Published in final edited form as:

Crit Care Med. 2009 April ; 37(4): 1348–1354. doi:10.1097/CCM.0b013e31819cee38.

Norepinephrine Increases Blood Pressure but not Survival with Anthrax Lethal Toxin in Rats

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Abstract

Introduction—The response of anthrax lethal toxin (LeTx) induced shock and lethality to conventional therapies has received little study. Previously, fluids worsened outcome in LeTx challenged rats in contrast to its benefit with LPS or *E. coli*. The present study investigated norepinephrine treatment.

Methods and Results—Sprague-Dawley rats (n=232) weighing between 230 and 250 gm were challenged with similarly lethal (80%) 24h infusions of either LPS or LeTx or with diluent only. Toxin challenged animals were also randomized to receive 24 h infusions with 1 of three doses of norepinephrine (0.03, 0.3, or 3.0 µg/kg/min) or placebo starting 1 h after initiation of challenge. All toxin animals received similar volumes of fluid over the 24 h (equivalent to 4.0 to 4.3 ml/kg/h). Although the intermediate norepinephrine dose (0.3 µg/kg/min for 24h) improved survival with LPS (p=0.04) and increased blood pressure prior to the onset of lethality with LeTx (p<0.0001) it did not improve survival with the latter (p=ns). Furthermore, neither increasing nor decreasing norepinephrine doses improved survival with LeTx.

Conclusion—Hypotension with LeTx may not be a primary cause of lethality in this model. Rather, LeTx may cause direct cellular injury insensitive to vasopressors. These findings suggest that during anthrax infection and shock, along with hemodynamic support, toxin directed treatments may be necessary as well.

Keywords

B. anthracis; lethal toxin; sepsis; vasopressor; treatment; rodent

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Footnotes The authors do not have associations that pose a conflict of interest.

Data from this manuscript was presented at the 2007 American Thoracic Society meeting in San Francisco, CA.

Introduction

Bacillus anthracis lethal toxin (LeTx) is comprised of protective antigen (PA), necessary for the uptake of toxin by host cells, and lethal factor (LF), the toxic moiety. Lethal toxin (LeTx) is closely associated with the shock and lethality occurring during anthrax infection (1,2). Purified LeTx decreases blood pressure and survival in animal models and strains of *B. anthracis* in which PA or LF have been inactivated are 1000 times less lethal than fully active strains (3-5). Finally, agents that directly inhibit PA or LF are protective in anthrax models (6-8).

Despite the importance of LeTx, the mechanisms underlying the shock and lethality it produces are unclear. Lethal factor is a zinc protease that inactivates mitogen-activated protein kinase kinases (MAPKKs) 1 to 4, 6 and 7 thereby inhibiting downstream mitogen-activated protein kinases (MAP kinases) such as ERK1/2, P38 and JNK (9-12). Consistent with its inhibitory effects on these stress kinase pathways, LeTx has not been associated with the excessive inflammatory cytokine and nitric oxide release that contributes to shock caused by other bacterial toxins (13). However, LeTx can produce direct endothelial cell apoptosis and increase endothelial permeability (14-16). Such endothelial dysfunction with LeTx could lead to shock via the extravasation of intravascular fluid (17). The hemoconcentration noted in patients with anthrax and animals challenged with LeTx support this possibility (13,18). Alternatively however, endothelial dysfunction caused by LeTx could inhibit the response of the systemic vasculature to either endogenous or exogenous catecholamine or other vasoregulatory molecules. Finally, LeTx could produce direct cellular and tissue injury leading to lethality via mechanisms independent of its effects on hemodynamic function.

Whatever its mechanisms, how LeTx induced shock responds to conventional hemodynamic support has received little investigation. This question appears important clinically however. During the 2001 anthrax outbreak, the uniform fatality in patients with shock despite aggressive hemodynamic support, as well as the pronounced hemoconcentration and recurrent pleural effusions have suggested that both the pathophysiology and response to treatment with this infection may differ from more common types of sepsis. Using a rat model in which LeTx was infused over 24 h to simulate its release during infection, we found that normal saline treatment actually decreased blood pressure and survival in patterns different from its effects with lipopolysaccharide (LPS) or *E. coli* challenge (19). The adverse effect of fluid with LeTx appeared to be related in part to worsened oxygenation. Moreover, fluid treatment negated the otherwise highly beneficial effects of a protective antigen directed monoclonal antibody tested in the model. The purpose of the present investigation was to test the influence of norepinephrine, another conventional hemodynamic therapy, on shock and lethality caused by LeTx. We hypothesized that norepinephrine compared to no treatment would increase blood pressure and improve survival with LeTx challenge.

Materials and Methods

Animal Care

The protocol used in this study was approved by the Animal Care and Use Committee of the Clinical Center of the National Institutes of Health.

Study Design

Sprague-Dawley rats (n=195) weighing between 230 and 250 gm, with central venous and systemic arterial catheters were briefly anesthetized with isoflurane for connection to infusion lines and transducers. They were then awakened and challenged with either LeTx

[lethal factor 150 µg/kg with protective antigen 300 µg/kg in 12 ml phosphate buffered saline (PBS) with rat albumin (25 µg/ml)] or *E. coli* lipopolysaccharide (LPS) (*E. coli* 0111:B4, Sigma, 8 mg/kg bw, St. Louis, MO) as 24 h infusions at a rate of 0.5 ml/h (6) (Figure 1, Panel A). After 1 h of challenge, 24 h infusions with either low, intermediate or high doses of norepinephrine (Ben Venue Laboratories, Inc., Bedford, OH) (0.03, 0.3 or 3 µg/kg/min) or dextrose 5% diluent (placebo) were started at a rate of 0.5 ml/h. Animals received similar volumes of toxin and treatment (i.e. 2.0 to 2.3 ml/kg/h for the first hour, 4.0 to 4.3 ml/kg/h for the next 23 h and then 2.0 to 2.3 ml/kg/h for the final h). Thus fluid administration during this period consisted of equal parts PBS and dextrose 5%. Immediately before LeTx or LPS and at 2 h intervals until death or catheter disconnection (24 h), mean arterial blood pressures (MBP) and heart rates (HR) were recorded. Also, at 4, 8, and 24 h animals had arterial blood gas and complete blood counts measured. All animals had similar volumes (0.5 ml) of blood removed and normal saline replaced with procedures. Animals were observed for 168 h. To determine the effects of LPS and LeTx alone, measures from Sprague-Dawley rats challenged with diluent (PBS) in the present (n=3) and concurrent studies (n=34) were employed together as a control group. These concurrent studies were part of the same approved protocol to investigate hemodynamic support with LeTx and LPS (20). Care and study of all control animals, including the total fluid volumes administered, were the same as in animals challenged with LeTx or LPS. Animals were observed q2h for the first 24 h, then q4h from 24 to 48 h, then q 12h for the remainder of the study and had access to water and feed throughout.

Additional animals (n=59) in the present study were challenged with LeTx, LPS or diluent as outlined above and then randomly selected at 4 or 8 h to first be anesthetized for plasma catecholamine, superoxide dismutase (SOD), nitric oxide (NO) and cytokine measures and then sacrificed for lung and liver SOD measurements (Figure 1, Panel B). Protective antigen, lethal factor and LPS for all experiments were prepared as previously described (13,21,22).

Hemodynamic, Blood and Tissue Measurements

Hemodynamic, arterial blood gas, lactate, norepinephrine, epinephrine, NO and cytokine measures were performed as previously published (6,13,16,23). Briefly, arterial blood gases and lactates were measured with iSTAT Clinical Analyzer (Abbott, East Windsor, NJ). Plasma norepinephrine and epinephrine levels were measured using HPLC with electrochemical detection (HPLC-EC) (Bedford, MA). Plasma nitric oxide levels were measured with the Cayman Nitrite/nitrate (NO) Colorimetric Kit (Cayman Chemical, Ann Arbor, Michigan). The serum cytokines TNFα, IL-1α and β, IL-2, IL-4, IL-6, IL-10, IFNγ, GM-CSF, 3 migratory inhibitory proteins (MIP-1α, MIP-2, and MIP-3α), monocyte chemoattractant protein (MCP-1) and RANTES (regulated on activation, normal T-cell expressed and secreted) were measured using a multiplexed sandwich ELISA (The SearchLight Rat Cytokine Array, Pierce, Rockford, IL). Serum, lung and liver total SOD activities (Cu/Zn-, Mn-, and Fe-SOD) were measured by quantification of the dismutation of superoxide radicals generated by the combination of xanthine oxidase and hypoxanthine (Cayman Chemical Superoxide Dismutase Assay kit, Cayman Chemical).

Statistical Analysis

Weekly experiments including 24 animals each were performed in which a toxin and treatment combination were assigned using numbers selected randomly. The experiments were designed to detect a 50% reduction in mortality with toxin doses producing an 80% lethality rate. To have 80% power to detect such a difference with an α=0.05 using a two-tailed test, we estimated 27 animals would be required per group. However, it was evident after initial cycles that the low and high doses of norepinephrine were having negligible or

harmful effects on blood pressure and outcome and it was not justified to expend further animals studying these doses. Kaplan-Meier survival curves were compared using Gehan's version of the Wilcoxon test, as has been reported previously (24). All other parameters were analyzed with repeated measures ANOVA using PROC MIXED in Statistical Analysis System Version 9.1 software (SAS Institute, Inc, Cary, NC), and least squares means and associated standard errors were reported. Our interest was focused on the overall group difference and its possible interaction with time. We were not interested in the factor of time, the repeated measure. Similar to prior studies in this model, mean arterial blood pressure and heart rate data were analyzed during the early period before lethality was evident (i.e. the initial 6 h in this study) with LeTx (the primary challenge of interest) and then over the later period of observation (8 to 24 h) (20). P-values were adjusted by the Bonferroni method for the multiple comparisons arising from this early-late dichotomy. Although evaluations of multiple physiological parameters were performed, no other adjustments to the p-values were made. Data were log transformed where appropriate. All results are expressed as least square means $\pm$ sem, and 2-sided $p < 0.05$ was considered significant.

Results

Survival

The doses of LPS and LeTx infused both resulted in similar lethality rates (Figure 2). With LPS challenge, the intermediate norepinephrine dose (0.3 $\mu\text{g/kg/min}$) was beneficial and significantly increased survival ($p=0.04$) (Figure 2). Lowering or increasing the norepinephrine dose (0.03 and 3.0 $\mu\text{g/kg/min}$) with LPS was not associated with beneficial effects. In contrast to LPS however, with anthrax LeTx challenge neither the intermediate norepinephrine dose nor the higher or lower doses improved survival. Since the intermediate norepinephrine dose (0.3 $\mu\text{g/kg/min}$) but not low (0.03 $\mu\text{g/kg/min}$) or high (3.0 $\mu\text{g/kg/min}$) doses altered survival (i.e. with LPS), analysis is presented for all other laboratory parameters comparing the effects of the intermediate dose alone with LPS versus LeTx.

Hemodynamic Measurements

Compared to animals challenged with diluent, LPS without norepinephrine decreased MBP throughout. These decreases were statistically significant over the early time period ($p=0.04$ for the differences comparing LPS alone and diluent) and the magnitude of these differences varied with time over both periods ($p=0.003$ and <0.001 respectively for the interaction with time) (Figure 3, Panel A). Compared to diluent animals, LeTx alone was not statistically different during the early period and had variable effects on MBP ($p<0.001$ for the interaction of time) (Figure 3, Panel B). However, late, LeTx decreased MBP significantly throughout ($p<0.001$ for the differences comparing LeTx alone and diluent) and the magnitude of these effects varied with time ($p<0.001$ for the interaction with time). Compared to toxin challenge alone, early norepinephrine treatment increased MBP with both toxins but this effect was only significant with LeTx ($p<0.001$) (Figure 3, Panels C and D). With both toxins, the early increase with norepinephrine continued into the later time period but did not persist and was not statistically significant.

Compared to animals challenged with diluent, LPS alone increased HR early and late it decreased it ($p<0.001$ for differences between LPS alone and diluent over both periods) (Figure 4, Panel A). Reductions in HR with LPS later were greater with time ($p<0.001$ for interaction with time). Early, LeTx alone had variable effects on HR ($p<0.001$ for the interaction with time) (Figure 4, Panel B). Late, LeTx decreased HR throughout ($p<0.001$ for the differences between LeTx alone and diluent) but these decreases varied over time ($p<0.001$ for the interaction with time). Compared to LPS alone, treatment with

norepinephrine did not alter HR significantly early or late but over the 24 h was associated with progressive increases in a trend approaching significance ($p=0.10$) (Figure 4, Panel C). With LeTx, norepinephrine did not alter HR significantly early or late.

Arterial Blood Gas and Lactate Measurements

Compared to diluent challenge, LPS alone decreased arterial pH and base excess and increased lactate ($p<0.001$ for the differences comparing LPS alone and diluent) but these changes were greater at later time points ($p<0.001$ for the interaction with time) (Figure 5). Compared to diluent, LeTx decreased arterial pH and base excess significantly ($p<0.001$ and $p=0.002$ respectively for the differences comparing LeTx alone and diluent). Norepinephrine with LPS challenge significantly increased arterial pH and base excess and decreased lactate compared to LPS alone ($p=0.04$ for all differences comparing LPS with norepinephrine to LPS alone) and changes in pH were greater at later time points ($p=0.04$ for the interaction with time). Compared to LeTx challenge alone, treatment with norepinephrine did not alter arterial pH, base excess or lactate significantly throughout.

Plasma Catecholamines, Nitric Oxide, Cytokine and SOD Measurements

Compared to animals challenged with diluent only, LPS increased plasma catecholamine (norepinephrine and epinephrine) and nitric oxide levels across the 4 and 8 h measurement times ($p<0.001$ for each) (Figure 6). LPS also increased cytokine levels significantly at these time points ($p=0.05$ for all except IL-1 β and TNF α at 4 h, data not shown). LeTx did not cause significant changes in any of these parameters throughout. Compared to animals challenged with diluent only, with the exception of lung SOD measures that were decreased with LeTx challenge at 8 h ($p=0.02$), no other SOD measure was altered significantly with either toxin during the study (data not shown).

Discussion

Although norepinephrine treatment increased blood pressure significantly prior to the onset of lethality with LeTx, it did not improve survival. In contrast, early increases in blood pressure with norepinephrine in LPS challenged animals, although not reaching significance, were associated with beneficial survival effects. These and other findings suggest that the relationship between hypotension, tissue hypoperfusion and lethality for LPS and LeTx may differ.

As observed in this model, LPS is known to increase nitric oxide and inflammatory cytokine levels, both of which are associated with arterial dilation and myocardial dysfunction (25-28). These same mediators have also been implicated in loss of responsiveness to the vasopressor effects of endogenous catecholamines (29,30). Together these abnormalities contribute to the tissue hypo-perfusion and injury leading to lethality with LPS (23,30). Possibly as a compensatory response, endogenous epinephrine and norepinephrine levels were increased with LPS in this study. Although hypotension with LPS may have been reduced due to hourly fluid administration (i.e. 4 ml/kg/h during toxin and treatment administration), metabolic acidosis and increased lactate levels were prominent in LPS challenged animals. While this acidosis may be due in part to a direct effect of LPS on mitochondrial function, it likely also reflects tissue hypoperfusion. Under these circumstances, norepinephrine's vasoconstrictive and inotropic effects would likely have benefit. Both of these effects can improve tissue perfusion and are a basis for norepinephrine's use during shock related to the systemic inflammatory response during sepsis (31,32). In keeping with these actions, norepinephrine during LPS challenge was associated with decreased acidosis and lactate levels and improved survival. Gradual increases in heart rate with norepinephrine in LPS challenged animals suggest that

chronotropic effects may have also contributed to its beneficial effects. Finally, norepinephrine may have anti-inflammatory effects via the beta-2 receptor which could also be beneficial with LPS challenge (33).

In contrast to LPS however, hypotension and lethality with LeTx challenge were not associated with increases in nitric oxide, inflammatory cytokine, or endogenous catecholamine levels. Furthermore, evidence of metabolic acidosis with lactate increases was small with LeTx compared to LPS. Finally and most noticeably, although norepinephrine increased blood pressure with LeTx, it did not alter survival or acid-base parameters. These findings together raise the possibility that in contrast to LPS, hypotension with LeTx in this model may be a secondary phenomenon and not a primary cause of lethality. Instead, LeTx may have produced direct cellular injury leading to lethality via mechanisms insensitive to increases in blood pressure or tissue perfusion with norepinephrine. The downstream MAP kinases inhibited after LF cleavage of MKKs include p38 (α , β , γ , and δ), extracellular-signal-regulated kinase (ERK1 and 2), and c-Jun N-terminal kinase (JNK 1, 2 and 3). These molecules control a wide range of critical cellular functions (34). In addition to effects on host defense, inhibition of these pathways by LF has been shown to cause apoptosis of macrophage and endothelial cells and disruption of endothelial and neutrophil cytoskeleton function (14-16,35,36). Genomic and proteomic analysis and direct *in vitro* studies have also suggested that LeTx may alter energy metabolism and impair mitochondrial function (37-39). Such changes might secondarily alter vascular integrity or myocardial function and cause hypotension the reversal of which would not alter outcome.

It is also possible however, that increased blood pressure with norepinephrine during LeTx challenge had maladaptive effects that negated its beneficial ones. Notably, if LeTx caused myocardial dysfunction out of proportion to injury to the peripheral vasculature, the vasopressor effects of norepinephrine might have increased afterload and worsened cardiac output. LeTx has been reported to alter myocardial function (40). However this study is difficult to interpret because the bolus administration of toxin in animals resulted in profound reductions in systemic blood pressures over very rapid periods as has been seen in similar models (40,41). Increased after load resulting in impaired cardiac output with norepinephrine in the present study might have also been expected to produce more severe tissue acidosis.

Absence of a beneficial survival effect of norepinephrine with LeTx does not appear to be related to the treatment doses that were studied. The intermediate dose protective with LPS is comparable to a norepinephrine dose of 20 μ g/min in a 70 kg human. This dose is frequently employed clinically during severe sepsis as well as in LPS challenged rat models (42,43). Furthermore, neither increasing nor decreasing the norepinephrine dose administered resulted in improved survival with LeTx. Whether the titration of norepinephrine based on hemodynamic measures during LeTx infusion or the use of alternate vasopressors or agents with greater inotropic effects would have beneficial effects with LeTx requires further study. Finally, although fluid administration in volumes (10 and 20 ml/kg/h) greater than that employed in the present study worsened outcome with LeTx in this rat model, it is possible that norepinephrine in combination with these higher fluid amounts would have had benefit (19). Studies to examine this possibility would ideally employ measures of preload to adjust fluids and measures of perfusion to titrate norepinephrine as is done clinically. Such studies may only be possible in a large animal model however.

Although LeTx is important in the pathogenesis of *B. anthracis*, other products likely contribute as well. Recent *in vivo* studies have shown that edema toxin may have a

substantial role in the shock and lethality associated with anthrax (19,44). Cell wall components from anthrax may also contribute to shock (45). Whether hemodynamic dysfunction related to these other mediators will be more responsive to conventional hemodynamic support than LeTx in the present study requires investigation. On the one hand, edema toxin is an adenyl cyclase that has the potential to actually negate the vasopressor effects of agents like norepinephrine (46). On the other hand, if cell wall plays a role in the shock caused by anthrax, it likely does so via some of the same inflammatory response pathways LPS does (45). In this case, fluid and vasopressors might have beneficial effects.

The present findings should not be interpreted to suggest that patients presenting with suspected anthrax infection and shock not receive aggressive hemodynamic support to maintain blood pressure and organ perfusion. Such therapies are necessary regardless of the underlying etiologic agent. These findings do suggest however that normalization of blood pressure with pressor agents may mask ongoing cellular injury related to LeTx.

This study has several limitations. As already noted, norepinephrine was not titrated in individual animals and direct measures of myocardial function were not performed. This study also did not include mechanical ventilation which would typically be applied to a critically ill patient nor did it examine the effects of vasopressor therapy in combination with a toxin directed agent. Therefore, whether the findings from this small animal model can be extrapolated clinically is not clear. Regarding a methodologic point, although it would have been ideal to have randomized an independent control group (i.e. diluent challenged) for these experiments, the use of such animals could not be justified since a similarly treated group was undergoing concurrent study in another protocol.

Both in this study and a prior one, treatment with fluid or norepinephrine, while beneficial with LPS or bacteria challenge, was either not effective or actually worsened outcome in animals challenged with LeTx (19). Whether these findings relate to the extremely poor outcome (e.g. 100% lethality) in patients with shock receiving conventional hemodynamic support during the 2001 United States anthrax outbreak is unclear. They do however emphasize the need to better define the pathogenic mechanisms leading to death with anthrax and the effectiveness of conventional treatments .

Acknowledgments

We greatly appreciate Dr. Deloris Koziol's and Dr. Robert Wesley's consultation and advice regarding statistical analysis for the revised manuscript.

This research was funded by NIH intradepartmental funds.

References

1. Mock M, Fouet A. Anthrax. *Annu Rev Microbiol.* 2001; 55:647–671. [PubMed: 11544370]
2. Leppla, SH. Anthrax toxin. In: Aktories, K.; Just, I., editors. *Bacterial proteins*. Springer; New York: 2000. p. 445-472.
3. Ezzell JW, Ivins BE, Leppla SH. Immunoelectrophoretic analysis, toxicity, and kinetics of in vitro production of the protective antigen and lethal factor components of *Bacillus anthracis* toxin. *Infect Immun.* 1984; 45:761–767. [PubMed: 6432700]
4. Molnar DM, Altenbern RA. Alterations in the biological activity of protective antigen of *Bacillus anthracis* toxin. *Proc Soc Exp Biol Med.* 1963; 114:294–297. [PubMed: 14101169]
5. Pezard C, Berche P, Mock M. Contribution of individual toxin components to virulence of *Bacillus anthracis*. *Infect Immun.* 1991; 59:3472–3477. [PubMed: 1910002]

6. Cui X, Li Y, Moayeri M, et al. Late treatment with a protective antigen derived monoclonal antibody improves hemodynamic function and survival in a lethal toxin-infused rat model of anthrax sepsis. *J Infect Dis.* 2005; 191:422–434. [PubMed: 15633102]
7. Mohamed N, Clagett M, Li J, et al. A high-affinity monoclonal antibody to the anthrax protective antigen passively protects rabbits before and after aerosolized *Bacillus anthracis* spore challenge. *Infect Immun.* 2005; 73:795–802. [PubMed: 15664918]
8. Subramanian GM, Cronin PW, Poley G, et al. A phase 1 study of PAmAb, a fully human monoclonal antibody against *Bacillus anthracis* protective antigen, in healthy volunteers. *Clin Infect Dis.* 2005; 41:12–20. [PubMed: 15937757]
9. Duesbery NS, Webb CP, Leppla SH, et al. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science.* 1998; 280:734–737. [PubMed: 9563949]
10. Vitale G, Pellizzari R, Recchi C, et al. Anthrax lethal factor cleaves the N-terminus of MAPKKs and induces tyrosine/threonine phosphorylation of MAPKS in cultured macrophages. *J Appl Microbiol.* 1999; 87:288. [PubMed: 10475970]
11. Ho DT, Bardwell AJ, Abdollahi M, et al. A docking site in MKK4 mediates high affinity binding to JNK MAPKs and competes with similar docking sites in JNK substrates. *J Biol Chem.* 2003; 278:32662–32672. [PubMed: 12788955]
12. Bardwell AJ, Abdollahi M, Bardwell L. Anthrax lethal factor-cleavage products of MAPK kinases exhibit reduced binding to their cognate MAPKs. *Biochem J.* 2004; 378:569–577. [PubMed: 14616089]
13. Cui X, Moayeri M, Li Y, et al. Lethality during continuous anthrax lethal toxin infusion is associated with circulatory shock but not inflammatory cytokine or nitric oxide release in rats. *Am J Physiol Regul Integr Comp Physiol.* 2004; 286:R699–709. [PubMed: 14715494]
14. Kirby JE. Anthrax toxin induces human endothelial cell apoptosis. *Infect Immun.* 2004; 72:430–439. [PubMed: 14688124]
15. Warfel JM, Steele AD, D'Agnillo F. Anthrax lethal toxin induces endothelial barrier dysfunction. *Am J Pathol.* 2005; 166:1871–1881. [PubMed: 15920171]
16. Gozes Y, Moayeri M, Wiggins JF, et al. Anthrax lethal toxin induces ketotifen-sensitive intradermal vascular leakage in certain inbred mice. *Infect Immun.* 2006; 74:1266–1277. [PubMed: 16428776]
17. Sherer K, Li Y, Cui X, Eichacker PQ. Lethal and edema toxins in the pathogenesis of *Bacillus anthracis* septic shock: implications for therapy. *Am J Respir Crit Care Med.* 2007; 175:211–221. [PubMed: 17095744]
18. Borio L, Frank D, Mani V, et al. Death due to bioterrorism-related inhalational anthrax: report of 2 patients. *JAMA.* 2001; 286:2554–2559. [PubMed: 11722269]
19. Sherer K, Li Y, Cui X, et al. Fluid support worsens outcome and negates the benefit of protective antigen-directed monoclonal antibody in a lethal toxin-infused rat *Bacillus anthracis* shock model. *Crit Care Med.* 2007; 35:1560–1567. [PubMed: 17452924]
20. Cui X, Li Y, Li X, et al. *Bacillus anthracis* edema and lethal toxin have different hemodynamic effects but function together to worsen shock and outcome in a rat model. *J Infect Dis.* 2007; 195:572–580. [PubMed: 17230417]
21. Park S, Leppla SH. Optimized production and purification of *Bacillus anthracis* lethal factor. *Protein Expr Purif.* 2000; 18:293–302. [PubMed: 10733882]
22. Ramirez DM, Leppla SH, Schneerson R, et al. Production, recovery and immunogenicity of the protective antigen from a recombinant strain of *Bacillus anthracis*. *J Ind Microbiol Biotechnol.* 2002; 28:232–238. [PubMed: 11986925]
23. Macarthur H, Westfall TC, Riley DP, et al. Inactivation of catecholamines by superoxide gives new insights on the pathogenesis of septic shock. *Proc Natl Acad Sci U S A.* 2000; 97:9753–9758. [PubMed: 10944234]
24. Quezado Z, Parent C, Karzai W, et al. Acute G-CSF therapy is not protective during lethal *E. coli* sepsis. *Am J Physiol Regul Integr Comp Physiol.* 2001; 281:R1177–1185. [PubMed: 11557626]
25. Cobb JP, Danner RL. Nitric oxide and septic shock. *JAMA.* 1996; 275:1192–1196. [PubMed: 8609688]

26. Dinarello CA. Proinflammatory and anti-inflammatory cytokines as mediators in the pathogenesis of septic shock. *Chest*. 1997; 112:321S–329S. [PubMed: 9400897]
27. Zhu X, Bagchi A, Zhao H, et al. Toll-like receptor 2 activation by bacterial peptidoglycan-associated lipoprotein activates cardiomyocyte inflammation and contractile dysfunction. *Crit Care Med*. 2007; 35:886–892. [PubMed: 17255871]
28. Price S, Anning PB, Mitchell JA, et al. Myocardial dysfunction in sepsis: mechanisms and therapeutic implications. *Eur Heart J*. 1999; 20:715–724. [PubMed: 10329063]
29. Takakura K, Xiaohong W, Takeuchi K, et al. Deactivation of norepinephrine by peroxynitrite as a new pathogenesis in the hypotension of septic shock. *Anesthesiology*. 2003; 98:928–934. [PubMed: 12657855]
30. Macarthur H, Couri DM, Wilken GH, et al. Modulation of serum cytokine levels by a novel superoxide dismutase mimetic, M40401, in an *Escherichia coli* model of septic shock: correlation with preserved circulating catecholamines. *Crit Care Med*. 2003; 31:237–245. [PubMed: 12545022]
31. Morimatsu H, Singh K, Uchino S, et al. Early and exclusive use of norepinephrine in septic shock. *Resuscitation*. 2004; 62:249–254. [PubMed: 15294412]
32. Hernandez G, Bruhn A, Romero C, et al. Management of septic shock with a norepinephrine-based haemodynamic algorithm. *Resuscitation*. 2005; 66:63–69. [PubMed: 15993731]
33. Kalinichenko VV, Mokyr MB, Graf LH, et al. Norepinephrine-mediated inhibition of anti-tumor cytotoxic T lymphocyte generation involves a beta-adrenergic receptor mechanism and decreased TNF-alpha gene expression. *J Immunol*. 1999; 163:2492–2499. [PubMed: 10452985]
34. Turk BE. Manipulation of host signaling pathways by anthrax toxins. *Biochem J*. 2007; 402:405–417. [PubMed: 17313374]
35. During RL, Gibson BG, Li W, et al. Anthrax lethal toxin paralyzes actin-based motility by blocking Hsp27 phosphorylation. *EMBO J*. 2007; 26:2240–2250. [PubMed: 17446863]
36. Park JM, Greten FR, Li ZW, et al. Macrophage apoptosis by anthrax lethal factor through p38 MAP kinase inhibition. *Science*. 2002; 297:2048–2051. [PubMed: 12202685]
37. Comer JE, Galindo CL, Chopra AK, et al. GeneChip analyses of global transcriptional responses of murine macrophages to the lethal toxin of *Bacillus anthracis*. *Infect Immun*. 2005; 73:1879–1885. [PubMed: 15731093]
38. Sapra R, Gaucher SP, Lachmann JS, et al. Proteomic analyses of murine macrophages treated with *Bacillus anthracis* lethal toxin. *Microb Pathog*. 2006; 41:157–167. [PubMed: 16950595]
39. Alileche A, Squires RC, Muehlbauer SM, et al. Mitochondrial impairment is a critical event in anthrax lethal toxin-induced cytolysis of murine macrophages. *Cell Cycle*. 2006; 5:100–106. [PubMed: 16357526]
40. Watson LE, Kuo SR, Katki K, et al. Anthrax toxins induce shock in rats by depressed cardiac ventricular function. *PLoS ONE*. May 23.2007 2:e466. [PubMed: 17520025]
41. Beall FA, Taylor MJ, Thorne CB. Rapid lethal effect in rats of a third component found upon fractionating the toxin of *Bacillus anthracis*. *J Bacteriol*. 1962; 83:1274–1280. [PubMed: 13866126]
42. Bellomo R. Noradrenaline: friend or foe? *Heart Lung Circ*. 2003; 12:S42–48. [PubMed: 16352145]
43. Bennett T, Mahajan RP, March JE, et al. Regional and temporal changes in cardiovascular responses to norepinephrine and vasopressin during continuous infusion of lipopolysaccharide in conscious rats. *Br J Anaesth*. 2004; 93:400–407. [PubMed: 15220167]
44. Firoved AM, Miller GF, Moayeri M, et al. *Bacillus anthracis* edema toxin causes extensive tissue lesions and rapid lethality in mice. *Am J Pathol*. 2005; 167:1309–1320. [PubMed: 16251415]
45. Van Amersfoort ES, Van Berkel TJ, Kuiper J. Receptors, mediators, and mechanisms involved in bacterial sepsis and septic shock. *Clin Microbiol Rev*. 2003; 16:379–414. [PubMed: 12857774]
46. Schutte F, Burgdorf C, Richardt G, et al. Adenosine A1 receptor-mediated inhibition of myocardial norepinephrine release involves neither phospholipase C nor protein kinase C but does involve adenylyl cyclase. *Can J Physiol Pharmacol*. 2006; 84:573–577. [PubMed: 16902603]

Figure 1.

Panel A shows the time course of catheter placement, challenge with lipopolysaccharide (LPS), lethal toxin (LeTx) or diluent, treatment with norepinephrine or placebo, hemodynamic measures including mean arterial blood pressure (MBP) and heart rate (HR), and arterial blood gas measures in animals observed for up to 168 h. Panel B shows the time course of catheter placement, challenges with LPS, LeTx or diluent, and measures of plasma catecholamines, nitric oxide (NO), cytokine, and superoxide dismutase (SOD) and lung and liver tissue SOD in animals observed for up to 8h. For the procedures shown in Panel A and B, a total of 232 and 59 animals were employed respectively as outlined in the methods.

Figure 2.

Proportion of animals surviving over time with one of three doses of norepinephrine (NE, 0.03, 0.3 or 3 $\mu\text{g/kg/min}$ for 24 h) or placebo (dose 0) with lipopolysaccharide (LPS) or lethal toxin (LeTx) challenge.

Figure 3.

Panels A and B show the serial mean changes (±SEM) from baseline in mean arterial blood pressure (MBP, mmHg) in animals challenged with a 24 h infusion of lipopolysaccharide (LPS, Panel A) or lethal toxin (LeTx, Panel B) alone or diluent over early (2 to 6 h) or late (8 to 24 h) time periods. Panels C and D show the serial mean (±SEM) changes from baseline in MBP in animals receiving LPS or LeTx with norepinephrine treatment (Panels C and D respectively) or animals receiving toxin alone. *p-value for the differences between toxin and diluent or between toxin with norepinephrine and toxin alone over the time period; **p value for the interaction of time and the effect of toxin.

Figure 4.

Panels A and B show the serial mean changes (±SEM) from baseline in heart rate (HR, bpm) in animals challenged with a 24 h infusion of lipopolysaccharide (LPS, Panel A) or lethal toxin (LeTx, Panel B) alone or diluent over early (2 to 6 h) or late (8 to 24 h) time periods. Panels C and D show the serial mean (±SEM) changes from baseline in HR in animals receiving LPS or LeTx with norepinephrine (0.3 µg/kg/h) treatment (Panels C and D respectively) or animals receiving toxin alone. *p-value for the differences between toxin and diluent; **p value for the interaction of time and the effect of toxin.

Figure 5.

Serial mean (±SEM) arterial pH (Panel A), base excess (ABE, Panel B) and lactate (LACT, panel C)) and in animals challenged with diluent alone, lipopolysaccharide (LPS) with or without norepinephrine (0.3μg/kg/min, NE) treatment or lethal toxin (LeTx) with or without norepinephrine, at 4, 8 and 24 h after the start of challenge. * p-value for differences between toxin alone and diluent over the time points; ** p-value for the interaction of time and the effect of toxin; † p-value for the differences between toxin with norepinephrine and toxin alone over the time points; †† p-value for the interaction of time and the effect of norepinephrine.

Figure 6.

Serial mean ($\pm$ SEM) plasma norepinephrine (Panel A), epinephrine (Panel B, and nitric oxide (Panel C) levels at 4 and 8 h following the start of lipopolysaccharide (LPS), lethal toxin (LeTx) or diluent challenge. * p-value for the difference between LPS and diluent over the time points; ** p-value for the interaction of time and the effect of LPS.