

POSTER #

692-T

Abstract

Background: Nucleoside reverse transcriptase inhibitors (NRTIs), such as zidovudine (AZT), are an integral component of HAART therapy. We have previously reported that AZT treatment of mice results in abnormal endothelium dependent relaxation. NRTI-mediated oxidative damage has been reported in both the heart and skeletal muscle. Generation of reactive oxygen species may have significant effects on the availability of NO for endothelium-dependent relaxation and therefore result in impaired endothelium-dependent relaxation. The present study tests the hypothesis that the effects of AZT on endothelium-dependent relaxation are related to increased superoxide generation.

Methods: Free radical generation was examined in both *ex vivo* isolated aorta preparations and *in vivo* cellular preparations. *Ex vivo* functional assays of contraction and relaxation were performed on isolated mouse aorta segments obtained from FVB/n wild-type mice exposed to AZT (100 mg/kg/day) or water for 35 days. *In vitro* studies utilized electron spin resonance to examine superoxide dismutase-inhibitable production of superoxide in cells exposed to AZT (1 mM) or media for 7 and 14 days.

Results: AZT-treatment significantly reduced aortic sensitivity to the endothelium-dependent vasorelaxant acetylcholine ($E.C_{.50} = 22.3 \pm 1.7$ v. 49.4 ± 4.5 nM in WT and WT+AZT respectively). Addition of tiron (1 mM), a free radical scavenger, eliminated the difference in endothelium-dependent relaxation produced by AZT in WT mice ($E.C_{.50} = 27.4 \pm 5.5$ nM). Exposure of BAECs to AZT for 7 days did not affect superoxide production. However, exposure of BAECs to AZT for 14 days resulted in a significant increase in superoxide production.

Conclusions: These results demonstrate that prolonged AZT exposure increases endothelial superoxide production. The increase in superoxide production results in impaired endothelium-dependent relaxation. These results indicate that AZT alters arterial function and suggest that NRTI therapy may contribute to cardiovascular complications in AIDS.

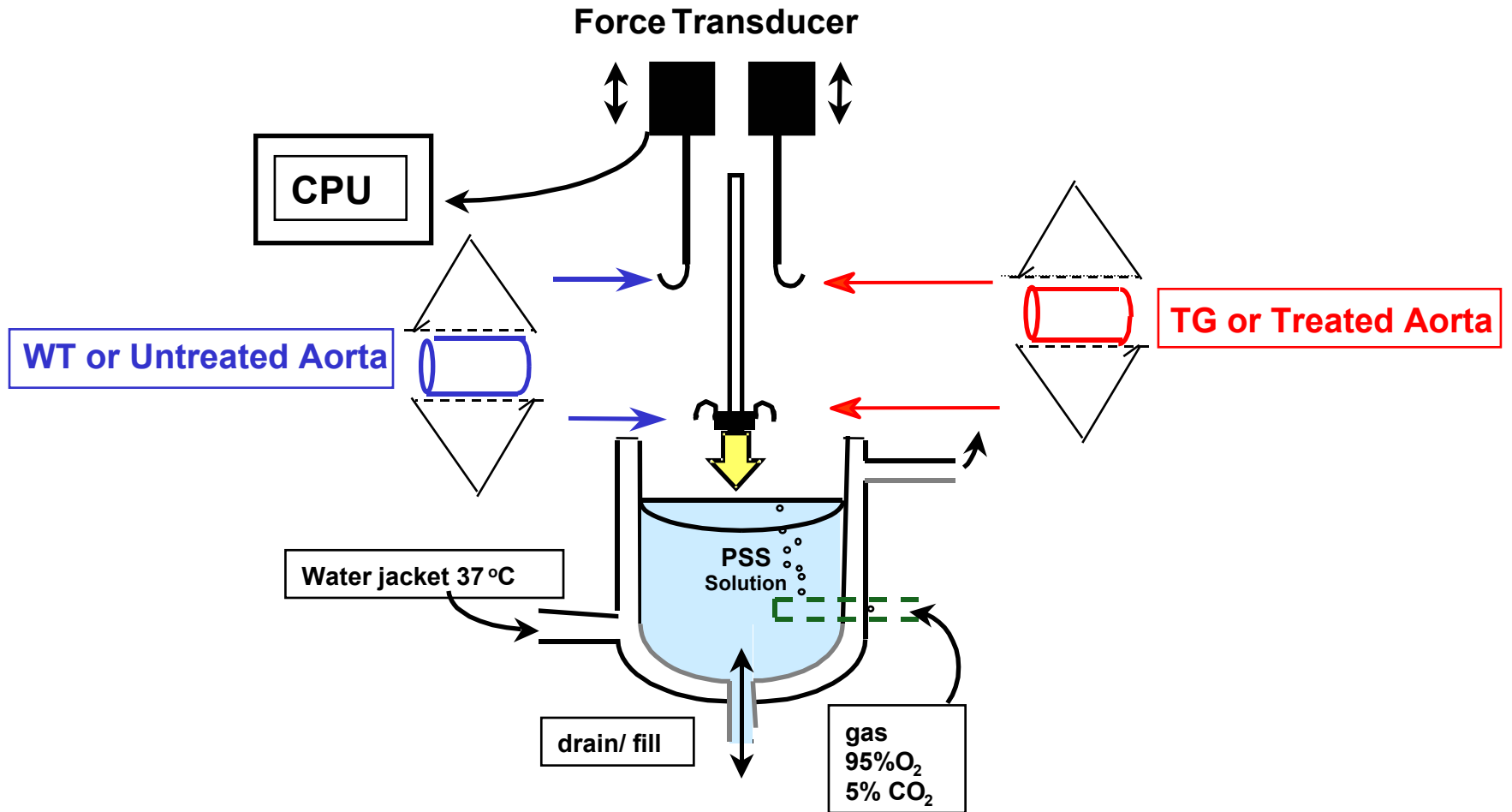
Introduction

There is ample evidence of disturbed vascular function in HIV-1 infected patients (for review see (Krishnaswamy et al., 2000)). HIV-1 infection is associated with vasculitis in small blood vessels (Cebrian et al., 1997;Mandell and Calabrese, 1998) and with aneurysms in medium or large arteries (Maniker and Hunt, 1996). A study examining the capacity of arteries from HIV positive patients to vasodilate established that post-ischemic reactive hyperemia, a measure of endothelial function, is impaired in HIV-infected patients (Monsuez et al., 2000). Similar impaired endothelium-dependent relaxation has been consistently demonstrated in patients with hypercholesterolemia and coronary artery disease (Stroes et al., 1995;Vita et al., 1990). Free radical generation is widely implicated as a major cause for impaired endothelium-dependent relaxation (for reviews see (Maytin, Leopold and Loscalzo, 1999;Napoli and Lerman, 2001)).

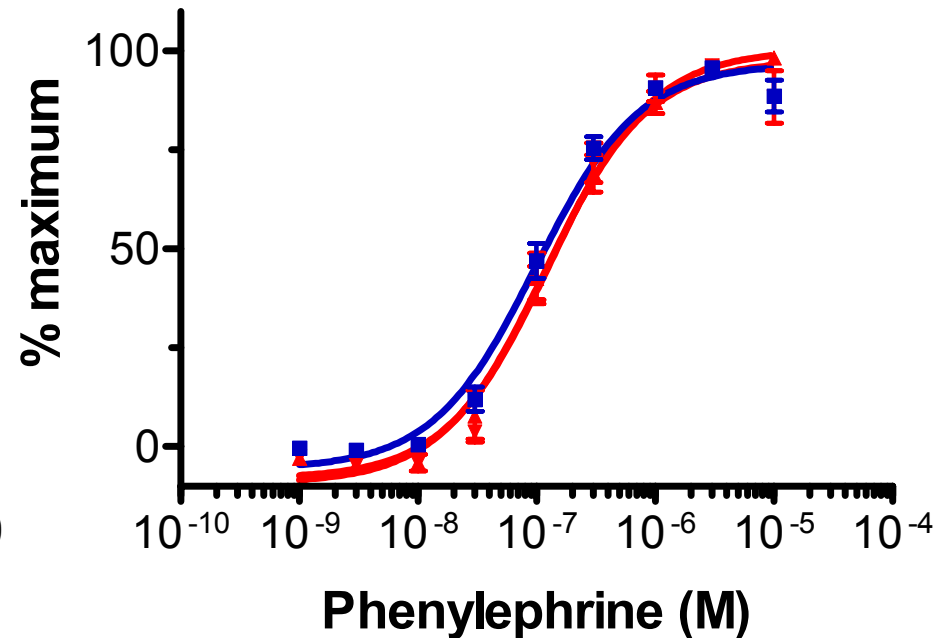
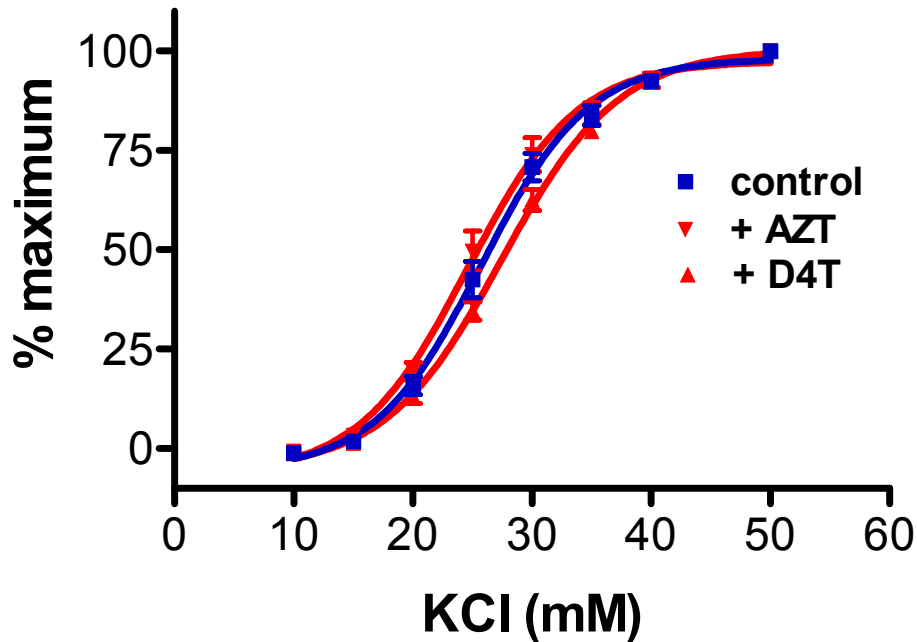
Highly active antiretroviral therapy (HAART) has significantly reduced morbidity and mortality of AIDS (Palella et al., 1998). Nucleoside reverse transcriptase inhibitors (NRTI) such as zidovudine (AZT, 3'-azido-2',3'-dideoxythymidine) and stavudine (2',3'-didehydro-3'-dideoxythymidine, D4T) are integral to HAART. NRTI therapy has been linked to the generation of free radicals in a number of tissue types. NRTI-mediated oxidative damage has been reported in the heart, skeletal muscle, and liver (de la Asuncion et al., 1998;Hayakawa et al., 1991;Skuta et al., 1999;Szabados et al., 1999). Generation of reactive oxygen species in vascular tissues may have significant effects on the availability of NO for endothelium-dependent relaxation and, therefore, result in impaired endothelium-dependent relaxation (Kojda et al., 1999). The goal of the present study was to examine the hypothesis that NRTI-mediated free radical generation contributes to impaired endothelium-dependent relaxation and that NRTI effects can be ameliorated by treatment with a free radical scavenger.

- Cebrian, M., Miro, O., Font, C., Coll-Vincent, B., and Grau, J. M. (1997). *AIDS Patient Care & STDS* **11**, 245.
- de la Asuncion, J. G., del Olmo, M. L., Sastre, J., Millan, A., Pellin, A., Pallardo, F. V., and Vina, J. (1998). *J Clin Invest* **102**, 4.
- Hayakawa, M., Ogawa, T., Sugiyama, S., Tanaka, M., and Ozawa, T. (1991). *Biochem Biophys Res Commun* **176**, 87.
- Kojda, G., Laursen, J. B., Ramasamy, S., Kent, J. D., Kurz, S., Burchfield, J., Shesely, E. G., and Harrison, D. G. (1999). *Cardiovasc Res* **42**, 206.
- Krishnaswamy, G., Chi, D. S., Kelley, J. L., Sarubbi, F., Smith, J. K., and Peiris, A. (2000). *Cardiol Rev* **8**, 260.
- Mandell, B. F., and Calabrese, L. H. (1998). *Curr Opin Rheumatol* **10**, 51.
- Maniker, A. H., and Hunt, C. D. (1996). *Surg Neurol* **46**, 49.
- Maytin, M., Leopold, J., and Loscalzo, J. (1999). *Curr Atheroscler Rep* **1**, 156.
- Monsuez, J. J., Dufaux, J., Vittecoq, D., and Vicaut, E. (2000). *J Acquir Immune Defic Syndr* **25**, 434.
- Napoli, C., and Lerman, L. O. (2001). *Mayo Clin Proc* **76**, 619.
- Palella, F. J., Jr., Delaney, K. M., Moorman, A. C., Loveless, M. O., Fuhrer, J., Satten, G. A., Aschman, D. J., and Holmberg, S. D. (1998). *N Engl J Med* **338**, 853.
- Skuta, G., Fischer, G. M., Janaky, T., Kele, Z., Szabo, P., Tozser, J., and Sumegi, B. (1999). *Biochem Pharmacol* **58**, 1915.
- Stroes, E. S., Koomans, H. A., de Bruin, T. W., and Rabelink, T. J. (1995). *Lancet* **346**, 467.
- Szabados, E., Fischer, G. M., Toth, K., Csete, B., Nemeti, B., Trombitas, K., Habon, T., Endrei, D., and Sumegi, B. (1999). *Free Radic Biol Med* **26**, 309.
- Vita, J. A., Treasure, C. B., Nabel, E. G., McLenachan, J. M., Fish, R. D., Yeung, A. C., Vekshtein, V. I., Selwyn, A. P., and Ganz, P. (1990). *Circulation* **81**, 491.

Contractility Measurements



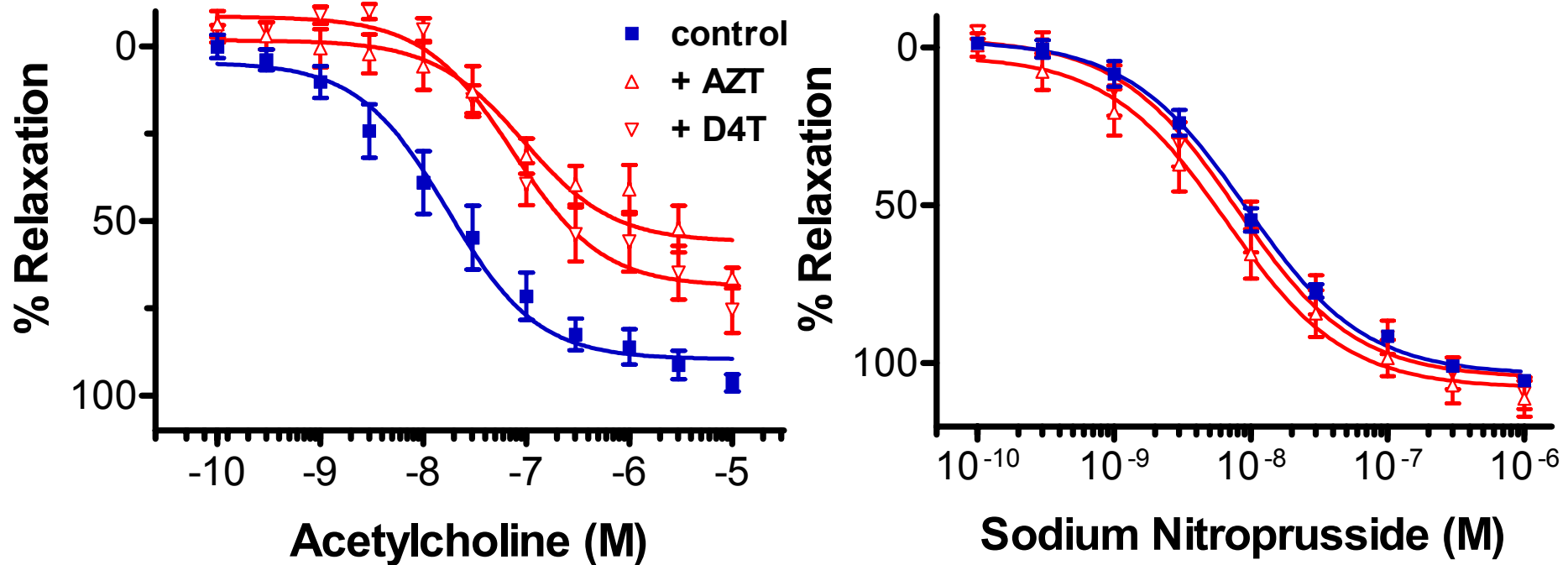
Effects of AZT and D4T on Aorta Contractility



Contractility of aortas from water AZT and D4T -treated mice. Aortas were isometrically mounted and concentration-isometric force curves generated in response to KCl (A) and phenylephrine (B). Neither the presence of AZT nor D4T treatment significantly affected sensitivity of aortas to KCl or phenylephrine. N=6.

Neither AZT nor D4T treatment affected contractile sensitivity to KCl or phenylephrine .

Effects of AZT and D4T on Endothelium-Dependent Relaxation



Acetylcholine and sodium nitroprusside concentration relaxation relations of aortas from treated with water, AZT and D4T. Aortas were precontracted with 300 nM phenylephrine and exposed to increasing concentrations of acetylcholine or sodium nitroprusside.

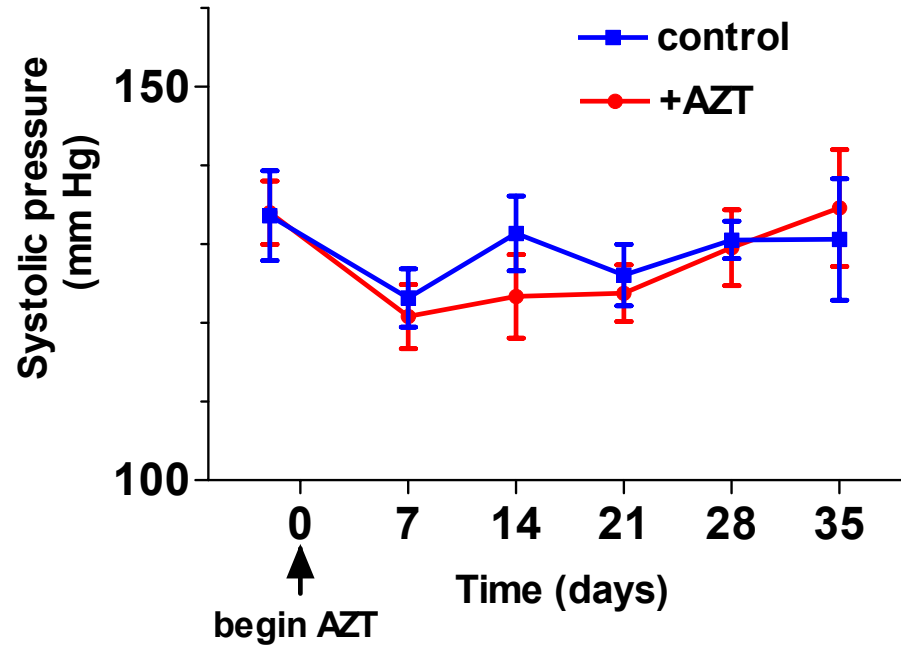
AZT and D4T treatment impair endothelium-dependent relaxation. Smooth muscle relaxation to an NO donor is unaffected.

Effects of AZT and D4T treatment on aortic relaxation

| Treatment | <u>Acetylcholine</u> | <u>Sodium Nitroprusside</u> |
|-------------------|-------------------------|-----------------------------|
| | E.C. ₅₀ (nM) | E.C. ₅₀ (nM) |
| | Maximum relaxation (%) | Maximum relaxation (%) |
| H ₂ O | 22.6 ± 9.6 | 9.6 ± 1.1 |
| (N=10) | 92.0 ± 3.0 | 103.9 ± 1.4 |
| AZT (100 mg/kg/d) | 110.5 ± 26.2* | 7.6 ± 1.5 |
| (N=6) | 58.1 ± 5.0* | 108.6 ± 5.4 |
| D4T (10 mg/kg/d) | 80.7 ± 9.2* | 7.7 ± 1.0 |
| (N=6) | 69.1 ± 7.8 | 102.2 ± 2.5 |

Aortas from AZT and D4T-treated mice have reduced maximal relaxation and sensitivity to acetylcholine but not sodium nitroprusside.

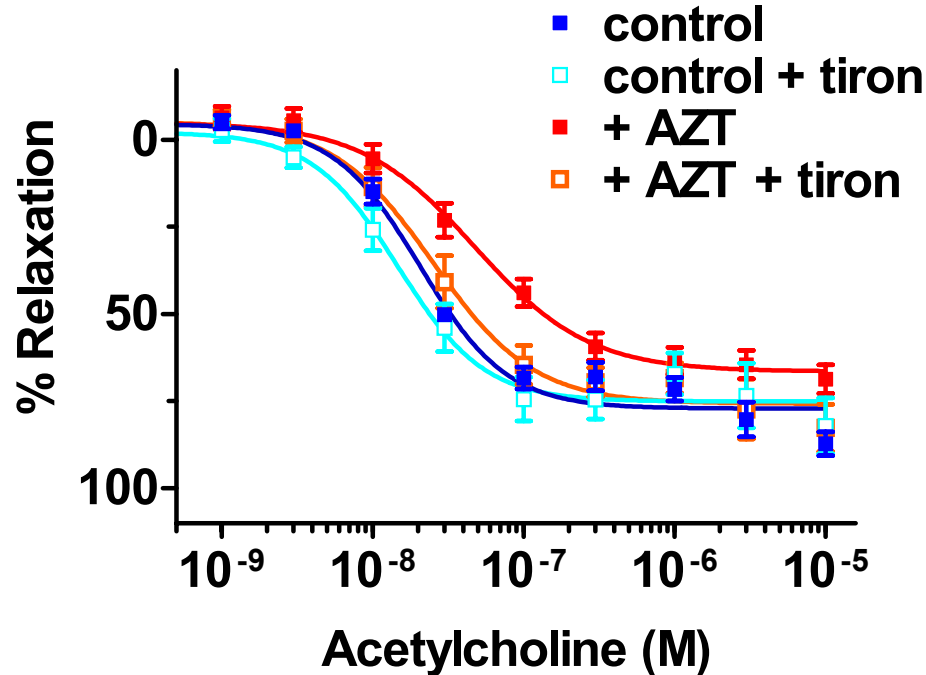
Effects of AZT on Systolic Pressure



Effects of AZT on systolic blood pressure. Animals were trained to an automated tail cuff system for 1 week and baseline systolic pressure determined for three consecutive days. AZT treatment (100 mg/kg/day) was initiated and blood pressure was monitored during the treatment. N=8.

Despite the observed effects of AZT treatment on endothelium-dependent relaxation *ex vivo*, concomitant alterations in systolic blood pressure in the whole animal were not observed.

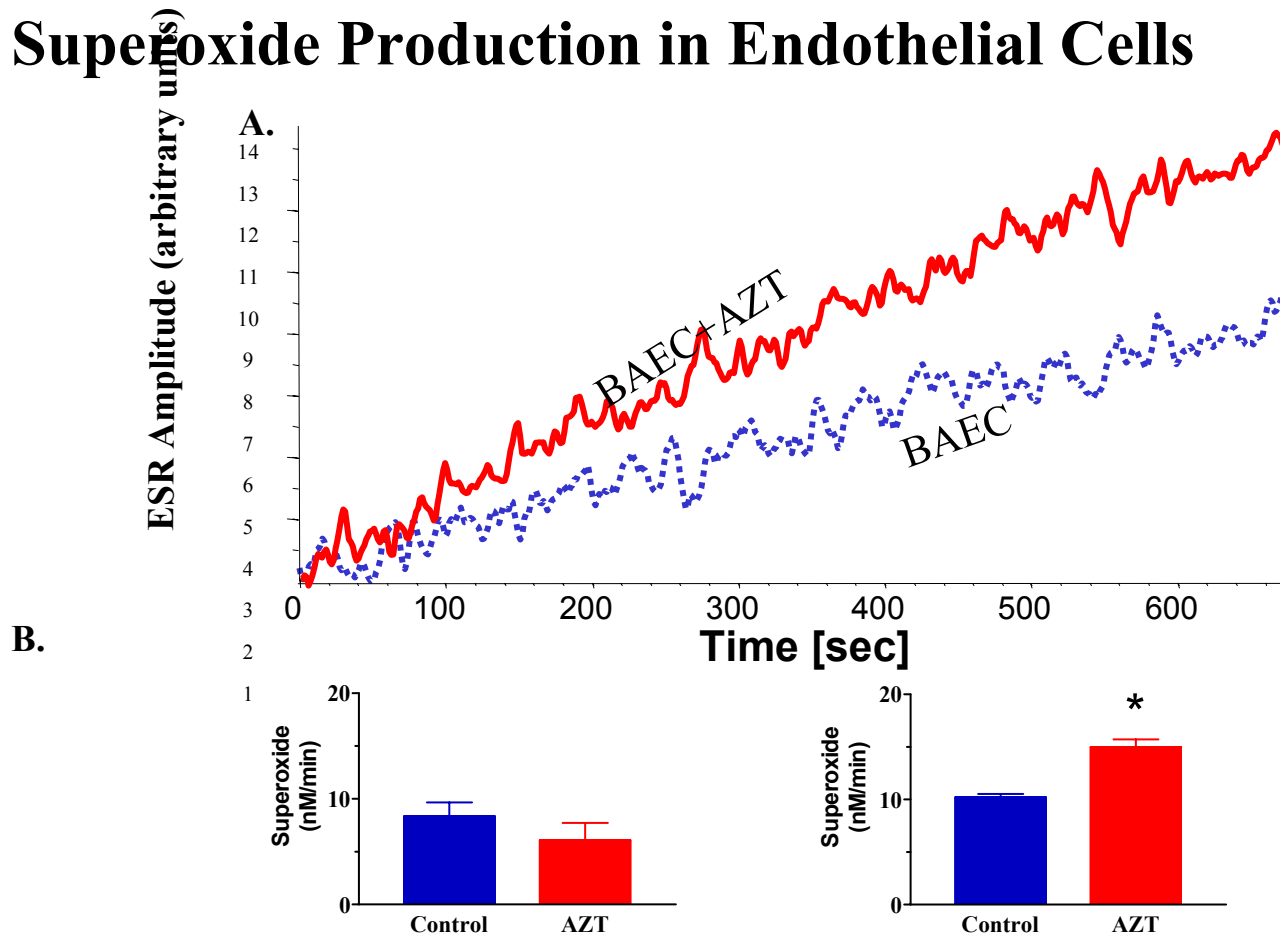
Effects of Tiron Pretreatment



Concentration-relaxation relations to acetylcholine (ACh) were generated following pretreatment with 1 mM tiron, a superoxide scavenger. N=5; mean \pm sem.

The effects of AZT on endothelium-dependent relaxation are eliminated by pretreatment with a free radical scavenger.

Superoxide Production in Endothelial Cells



Superoxide production following exposure of BAECs to 1 mM AZT for 7 and 14 days. A. Representative tracings of ESR time scans of SOD-inhibitable CP formation for control BAECs and BAECs exposed to AZT for 14 days. B. Summary of superoxide production in control BAECs and BAECs treated with AZT for 7 (left panel) and 14 days (right panel). (N=4; * p<0.05 compared to control BAECs).

AZT causes a time-dependent increase in superoxide production in endothelial cells

SUMMARY

NRTI TREATMENT:

- Does not affect aortic contractile sensitivity
- Reduces sensitivity to acetylcholine-mediated relaxation
 - Pretreatment with a free radical scavenger eliminated this effect
- Elevates superoxide staining in aortas from AZT-treated mice
- Increases superoxide production in BAECs

AZT treatment increases superoxide production. This effect results in impaired endothelium-dependent relaxation. The effects of NRTI therapy on endothelium-dependent relaxation may contribute pathophysiologically to cardiovascular complications in AIDS.

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