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EDITORIAL

Anthracycline cardiotoxicity: the importance of horizontally integrating pre-clinical and clinical research

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This editorial refers to 'Diverging effects of enalapril or eplerenone in primary prevention against doxorubicin-induced cardiotoxicity' by R. Hullin *et al.*, pp. 272–281 and 'Inhibition of the cardiac myocyte mineralocorticoid receptor ameliorates doxorubicin-induced cardiotoxicity' by A. Lother *et al.*, pp. 282–290.

1. Anthracycline cardiotoxicity and cardioprotection in humans

Cardiotoxicity is an established complication of cancer therapies; the cardiomyopathy caused by anthracyclines is a classic example.[1] However, some new therapies used in conjunction with anthracyclines have introduced an assortment of cardiovascular complications.[1] An in-depth understanding of the early- and long-term cardiovascular complications of anti-neoplastics is essential.[1]

Anti-neoplastic agents, by design, work most effectively by killing rapidly dividing malignant cells. However, they also cause toxicity by damaging normal cells with high division rates, a state that is more common for human hearts early in life. Cardiomyocytes, unlike bone marrow and other cells, are often terminally differentiated cells with a limited capacity to regenerate; hence, they are vulnerable to long-term damage from these types of medications.

Therefore, cardiomyocytes are particularly sensitive to anthracyclines, even though they are a relatively mitotically quiescent cell population. The susceptibility of cardiomyocytes to anthracyclines is manifold and not hinged on a single theory.[2] Once damaged by chemotherapeutic agents, cardiomyocytes may never recover. We speculate based on our work that this may be for at least three reasons, as noted below: First, with anthracycline-associated free radical injury there is a loss of cardiomyocytes, as measured by serum cardiac troponin elevations,[3] which results in human children as they grow with increasing age having hearts that are too small for their body-surface areas due to this loss of cardiomyocytes, resulting in the remaining cardiomyocytes being enlarged but of inadequate number.[4] This results in the inadequate left ventricular mass for body-surface area that we have observed in long-term survivors.[5] Second, because the heart is one of the most energy dependent organs, and anthracycline therapy can lead to an irreversible mitochondriopathy in both rodents and humans,[6,7] the anthracycline mitochondrial effects may result in disproportionate toxicity for cardiomyocytes compared with other cells. Third, the limited regenerative stem cell populations that could serve as precursors for differentiated cardiomyocytes may be disproportionately more sensitive to the toxic effects of anthracyclines than are the non-cardiomyocyte stem cell populations.

Dexrazoxane provides significant protection against anthracycline cardiotoxicity without reducing oncologic efficacy.[8–10] However, this drug does not completely suppress anthracycline cardiotoxicity. Strategies other than dexrazoxane such as continuous (vs. bolus) anthracycline infusions,[11,12] angiotensin-converting enzyme inhibitor therapy,[13] tailoring chemotherapy to a patient's left ventricular systolic performance,[14–16] growth hormone replacement therapy,[17] and phosphocreatine with a control treatment (vitamin C, adenosine triphosphate, vitamin E, and oral coenzyme Q10)[18] have thus far not provided both long-term cardioprotection while simultaneously maintaining or enhancing oncologic treatment efficacy in pediatrics patients.

2. Pre-clinical models of anthracycline cardioprotection

Developing animal models of human pediatric anthracycline cardiotoxicity that include realistic treatment courses, risk factors, and validated biomarkers are essential for relevant cardioprotection studies.[19] Therefore, the potential to explore divergent cardioprotective strategies can be addressed, which is why the two studies published in this issue of the Journal are so important. These studies examined the impact of modulating mineralocorticoid receptor activity, either pharmacologically or genetically, on anthracycline-induced cardiotoxicity. The outcome of these studies revealed divergent results. Lother *et al.*[20] found that the

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mineralocorticoid receptor pathway is cardioprotective, whereas studies by Hullin *et al.*[21] failed to demonstrate comparable results.

It was a surprise that these two respected groups generated discrepant results. We find it difficult to concoct explanations for these differences but could perceive situations after the fact, thereby making it explainable and predictable with the benefit of hindsight. However, what we don't know is far more relevant than what we do know. This illustrates a severe limitation to our current state of learning from observations or experience and the fragility of our knowledge on this topic. Inductive reasoning by editorialists who make broad generalizations from specific observations may be dangerous. In this editorial, we have therefore taken the approach of highlighting some of the common factors that may contribute individually or cumulatively to the outcome of the present studies. By considering these factors, which may be responsible for the observed discrepancies, more relevant experimental models can be developed.

The influence of mineralocorticoid receptor activity, and its potential anthracycline cardioprotective activity, differs in these two experimental studies, as well as in other animal and human studies.[22–24] The reasons for such differences may be due to the variety of animal models that are utilized to examine the cardioprotective activity. The effects of anthracyclines in the laboratory can, but do not always, reproduce the clinical situation.[19] A consistent clinical observation is that doxorubicin cardiotoxicity develops chronically over-time. The wisdom of compressing the time to injury by acute high-dose studies in animals to mimic the chronic *in vivo* situation is thus questionable.

As noted in the papers of Lother *et al.*[20] and Hullin *et al.*[21] myocardial fibrosis was a consistent observation following doxorubicin treatment. In our anthracycline cardiotoxicity studies in children, we have observed minimal fibrosis compared to a substantial loss of cardiomyocytes, resulting in an inadequate number of cardiomyocytes for body-surface area.[4,25] Studies with doxorubicin have shown this agent has strong effects on reducing scar and connective tissue formation, fibrosis, collagen and matrix deposition, and fibroblast proliferation and survival. In rats, a single exposure to doxorubicin caused a progressive loss and redistribution of myocardial interstitial collagen matrix.[26] If this anti-fibrotic finding represents a true anthracycline action, the decrease in myocardial fibrosis associated with mineralocorticoid receptor antagonist treatment may be of limited cardioprotective value. Appropriate animal models are of considerable value in elucidating important cardioprotective activities.[19]

3. Considerations for animal models of anthracycline cardiotoxicity

Many animals receiving anthracyclines experience lesions that are morphologically similar to those occurring in patients taking these drugs.[27,28] Studies exploring the possibility that certain drugs could protect the myocardium were begun once appropriate animal models of anthracycline cardiotoxicity were developed.[27,29]

At present, the most consistent cardioprotective agent identified in animal models and confirmed in clinical studies is provided by dexrazoxane.[8–10,29] Although dexrazoxane significantly suppresses anthracycline cardiotoxicity, the cardioprotective activity is not complete. This may be due to the multiple potential cardiotoxic actions elicited by exposure to anthracyclines.[2,30] Dexrazoxane may interfere with some but not all of the cardiotoxic activity. As evidenced by the papers of Lother *et al.*[20] and Hullin *et al.*[21] in this issue there continues to be a need for appropriate animal models with which investigators can examine the potential multiple cardiotoxic anthracycline pathways. These models would be the basis for identifying potential cardioprotectant agents. To increase the likelihood of successful identification, pre-clinical studies searching for new cardioprotective agents should consider utilizing procedures that could be reproducible between experimental sites. Some of the variables affecting experimental reproducibility are summarized below and in *Table 1*.

3.1 Dosing regimen

Many studies have used regimens in which animals were treated with single or multiple high doses of an anthracycline. High doses cause toxicity in non-cardiac tissues, such as the gastrointestinal tract and bone marrow, and the animals may die before myocardial changes occur.[31] A more realistic approach would be to use smaller repeated doses that result in myocardial changes that develop gradually over weeks to months.[32] The changes induced by this chronic treatment are morphologically similar to those observed in patients. Dosages in such studies should be matched as closely as possible to those used clinically.

3.2 Animal models

The advantages and disadvantages of animal models of chronic anthracycline cardiotoxicity vary according to species. In some animals (mice, rats, and rabbits), nephrotic lesions develop simultaneously with cardiac lesions, whereas in other animals (dogs and miniature pigs), anthracyclines primarily affect the heart.[27,28]

3.3 Species variability

Sex and species strain can influence anthracycline cardiotoxicity studies. Male rodents are more sensitive to anthracycline cardiotoxicity than are female rodents.[33,34] Thus, studies should include both male and female animals. Cardiac lesions also develop at lower doses in certain strains of mice and rats than in other strains.[35]

3.4 Route of administration

Clinically, anthracyclines are administered intravenously, so pre-clinical studies intended to replicate the clinical situation should also administer them intravenously.

3.5 Duration of the study

Serious myocardial changes can occur during and after anthracycline therapy. Studies examining potential cardioprotective agents should compare the severity of myocardial lesions at the end of treatment with that evident after a prolonged drug-free period.[36]

Table 1 Design of relevant pre-clinical anthracycline cardioprotection studies

Consideration	Suggestion
Animal species	Rodent (initial)/non-rodent (dog)
Age	Adult animal-rodent/young animal-to be determined
Gender	Male/female
Dosing route	Intravenous
Dosing regimen	Weekly
Study duration	Multiple weeks
Cardioprotectant regimen	Prior to anthracycline dose
Cardioprotection persistency	Determination after drug free period
Useful cardiac biomarkers	Cardiac troponins/NT-proBNP

3.6 Biomarkers

To evaluate the progression of myocardial alterations, concentrations of cardiac biomarkers for anthracycline cardiotoxicity should be measured at baseline, pre-treatment, during, and after treatment.[37,38] Measurements of blood cardiac troponins are sensitive and specific biomarkers of cardiac injury in both animals and humans.[39–41] Therefore, they may be useful in the pre-clinical characterization of cardiac risk as biomarkers of that risk, which can guide relevant clinical application and interpretation.[39–41] However, cardiac troponin blood levels can vary according to the strain, gender, and age of the animal.[39–41] As a result, they may need adjustment based on the kinetics of the test article, the kinetics of the biomarker in the test species, and also based on the severity of the anticipated cardiovascular perturbation.[39–41]

In summary, pre-clinical studies of anthracycline cardiotoxicity are necessary and would be of more clinical value by considering:

(1) Being designed to incorporate a chronic component because the human cardiac phenotype in children with anthracycline cardiomyopathy shifts from an early dilated cardiomyopathy to a later progressive restrictive cardiomyopathy.[5] These models have also revealed an irreversible mitochondriopathy similar to that found in exposed humans.[6,7] For example, a recent study showed that the mitochondria from adults are 'apoptosis refractory'.[42] In contrast, the mitochondria from the heart and brain tissues in young mice and humans are primed for apoptosis, pre-disposing them to undergo cell death in response to genotoxic damage.[42] This result supports the hypothesis that children with cancer may be more pre-disposed to the severe side effects of toxic chemotherapy than are adult cancer patients, in whom this apoptotic machinery is almost absent[42];

(2) Anthracycline chemotherapy is virtually always given as part of multi-agent chemotherapy in humans, so testing these drug combinations in animal models may improve translational understanding;

(3) Multi-agent cardioprotection against multi-agent chemotherapy should be tested in pre-clinical models. New animal studies should examine new agents, such as mineralocorticoid receptor antagonists, with or against dexrazoxane to determine whether cardioprotection is incremental;

(4) Use of validated cardiac biomarkers should be incorporated into pre-clinical animal studies as surrogate endpoints in assessing attenuation of anthracycline cardiotoxicity[43] and;

(5) We, and others, have identified and validated genetic pre-disposition for anthracycline cardiotoxicity. On the basis of animal model findings[44] we determined in human children that having certain mutations of the hemochromatosis gene resulted in a nearly nine-fold increased risk of having dead and dying cardiomyocytes after having received anthracycline treatment for childhood cancer when compared to children receiving anthracyclines who did not have this mutation.[45] This enables us clinically to identify children at high-risk for cardiotoxicity prior to their receiving anthracyclines to target cardioprotective strategies. Using animal models to define high-risk groups for cardiotoxicity, and to also illuminate important mechanisms of cardiotoxicity and its protection, should be encouraged.

4. Why might mineralocorticoid receptor antagonist therapy be encouraging in addition to dexrazoxane for anthracycline cardioprotection?

Cardiac mineralocorticoid receptor activation in cardiomyocytes, macrophages, endothelial cells, and vascular smooth muscle cells in the heart is important in developing cardiac ventricular dysfunction, conduction abnormalities, tissue inflammation, oxidative stress, fibrosis, aging, and heart failure through direct signal mediation and paracrine activities.[46–52] Mineralocorticoid receptor antagonist blockade, such as with the potassium sparing diuretics, suppresses fibrosis and effectively treats chronic heart failure and post-myocardial infarction, improving clinical outcomes and supporting the function of aldosterone signalling in extra-renal organs.[46–52]

Major clinical considerations in anthracycline cardiotoxicity in children are the differences between boys and girls in the incidence, course, and outcome of this type of cardiotoxicity, with girls being significantly more sensitive to anthracycline cardiotoxicity[25] and deriving significantly more cardioprotection from dexrazoxane than do boys with equivalent dosing based on body-surface area.[9] Unexpectedly, our rodent anthracycline cardiotoxicity models exhibited the opposite gender susceptibility compared with human children.[33,34]

However, this human anthracycline cardiotoxicity gender relationship is similar to that reported for mineralocorticoid receptors, in which clinical evidence indicates that females are also more sensitivity to endogenous mineralocorticoid receptor activity and experimental evidence indicates that mineralocorticoid receptor-targeted interventions may be more efficacious in females.[47] This similarity suggests that the mechanisms of mineralocorticoid receptor-related activities need to be evaluated in a sex-specific manner, similar to what we have done in human anthracycline and dexrazoxane studies, to help provide a basis for developing cardiac-specific anthracycline cardioprotective therapies, which may also be sex-specific.[47]

The gender differences were not specifically explored in the Lother *et al.*[20] and Hullin *et al.*[21] studies. Yet, the mineralocorticoid receptor antagonist literature shows gender differences in terms of adverse cardiac effects related to excess mineralocorticoid receptor activity that again is very similar to what has been observed with human children receiving anthracycline chemotherapy. We suggest this as an area for future study. The gender similarity creates enthusiasm to encourage further studies of the manipulation of the mineralocorticoid receptor to determine if it may reduce anthracycline cardiotoxicity in a clinically meaningful way.

In conclusion, the importance of horizontally integrating pre-clinical and clinical discoveries cannot be overstated when considering safer ways to treat cancer patients with anthracyclines.[53,54] These needed synergies will facilitate the more rapid implementation of cardioprotective strategies to this population at high-risk for cardiovascular morbidity and mortality. But, as investigators we must ensure that these translational approaches to anti-cancer therapy require a quality-of-life-driven agenda if we are to achieve the optimal patient benefit.[55]

Author's contributions

SEL and EHH wrote this editorial.

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