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Doxorubicin pathways: pharmacodynamics and adverse effects

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ABCC1; ABCC2; anthracyclines; cardiotoxicity; CAT; CBR3; CYBA; doxorubicin; drug resistance; NCF4; pharmacogenomics; PharmGKB pathways; RAC2

Introduction

The goal of this study is to give a brief background on the literature supporting the PharmGKB pathway about doxorubicin action, and provides a summary of this active area of research. The reader is referred to recent in-depth reviews [1–4] for more detailed discussion of this important and complex pathway. Doxorubicin is an anthracyline drug first extracted from *Streptomyces peucetius var. caesius* in the 1970's and routinely used in the treatment of several cancers including breast, lung, gastric, ovarian, thyroid, non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, sarcoma, and pediatric cancers [5–7]. A major limitation for the use of doxorubicin is cardiotoxicity, with the total cumulative dose being the only criteria currently used to predict the toxicity [4,8]. As there is evidence that the mechanisms of anticancer action and of cardiotoxicity occur through different pathways there is hope for the development of anthracycline drugs with equal efficacy but reduced toxicity [4]. Knowledge of the pharmacogenomics of these pathways may eventually allow for future selection of patients more likely to achieve efficacy at lower doses or able to withstand higher doses with lesser toxicity. We present here graphical representations of the candidate genes for the pharmacogenomics of doxorubicin action in a stylized cancer cell (Fig. 1) and toxicity in cardiomyocytes (Fig. 2), and a table describing the key variants examined so far.

Mechanisms of anticancer pharmacodynamics

There are two proposed mechanisms by which doxorubicin acts in the cancer cell (i) intercalation into DNA and disruption of topoisomerase-II-mediated DNA repair and (ii) generation of free radicals and their damage to cellular membranes, DNA and proteins

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(shown in Fig. 1) [9]. In brief, doxorubicin is oxidized to semiquinone, an unstable metabolite, which is converted back to doxorubicin in a process that releases reactive oxygen species. Reactive oxygen species can lead to lipid peroxidation and membrane damage, DNA damage, oxidative stress, and triggers apoptotic pathways of cell death [10]. Candidate genes that may modulate this pathway involve those capable of the oxidation reaction (NADH dehydrogenases, nitric oxide synthases, xanthine oxidase) [11,12] and those capable of deactivating the free radicals such as glutathione peroxidase, catalase, and superoxide dismutase. Alternatively, doxorubicin can enter the nucleus and poison topoisomerase-II, also resulting in DNA damage and cell death [13]. Candidate pharmacogenes for this part of the pathway include the enzymes involved in the DNA repair mechanisms and the cell cycle control (*TOP2A, MLH1, MSH2, TP53,* and *ERCC2* genes). Although the evidence for some of these candidate genes is unrefutable (*TOP2A*) [13,14] others are included based on the data from model systems but the polymorphic nature may be worth exploring in PGx studies [15,16].

Mechanisms of cardiotoxicity

The exact mechanism of cardiotoxicity of doxorubicin is somewhat controversial. There are two main theories (i) iron-related free radicals and formation of doxorubicinol metabolite and (ii) mitochondrial disruption, which is somewhat intertwined (reviewed in [17] and [18] and shown in Fig. 2). One of the strongest pieces of supporting evidence for the iron hypothesis is that the iron chelator, dexrazoxane is protective against doxorubicin-induced toxicity *in vivo* [19]. The best evidence supporting the mitochondrial hypothesis is in the association of the genetic variants in several component genes of the mitochondrial NAD(P)H oxidase complex with doxorubicin cardiotoxicity in the pharmacogenetic studies (see pharmacogenetics section for details) [20,21].

In brief, doxorubicin can be reduced to doxorubicinol, a metabolite that interferes with iron (by ACO1) and calcium regulations (by the calcium pump of sarcoplasmic reticulum, ATP2A2 and the Na+/K+ pump of sarcolemma, RYR2) and the F0F1 proton pump of mitochondria (coded by the *ATP5* gene family) [3,22,23]. Candidate genes for the formation of doxorubicinol are *AKR1C3*, *AKR1A1*, *CBR1*, and *CBR3*. (For more details of the doxorubicin metabolism see the doxorubicin pharmacokinetics (PK) pathway at PharmGKB). Candidate genes involved in the generation of reactive oxygen species or reactive nitrogen species from doxorubicin metabolism include nitric oxide synthases [24] and NAD(P)H oxidase complex genes *NCF4*, *CYBA*, and *RAC2* [20]. Metabolism of doxorubicin within the mitochondria can disrupt respiration and leads to the release of cytochrome-C initiating apoptosis [25].

The mechanism of the action of dexrazoxane protection against cardiotoxicity may be by sequestration of iron preventing free radical formation. However, as other iron chelators, such as deferasirox, fail to exert the protective effects of dexrazoxane [26] an alternative mechanism is by the interaction with TOP2B that prevents doxorubicin from inducing DNA damage [3,27].

Finding a way to maintain the efficacy and reduce toxicity has been one of the major areas of focus of anthracycline research. Although dexrazoxane reduces toxicity when given with doxorubicin, efficacy is also reduced so this combination is used only after a cumulative dose threshold has been reached [28]. Other anthracyclines, daunorubicin, epirubicin, and idarubicin also result in cardiotoxicity to varying degrees [7]. Daunorubicin is also considered as cardiotoxic as doxorubicin [29]. Epirubicin was less toxic than doxorubicin in animal models [29] and some in-vivo data showed less cardiotoxicity for epirubicin [7]. Although a recent Cochrane review and metaanalysis concluded that there was no

significant difference between occurrence of clinical heart failure between doxorubicin and epirubicin when looking at the randomized clinical trial data [28]. However, liposomal formulation of doxorubicin was shown to be less cardiotoxic than traditional doxorubicin without compromising efficacy in adults with solid tumors [28]. Idarubicin was also less cardiotoxic in animal models [30] but the Cochrane review did not find sufficient evidence from randomized studies to support any direct comparison *in vivo* [28].

As with most cancer treatments doxorubicin is rarely given in isolation. Most of the in-vivo studies involve cotreatment with other antineoplastic agents such as taxanes, platinum drugs, nitrogen mustard analogs, fluoropyrimidines, and vinca alkaloids, which can complicate the association of variants with a particular treatment. The reports of drug–drug interaction have been shown for doxorubicin with phenytoin [31,32] and cyclosporine [33], likely by ABCB1, and sorafenib by RALBP1 [34]. More clinically relevant are the drug–drug interactions resulting in the cardiotoxicity from cotreatment with doxorubicin and trastuzumab or taxanes such as paclitaxel and docetaxel [35]. Trastuzumab has Food and Drug Administration black box warning for cardiomyopathy that cautions against concurrent use of anthracyclines. Trastuzumab blocks the ERBB2 signaling of NRG1, a cardioprotective pathway that protects against stress. Without this endogenous cardioprotection, doxorubicin treatment can be more damaging [35]. The interaction with taxanes is through a different mechanism. In model systems, paclitaxel potentiates the cardiotoxicity of doxorubicin; this is less pronounced for docetaxel. Paclitaxel also potentiates the cardiotoxicity of epirubicin, with epirubicin plus docetaxel being the least toxic combination [29]. The mechanism for the taxane-induced increase in cardiotoxicity is by increased formation of doxorubicinol, by the modulation of the catalytic activity of aldehyde reductase [35].

Resistance

Although doxorubicin is a valuable clinical antineoplastic agent, in addition to problems with cardiotoxicity, resistance is also a problem limiting its use [7,36]. The mechanism of resistance involves ABCB1 (MDR1, Pgp) [37] and ABCC1 (MRP1) [38] and other transporters (ABCC2, ABCC3, ABCG2, and RALBP1) [1,39,40]. Another mechanism of doxorubicin resistance is the amplification of TOP2A [41], which has been shown to affect the treatment response [14,42]. The amplification of TOP2A has a complicated relationship to neighboring gene *HER-2* (*ERBB2*), used as a marker for breast cancer treatments in particular the HER-2-targeted trastuzumab (reviewed in [14]). The amplification of *ERBB2* gene also affects the doxorubicin response (see below) [14].

Pharmacogenomics

There are considerable interindividual variations in the pharmacokinetic parameters of doxorubicin and doxorubicinol [43]. However, the impact of genetic variants on doxorubicin response has only been studied recently. Cumulative anthracycline dose is the only confirmed significant risk factor for doxorubicin-induced cardiotoxicity [44]. So far, most studies have looked at the effects of variation on PK or resistance with only a few examining clinical outcomes such as cardiotoxicity or survival (summarized in Table 1). Below, we highlight variants associated with clinical phenotypes.

Variants in ABCC1 (rs45511401), ABCC2 (rs8187694, rs8187710), CAT (rs10836235), CBR3 (rs1056892), CYBA (rs4673), NCF4 (rs1883112), and RAC2 (rs13058338) are associated with cardiotoxicity *in vivo* [20,21,50,56]. Wojnowski *et al.* [20] in a study of single nucleotide polymorphisms of 82 genes from 1697 patients, 3.2% of whom developed either acute or chronic doxorubicin-induced cardiotoxicity, found five significant associations between cardiotoxicity and polymorphisms of the NAD(P)H oxidase complex

(CYBA, NCF4, and RAC2), and doxorubicin transporters. Consistent with this, mice deficient in NAD(P)H oxidase activity, unlike wild-type mice, were resistant to the chronic doxorubicin treatment [20]. A recent study by Rossi *et al.* [21] in lymphoma patients treated with doxorubicin-containing chemotherapy also showed an association of CYBA (rs4673) and NCF4 (rs1883112) with toxicity.

In a small study, Blanco *et al.* [56] suggested the CBR3 Val244Met polymorphism (rs1056892) might have an impact on the risk of anthracycline-related congestive heart failure among childhood cancer survivors. This variant was also associated with higher doxorubicinol area under the curve and higher CBR3 expression in the tumor tissue from Asian breast cancer patients [54]. However, another study of Asian breast cancer patients showed no effect of this variant on PK [52]. The in-vitro studies of this variant are conflicting with some showing decreased activity of the variant protein with doxorubicin as a substrate [56,62] and others showing increased activity using menadione as a substrate [57].

A variant in *PXR* (*NR1I3*) affects doxorubicin clearance *in vivo* [60] and may be a candidate gene for the doxorubicin resistance because of its role in regulating the transporter expression [1]. Lal *et al.* [1] suggest additional transporters as candidate genes in their recent review, including *ABCB5*, *ABCB8*, and *ABCC5*, although none have yet been shown to have doxorubicin-related phenotypes.

In addition to studies of single nucleotide polymorphisms, there have also been PGx studies of gene copy number for *ERBB2, TOP2A*, and *GST*s. Metaanalyses suggest that anthracycline-containing regimens provide more benefit than nonanthracycline-containing regimens in women whose tumors have multiple copies of *ERBB2* gene [14,42] (approximately half of the studies examined had regimens that contained doxorubicin, the rest were epirubicin). Amplification of *TOP2A* gene is also proposed as a biomarker for doxorubicin response although some results have been contradictory [14,42].

Conclusion

There have been substantial studies on the doxorubicin mechanism of action and so we know many of the genes that modulate the doxorubicin response. However, PGx studies that implicate variants in these genes are still in their infancy. As with many antineoplastic drugs, the PGx can be complicated by combined treatments. However, there are clear benefits for identifying individuals at risk for toxicity and response. Assembling the PGx candidates should help us learn how to identify individuals at elevated risk for doxorubicin-related cardiotoxicity or treatment failure. There remains a need for large studies that can simultaneously examine expression and copy number biomarkers and single nucleotide polymorphisms in genes related to all aspects of doxorubicin PD and PK to clarify the complete picture of doxorubicin PGx.

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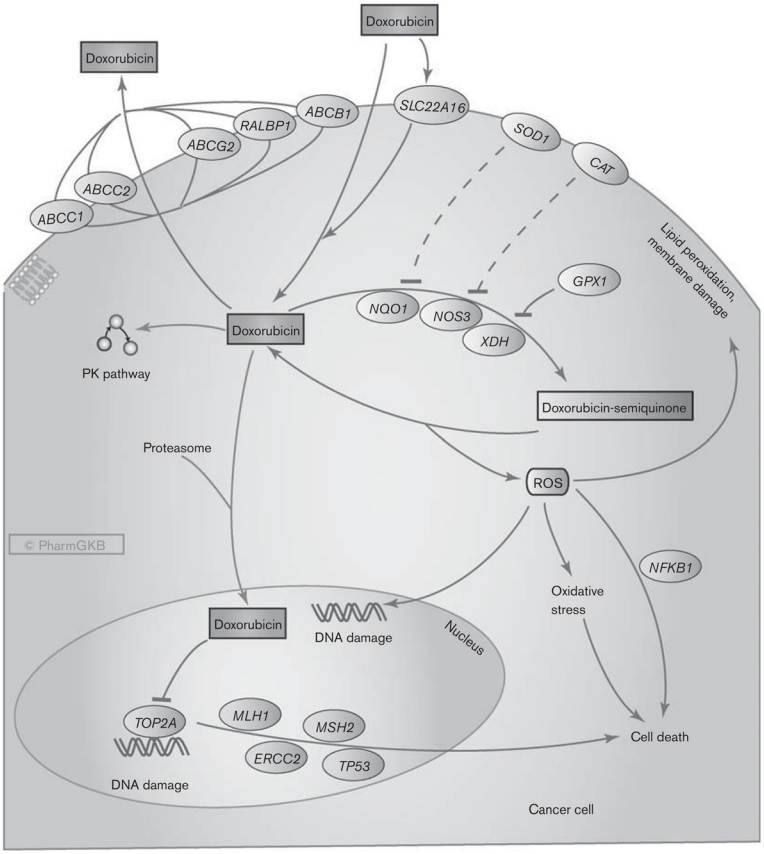

Fig. 1.

Graphical representation of the candidate genes involved in the pharmacodynamics of doxorubicin in a stylized cancer cell. A fully interactive version of this pathway is available online at PharmGKB at http://www.pharmgkb.org/do/serve?objId=PA165292163&objCls=Pathway. PK pathway, pharmacokinetics pathway; ROS, reactive oxygen species.

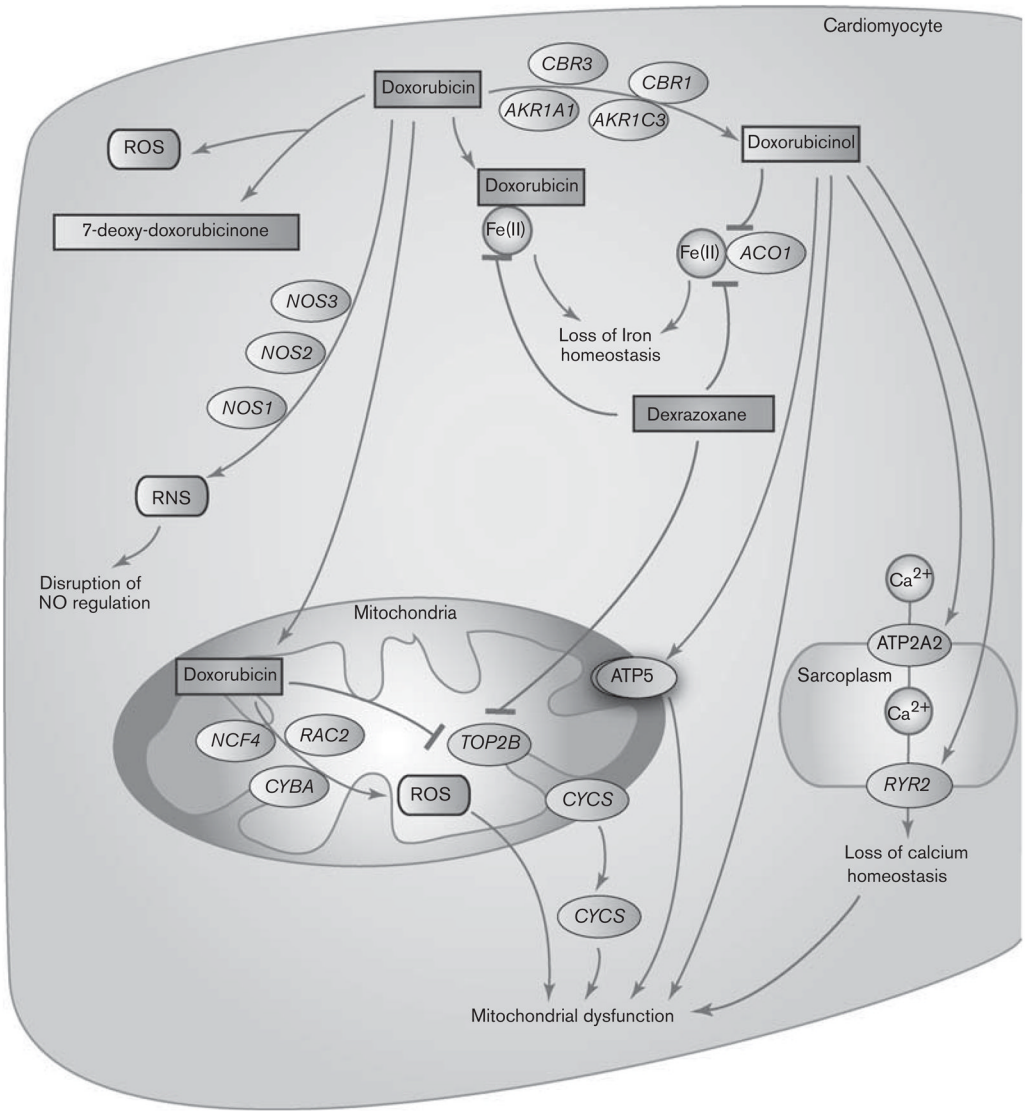

Fig. 2.

Graphical representation of the candidate genes involved in the cardiotoxicity of doxorubicin. A fully interactive version of this pathway is available online at PharmGKB at http://www.pharmgkb.org/do/serve?objId=PA165292164&objCls=Pathway. NO, nitrogen oxide; RNS, reactive nitrogen species; ROS, reactive oxygen species.

Table 1

Pharmacogenomic studies of doxorubicin

Gene	rs#	Common names	Variant	Phenotype *in vivo*	Phenotype *in vitro*	PMIDs
ABCB1	rs1128503	1236C> T	T	Increased drug exposure, reduced clearance[a]		18377430 [45]
ABCB1	rs2032582	2677G>A/T	A/T	Increased drug exposure, reduced clearance[a]		18377430 [45]
ABCB1	rs1045642	3435C > T	T	Increased drug exposure, reduced clearance[a]		18377430 [45]
ABCB1	rs35810889	Met89Thr	C		Increased resistance	17352537[b] [46]
ABCB1	rs35657960	Leu662Arg	G		Increased resistance	17352537[b] [46]
ABCB1	rs35023033	Arg669Cys	T		Increased resistance	17352537[b] [46]
ABCB1	rs2229107	Ser1141Thr	A		Increased resistance	17352537[b] [46]
ABCB1	rs35730308	Trp1108Arg	C		Reduced resistance	17352537[b] [46]
ABCC1	rs45511401	Gly671Val	T	Cardiotoxicity	No difference in transport	16330681 [20], 11721885 [47]
ABCC1	rs60782127	Arg433Ser	T		Increased resistance	12042670 [48]
ABCC1	rs4148356	Arg723Gln	A		Reduced resistance	19214144 [49]
ABCC2	rs17222723	Val1188Glu	A	Acute cardiotoxicity[a]		16330681 [20]
ABCC2	rs8187710	Cys1515Tyr	A	Acute cardiotoxicity[a]		16330681 [20]
ABCG2	rs2231142	421C > A	C/A	No difference in PK		18377430 [45]
CAT	rs10836235	c.66 + 78C> T	T	Cardiotoxicity		19863340 [50]
CAT	rs1001179	−262C > T	T	No association		19863340 [50]
CBR1	rs1143663	Val88Ile	A		Reduced activity	19204081 [51]
CBR1	rs41557318	Pro131Ser	T		Reduced activity	19204081 [51]
CBR1	rs20572	c.627C> T	T	Lower clearance, higher exposure[a]		19016765 [52]
CBR1	rs9024	1096G>A	A	Lower clearance, higher exposure[a]	Reduced activity	19016765 [52], 19022938[b] [53]
CBR3	rs8133052	Cys4Tyr	A	Lower ratio of doxorubicinol plasma AUC/doxorubicin AUC; lower expression in breast tumor, no effect	Higher V_{max} lower K_m	18551042 [54], 20007405 [55], 19016765 [52]
CBR3	rs1056892	Val244Met	A	Higher doxorubicinol AUC; higher expression in tumor tissue; risk of heart failure (trend); no effect	Conflicting reports of reduced activity and higher activity	18551042 [54], 18457324 [56], 20007405 [55], 15537833 [57], 19016765 [52]
CBR3	rs2835285	Val93Ile	T		Reduced activity	20007405 [55]
CBR3	rs4987121	Met235Leu	T		Reduced activity	20007405 [55]
CYBA	rs4673	His72Tyr	T	Acute cardiotoxicity, poorer event-free survival		16330681 [20], 19448608 [21]

Gene	rs#	Common names	Variant	Phenotype *in vivo*	Phenotype *in vitro*	PMIDs
GSTM1		0[a]	Null	No association		19863340 [50]
GSTT1		0[a]	Null	No association		19863340 [50]
ERBB2		HER-2 Amplification	Multi	Improved disease-free survival, improved overall survival 1		8258981 [42], 19758759 [14]
NCF4	rs1883112	−212A >G	G	Chronic cardiotoxicity, hematological toxicity		16330681 [20], 19448608 [21]
NOS3	rs2070744	−786T >C	C	Increased risk of cancer recurrence after chemotherapy that included doxorubicin		19671875 [58]
NOS3	rs1799983	894G> T Glu289Asp	T	Increased risk of cancer recurrence after chemotherapy that included doxorubicin		19671875 [58]
NQO1	rs1800566	NQO1*2	T	No association	Very low activity	18457324 [56], 15047100 [59]
NR1I2	n/a	PXR*1B, 2654T >C	C	Lower doxorubicin clearance	Reduced mRNA expression of PXR, CYP3A4, and ABCB1	18981011 [60]
RAC2	rs13058338	7508T > A	A	Acute cardiotoxicity		16330681 [20]
SLC22A16	rs714368	146A >G His49Arg	G	Increased drug exposure		17559346 [61]
SOD2	rs4880	Val16Ala	C	No association		19863340 [50]
TOP2A		topo II alpha Amplification	Multi	Increased response		19758759 [14]

PMIDs, PubMed Identifiers.

[a]As part of haplotype.

[b]Primary data available on PharmGKB at (http://www.pharmgkb.org/do/serve?objCls=Drug&objId=PA449412&tabType=tabGenetics#tabview=tab7).