot JC, et al., Novel approach to early detection of doxorubicin cardiotoxicity by gadolinium-enhanced cardiovascular magnetic resonance imaging in an experimental model. *Circ Cardiovasc Imaging*, 2010 3(5): p. 550–8. [PubMed: 20622140]
31. Cove-Smith L, et al., An integrated characterization of serological, pathological, and functional events in doxorubicin-induced cardiotoxicity. *Toxicol Sci*, 2014 140(1): p. 3–15. [PubMed: 24675088]
32. Keller AM, et al., Nuclear magnetic resonance study of high-energy phosphate stores in models of adriamycin cardiotoxicity. *Magn Reson Med*, 1986 3(6): p. 834–43. [PubMed: 3821462]
33. Dekker T, et al., Chronic cardiotoxicity of adriamycin studied in a rat model by ³¹P NMR. *NMR Biomed*, 1991 4(1): p. 16–24. [PubMed: 2029456]

34. Carvalho RA, et al., Metabolic remodeling associated with subchronic doxorubicin cardiomyopathy. *Toxicology*, 2010 270(2–3): p. 92–8. [PubMed: 20132857]
35. Maslov MY, et al., Reduced in vivo high-energy phosphates precede adriamycin-induced cardiac dysfunction. *Am J Physiol Heart Circ Physiol*, 2010 299(2): p. H332–7. [PubMed: 20495142]
36. Santacruz L, et al., Creatine supplementation reduces doxorubicin-induced cardiomyocellular injury. *Cardiovasc Toxicol*, 2015 15(2): p. 180–8. [PubMed: 25253560]
37. Andreadou I, et al., Metabonomic identification of novel biomarkers in doxorubicin cardiotoxicity and protective effect of the natural antioxidant oleuropein. *NMR Biomed*, 2009 22(6): p. 585–92. [PubMed: 19308947]
38. Li Y, et al., Screening, verification, and optimization of biomarkers for early prediction of cardiotoxicity based on metabolomics. *J Proteome Res*, 2015 14(6): p. 2437–45. [PubMed: 25919346]
39. Ponde NF, Lambertini M, and de Azambuja E, Twenty years of anti-HER2 therapy-associated cardiotoxicity. *ESMO Open*, 2016 1(4): p. e000073. [PubMed: 27843627]
40. Moslehi JJ, Cardiovascular Toxic Effects of Targeted Cancer Therapies. *N Engl J Med*, 2016 375(15): p. 1457–1467. [PubMed: 27732808]
41. Jones AL, et al., Management of cardiac health in trastuzumab-treated patients with breast cancer: updated United Kingdom National Cancer Research Institute recommendations for monitoring. *Br J Cancer*, 2009 100(5): p. 684–92. [PubMed: 19259090]
42. Fallah-Rad N, et al., The utility of cardiac biomarkers, tissue velocity and strain imaging, and cardiac magnetic resonance imaging in predicting early left ventricular dysfunction in patients with human epidermal growth factor receptor II-positive breast cancer treated with adjuvant trastuzumab therapy. *J Am Coll Cardiol*, 2011 57(22): p. 2263–70. [PubMed: 21616287]
43. Gong IY, et al., Early diastolic strain rate measurements by cardiac MRI in breast cancer patients treated with trastuzumab: a longitudinal study. *Int J Cardiovasc Imaging*, 2019 35(4): p. 653–662. [PubMed: 30390170]
44. Wang DY, et al., Cardiovascular Toxicities Associated with Cancer Immunotherapies. *Curr Cardiol Rep*, 2017 19(3): p. 21. [PubMed: 28220466]
45. Martin Huertas R, et al., Cardiac toxicity of immune-checkpoint inhibitors: a clinical case of nivolumab-induced myocarditis and review of the evidence and new challenges. *Cancer Manag Res*, 2019 11: p. 4541–4548. [PubMed: 31191015]
46. Mak IT, et al., EGFR-TKI, erlotinib, causes hypomagnesemia, oxidative stress, and cardiac dysfunction: attenuation by NK-1 receptor blockade. *J Cardiovasc Pharmacol*, 2015 65(1): p. 54–61. [PubMed: 25343568]
47. Quagliarello V, et al., Cardiotoxicity and pro-inflammatory effects of the immune checkpoint inhibitor Pembrolizumab associated to Trastuzumab. *Int J Cardiol*, 2019 292: p. 171–179. [PubMed: 31160077]
48. Kerkela R, et al., Sunitinib-induced cardiotoxicity is mediated by off-target inhibition of AMP-activated protein kinase. *Clin Transl Sci*, 2009 2(1): p. 15–25. [PubMed: 20376335]
49. Greineder CF, Kohnstamm S, and Ky B, Heart failure associated with sunitinib: lessons learned from animal models. *Curr Hypertens Rep*, 2011 13(6): p. 436–41. [PubMed: 21826469]
50. Blyszczuk P, Myocarditis in Humans and in Experimental Animal Models. *Front Cardiovasc Med*, 2019 6: p. 64. [PubMed: 31157241]
51. Barron CC, et al., An evaluation of the safety of continuing trastuzumab despite overt left ventricular dysfunction. *Curr Oncol*, 2019 26(4): p. 240–246. [PubMed: 31548803]
52. Upshaw JN, et al., Comprehensive Assessment of Changes in Left Ventricular Diastolic Function With Contemporary Breast Cancer Therapy. *JACC Cardiovasc Imaging*, 2019.
53. Hahn VS, Lenihan DJ, and Ky B, Cancer therapy-induced cardiotoxicity: basic mechanisms and potential cardioprotective therapies. *J Am Heart Assoc*, 2014 3(2): p. e000665. [PubMed: 24755151]
54. Sawyer DB, Anthracyclines and heart failure. *N Engl J Med*, 2013 368(12): p. 1154–6. [PubMed: 23514294]
55. Johnson DB, et al., Fulminant Myocarditis with Combination Immune Checkpoint Blockade. *N Engl J Med*, 2016 375(18): p. 1749–1755. [PubMed: 27806233]

CLINICAL RELEVANCE

Myocardial dysfunction induced by cancer therapies is an emerging healthcare concern and its diagnosis often denotes an irreversible pathophysiological remodeling of the LV tissue. Novel CMR tissue characterization techniques offer cardiac imaging markers that may facilitate the early identification of the underlying causes, including interstitial fibrosis, myocardial edema and myocellular atrophy and allows the development therapeutic interventions to prevent and treat CTRCD.

Fig. 1: Depiction of the Histopathological Mechanisms Leading to CMR-derived Extracellular Volume Fraction Expansion after Chronic Receipt of Doxorubicin.

Extracellular volume (ECV) increase can be caused by factors that expand the extracellular space such as diffuse fibrosis and/or edema, or factors that decrease the myocardial volume such as decreases in cardiomyocyte size and/or number.

Table 1:
Examples of human equivalent doses of Dox frequently used in murine models of cardiotoxicity.

These conversions were calculated with an average male mouse weight of 0.025 kg and human with weight 89.7 kg (average for men).

Dox doses for mice (mg/kg)	HED (mg/kg)	HED (mg/m ²)
1	0.081	2.997
2	0.162	5.994
5	0.405	14.985
10	0.810	29.970
15	1.215	44.955
20	1.620	59.940

Dox: Doxorubicin, HED: Human Equivalent Doses

CMR-derived tissue characterization changes representative of cardiomyocyte and interstitial remodeling in response to potential cardiotoxic chemotherapy

Table 2:

CMR Tissue Characterization Technique	Cardiomyocyte Atrophy & Edema	Cardiomyocyte Atrophy & Fibrosis	Cardiomyocyte Hypertrophy & Fibrosis
T1	↑	↑	↑
ECV	↑	↑	↑
T2	↑	-	↓/-
τic	↓	↓	↑

CMR parameters (T1, ECV, T2, τic) and their direction of change associated with cardiomyocyte and interstitial features as indicated by an increase (up arrow), decrease (down arrow), or no change (dash). Arrow and dashes in bold represent the tissue characteristic that determines the nature of the remodeling process and ECV expansion.

Table 3.

Summary of cardiotoxicity animal model studies employing CMR imaging

Ref. #	Species	Anti-cancer agent and administration route	Chemotherapy dose and frequency	MRI field strength	CMR tissue findings	Histopathology findings
Large animals						
Galan-Arriola et al. [8]	Pig	Dox, ICA	0.45mg/kg 2× weekly for 3–5 weeks	3.0 T	Increased T2 that preceded T1 increase and LVEF decrease	Cardiomyocyte vacuolization and interstitial fibrosis
Hong et al. [21]	Rabbit	Dox, IV	1.0 mg/kg 2× weekly for 16 weeks	3.0 T	Increase native T1 & ECV	Interstitial fibrosis, loss of myofibrils, & cardiomyocyte intracellular vacuolization
Psaltis et al. [22]	Sheep	Dox, ICA	1mg/kg twice weekly	-	Increased LGE	Increased fibrosis
Meléndez et al. [24]	Monkey	Dox, IV	30 to 60 mg/m ² biweekly up to 240 mg/m ²	3.0 T	LVEF decline, increased LV mass index, ECV fraction, T2	Increased LV collagen deposition; fibrosis, cardiomyocyte cross-section area. Decrease in the number of cardiomyocytes.
Small animals						
Farhad et al. [25]	Mouse	Dox, SubQ pellet	5 mg/kg/wk for 5 weeks	9.4T	Increase ECV; T2 prolongation	Interstitial myocardial fibrosis and cardiac water
Lightfoot et al. [28]	Rat	Dox, IV	1.5 to 2.5 mg/kg/wk for 10 weeks	1.5T	Increase in gadolinium enhancement	Cardiomyocyte vacuolization and degeneration, inflammatory cell infiltration, edema, diffuse myocardial fibrosis
Cove-Smith et al. [29]	Rat	Dox, IV	1.25 mg/kg for 8 weeks	4.7T	Decreased LVEF, Increased peak and LGE	Cardiomyocyte degeneration, extensive vacuolation & myocardial fibrosis

Dox: Doxorubicin; ICA: Intracoronary administration; IV: intravenous; SubQ: subcutaneous; T: Tesla; LVEF: left ventricular ejection fraction; ECV: extracellular volume; LGE: late gadolinium enhancement.

Table 4.

Anthracycline-induced animal models of cardiotoxicity using NMR spectroscopy

Ref. #	Species	Dox dose regimen	NMR isotope	Main findings
Keller et al. [30]	Rabbit	5 mg/kg daily × 5 days (acute) and 1.2–1.5 mg/kg twice a week × 7–10 weeks (chronic)	³¹ P	Decrease in PCR-to-ATP ratio in acute dosing; no change in PCR-to-ATP ratio in chronic dosing
Dekker et al. [31]	Rat	6,8,10,12, and 13 mg/kg cumulative doses weekly × 5 weeks	³¹ P	Decrease in PCR-to-ATP ratio at all doses and increased inorganic phosphate to PCR ratio at higher (12 and 13 mg/kg) doses
Carvalho et al. [32]	Rat	2mg/kg weekly × 6 weeks	¹³ C	Impaired long-chain fatty acid oxidation and increased anaerobic metabolism
Li et al. [36]	Rat	20mg/kg single dose	¹ H	Increased lactate, succinate, alanine, and acetate in Dox treated rats; oleuropein co-treated rats had no increase in these metabolites
Maslov et al. [33]	Mouse	5mg/kg weekly × 5 weeks	³¹ P and ¹ H	Decreased PCR-to-ATP ratio that preceded LV EF decrease in Dox treated mice; creatine kinase overexpression protective against PCR-to-ATP ratio alteration and functional decline
Santacruz et al. [34]	Mouse	5mg/kg weekly × 5 weeks	³¹ P and ¹ H	Decreased PCR-to-ATP ratio that preceded systolic and diastolic dysfunction

Dox: Doxorubicin; NMR: nuclear magnetic resonance; ATP: adenosine triphosphate; PCR: phosphocreatine