N-acetylcysteine reverses cardiac myocyte dysfunction in HIV-Tat proteinopathy

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Chen F, Lewis W, Hollander JM, Baseler W, Finkel MS. N-acetylcysteine reverses cardiac myocyte dysfunction in HIV-Tat proteinopathy. J Appl Physiol 113: 105–113, 2012. First published May 3, 2012; doi:10.1152/japplphysiol.00068.2012.—HIV cardiomyopathy remains highly prevalent among the estimated 33 million HIV-infected individuals worldwide. This is particularly true in developing countries. Potential mechanisms responsible for myocardial dysfunction following HIV infection include direct effects of HIV proteins. We have previously reported that cardiac myocyte-specific expression of HIV-Tat (Tat) results in a murine cardiomyopathy model. We now report that Tat exhibits decreased myocardial ATP (wild type (WT) vs. Tat transgenic (TG), P < 0.01) and myocyte GSH levels (WT vs. TG, P < 0.01), decreased GSH/GSSG ratio (WT vs. TG, P < 0.01), increased H2O2 levels (WT vs. TG, P < 0.05), and increased catalase (TG vs. WT, P < 0.05), with no effect on GSH and GSH/GSSG. NAC (10–4 M) reverses both the positive and negative inotropic defects in Tat; increased GSH (P < 0.01) and GSH/GSSG (P < 0.01); reversed H2O2 level (P < 0.05) and GPX1 activity (P < 0.05); and normalized the blunted inotropic response to Ca2+ (P < 0.01, for each) and shortened anatomical and functional survival in vitro (P < 0.01). The sulfhydryl donor, N-acetylcysteine (NAC; 10–4 M), completely reversed both the positive and negative inotropic defects in Tat; increased GSH (P < 0.01) and GSH/GSSG (P < 0.01); reversed H2O2 level (P < 0.05) and GPX1 activity (P < 0.05); and normalized the blunted inotropic response to Ca2+ (P < 0.01). NAC (10–7 M) normalized duration of contractile function from <40 min to >120 min (P < 0.01), with no effect on GSH and GSH/GSSG. NAC (10–4 M) reverses cardiac myocyte dysfunction and markers of oxidative stress. NAC (10–7 M) enhances myocyte function independent of changes in glutathione. Elucidating the molecular mechanisms involved in the GSH-dependent and GSH-independent salutary effects of NAC should identify novel therapeutic targets for myocardial proteinopathies recently appreciated in human cardiomyopathies.

HIV CARDIOMYOPATHY remains highly prevalent among the estimated 33 million HIV-infected individuals worldwide (UNAIDS Report on the global AIDS epidemic 2010). This is particularly true in developing countries where HAART (highly active antiretroviral therapy) is not usually available (15, 27, 29). HAART has been reported to reduce the incidence of HIV-associated myocardial dysfunction by at least 30% (4). However, a recent report from India on HIV-infected children treated with HAART indicates that HIV cardiomyopathy remains a serious concern in this particularly vulnerable population (36).

Potential mechanisms proposed by us and others to contribute to myocardial dysfunction include direct effects of HIV proteins (e.g., Tat, gp120), as well as indirect effects of inflammatory mediators, coinfection, nutritional deficiencies, pharmacotherapies, and behavioral stress (3, 7, 11, 30, 49, 56). A pathogenic role for HIV proteins has been supported by evidence of HIV nucleic acids in the myocardium of HIV cardiomyopathy patients (13).

Lewis generated a transgenic mouse model with HIV Tat targeted exclusively to the myocardium (Tat Tg) to explore the possible specific contribution of HIV Tat to HIV cardiomyopathy (43). He first reported echocardiographic evidence of myocardial dysfunction and ultrastructural defects in mitochondria in his Tat Tg cardiomyopathy model. In addition, Tat Tg demonstrated depletion of GSH in liver (8) and heart tissue (43), which renders cells more susceptible to oxidative stress. We subsequently quantified both systolic and diastolic dysfunction in this model in the awake state using catheter-based hemodynamic techniques (11). We now report our findings of a pathogenic role for oxidative stress in reversible myocardial dysfunction in this Tat Tg consistent with other genetic cardiomyopathy models (14, 54).

Both in vivo and in vitro administration of N-acetylcysteine (NAC) has been shown to raise intracellular levels of reduced glutathione by providing an additional source of cysteine as a precursor (9, 18, 26). NAC injected intraperitoneally into rodents has been reported to increase glutathione levels in the brain and protect the brain against the damaging effects of hydroxyl radicals, lipid peroxidation, and oxidative stress induced by gp120 and Tat (51). In vitro, NAC reverses gp120- and Tat-induced oxidative stress in immortalized endothelial cells and inhibits viral replication in human monocyte-derived macrophages and lymphocytes (21). Clinical studies in HIV-infected patients reveal evidence of impaired glutathione metabolism responsive to NAC supplementation (20). Accordingly, we further report the salutary effects of NAC on our Tat Tg cardiomyopathy model.

MATERIALS AND METHODS

Materials. All reagents were purchased from Sigma Chemical (St. Louis, MO) unless otherwise indicated.

Statistical methods. Values are expressed as means ± SE. Myocyte data represent the means ± SE of 15 different determinations derived from 3 individual myocytes from 5 separate myocyte preparations from 5 different mice. Comparisons among the groups were analyzed by ANOVA followed by a post hoc test. Two-way ANOVA with repeated measures was used to compare the values measured in the groups. Statistical significance is accepted at the level of P < 0.05.

Animal preparation. Wild-type (WT) FVB inbred female mice (Harlan) were paired with Tat Tg mice (TG) originally obtained from Dr.
William Lewis at Emory University (+/− tat Tag high). The TG littermates were genotyped by PCR as we previously described (43). Briefly, fifty nanograms of mouse genomic tail DNA was used along with primers TAT1 (5′-GGAGCGATAGATCAGTCTAGGAC-3′) and TAT2 (5′-CCTCCACCCA GCTCAGTTGTCG-3′) (Sigma), which were designed to detect the presence of the targeted TG. They produced a product of 1.1 kb. PCR was performed in a 20-μl volume with Taq DNA polymerase (Qiagen) in a DNA thermal cycler 480 (Perkin Elmer) with the following conditions: 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 30 s at 55°C, 1 min at 72°C, ending with a 3-min extension at 72°C and then 4°C. The PCR products were loaded onto a 1% agarose electrophoresis gel. We previously reported both systolic and diastolic dysfunction in vivo by 6 mo of age in Tat (32). Accordingly, both WT and TG mice were studied after reaching 6 mo of age. Mice were housed in a room specifically dedicated for transgenic mice in the Animal Care Facility of the Robert C. Byrd Health Sciences Center of West Virginia University. Strict adherence to the protocol approved by the West Virginia University Animal Care and Use Committee was maintained throughout.

Adult mouse cardiac myocytes. Mice were anesthetized with pentobarbital sodium and the hearts removed rapidly and perfused for 25 min with Krebs-Henselet bicarbonate (KHB) containing 0.1% collagena (Worthington type II), 0.5 mg protease (Sigma), and 10 mM 2,3-butanediol monoxime (BDM), according to the method of Langendorff at a constant rate of 4 ml/min using a peristaltic pump, as we previously reported (19). All buffer and enzyme solutions were maintained throughout. A coverslip with attached cells was placed in a plastic transfer pipette, cardiac tissue was aspirated until most of the heart softened, left ventricular (LV) tissue was sliced and placed in 4°C KHB, and then RIPA lysis buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM beta-glycerophosphate, 1 mM Na3VO4, 1 μg/ml leupeptin; Cellsignaltech) was added followed by sonication and centrifuging at 14,000 g for 10 min at 4°C. Supernatants were used to measure GPx activity by commercial glutathione assay kit (Cayman Chemical). In brief, oxidation of NADPH to NADP+ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the A340 is directly proportional to the GPx activity in the sample. H2O2 concentration was detected in supernatants using Amplex Red Hydrogen Peroxide/Peroxidase Assay Kit (Invitrogen). Fifty microliters of the supernatant was incubated with 0.2 U/ml horseradish peroxidase and 100 μM Amplex Red reagent (10-acetyl-3,7-dihydrophenoxazine) at room temperature for 30 min in darkness. The fluorescence was quantified using FLUOStar Optima (BMG Lab Technologies) with excitation at 560 nm and emission at 590 nm. Catalase concentration was assayed in 50 μl fivefold-diluted supernatant by Amplex Red Catalase Assay Kit (Invitrogen). After 30-min incubation of 50 μl supernatant with 50 μl 40 μM H2O2 at room temperature, 50 μl of the Amplex Red/HRP working solution was added and followed by 30-min incubation at 37°C in the dark. The fluorescence was read by FLUOStar Optima (BMG Lab Technologies) with excitation at 560 nm and emission at 590 nm.

Cardiac myocyte viability. Trypan blue exclusion was used to assess cardiac myocyte viability. Isolated cardiac myocytes were incubated with KHB alone or KHB with NAC for 60 min. Trypan blue exclusion tests were performed initially, or 40 min or 120 min after incubation. Trypan blue 0.4% solution (MP Biomedicals), 0.1 ml, was added into 1 ml cardiac myocytes and incubated for 3 min. The number of blue staining cells and the number of total cells were counted. The percentage of viable cells = [1.00 − (number of blue cells + number of total cells)] × 100. Myocytes were photographed using phase-contrast microscopy (10X) (Olympus, IX70-S1F2).

RESULTS

In vitro cardiac myocyte contractile dysfunction. The presence of the transgene was confirmed by PCR of litters from FVB females crossed with HIV Tat transgenic males, as we previously described (11). We also reported echocardiographic data in anesthetized mice and hemodynamic data in awake mice that revealed significant defects in both systolic and diastolic myocardial function in this animal model in vivo by 6 mo of age (11). We now confirm the presence of defects by 6 mo in both positive and negative inotropy in cardiac myocytes isolated from Tat vs. WT reflected in decreased % shortening (Fig. 1A; %PS, 8.78 ± 0.95% vs. 5.06 ± 0.73%, WT vs. TG, P < 0.01; n = 15, respectively), positive inotropic (Fig. 1B; +dI/dt, 309.7 ± 17.6 vs. 118.5 ± 11.7 μm/s, WT vs. TG, P < 0.01; n = 15, respectively), and negative inotropic function (Fig. 1C; −dI/dt, −322.1 ± 12.4 vs. −228.9 ± 16.6 μm/s, WT vs. TG, P < 0.01; n = 15, respectively).
μm/s, WT vs. TG, P < 0.01; n = 15, respectively) using automated edge detection.

Enhanced oxidative stress. Enhanced oxidative stress from mitochondrial dysfunction has been proposed to play a central pathogenic role in HIV cardiomyopathy in patients and animal models (33). Anti-HIV medications have also been associated with mitochondrial toxicity that may augment oxidative stress and cardiac dysfunction in HIV patients (34). Ultrastructural changes in the mitochondria were described in the initial report and cardiac dysfunction in HIV patients (34). Exposure of TG myocytes to increasing concentrations of NAC revealed reversal of depression in %PS to WT levels at 10⁻⁴ M NAC (Fig. 5D).

Further experiments revealed that the addition of NAC (10⁻⁴ M) to TG completely ameliorated the positive and negative inotropic dysfunction compared with WT (Fig. 6A: %PS, 5.06 ± 0.73% vs. 9.15 ± 0.68%, TG vs. TG + NAC, P < 0.01; Fig. 6B: +d/dt, 118.5 ± 11.7 vs. 272.3 ± 27.4 μm/s, WT vs. TG, P < 0.01; Fig. 6C: −d/dt, −228.9 ± 16.6 vs. −396.4 ± 43.7 μm/s, TG vs. TG + NAC, P < 0.01; n = 15 for each).

NAC reduces oxidative stress. A potential mechanism for the beneficial effects of NAC on WT cardiac myocyte dysfunction may be the repletion of GSH (37). This interpretation is supported by the reversal of the depletion in GSH (Fig. 7A; GSH, 3.87 ± 0.27 vs. 8.99 ± 0.78 μM/mg protein, WT vs. TG, P < 0.01; n = 6, respectively) and GSH/GSSG (Fig. 7B; GSH/GSSG, 3.22 ± 0.29 vs. 16.58 ± 3.02, TG vs. TG + NAC, P < 0.01; n = 8–9, respectively) by the same concentration of NAC (10⁻⁴ M) that reversed the inotropic defects. NAC (10⁻⁴ M) also decreased the level of H₂O₂ and the activity of GPX1 in TG myocytes (Fig. 8A, H₂O₂ level, 8.8 ± 0.7 vs. 7.0 ± 0.5 μM/mg protein, TG vs. TG + NAC, P < 0.05; Fig. 8B, GPX1 activity, 11.8 ± 0.5 vs. 7.6 ± 0.9 nM-min⁻¹·ml⁻¹, TG vs. TG + NAC, P < 0.05; n = 6).

**Fig. 1. Bar graph depicting the changes in percentage peak shorting [%PS; 8.78 ± 0.95% vs. 5.06 ± 0.73%, wild type (WT) vs. Tat transgenic (TG), **P < 0.01; n = 15] (A), positive inotropy (+d/dt, 309.7 ± 17.6 vs. 118.5 ± 11.7 μm/s, WT vs. TG, **P < 0.01; n = 15, respectively) (B), and negative inotropy (−d/dt, 322.1 ± 12.4 vs. −228.9 ± 16.6 μm/s, WT vs. TG, **P < 0.01; n = 15, respectively) (C) in cardiac myocytes isolated from TG vs. WT.

**Fig. 2. Bar graph depicting the ATP levels in heart tissue (9.76 ± 1.55 vs. 0.721 ± 0.611 μM/g, WT vs. TG, **P < 0.01; n = 8–9, respectively) from TG vs. WT.

**Fig. 3. Bar graph depicting the concentrations of GSH (9.68 ± 0.58 vs. 3.87 ± 0.27 μM/mg, WT vs. TG, **P < 0.01; n = 8–9, respectively) (A), and GSH/GSSG (12.53 ± 3.76 vs. 3.22 ± 0.29, WT vs. TG, **P < 0.01; n = 8–9, respectively) (B) in cardiac myocytes isolated from TG vs. WT.
NAC reverses attenuated calcium response. HIV Tat has been reported to alter calcium signaling in neurons (39). Accordingly, we demonstrated that NAC (10^{-4} M) reversed the attenuated positive inotropic effect of increasing concentrations of calcium on cardiac myocyte peak shortening (%PS) (Fig. 9A; %PS, WT + NAC, TG + NAC, vs. TG, P < 0.05; n = 15, respectively for each) and contractility (+dI/dt) (Fig. 9B; +dI/dt, WT + NAC, TG + NAC, vs. TG, P < 0.05; n = 15, respectively for each) in TG vs. WT.

NAC preserves myocyte function over time. Millimolar concentrations of NAC have generally been used to restore millimolar concentrations of intracellular GSH (16, 28). Our physiological and biochemical data in Figs. 5–9 using 10^{-4} M NAC are consistent with this mechanism. We also demonstrated that much lower concentrations of NAC (10^{-7} M) did not change the GSH level and the ratio of GSH/GSSG in TG myocytes (Fig. 7, A and B). We have previously proposed an additional GSH-independent mechanism through which NAC may mediate salutary inotropic effects by sulfhydryl regulation of the ryanodine sensitive SR calcium release channel (RyR2) (10). The presence of maximal inotropic and GSH effects of NAC at 10^{-4} M and the absence of GSH effects of NAC at 10^{-7} M provides a potential opportunity to distinguish between the GSH-dependent and GSH-independent effects of NAC.

Accordingly, we explored potential salutary physiologic effects of 10^{-7} M NAC on TG vs. WT myocytes subjected to electrical field stimulation that mildly stressed the RyR2. WT myocytes retained their capacity to contract for at least 120 min following three pulses of electrical field stimulation at 0, 5, 10, 20, 30, 40, 60, 90, and 120 min (Fig. 10). Similarly stimulated TG myocytes significantly decreased the amplitude of their contractions at 30 min and completely stopped contracting at 40 min (P < 0.01). The addition of NAC (10^{-7} M) was sufficient to retain contractile responses to electrical field stimulation for at least 120 min in TG (Fig. 10). NAC (10^{-7} M) had no effect on the contraction of WT myocytes.

NAC preserves myocyte morphology over time. Morphologic findings were consistent with the physiological data. Uptake of trypan blue (i.e., loss of cell viability) was greater in TG vs. WT myocytes after stimulation at 40 and 120 min following cell isolation (Fig. 11). The addition of a low concentration of NAC (10^{-7} M) was sufficient to prevent the loss of cell viability in isolated TG myocytes at 40 min (24.9 ± 5.3% vs. 52.3 ± 4.4%, TG vs. TG + NAC, P < 0.01; n = 6, respectively) and 120 min (7.3 ± 1.3% vs. 33.6 ± 3.3%, TG vs. TG + NAC, P < 0.01; n = 6 views, respectively).

DISCUSSION

HIV Tat (Tat) is a small cysteine (sulfhydryl)- and arginine (membrane permeable)-rich nuclear protein that is required for efficient RNA polymerase II transcription of the integrated provirus (46). Tat circulates in the blood of HIV-infected individuals and has been reported to have biological effects on...
different organs and host systems (e.g., myocardium, kidney, liver, and CNS) via regulation of multiple signaling pathways (3, 19). The substantial experimental evidence supporting a role for Tat in HIV-associated neurocognitive disorders (HAND) raised the possibility of its involvement in HIV cardiomyopathy, as well (28). Accordingly, Lewis developed a transgenic mouse model with Tat targeted to the myocardium using a cardiac-specific α-myosin heavy chain promoter (Tat Tg) (43).

We have previously reported in vivo physiological evidence of both systolic and diastolic myocardial dysfunction in this Tat Tg cardiomyopathy model (11). Whether these changes resulted from intrinsic defects in cardiac myocytes or altered neurohumoral regulation of the myocardium was not addressed using in vivo techniques. Accordingly, we now provide compelling in vitro evidence of intrinsic systolic (+d/l/dt) and diastolic (−d/l/dt) dysfunction in cardiac myocytes isolated from this Tat Tg cardiomyopathy model (Fig. 1). Myocardial dysfunction in this model is also unlikely secondary to noncardiac systemic mediators since the HIV Tat protein is genetically expressed directly within the cardiac myocytes themselves (13).

Lewis originally reported ultrastructural defects in mitochondria and lower GSH levels in the myocardium of this Tat Tg model (43). We now extend and expand these observations by reporting decreased myocardial ATP and cardiac myocyte GSH and GSH/GSSG levels in Tat Tg (Figs. 2 and 3). We further report elevated levels of H2O2 and increased catalase and glutathione peroxidase 1 (GPX1) activities in Tat Tg cardiac myocytes (Fig. 4). The relative increase in antioxidant enzyme activity of 30–50% was unlikely to be sufficient to compensate for the relative decrease of 60–75% in GSH and GSH/GSSG levels measured. It is also unlikely that the increase in catalase activity resulted from its own oxidation since catalase activity was previously shown to be unchanged by singlet oxygen in vitro (35). Taken together, our current myocardite and previous myocardial data support the presence of oxidative stress in this cardiomyopathy model that results from a lack of either ATP or GSH production or enhanced consumption of either ATP or GSH, or both.

A compelling body of basic and clinical literature supports a pathogenic role for oxidative stress in animal models and humans with cardiomyopathies and heart failure (1, 30). Oxidative stress is said to occur when there is an imbalance between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell. The most widely accepted predominant source of ROS is the mitochondrial electron transport chain. Electrons are transferred in a series of thermodynamically favorable steps to form H2O and ATP from O2. This highly efficient system still leads to the production of oxygen with highly reactive unpaired electrons (e.g., superoxide, hydroxyl radical) and hydrogen peroxide. In addition oxygen can interact with nitric oxide (NO) to form peroxynitrite and other reactive nitrogen species. NO appears to play a critical role in oxidative stress and reversible myocardial dysfunction, stunning, hibernating myocardium, and myocardial preconditioning (6, 12). Other potential sources of ROS include NADPH oxidase, cytochrome P-450, and xanthine oxidase. Potent antioxidant enzyme systems have evolved to prevent oxidative damage. These include superoxide dismutase, catalase, and glutathione peroxidase. A delicate balance exists between the production of intracellular oxidants and antioxidants to maintain cellular homeostasis. Enhanced oxidative stress and/or inadequate reducing capacity result in oxidative stress from uncontrolled free radical activation. Such free radical activation in the heart can cause myocardial contractile dysfunction (1, 16). A recent report of Tat inhibition of K channels in cardiac myocytes associated with enhanced oxida-
Fig. 8. Bar graph depicting the effects of $10^{-4}$ M NAC on H$_2$O$_2$ levels (8.8 ± 0.7 vs. 7.0 ± 0.5 μM/mg protein, TG vs. TG + NAC, #P < 0.05, n = 6, respectively) (A) and glutathione peroxidase (GPX1) activity (11.8 ± 0.5 vs. 7.6 ± 0.9 nM·min$^{-1}$·ml$^{-1}$, TG vs. TG + NAC, #P < 0.05, n = 6, respectively) (B) in cardiac myocytes isolated from TG vs. WT. *P < 0.05 vs. WT; #P < 0.05 vs. TG.

Fig. 9. Graphic depiction of the effects of increasing concentrations of extracellular Ca$^{2+}$ alone and following incubation with NAC ($10^{-4}$ M) on WT vs. TG %PS (P < 0.05, WT vs. TG, n = 15, respectively; P < 0.05, TG vs. TG + NAC, n = 15, respectively) (A), positive inotropy (+d/dt) (P < 0.05, WT vs. TG, n = 15, respectively, P < 0.05; TG vs. TG + NAC, n = 15, respectively) (B), and negative inotropy (−d/dt) (P < 0.05, WT vs. TG; P = 0.07, TG vs. TG + NAC, n = 15, respectively) (C).
We have also previously reported a defect in sulfhydryl regulation of the RyR2 in a genetic hamster cardiomyopathy model using NAC as a sulfhydryl donor (14). A defect in the delta sarcoglycan component of the dystrophin-associated complex was subsequently shown by others to be responsible for the genetic hamster cardiomyopathy (38, 47). NAC has also recently been shown to be cardioprotective in the mdx dystrophin mouse model of muscular dystrophy (53). The mechanism of the benefit of NAC was not explained. However, NAC did not reduce free radical generation by NADPH (39, 54). An alternative possibility that was not explored by the authors could be an effect of NAC on sulfhydryl regulation of RyR2. This possibility is further supported by emerging evidence that HIV Tat regulates a ryanodine receptor in neuronal tissues (39, 40, 55). This hypothesis has been bolstered by recent reports of the pathogenic role of phosphorylation, nitrosylation, and oxidation of RyR2 in cardiomyopathies and heart failure (31, 42).

Therefore, we studied the effect of NAC on calcium signaling in Tat Tg (Fig. 9). Cardiac myocytes isolated from Tat Tg revealed an attenuated response to the addition of extracellular calcium (Fig. 9). NAC normalized the inotropic response of Tat Tg myocytes to calcium (Fig. 9). These data support an effect of NAC on calcium handling in this cardiomyopathy model. Defective calcium handling could result from changes in any number of proteins, including RyR2 or SERCA2. Considerably more detailed physiological and biochemical studies will be necessary before drawing any definitive conclusions regarding the role of NAC on RyR2 in this model, however.

We further explored the possibility that stressing the cardiac myocytes in vitro might enhance the sensitivity of our system to identify more subtle effects of NAC concentrations too low to affect glutathione levels. We first confirmed that NAC (10⁻⁷ M) did not increase GSH or GSH/GSSG levels in our model (Fig. 7). We subsequently “stressed” our myocytes by performing repetitive pulses of electrical field stimulation over time. Electrical field stimulation depolarizes the sarcolemmal membrane to activate the physiological process of E-C coupling described above (24). Cardiac myocytes isolated from WT mice tolerate repeated bursts of electrical stimulation with maintenance of contractility for at least 2 h (Fig. 10). However, Tat myocytes significantly decreased their contractile response at 30 min and completely stop beating at 40 min of the same repeated bursts of stimulation (Fig. 10). A very low concentration of NAC (10⁻⁷ M) completely prevented this loss of contractile response to stimulation (Fig. 10). In addition, this low concentration of NAC was sufficient to retain sarcolemmal membrane integrity, as reflected in trypan blue exclusion (Fig. 11). These data suggest potential concentration-dependent differences in the mechanisms responsible for the salutary effects of NAC in Tat, and possibly other cardiomyopathy models.

In summary, we provide compelling evidence of reversal of cardiac myocyte dysfunction and oxidative stress by NAC in a cardiac myocyte-specific HIV Tat transgenic mouse cardiomyopathy model. We also reveal preservation of morphologic integrity and inotropic function by much lower concentrations of NAC under conditions of enhanced calcium release from repetitive electrical stimulation.

Potential clinical implications of our data include the possibility that HIV cardiomyopathy may respond to NAC therapy. NAC therapy is already being explored for treating HIV infection (45). These studies are being conducted based on data indicating enhanced oxidative stress and decreased glutathione levels following HIV infection (37). These clinical implications are independent of whether HIV Tat directly contributes to HIV cardiomyopathy. This is particularly relevant to consider since a potential limitation of our studies includes the relative paucity of reports of Tat protein in human HIV cardiomyopathy (13). The absence of published data may suggest the failure to find Tat and/or the lack of enthusiasm to search for it. Our present and previous reports will hopefully stimulate a more aggressive search for more direct evidence for HIV proteins in patients with HIV cardiomyopathy.

However, this HIV Tat cardiomyopathy model does possess other features that make it particularly suitable to study HIV and other cardiomyopathies. We have previously reported that NAC reversed myocardial dysfunction in the Syrian hamster genetic cardiomyopathy model (14). Others have shown similar salutary effects of NAC on sarcolemmal integrity, as reflected in trypan blue exclusion (Fig. 11). These data suggest potential concentration-dependent differences in the mechanisms responsible for the salutary effects of NAC in Tat, and possibly other cardiomyopathy models.

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Potential clinical implications of our data include the possibility that HIV cardiomyopathy may respond to NAC therapy. NAC therapy is already being explored for treating HIV infection (45). These studies are being conducted based on data indicating enhanced oxidative stress and decreased glutathione levels following HIV infection (37). These clinical implications are independent of whether HIV Tat directly contributes to HIV cardiomyopathy. This is particularly relevant to consider since a potential limitation of our studies includes the relative paucity of reports of Tat protein in human HIV cardiomyopathy (13). The absence of published data may suggest the failure to find Tat and/or the lack of enthusiasm to search for it. Our present and previous reports will hopefully stimulate a more aggressive search for more direct evidence for HIV proteins in patients with HIV cardiomyopathy.

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effects in the mdx mouse cardiomyopathy model (54). This HIV Tat cardiomyopathy model also responds favorably to NAC. Both the Syrian hamster and mdx mouse have been shown to possess defects in the dystrophin-associated protein complex (14, 38, 47, 54). The Tat protein may play a role similar to that played by these dystrophin-associated proteins in these other genetic cardiomyopathy models. Recent reports link abnormal myocyte proteins to cardiomyopathies through activation of the unfolded protein response (17). Therefore, future studies in this HIV Tat cardiomyopathy model should provide new insights into the pathogenic roles of oxidative stress, sulfhydryl regulation of RyR2, and possibly the unfolded protein response recently noted as important mediators of myocardial dysfunction in animal models and patients (39).

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author Contributions: F.C. and M.S.F. conception and design of research; F.C. and W.A.B. performed experiments; F.C. and W.A.B. analyzed data; F.C., W.L., J.M.H., W.A.B., and M.S.F. interpreted results of experiments; F.C. and W.A.B. performed experiments; F.C. and W.A.B. analyzed data; F.C., W.L., J.M.H., W.A.B., and M.S.F. edited and revised manuscript; M.S.F. edited and revised manuscript; M.S.F. approved final version of manuscript.

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