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RESEARCH LETTER

Anthracycline Cardiotoxicity Is Associated With Elevated β1-Adrenergic Receptor Density

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β1AR (β1-adrenergic receptor) is a principal regulator of cardiac function that is downregulated in human dilated cardiomyopathy (DCM/heart failure [HF]) hearts.[1] Anthracyclines that are a mainstay therapy for cancer are associated with cardiotoxicity,[2] but less is known about β1ARs in anthracycline cardiotoxicity. We investigated whether β1AR is altered in anthracycline-mediated human HF (ANTH-HF) by performing radioligand binding on nonfailing (NF), HF (DCM), or ANTH-HF human samples. Unexpectedly, ANTH-HF samples showed a significant increase of β1ARs at the plasma membrane in contrast to their downregulation in HF (Figure – Panel A, plasma membrane, left panel). There were no apparent differences in β2AR (β2-adrenergic receptor density) (Figure – Panel A, plasma membrane, right panel). Endosomal βAR (β-adrenergic receptor) densities were measured to determine whether internalization could account for differential distribution. Although no differences were observed in endosomal β1AR density with the NF or ANTH-HF samples, there was significant accumulation of endosomal β1ARs in HF (Figure – Panel A, endosome, left panel) with no changes in endosomal β2ARs (Figure – Panel A, endosome, right panel). This shows that internalization or recycling of β1AR is not altered in human ANTH-HF samples and is similar to NF. To test whether increased transcription in ANTH-HF underlies elevated β1AR density, quantitative real-time polymerase chain reaction was performed. Mild yet significant reduction of β1ARs was observed in ANTH-HF compared with NF (Figure – Panel B), showing that transcription may not contribute to elevated β1ARs in ANTH-HF. βARs undergo ubiquitination for proteolytic degradation,[3] and immunoblotting showed marked reduction of global ubiquitination in the ANTH-HF compared with NF (Figure – Panel C, lower panel, densitometry), which was significantly elevated in HF (Figure – Panel C, lower panel, densitometry). This observation is distinct from mouse studies where doxorubicin treatment increases ubiquitin proteosome,[4] reflecting a complex etiology in humans compared with mice. GRK2 (G-protein coupled receptor kinase 2) is a key determinant of β1AR function,[1] and therefore immunoblotting was performed to assess for changes in G-protein coupled receptor kinases (GRK2, GRK3 [G-protein coupled receptor kinase 3], GRK5 [G-protein coupled receptor kinase 5], and GRK6 [G-protein coupled receptor kinase 6]). GRK2, GRK3, or GRK6 levels were not appreciably altered in ANTH-HF compared with NF (Figure – Panel D), whereas GRK5 was significantly upregulated in ANTH-HF (Figure – Panel D, right panel, densitometry), indicating that ANTH-HF etiology is different from HF despite similar patient characteristics (Figure – Panel E). Thus, ANTH-HF is characterized

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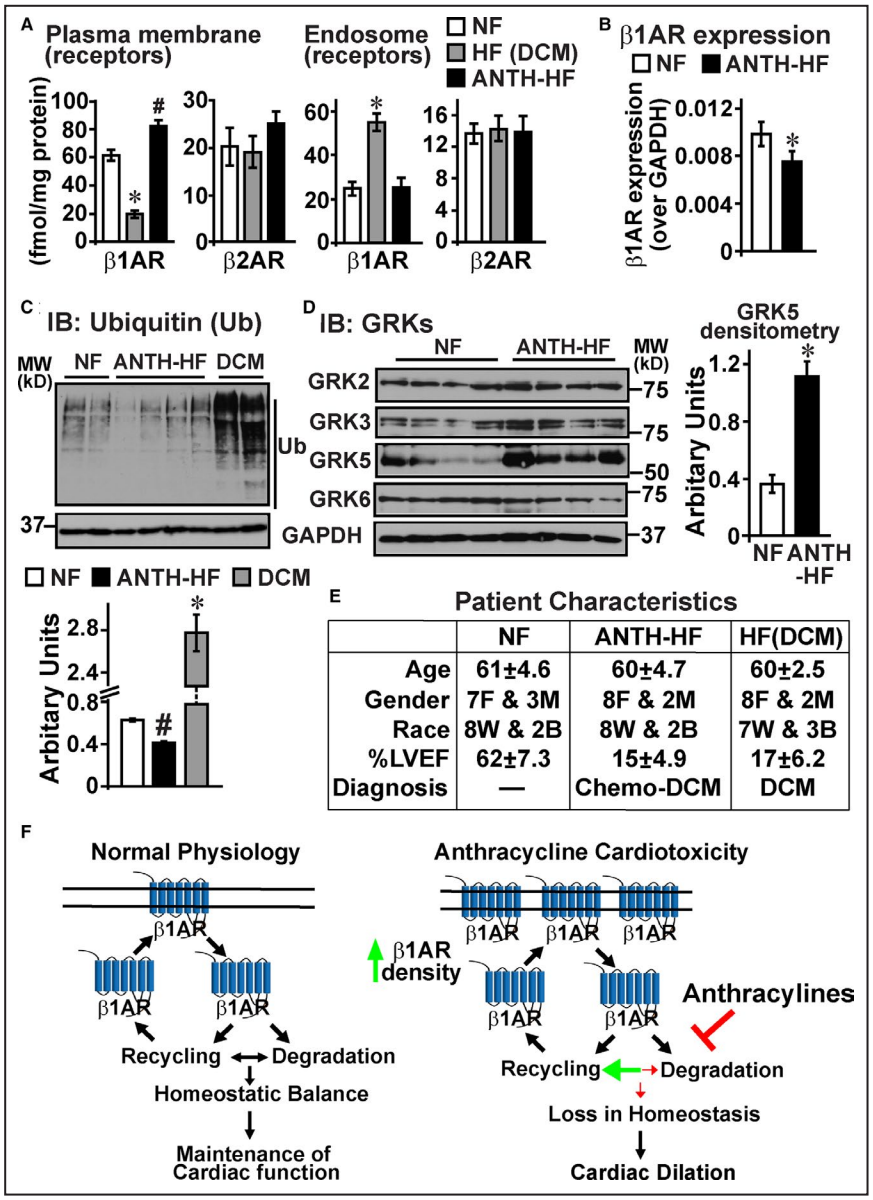

	NF	ANTH-HF	HF(DCM)
Age	61±4.6	60±4.7	60±2.5
Gender	7F & 3M	8F & 2M	8F & 2M
Race	8W & 2B	8W & 2B	7W & 3B
%LVEF	62±7.3	15±4.9	17±6.2
Diagnosis	—	Chemo-DCM	DCM

Figure 1. Unique βAR and GRK changes associated with anthracycline cardiotoxicity.
A, Plasma membranes from NF (n=10), DCM (or HF) (n=10), or HF attributed to ANTH-HF (n=10) were subjected $^{[125]}$I-cyanopindalol radioligand binding. **Left**, Plasma membrane: β1AR density (**P*<0.001 vs NF and ANTH-HF, $^{\#}P$<0.005 vs NF and HF [DCM]) and β2AR density. **Right**, Endosomes: β1AR density (**P*<0.001 vs NF and ANTH-HF) and β2AR density. **B**, β1AR quantitative real-time polymerase chain reaction performed on RNA isolated from NF (n=10) or ANTH-HF (n=10) normalized to GAPDH (**P*<0.05 vs NF). **C**, Western immunoblotting of the cardiac lysates from NF, ANTH-HF, or HF with anti-Ub antibody followed by GAPDH. Densitometry data (n=8–10): **P*<0.01 vs NF and ANTH-HF; $^{\#}P$<0.01 vs NF. **D**, NF and ANTH-HF cardiac lysates immunoblotted with anti-GRK2, anti-GRK3, anti-GRK5, or anti-GRK6 antibody followed by GAPDH. Densitometry data (n=10): **P*<0.01 vs NF. **E**, Patient characteristics. **F**, Schematic showing anthracycline-induced cardiotoxicity is associated with elevated β1AR density and reduced ubiquitination that may underlie impaired proteasomal degradation with a potential for increased β1AR signaling, leading to deleterious cardiac remodeling. %LVEF indicates percentage left ventricular ejection fraction; ANTH-HF, anthracycline-mediated human heart failure; B, Black race; β1AR, β1-adrenergic receptor; β2AR, β2-adrenergic receptor; Chemo-DCM, chemotoxicity-mediated dilated cardiomyopathy; DCM, dilated cardiomyopathy; F, female; GRK, G-protein coupled receptor kinase; GRK2, G-protein coupled receptor kinase 2; GRK3, G-protein coupled receptor kinase 3; GRK5, G-protein coupled receptor kinase 5; GRK6, G-protein coupled receptor kinase 6; HF, heart failure; IB, immunoblotting; M, male; MW, molecular weight; NF, nonfailing; Ub, ubiquitin; and W, White race.

by elevated GRK5 and impaired ubiquitination that may underlie elevated β1AR density (Figure – Panel F) in contrast to known β1AR downregulation in HF.[1] As β1AR is proapoptotic,[5] it is tempting to speculate that elevated β1ARs in part might underlie anthracycline-induced cardiotoxicity, suggesting that selective β1AR blockers may alleviate cardiotoxicity. Also, patients on anthracyclines are prophylactically given pan-β-blockers targeting β1AR and β2AR, wherein blocking the ubiquitously expressed β2AR function in other healthy organs/tissues could have unintended consequences.

The data supporting the findings of this study are available from the corresponding author upon reasonable request. All primary data is available with the corresponding author.

The institutional review board approved the studies, and the participants gave informed consent. Patients' characteristics are shown in Figure Panel E. All patients (NF, HF [DCM], or ANTH-HF [Adriamycin]) were deidentified and age and sex matched.

Plasma membranes and endosomes were isolated, and βAR density was determined on cardiac samples from the left ventricle close to the apex.[1] For nonspecific binding, 100 μmol/L propranolol was used, and ICI 181 551 was used for determining β2AR density, which was subtracted from the nonspecific propranolol values to determine β1AR density.

RNA was isolated as described previously.[1] Quantitative real-time polymerase chain reaction was performed using iQ SYBR Green (BioRAD CFX96 cycler) and human β1AR primers (forward-5′-AATCGATCATCGTGGCTCCC-3′ and reverse-5′-GGGTTTGCCCTACACAAGGA-3′) or GAPDH as internal control, wherein 2-ΔΔCT was used for data analysis.

Immunoblotting was performed on the cardiac lysates (100 μg)[1] with anti-ubiquitin antibody (Santa-Cruz; 1:500) and anti-GRK2, anti-GRK3, anti-GRK5, or anti-GRK6 antibodies (Santa-Cruz)[1] and probed with anti-GAPDH (Santa-Cruz; 1:2000) antibody. Image J software was used for densitometry. Data are expressed as mean±SEM. ANOVA was used for comparison of multiple groups (NF, HF, or ANTH-HF). Post hoc analysis was performed with the Scheffe test. A value of $P<0.05$ was considered significant.

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Disclosures

None.

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