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Overexpression of mitochondrial antioxidant manganese superoxide dismutase (MnSOD) provides protection against AZT- or 3TC-induced endothelial dysfunction

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Abstract

Nucleoside reverse transcriptase inhibitors (NRTIs) are considered the backbone of current combination therapies for HIV. These therapies have significantly decreased mortality and morbidity in HIV-infected patients, but some are associated with cardiovascular complications, including endothelial dysfunction, an early marker for atherosclerosis. Our prior studies demonstrated that co-treatment of cells with an antioxidant therapy reversed NRTI-induced endothelial injury. Thus, as a proof of concept that mitochondrially-targeted antioxidants may be useful in preventing NRTI toxicity, in the current study, mice overexpressing a mitochondrial antioxidant, manganese superoxide dismutase (MnSOD), were compared with wild-type (WT) mice. Mice were treated chronically with either zidovudine (AZT), lamivudine (3TC), or tenofovir (TDF) to determine whether overexpression of MnSOD protected them from endothelial dysfunction. Endothelial function was assessed using vessel reactivity experiments on thoracic aortas as well as measures of endothelium derived factors nitric oxide (NO), endothelin-1 (ET-1), and prostacyclin. Oxidative stress was evaluated as levels of plasma 8-isoprostane. Alterations in vessel reactivity, NO, and ET-1 in WT mice treated with AZT or 3TC were noted. Overexpression of MnSOD offered protection from decreases in vessel reactivity and increases in ET-1. These findings indicate that mitochondrial oxidative stress induced by AZT or 3TC plays a major role in mediating NRTI-induced endothelial dysfunction, and suggest that the use of targeted antioxidants administered in conjunction with NRTIs may attenuate these effects.

Keywords

endothelial dysfunction; NRTI; atherosclerosis; antioxidants

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1. Introduction

Nucleoside reverse transcriptase inhibitors (NRTIs) are a major component of the current combination therapy for HIV. These combination therapies have significantly decreased mortality (Hammer et al., 1997) and reduced viral load in HIV-infected patients (Hirsch et al., 1999). Long term treatment with these drugs, however, has also been associated with cardiovascular complications including endothelial dysfunction, an early marker of atherosclerosis (Jiang et al., 2006; Lewis, 2004; Sutliff et al., 2002). Endothelial dysfunction is characterized by an imbalance of endothelium-derived contracting (EDCF) and relaxing factors (EDRF). This imbalance may be due to decreased EDRF, increased EDCF, or a combination of the two (Furchgott and Vanhoutte, 1989; Furchgott and Zawadzki, 1980). Two of the primary EDRF are nitric oxide (NO) and prostacyclin, and a major EDCF is endothelin-1 (ET-1). Studies have demonstrated that oxidative stress can alter the EDRF/EDCF balance *via* effects on any or all of these factors (Feletou and Vanhoutte, 2006).

In vitro analyses in our lab have demonstrated that NRTI-treated endothelial cells produce decreased NO and increased ET-1 levels, indicative of an EDRF/EDCF imbalance and endothelial dysfunction. Additionally, these cells generated increased reactive oxygen species (ROS), reduced ATP production, and decreased electron transport chain activity, suggesting that mitochondrial oxidative stress is a key player in this dysfunction (Xue et al., 2013). Oxidative stress occurs when the production of ROS exceeds the ability of antioxidants to scavenge and neutralize them. Superoxide radical, generated primarily in mitochondria as a consequence of normal metabolic processes, is the primary oxygen radical produced in the cell, and its production may be increased in pathological conditions (Turrens and Boveris, 1980). The cell has a number of protective mechanisms to prevent toxicity from superoxide (Madamanchi et al., 2005), with the superoxide dismutases (SOD) acting as the primary scavenging enzymes. These enzymes catalyze the conversion of superoxide to molecular oxygen and hydrogen peroxide (Halliwell and Gutteridge, 1984), and exist in three major forms, each localized to specific intracellular and extracellular compartments: extracellular Cu/ZnSOD, mitochondrial MnSOD, and cytosolic Cu/ZnSOD (Crapo et al., 1992; Weisiger and Fridovich, 1973). Studies have indicated that with aging, insufficient MnSOD alters endothelium-dependent vessel reactivity, a well-established measure of endothelial function (Brown et al., 2007).

Animal studies have demonstrated that a number of NRTIs induce endothelial dysfunction, and that this dysfunction is associated with increased ROS (Jiang et al., 2007; Jiang et al., 2006; Sutliff et al., 2002). The current study utilized mice overexpressing MnSOD to determine the protective effects of this mitochondrial enzyme on endothelial function *in vivo* after chronic administration with zidovudine (AZT), lamivudine (3TC), or tenofovir disoproxil fumarate (TDF). Following treatment, vessel reactivity experiments utilizing thoracic aortas were employed to evaluate endothelial function, and plasma was collected to evaluate endothelium derived factors and oxidative stress. The use of NRTIs from differing subclasses, i.e., a thymidine (AZT) and a cytidine (3TC) analog, compared to a nucleotide reverse transcriptase inhibitor (TDF), enabled us to assess any differential toxic effects of the drugs on the endothelium, as well as any differential protective effects of the overexpressed antioxidant enzyme between groups. We hypothesized that overexpression of

MnSOD would protect against oxidative stress, and would, by extension protect against endothelial dysfunction.

Our findings indicate that two of the drugs tested induced endothelial dysfunction, and that MnSOD overexpression offered variable degrees of protection for these treatments. It is not clear whether these differences are due to additional toxic mechanisms or simply a greater degree of mitochondrial-induced oxidative stress for one drug compared to another. Further studies are needed to investigate the possibility of additional mechanisms of toxicity. However, the findings reported here suggest a possible benefit of mitochondrially-targeted antioxidants as an adjunct therapy for preventing the endothelial dysfunction induced by NRTI treatments.

2. Materials and methods

2.1 Drugs

AZT, 3TC, and TDF were purchased from Morris & Dickson Co. (Shreveport, LA).

2.2 Animals

Transgenic mice overexpressing human MnSOD were a generous gift from Yunfeng Zhao at LSU Health Sciences Center. These mice were developed at the University of Kentucky (Yen et al., 1996). Animals were bred on a C57BL/6 background and were maintained in an AALAC-accredited animal facility. Animal care and use was in accordance with NIH guidelines and all procedures were approved in advance by the LSUHSC Institutional Animal Care and Use Committee. Both male and female mice were utilized for experiments, with mice of each sex stratified as evenly as possible across treatment groups. Dosing of animals was initiated at 6-10 wk of age.

2.3 NRTI administration

Mice were treated with AZT (100 mg/kg/day), TDF (50 mg/kg/day), or 3TC (50 mg/kg/day) in their drinking water for 6-8 wk. These doses were selected by extrapolation from human dosages, using normalization to body surface area (Reagan-Shaw et al., 2008). The method is based on FDA guidelines for estimating doses for clinical trials following animal experimentation (FDA, 2005). To maximize the use of animals and to ensure that our analyses were representative of a human population, male and female mice were randomized as evenly as possible across all groups. Following treatment, mice were anesthetized with pentobarbital (50 mg/kg, i.p.). Anesthesia was confirmed by lack of toe pinch response, and animals were sacrificed by pneumothorax. Blood was collected from the inferior vena cava, and thoracic aortas were excised.

2.4 Measurement of plasma drug levels

Blood for measuring drug levels in mice was collected in EDTA. Plasma drug levels were determined by HPLC using a modification of a previously described method (Jiang et al., 2006; Notari et al., 2006).

2.5 Measurement of plasma biomarkers

Blood for measuring NO metabolites was collected in heparin. After centrifugation, plasma was mixed 1:5 (v/v) with a stabilizing buffer containing 800 mM potassium ferricyanide, 100 mM N-ethylmaleimide, and 10% Nonidet-P40, to preserve nitrite. Samples (were injected into a purge vessel containing a solution with potassium iodide (66.8 mM), iodine (28.5 mM) and acetic acid (78% v/v) to convert nitrite to NO for analysis using a Sievers 280i Nitric Oxide Analyzer (GE, Boulder, CO).

Blood for measuring all other biomarkers was collected in EDTA. Plasma ET-1 and 6-keto prostaglandin F1 were measured using ELISA kits from Enzo Life Sciences (Farmingdale, NY) and Cayman Chemical (Ann Arbor, MI), respectively. Alanine aminotransferase (ALT) and Blood Urea Nitrogen (BUN) were measured using kits from Eagle Diagnostic (DeSoto, TX). Plasma creatinine was measured using a BioVision fluorometric assay kit (Milpitas, CA).

The measurement of 8-isoprostane (8-isoPGF$_{2\alpha}$) required saponification and extraction of F_2-isoprostanes from the plasma (Jiang et al., 2009). 8-isoPGF$_{2\alpha}$ was then measured in the extracts using an ELISA kit from Cayman.

2.6 Vessel reactivity experiments

Thoracic aortas were kept at 37° C in Krebs Henseleit solution (118 mM NaCl, 4.8 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.0 mM KH_2PO_4, 27 mM $NaHCO_3$, and 11.1 mM D-glucose), pH 7.4, and the solution was aerated with 95% O_2 and 5% CO_2 for the duration of the experiments. Aortas were cut into rings and were connected to a force displacement transducer interfaced to a Power Lab data acquisition system (ADInstruments). Baseline tension was set at 1 g and vessels were equilibrated for 1 h before each treatment. Rings were pre-contracted with phenylephrine to achieve 70-80% maximal relaxation, followed by relaxation using increasing doses of acetylcholine (to measure endothelium-dependent relaxation) or sodium nitroprusside (to measure endothelium-independent relaxation).

2.7 Statistics

Data are means ± SD. Statistical analyses were performed using GraphPad Prism software. Treatment and strain were compared by 2-way ANOVA, followed by Fisher LSD posthoc tests. Levels of individual drugs were analyzed between mouse strains using unpaired t-test. $P < 0.05$ was considered significant.

3. Results

3.1 Plasma drug levels

Drug levels measured in the plasma of treated animals were within the reported C_{min} and C_{max} for humans [AZT: Cmin = 3 ng/mL and Cmax = 635 ng/mL; 3TC: Cmin = 78 ng/mL and Cmax = 1195 ng/mL (van Praag et al., 2002); TDF: Cmin = 52 ng/mL and Cmax = 262 ng/mL (Luber et al., 2010)]. For the most part, drug levels were not altered between strains. However, plasma 3TC levels were lower in transgenic compared to WT mice. Studies in humans have indicated that wide discrepancies in mean plasma concentrations of NRTIs

occur at different time points after dosing. Importantly, in most cases, these drug levels peak within 0.5-1 h after dosing (Hemanth Kumar et al., 2009; Ramachandran et al., 2006; Vourvahis et al., 2008). Thus, the lower plasma 3TC levels in transgenic compared to WT mice are likely due to timing of blood collection rather than metabolic differences between the two groups (Table 1).

3.2 NRTI induced oxidative stress and tissue damage in WT and MnSOD transgenic mice

Wild-type mice treated chronically with any one of the three drugs demonstrated increased levels of plasma 8-isoprostane, an indicator of oxidative stress. These increases were attenuated in MnSOD transgenic mice treated with AZT and TDF, suggesting some protection against oxidative stress (Fig. 1).

Elevated plasma 8-isoprostane levels in the absence of prior evidence of TDF-associated cardiovascular problems prompted us to investigate, to a limited extent, whether the oxidative stress may be an indication of toxicity in other tissues (Izzedine et al., 2003; Lund et al., 2007; Sutliff et al., 2002). Plasma ALT levels were elevated in 3TC and in TDF-treated WT mice, but not in MnSOD transgenic mice treated with either drug (Table 1). Plasma BUN levels were also elevated in WT mice treated with AZT, 3TC, or TDF, but not in transgenic mice (Table 1). Plasma creatinine levels were not altered in any groups tested.

3.3 Endothelium-derived factors in WT and MnSOD transgenic mice

NO levels are difficult to assess in plasma, due to its conversion to nitrite and nitrate in blood. For this reason, levels of nitric oxide metabolites, nitrite and nitrate, are typically used to assess NO in plasma. Experimental studies, however, have demonstrated that only nitrite levels are actual indicators of eNOS activity (Lauer et al., 2001). For this reason, our studies utilized conversion of nitrite to NO for the estimation of NO levels. Wild-type mice treated with AZT or 3TC exhibited decreased plasma nitrite levels. Plasma nitrite levels in AZT-treated transgenic mice were also significantly reduced, with a similar trend in 3TC-treated transgenics. These findings indicate that overexpression of MnSOD offers no protection against AZT-induced decreases in NO, and little or no protection against 3TC-induced decreases (Fig. 2).

Plasma levels of 6-keto prostaglandin F1 , the stable metabolite of prostacyclin, were unaffected by chronic treatment with AZT, 3TC, or TDF. Additionally, there were no significant differences between levels in transgenic and WT mice (Fig. 3).

On the other hand, plasma ET-1 levels were increased in WT mice treated with AZT or 3TC. These increases were not evident in transgenic mice, indicating that overexpression of MnSOD protects against AZT-and 3TC-induced increases in plasma ET-1 (Fig. 4).

3.4 Endothelium-dependent vasorelaxation in WT and MnSOD transgenic mice

Vessel reactivity experiments are considered the gold standard for measuring endothelial function. Decreased endothelium-dependent vasorelaxation is a marker for endothelial dysfunction. In this study, WT mice treated chronically with either AZT or 3TC, demonstrated decreases in endothelium-dependent vasorelaxation compared to controls.

Transgenic mice treated with AZT or 3TC did not demonstrate diminished endothelium-dependent vessel reactivity responses, suggesting a protective effect of MnSOD overexpression on the endothelium. Additionally, vessel reactivity of AZT- or 3TC-treated transgenic mice was significantly greater than that of treated WT mice. TDF-treated mice demonstrated no significant difference in vessel reactivity in either treated WT or transgenics (Fig. 5). No alterations in endothelium-independent vasorelaxation were demonstrated in any groups (data not shown).

4. Discussion

AZT was the first antiretroviral drug approved for treatment of acquired immune deficiency syndrome (AIDS) in 1987. Through the years, other NRTIs have been developed and many, including AZT, remain integral components of HAART for HIV infected patients. Although AZT treatment helped slow the progression of AIDS, it was soon discovered that long- term use of AZT induced mitochondrial myopathy in many patients (Dalakas et al., 1990; Moore et al., 1991). In fact, long term treatment with NRTIs has revealed a number of tissue-specific toxicities associated with the different drugs, including endothelial dysfunction, an early event in the development of atherosclerosis (Izzedine et al., 2003; Jiang et al., 2006; Lewis and Dalakas, 1995; Sutliff et al., 2002). Consequently, some NRTIs are no longer in use due to their toxicities. The drugs in this study, however, are currently in use, either as primary or alternative backbones of therapy in adult, pediatric, or perinatal regimens (NIH Adults, 2012; NIH Pediatric, 2012; NIH Perinatal, 2012). Additionally, current US Public Health Service recommendations for post-exposure prophylaxis (PEP), call for treatment with 3 or more antiretroviral drugs, which may include any of the drugs tested here (Kuhar et al., 2013).

Isoprostanes are produced by free radical induced peroxidation of membrane phospholipids, and plasma 8-isoprostane levels are widely used as indicators of systemic oxidative stress in body fluids. Increases in oxidative stress are associated with a number of pathologies, including neurodegenerative diseases, cancer, and atherosclerosis (Coyle and Puttfarcken, 1993; Montuschi et al., 2004; Reuter et al., 2010). In addition to serving as markers of oxidative stress, 8-isoprostanes stimulate monocyte adhesion in endothelial cells, an early event in the development of atherosclerosis (Leitinger et al., 2001). In our study, plasma levels of 8-isoprostane in mice treated with NRTIs were elevated, indicating systemic oxidative stress, and as anticipated, overexpression of MnSOD offered some protection.

Systemic oxidative stress is not limited to the endothelium, and NRTIs have demonstrated the capacity to induce oxidative stress in brain, kidney, liver, and other tissues (Abraham et al., 2013; Adaramoye et al., 2012; Opii et al., 2007; Velsor et al., 2004; Vigouroux et al., 2011). Liver toxicity has been associated with NRTI therapies and although TDF is not generally considered hepatotoxic (Rodriguez-Rosado et al., 1998; Rotger et al., 2007), our WT mice treated with 3TC or TDF exhibited slightly elevated ALT compared to control mice. TDF and other NRTIs have been linked to nephrotoxicity, specifically in renal proximal tubule cells (Fernandez-Fernandez et al., 2011; Izzedine et al., 2003; Kohler et al., 2009). In this study, BUN and creatinine levels were measured to assess kidney toxicity. Slight elevations in BUN were demonstrated in WT mice treated with all three drugs, but

not in transgenic mice. It is not clear whether a longer duration of treatment would have had a greater impact on these markers of tissue toxicity.

Nitric oxide reacts rapidly with superoxide to form peroxynitrite, reducing the bioavailability of NO (Feletou and Vanhoutte, 2006). In addition, oxidative stress can alter endothelial nitric oxide synthase (eNOS) functionality by depleting the cofactor tetrahydrobiopterin (BH_4), in a process known as eNOS uncoupling. This uncoupling triggers increased ROS formation and decreased NO levels (Dumitrescu et al., 2007; Landmesser et al., 2003; Santhanam et al., 2012). In this study, treatment with AZT and 3TC reduced plasma nitrite levels by 31.4 and 28.5%, respectively. To put this in perspective, prior reports utilizing known atherogenic conditions have shown similar reductions in plasma nitrite levels. For example, treatment of mice with an atherogenic diet for three weeks induced no significant change in plasma nitrite levels (Stokes et al., 2009), whereas treatment of mice for 20 weeks with a similar high fat diet produced a 42% reduction in levels of plasma nitrite (Rajapakse et al., 2014). On the other hand, induction of diabetes in mice using streptozotocin produced only a 17% reduction in plasma nitrite levels (Sasaki et al., 2008). Thus, our observation of NRTI-induced 28-31% reductions in plasma nitrite levels are likely biologically significant. However, overexpressed MnSOD did not protect against decreased nitrite levels in AZT-treated mice, even with the reduced levels of oxidative stress indicated by 8-isoprostane measurements. This suggests an alternative and/or additional mechanism underlying the AZT-induced nitrite reductions.

Normally, the potent vasoconstrictor ET-1 is produced and maintained at low levels, primarily by endothelial cells. In pathological conditions, however, ET-1 production may be increased, not only by endothelial cells, but also in other types of cells, including smooth muscle cells and macrophages (Bohm and Pernow, 2007). Prior work in our laboratory demonstrated that treatment of endothelial cells with AZT, 3TC, or tenofovir induces increases in ET-1 *in vitro* and that co-treatment with the antioxidant coenzyme Q10 prevents this increase (Xue et al., 2013). In the current study, WT and transgenic mice treated with AZT and 3TC paralleled the *in vitro* results. Unlike the observed decreases in nitrite levels, ET-1 increases appear totally dependent upon mitochondrial oxidative stress, as all increases were abolished in the MnSOD transgenic animals.

In conclusion, these studies implicate mitochondrial oxidative stress as the major culprit in AZT- and 3TC-induced alterations in ET-1, but not in NO levels. Vessel reactivity experiments demonstrate the importance of a balance between these endothelial derived mediators. The endothelial protection offered by overexpression of a mitochondrial antioxidant suggests that targeted antioxidants may help prevent some of the cardiovascular toxicity associated with NRTIs. No endothelial dysfunction, however, was evident in mice treated with TDF, a drug that has been linked to renal rather than cardiovascular toxicity (Izzedine et al., 2003). However, while renal and other tissue toxicities are believed to be mediated by mitochondrial oxidative stress (Abraham et al., 2013; Duong Van Huyen et al., 2003; Jiang et al., 2009; Kohler et al., 2009), our studies indicate that targeted antioxidants may also be useful in preventing other NRTI side effects. Our *in vitro* studies support the concept of adding coenzyme Q10 to current therapies (Xue et al., 2013). Unfortunately, variable results in effects of CoQ10 treatments for mitochondrial diseases have been

reported, presumably due to low bioavailability in plasma and tissues after administration (Molyneux et al., 2008; Wyman et al., 2010). Dramatic progress toward improving bioavailability of CoQ10 has been made in recent years (Beg et al., 2010), making a stronger case for this preventive treatment. Alternatively, mitochondrially-targeted antioxidants have also been developed, and results from early testing have been promising (Murphy and Smith, 2007; Szeto, 2006). Further testing with these antioxidants in combination with NRTIs is still needed to confirm protective effects.

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References Cited

Abraham P, Ramamoorthy H, Isaac B. Depletion of the cellular antioxidant system contributes to tenofovir disoproxil fumarate - induced mitochondrial damage and increased oxido-nitrosative stress in the kidney. Journal of biomedical science. 2013; 20:61. [PubMed: 23957306]

Adaramoye OA, Adewumi OM, Adesanoye OA, Faokunla OO, Farombi EO. Effect of tenofovir, an antiretroviral drug, on hepatic and renal functional indices of Wistar rats: protective role of vitamin E. Journal of basic and clinical physiology and pharmacology. 2012; 23:69–75. [PubMed: 22944656]

Beg S, Javed S, Kohli K. Bioavailability enhancement of coenzyme Q10: an extensive review of patents. Recent patents on drug delivery & formulation. 2010; 4:245–255. [PubMed: 20863275]

Bohm F, Pernow J. The importance of endothelin-1 for vascular dysfunction in cardiovascular disease. Cardiovascular research. 2007; 76:8–18. [PubMed: 17617392]

Brown KA, Didion SP, Andresen JJ, Faraci FM. Effect of aging, MnSOD deficiency, and genetic background on endothelial function: evidence for MnSOD haploinsufficiency. Arteriosclerosis, thrombosis, and vascular biology. 2007; 27:1941–1946.

Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science. 1993; 262:689–695. [PubMed: 7901908]

Crapo JD, Oury T, Rabouille C, Slot JW, Chang LY. Copper,zinc superoxide dismutase is primarily a cytosolic protein in human cells. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89:10405–10409. [PubMed: 1332049]

Dalakas MC, Illa I, Pezeshkpour GH, Laukaitis JP, Cohen B, Griffin JL. Mitochondrial myopathy caused by long-term zidovudine therapy. The New England journal of medicine. 1990; 322:1098–1105. [PubMed: 2320079]

Dumitrescu C, Biondi R, Xia Y, Cardounel AJ, Druhan LJ, Ambrosio G, Zweier JL. Myocardial ischemia results in tetrahydrobiopterin (BH4) oxidation with impaired endothelial function ameliorated by BH4. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104:15081–15086. [PubMed: 17848522]

Duong Van Huyen JP, Landau A, Piketty C, Belair MF, Batisse D, Gonzalez-Canali G, Weiss L, Jian R, Kazatchkine MD, Bruneval P. Toxic effects of nucleoside reverse transcriptase inhibitors on the liver. Value of electron microscopy analysis for the diagnosis of mitochondrial cytopathy. American journal of clinical pathology. 2003; 119:546–555. [PubMed: 12710127]

FDA. Estimating the safe starting dose in clinical trials for therapeutics in adult healthy volunteers. Center for Drug Evaluation and Research, U. S. Food and Drug Administration; Rockville, Maryland, USA: 2005.

Feletou M, Vanhoutte PM. Endothelial dysfunction: a multifaceted disorder (The Wiggers Award Lecture). American journal of physiology. Heart and circulatory physiology. 2006; 291:H985–1002. [PubMed: 16632549]

Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Nino MD, Izquierdo MC, Poveda J, Sainz-Prestel V, Ortiz-Martin N, Parra-Rodriguez A, Selgas R, Ruiz-Ortega M, Egido J, Ortiz A.

Tenofovir nephrotoxicity: 2011 update. AIDS research and treatment. 2011; 2011:354908. [PubMed: 21716719]

Furchgott RF, Vanhoutte PM. Endothelium-derived relaxing and contracting factors. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 1989; 3:2007–2018. [PubMed: 2545495]

Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature. 1980; 288:373–376. [PubMed: 6253831]

Halliwell B, Gutteridge JM. Oxygen toxicity, oxygen radicals, transition metals and disease. The Biochemical journal. 1984; 219:1–14. [PubMed: 6326753]

Hammer SM, Squires KE, Hughes MD, Grimes JM, Demeter LM, Currier JS, Eron JJ Jr. Feinberg JE, Balfour HH Jr. Deyton LR, Chodakewitz JA, Fischl MA. A controlled trial of two nucleoside analogues plus indinavir in persons with human immunodeficiency virus infection and CD4 cell counts of 200 per cubic millimeter or less. AIDS Clinical Trials Group 320 Study Team. The New England journal of medicine. 1997; 337:725–733. [PubMed: 9287227]

Hemanth Kumar AK, Ramachandran G, Rajasekaran S, Padmapriyadarsini C, Narendran G, Anitha S, Subramanyam S, Kumaraswami V, Swaminathan S. Pharmacokinetics of lamivudine & stavudine in generic fixed-dose combinations in HIV-1 infected adults in India. The Indian journal of medical research. 2009; 130:451–457. [PubMed: 19942751]

Hirsch M, Steigbigel R, Staszewski S, Mellors J, Scerpella E, Hirschel B, Lange J, Squires K, Rawlins S, Meibohm A, Leavitt R. A randomized, controlled trial of indinavir, zidovudine, and lamivudine in adults with advanced human immunodeficiency virus type 1 infection and prior antiretroviral therapy. The Journal of infectious diseases. 1999; 180:659–665. [PubMed: 10438352]

Izzedine H, Launay-Vacher V, Isnard-Bagnis C, Deray G. Drug-induced Fanconi's syndrome. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2003; 41:292–309. [PubMed: 12552490]

Jiang B, Hebert VY, Khandelwal AR, Stokes KY, Dugas TR. HIV-1 antiretrovirals induce oxidant injury and increase intima-media thickness in an atherogenic mouse model. Toxicology letters. 2009; 187:164–171. [PubMed: 19429260]

Jiang B, Hebert VY, Li Y, Mathis JM, Alexander JS, Dugas TR. HIV antiretroviral drug combination induces endothelial mitochondrial dysfunction and reactive oxygen species production, but not apoptosis. Toxicology and applied pharmacology. 2007; 224:60–71. [PubMed: 17669453]

Jiang B, Hebert VY, Zavecz JH, Dugas TR. Antiretrovirals induce direct endothelial dysfunction in vivo. Journal of acquired immune deficiency syndromes. 2006; 42:391–395. [PubMed: 16810107]

Kohler JJ, Hosseini SH, Hoying-Brandt A, Green E, Johnson DM, Russ R, Tran D, Raper CM, Santoianni R, Lewis W. Tenofovir renal toxicity targets mitochondria of renal proximal tubules. Laboratory investigation; a journal of technical methods and pathology. 2009; 89:513–519.

Kuhar DT, Henderson DK, Struble KA, Heneine W, Thomas V, Cheever LW, Gomaa A, Panlilio AL, Group USPHSW. Updated US Public Health Service guidelines for the management of occupational exposures to human immunodeficiency virus and recommendations for postexposure prophylaxis. Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America. 2013; 34:875–892.

Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. The Journal of clinical investigation. 2003; 111:1201–1209. [PubMed: 12697739]

Lauer T, Preik M, Rassaf T, Strauer BE, Deussen A, Feelisch M, Kelm M. Plasma nitrite rather than nitrate reflects regional endothelial nitric oxide synthase activity but lacks intrinsic vasodilator action. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:12814–12819. [PubMed: 11606734]

Leitinger N, Huber J, Rizza C, Mechtcheriakova D, Bochkov V, Koshelnick Y, Berliner JA, Binder BR. The isoprostane 8-iso-PGF(2alpha) stimulates endothelial cells to bind monocytes: differences from thromboxane-mediated endothelial activation. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2001; 15:1254–1256. [PubMed: 11344105]

Lewis W. Cardiomyopathy, nucleoside reverse transcriptase inhibitors and mitochondria are linked through AIDS and its therapy. Mitochondrion. 2004; 4:141–152. [PubMed: 16120379]

Lewis W, Dalakas MC. Mitochondrial toxicity of antiviral drugs. Nature medicine. 1995; 1:417–422.

Luber AD, Condoluci DV, Slowinski PD, Andrews M, Olson K, Peloquin CA, Pappa KA, Pakes GE, Team COLS. Steady-state amprenavir and tenofovir pharmacokinetics after coadministration of unboosted or ritonavir-boosted fosamprenavir with tenofovir disoproxil fumarate in healthy volunteers. HIV medicine. 2010; 11:193–199. [PubMed: 19863619]

Lund KC, Peterson LL, Wallace KB. Absence of a universal mechanism of mitochondrial toxicity by nucleoside analogs. Antimicrobial agents and chemotherapy. 2007; 51:2531–2539. [PubMed: 17470651]

Madamanchi NR, Vendrov A, Runge MS. Oxidative stress and vascular disease. Arteriosclerosis, thrombosis, and vascular biology. 2005; 25:29–38.

Molyneux SL, Young JM, Florkowski CM, Lever M, George PM. Coenzyme Q10: is there a clinical role and a case for measurement? The Clinical biochemist. Reviews / Australian Association of Clinical Biochemists. 2008; 29:71–82.

Montuschi P, Barnes PJ, Roberts LJ 2nd. Isoprostanes: markers and mediators of oxidative stress. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2004; 18:1791–1800. [PubMed: 15576482]

Moore RD, Hidalgo J, Sugland BW, Chaisson RE. Zidovudine and the natural history of the acquired immunodeficiency syndrome. The New England journal of medicine. 1991; 324:1412–1416. [PubMed: 2020297]

Murphy MP, Smith RA. Targeting antioxidants to mitochondria by conjugation to lipophilic cations. Annual review of pharmacology and toxicology. 2007; 47:629–656.

NIH Adults, P.. Panel on antiretroviral guidelines for adults and adolescents.. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. 2012. AIDSinfo.nih.gov/guidelines

NIH Pediatric, P.. Panel on antiretroviral therapy and medical management of HIV-infected children.. Guidelines for the use of antiretroviral agents in pediatric HIV infection. 2012. AIDSinfo.nih.gov/guidelines, http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf

NIH Perinatal, P.. Panel on treatment of HIV-infected pregnant women and prevention of perinatal transmission.. Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States. 2012. AIDSinfo.nih.gov/guidelines, http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf

Notari S, Bocedi A, Ippolito G, Narciso P, Pucillo LP, Tossini G, Donnorso RP, Gasparrini F, Ascenzi P. Simultaneous determination of 16 anti-HIV drugs in human plasma by high-performance liquid chromatography. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 2006; 831:258–266.

Opii WO, Sultana R, Abdul HM, Ansari MA, Nath A, Butterfield DA. Oxidative stress and toxicity induced by the nucleoside reverse transcriptase inhibitor (NRTI)--2',3'-dideoxycytidine (ddC): relevance to HIV-dementia. Experimental neurology. 2007; 204:29–38. [PubMed: 17069802]

Rajapakse NW, Karim F, Straznicky NE, Fernandez S, Evans RG, Head GA, Kaye DM. Augmented endothelial-specific L-arginine transport prevents obesity-induced hypertension. Acta physiologica. 2014 2014 Jul 10. doi: 10.1111/apha.12344. [Epub ahead of print].

Ramachandran G, Hemanthkumar AK, Kumaraswami V, Swaminathan S. A simple and rapid liquid chromatography method for simultaneous determination of zidovudine and nevirapine in plasma. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences. 2006; 843:339–344.

Reagan-Shaw S, Nihal M, Ahmad N. Dose translation from animal to human studies revisited. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2008; 22:659–661. [PubMed: 17942826]

Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? Free radical biology & medicine. 2010; 49:1603–1616. [PubMed: 20840865]

Rodriguez-Rosado R, Garcia-Samaniego J, Soriano V. Hepatotoxicity after introduction of highly active antiretroviral therapy. Aids. 1998; 12:1256. [PubMed: 9677182]

Rotger M, Colombo S, Furrer H, Decosterd L, Buclin T, Telenti A. Does tenofovir influence efavirenz pharmacokinetics? Antiviral therapy. 2007; 12:115–118. [PubMed: 17503755]

Santhanam AV, d'Uscio LV, Smith LA, Katusic ZS. Uncoupling of eNOS causes superoxide anion production and impairs NO signaling in the cerebral microvessels of hph-1 mice. Journal of neurochemistry. 2012; 122:1211–1218. [PubMed: 22784235]

Sasaki N, Yamashita T, Takaya T, Shinohara M, Shiraki R, Takeda M, Emoto N, Fukatsu A, Hayashi T, Ikemoto K, Nomura T, Yokoyama M, Hirata K, Kawashima S. Augmentation of vascular remodeling by uncoupled endothelial nitric oxide synthase in a mouse model of diabetes mellitus. Arteriosclerosis, thrombosis and vascular biology. 2008; 28:1068–1076.

Stokes KY, Dugas TR, Tang Y, Garg H, Guidry E, Bryan NS. Dietary nitrite prevents hypercholesterolemic microvascular inflammation and reverses endothelial dysfunction. American journal of physiology. Heart and circulatory physiology. 2009; 296:H1281–1288. [PubMed: 19252084]

Sutliff RL, Dikalov S, Weiss D, Parker J, Raidel S, Racine AK, Russ R, Haase CP, Taylor WR, Lewis W. Nucleoside reverse transcriptase inhibitors impair endothelium-dependent relaxation by increasing superoxide. American journal of physiology. Heart and circulatory physiology. 2002; 283:H2363–2370. [PubMed: 12388299]

Szeto HH. Cell-permeable, mitochondrial-targeted, peptide antioxidants. The AAPS journal. 2006; 8:E277–283. [PubMed: 16796378]

Turrens JF, Boveris A. Generation of superoxide anion by the NADH dehydrogenase of bovine heart mitochondria. The Biochemical journal. 1980; 191:421–427. [PubMed: 6263247]

van Praag RM, van Weert EC, van Heeswijk RP, Zhou XJ, Sommadossi JP, Jurriaans S, Lange JM, Hoetelmans RM, Prins JM. Stable concentrations of zidovudine, stavudine, lamivudine, abacavir, and nevirapine in serum and cerebrospinal fluid during 2 years of therapy. Antimicrobial agents and chemotherapy. 2002; 46:896–899. [PubMed: 11850283]

Velsor LW, Kovacevic M, Goldstein M, Leitner HM, Lewis W, Day BJ. Mitochondrial oxidative stress in human hepatoma cells exposed to stavudine. Toxicology and applied pharmacology. 2004; 199:10–19. [PubMed: 15289086]

Vigouroux C, Caron-Debarle M, Le Dour C, Magre J, Capeau J. Molecular mechanisms of human lipodystrophies: from adipocyte lipid droplet to oxidative stress and lipotoxicity. The international journal of biochemistry & cell biology. 2011; 43:862–876. [PubMed: 21392585]

Vourvahis M, Tappouni HL, Patterson KB, Chen YC, Rezk NL, Fiscus SA, Kearney BP, Rooney JF, Hui J, Cohen MS, Kashuba AD. The pharmacokinetics and viral activity of tenofovir in the male genital tract. Journal of acquired immune deficiency syndromes. 2008; 47:329–333. [PubMed: 18197124]

Weisiger RA, Fridovich I. Superoxide dismutase. Organelle specificity. The Journal of biological chemistry. 1973; 248:3582–3592. [PubMed: 4702877]

Wyman M, Leonard M, Morledge T. Coenzyme Q10: a therapy for hypertension and statin-induced myalgia? Cleveland Clinic journal of medicine. 2010; 77:435–442. [PubMed: 20601617]

Xue SY, Hebert VY, Hayes DM, Robinson CN, Glover M, Dugas TR. Nucleoside Reverse Transcriptase Inhibitors Induce a Mitophagy-Associated Endothelial Dysfunction That Is Reversed by Coenzyme Q10 Co-Treatment. Toxicological sciences : an official journal of the Society of Toxicology. 2013

Yen HC, Oberley TD, Vichitbandha S, Ho YS, St Clair DK. The protective role of manganese superoxide dismutase against adriamycin-induced acute cardiac toxicity in transgenic mice. The Journal of clinical investigation. 1996; 98:1253–1260. [PubMed: 8787689]

Highlights

- NRTI-induced endothelial dysfunction was evaluated in wild-type mice and mice overexpressing a mitochondrial antioxidant.
- Endothelial dysfunction in WT mice was marked by alterations in nitric oxide and endothelin-1, as well as the oxidative stress marker 8-isoprostane.
- Overexpression of a mitochondrial antioxidant offered protection
- This study presents a strong case for the use of mitochondrially-targeted antioxidants as adjunct therapy for NRTIs.

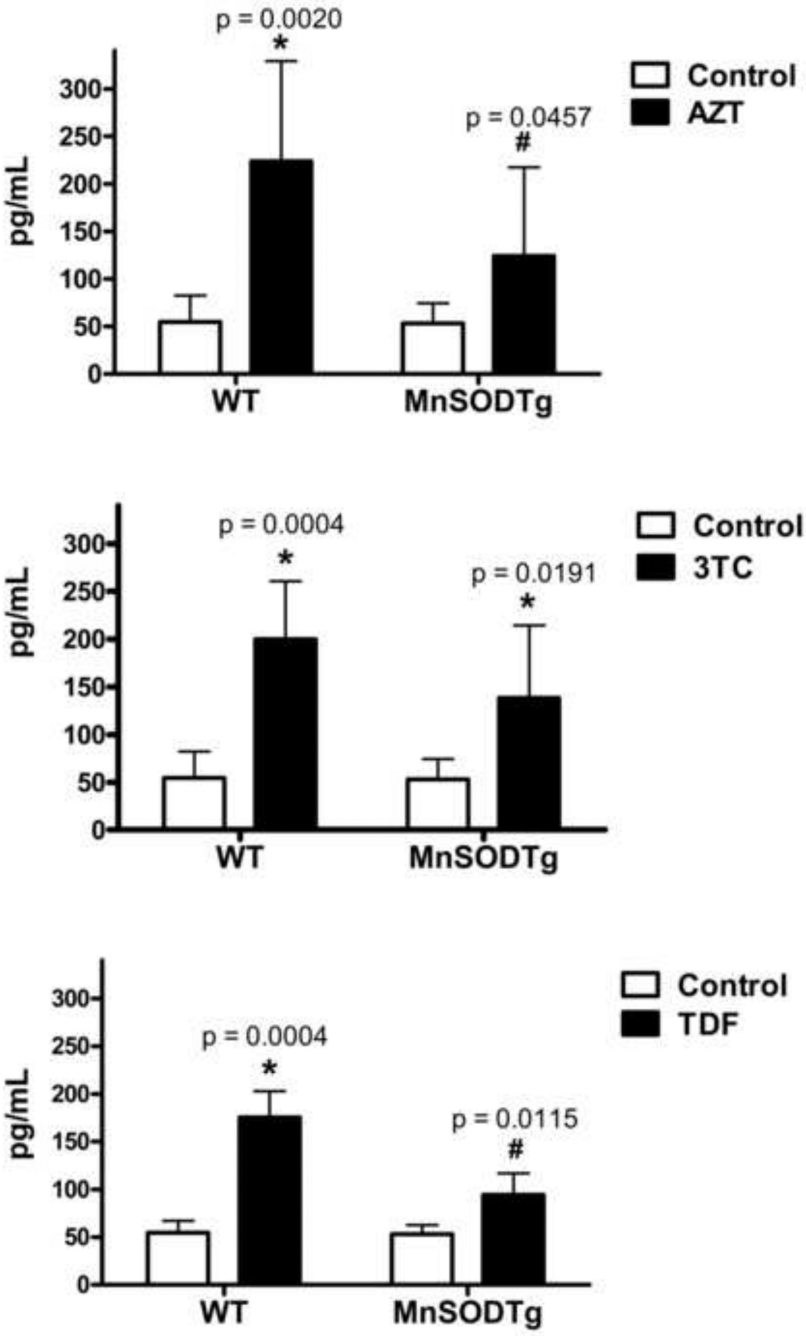

Figure 1.

Plasma 8-isoprostane levels in wild-type (WT) and transgenic mice overexpressing MnSOD (MnSOD Tg). Mice were treated for 6-8 weeks with AZT (100 mg/kg/day), 3TC (50 mg/kg/day), or TDF (50 mg/kg/day) and plasma levels of 8-isoprostane were compared with controls. Data are means ± standard deviation. * Denotes significant difference ($p < 0.05$) from matched controls. # Indicates significant difference ($p < 0.05$) between treated MnSOD Tg and treated WT. n = 5/group.

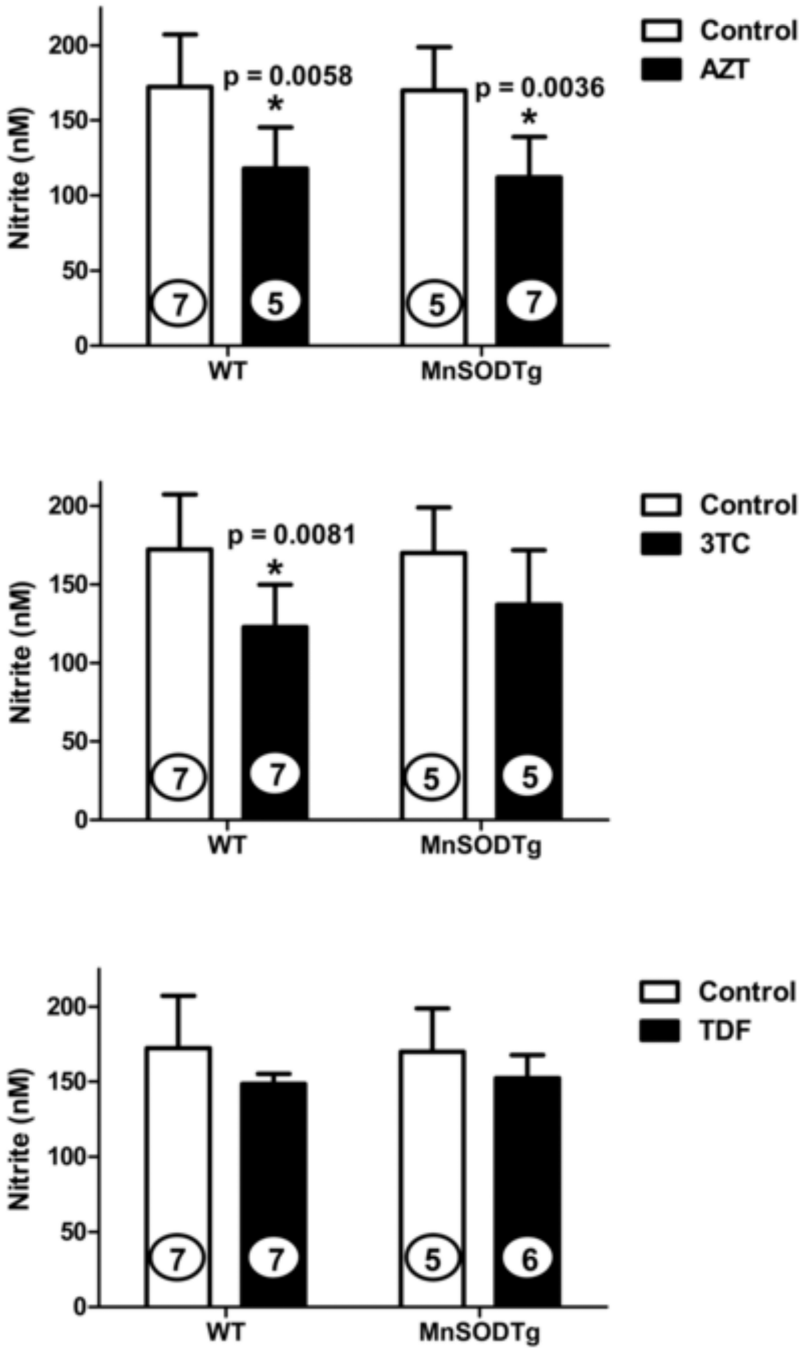

Figure 2.

Plasma nitrite levels in wild-type (WT) and transgenic mice overexpressing MnSOD (MnSOD Tg). Mice were treated for 6-8 weeks with AZT (100 mg/kg/day), 3TC (50 mg/kg/day), or TDF (50 mg/kg/day) and plasma nitrite levels were compared with controls. *$P <$ 0.05 compared to matched controls. Data are means ± SD. #$p < 0.05$ between treated MnSOD Tg and treated WT. n = 5-7/group, as indicated within each bar.

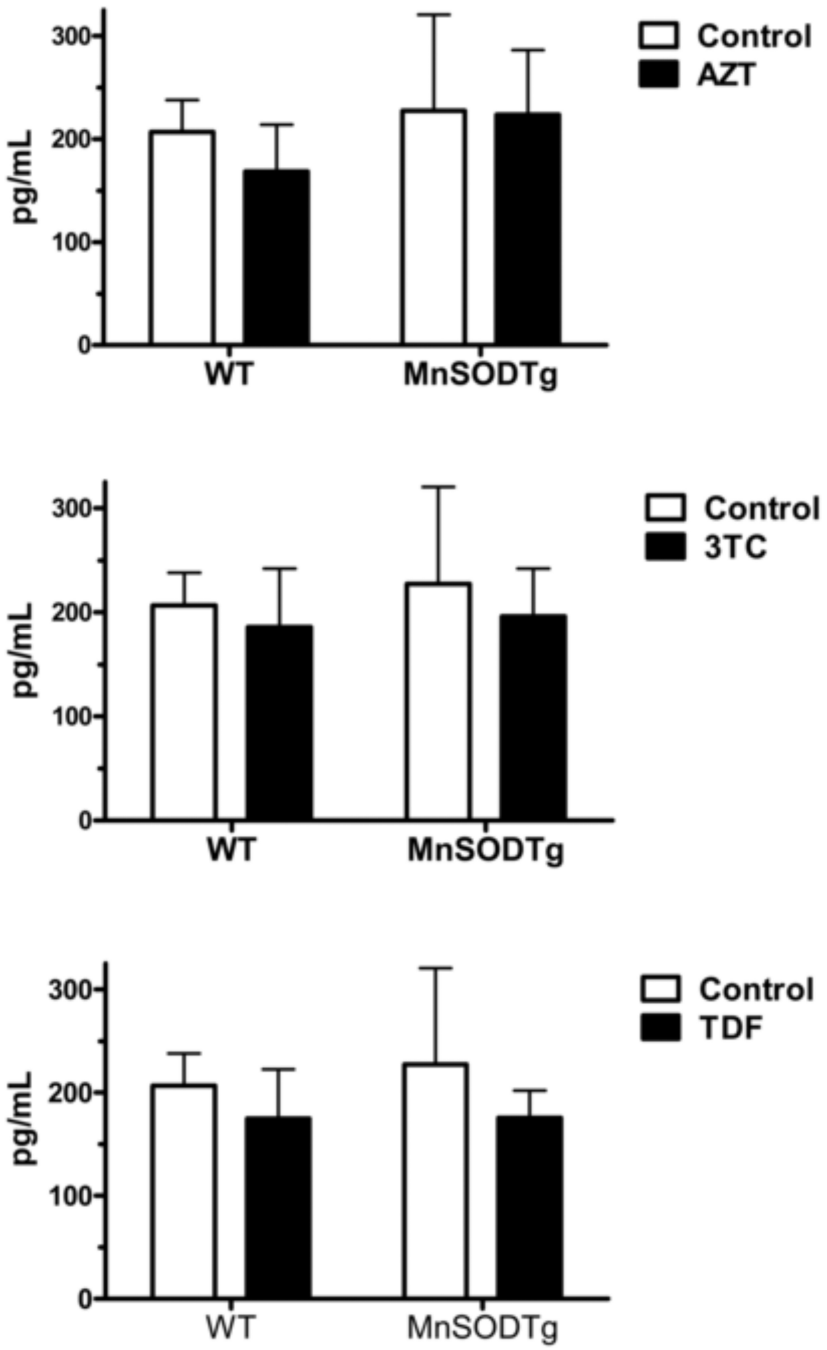

Figure 3.

Plasma 6-keto prostaglandin F1 levels in wild-type (WT) and transgenic mice overexpressing MnSOD (MnSOD Tg). Mice were treated for 6-8 weeks with AZT (100 mg/kg/day), 3TC (50 mg/kg/day), or TDF (50 mg/kg/day) and plasma 6-keto prostaglandin F1 levels were compared with controls. Data are means ± SD. *Significance ($p < 0.05$) compared to matched controls. #$p < 0.05$ between treated MnSOD Tg and treated WT. n = 5/group.

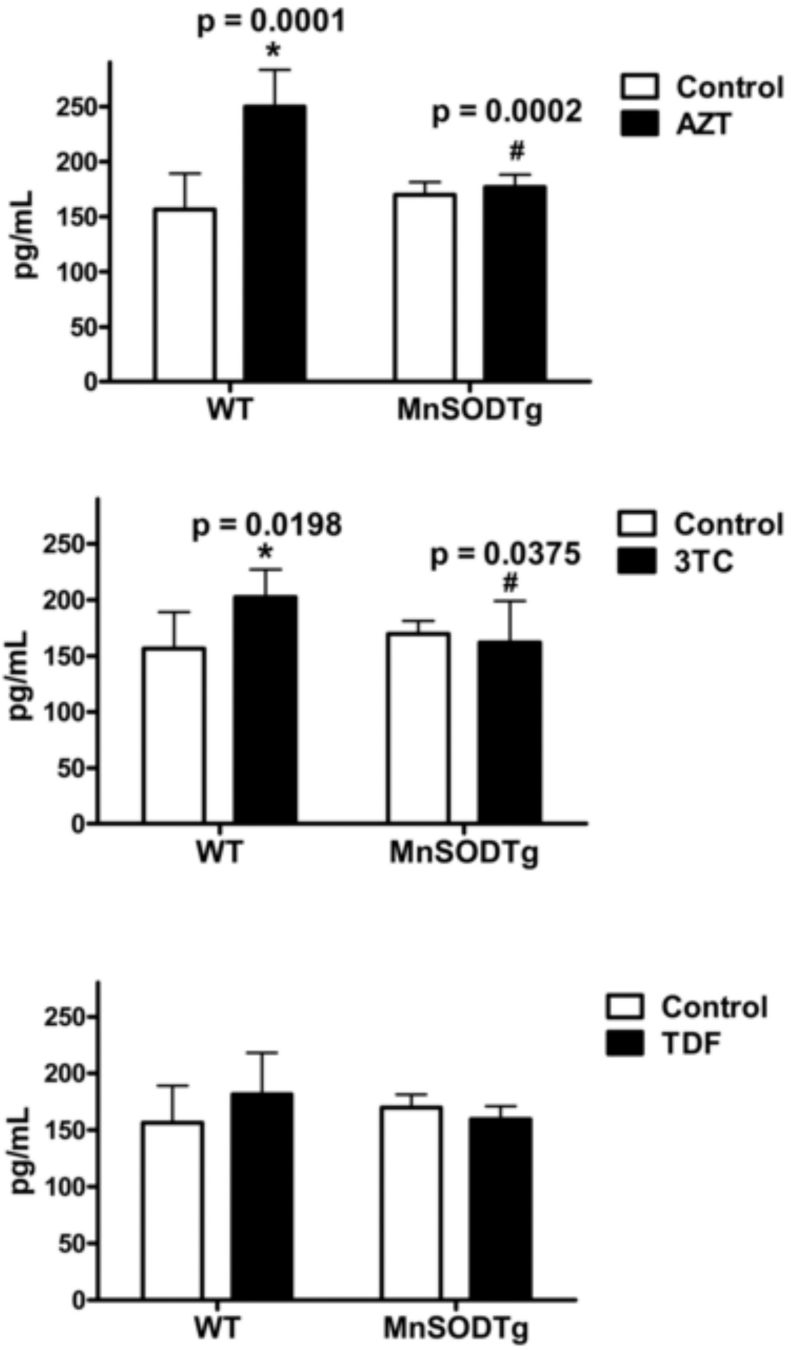

Figure 4.

Plasma ET-1 levels in wild-type (WT) and transgenic mice overexpressing MnSOD (MnSOD Tg). Mice were treated for 6-8 weeks with AZT (100 mg/kg/day), 3TC (50 mg/kg/day), or TDF (50 mg/kg/day) and plasma ET-1 levels were compared with controls. Data are means ± SD. *Denotes significance ($p < 0.05$) compared to matched controls. # Indicates $p < 0.05$ between treated MnSOD Tg and treated WT. n = 5/group.

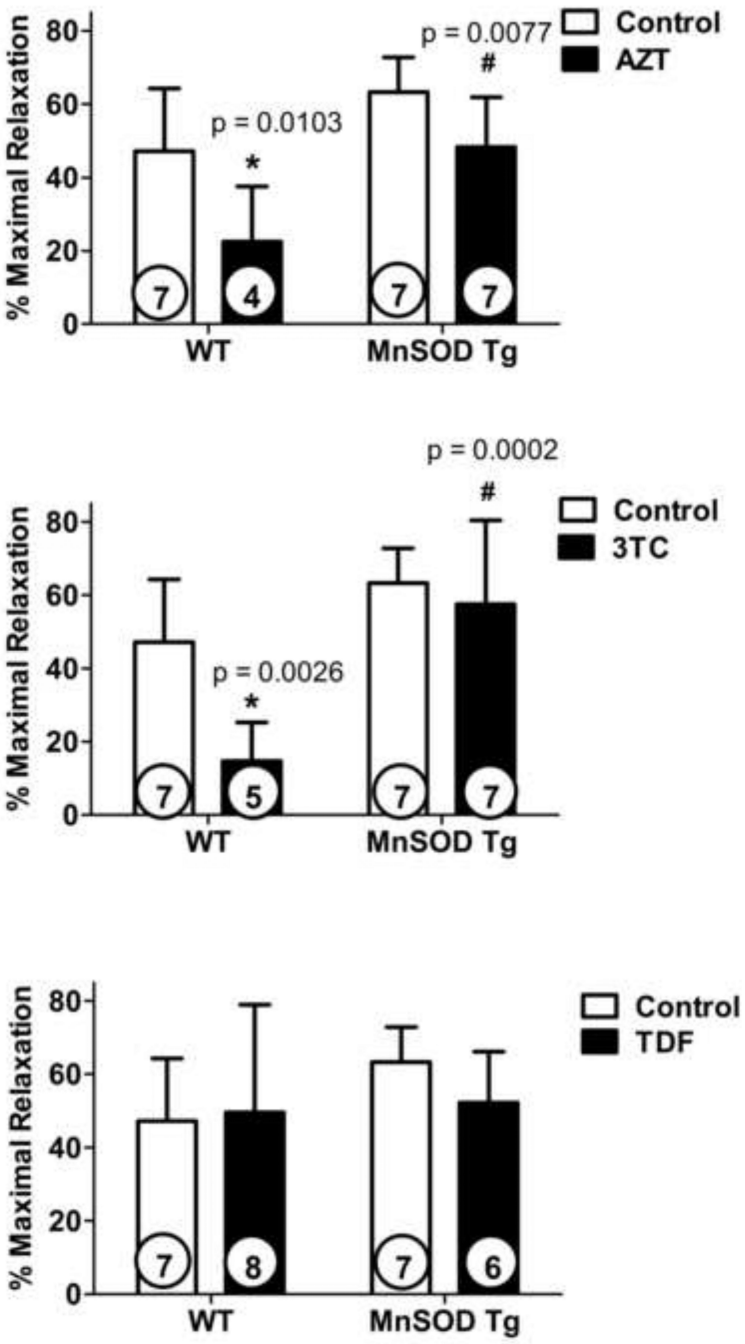

Figure 5.

Endothelium-dependent vasorelaxation in wild-type (WT) and transgenic mice overexpressing MnSOD (MnSOD Tg). Mice were treated for 6-8 weeks with AZT (100 mg/kg/day), 3TC (50 mg/kg/day), or TDF (50 mg/kg/day) and endothelium-dependent vasorelaxation results were compared with controls. Data are means ± SD. *Denotes $p < 0.05$ compared to matched controls. #Indicates significant difference ($p < 0.05$) between treated MnSOD Tg and treated WT. n = 4-9/group, as indicated within each bar.

Table 1

Drug levels and markers of tissue damage in mouse plasma.

	Control WT	Control Tg	AZT WT	AZT Tg	3TC WT	3TC Tg	TDF WT	TDF Tg
Drug levels (ng/mL)			131 ± 6.81	145 ± 51.5	184 ± 30.0	*130 ± 13.8 p=0.0472	243 ± 79.1	202 ± 46.3
Plasma Creatinine (μM)	178 ± 74.5	198 ±154	135 ± 14.3	133 ± 38.7	125 ± 14.6	124 ± 19.5	109 ± 17.2	120 ± 31.3
Plasma BUN (mg/dL)	17.7 ± 2.83	19.9 ± 3.09	*25.5±4.19 p=0.0031	19.7 ± 1.58	*23.5 ± 9.94 p=0.0251	20.3 ± 3.39	*25.6 ± 0.95 p=0.0028	20.5 ± 1.25
Plasma ALT (IU/L)	11.7 ± 1.20	15.5 ± 6.45	16.1 ± 3.13	22.8 ± 5.60	*31.6 ± 16.5 p=0.0019	12.2 ± 0.91	*44.9 ± 18.0 *p*<0.0001	12.0 ± 2.35

Values represent mean ± standard deviation.

Abbreviations: Tg = MnSOD transgenic mice; WT = wild type mice; BUN = blood urea nitrogen; and ALT = alanine aminotransferase.

*Significant difference from control, ($p < 0.05$) denoted by.