JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

TITLE PAGE

Low dose anthracycline and risk of heart failure in a pharmacokinetic model of human myocardium exposure: Analog specificity and role of secondary alcohol metabolites

Emanuela Salvatorelli, Pierantonio Menna, Massimo Chello, Elvio Covino, and Giorgio Minotti

Drug Sciences (E.S., P.M., G.M.) and Cardiac Surgery (M.C., E.C.), Department of Medicine and Center for Integrated Research, University Campus Bio-Medico, Rome, Italy

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

RUNNING TITLE PAGE

RUNNING TITLE: Anthracyclines and heart failure

CORRESPONDING AUTHOR ADDRESS:

Giorgio Minotti
Department of Medicine
University Campus Bio-Medico
Via Alvaro del Portillo, 21
00128 Rome - ITALY

TELEPHONE: 011-39-06-225419109
FAX: 011-39-06-22541456
E-MAIL: g.minotti@unicampus.it

NUMBER OF TEXT PAGES: 16

NUMBERS OF TABLES: 6

NUMBER OF FIGURES: 5

NUMBER OF REFERENCES: 38

NUMBER OF WORDS Abstract: 249

Introduction: 729

Discussion: 1499

NONSTANDARD ABBREVIATIONS: DOX(OL), doxorubicin(ol); EPI(OL), epirubicin(ol); DNR(OL), daunorubicin(ol); IDA(OL), idarubicin(ol); HF, heart failure; C_{max}, peak concentration; HPLC, high performance liquid chromatography; log P, partition coefficient; log D, distribution coefficient.

RECOMMENDED SECTION ASSIGNMENT : Metabolism, transport, and pharmacogenomics.

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

ABSTRACT

Cumulative doses of doxorubicin and other antitumor anthracyclines may cause heart failure (HF). Cardiotoxicity is determined by cardiac exposure to anthracyclines and to more toxic secondary alcohol metabolites that are formed inside cardiomyocytes or diffuse from the bloodstream. Concerns exist that HF might be caused by cumulative anthracycline doses that were thought to be safe. Patients with gain-of-function polymorphism of carbonyl reductase 3 (CBR3), which converts anthracyclines to secondary alcohol metabolites, would be at a higher risk of HF. Recently a pharmacokinetic model was developed that simulated clinical exposure of human myocardium to anthracyclines and incorporated simulations of CBR3 polymorphism. It was shown that HF risk could occur after lower doxorubicin doses than previously reported, particularly for patients with CBR3 polymorphism (Salvatorelli et al., J. Pharmacol. Exp. Ther. 362:263-270, 2017). Here we show that also daunorubicin and idarubicin, but not epirubicin, might cause HF after reportedly safe cumulative doses. CBR3 polymorphism increased HF risk from daunorubicin and idarubicin to a greater extent as compared to doxorubicin. This was caused by daunorubicin and idarubicin forming higher levels of toxic metabolites in human myocardium; moreover, daunorubicin and idarubicin metabolites diffused from plasma and accumulated in cardiac tissue, while doxorubicin metabolite did not. CBR3 polymorphism did not aggravate HF risk from epirubicin, which was caused by the very low levels of formation of its toxic metabolite. These results support concerns about HF risk from low dose anthracycline, characterize the analog specificity of HF risk, and illuminate the role of secondary alcohol metabolites.

INTRODUCTION

Clinical use of the antitumor anthracycline, doxorubicin (DOX), is limited by the possible development of cardiomyopathy and heart failure (HF). A remarkable characteristic of cardiotoxicity pertains to its dependence on the cumulative dose of DOX; in fact, HF risk increases slowly over a range of cumulative doses but increases exponentially once an apparent threshold is exceeded (Ewer, 2013).

In early clinical studies a cumulative dose of 550 mg of DOX/m^2 caused HF in 5% of otherwise healthy adults (von Hoff et al., 1979). Subsequent studies refined the 5% risk dose to 400 mg of DOX/m^2 (Swain et al., 2003), which is currently accepted as the reference threshold for HF after DOX administration (Ewer, 2013). Concerns about HF development after lower cumulative doses than 400 mg of DOX/m^2 have nonetheless been raised (Limat et al., 2003). Some reports suggest that any dose between 50 and 375 mg/m^2 might cause asymptomatic abnormalities that progress toward HF (Drafts et al., 2013).

Anthracyclines are used for many cancer patients. Clinical studies that prospectively re-explore dose relations for HF risk would be much needed for optimizing cardiovascular surveillance or treatment of cancer patients, but unfortunately anthracyclines were developed decades ago and do not longer attract financial support for *ad hoc* studies. Translational models that surrogate for clinical studies are therefore needed. Any such model should avoid pitfalls of animal or cellular models and should be based on the following pharmacokinetic concepts: i) HF risk correlates with anthracycline plasma C_{max} and diffusion in the heart ii), DOX is only partially eliminated from cardiac tissue iii), each anthracycline infusion generates a cardiac anthracycline pool that accumulates and induces cardiotoxicity (Minotti et al., 2010; Stewart et al., 1993). According to these concepts HF would occur when repeat infusions cause the size of anthracycline pools to exceed the defense mechanisms of heart (Minotti et al., 2010).

The composition of cardiac anthracycline pools is also important. Two-electron reduction of a carbonyl group in the anthracycline side chain generates secondary alcohol metabolites (R-CO-

R' → R-CHOH-R'). These metabolites are ~40 times more potent than their parent drugs at inhibiting ATPases that govern systolic contraction and diastolic relaxation (Boucek et al., 1987; Mushlin et al., 1993; Olson et al., 1990). Patients with an individual predisposition to generate high levels of secondary alcohol metabolites should therefore be considered to carry an increased risk of anthracycline cardiotoxicity (Blanco et al., 2008; Blanco et al., 2012).

Recently a translation model was developed to explore correlations between cardiac anthracycline pools and risk of HF. This model consisted of ex vivo human myocardial samples exposed to simulations of rapid infusions of DOX. The size of the anthracycline pool induced by a single DOX infusion was adjusted for the higher toxicity of its secondary alcohol metabolite and was incorporated into equations from which exponential risk versus dose curves for multiple infusions were obtained. This approach allowed to slightly revise the 5% risk dose of DOX from 400 to 380 mg/m^2. More importantly, it was shown that 1-2% of patients might develop HF after ~20% lower cumulative doses than characterized for historical cohorts of low risk patients (220-280 mg/m^2 versus 300-340 mg/m^2, respectively) (Salvatorelli et al., 2017). Risk from low dose DOX was aggravated by simulations of gain-of-function polymorphism of carbonyl reductase (CBR) 3, one of the enzymes that convert anthracyclines to secondary alcohol metabolites (Salvatorelli et al., 2017). These figures supported concerns about HF risk from low dose DOX.

Here the pharmacokinetic model was adopted to reassess HF risk from other widely used anthracyclines (epirubicin/EPI, daunorubicin/DNR, idarubicin/IDA). Whereas EPI is considered as an alternative to DOX for the treatment of e.g., breast cancer, DNR and IDA are used primarily to treat leukemias. Cumulative doses associated with 5% HF risk from EPI, DNR or IDA have been reported (Anderlini et al., 1995; Ryberg et al., 2008; Von Hoff et al., 1977; Winthrop Pharmaceuticals, 2010), but evidence for HF development after lower doses is available, particularly for DNR and IDA (Bonadonna and Monfardini, 1969; Lo-Coco et al., 2013; Ohtake et al., 2011). Moreover, the propensity of these anthracyclines to generate secondary alcohol metabolites may be quite different from that of DOX (Salvatorelli et al., 2007; Mordente et al.,

2003), possibly resulting in major changes of the cardiotoxic potential of anthracycline pools. The rationale for probing EPI, DNR and IDA was therefore provided by a number of pharmacological and clinical premises.

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

EXPERIMENTAL PROCEDURES

Myocardial Samples

For experiments with EPI and DNR or IDA we used myocardial samples from 44 patients (median age 73 years, range 36-86) undergoing aorto-coronary bypass grafting or valve replacement. For comparisons with DOX we used data from the recent study that probed DOX in the pharmacokinetic model. The latter included samples from 42 similar patients (median age 74 years, range 44-85) (Salvatorelli et al., 2017). All samples were taken during cannulation of beating nonischemic right atrium for preparation of cardiopulmonary bypass. This protocol was approved by the Ethics Committee of University Campus Bio-Medico of Rome. Patient characteristics are reported in **TABLE 1**. Patients >60 years of age were significantly more numerous than patients ≤60 years ($P<0.001$), and males were significantly more numerous than females in either age group ($P<0.001$). The two groups were balanced for coronary or valvular surgery ($P=0.576$), with the former always prevailing on the latter (56% versus 44% for patients ≤60 years, 64% versus 36% for patients >60 years).

Given the age range and the underlying cardiac disease, patients from both groups presented with comorbidities such as diabetes, dyslipidemia, and especially hypertension. Fewer patients presented with atrial fibrillation ($P<0.001$ versus dyslipidemia or hypertension). Patients ≤60 or >60 years of age were balanced for all comorbidities but hypertension, which occurred more frequently for patients >60 years of age (see also Table1). On average, there were 2 comorbidities per patient in each group. Only a couple of patients <60 years were comorbid-free.

Myocardial Exposure to Anthracyclines

Myocardial samples were processed to obtain thin myocardial strips that were placed in 2 ml of human plasma as described (Salvatorelli et al., 2017). The strips were then exposed to anthracyclines, which were used at the C_{max} values occurring during clinical infusions for treatment of breast cancer by DOX or EPI (Danesi et al., 2002; Gianni et al., 1997), treatment of acute lymphoblastic leukemia by DNR (De Gregorio et al., 1982), treatment of acute myeloid leukemia by

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

IDA (Eksborg et al., 1997). Myocardial exposure was limited to the time at C_{max} (i.e., the clinical time interval over which C_{max} was shown to decrease by approximately 50% in the exposed patients). Unless otherwise indicated the strips were subsequently placed in anthracycline-free plasma to simulate systemic anthracycline clearance and to promote myocardial efflux of unmetabolized drugs or of secondary alcohol metabolites that had been generated inside cardiac tissue ("endogenous metabolites"). Because many tissues generate anthracycline secondary alcohol metabolites and release them in bloodstream ("exogenous metabolites") the strips were separately exposed to purified metabolites (doxorubicinol/DOXOL, epirubicinol/EPIOL, daunorubicinol/DNROL, idarubicinol/IDAOL), which were used at clinically reported C_{max} and time at C_{max} values. All myocardial samples were therefore exposed to anthracycline and metabolite levels that in patients were determined by drug equilibration between a central compartment (plasma) and excretory organs (liver, kidney). Pharmacokinetic details are reported in **TABLE 2**.

Myocardial strips were exposed to anthracyclines only once. All experiments therefore reproduced single anthracycline infusions. Total incubation time (anthracycline treatment + anthracycline elimination) never exceeded 4 hours. This was necessary to preserve myocardial strip integrity and functions (Salvatorelli et al., 2017). Preservation of myocardial viability was evidenced by the lack of troponin release during the course of incubations (<2.5% of total myocardial troponin I even when DOX was used at C_{max} for 4 hours without clearance in anthracycline-free plasma) (see also Salvatorelli et al., 2012).

Anthracycline Pharmacokinetics

For the experiments of strips exposed to parent anthracyclines and then subjected to drug elimination in anthracycline-free plasma, metabolite formation was determined by the sum of metabolites retained in the strips with metabolites released in plasma. Control experiments showed that plasma per se was unable to metabolize anthracyclines, secondary alcohol metabolites always being below the limit of detection. This excluded interferences with measurements of metabolites that were released from strips. For the same experiments anthracycline uptake was therefore

determined as [(anthracycline in strips + anthracycline in plasma) + (metabolite formation)]. For the experiments of strips exposed to exogenous metabolites and then subjected to drug elimination in metabolite-free plasma, uptake was determined by the sum of metabolites retained in the strips with metabolites released in plasma.

Assays

Strips and plasma were extracted and assayed for parent anthracyclines and metabolites by HPLC with fluorescence detection, as described in detail (Salvatorelli et al., 2017). Retention times were: 15.3 and 13.9 min for DOX and DOXOL, 15.5 and 14.0 min for EPI and EPIOL, 17.1 and 15.7 min for DNR and DNROL, 18.8 and 17.4 min for IDA and IDAOL. Anthracyclines and metabolites were quantified against standard curves of authentic standards of each analyte (lowest quantification limit of 0.001 μM or 0.005 μM for anthracyclines and metabolites, respectively). Inasmuch as cardiac tissue shows essentially the same density as that of water (Mushlin et al., 1993) all values were normalized to the weight of the strips and were expressed as micromolar equivalents.

Quantification of Cardiac Anthracycline Pools

Cardiac anthracycline pools were quantified by the sum of anthracyclines and endogenous or exogenous metabolites that were recovered from the strips at the end of incubations. Metabolite levels were routinely multiplied by 40 to normalize for their stronger negative effects on cardiac contraction and relaxation (Salvatorelli et al., 2017). For DOX or EPI or DNR, which are administered once every a given number of days or weeks, the cardiac pools induced by single infusions were determined by the formula

$$pool_{DOX,\ EPI,\ DNR} = \text{anthracycline} + [40 \times (\text{endogenous} + \text{exogenous metabolite})]$$

A different approach was used for IDA, which is given in courses of three infusions over three consecutive days. Cardiac pools induced by simulations of a single infusion were calculated by adding IDA with endogenous IDAOL. Next, the sum of IDA and endogenous IDAOL was multiplied by a factor of three to reproduce anthracycline accumulation after three infusions. These cardiac

pools were eventually added with myocardial retention of three increasing concentrations of exogenous IDAOL, which reproduced stepwise increases of circulating IDAOL during a three-days course of IDA (Eksborg et al., 1997). The cardiac pool induced by a three-days course of IDA was therefore determined by the formula

$$pool_{IDA} = 3x\ [IDA + (40 \times \text{endogenous IDAOL})] + (40 \times \text{cumulative exogenous IDAOL})$$

Where indicated the experimental data were used to simulate gain-of-function polymorphism of CBR3. We did not preliminarily search if one or more samples carried a polymorphism; however, we characterized that for each anthracycline the levels of endogenous metabolite correlated with the levels of myocardial uptake of the parent drug ($P<0.01$ for DOX or EPI, $P<0.001$ for DNR or IDA). This showed that metabolite formation only depended on substrate availability. No deviating effect from high metabolizing outliers was observed. CBR polymorphism was therefore simulated over a rather homogenous metabolic background by assuming that catalytic variants cause a twofold increased formation of the alcohol metabolite (Blanco et al., 2008; Blanco et al., 2012). The levels of parent anthracyclines were assumed to decrease by the amount required to generate excess metabolite (Salvatorelli et al., 2017). Myocardial exposure to exogenous metabolites was also assumed to increase twofold in presence of CBR3 polymorphism.

Five Percent Risk Pools and Risk versus Dose Curves

The cardiac pool of DOX associated with 5% risk of HF, 31.3 μM, was previously calculated by the formula

$$5\%\ \text{risk pool} = [\text{post-infusion pool} \times (5\%\ \text{risk dose} : \text{infusion dosage})]$$

where the ratio of 5% risk pool to single infusion dosage gave the number of DOX infusions associated with the 5% risk dose (Salvatorelli et al., 2017). Having assumed cause-and-effect relations between cardiac anthracycline accumulation and HF risk, we assumed that a cardiac pool of 31.3 μM caused 5% risk of HF from all other anthracyclines and was therefore referred to as 5% risk pool (Salvatorelli et al., 2017). Five percent risk pools of EPI, DNR and IDA were then incorporated into Equation [1], which was previously developed to obtain exponential risk versus

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

dose curves

$$\text{HF risk (\%)} = (\text{pool}_n : \text{5\% risk pool})^2 \times c\text{n}^2 \quad [1]$$

In Equation [1] pool_n was the cardiac pool induced by n infusions of a given anthracycline, and *c* was a correction constant specific to that anthracycline (Salvatorelli et al., 2017) (**Table 3**). Equation [1] was also used for simulations of CBR3 polymorphism, subject to incorporation of the appropriate correction constants (see also Table 3).

Other experimental conditions

HPLC-grade standards of parent anthracyclines, and of all metabolites but IDAOL, were obtained through the courtesy of Nerviano Medical Sciences (Milan Italy). IDAOL was prepared by us through whole blood enzymatic reduction of IDA, followed by HPLC purification and chemical assignment by negative ionization electron spray mass spectrometry. Data were expressed as means ± standard deviations (SD) (nine experiments for EPI, sixteen experiments for IDA, twenty-three experiments for DNR). Cumulative doses of EPI, DNR and IDA were converted to doxorubicin myelotoxic equivalents by means of isotoxic conversion factors (0.67 for EPI and DNR, 5.0 for DNR) (Ewer, 2013). Differences between data were analyzed by one way ANOVA with Bonferroni's test for multiple comparisons, Student's t test or Fisher exact test, as appropriate. Slope analyses were performed by receiver operator characteristics (ROC). All analyses were performed by Prism 5®, version 5.01 (GraphPad Software Inc., La Jolla, CA). Other details are reported in legends for Figures and Tables.

RESULTS

Post-infusion Cardiac Anthracycline Pools

Under the experimental conditions that reproduced clinical anthracycline infusions, DOX and EPI exhibited high levels of myocardial uptake and retention and low levels of formation of endogenous metabolites, which were retained in the strips. Daunorubicin and IDA exhibited lower levels of uptake and retention but caused higher levels of formation of endogenous metabolites, which were partially retained in myocardial strips. Simulations of CBR polymorphism augmented anthracycline conversion to endogenous metabolite, which was more evident for DNR and IDA (**Table 4**). Human myocardium incorporated exogenous DNROL and IDAOL but not DOXOL or EPIOL. Myocardial accumulation of exogenous DNROL and IDAOL increased under conditions that simulated CBR polymorphism (**Table 5**).

The cardiac pools of DOX and EPI and DNR or IDA were quantified as described in Experimental Procedures and were shown to be similar in size but different in composition. For DOX and EPI the pools were composed primarily of parent drugs, with the endogenous metabolites accounting for ≤15% of the size of the pools. For DNR and IDA endogenous and exogenous metabolites accounted for >90% of the size of the pools (**Figure 1**). Simulations of CBR polymorphism expanded the cardiac pool of each anthracycline but the effect size was more evident for DNR and IDA. In the pool of IDA the exogenous metabolite was always more abundant than the endogenous metabolite (see also Figure 1).

Risk versus dose curves

Cardiac anthracycline pools were incorporated into Equation [1] to obtain curves that correlated HF risk with multiple infusions and cumulative doses. Exponential curves were obtained for all anthracyclines. Simulations of CBR polymorphism caused leftward shifts of the curves of DOX, DNR and IDA, which denoted an increased HF risk at lower cumulative doses of these anthracyclines. Significant aggravation of HF risk was not observed over a broad range of cumulative doses of EPI (**Figure 2**).

Risk versus dose curves were used to extrapolate cumulative anthracycline doses associated with 5% or 1-2% risk of HF. The experimental values were then compared to clinical vaues for low risk adult patients (Anderlini et al., 1995; Ryberg et al., 2008; Swain et al., 2003; Von Hoff et al., 1977; Winthrop pharmaceuticals, 2010) (**Table 6**). The experimental 5% risk doses of DOX and DNR and IDA were lower than the clinical values, which was more evident for DNR and IDA. Simulations of CBR polymorphism decreased the 5% risk doses of DOX and DNR and IDA, which also was more evident for DNR and IDA. As for 1-2% risk doses, DOX showed lower values than reported for patients, and these values were further decreased by CBR polymorphism. Clinical doses of DNR or IDA associated with 1-2% risk were not firmly established in the literature, and hence reliable comparisons between experimental and clinical values could not be determined. The experimental doses nonetheless denoted that 1-2% risk could occur after very low doses of DNR or IDA, particularly if CBR polymorphism was simulated. The experimental risk doses of EPI were congruent with the clinical values, and simulations of CBR polymorphism only caused marginal effects as compared with DOX, DNR or IDA (see also Table 6).

Dose and Analog Dependence of Risk Aggravation by CBR polymorphism

Slope analyses of risk versus dose curves showed that the effects of CBR polymorphism on increasing HF risk was both analog and dose dependent. For each anthracycline excess risk increased over a dose range but decreased at higher doses. Maximum aggravation of HF risk was noticeably higher for DNR and IDA than DOX or EPI, which was consistent with the propensity of DNR and IDA to generate higher levels of secondary alcohol metabolites. Cumulative doses of EPI, DNR and IDA were also expressed as doxorubicin myelotoxic equivalents (Ewer, 2013). This was done to evaluate excess risk when different anthracyclines were given in doses that caused a comparable degree of myelotoxicity. Under such defined conditions excess risk from DNR and EPI peaked at 200 or 600 mg/m^2, respectively, while excess risk from IDA began increasing at 400 mg/m^2 and reached its maximum at higher doses than 1000 mg/m^2 (**Figure 3**).

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

We next simulated HF risk in patient cohorts in which half of patients received doxorubicin equivalents of one analogue and the remaining half received doxorubicin equivalents of another analogue. Over a dose range from 50 to 600 mg/m^2, which includes the majority of past and contemporary oncologic protocols, CBR polymorphism caused excess risk in some cohorts (DOX-DNR, EPI-DNR, DNR-IDA) but not in others (DOX-EPI, DOX-IDA, EPI-IDA). When observed, excess risk occurred at doses between 150-350 mg of doxorubicin equivalents/m^2, with a peak occurring at 200-220 mg/m^2 (**Figure 4**). Excess risk did not occur for patient cohorts in which patients were equally distributed to receive DOX or doxorubicin equivalents of EPI, DNR or IDA (**Figure 5**).

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

DISCUSSION

Analog-Specificity of HF Risk from Low Dose Anthracycline

Molecular mechanisms of anthracycline cardiotoxicity, such as iron-catalyzed oxidative stress or topoisomerase 2β-mediated DNA damage, await clinical validation (Minotti et al., 2004; Vejpongsa and Yeh, 2014). Cardiac anthracycline accumulation is a better defined culprit of cardiotoxicity (Minotti et al., 2010). A pharmacokinetic model of cardiac anthracycline pools showed that DOX could induce HF after lower than clinically reported doses (Salvatorelli et al., 2017). Here the same model showed that the experimental 5% risk doses of DNR or IDA were ~50% or ~40% lower than reported for historical patient cohorts (380 versus 800 mg of DNR/m^2, 180 versus 290 mg of IDA/m^2) (von Hoff et al., 1977; Anderlini et al., 1995). Moreover, 1-2% risk doses of DNR and IDA were low enough to caution against HF risk for any patient exposed to these anthracyclines (170-270 mg of DNR/m^2, 100-130 mg of IDA/m^2). These findings support concerns about cardiac events in patients treated by much lower doses than 800 mg of DNR/m^2 or 290 mg of IDA/m^2 (Bonadonna and Monfardini, 1969; Lo-Coco et al., 2013; Ohtake et al., 2011). On the other hand, the experimental risk doses of EPI were congruent with clinical reports (Ryberg et al., 1998). Thus, HF risk from low dose anthracycline is analog dependent.

Role of Secondary Alcohol Metabolites

Post-infusion cardiac pools of DOX, EPI, DNR and IDA were similar in size but different in composition. This was caused by DNR and IDA forming higher levels of endogenous metabolites. Myocardial accumulation of endogenous DNROL and IDAOL was partially limited by the ease with which either metabolite diffused from strips in plasma; however, the strips accumulated exogenous DNROL and IDAOL that diffused from plasma in strips. Bidirectional movements across strips and plasma were not observed for endogenous or exogenous DOXOL and EPIOL. Partition coefficients show that DNROL and IDAOL are in fact more lipophilic than DOXOL and EPIOL (1.01 for DNROL, 1.18 for IDAOL, -0.03 for DOXOL and EPIOL, at pH 7.4). A similar information is offered by distribution coefficients (0.35 for DNROL, 0.49 for IDAOL, -0.69 for DOXOL and EPIOL, at pH

7.4) (ChemAxon, 1998–2017, https://chemicalize.com). The cardiac pools of DNR and IDA were therefore composed primarily of endogenous and exogenous metabolites, while the pools of DOX and EPI contained high amounts of parent anthracycline and low amounts of endogenous metabolite.

The slope of risk versus dose curves was determined by the size of anthracycline pools and by factors like the anthracycline infusion dosage and the number of infusions needed to form the 5% risk pool (see Equation 1). The composition of anthracycline pools was unimportant at this stage of HF risk assessment. The propensity of a given anthracycline to generate secondary alcohol metabolites became important when gain-of-function CBR3 polymorphism was simulated. The higher propensity of DNR and IDA to generate endogenous metabolites and to accumulate exogenous metabolites in myocardial strips caused a more remarkable aggravation of HF risk from these anthracyclines as compared to DOX or EPI. For EPI, marginal increases of its alcohol metabolite did not cause excess risk over a broad range of cumulative doses.

Study Limitations and Strengths

Only atrial samples were used in this study. We were in fact unable to retrieve ventricular samples from patients undergoing e.g., heart transplant or ventriculoplasty. Major differences in anthracycline metabolisation by atrial or ventricular tissues should nonetheless be excluded. Previous studies of post-mortem left ventricle samples did in fact demonstrate the same pattern of DOXOL and DNROL formation as that observed in this present study of atrial samples (Mordente et al., 2003).

The majority of myocardial samples were from male donors >60 years of age, carrying comorbidities that are common in advanced aged. These factors too might have biased formation of anthracycline pools and risk assessment for females or younger patients without comorbidities. However, we previously reported that cardiac anthracycline accumulation and metabolisation were not significantly influenced by gender and age in a similar patient population (Salvatorelli et al., 2017).

The pharmacokinetic model neither predicts how many months or years would elapse between anthracycline administration and HF development, nor incorporates the weight of comorbidities on precipitating anthracycline cardiotoxicity. This model only defines the absolute potential for human myocardium to accumulate and metabolize anthracyclines. Likewise, this model assumes that the size of cardiac anthracycline pools would not change upon multiple infusions but we cannot exclude that it actually changed through e.g., CBR3 upregulation (Blanco et al., 2012). Cardiotoxicity would increase and risk doses would decrease if that were the case. We also acknowledge that CBR polymorphism is just one of many genetic that modifiers that possibly aggravate anthracycline cardiotoxicity (Salvatorelli et al., 2015).

These limitations having been acknowledged, we suggest that our study has many strengths. For example, it may assist cardio-oncologists in reconciling conflicting reports on HF risk aggravation by CBR polymorphism. Some reports showed that the gain-of-function CBR3 polymorphism, V244M, did not aggravate anthracycline cardiotoxicity (Visscher et al., 2012). In other studies the same polymorphism increased HF risk in patients exposed to low-moderate anthracycline doses but not in patients exposed to higher doses (Blanco et al., 2012). Here we have shown that HF risk aggravation by CBR polymorphism only occurs over dose ranges that are specific to each anthracycline. The effect size of CBR polymorphism depends on the ease with which anthracyclines are converted to secondary alcohol metabolites, DNR and IDA showing more remarkable effect sizes. These findings anticipate that the chances of detecting HF risk aggravation by CBR polymorphism would depend on which anthracycline and cumulative doses were used in a patient cohort. Our simulations show that a broad spectrum of clinical situations can in fact occur, from a lack of excess risk to a significant excess risk over 150-350 mg of doxorubicin equivalents/m^2. When observed, excess risk peaked after as low cumulative doses as 200-220 mg/m^2, which denotes how unpredictably HF may occur in some patients.

The bell-shaped effects of CBR polymorphism on HF risk require further considerations. For the clinical study that characterized excess risk only for patients exposed to low-moderate dose

anthracycline it was suggested that high dose anthracycline saturated cardiac CBRs, such that unmetabolized anthracylines accumulated over metabolites and caused cardiotoxicity to progress independent of CBR polymorphism (Blanco et al., 2012). Here we suggest that secondary alcohol metabolites, being more toxic than parent anthracyclines, accelerate the course of development of cardiotoxicity. It follows that for each anthracycline there is a dose range over which CBR polymorphism increases the cardiac levels of toxic metabolite, dissipates defense mechanisms, and aggravates HF risk. At higher cumulative doses cardiac defense mechanisms collapse and cardiotoxicity progresses regardless of whether CBR is saturated or liable to a continued generation of metabolites.

From Pharmacology to Clinical Implications

This study supports and modifies the concept that there might be no safe dose of anthracyclines. It shows that HF risk from “safe” doses may be higher for DOX and DNR or IDA as compared to EPI. The pharmacokinetic model was nonetheless tailored to the dose of DOX that caused 5% risk in low risk adult subjects (Salvatorelli et al., 2017). Further risk aggravation should therefore be considered if anthracyclines, including EPI, were given to more vulnerable patients such as children-adolescents or the elderly. Comorbidities (hypertension, dyslipidemia, diabetes) would also aggravate HF risk by introducing hemodynamic stress or microvasculature dysfunction. Patients with CBR polymorphism would be exposed to the highest risk, particularly in cases when they were given DNR or IDA. Inasmuch as genotyping is not routinely performed, this information introduces concerns.

Contemporary anthracycline dosages rank low or very low compared to earlier seminal regimens. We would not recommend further dose reductions that might compromise oncologic efficacy. Strategies that diminish anthracycline C_{max} (prolonged infusions, liposomal anthracyclines) might be considered for reducing HF risk but the disadvantages of such strategies should be considered as well (Menna and Salvatorelli, 2017; Salvatorelli et al., 2015). Moreover, slow infusions did not diminish the circulating levels of DNROL (DeGregorio et al., 1982) or IDAOL

(Eksborg et al., 1997), which in fact represent a sizeable fraction of post-infusion cardiac pools induced by DNR, and by IDA in particular. Our findings rather suggest that all patients should receive a rigorous control of risk factors and an adequate cardiac surveillance.

Prophylactic administration of cardiovascular drugs, like β blockers or inhibitors of the renin-angiotensin II system, has been recommended also for low risk patients (Cardinale et al., 2015). The result of this study, showing a higher HF risk than previously reported, lend support to this recommendation. Also the iron chelator and topoisomerase 2β inhibitor, dexrazoxane, should receive a wider attention. Neither uncertainties about its mode of action (Doroshow, 2012; Zhang et al., 2012) nor unconfirmed safety concerns (Asselin et al., 2016) should limit the clinical use of dexrazoxane.

In conclusion, a pharmacokinetic model of human myocardium exposure shows that “safe” anthracycline doses may cause HF, with some analogues causing a higher risk compared to others. All patients candidate for anthracyclines should be given adequate strategies of cardioprotection and surveillance.

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

AUTHORSHIP CONTRIBUTIONS

Participated in research design: Salvatorelli, Menna, and Minotti

Conducted experiments: Salvatorelli and Minotti

Contributed new reagents or analytic tools: Menna, Chello and Covino

Performed data analysis: Salvatorelli, Menna, and Minotti

Wrote or contributed to the writing of the manuscript: Salvatorelli, Menna, Chello, Covino, and Minotti

REFERENCES

Anderlini P, Benjamin RS, Wong FC, Kantarjian HM, Andreeff M, Kornblau...S O'Brien SM, Mackay B, Ewer MS, and Pierce SA (1995) Idarubicin cardiotoxicity: a retrospective study in acute myeloid leukemia and myelodysplasia. *J Clin Oncol* **13**: 2827-2834.

Asselin BL, Devidas M, Chen L, Franco VI, Pullen J, Borowitz MJ, Hutchison RE, Ravindranath Y, Armenian SH, Camitta BM, and Lipshultz SE (2016) Cardioprotection and safety of dexrazoxane in patients treated for newly diagnosed T-cell acute lymphoblastic leukemia or advanced-stage lymphoblastic non-hodgkin lymphoma: A report of the Children's Oncology Group Randomized Trial Pediatric Oncology Group 9404. *J Clin Oncol* **34**: 854-862.

Blanco JG, Leisenring WM, Gonzalez-Covarrubias VM, Kawashima TI, Davies SM, Relling MV, Robison LL, Sklar CA, Stovall M, and Bhatia S (2008) Genetic polymorphisms in CBR3 and NQO1 in patients who developed anthracycline-related congestive heart failure after childhood cancer. *Cancer* **112**: 2789-2795.

Blanco JG, Sun CL, Landier W, Chen L, Esparza-Duran D, Leisenring W, Mays A, Friedman DL, Ginsberg JP, Hudson MM, Neglia JP, Oeffinger KC, Ritchey AK, Villaluna D, Relling MV, and Bhatia S (2012) Anthracycline-related cardiomyopathy after childhood cancer: role of polymorphisms in carbonyl reductase genes: A report from the Children's Oncology Group. *J Clin Oncol* **30**:1415-1421.

Bonadonna G and Monfardini S (1969) Cardiac toxicity of daunorubicin. *Lancet* **1**:837.

Boucek RJ, Olson RD, Brenner DE, Ogunbunmi EM, Inui M, and Fleischer S (1987) The major metabolite of doxorubicin is a potent inhibitor of membrane-associated ion pumps. *J Biol Chem* **262**:15851-15856.

Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, Civelli M, Lamantia G, Colombo N, Curigliano G, Fiorentini C, and Cipolla CM (2015) Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. *Circulation* **131**: 1981-8.

ChemAxon Ltd. (1998-2017) https://chemicalize.com

Danesi R, Innocenti F, Fogli S, Gennari A, Baldini E, Di Paolo A, Salvadori B, Bocci G, Conte PF, and Del Tacca M (2002) Pharmacokinetics and pharmacodynamics of combination chemotherapy with paclitaxel and epirubicin in breast cancer patients. *Br J Clin Pharmacol* **53**: 508-518.

DeGregorio MW, Carrera CJ, Klock JC, Pegelow CH, and Wilbur JR (1982) Cellular and plasma kinetics of daunorubicin given by two methods of administration in a patient with acute leukemia. *Cancer Treat Rep* **66**: 2085-2088.

Doroshow JH (2012) Dexrazoxane for the prevention of cardiac toxicity and treatment of extravasation injury from the anthracycline antibiotics. *Curr Pharm Biotechnol* **13**: 1949-1956.

Drafts BC, Twomley KM, D'Agostino R Jr, Lawrence J, Avis N, Ellis LR, Thohan V, Jordan J, Melin SA, Torti FM, Little WC, Hamilton CA, and Hundley WG (2013) Low to moderate dose anthracycline-based chemotherapy is associated with early noninvasive imaging evidence of subclinical cardiovascular disease. *J Am Coll Cardiol Cardiovasc Imaging* **6**:877-885.

Eksborg S, Björkholm M, Hast R, and Fagerlund E (1997) Plasma pharmacokinetics of idarubicin and its 13-dihydro metabolite-a comparison of bolus versus 2 h infusion during a 3 day course. *Anticancer Drugs* **8**: 42-47.

Ewer MS (2013) Anthracycline cardiotoxicity: Clinical aspects, recognition, monitoring, treatment, and prevention, in *Cancer and the Heart* (Ewer MS and Yeh ET eds) pp 11-41, People's Medical Publishing House, Shelton.

Gianni L, Vigano' L, Locatelli A, Capri G, Giani A, Tarenzi E, and Bonadonna G (1997) Human pharmacokinetic characterization and in vitro study of the interaction between doxorubicin and paclitaxel in patients with breast cancer. *J Clin Oncol* **15**:1906-1915.

Limat S, Demesmay K, Voillat L, Bernard Y, Deconinck E, Brion A, Sabbah A, Woronoff-Lemsi MC, and Cahn JY (2003) Early cardiotoxicity of the CHOP regimen in aggressive non-Hodgkin's lymphoma. *Ann Oncol* **14**:277-281.

Lo-Coco F, Avvisati G, Vignetti M, Thiede C, Orlando SM, Iacobelli S, Ferrara F, Fazi P, Cicconi L, Di Bona E, Specchia G, Sica S, Divona M, Levis A, Fiedler W, Cerqui E, Breccia M, Fioritoni G, Salih HR, Cazzola M, Melillo L, Carella AM, Brandts CH, Morra E, von Lilienfeld-Toal M, Hertenstein B, Wattad M, Lübbert M, Hänel M, Schmitz N, Link H, Kropp MG, Rambaldi A, La Nasa G, Luppi M, Ciceri F, Finizio O, Venditti A, Fabbiano F, Döhner K, Sauer M, Ganser A, Amadori S, Mandelli F, Döhner H, Ehninger G, Schlenk RF, and Platzbecker U; Gruppo Italiano Malattie Ematologiche dell'Adulto; German-Austrian Acute Myeloid Leukemia Study Group; Study Alliance Leukemia (2013) Retinoic acid and arsenic trioxide for acute promyelocytic leukemia. *N Engl J Med* **369**: 111-121

Menna P, Paz OG, Chello M, Covino E, Salvatorelli E, and Minotti G (2012) Anthracycline cardiotoxicity. *Expert Opin Drug Saf* **11** (Suppl 1): 21-36.

Menna P and Salvatorelli E (2017) Primary prevention strategies for anthracycline cardiotoxicity: A brief overview. *Chemotherapy* **62**: 159-168.

Minotti G, Menna P, Salvatorelli E, Cairo G, and Gianni L (2004) Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacol Rev* **56**:185-229.

Minotti G, Salvatorelli E, and Menna P (2010) Pharmacological foundations of cardio-oncology. *J Pharmacol Exp Ther* **334**:2-8.

Mordente A, Minotti G, Martorana GE, Silvestrini A, Giardina B, and Meucci E (2003) Anthracycline secondary alcohol metabolite formation in human or rabbit heart: biochemical aspects and pharmacologic implications. *Biochem Pharmacol* **66**:989-998.

Mushlin PS, Cusack BJ, Boucek RJ, Andrejuk T, Li X, and Olson RD (1993) Time-related increases in cardiac concentrations of doxorubicinol could interact with doxorubicin to depress myocardial contractile function. *Br J Pharmacol* **110**:975-982.

Ohtake S, Miyawaki S, Fujita H, Kiyoi H, Shinagawa K, Usui N, Okumura H, Miyamura K, Nakaseko C, Miyazaki Y, Fujieda A, Nagai T, Yamane T, Taniwaki M, Takahashi M, Yagasaki F, Kimura Y, Asou N, Sakamaki H, Handa H, Honda S, Ohnishi K, Naoe T, and Ohno R (2011) Randomized study of induction therapy comparing standard-dose idarubicin with high-dose daunorubicin in adult patients with previously untreated acute myeloid leukemia: the JALSG AML201 Study. *Blood* **117**: 2358-2365.

Olson RD and Mushlin PS (1990) Doxorubicin cardiotoxicity: analysis of prevailing hypotheses. *FASEB J* **4**:3076-3086.

Ryberg M, Nielsen D, Skovsgaard T, Hansen J, Jensen BV, and Dombernowsky P (1998) Epirubicin cardiotoxicity: an analysis of 469 patients with metastatic breast cancer. J Clin Oncol **16**: 3502-3508.

Salvatorelli E, Menna P, Gianni L, and Minotti G (2007) Defective taxane stimulation of epirubicinol formation in the human heart: Insight into the cardiac tolerability of epirubicin-taxane chemotherapies. *J Pharmacol Exptl Ther* **320**: 790-800.

Salvatorelli E, Menna P, Gonzalez Paz O, Surapaneni S, Aukerman SL, Chello M, Covino E, Sung V, and Minotti G (2012) Pharmacokinetic characterization of amrubicin cardiac safety in an ex vivo human myocardial strip model. II. Amrubicin shows metabolic advantages over doxorubicin and epirubicin. *J Pharmacol Exp Ther* **341**: 474-483.

Salvatorelli E, Menna P, Cantalupo E, Chello M, Covino E, Wolf FI, and Minotti G (2015) The concomitant management of cancer therapy and cardiac therapy. *Biochim Biophys Acta* **1848**:2727-2737.

Salvatorelli E, Menna P, Chello M, Covino E, and Minotti G (2017) Modeling human myocardium exposure to doxorubicin defines the risk of heart failure from low-dose doxorubicin. *J Pharmacol Exp Ther* **362**: 263-270.

Stewart, DJ, Grewaal D, Green RM, Mikhael N, Goel R, Montpetit VA, and Redmond MD (1993) Concentrations of doxorubicin and its metabolites in human autopsy heart and other tissues. *Anticancer Res* **13**: 1945-1952.

Swain SM, Whaley FS, and Ewer MS (2003) Congestive heart failure in patients treated with doxorubicin: a retrospective analysis of three trials. *Cancer* **97**:2869-2879.

Vejpongsa P and Yeh ET (2014) Prevention of anthracycline-induced cardiotoxicity: challenges and opportunities. *J Am Coll Cardiol* **64**: 938-945.

Visscher H, Ross CJ, Rassekh SR, Barhdadi A, Dubé MP, Al-Saloos H, Sandor GS, Caron HN, van Dalen EC, Kremer LC, van der Pal HJ, Brown AM, Rogers PC, Phillips MS, Rieder MJ, Carleton BC, and Hayden MR; Canadian Pharmacogenomics Network for Drug Safety Consortium (2012) Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. *J Clin Oncol* **30**: 1422-1428.

von Hoff DD, Rozencweig M, Layard M, Slavik M, Muggia FM (1977) Daunomycin-induced cardiotoxicity in children and adults: A review of 110 cases. *Am J Med* **62**:200-208.

Von Hoff DD, Layard MW, Basa P, Davis HL Jr, Von Hoff AL, Rozencweig M, Muggia FM (1979) Risk factors for doxorubicin-induced congestive heart failure. *Ann Intern Med* **91**: 710-717.

Winthrop pharmaceuticals (2010) Daunorubicin. Summary of Product Characteristics.

Zhang S, Liu X, Bawa-Khalfe T, Lu LS, Lyu YL, Liu LF, and Yeh ET (2012) Identification of the molecular basis of doxorubicin-induced cardiotoxicity. *Nat Med* **18**:1639-1642.

FOOTNOTES

This work was supported by University Campus Bio-Medico Special Project “Cardio-Oncology” (to G.M.)

Reprints request to:

Giorgio Minotti, MD
CIR and Drug Sciences
University Campus Bio-Medico
Via Alvaro del Portillo 21
00128 Rome-ITALY
Phone: 011-39-06-225419109
FAX 011-39-06-22541456
g.minotti@unicampus.it

LEGENDS FOR FIGURES

Figure 1 Post-infusion cardiac anthracycline pools

Cardiac anthracycline pools were quantified as described in Experimental Procedures. Absent CBR polymorphism, the pools of DOX, EPI and DNR or IDA were not significantly different ($P>0.05$ by ANOVA with Bonferroni's post test). In the presence of CBR polymorphism the pools of DNR and IDA were significantly larger than DOX or EPI ($P<0.001$). For each analog CBR polymorphism caused a significant increased anthracycline accumulation (paired Student's t test).

CBR^+, CBR polymorphism.

Figure 2 Risk versus dose curves and effects of CBR polymorphism

Risk versus dose curves were obtained by Equation [1] as described in Experimental Procedures.

CBR^+, CBR polymorphism

Figure 3 Analog and dose dependence of HF excess risk from CBR polymorphism

For each anthracycline HF excess risk from CBR polymorphism was determined by subtracting risk versus dose curves without CBR polymorphism from the corresponding curve with CBR polymorphism. Where indicated cumulative doses of EPI, DNR and IDA were converted to doxorubicin myelotoxic equivalents.

CBR^+, CBR polymorphism.

Figure 4. Dose-related HF risk in cohorts of patients equally distributed to receive myelotoxic equivalents of one of two different anthracyclines

Risk of HF was simulated for cohorts of patients with or without CBR polymorphism. Curves were obtained by point-by-point medians of risk values induced by the two anthracyclines represented in each cohort. Slopes were analyzed by ROC over 50 mg increments/m^2.

CBR^+, CBR polymorphism.

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

Figure 5. Dose-related HF risk in cohorts of patients equally distributed to receive myelotoxic equivalents of one of four different anthracyclines

Experimental conditions were as described in the legend for Figure 4.

CBR^+, CBR polymorphism.

Table 1

Clinical characteristics of myocardial sample donors

Patients	Age	Male	Female	Diabetes mellitus	Dyslipidemia	Hypertension	Atrial fibrillation	Comorbid-free patients
≤60 years (n=16)	57 (36-60)	14 (88%)	2 (12%)	4 (25%)	11 (69%)	13 (81%)	2* (13%)	2 (13%)
>60 years (n=70)	76 (61-86)	51 (73%)	19 (27%)	22 (31%)	56 (80%)	68 (97%)	9* (13%)	0
P	<0.001	0.336	0.334	0.761	0.332	0.043	1.000	0.033

Patient population (n=86) was composed of 44 sample donors for experiments with EPI, DNR or IDA (this study), and 42 sample donors for experiments with DOX (Salvatorelli et al., 2017).

Data were analysed by two-tailed unpaired Student's t test or Fisher exact test.

*P<0.001 versus dyslipidemia or hypertension

Table 2

Clinically-modelled exposure of human myocardium to anthracyclines and exogenous metabolites

Anthracycline	Infusion dose	Infusion duration	C_{max} (parent anthracycline)	Time at C_{max} (parent anthracycline)	C_{max} (exogenous metabolite)	Time at C_{max} (exogenous metabolite)	Reference
	mg/m^2	min	μM	min	μM	min	
DOX	60	5	10	15	0.1	30	Gianni et al., 2007
EPI	90	5	10	5	0.1	10	Danesi et al., 2002
DNR	50	<5-10	0.5	25	0.2	120	De Gregorio et al., 1982
IDA	10	3-10	day 1 0.3 day 2 0.3 day 3 0.3	12 12 12	0.02 0.04 0.06	240 240 240	Eksborg et al., 1997

IDAOL was used at three concentration levels that simulated stepwise increases of its C_{max} during the course of three infusions of IDA over three consecutive days. Because exogenous DNROL and IDAOL remain at C_{max} for ~12 or ~24 hours, respectively, time at C_{max} was simulated by normalizing 24-hour exposure in vivo to 4-hour incubation in vitro. Strips were therefore exposed to DNROL for 2h and to IDAOL for 4h. For DOXOL and EPIOL time at C_{max} was simulated in accordance to such normalization.

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

Table 3

Correction constants for equation-based HF risk calculation

CBR polymorphism	DOX	EPI	DNR	IDA
-	0.113	0.049	0.113	0.128
+	0.159	0.059	0.404	0.423

Correction constants were extrapolated by setting Equation [1] at 5% risk of HF. In the same equation the number of infusions associated with 5% risk of HF was expressed by the ratio of 5% risk pool to post-infusion infusion pool.

Table 4

Myocardial retention of parent anthracyclines and endogenous metabolites

Anthracycline	CBR polymorphism	Anthracycline uptake μM	Metabolite formation μM	Anthracycline retention μM	Metabolite retention μM
DOX	-	5.9 ± 2.0	0.02 ± 0.02	4.0 ± 1.8	0.02 ± 0.02
	+	5.9 ± 2.0	0.04 ± 0.05	3.9 ± 1.8	0.04 ± 0.05
EPI	-	4.2 ± 0.3	0.01 ± 0.01	2.9 ± 0.2	0.01 ± 0.01
	+	4.2 ± 0.3	0.014 ± 0.011	2.9 ± 0.2	0.014 ± 0.011
DNR	-	0.7 ± 0.3	0.33 ± 0.35	0.2 ± 0.1	0.06 ± 0.01
	+	0.7 ± 0.3	0.50 ± 0.40	0.1 ± 0.1	0.12 ± 0.11
IDA	-	1.3 ± 0.4	0.15 ± 0.08	0.5 ± 0.3	0.03 ± 0.01
	+	1.3 ± 0.4	0.30 ± 0.20	0.2 ± 0.3	0.06 ± 0.04

Values were means ± SD of 9 experiments for EPI, 16 experiments for IDA, 23 experiments for DNR. For DOX, data (n=9) were from Salvatorelli et al., 2017. Data were analysed by ANOVA with Bonferroni's post test for multiple comparisons. In the absence of CBR polymorphism i) anthracycline uptake and retention were significantly higher for DOX and EPI compared to DNR or IDA ($P<0.001$) ii), metabolite formation was significantly higher for DNR and IDA than DOX or EPI ($P<0.01$) iii), metabolite retention was significantly higher for DNR than DOX or EPI ($P<0.01$) and for IDA compared to DOX or EPI ($P<0.05$, unpaired Student's t test). In the presence of CBR polymorphism i) metabolite formation was significantly higher for DNR and IDA than DOX or EPI ($P<0.01$) ii), metabolite retention was higher for DNR and IDA than DOX or EPI ($P<0.05$, unpaired Student's t test).

JPET Fast Forward. Published on December 8, 2017 as DOI: 10.1124/jpet.117.246140
This article has not been copyedited and formatted. The final version may differ from this version.

Table 5

Myocardial uptake and retention of exogenous metabolites.

Metabolite	CBR polymorphism	Uptake μM	Retention μM
DOXOL	- +	n.d. n.d.	n.d. n.d.
EPIOL	- +	n.d. n.d	n.d. n.d.
DNROL	- +	0.110 ± 0.032 0.221 ± 0.065	0.048 ± 0.010 0.096 ± 0.018
IDAOL			
day 1	- +	n.a. n.a.	0.015 ± 0.004 0.029 ± 0.003
day 2	- +	n.a. n.a.	0.023 ± 0.004 0.035 ± 0.011
day 3	- +	n.a. n.a.	0.041 ± 0.013 0.081 ± 0.030
cumulative	- +	n.a. n.a.	0.078 ± 0.010 0.160 ± 0.019

Number of replicates were as described in the legend to Table 4. Cumulative retention of IDAOL was significantly higher than DNROL retention both in absence and presence of polymorphism ($P<0.001$, unpaired Student's t test). For IDAOL, time at Cmax was 4 hours, and therefore separate determinations of uptake and retention were not assessable (n.a.) (see also legend to Table 4).

n.d., not detectable.

Table 6

Experimental versus clinical anthracycline doses associated with 5% or 1-2% HF risk

HF risk	CBR polymorphism	DOX		EPI		DNR		IDA	
		experimental	clinical	experimental	clinical	experimental	clinical	experimental	clinical
5%	-	380	400	920	900	360	600-800	180	290
	+	330 (-13%)	-	840 (-9%)	-	190 (-47%)	-	105 (-42%)	-
1-2%	-	220-280	300-340	580-720	600-700	170-270	-	100-130	-
	+	180-230 (-18%) (-18%)	-	510-650 (-12%) (-10%)	-	120-160 (-29%) (-41%)	-	65-80 (-35%) (-38%)	-

Clinical values were obtained or extrapolated from Anderlini et al. (1995), Ryberg et al. (1998), Swain et al. (2003), von Hoff et al. (1977), Winthrop pharmaceuticals (2010). Bracketed value indicate risk dose percentage decrements induced by CBR polymorphism. Missing data denote a lack of firmly established clinical doses.

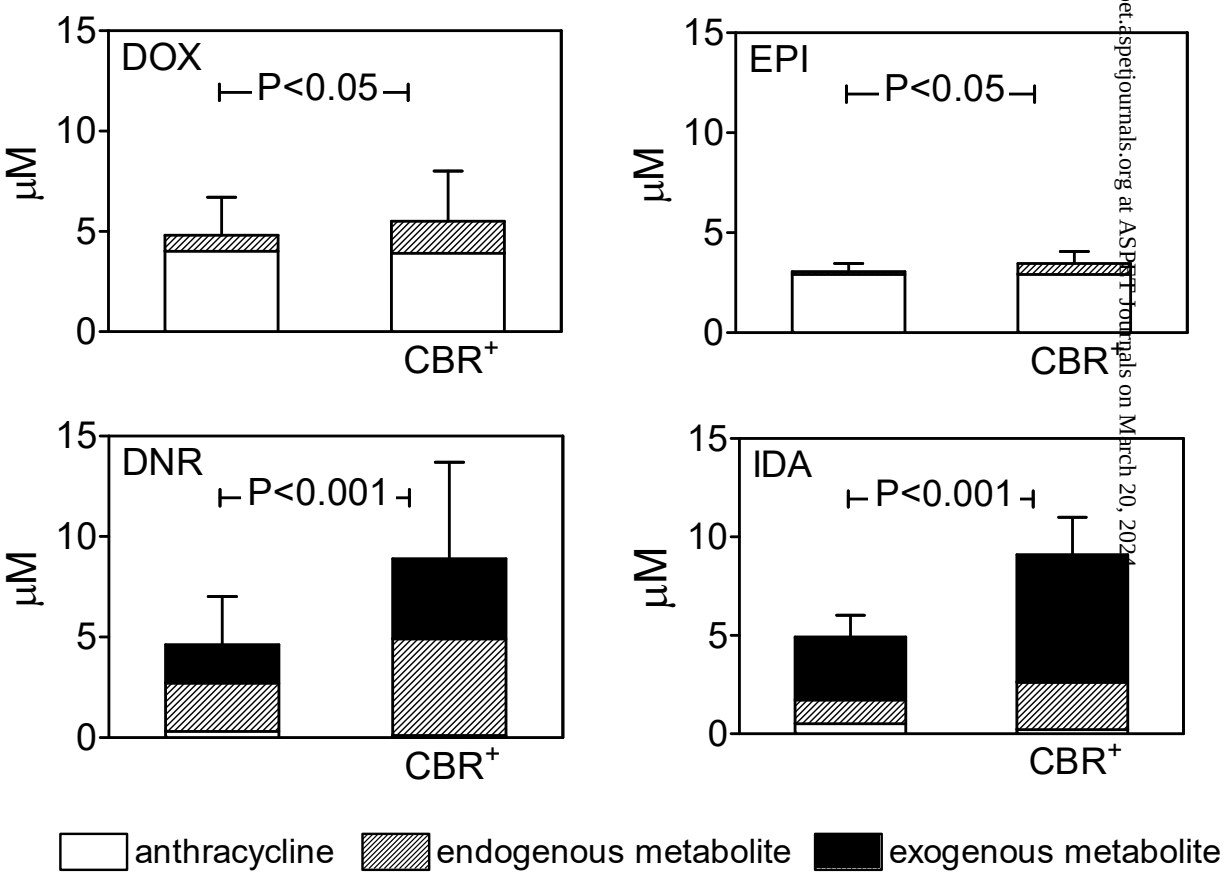

Figure 1

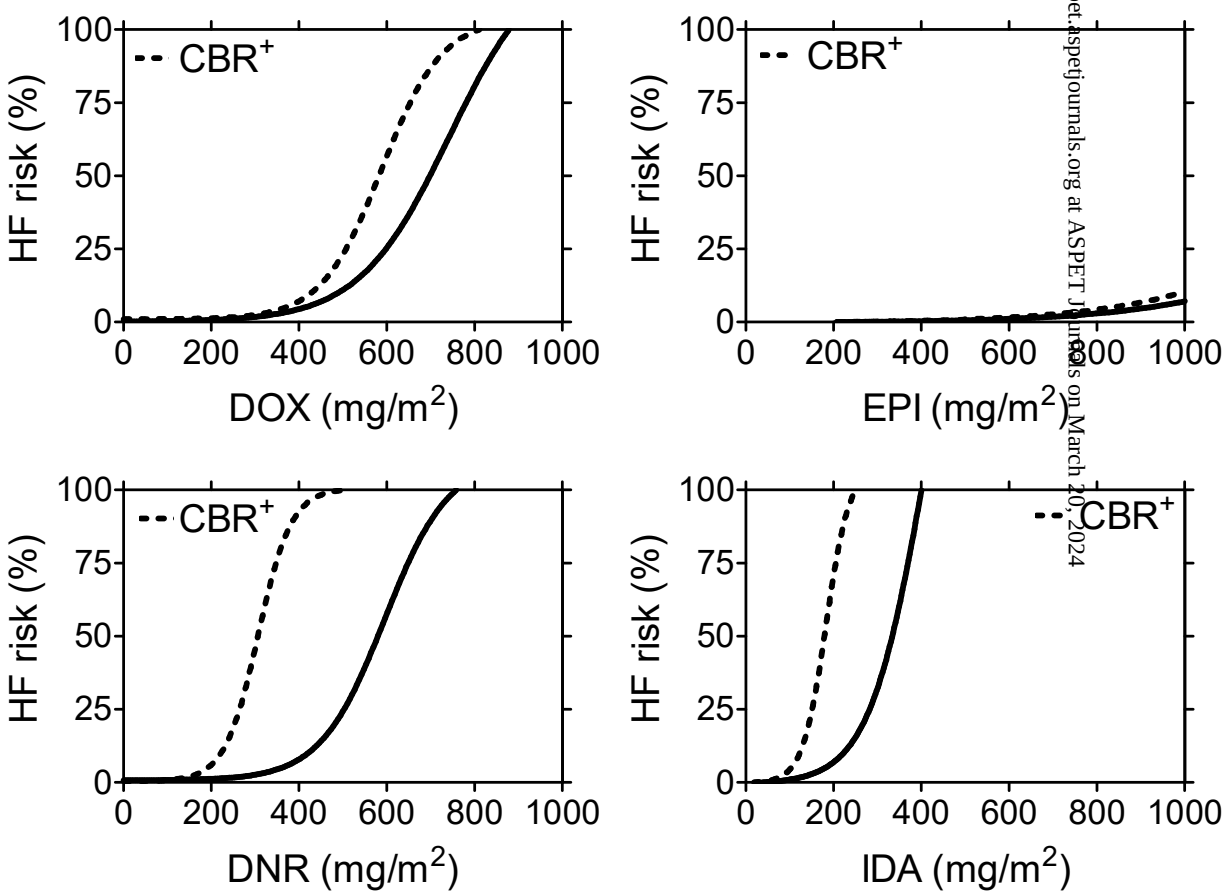

Figure 2

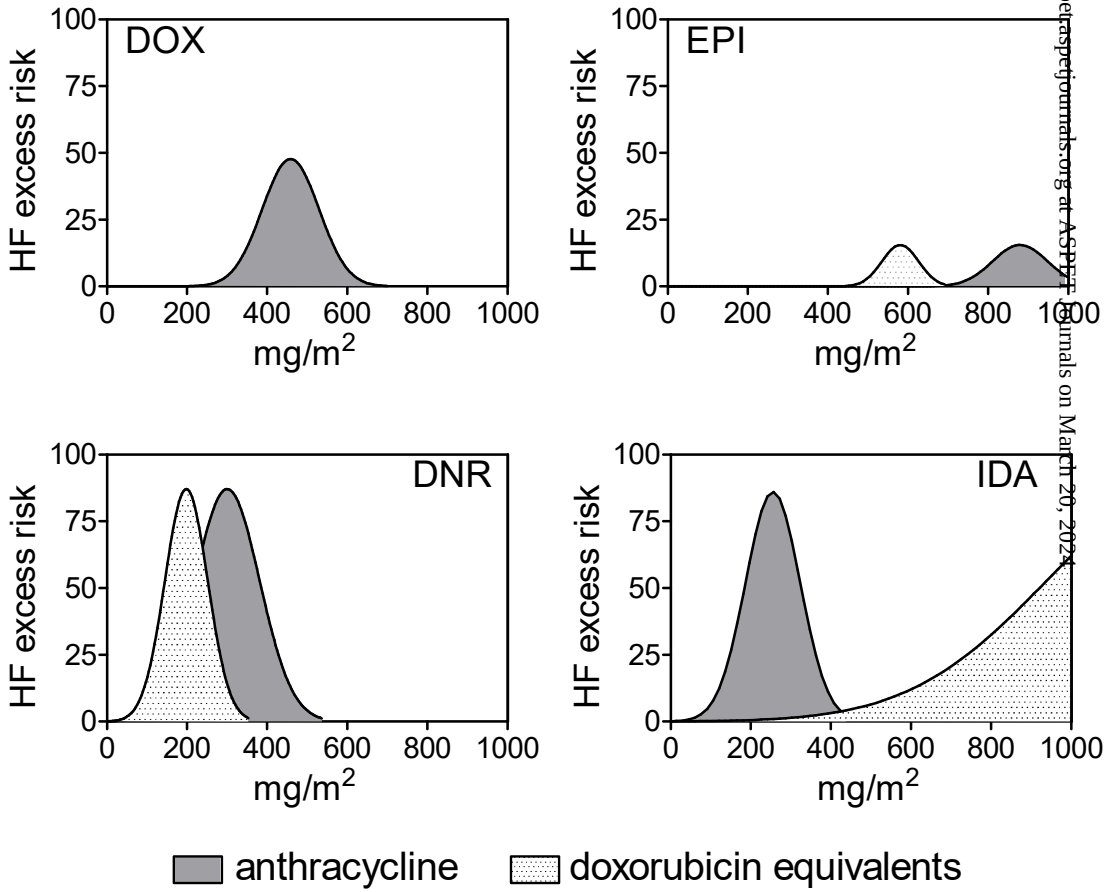

Figure 3

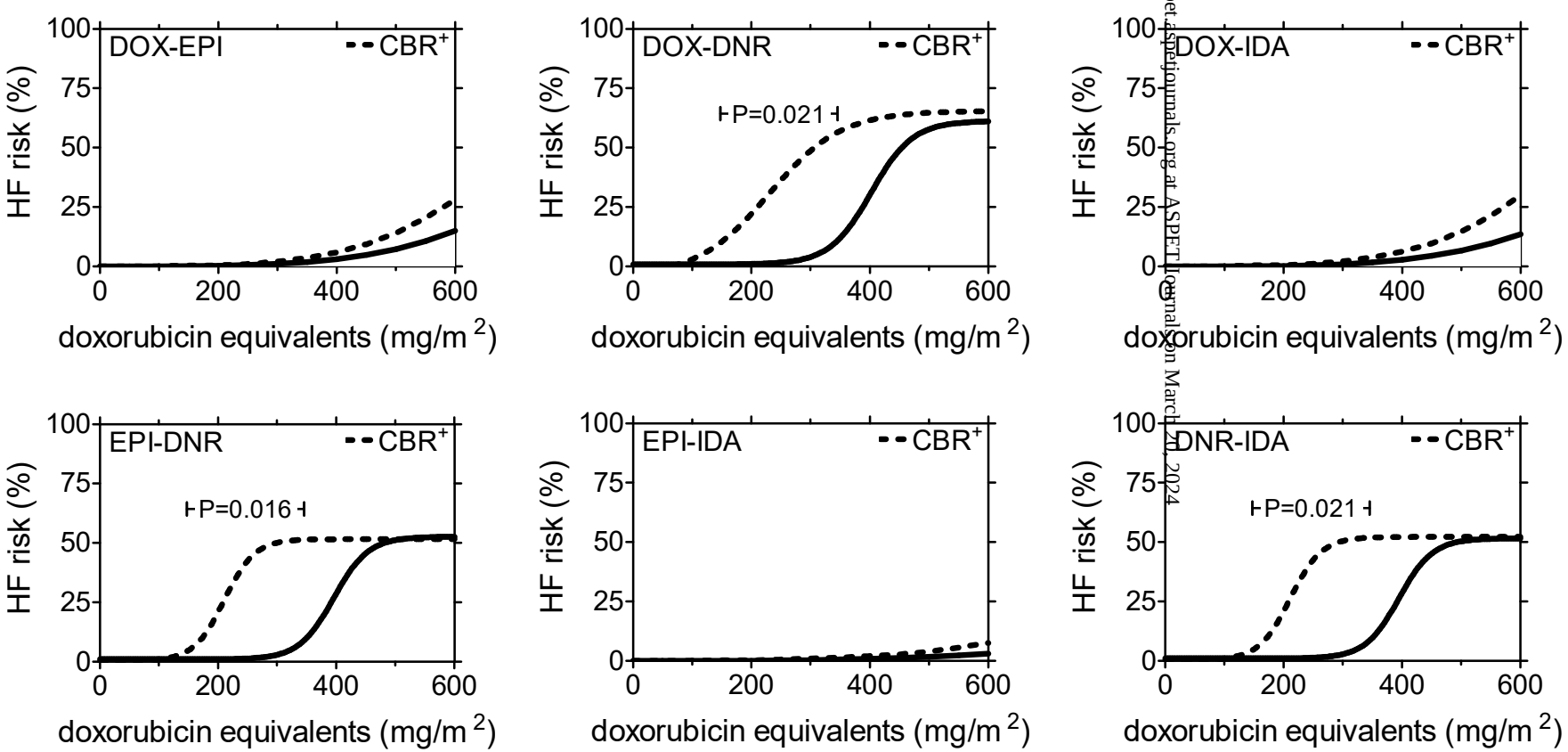

Figure 4

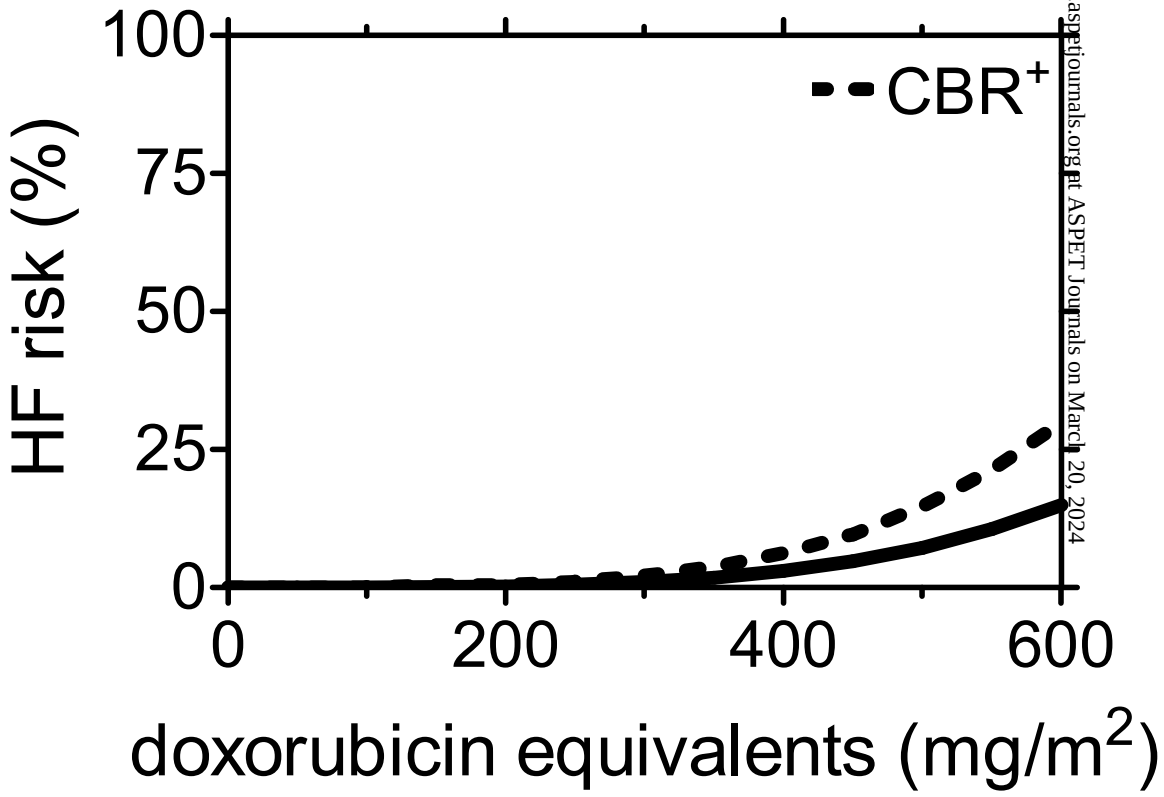

Figure 5