International Journal of
Translational Medicine

MDPI

Article

Efficacy of Doxycycline as Monotherapy or in Combination with Levofloxacin in Treating Late-Stage Systemic Anthrax in Non-Human Primates

Amir Ben-Shmuel †, Assa Sittner †, Itai Glinert, Elad Bar-David, Josef Schlomovitz, Haim Levy * and Shay Weiss *

Department of Infectious Diseases, Israel Institute for Biological Research, Ness-Ziona 7410033, Israel; amirb@iibr.gov.il (A.B.-S.); assas@iibr.gov.il (A.S.); itaig@iibr.gov.il (I.G.); eladb@iibr.gov.il (E.B.-D.); yosi.shlo@gmail.com (J.S.)

* Correspondence: haiml@iibr.gov.il (H.L.); shayw@iibr.gov.il (S.W.)

† These authors contributed equally to this work.

Abstract

Background: *Bacillus anthracis* spores pose a major biosecurity threat, in light of their use in several documented bioterror attacks and the fact that it was weaponized. The absence of specific and accurate treatment instructions in the 2001 anthrax letter attacks and the poor prognosis of patients receiving ineffective treatment emphasized the need for reliable guidelines for treating exposed (prophylaxis) or symptomatic populations. The recent 2023 CDC guidelines for treating systemic anthrax recommend a combined treatment of a tetracycline (minocycline or doxycycline) with meropenem or a fluoroquinolone. These recommendations differ from the 2001 and 2015 updates, and in the absence of significant clinical data, rely on animal studies. Previously, we demonstrated, based on a rabbit model, that these recommendations are effective in treating various stages of anthrax, ranging from post-exposure prophylaxis to systemic involvement and involvement of the CNS. Methods: We used a trigger-to-treat type of experiment using non-human primates (NHP) infected by nasal spray of a lethal dose of *B. anthracis* spores. Results: We confirmed our rabbit results by demonstrating the efficacy of doxycycline as a monotherapy or in combination with levofloxacin in treating late-stage systemic anthrax in non-human primates (NHP), in a trigger-to-treat type of experiment. Our experiments show high efficacy of the mono or combined therapy. Conclusions: Our results support the CDC guidelines placing doxycycline as first-choice therapeutics by demonstrating the efficacy of using it as a monotherapy or in combination with levofloxacin, in a second highly relevant NHP model.

Keywords: *Bacillus anthracis*; doxycycline; levofloxacin; non-human primates; anthrax; treatment; trigger to treat

Check for updates

Academic Editor: Simone Brogi

Received: 6 July 2026
Revised: 24 August 2026
Accepted: 25 August 2026
Published: 27 August 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Bacillus anthracis, a Gram-positive spore-forming bacterium, is the etiological cause of anthrax, a lethal zoonotic disease that is naturally transmitted to humans by contact with sick animals or contaminated animal products [1–3]. In most cases, the spores (the infectious form) penetrate the body through skin lesions or the gastrointestinal or respiratory tracts [1]. The route of infection determines the type of anthrax that develops, i.e., local eschar, gastrointestinal and systemic disease. Systemic disease is almost always fatal without treatment and will always develop after inhalational and oral infections [4]. The cutaneous form is the most obvious of the three and will resolve in 80–95% of the cases [5]. A

relatively new form of anthrax, deep tissue infection, resulting from the injection of spore-contaminated heroin, causes extended edema and inflammation of the injected limb [6]. After penetrating the host, the germinating bacterial cell produces two major virulence factors: capsule and tripartite toxin. The poly-γ-D-glutamic acid capsule protects the bacteria from phagocytosis and acts as an immunomodulator [7,8]. The toxin is composed of two enzymes: Lethal Factor (LF), a protease that cleaves specifically enzymes of the MAP kinase regulatory pathway [9,10], Edema Factor (EF), a calmodulin-dependent adenylate cyclase [11–13] and Protective antigen (PA), a transporting protein that binds to a specific cellular receptor and delivers the two enzymes into the host cell [14,15]. The capsule and toxins are secreted into the host blood and can serve as biomarkers for the disease. Since the PA is the most abundant, it is used as a sensitive indicator of the development of bacteremia [16,17].

Historically, anthrax patients were treated with injections of penicillin G, which was highly effective but of limited applicability in the case of mass exposure events [1]. Following the 2001 anthrax mail attacks and the confusion in treatment of systemic patients, the Centers for Disease Control and Prevention (CDC) recommended treatment based on ciprofloxacin or doxycycline in combination with up to four additional substances [18]. The CDC guideline addressed two major anthrax stages: early-stage post-exposure asymptomatic population (post-exposure prophylaxis—PEP) and symptomatic, most likely systemic patients. The systemic patients' symptoms could range from flu-like to an acute condition presenting high fever, pleural fluid accumulation and meningitis [19]. For PEP, the CDC recommended 60 days of oral treatment with ciprofloxacin or doxycycline. For symptomatic patients, the CDC recommended combined treatment based on ciprofloxacin or doxycycline together with one or more substances from a list. Eventually, the combination of ciprofloxacin with clindamycin was the treatment of choice and was successfully used in several anthrax cases since [19].

Since the original publication of guidelines (in 2001), the CDC updated its recommendations twice-in 2015 and 2023 [20,21]. The 2015 recommendations divided the symptomatic patients into two groups based on the physician's ability to exclude CNS involvement. The treatment in this case was based on the fluoroquinolone (ciprofloxacin or levofloxacin) supplemented with a protein synthesis inhibitor (clindamycin or linezolid). In the case where meningitis could not be excluded, the CDC recommended combining three antibiotics: a fluoroquinolone, a protein synthesis inhibitor (Linezolid as first choice) and a β-lactam (meropenem). Unlike the 2015 recommendations that were based mainly on data from other CNS diseases, the 2023 update was based on comprehensive analysis of all the available data (published and unpublished) on anthrax treatment in relevant animal models [22]. The recommended treatment is based on a tetracycline (Minocycline or doxycycline) combined with meropenem or a fluoroquinolone (ciprofloxacin or levofloxacin) [21]. Previously, we reported the high efficacy of doxycycline in treating late stages of systemic anthrax in rabbits as a monotherapy or in combination with a fluoroquinolone or meropenem [23–26]. We also demonstrated that, unlike levofloxacin, ciprofloxacin was not effective in treating advanced-stage anthrax in rabbits and NHP [23]. In this work, we validate the rabbit results in non-human primates (NHP) that were infected via intranasal spray of spores in trigger-to-treat experiments with doxycycline alone or with levofloxacin.

2. Materials and Methods

This study was carried out by trained personnel, in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Research Council. All protocols were approved by the IIBR committee on the Ethics of Animal Experiments (Protocol numbers: RM-01-18, approved on 13 September 2018, and

RM-03-19, approved on 23 September 2019). Animals had free access to food and water, maintained in a controlled environment with a constant fresh air supply and an ambient room temperature of 24 °C. Enrichment was supplied in the form of specialized toys. For each experiment, animals were randomly selected, sedated using 100 mg ketamine and 10 mg xylazine and transferred from the general animal facility to the BSL3 facility for acclimation. As the animal transfer between the general animal facility and the BSL3 facility is unidirectional, to avoid euthanizing naïve animals, the negative control animals were maintained in the general animal facility and were monitored daily by the facility staff.

The *B. anthracis* strain used in this study was the Vollum strain (ATCC14578). The MICs of this strain to doxycycline are 0.016 μg/mL and to levofloxacin are 0.047 μg/mL.

Animals (female Rhesus macaque) were anesthetized using 100 mg ketamine and 10 mg xylazine and infected by spraying 1×10^6 CFU ($10 \times LD_{50}$) *B. anthracis* Vollum spore suspension [24] into their nasal cavity with a MicroSprayer® Aerosolizer for Rats—Model IA-1B-R (PennCentury™ Wyndmoor, PA, USA). Blood samples (3 mL) were drawn from a limb vein every 4 h starting 24 h post-inoculation, and serum protective antigen (PA) concentration was determined using a home-made rapid ELISA [25] as a correlate for bacteremia level (Table 1). When bacteremia was estimated as $>10^4$ CFU/mL, blood was drawn and plated to determine the exact bacteremia (CFU/mL) and treatment was initiated. As we previously demonstrated in rabbit and guinea pig experiments, this level of bacteremia represents progressed systemic disease at a stage that effective antibiotic treatment will be successful in >95% of the cases and drops dramatically when bacteremia is higher than 1×10^6 CFU/mL.

Table 1. Serum PA and bacteremia at treatment initiation.

Bacteremia (CFU/mL)	Serum PA (μg/mL)
3×10^8	>50
$1.2–4 \times 10^7$	20–30
$1–2 \times 10^6$	3–5
$3–5 \times 10^5$	0.3–0.5
$1–2 \times 10^4$	0.05

Animals were treated SC with doxycycline 15 mg/kg (Vibramycin SF 100 mg/5 mL, Pfizer, New York, NY, USA) as a monotherapy (n = 6) or combined (n = 5) with levofloxacin 20 mg/kg (Tavanic 500 mg/100 mL, Aventis, Paris, France) twice a day for three days, followed by doxycycline alone, SC twice a day for four days. The treatment regimen was based on trough antibiotic inhibitory concentration at 12 h post-treatment [27,28]. Animals were monitored twice a day for 14 days post-inoculation for behavioral signs and body temperature using a subcutaneous RFID chip in the first 7–8 days. Animals unable or unwilling to drink were injected with 20–100 mL of saline or isotonic dextrose solution SC. Animals were euthanized immediately by a 120 mg/kg sodium pentobarbitone injection when one of the following symptoms was detected: severe respiratory distress or the loss of righting reflex. Since in most cases anthrax symptoms are detectable only in close proximity to death, both animals succumbed to the disease. To reduce the use of NHP, these two animals, one from each treatment group, serve as positive controls.

The use of *B. anthracis* was performed according to the Israeli select agent law. The experiments were regulated and monitored by the head of the biosafety unit and by the institutional regulatory committee at the IIBR. All experiments were conducted in a BSL3 laboratory.

3. Results

The doxycycline monotherapy group consisted of six animals. The animals were sedated and infected by spraying 1×10^6 CFU ($10 \times LD_{50}$) *B. anthracis* Vollum spore suspension [24] into their nasal cavity with a MicroSprayer® Aerosolizer. 24 h post-infection, blood samples were obtained, and PA serum concentration was estimated using a previously reported rapid ELISA [29]. In this specific experiment, all the animals were positive for serum PA at levels that indicate bacteremia at the desired levels (Table 1). At this point, we decided to start treating all the animals in this group, although our predictions indicated that bacteremia would be lower than the target of 1×10^4 CFU/mL, since according to our experience at these bacteremia levels, deterioration could be fast. The precise bacteremia of each of the animals at treatment initiation was determined by plating diluted blood samples. In perspective, we treated animals with bacteremia between 200 and 3×10^8 CFU/mL (Figure 1A). Animals were treated twice a day SC with 15 mg/kg of doxycycline. Animal posture and general behavior were observed from a distance using an in-room camera, and body temperature was recorded daily. Similar to the efficacy reported in the rabbit model [25], doxycycline monotherapy was highly effective, protecting all the animals with bacteremia of up to 10^7 CFU/mL, but failing to protect the animal with bacteremia of 3×10^8 CFU/mL. Body temperature in all surviving animals (Figure 2A) showed minimal differences throughout the treatment period. In contrast, there was a rapid decrease in the temperature of the animal that succumbed to the infection. This animal died within 36 h from treatment initiation; its body temperature dropped to 32 °C within 24 h and succumbed within the following 12 h, receiving two rounds of antibiotic treatment in the duration. Gross necropsy revealed high accumulation of pleural fluids and high intracranial tension that included hemorrhage, estimated by the excessive amount of bloody CSF discharged during brain necropsy. Both blood and brain were sterile, which is similar to what we had previously demonstrated for clindamycin in the rabbit model [29]. We cannot exclude the presence of bacteria in the organs; however, the fact that the blood and CSF were sterile indicates that the death was not due to treatment failure.

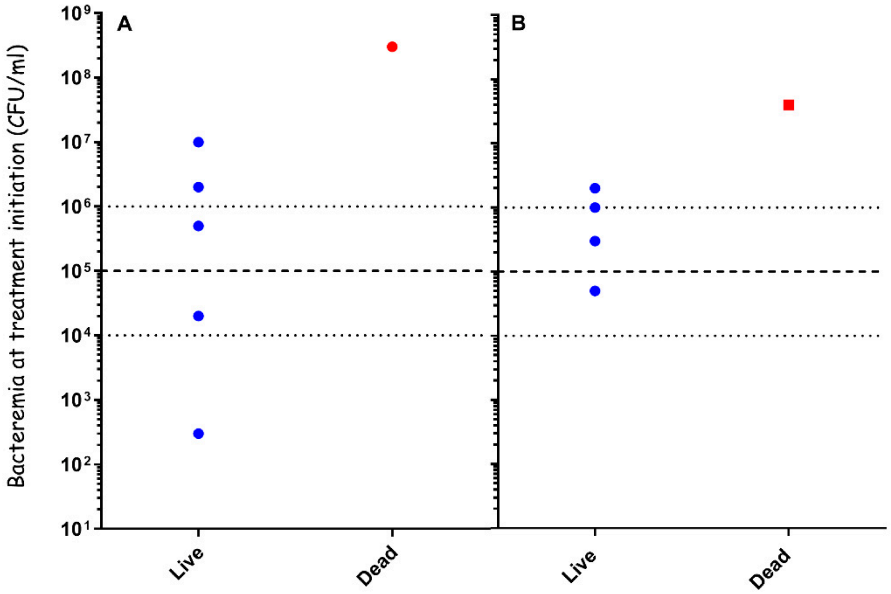


Figure 1. Outcome of inhalation anthrax treatment with doxycycline alone or in combination with levofloxacin. Animals were infected intranasally with Vollum spores using a PennCentury™ device. Disease progression was monitored by determining serum PA concentration which served as the trigger-to-treat. Bacteremia (CFU/mL) at treatment initiation and outcome of the treatment (Live or Dead) is indicated for (**A**)—doxycycline and (**B**)—doxycycline + levofloxacin.

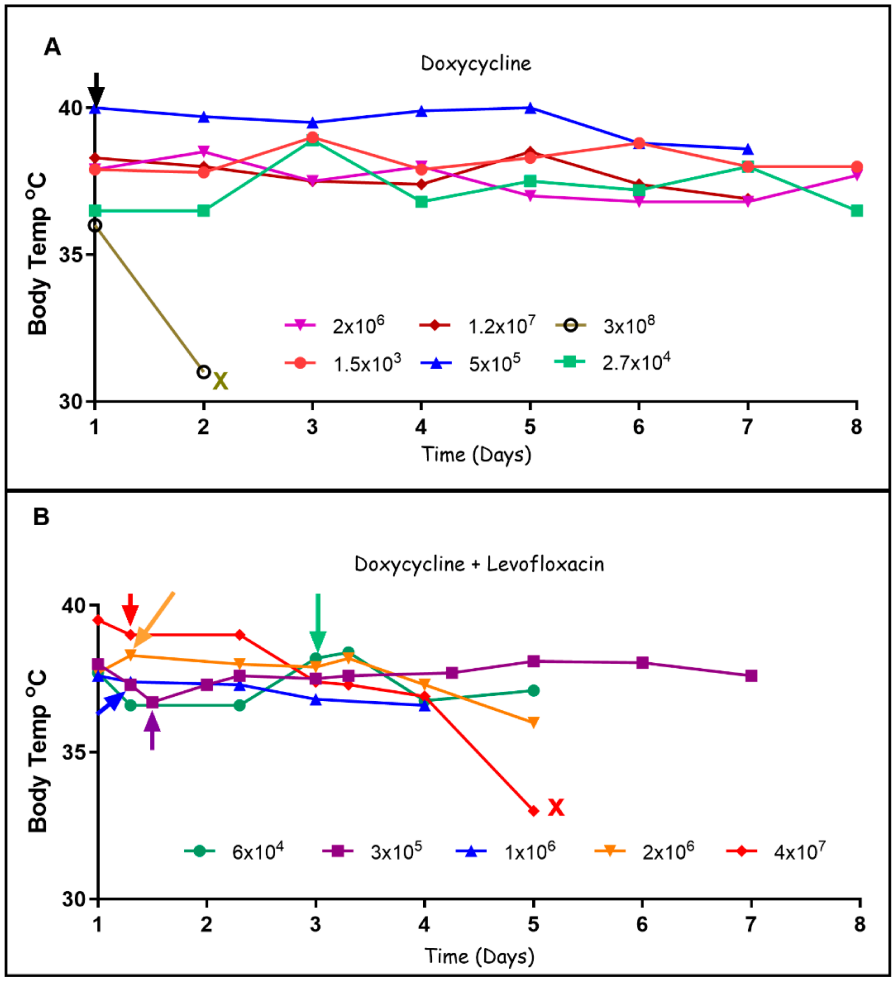


Figure 2. Body temperature of individual animals during the treatment period. A subcutaneous chip was used to record body temperature throughout the treatment period. (**A**)—doxycycline and (**B**)—doxycycline + levofloxacin. Arrows indicate treatment initiation and X indicates that the specific animal succumbed to the disease. The doxycycline treatment was initiated at 24 h (black arrow) post-infection, while in the doxycycline + levofloxacin experiment the treatment onset variation was high and the arrows are color coded according to the specific animal treated. The bacteremia at treatment initiation is indicated in the legend.

Since in the case of systemic disease the combination of at least two antibiotics is recommended [21], we tested the effect of adding levofloxacin to the treatment. For this experiment, five animals were exposed to *B. anthracis* Vollum spores as described for the doxycycline mono-treatment. Serum PA was estimated every 4 h starting 24 h post-infection. Unlike the first experiment, the disease progressed differently in each of the animals, resulting in treatment initiation at different time points, as shown by collared arrows in Figure 2B. This treatment group consists of five animals with bacteremia of 5×10^4 to 4×10^7 CFU/mL. Animals were treated twice a day SC with 15 mg/kg of doxycycline and 20 mg/kg of levofloxacin for three days, followed by a four-day treatment with doxycycline as monotherapy. Animals were monitored as above. This combined treatment was highly effective, protecting animals with a bacteremia of 5×10^4 to 2×10^6 CFU/mL (Figure 1B).

This treatment failed to protect the single animal with 4×10^7 CFU/mL. Body temperature monitoring (Figure 2B) again showed that in surviving animals no major changes were observed, while there was a slight decrease in temperature during the four days from treatment initiation to death in the animal that succumbed. Once this was detected, a warming infrared lamp was added, and 200 mL of saline was administered SC twice a day.

This limited supportive treatment, on top of the therapeutic protocol, did not prevent the animal's death. Perhaps an intensive care level of treatment might have been more effective in preventing the animal's death. Gross necropsy did not reveal any obvious damage to internal organs and there was no bacterial growth in blood or brain samples.

4. Discussion

Anthrax is a rare lethal zoonotic disease in which we rely exclusively on animal models for the development of clinical strategies [3]. The recent CDC recommends a combined treatment of tetracycline (minocycline or doxycycline) with meropenem or fluoroquinolone (levofloxacin) for the treatment of systemic anthrax [21]. Since previously, tetracycline was limited to PEP [20], most of the published data refers to the efficacy of this drug in treating animals in 24 h post-exposure [30]. At this time point, most animals are symptom-free and likely not bacteremic. Since animals do not show any disease signs, the only reliable marker is blood bacteremia, which increases with the progression of the disease and can reach 10^8–10^9 CFU/mL close to death [31]. Therefore, when testing treatment efficacy, a trigger-to-treat type of experiment is used. Since live counts are time-consuming and are relevant only in retrospect, in most experiments, plasma toxins, mainly PA, are used as a trigger-to-treat marker [17,25,32,33]. Since the correlation of bacteremia to PA is not perfect, in most of these experiments, treatment is initiated immediately once PA is detected and is usually based on a large cohort of animals to achieve statistical significance [34–36]. Therefore, the accepted model for such experiments is the rabbit; however, NHPs are still considered the relevant animal model when translating scientific data to human recommendations [37].

The efficacy of anthrax treatment is inversely correlated to the time from infection, where the efficacy decreases as the disease progresses. Treating exposed individuals (non-symptomatic) is highly effective. During the 2001 anthrax letter attack, thousands of suspected and confirmed people received ciprofloxacin or doxycycline prophylaxis, and none developed anthrax symptoms [38]. Modeling treatment of symptomatic individuals is more complicated since it includes variation in patients exhibiting a variety of signs ranging from flu-like to acute phase, which includes central nervous system (CNS) involvement [19]. The aim of this work was to evaluate the efficacy of the recent CDC guidelines in treating systemic anthrax patients, in the presence or absence of indications of CNS involvement [21]. For this purpose, we can use two types of experiments: a direct CNS infection model [39] and a trigger-to-treat type of experiment following airway spore infection. In the trigger-to-treat type of experiments, we aim to achieve bacteremia ranging from 0 to 10^8 CFU/mL, one that will allow us to distinguish between different stages of the systemic disease. Based on our previous finding indicating that from a bacteremia of 10^5 CFU/mL bacteria could be detected in the brain tissue [31], we defined bacteremia of 10–10^4 CFU/mL as flu-like and 10^4–10^6 CFU/mL as acute stages of the disease. In our rabbit experiments, we defined the treatment as efficient when all the animals with bacteremia of up to 10^4 at treatment initiation were saved [26,29].

Previously, we demonstrated that doxycycline, both as monotherapy or combined with levofloxacin, is effective in treating late-stage systemic disease or following direct CNS infection in rabbits [23,25]. We also demonstrated that this treatment is effective in NHP following direct CNS infection [23]. Since the CDC guidelines refer to the treatment of patients with progressed systemic anthrax, the NHP experiment had to simulate this condition. The disease that develops following CSF injection is mainly a CNS disease [39], while the systemic organ damage is secondary and results from transfer from the CNS to the bloodstream (the opposite direction of the natural disease). This model is great in testing the ability of antibiotics to cross the BBB and be effective in resolving the infection [39,40]. In this experiment, we started with a pulmonary infection of the animal and monitored the

PA level carefully in an attempt to get maximal bacteremia variation using a limited number of animals, since we are dealing with NHP. All the animals presented with bacteremia at treatment initiation, indicating that they were all successfully infected and developed a disease that was lethal in the absence of effective treatment. The fact that one animal from each treatment group succumbed to the infection, while being treated with antibiotics, supports this statement and can be considered a positive control. Herein, we tested the efficacy of doxycycline as a mono treatment or in combination with levofloxacin as a treatment of late-stage systemic disease following airway spore infection in NHP. The two treatments were highly effective, protecting animals with bacteremia as high as 10^6–10^7 CFU/mL. The fact that there is one animal in each bacteremia level restricts our ability to make any claims regarding the differences between the two groups. However, the fact that five animals in the doxycycline group, with bacteremia of up to 1×10^7 CFU/mL and four animals from the group treated with doxycycline and levofloxacin, with bacteremia of up to 2×10^6 CFU/mL, survived, indicates high treatment efficacy. Our treatment failed to protect one animal from each group. Doxycycline failed to protect an animal that had a very high bacteremia level of 3×10^8 CFU/mL. This bacteremia level is typical of moribund animals and is impossible to treat with antibiotic treatment. The finding of large quantities of pleural fluid and high intracranial pressure due to CSF accumulation, similar to that reported for human anthrax patients, supports this assumption. The fact that the blood and CSF were sterile after two doses of antibiotics points to the potency of doxycycline and not that the animal is actually sterile. The animal that died during the doxycycline and levofloxacin treatment had a high bacteremia of 4×10^7 CFU/mL at treatment initiation and actually improved during the first three days of treatment. However, the overall condition of the animal deteriorated and our supportive treatment of fluids and a heating lamp was insufficient, and the animal died without any obvious necropsy findings. Since this animal received five days of antibiotic treatment, we can assume that the animal was sterile. In these two cases, we can assume that intensive care treatment would prolong the survival of these animals, especially the one that died on day six.

Due to the nature of the NHP model, we limited the number of animals in each experiment, resulting in one animal in each bacteremia group. This situation limits our ability to make direct comparisons between the two treatments, but rather suggests non-inferiority. Overall, these findings support our previous rabbit results and the recent CDC recommendations for using doxycycline as a first-line choice in treating this stage of the disease.

Author Contributions: Conceptualization, H.L., S.W. and A.B.-S.; methodology, E.B.-D., J.S. and I.G.; validation, S.W., A.S. and H.L.; formal analysis, H.L. and S.W.; investigation, A.B.-S., A.S., I.G., E.B.-D., J.S., H.L. and S.W.; writing—original draft preparation, H.L., S.W., A.B.-S. and I.G.; writing—review and editing, A.S., E.B.-D. and J.S.; visualization, H.L. and S.W.; supervision, H.L.; project administration, H.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study was carried out by trained personnel, in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Research Council. All protocols were approved by the IIBR committee on the Ethics of Animal Experiments (Protocol numbers, RM-01-18, approved on 13 September 2018 and RM-03-19, approved on 23 September 2019).

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Dixon, T.C.; Meselson, M.; Guillemin, J.; Hanna, P.C. Anthrax. *N. Engl. J. Med.* **1999**, *341*, 815–826. [CrossRef] [PubMed]
2. Hanna, P. Anthrax pathogenesis and host response. *Curr. Top. Microbiol. Immunol.* **1998**, *225*, 13–35. [CrossRef] [PubMed]
3. WHO. *Anthrax in Humans and Animals*; WHO: Geneva, Switzerland, 2008.
4. Sirisanthana, T.; Brown, A.E. Anthrax of the gastrointestinal tract. *Emerg. Infect. Dis.* **2002**, *8*, 649–651. [CrossRef] [PubMed]
5. Doganay, M.; Metan, G.; Alp, E. A review of cutaneous anthrax and its outcome. *J. Infect. Public Health* **2010**, *3*, 98–105. [CrossRef] [PubMed]
6. Hanczaruk, M.; Reischl, U.; Holzmann, T.; Frangoulidis, D.; Wagner, D.M.; Keim, P.S.; Antwerpen, M.H.; Meyer, H.; Grass, G. Injectional anthrax in heroin users, Europe, 2000–2012. *Emerg. Infect. Dis.* **2014**, *20*, 322–323. [CrossRef] [PubMed]
7. Candela, T.; Fouet, A. Poly-gamma-glutamate in bacteria. *Mol. Microbiol.* **2006**, *60*, 1091–1098. [CrossRef] [PubMed]
8. Ezzell, J.W.; Welkos, S.L. The capsule of *Bacillus anthracis*, a review. *J. Appl. Microbiol.* **1999**, *87*, 250. [CrossRef] [PubMed]
9. Fang, H.; Cordoba-Rodriguez, R.; Lankford, C.S.R.; Frucht, D.M. Anthrax Lethal Toxin Blocks MAPK Kinase-Dependent IL-2 Production in $CD4^+$ T Cells. *J. Immunol.* **2005**, *174*, 4966–4971. [CrossRef] [PubMed]
10. Tonello, F.; Montecucco, C. The anthrax lethal factor and its MAPK kinase-specific metalloprotease activity. *Mol. Asp. Med.* **2009**, *30*, 431–438. [CrossRef] [PubMed]
11. Leppla, S.H. *Bacillus anthracis* calmodulin-dependent adenylate cyclase: Chemical and enzymatic properties and interactions with eucaryotic cells. *Adv. Cycl. Nucleotide Protein Phosphorylation Res.* **1984**, *17*, 189–198.
12. Labruyere, E.; Mock, M.; Ladant, D.; Michelson, S.; Gilles, A.M.; Laoide, B.; Barzu, O. Characterization of ATP and calmodulin-binding properties of a truncated form of *Bacillus anthracis* adenylate cyclase. *Biochemistry* **1990**, *29*, 4922–4928. [CrossRef] [PubMed]
13. Sarfati, R.S.; Kansal, V.K.; Munier, H.; Glaser, P.; Gilles, A.M.; Labruyere, E.; Mock, M.; Danchin, A.; Barzu, O. Binding of 3′-anthraniloyl-2′-deoxy-ATP to calmodulin-activated adenylate cyclase from *Bordetella pertussis* and *Bacillus anthracis*. *J. Biol. Chem.* **1990**, *265*, 18902–18906. [CrossRef]
14. Collier, R.J. Membrane translocation by anthrax toxin. *Mol. Asp. Med.* **2009**, *30*, 413–422. [CrossRef] [PubMed]
15. Brossier, F.; Levy, M.; Landier, A.; Lafaye, P.; Mock, M. Functional analysis of *Bacillus anthracis* protective antigen by using neutralizing monoclonal antibodies. *Infect. Immun.* **2004**, *72*, 6313–6317. [CrossRef] [PubMed]
16. Quinn, C.P.; Semenova, V.A.; Elie, C.M.; Romero-Steiner, S.; Greene, C.; Li, H.; Stamey, K.; Steward-Clark, E.; Schmidt, D.S.; Mothershed, E.; et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin protective antigen. *Emerg. Infect. Dis.* **2002**, *8*, 1103–1110. [CrossRef] [PubMed]
17. Kobiler, D.; Weiss, S.; Levy, H.; Fisher, M.; Mechaly, A.; Pass, A.; Altboum, Z. Protective antigen as a correlative marker for anthrax in animal models. *Infect. Immun.* **2006**, *74*, 5871–5876. [CrossRef] [PubMed]
18. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for exposure management and antimicrobial therapy, October 2001. *MMWR Morb. Mortal. Wkly. Rep.* **2001**, *50*, 909–919. [PubMed]
19. Jernigan, J.A.; Stephens, D.S.; Ashford, D.A.; Omenaca, C.; Topiel, M.S.; Galbraith, M.; Tapper, M.; Fisk, T.L.; Zaki, S.; Popovic, T.; et al. Bioterrorism-related inhalational anthrax: The first 10 cases reported in the United States. *Emerg. Infect. Dis.* **2001**, *7*, 933–944. [CrossRef] [PubMed]
20. Bower, W.A.; Hendricks, K.; Pillai, S.; Guarnizo, J.; Meaney-Delman, D.; Centers for Disease Control and Prevention. Clinical Framework and Medical Countermeasure Use During an Anthrax Mass-Casualty Incident. *MMWR Recomm. Rep.* **2015**, *64*, 1–22. [CrossRef] [PubMed]
21. Bower, W.A.; Yu, Y.; Person, M.K.; Parker, C.M.; Kennedy, J.L.; Sue, D.; Hesse, E.M.; Cook, R.; Bradley, J.; Bulitta, J.B.; et al. CDC Guidelines for the Prevention and Treatment of Anthrax, 2023. *MMWR Recomm. Rep.* **2023**, *72*, 1–47. [CrossRef] [PubMed]
22. Pillai, S.K.; Huang, E.; Guarnizo, J.T.; Hoyle, J.D.; Katharios-Lanwermeyer, S.; Turski, T.K.; Bower, W.A.; Hendricks, K.A.; Meaney-Delman, D. Antimicrobial Treatment for Systemic Anthrax: Analysis of Cases from 1945 to 2014 Identified Through a Systematic Literature Review. *Health Secur.* **2015**, *13*, 355–364. [CrossRef] [PubMed]
23. Ben-Shmuel, A.; Glinert, I.; Sittner, A.; Bar-David, E.; Schlomovitz, J.; Levy, H.; Weiss, S. Doxycycline, levofloxacin, and moxifloxacin are superior to ciprofloxacin in treating anthrax meningitis in rabbits and NHP. *Antimicrob. Agents Chemother.* **2024**, *68*, e0161023. [CrossRef] [PubMed]
24. Altboum, Z.; Gozes, Y.; Barnea, A.; Pass, A.; White, M.; Kobiler, D. Postexposure prophylaxis against anthrax: Evaluation of various treatment regimens in intranasally infected guinea pigs. *Infect. Immun.* **2002**, *70*, 6231–6241. [CrossRef] [PubMed]
25. Weiss, S.; Kobiler, D.; Levy, H.; Pass, A.; Ophir, Y.; Rothschild, N.; Tal, A.; Schlomovitz, J.; Altboum, Z. Antibiotics cure anthrax in animal models. *Antimicrob. Agents Chemother.* **2011**, *55*, 1533–1542. [CrossRef] [PubMed]
26. Sittner, A.; Bar-David, E.; Glinert, I.; Ben-Shmuel, A.; Schlomovitz, J.; Levy, H.; Weiss, S. Closing the Gaps: Testing the Efficacy of Carbapenem and Cephalosporin Treatments of Late-Stage Anthrax in Rabbits. *Pathogens* **2024**, *13*, 936. [CrossRef] [PubMed]

27. Embers, M.E.; Hasenkampf, N.R.; Embers, D.G.; Doyle, L.A. Pharmacokinetic analysis of oral doxycycline in rhesus macaques. *J. Med. Primatol.* **2013**, *42*, 57–61. [CrossRef] [PubMed]
28. Kao, L.M.; Bush, K.; Barnewall, R.; Estep, J.; Thalacker, F.W.; Olson, P.H.; Drusano, G.L.; Minton, N.; Chien, S.; Hemeryck, A.; et al. Pharmacokinetic Considerations and Efficacy of Levofloxacin in an Inhalational Anthrax (Postexposure) Rhesus Monkey Model. *Antimicrob. Agents Chemother.* **2006**, *50*, 3535–3542. [CrossRef] [PubMed]
29. Weiss, S.; Altboum, Z.; Glinert, I.; Schlomovitz, J.; Sittner, A.; Bar-David, E.; Kobiler, D.; Levy, H. Efficacy of Single and Combined Antibiotic Treatments of Anthrax in Rabbits. *Antimicrob. Agents Chemother.* **2015**, *59*, 7497–7503. [CrossRef] [PubMed]
30. Kennedy, J.L.; Bulitta, J.B.; Chatham-Stephens, K.; Person, M.K.; Cook, R.; Mongkolrattanothai, T.; Shin, E.; Yu, P.; Negron, M.E.; Bower, W.A.; et al. Postexposure Prophylaxis and Treatment of *Bacillus anthracis* Infections: A Systematic Review and Meta-analyses of Animal Models, 1947–2019. *Clin. Infect. Dis.* **2022**, *75*, S379–S391. [CrossRef] [PubMed]
31. Levy, H.; Glinert, I.; Weiss, S.; Bar-David, E.; Sittner, A.; Schlomovitz, J.; Altboum, Z.; Kobiler, D. The central nervous system as target of Bacillus anthracis toxin independent virulence in rabbits and guinea pigs. *PLoS ONE* **2014**, *9*, e112319. [CrossRef] [PubMed]
32. Migone, T.S.; Subramanian, G.M.; Zhong, J.; Healey, L.M.; Corey, A.; Devalaraja, M.; Lo, L.; Ullrich, S.; Zimmerman, J.; Chen, A.; et al. Raxibacumab for the treatment of inhalational anthrax. *N. Engl. J. Med.* **2009**, *361*, 135–144. [CrossRef] [PubMed]
33. Lawrence, W.S.; Peel, J.E.; Sivasubramani, S.K.; Baze, W.B.; Whorton, E.B.; Beasley, D.W.C.; Comer, J.E.; Hughes, D.E.; Ling, L.L.; Peterson, J.W. Teixobactin Provides Protection against Inhalation Anthrax in the Rabbit Model. *Pathogens* **2020**, *9*, 773. [CrossRef] [PubMed]
34. Migone, T.S.; Bolmer, S.; Zhong, J.; Corey, A.; Vasconcelos, D.; Buccellato, M.; Meister, G. Added benefit of raxibacumab to antibiotic treatment of inhalational anthrax. *Antimicrob. Agents Chemother.* **2015**, *59*, 1145–1151. [CrossRef] [PubMed]
35. Henning, L.N.; Carpenter, S.; Stark, G.V.; Serbina, N.V. Development of Protective Immunity in New Zealand White Rabbits Challenged with *Bacillus anthracis* Spores and Treated with Antibiotics and Obiltoxaximab, a Monoclonal Antibody against Protective Antigen. *Antimicrob. Agents Chemother.* **2018**, *62*, e01590-17. [CrossRef] [PubMed]
36. Hesse, E.M.; Godfred-Cato, S.; Bower, W.A. Antitoxin Use in the Prevention and Treatment of Anthrax Disease: A Systematic Review. *Clin. Infect. Dis.* **2022**, *75*, S432–S440. [CrossRef] [PubMed]
37. Goossens, P.L. Animal models of human anthrax: The Quest for the Holy Grail. *Mol. Asp. Med.* **2009**, *30*, 467–480. [CrossRef] [PubMed]
38. Jernigan, D.B.; Raghunathan, P.L.; Bell, B.P.; Brechner, R.; Bresnitz, E.A.; Butler, J.C.; Cetron, M.; Cohen, M.; Doyle, T.; Fischer, M.; et al. Investigation of bioterrorism-related anthrax, United States, 2001: Epidemiologic findings. *Emerg. Infect. Dis.* **2002**, *8*, 1019–1028. [CrossRef] [PubMed]
39. Ben-Shmuel, A.; Glinert, I.; Sittner, A.; Bar-David, E.; Schlomovitz, J.; Brosh, T.; Kobiler, D.; Weiss, S.; Levy, H. Treating Anthrax-Induced Meningitis in Rabbits. *Antimicrob. Agents Chemother.* **2018**, *62*, e00298-18. [CrossRef] [PubMed]
40. Sittner, A.; Ben-Shmuel, A.; Glinert, I.; Bar-David, E.; Schlomovitz, J.; Kobiler, D.; Weiss, S.; Levy, H. Using old antibiotics to treat ancient bacterium-beta-lactams for *Bacillus anthracis* meningitis. *PLoS ONE* **2020**, *15*, e0228917. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.