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Transforming Growth Factor- β 1-mediated cardiac fibrosis: Potential role in HIV and HIV/ART-linked cardiovascular disease

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Abstract

HIV infection elevates the incidence of cardiovascular disease (CVD) independent of traditional risk factors. Autopsy series document cardiac inflammation and endomyocardial fibrosis in the HIV+ treatment naïve, and gadolinium enhancement magnetic resonance imaging has identified prominent myocardial fibrosis in the majority of HIV+ individuals despite use of suppressive antiretroviral therapies (ART). The extent of such disease may correlate with specific ART regimens. For example, HIV-infected patients receiving ritonavir (RTV)-boosted protease inhibitors have the highest prevalence of CVD, and RTV-exposed rodents exhibit cardiac dysfunction coupled with cardiac and vascular fibrosis, independent of RTV-mediated lipid alterations. We recently showed that platelet transforming growth factor (TGF)- β 1 is a key contributor to cardiac fibrosis in murine models. We hypothesize that in the HIV+/ART naïve, cardiac fibrosis is a consequence of proinflammatory cytokine and/or ART-linked platelet activation with release of TGF- β 1. Resultant TGF- β 1/Smad signaling would promote collagen synthesis and organ fibrosis. We document these changes in a pilot immunohistochemical evaluation of cardiac tissue from two ART-naïve pediatric AIDS patients. In terms of ART, we showed that RTV inhibits immunoproteasome degradation of TRAF6, a nuclear adapter signaling molecule critical to the regulation of proinflammatory cytokine signaling pathways involved in osteoclast differentiation and accelerated osteoporosis. We now present a model illustrating how RTV could similarly amplify TGF- β 1 signaling in the promotion of cardiac fibrosis and accelerated CVD. Supportive clinical data correlate RTV use with elevation of NT-proBNP, a biomarker for CVD. We discuss potential interventions involving intrinsic modulators of inflammation and collagen degradation, including carbon monoxide-based therapeutics.

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Conflicts of interest

There are no conflicts of interest.

Keywords

cardiovascular disease; fibrosis; inflammation; protease inhibitor; ritonavir; TGF- β 1

Introduction

HIV infection elevates the incidence of cardiovascular disease (CVD) and osteoporosis independent of traditional risk factors [1-4]. A heightened proinflammatory state, including elevated levels of tumor necrosis factor (TNF)- α , interleukin (IL)-6, C-reactive protein (CRP), and receptor activator of nuclear factor κ B ligand (RANKL), is associated with accelerated CVD and bone mineral density loss in HIV- and HIV+ individuals [5,6], and persists in the latter despite suppressive antiretroviral therapy (ART) [7]. Use of HIV protease inhibitors (PI), particularly ritonavir (RTV), predicts increased carotid intima-media thickness, a subclinical marker of atherosclerosis [8]. Duration of RTV-boosted PI use was the only significant association for CVD among HIV-infected adolescents [9]. Osteoporosis is similarly increased in patients on RTV-boosted PIs [10,11], an important observation given the strong link between CVD and osteoporosis in the general population [12].

Mechanisms through which HIV/ART could promote CVD include induction of hyperlipidemia and proinflammatory cytokines with oxidative stress, endothelial damage, and hypercoagulability [8]. In our opinion, certain ART regimens could also facilitate cytokine *activity* via inhibition of physiologic regulators of inflammatory signaling, regardless of their effects on cytokine *levels*. As one example, we found that RTV abrogates a physiologic block to the activity of RANKL, the primary mediator of osteoclast differentiation, via inhibition of degradation of the nuclear signaling adaptor TNF receptor associated factor (TRAF)-6 [6,13,14]. This led to accelerated osteoclast formation in HIV+ women receiving RTV, compared to those on non-RTV based regimens [11]. In terms of CVD, we note that cardiac fibrosis is a prominent feature of untreated HIV infection [15], but the prevalence of fibrosis increases, or at least is not suppressed, by ART [16], and we showed that platelet transforming growth factor (TGF)- β 1 is a key contributor to cardiac fibrosis in a mouse model of heart failure [17]. We therefore hypothesize that TGF- β 1, primarily as released from activated platelets but also potentially from other cells such as monocytes, initiates cardiac fibrosis in untreated HIV infection, and that its activity is augmented by RTV-mediated enhancement of TRAF-6. We further postulate that these processes can be targeted by modulators of TGF- β .

Cardiac fibrosis in the setting of HIV

Cardiac fibrosis was an early feature of untreated HIV disease [15,18,19]. All of 6 HIV+ children in one autopsy series had prominent ventricular conduction system fibrosis [20]. This pathology persists or increases despite ART. For example, hyaluronic acid levels, a biomarker of fibrosis, are elevated in the HIV+/ART-naïve, and did not reach control concentrations despite 12 months of ART [21]. Gadolinium enhancement magnetic resonance imaging documented prominent myocardial fibrosis in 76% of 90 HIV/ART subjects, while only 13% of controls had evidence of *any* myocardial fibrosis [16].

Cardiac fibrosis in animal models for HIV infection and RTV exposure

19 of 85 rhesus macaques infected with simian immunodeficiency virus (SIV) developed a fibrotic vasculopathy, lesions “similar [to those] described in children with AIDS” [22]. In a second study, 9 of 18 SIV-infected macaques had cardiac involvement, with up to a 4-fold increase in collagen content, compared to animals infected with a non-pathogenic SIV strain [23].

LBNF1 rats developed systolic dysfunction after 5 weeks of RTV exposure, with a 2-fold increase in left ventricle fibrosis by week 8 [24]. RTV also promoted atherosclerosis in ApoE^{-/-} mice in conjunction with increased TGF- β in plasma and cardiac tissue [25]. Wistar rats exposed to RTV-boosted lopinavir showed cardiac contractile dysfunction, albeit fibrosis was not documented [26].

In terms of potential confounding factors, RTV and RTV-boosted PIs may induce hyperlipidemia, complicating dissection of primary etiologic events for CVD [27]. However, cardiac fibrosis in RTV-exposed rodents, and elevation of inflammatory markers and preclinical indices of atherosclerosis in RTV treated HIV+ patients, have been documented independent of those processes. Co-exposure of rodents to the CCR5 HIV co-receptor antagonist and anti-inflammatant maraviroc also helped distinguish the lipogenic effect of RTV from RTV-associated structural cardiovascular changes [25].

Modeling HIV involvement in cardiac fibrosis

Under physiologic conditions (Fig. 1A), collagen synthesis is controlled by two distinct TGF- β signaling pathways, Smad and TAK1/MKK3/p38 [28]. TRAF6 regulates both canonical Smad pathways via cytokines that are elevated in HIV infection and induce TGF- β 1 [29], and Smad-independent pathways involving TAK1/MKK3/p38 [30]. The latter includes a negative feedback loop involving Beclin 1. Beclin 1 is involved in autophagy-dependent collagen degradation in cardiac tissues [31].

In untreated, asymptomatic HIV infection (Fig. 1B), plasma levels of TGF- β are increased ~2-fold, and in advancing HIV disease a further rise in TGF- β levels was observed [32,33]. In our opinion this is a consequence of platelet activation via three possible mechanisms: binding of viral envelope to platelet DC-SIGN [34]; cytokine stimulation involving IL-6 and IL-8 [35]; and monocyte tissue factor (TF)-mediated thrombin generation [36]. All of these processes could result in platelet activation and TGF- β 1 release.

TGF- β function controlled through autophagy [37] may also be amplified by suppression of Beclin 1 by certain HIV gene products [38] (Fig. 1B). Beclin 1 inactivation led to a 3-fold increase in fibrosis in a murine cardiomyopathy model [39]. However, other HIV proteins may have different effects, which may be cell type specific [38].

Modeling ART involvement in cardiac fibrosis

ART suppresses HIV viral load (Fig. 1C), but levels of key proinflammatory cytokines are not normalized, and may still have pathologic consequences [7,40]. As noted above, we

demonstrated that RTV enhanced RANKL signaling via inhibition of TRAF6 degradation in the immunoproteasome [6,13,14], resulting in increased monocyte differentiation into bone-resorbing osteoclasts [11]. TRAF6 also mediates TGF- β 1 signaling [29-31], so that in our opinion RTV should facilitate TGF- β activity in a manner similar to RANKL. Cytokines apart from TGF- β 1 that are involved in cardiac fibrosis, including IL-13 [41], also signal via TRAF6 [42] and may be similarly enhanced by RTV. There is also the potential for RTV to directly activate platelets with TGF- β 1 release, as demonstrated for abacavir in vivo [43]; we have preliminary in vitro data supporting such a role, using concentrations $<1\mu\text{M}$ (unpublished observation).

One unknown is the potential for mitigation of the effect of TRAF6-mediated increases in TGF- β 1 activity and promotion of collagen production if concomitant facilitation of TAK1/MKK3/p38 signaling leads to enhanced autophagy (Fig. 1C). But RTV had no direct impact on autophagy, at least in adipocytes [38]. Another PI, atazanavir, actually triggered autophagy in those cells [44], consistent with the lack of association of atazanavir with cardiac fibrosis or enhanced TRAF6 activity in our system [14, and unpublished observations]. Given the RTV-induced cardiac fibrosis seen in rodent models, and persistent cardiac fibrosis in patients on ART, we hypothesize that this secondary pathway does not predominate.

Enhanced fibrosis and TGF- β 1 signaling in cardiac tissue from ART-naïve HIV+ children

We initiated a pilot study to evaluate fibrosis and a canonical TGF- β signaling event, phosphorylation of Smad2/3, in cardiac tissue of ART-naïve HIV+ children, using previously described methodology [17,45]. Cardiac sections were obtained from a healthy 3 year old infant who had died accidentally and two children with perinatal HIV infection, 2 and 9 years of age (Fig. 2A,B). Neither control nor HIV-infected children had evidence of myocarditis or infections linked to accelerated CVD. Trichrome staining of cardiac sections of control and HIV+ infants showed deposition of collagen and increased phosphorylated (p) Smad2 in the latter (Fig. 2B). Quantitation of the average area of staining for trichrome and pSmad2 showed a marked increase in the HIV+ subjects, with strong correlation between fibrosis score and level of pSmad2 staining ($r=0.8531$; Fig. 2C).

Biomarkers for CVD in HIV/ART

We evaluated levels of the N-terminal fragment of the prohormone B-type natriuretic peptide (NT-proBNP) in plasmas from a cohort of HIV- and HIV+ postmenopausal women involved in an investigation of ART-linked osteoporosis [4,11]. NT-proBNP is a biomarker for left ventricle dysfunction, and a prognostic factor for CVD in the general population [46] and in HIV+ individuals [47]. Only those HIV+ women on RTV-based ART had a significant increase in the highest quartile of NT-proBNP (Fig. 3).

Interventions to reduce HIV/RTV-linked fibrosis

In Fig. 1D we propose a strategy to mitigate the fibrotic effects of TGF- β 1 by exploiting natural inhibitors of TGF- β function. Carbon monoxide (CO) is generated endogenously in mammalian cells through the catalysis of heme, modulating vascular contractility and protecting against oxidative stress [49]. We, and others, have shown that CO and the CO releasing molecules CORM-2,-3 suppress basal and TGF- β 1 stimulated collagen expression [31,48]. CO and CORMs are active in animal models of pulmonary and renal fibrosis [48,49], and a multi-center study of CO inhalation treatment in idiopathic pulmonary fibrosis, administered two times weekly, two hours per dose, for 12 weeks, is in progress (ClinicalTrials.gov NCT01214187). We hypothesize that CO will inhibit fibrosis by suppressing inflammation through its anti-oxidant and anti-apoptotic properties as well as by promoting autophagy.

Broad benefits of our model for additional fibrosis-related dysfunctions characteristic of HIV/ART are possible. Clinical studies suggest that HIV/ART may accelerate fibrosis in lung, kidney and liver, and clinically relevant, if modest, inhibitors of fibrosis such as pirfenidone, possibly acting via inhibition of TGF- β 1, have mitigated SIV-linked lymph node fibrosis [50].

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J.L., M.C. and J.A. conceived the idea and drafted the manuscript. J.L. obtained the tissue specimens, J.A. performed the immunohistochemistry studies, and H.T. performed the cytokine analyses. We thank Brennah Murphy for assisting with fibrosis quantification. All authors reviewed the manuscript and contributed to the conclusions.

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Fig. 1.

(A) TGF-β1 regulates collagen synthesis and accumulation to maintain homeostasis under physiological conditions. This involves positive signaling pathways initiating collagen synthesis mediated by the nuclear signaling adapter protein TRAF6 via Smad2,3 and TAK1/MEK/p38, and mechanisms for collagen degradation via autophagy linked to TAK1/MEK/p38 activation. These pathways have been documented in murine and human cardiac myocytes.

(B) HIV infection leads to increases in TGF-β1 levels, which may induce pathologic fibrosis. HIV induces a variety of proinflammatory cytokines which elevate TGF-β1 and, through various mechanisms, including direct binding of HIV envelope to platelet DC-SIGN, induction of IL-6 and IL-8, and induction of tissue factor, can activate platelets, leading, we hypothesize, to their release of TGF-β1. Other cells involved in this inflammatory milieu, such as monocytes, may also contribute to TGF-β release. In addition, certain circulating HIV proteins can interfere with regulation of collagen accumulation by blocking Beclin 1. Beclin 1 has been documented to promote autophagy, alone and in association with an LC3 autophagocytic vesicle, in cardiac myocytes.

(C) Treatment of HIV infection with ritonavir-based ART lowers circulating levels of TGF-β1 and other proinflammatory cytokines, but concentrations are not normalized. RTV itself may also be able to activate platelets, an effect thus far shown only for abacavir. In addition, the activity of TGF-β1 may be enhanced by RTV-mediated facilitation of TRAF6 activity through specific inhibition of immunoproteasome function, with resultant suppression of TRAF6 degradation. This could lead to increased collagen accumulation mediated through the TAK1/MKK3/p38 pathway. The role of autophagy in terms of overall collagen accumulation in this system is unknown. Its potential facilitation, via suppression of HIV proteins which block Beclin 1, and the contribution of TRAF6 mediated potentiation of TAK1/MEK/p38-associated autophagy, awaits further investigation.

(D) Points of possible intervention to mitigate HIV/RTV-induced cardiac fibrosis. Carbon monoxide (CO) has been shown to have a dual role in many cell types, blocking production of TGF- β 1 and other inflammatory cytokines as well as facilitating Beclin 1 activity, thereby promoting autophagy-mediated collagen degradation. We hypothesize that all of these CO-related mechanisms interact to mitigate TGF- β related cardiac fibrosis.

Fig. 2.

Fibrosis and heightened Smad2 activation in cardiac tissue from ART-naïve HIV+ children. (A,B) Cardiac sections from a healthy 3 year old male who died in a motor accident (A), and an HIV-infected 2 year old male (B), were stained with trichrome, as a measure of fibrosis (note areas of blue deposits) or antibody against phosphorylated Smad2 (Phospho-Smad2), as a measure of TGF- β signaling (note intensity of brown nuclear deposits). Photomicrographs were taken with 20X, 100X and 200X magnifications. (C) The bar graphs represent arbitrary units of trichrome staining intensity as a measure of collagen deposition, and phosphorylated Smad2 staining as a measure of Smad2 activation in cardiac sections from two HIV+ children and 1 HIV- control. A high degree of correlation between fibrosis score and Smad2 staining was seen (line graph).

Fig. 3.

Plasmas collected during a study of bone mineral density loss in HIV– and HIV+ postmenopausal women, predominantly Hispanic and African-American and matched for age and tobacco, ethanol and steroid hormone use, were assayed for NT-proBNP utilizing a competitive ELISA (Biomedica, Vienna, Austria). Samples were run in duplicate and statistics performed using SAS Software (version 9.4), unpaired Student t tests, and one way analysis of variance. Univariate distribution provided quartile cutoffs. There was no difference in NT-proBNP levels for the lowest (10th) percentile or quartiles 1 through 3 for any groups. However, there was a difference ($p < 0.05$) for elevation of NT-proBNP for HIV + women on ritonavir (RTV)-based ART ($n = 19$) vs. HIV– controls ($n = 19$), and a trend for higher NT-proBNP among women on RTV vs. the HIV+ ART naïve ($n = 16$; $p < 0.10$). There was no increase in NT-proBNP levels between the controls and HIV+ women on non-protease inhibitor based regimens ($n = 28$; $p = 0.86$).