Published in final edited form as:

J Oncol Pharm Pract. 2013 September ; 19(3): 269–272. doi:10.1177/1078155212461022.

Unexpected Doxorubicin-Mediated Cardiotoxicity in Sisters – Possible Role of Polymorphisms in Histamine N-Methyl Transferase

Kamakshi Sachidanandam, PhD¹, Arlene A. Gayle, MD², H. Ian Robins, MD, PhD^{2,3}, and Jill M. Kolesar, PharmD^{1,3}

¹University of Wisconsin School of Pharmacy, Madison, WI 53705, USA

²University of Wisconsin School of Medicine and Public Health, Madison, WI 53705, USA

³University of Wisconsin Carbone Cancer Center, Madison, WI 53792, USA

Abstract

The anthracycline anticancer agent doxorubicin (DOX) has long been recognized to induce a dose-limiting cardiotoxicity and may be associated with genes relevant to doxorubicin disposition. Recent reports suggest a role for a number of single nucleotide polymorphisms in anthracycline cardiotoxicity (ACT) in children. We describe two adult sisters with ACT who developed after relatively low dose doxorubicin. One sister carried the variant genotype for histamine N-ethyl transferase (HNMT, rs17583889) while the other was heterozygous, suggesting a similar role for these genotypes in adults with ACT. Although this requires further study, these genotypes may be important in the clinical dosing, or use of the liposomal formulation of doxorubicin.

Keywords

Doxorubicin; Cardiotoxicity; Polymorphism

Background

The anthracycline anticancer agent doxorubicin (DOX) has long been recognized to induce a dose-limiting cardiotoxicity ¹. Anthracycline cardiotoxicity (ACT) may be acute or chronic. Recent studies postulate a distinct late-onset cardiotoxicity, which develops after years or even decades of asymptomatic survival ^{2, 3}. Transgenic mouse models have demonstrated a genetic etiology in the development of chronic ACT ^{3, 4}. The overexpression of the multiple drug-resistant gene, MDR1, protects from the cardiotoxic effect of doxorubicin ^{4, 5}. In humans, the presence of a genetic component is suggested by the wide variation and individual sensitivities to anthracyclines ^{6, 7}. Wojnowski et al. identified that genetic variants in the transport of doxorubicin and free radical metabolism may be pivotal in modulating individual risk of ACT ³. Carbonyl reductases (CBR) catalyze the NADPH-dependent

Please send correspondence to: Jill M. Kolesar, PharmD, 600 Highland Ave., Room K4/554, Madison, WI 53792-5669, USA, Tel: +1 608 263 5369; Fax: +1 608 265 8133; jmkolesar@pharmacy.wisc.edu.

Conflict of interest: The authors declare that no conflicts of interests exist.

reduction of anthracyclines and resulting metabolites are implicated in ACT⁸⁻¹¹. In this regard, it was concluded that the molecular basis of adriamycin dependent development of cardiotoxicity is still far from being clear.

Relevant to this background, we had reported two unexpected cases (on the basis of low DOX exposure) of severe presumed doxorubicin (DOX)-induced cardiac toxicity in two sisters receiving adjuvant breast cancer therapy¹². Twelve single nucleotide polymorphisms (SNPs) in ATP Binding Cassette subfamily B member 1 (ABCB1), Nicotinamide Adenine Dinucleotide Phosphate (NADPH) oxidase and Carbonyl Reductase (CBR) 1 and 3 that were previously reported to have an association with ACT were initially evaluated in the two patients and no correlations were found¹². Visscher et al. and Blanco et al. recently reported that there was an association of polymorphisms in CBR1, histamine N-ethyl transferase (HNMT) and ABCB4 in children with ACT^{13, 14}. The goal of this study was to examine whether the same relationships held true in adults with ACT.

Case Reports

Detailed case reports of these two sisters have been previously published¹².

Experimental Methods

The genotyping analysis of this retrospective study was performed in the Analytical Instrumentation Laboratory for Pharmacokinetics, Pharmacodynamics and Pharmacogenetics, University of Wisconsin Comprehensive Cancer Center, Madison, Wisconsin, USA). The patients' consent to participate in this study was obtained in compliance with existing institutional review board requirements. Briefly, genomic DNA was extracted from the patients' whole blood using standardized extraction procedures and quantified using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA). PCR was used to amplify the regions of the genomic DNA containing the selected SNPs. The final PCR reaction mixture contained forward and reverse primers (10 pmol each; one of the primers is biotinylated at the 5' end), template DNA, 1X PCR master mix (Promega, Madison Wisconsin, USA) and nuclease-free water. The primers (sequence information available upon request) were designed using the Pyromark Assay Design 2.0 software (Qiagen, Valencia, California, USA) and synthesized by Integrated DNA Technologies (Coralville, Iowa, USA; primer sequences available upon request). The biotinylated PCR product was immobilized on streptavidin-coated sepharose beads (GE Healthcare, Piscataway, NJ), fixed with 70% ethanol, denatured with 10 mM NaOH, washed with 200 mM Tris-acetate, pH 7.6, and finally annealed to the specific sequencing primer. The samples were subsequently analyzed using PyroGold reagents (Qiagen, Valencia, CA) on a PSQ96 MD pyrosequencer (Qiagen). Genotyping was performed for SNPs in the CBR1 (rs9024), HNMT (rs17583889) and ABCB4 (rs1149222) genes respectively.

Results

There were no significant correlations with CBR1 and ABCB4 SNPs. However, one sister carried the variant genotype for HNMT rs17583889 while the other was heterozygous,

which classified them as high-risk and intermediate risk respectively, for the development of ACT. A summary of genotyping results are outlined in Table 1.

Discussion

Anthracycline mediated cardiotoxicity is oftentimes associated with polymorphisms in genes involved in its transport and metabolism ²⁻¹¹. Our previous study involving two sisters who presented with cardiotoxicity after prolonged exposure to doxorubicin aimed to identify associations between genetic polymorphisms and their risk of developing ACT. Genomic DNA from these patients were previously examined for SNPs in ABCB1, NADPH oxidase and CBRs 1 and 3, based on strong evidence suggesting a role for these SNPs in mediating these events. However, no such associations were found. Our conclusions at that time were that the mechanisms investigated in the study were not associated with ACT in these patients, suggesting a potentially different, unknown etiology ¹².

Recently, Visccher et al. and Blanco et al. reported a role for another SNP in CBR1, as well as SNPs in ABCB4 and HNMT in ACT in children ^{13, 14}. A panel of SNPs were identified by microarray studies and classified by whether they were risk or protective factors for cardiotoxicity involving anthracycline administration ¹³. With the mechanisms for ACT in our study still being unknown, our goal was to examine whether these associations could be extended to adult patients. While there were no variations in ABCB4 and CBR1 genotypes, variant and heterozygous genotypes were observed in HNMT, which were high risk and intermediate risk genotypes for the development of ACT (Table 1). In this context, it is important to note the differences in dosage of doxorubicin between our study in adults and the two relevant studies in children. In both studies in children, the cardiotoxicity groups received a high dose of doxorubicin (median doses ranging between 270 and 360 mg/m²) ^{13, 14}, whereas the two patients in our study received a fairly low adult doses (280 and 158 mg/m² respectively).

The exact association between HNMT and cardiotoxicity is still unknown, but antihistamines have been shown to reverse multidrug resistance in breast cancer cells in vitro ¹⁵. They have also been shown to decrease cardiotoxicity mediated by doxorubicin in vivo ¹⁵. Moreover, the SNP rs17583889 is in the intronic region of HNMT and its functional significance in relation to histamine metabolism is unknown. Further studies need to be performed in order to elucidate the role of HNMT in mediating ACT.

Conclusion

This is the first case report to demonstrate a role for HNMT in adults with ACT. We recognize that given the small number of samples in our study, it is difficult to draw strong correlations between genotype and clinical outcome. However, our study demonstrates the importance of the genetic component in understanding toxicities associated with chemotherapy and while further study is needed prior to clinical implementation, it is well accepted that doxorubicin cardiotoxicity may be decreased by dose reductions, use of liposomal doxorubicin, or use of dexrazoxane; therefore polymorphisms associated with

doxorubicin cardiotoxicity may be helpful for selection of candidates for these interventions in the future.

References

1. Gianni L, Herman E, Lipshultz S, et al. Anthracycline cardiotoxicity: from bench to bedside. *J Clin Oncol.* 2008; 26(22):3777. [PubMed: 18669466]
2. Berthiaume JM, Wallace KB. Adriamycin-induced oxidative mitochondrial cardiotoxicity. *Cell Biol Toxicol.* 2007; 23(1):15–25. [PubMed: 17009097]
3. Wojnowski L, Kulle B, Schirmer M, et al. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. *Circulation.* 2005; 112(24):3754–3762. [PubMed: 16330681]
4. Couture L, Nash JA, Turgeon J. The ATP-binding cassette transporters and their implication in drug disposition: a special look at the heart. *Pharmacol Rev.* 2006; 58(2):244–258. [PubMed: 16714487]
5. Dell'Acqua G, Polishchuck R, Fallon JT, et al. Cardiac resistance to adriamycin in transgenic mice expressing a rat alpha-cardiac myosin heavy chain/human multiple drug resistance 1 fusion gene. *Hum Gene Ther.* 1999; 10(8):1269–1279. [PubMed: 10365658]
6. Deng S, Wojnowski L. Genotyping the risk of anthracycline-induced cardiotoxicity. *Cardiovasc Toxicol.* 2007; 7(2):129–134. [PubMed: 17652817]
7. Gille L, Nohl H. Analyses of the molecular mechanism of adriamycin-induced cardiotoxicity. *Free Radic Biol Med.* 1997; 23(5):775–782. [PubMed: 9296455]
8. Ambrosone CB, Ahn J, Singh KK, et al. Polymorphisms in genes related to oxidative stress (MPO, MnSOD, CAT) and survival after treatment for breast cancer. *Cancer Res.* 2005; 65(3):1105–1111. [PubMed: 15705913]
9. Forrest GL, Gonzalez B, Tseng W, et al. Human carbonyl reductase overexpression in the heart advances the development of doxorubicin-induced cardiotoxicity in transgenic mice. *Cancer Res.* 2000; 60(18):5158–5164. [PubMed: 11016643]
10. Slupe A, Williams B, Larson C, et al. Reduction of 13-deoxydoxorubicin and daunorubicinol anthraquinones by human carbonyl reductase. *Cardiovasc Toxicol.* 2005; 5(4):365–376. [PubMed: 16382174]
11. Olson LE, Bedja D, Alvey SJ, et al. Protection from doxorubicin-induced cardiac toxicity in mice with a null allele of carbonyl reductase 1. *Cancer Res.* 2003; 63(20):6602–6606. [PubMed: 14583452]
12. Gayle AA, Sachidanandam K, Kolesar JM, et al. Unexpected doxorubicin-induced cardiomyopathy in sisters. *Int Canc Conf J.* 2012; 1:15–18.
13. Visscher H, Ross CJD, Rassekh SR, et al. Pharmacogenomic prediction of anthracycline-induced cardiotoxicity in children. *J Clin Oncol.* 2012; 30:1422–28. [PubMed: 21900104]
14. Blanco JG, Sun CL, Landier W, et al. Anthracycline-related cardiomyopathy after childhood cancer: role of polymorphisms in carbonyl reductase genes – a report from the Children's Oncology Group. *J Clin Oncol.* 2012; 30:1415–21. [PubMed: 22124095]
15. Zhang Y, Berger SA. Ketotifen reverses MDR1-mediated multidrug resistance in human breast cancer cells in vitro and alleviates cardiotoxicity induced by doxorubicin in vivo. *Cancer Chemother Pharmacol.* 2003; 51:407–414. [PubMed: 12687290]

Table 1

Summary of genotyping results of the selected SNPs.

Gene	SNP	Frequency in Caucasians ^{a,b}	SNP type ^c	Genotype		
				Case 1	Case 2	Genotype with increased risk ^d
CBRI	rs9024	GG 0.775, GA 0.216, AA 0.009	Synonymous (G1096A)	G/G	G/G	A/A
HNMT	rs17583889	CC 0.708, CA 0.274, AA 0.018	Intronic (Intron 2)	C/A	A/A	A/A
ABCB4	rs1149222	TT 0.619, TA 0.345, AA 0.035	Intronic(Intron 14)	T/T	T/T	A/A

SNP: single nucleotide polymorphism; CBRI: carbonyl reductase I; HNMT: histamine N-methyl transferase; ABCB4: ATP binding cassette subfamily B member 4.

^aFrequencies in Caucasians were obtained from the HapMap CEU database.

^bThe wild-type genotype appears first, followed by the heterozygote and the variant.

^cA synonymous SNP is one in which there is no amino-acid change; an intronic SNP is located in the untranslated intronic region of the gene.

^dListed genotypes have been shown to be associated with an increased risk of doxorubicin-induced cardiotoxicity.