

Review

Cardio-Oncology Preclinical Models: A Comprehensive Review

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Abstract. *Cardiotoxicity is a common side effect induced by cancer therapies, which increases the risk of long-term morbidity and mortality in cancer survivors. To date, the mechanism leading to this toxicity is still unclear, thus complicating cardiac safety assessment and predictive factor identification. The advances in technology, particularly regarding radiation therapy and constant development of novel antineoplastic agents, require urgent development of efficient preclinical models to detect drug cardiotoxicity. A myriad of empirical preclinical models have been used to investigate cardiotoxicity, though with limited success. Recently, multicellular spheroid models have gained attention by mimicking the in vivo microenvironment. The aim of this review is to focus on the most relevant preclinical models used to assess antineoplastic drug- and radiotherapy- related cardiotoxicities, with an overview on their current use. It also aims to discuss the possible directions of translational research in the cardio-oncology field.*

Cardiotoxicity is a common side-effect induced by cancer therapies; it increases the risk of long-term morbidity and mortality in cancer survivors. Indeed, cardiotoxic side-effects caused by antineoplastic drugs can range from asymptomatic

reductions in left ventricular ejection fraction (LVEF), to tachycardia and arrhythmias, cardiomyopathy, myocardial infarction, and highly symptomatic congestive heart failure (1).

The real risk of cardiotoxicity was observed after the beginning of clinical use of antineoplastic drugs. Numerous clinical trials have highlighted the cumulative cardiac toxicities of anticancer treatments for hematological malignancies and solid tumors (2). However, the real frequency of cardiac toxicities has not been assessed yet. Indeed, patients with cardiac comorbidities are generally excluded from early phases of clinical trials.

Despite the large use of anthracyclines as cancer treatment, the incidence of doxorubicin acute cardiotoxicity within 2-3 days following its administration has been reported to be about 11% (3). Conversely, the incidence of chronic doxorubicin cardiotoxicity has been estimated at 1.7% (4). Likewise, new anticancer drugs, in particular vascular endothelial growth factor (VEGF) pathway inhibitors such as bevacizumab, sunitinib and sorafenib increase the occurrence of myocardial ischemia and have been correlated with hypertension and heart failure (5, 6). Moreover, congestive heart failure has been reported in approximately 8% of patients receiving sunitinib. In addition, administration of immune-checkpoint inhibitors (ICIs) has been shown to cause cardiovascular adverse events, particularly myocarditis (7), while, pericardial disease was the second most commonly reported adverse reaction (13.6% of patients treated with ICIs) in the Vigibase database (8, 9). Recent reviews have highlighted an increasing incidence of ICI-related cardiotoxicity, particularly acute myocardial infarction and takotsubo syndrome (10). Likewise, the use of chest radiation therapy to treat lymphoma, breast and lung cancer has been associated with an increasing risk of cardiotoxicity leading to cardiovascular complications (11-14), while the combination of antineoplastic drugs and radiotherapy may induce a potential risk of additional cardiac

This article is freely accessible online.

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Key Words: Cardiotoxicity, antineoplastic drugs, preclinical model, in vivo model, in vitro model, spheroid model, review.

toxicities (15). Therefore, it is important to identify the predictive factors of cardiotoxicities related to anti-cancer treatments, in order to adapt patient's treatment (16-18).

It has been shown that free radicals leading to oxidative stress, mitochondrial dysfunction, DNA damage, and apoptosis are involved in the antineoplastic treatment-induced cardiotoxicity (19); however, the exact mechanism remains unclear. Therefore, it is crucial to better understand the underlying molecular mechanism by using reliable experimental models.

A myriad of empirical preclinical models are used to predict cardiotoxicity, though with fairly limited success. Cardiac electrophysiological effects are assessed using cell lines overexpressing hERG (human ether-a-go-go-related gene) (20). *In vivo* models are also used for the assessment of the effects of kinase inhibitors (21). This review focuses on the most relevant preclinical models used to assess antineoplastic drug- and radiotherapy- related cardiotoxicities with an overview of their current use. Moreover, the possible directions of translational research in the cardio-oncology field are discussed.

***In Vitro* Models of Cardiotoxicity**

Cell line models represent prominent experimental models to evaluate pharmacokinetics, cell viability, cytotoxicity and drug efficacy for antineoplastic validation. Anticancer drugs have been observed to affect contractility, electrophysiological properties and induce structural toxicities. Almost all validation phases of antineoplastic therapies use cell models. Therefore, several *in vitro* models have been developed. The most relevant ones with regard to antineoplastic cardiotoxicity assessment will be discussed.

Primary cardiomyocytes. Most studies were performed with cultured primary cardiomyocytes, to assess contraction, ischemia, hypoxia, hypertrophy and drug toxicity. Neonatal rat cardiac ventricular myocytes (NRVM) served as *in vitro* models to study mechanisms of cardiac pathologies (22). Moreover, the cardiotoxicity of multiple tyrosine kinase inhibitors (TKIs) was assessed on NRVM primary culture (23). When used in the same model, sunitinib and dasatinib were more cytotoxic than other TKIs (23). Furthermore, evaluation assays of molecules protecting against doxorubicin cardiotoxicity were carried out in neonatal mouse cardiac ventricular myocyte (NRVM) cultures (24). Conversely, studies with NMVMs are limited to contractile function, handling calcium, and electrophysiology. In NMVM cultures, high levels of metallothionein were shown to induce an inhibition of doxorubicin toxicity (25). Likewise, testosterone was reported to antagonize NMVM senescence induced by doxorubicin (26).

Adult cardiomyocytes provide a powerful model for heart research. Nevertheless, the isolation of these cells remains a

very delicate process (27). The mechanism contributing to anthracycline late cardiotoxicity was studied on adult rat cardiomyocyte cultures. Indeed, doxorubicin caused an accumulation of poly-ubiquitinated proteins and autophagosomes causing damage on cardiomyocytes (28). Other studies assessed the doxorubicin effect on contractile function and oxidative stress status of the adult rat cardiomyocyte cells (29, 30). These studies showed that doxorubicin resulted in an increase in intracellular Ca^{2+} concentrations inducing reactive oxygen species (ROS) production (30). Neuregulin-1 β attenuated doxorubicin-induced alterations and reduced oxidative stress in adult rat ventricular myocytes (ARVM) (29). Antineoplastic-related cardiotoxicities assays on adult mouse cardiomyocytes are limited (21). However, adult cardiomyocytes offer an accurate model, which recapitulates cardiac function as it occurs in the adult heart *in vivo*.

Human cardiomyocytes provide a more precise model of drug-related cardiotoxicities, compared to animal-derived cardiomyocytes. Indeed, these cells conserve their morphological integrity and electrophysiological function. A few studies have investigated the cardiotoxic effect of antineoplastic therapy on human cardiomyocytes (31-33). These studies showed that these cells maintained their morphological integrity and electrophysiological properties for a short duration in culture. Due to the scarcity of human cardiac tissue donors and technical difficulties to extract and isolate these cell types, this model is less commonly used (34).

Primary cardiomyocytes are the most relevant model; however, their isolation is time-consuming and costly due to technical difficulties, such as the enzymatic digestion which can induce a disruption of the permeability of the cell membrane (31, 35).

Established cell lines. To assess cardiotoxicity, a number of human and rodent cell lines have been developed. One frequently used model to assess cardiac physiology is the H9C2 cardiomyoblast cell line. These cells, isolated from embryonic rat heart ventricle, have shown many similarities to primary cardiomyocytes. H9C2 cell line offers a valuable *in vitro* model for the investigation of drug metabolizing enzymes of the heart, drug-induced toxicities (36, 37) and transmembrane signal transduction (38). Furthermore, H9C2 cells have been validated as a valuable model for cardiac ischemia-reperfusion injury (39). However, these cells have multiple skeletal muscle characteristics and can differentiate into myotube-like structures (36, 38, 40). This is why the use of this model in cardiac studies is limited. Several studies have assessed doxorubicin-induced cardiotoxicity with H9C2 cultures (41-44). The cardiac toxicity of doxorubicin, fluorouracil together with adriamycin and cyclophosphamide (FAC), as well as of tyrosine kinase inhibitors has been widely assessed with H9C2 cell lines (45, 46).

Mouse atrial HL-1 cells are also immortalized cells with a cardiac phenotype and widely used as cardiac ischemia-reperfusion injury model. These cell lines provide a valuable *in vitro* model for cardiovascular disease states (47). HL-1 cells have been less used in the evaluation of cardiotoxicities related to antineoplastic molecules. Oxidative stress related to doxorubicin has been assessed through this model (48, 49). In a comparative study, Kuzenetsov *et al.* demonstrated that H9C2 cardiomyoblasts are more similar energetically to primary cardiomyocytes than HL-1, and therefore, constitute a more suitable model for cardiac ischemia-reperfusion injury studies (39).

The AC16 human cardiomyocyte cell line is derived from primary cells of human ventricular tissue (50). The cardiotoxic side effects of doxorubicin at cumulative doses were assessed on AC16 cell cultures (51). Regarding cardiac electrophysiological proprieties assessment, cell lines overexpressing hERG (human ether-a-go-go-related gene) are mostly used. Despite the fact that these cell lines are suitable for the study of electrophysiological properties, these immortal cells do not have the physiological and functional characteristics of cardiomyocytes. To our knowledge, no study has analyzed anti-neoplastic-induced cardiotoxicity using these cell lines so far.

Human pluripotent stem cell derived cardiomyocytes. Human induced pluripotent stem cell derived cardiomyocytes (hiPSC-CMs) are a revolutionary model in cardio-oncology preclinical research. Indeed, these cardiomyocytes are specific to the donor patient and their generation is unlimited. In this context, hiPSC-CMs derived from breast cancer patients suffering from doxorubicin-induced cardiotoxicity, showed a constant toxicity to doxorubicin (52). Thus, this model can be used to detect the population at greater risk of developing antineoplastic-induced cardiotoxicity. From a functional point of view, hiPSC-CMs exhibit calcium flux, conserve contractile function, and express the majority of ion channels and sarcomere proteins found in the adult heart. The contractile function of hiPSC by motion field imaging (MFI) was monitored along with antineoplastic drug exposure (53). In this model, doxorubicin induced much more cardiotoxicities than erlotinib, a TKI targeting EGFR. Another study using hiPSC-CMs to assess cardiotoxicity of 4 TKIs, demonstrated that each molecule has its own toxicity profile with different mechanisms that led to cardiotoxicity (54). Similarly, Wang *et al.* demonstrated that TKIs have distinct cardiac side effects in the hiPSC-CMs model (55). Indeed, following sorafenib treatment, oxidative phosphorylation was down-regulated resulting in mitochondrial defects (55). Recently, the effects of trastuzumab on the structural and functional properties of iPSC-CMs from healthy individuals and from patients were assessed (56). Cells derived from patients with severe cardiac dysfunction were more

vulnerable to trastuzumab treatment, than healthy iPSC-CMs (56). Despite all these advantages, the use of hiPSC-CM also has drawbacks. These cells remain at an immature state, substantially resembling the fetal phenotype, which induces a structural and electrophysiological variability, such as sarcomeric organization and ion channel density distribution (57). In addition, hiPSC generation remains very expensive and time consuming.

Co-cultured cell lines. Co-culture represents an effective model for drug research as it allows an in-depth monitoring of drug effects on cell-cell interactions. A number of studies have used this model to assess the putative cardiotoxic and pro-inflammatory effects of immunotherapy. Co-cultures of human cardiomyocytes and lymphocytes were exposed to ipilimumab or nivolumab (58). A significant cardiotoxic effect, related to these ICIs, was observed in co-cultures of lymphocytes and tumor or cardiac cells associated to leukotriene overexpression (58). Similarly, co-cultures of tumor cells or human fetal cardiomyocytes with lymphocytes exposed to pembrolizumab and trastuzumab induced a cardiotoxic effect associated with cardiomyocyte viability reduction (59). Cardiac pro-inflammatory effects were mediated by overexpression of NF- κ B and leukotriene B4 (59). However, even if cell-cell interaction is easier in co-cultures, the complex phenomenon of *in vivo* cardiophysiology cannot be accurately reproduced.

Preclinical Animal Models

Chemotherapy. Rodent models have been widely used to investigate the various forms of anthracycline-induced cardiotoxicity (60). Forty years ago, Herman *et al.* demonstrated that Wistar-Kyoto hypertensive rats were much more sensitive to the cardiotoxic effects of doxorubicin than normotensive rats (61). Mouse models were also used to investigate doxorubicin-induced cardiac dysfunction; the mechanisms contributing to this dysfunction were studied with a juvenile DBA/2J mouse model (62). Regarding doxorubicin cardiac toxicity, it has been reported to be different in male and female adult Wistar rats (63). After 7 weeks of doxorubicin administration, males developed major signs of cardiomyopathy with cardiac atrophy, reduced left ventricular ejection fraction and 50% mortality rate, while female left ventricular ejection was moderately affected (63). Furthermore, mouse xenograft tumor models were developed to assess doxorubicin cardiotoxicity (64). Similar to rodent model, doxorubicin showed more severe cardiotoxicity in the male xenograft model than in the female one (64).

Other animal species such as dogs, mini pigs and rabbits were used to assess anthracycline cardiac toxicities (60). In addition, doxorubicin-induced cardiotoxicity was investigated using a genetically modified mouse model (65). This study identified a potential predictive factor, reporting that patients

with higher expression of Top2 β in their cardiomyocytes may be more susceptible to doxorubicin cardiac toxicities. Zebrafish is another animal model for studying cardiac side effects. Zebrafish embryos exposed to anthracycline - daunorubicin, pirarubicin, doxorubicin (DOX), epirubicin and DOX-liposome developed heart defects (66). Early developing zebrafish embryos experienced the same effects as the mammalian models, offering a promising model of drug-induced cardiotoxicity (66). Moreover, zebrafish cardiomyocytes express voltage-gated sodium channels, L-type and T-type calcium channels and potassium channel, similarly to vertebrates (67). These electrophysiological characteristics of zebrafish model are useful to study drug-associated QT prolongation (68). Furthermore, with the advances in genome editing technology, transgenic zebrafish models were developed. Indeed, it has been shown that genetic mutation of CYP1a protected zebrafish against doxorubicin-induced cardiotoxicity (69).

Radiotherapy. Radiation therapy is administered to more than 50% of cancer patients. Animal models have been used to study cardiac toxicity induced by different radiotherapy regimens, ranging from whole thorax, whole heart and partial heart exposure (70). Lauk *et al.* showed that rat heart local irradiation with a single dose of over 10 Gy induced heart disease (71). Moreover, a study that used a genetically modified mouse model to identify the molecular basis of radiation-induced cardiotoxicity demonstrated that p53 functions in endothelial cells to prevent radiation-induced myocardial injury in mice (72). In another study, in which female rats received a 24 Gy localized whole-heart radiation, myocardial strain worsened post-RT, especially at 10-weeks and in lateral regions (68). Combined radiation therapy and antineoplastic drugs have been little studied through animal models. In the 70s, the cardiotoxic effects of adriamycin and radiation therapy were assessed using young New Zealand White rabbits (74). Animals in the combined group (radiation and adriamycin) developed diffuse myocardial fibrosis with greater frequency and severity than animals in the single therapy group (74). In another study, male Sprague-Dawley rats received local heart irradiation (9 Gy) and oral sunitinib. No early enhanced effects of radiation and sunitinib on cardiac function and structure were observed (75). Currently, patients receive several combinations of antineoplastic drugs and radiation therapy. More experiments are needed to study the impact of this antineoplastic association on cardiac functions.

Targeted therapy. In the era of personalized medicine, targeted therapy (TT) radically changed the outcomes of various cancers. However, TT drugs are associated with significant cardiac toxicity (76, 77). Indeed, bevacizumab and sunitinib were shown to induce systemic hypertension and left ventricular systolic dysfunction in young male

C57BL/6 mice (78). Experiments using ErbB2-deficient conditional mutant mice demonstrated cardiomyopathy related to trastuzumab (79). ErbB2 signaling in cardiomyocytes was found to be essential to prevent dilated cardiomyopathy (73). Zebrafish model was also used to predict sorafenib cardiotoxicity (80). Additionally, cardiotoxic effects of three antiangiogenic antineoplastic drugs - bevacizumab, endostar, and apatinib - were analyzed using transgenic zebrafish embryos and human lung cancer xenotransplantation models (81). Pericardial edema was associated with decreased heart rate with apatinib in this model. Conversely, no obvious side effects were observed with bevacizumab, which was not concordant with the toxicities experienced by cancer patients (81).

Immunotherapy. Nowadays, immunotherapy has emerged as a major antineoplastic treatment. Rare but clinically significant cardiotoxicity has been associated with ICIs. In murine models, programmed cell death-1 (PD-1) deficiency may induce dilated cardiomyopathy (82). Furthermore, C57BL/6 mice that were treated with ipilimumab (15 mg/kg) in compliance to the clinical use of this monoclonal antibody experienced cardiotoxic effects (58). The combined use of pembrolizumab and trastuzumab increased the expression of interleukins by 40-50%, in female C57BL/6 mice (59).

Taken together, animal models are widely used to assess cardiotoxicity of antineoplastic drugs. However, these models cannot faithfully recapitulate the complex pathogenesis of cardiotoxicity in cancer patients. Indeed, cancer physiopathology is complicated and animal models are not able to reproduce all patient comorbidities. Furthermore, animal models have different physiology, drug metabolism and gene expression. Genetically modified animals allow a better understanding of the mechanisms of cardiotoxicity induced by antineoplastic treatments. The zebrafish has emerged as a promising model due to its high degree of similarity with human cardiovascular function (68). More studies are needed to validate these models in the cardio-oncology field.

Three-dimensional (3D) Spheroid/Biosensor Model

Three-dimensional *in vitro* cell systems are a promising alternative to animal models to assess antineoplastic cardiotoxicity. Numerous types of 3D models have been developed in the cardiovascular field. Contrary to 2D-cultured cardiomyocytes, 3D-cardiac tissue allows high cell density and the interaction between cells and the extracellular matrix. Recent development of perfused 3D cells culture models and organ-on-chip models offers a promising alternative for the investigation of cardiac response to antineoplastic drugs. Polonchuk *et al.* demonstrated that cardiac spheroid mimics human heart microenvironment (83). This model was based on

the co-culture of hiPSC, endothelial cells and fibroblasts. Experiments using spheroid models demonstrated that doxorubicin induced oxidative stress associated with an increased endothelial nitric oxide synthase (83). In addition, doxorubicin induced a drastic decrease of cell outgrowth in spheroid-on-a-chip-device (84). Moreover, a recent study using 3D spheroid model demonstrated the protective role of NRF2 against doxorubicin induced cardiotoxicity (85). Nevertheless, spheroid chip format remains cost-effective and requires short training, thus presenting a convenient tool drug evaluation (84).

In a recent study, a cell-based biosensor was developed, integrating electrodes and microelectrodes for cell viability and electrophysiology monitoring, respectively (86). HeLa cells and cardiomyocytes of neonatal rats were cultured on this cell-based biosensor and cardiotoxicity assessment showed that taxol exposure induced a slight effect on cell viability and electrophysiological activity. By contrast, vinblastine had a strong effect on both cell viability and electrophysiological activity (86). To avoid drug absorption by the scaffold material, Arai *et al.* fabricated a scaffold-free cardiac construct using iPSCs-CMs and a bio-3D printer (87, 88). The same group presented a method to evaluate contractile force of these cardiac constructs (88). Despite the dazzling progress of 3D cell culture, and the evidence of the clinical pertinence, the use of this model remains limited in the cardio-oncology field. In addition to being less expensive, 2D culture is standardized with large data in the literature, while 3D culture requires technical equipment and expertise and still needs effort to improve accuracy, relevance, and reproducibility (89).

Discussion

Antineoplastic therapies have greatly improved cancer survival; nevertheless they are associated with acute and chronic cardiac toxicity. The constant development of novel antineoplastic agents and improvement of radiotherapy technologies require urgent development of efficient preclinical models for cardiotoxicity detection. Several *in vivo* and *in vitro* preclinical models have been used in cardio-oncology investigations. To date, none of these models has succeeded in reproducing the complex pathophysiology of cancer. Moreover, the results of preclinical evaluations of the toxic effects related to antineoplastic drugs using various models have been variable and they are rarely comparable to human data. Most of the studies investigating antineoplastic drug cardiac toxicities were designed with young healthy animals, complicating results transposition to elderly cancer patients (90).

In the era of personalized medicine, the main goal is to provide better treatment efficacy while avoiding toxicities. Patient-derived hiPSC-CMs have proven to be a reliable model for individual doxorubicin-induced cardiotoxicity

prediction (52, 91). Continuing research should override the immature nature of hiPSC-CM, which is the main drawback of this *in vitro* model. Furthermore, CRISPR/Cas9 technology has been largely applied to hiPSCs. Through CRISPR/Cas9 forward genetic screening in hiPSCs, Sapp *et al.* demonstrated that loss of function of transporter proteins SLCO1A2 and SLCO1B3 protects against doxorubicin-cardiotoxicity (92). Genetic engineering technologies in zebra fish model identified another cardioprotective pathways through CYP1 inhibition (69). Taken together these studies demonstrate that genome editing technologies are revolutionary tools in cardiooncology field. The use of mouse transgenic models allowed a better understanding of doxorubicin-induced cardiotoxicity. Indeed, mice overexpressing neuropeptide Y treated with doxorubicin had a lower ejection fraction. In this model, doxorubicin at relatively low doses caused significant cardiotoxicity, which was associated with neuropeptide Y overexpression (93). In addition, mice lacking muscle ring finger-1 (MuRF1) were protected from the cardiac atrophy induced by doxorubicin and exhibited no reduction in contractile function (94). Another study using mouse models showed that targeting RAC1 signaling is a promising approach to relieve acute and delayed doxorubicin-induced cardiac damage (95, 96).

A cellular model using H9C2 cell line, demonstrated that yangxin granules -a Chinese herbal- had a protective effect against doxorubicin-induced heart failure (97). However, more experimental and clinical studies are warranted to investigate and confirm the cardioprotective action of yangxin. Other preclinical models have demonstrated that fibronectin type III domain-containing 5 (FNDC5) overexpression or irisin treatment can alleviate doxorubicin-induced oxidative stress and cardiomyocyte apoptosis (98). All these preclinical models have identified potential targets to reduce and prevent the cardiotoxic effect induced by doxorubicin.

Cardiotoxicity should be predicted as early as possible and, ideally, before treatment initiation, or before clinical evidence of contractile dysfunction. Recently, the role of microRNA (miRNA) in doxorubicin-induced cardiotoxicity was highlighted (99). In addition to troponin – a circulating marker of heart damage, several *in vitro* and *in vivo* studies identified miRNA as diagnostic marker of doxorubicin cardiotoxicity (99). Recently, miRNA modulation was described as a promising therapeutic strategy to counteract cardiotoxicity induced by different oncologic treatments such as epirubicin, bevacizumab, ionizing radiation and radiotherapy (100). Likewise, exosomes in body fluids can serve as biomarkers in the early detection of doxorubicin-induced cardiotoxicity (101). However, exosome isolation remains a delicate process and its efficiency and purity are not guaranteed (102).

Lastly, the novel 3D cardiac cell cultures hold great potential in cardiotoxicology research. Spheroid models may

be a more rational alternative to cell culture, due to the more physiological growth of the cells, despite the limiting factors such as higher cost, more elaborate maintenance requirements and the unsuitability of some cell lines. Several cardiac constructs have emerged, integrating various organotypic microtissues in microfluidic systems. However, further studies are needed to ensure reproducibility and protocol standardization.

In conclusion, cancer therapy-associated cardiotoxicities represent common serious events that may imply fatal complications for patients. To date, none of preclinical models has succeeded in reproducing the complex physiopathology of cancer disease. The development of reliable and effective preclinical models for cardiotoxicity prediction and prevention is essential to improve the long-term cardiac health of cancer patients.

Conflicts of Interest

The Authors declare no competing financial interests.

Authors' Contributions

WB and NM contributed to the construction of the review. BM, ER, CR and ED contributed to drafting and revision of the manuscript. All Authors read and approved the final manuscript.

Acknowledgements

The Authors would like to thank Sandrine Sotton for English editing services.

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Received June 10, 2021

Revised September 27, 2021

Accepted September 29, 2021