MAJOR ARTICLE

Linezolid Dampens Neutrophil-Mediated Inflammation in Methicillin-Resistant *Staphylococcus aureus*–Induced Pneumonia and Protects the Lung of Associated Damages

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Background. Linezolid is considered as a therapeutic alternative to the use of glycopeptides for the treatment of pneumonia caused by methicillin-resistant *Staphylococcus aureus* (MRSA). Clinical studies reported a potent survival advantage conferred by the oxazolidinone and called into question the use of glycopeptides as first-line therapy.

Methods. In a mouse model of MRSA-induced pneumonia, quantitative bacteriology, proinflammatory cytokine concentrations in lung, myeloperoxidase activity, Ly6G immunohistochemistry, and endothelial permeability were assessed to compare therapeutic efficacy and immunomodulative properties of linezolid and vancomycin administered subcutaneously every 12 hours.

Results. Significant antibacterial activity was achieved after 48 hours of treatment for linezolid and vancomycin. Levels of interleukin 1β, a major proinflammatory cytokine, and macrophage inflammatory protein 2, a chemokine involved in the recruitment of neutrophils, were decreased by both antimicrobials. Only linezolid was able to dramatically reduce the production of tumor necrosis factor α. Analysis of myeloperoxidase activity and Ly6G immunostaining showed a dramatic decrease of neutrophil infiltration in infected lung tissues for linezolid-treated animals. A time-dependent increase of endothelial permeability was observed for the control and vancomycin regimens. Of interest, in the linezolid group, decreased endothelial permeability was detected 48 hours after infection.

Conclusions. Our results indicate that linezolid could be superior to vancomycin for the management of MRSA pneumonia by attenuating an excessive inflammatory reaction and protecting the lung from pathogen-associated damages.

Keywords. Oxazolidinones; glycopeptides; pneumonia; cytokines; neutrophil; *Staphylococcus aureus*; myeloperoxidase; animal model.

Managing methicillin-resistant *Staphylococcus aureus* (MRSA) in both healthcare and community settings continues to be a high priority for clinicians. If vancomycin is still considered for the treatment of MRSA infections, its superiority was challenged by the availability of new antimicrobials with promising activity in both experimental models and clinical practice, which should improve the management of MRSA in the coming years [1–3]. However, despite the use of effective antibiotics, lower respiratory tract bacterial infections continue to be a major cause of morbidity and mortality in both industrialized and developing countries [4].

Vancomycin resistance has emerged in the United States and the minimum inhibitory concentrations (MICs) tend to increase among *S. aureus* isolates [5]. An interesting alternative to the use of glycopeptides

Received 14 November 2013; accepted 3 March 2014; electronically published 11 March 2014.

Presented in part: 52nd Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, California, 9–12 September 2012 (abstract B-651); 53rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Denver, Colorado, 10–13 September 2013 (abstract B-491).

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The Journal of Infectious Diseases 2014;210:814–23

DOI: 10.1093/infdis/jiu145

is linezolid, the only marketed oxazolidinone, although others are in development [6]. Acting as a protein synthesis inhibitor [7], linezolid displays a time-dependent, nonbactericidal in vitro activity against staphylococci [8], contrary to glycopeptides exhibiting bactericidal activity by inhibition of the cell wall synthesis. If the distinction between agents exhibiting bacteriostatic and bactericidal activity is obvious in vitro, the theoretical superiority of bactericidal agents over bacteriostatic agents has to be demonstrated in clinical situations [9].

The respiratory system is continuously exposed to a variety of pathogens, suggesting the presence of an underlying multifaceted defense system [10]. One of the most important parts of the initial innate immune response in the lung against bacterial infection is the strong recruitment of neutrophils [11]. This leads to a complex relationship between innate immunity and bacterial infection during the pneumonia process. An appropriate inflammatory response is required for the clearance of the causative bacteria (positive effect), but an excessive neutrophil-mediated inflammation could lead to local or systemic damages (negative effect) [12]. As previously described for macrolide antibiotics [13], the addition of antibiotic therapy could affect the balance between favorable and unfavorable effects. Beyond the direct antibacterial activity of oxazolidinones, it has been shown that linezolid is able to exert additional properties, such as suppression of toxin production [14, 15] and inhibition of the expression of virulence factors [14].

On the basis of distinct mechanisms of action, we hypothesized that linezolid and vancomycin could exhibit different immunomodulatory properties in addition to their direct antibacterial activities. Using a mice pneumonia model, we aimed to assess the antibacterial activities of antistaphylococcal drugs against MRSA, as well as the consequences of therapy on the lung immune response to the infection.

MATERIAL AND METHODS

Bacterial Strain

MRSA strain ATCC 33 591 was grown overnight in brain heart infusion broth at 37°C (Becton-Dickinson, Franklin Lakes, NJ). Immediately before use, the bacteria pellet (centrifuged at 800 g for 10 minutes) was washed twice, using 0.9% NaCl. After the second wash, the pellet was resuspended in sterile saline, and the inoculum was calibrated by nephelometry.

Susceptibility Testing

The MICs were determined in cation-supplemented Mueller-Hinton (MH) broth [16]. Time-kill experiments were performed in glass flasks containing MH broth [17] with an inoculum of 5.10^6 colony-forming units (CFU)/mL in the presence of linezolid or vancomycin at various concentrations (1 and 32 times the MIC).

Animals

Six-week-old RjOrl:SWISS mice (weight, 20–24 g) were purchased from Janvier Laboratories (Le Genest Saint Isle, France). Mice were given food and water at libitum. Animals were treated in accordance with institutional policies and the guidelines stipulated by the animal welfare committee. The Committee of Animal Ethics of the University of Nantes approved all animal experimentation in this study.

Pharmacokinetic Studies

The doses used for drugs were designed to approximate antibiotic human plasma exposure obtained with intravenous formulations of linezolid 600 mg every 12 hours and vancomycin 1 g every 12 hours in humans [18, 19]. The amount of protein binding in mouse serum is similar to that of protein binding in human serum for linezolid and vancomycin [19]. Animals were assigned to 3 groups: no treatment (controls), subcutaneous injection every 12 hours of linezolid at a dose of 80 mg/kg, and subcutaneous injection every 12 hours of vancomycin at a dose of 110 mg/kg. Linezolid was assayed by high-performance liquid chromatography (lower detection limit, 0.1 mg/L; coefficient of variation, <10%) by a method adapted from Peng et al [20]. Vancomycin concentrations were determined by immunoenzymatic assay (lower detection limit, 2.5 mg/L; coefficient of variation, 4.1%–6.9%).

Pneumonia Model

Pneumonia was induced as previously described [21]. Mice were briefly anesthetized with isoflurane (Abbott, Chicago, IL) and placed in dorsal recumbency. A 24-gauge feeding needle was inserted transtracheally, and 75 μL of a bacterial suspension adjusted to 10^9 CFU/mL was injected. Treatment was started 2 hours after the bacterial challenge, and antibiotics were administered to the animals by the subcutaneous route for 2 days.

Quantitative Bacteriology in Infected Lung and Spleen (Bacterial Dissemination)

Lungs and spleen from each animal were removed, weighed, and homogenized in 1 mL of saline buffer (Mixer Mill MM 400, Retsch, Newtown, PA) and used for quantitative cultures on agar for 24 hours at 37°C. Viable counts, measured 48 hours after incubation, were expressed as mean (± SD) $\log_{10}$ CFU per gram of organ.

Preparation of Lung Homogenate for Enzyme-Linked Immunosorbent Assay (ELISA) and Determination of Cytokine Levels

Immediately after removal, weighed lung samples were mechanically homogenized in cold lysis buffer (1X phosphate buffered saline [pH 7.4] and 0.1% Triton X-100) containing 1 mM protease inhibitor cocktail (Sigma, St. Louis, MO). Tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), and macrophage inflammatory protein 2 (MIP-2) concentrations in lung homogenate were

quantified with ELISA kits according to manufacturer instructions (eBioscience, Paris, France; and R&D Systems, Lille, France). Protein concentration in each sample was determined using the BCA protein assay kit (Pierce, Rockford, IL).

Myeloperoxidase (MPO) Activity

At 2, 8, 24, and 48 hours after the bacterial challenge, animals were euthanized, and lungs were removed, frozen in liquid nitrogen, and stored at −80°C until assayed. The MPO assay was performed as previously described [21, 22].

Histologic and Immunohistochemical Analyses

After mice were euthanized, lungs were removed immediately by thoracotomy and immersed in 4% paraformaldehyde overnight. Paraffin sections were stained with hematoxylin-eosin-safran. Immunohistochemical analysis was performed after antigen retrieval with citrate buffer. Neutrophil staining was performed using Ly6G/Gr-1 monoclonal antibodies (clone 1A8; 1:100 [BioLegend, San Diego, CA]) followed by the Histofine rabbit-to-mouse kit (Nichirei Biosciences, distributed by Microm-Microtech, France). Diaminobenzidine was used as a chromogen. The percentage of the total lung area that was Ly6G positive was determined using the algorithm simple interactive object extraction (SIOX) as a plug-in in Fiji software.

Lung Endothelial Permeability

The determination of the lung endothelial permeability was performed as previously described by Boutoille et al [23]. Briefly, mice were given a 2-mg intraperitoneal injection of fluorescein isothiocyanate (FITC)–conjugated albumin (Sigma, Lyon, France). Two hours later, the lungs were removed, mechanically homogenized in 1 mL of 0.9% NaCl, and then centrifuged at 4000×*g* for 10 minutes. Blood was collected and centrifuged at 4000×*g* for 10 minutes. FITC-albumin was measured in supernatant obtained from lung homogenates and blood by fluorimetry at 480 nm.

Statistics

Statistical analyses were performed with GraphPad Prism Software (version 4.0; GraphPad Software, San Diego, CA). Normally distributed data were analyzed using analysis of variance to compare the effects between the different groups, followed by a Bonferroni test to compare the treated groups 2 by 2. Continuous nonparametric variables were expressed as

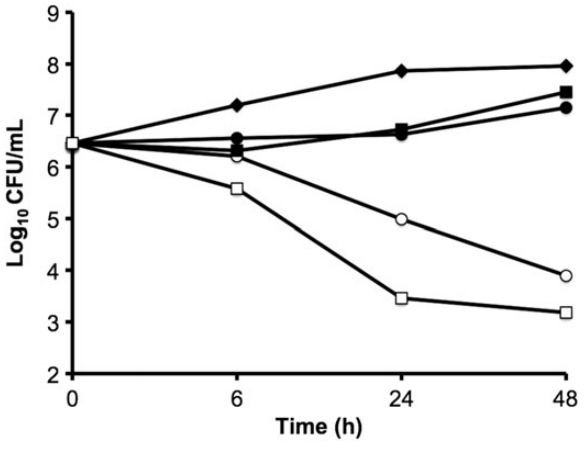

Figure 1. Killing curves for linezolid and vancomycin against methicillin-resistant *Staphylococcus aureus* strain ATCC 33 591. ◆, control; •, linezolid at 1 times the minimum inhibitory concentration [MIC]; ○, linezolid at 32 times the MIC; ■, vancomycin at 1 times the MIC; □, vancomycin at 32 times the MIC. Abbreviation: CFU, colony-forming units.

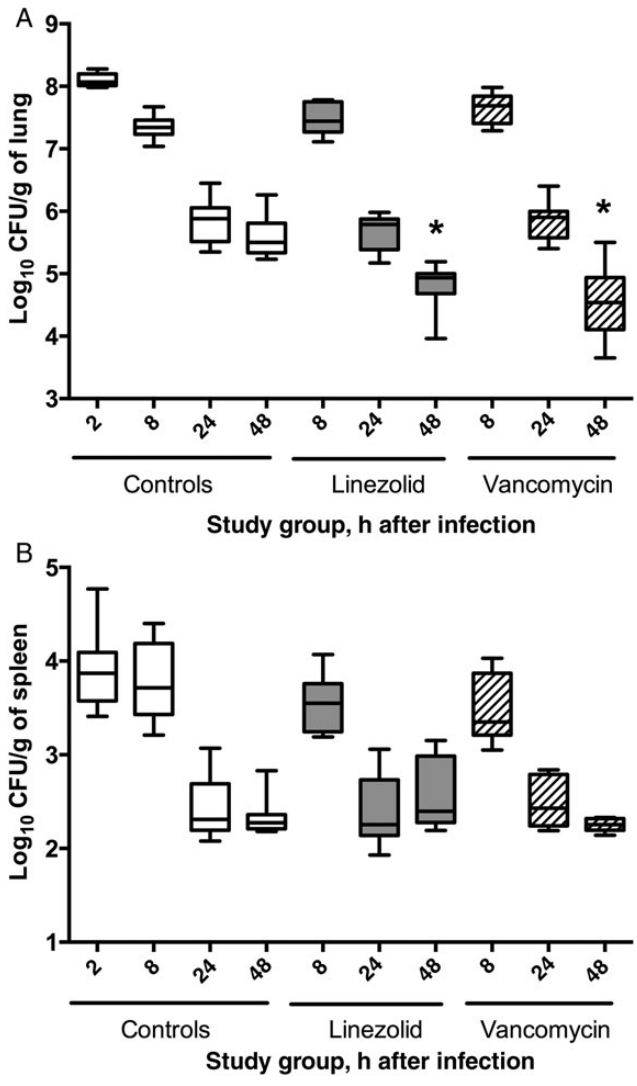

Figure 2. In vivo antibacterial efficacy of linezolid and vancomycin after 48 hours of treatment for pneumonia due to methicillin-resistant *Staphylococcus aureus*. *A*, Bacterial counts in lung. *B*, Bacterial counts in spleen. Three groups of mice were studied: untreated (control) mice, linezolid-treated mice, and vancomycin-treated mice. Boxes represent median (interquartile range). Data are representative of 2 independent experiments (6 mice/group). **P* < .01 vs all other groups. Abbreviation: CFU, colony-forming units.

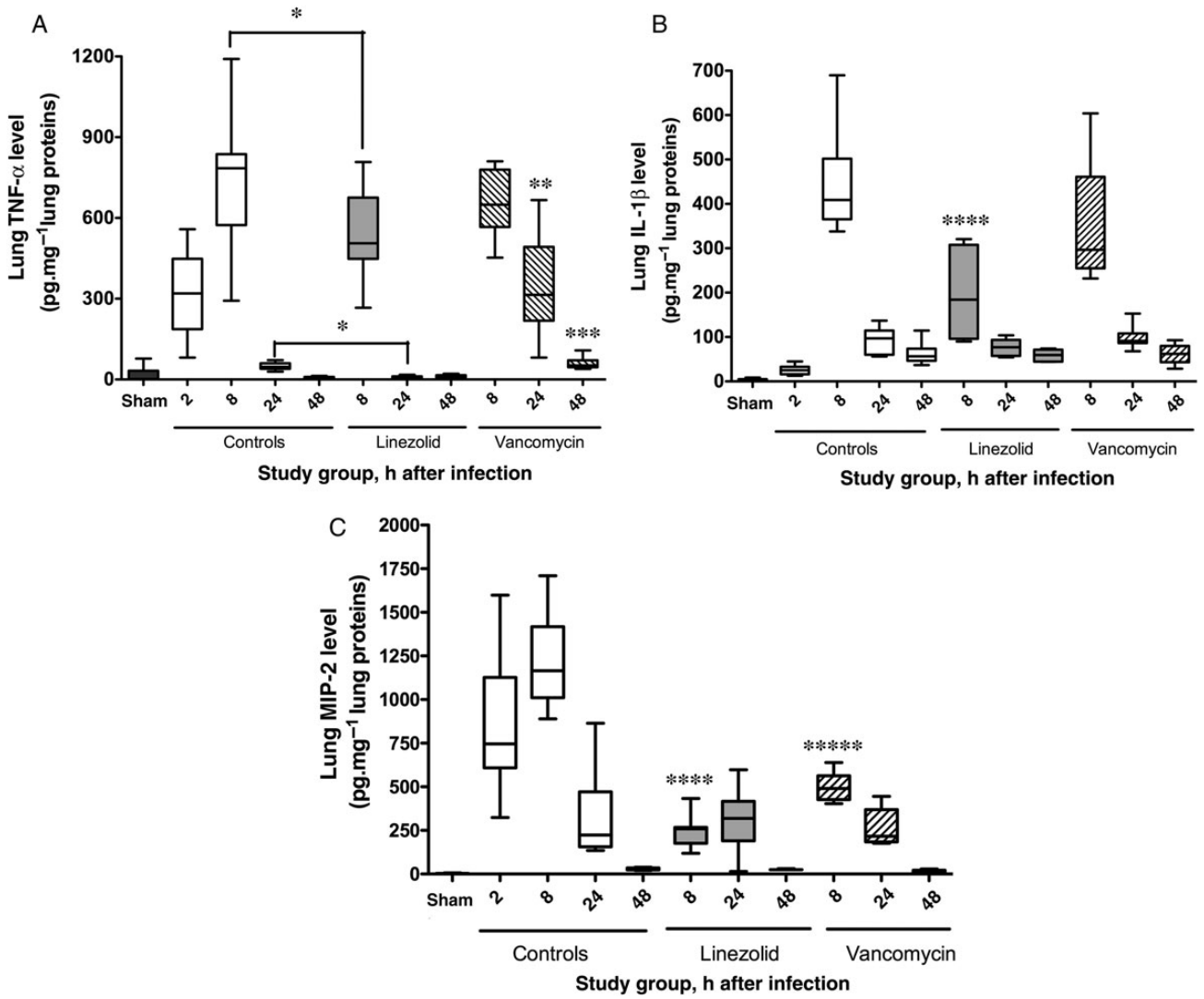

Figure 3. Linezolid and vancomycin modulate the production of proinflammatory cytokines during methicillin-resistant *Staphylococcus aureus*–induced pneumonia. Four groups of mice were studied: sham-treated (noninfected, nontreated) mice (Sham), untreated (control) mice (C), linezolid-treated mice (LZD), and vancomycin-treated mice (VAN). Boxes represent median (interquartile range). Data are representative of 3 independent experiments (6 mice/group). *$P < .05$; **$P < .001$ vs C (24 hours after infection) and LZD (24 hours after infection); ***$P < .05$ vs C (48 hours after infection) and LZD (48 hours after infection); ****$P < 0.05$ vs C (8 hours after infection) and VAN (8 hours after infection); *****$P < .05$ vs C (8 hours after infection) and LZD (8 hours after infection). Abbreviations: IL-1β, interleukin 1β; MIP-2, macrophage inflammatory protein 2; TNF-α, tumor necrosis factor α.

medians (interquartile ranges) and were compared using the Kruskal–Wallis test for multiple comparisons. In case of significance, the Mann–Whitney test was used for intergroup comparison. A P value of $< .05$ was considered statistically significant.

RESULTS

Linezolid and Vancomycin Show Similar Antibacterial Activity in MRSA-Induced Pneumonia

The MICs of MRSA 33 591 were 1 and 2 mg/L for vancomycin and linezolid, respectively. Using time-kill curves, we investigated the in vitro activity of both drugs with low and high concentrations (ie, 1 and 32 times the MIC, respectively). Linezolid showed only modest time-dependent activity against *S. aureus*. At 32 times the MIC, the decrease in the initial inoculum was close to 1.5 $\log_{10}$ after 24 hours (Figure 1). As expected, vancomycin displayed a superior activity, exhibiting a slow bactericidal activity (3-$\log_{10}$ decrease after 24 hours, compared with the initial inoculum).

Treatment with antistaphylococcal drugs was started 2 hours after the inoculation, and animals were euthanized at different time points to determine the time course of the in vivo response to antibacterial therapy (Figure 2*A*). Linezolid and vancomycin demonstrated a time-dependent activity in the infected lung

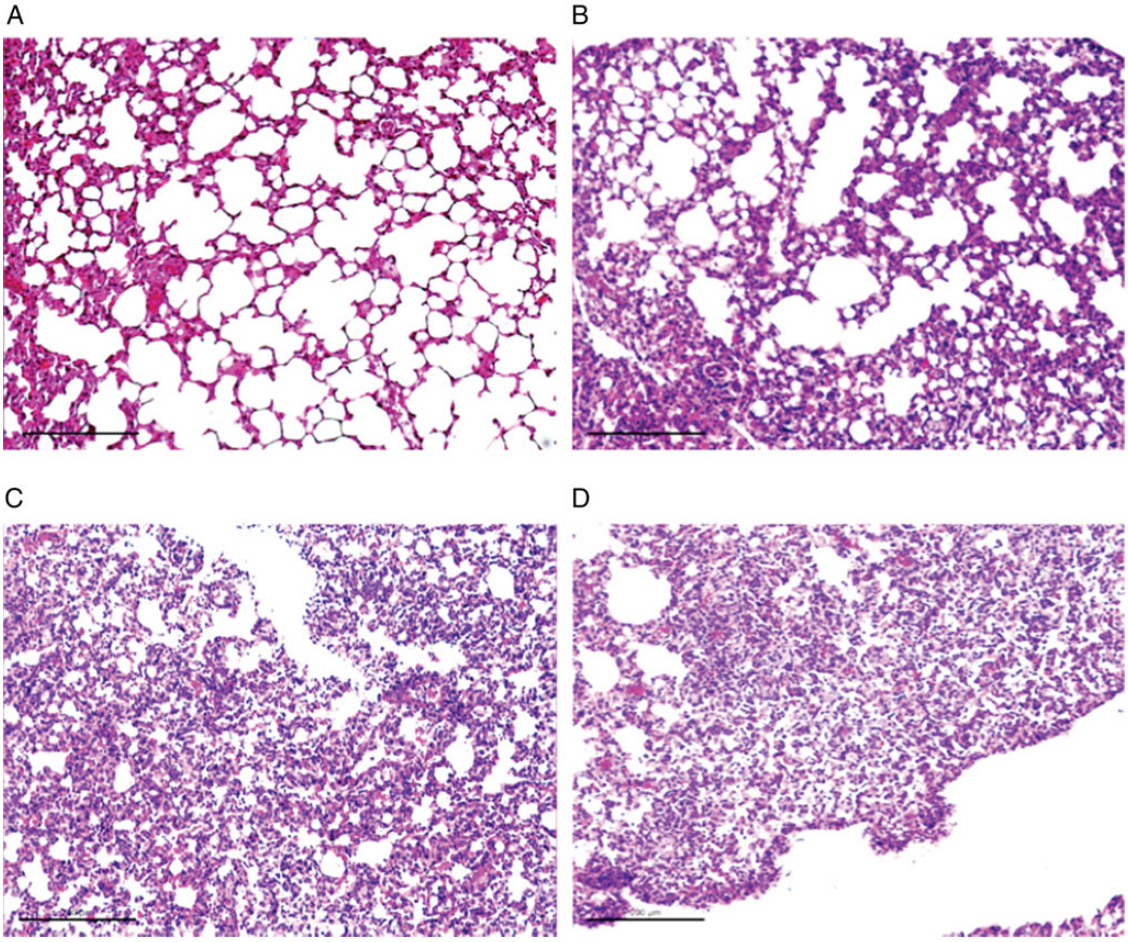

Figure 4. Lung histopathologic examination of *Staphylococcus aureus*–induced pneumonia in mice. Hematoxylin-eosin-safranin stain was applied to the sections (original magnification ×50). *A*, Sham-treated (ie, noninfected, nontreated) mice. *B*, *S. aureus*–induced pneumonia 8 hours after bacterial challenge. *C*, *S. aureus*–induced pneumonia 24 hours after bacterial challenge. *D*, *S. aureus*–induced pneumonia 48 hours after bacterial challenge.

tissues, with a significant decrease observed only after 48 hours of treatment. Both drugs were able to decrease the lung bacterial load by 1 $\log_{10}$ CFU/g of tissue. Spleen bacterial counts are considered an appropriate marker of the systemic dissemination of the infection. Antibacterial treatment did not result in lower bacterial counts as compared to those in untreated control animals ($P > .05$), with no difference observed in the spleen bacterial burden after 6, 24, and 48 hours of treatment (Figure 2*B*).

Antistaphylococcal Therapy Modulates the Production of Proinflammatory Cytokines and Chemokines During MRSA Pneumonia

Cytokines concentrations were determined in lung tissue homogenates 2, 8, 24, and 48 hours after infection to reflect the time course of expression of proinflammatory cytokines (TNF-α and IL-1β), major inducers of the inflammatory response, and of MIP-2, a chemokine involved in the recruitment of neutrophils within the infected site, during the pneumonia process (Figure 3). TNF-α (Figure 3*A*), IL-1β (Figure 3*B*), and MIP-2 (Figure 3*C*) levels increased significantly in the early stages of infection, with a peak obtained 8 hours after the bacterial challenge. After the fast increases in cytokine levels, the level of each cytokine declined rapidly, highlighting the transient and kinetic aspects of signaling molecules involved in the immune response. In treated animals, levels of IL-1β and MIP-2 were decreased, with an abolition of the peak observed in control (ie, infected, nontreated) mice (Figure 3*B* and *C*, respectively). Although MIP-2 and IL-1β levels in treated mice were less than those in untreated mice 8 hours after infection, the only statistically significant difference between treated and control mice involved the IL-1β level in the linezolid group ($P < .05$). Regarding TNF-α, linezolid was able to reduce its production in vivo 8 and 24 hours after infection (Figure 3*A*). Interestingly, vancomycin induced a significant increase in TNF-α production, compared with that in control and linezolid-treated animals, 24 and 48 hours after infection. This increase was not observed in uninfected animals treated with antibiotic alone: linezolid or vancomycin did not modify the expression of

TNF-α, nor of IL-1β or MIP-2, compared with expression in the sham (ie, uninfected, untreated) group, at the different times (data not shown).

Linezolid Therapy Dampened Neutrophil-Mediated Inflammation in Infected Lungs During MRSA Pneumonia

Considering the differences in cytokine levels associated with linezolid and vancomycin, we investigated whether these differences were correlated with a difference in pulmonary outcomes. Histologic examination of slides for noninfected mice displayed thin-walled air spaces with a single pneumocyte layer with no cell infiltrate within the alveoli (Figure 4). Lungs of infected mice showed a gradual increase in the accumulation of inflammatory cells within the alveoli, starting 24 hours of infection. All mice showed mild pulmonary inflammation that slightly increased at 48 hours, with a higher percentage of lungs affected.

The microbicidal role of neutrophils, through their MPO activity, has been described as a major line of defense against bacterial infection [24]. Most abundantly expressed in neutrophil granulocytes, the enzyme has been proposed to be a surrogate marker of the degree of neutrophil activation. High levels of MPO were observed 8 hours after bacterial challenge in MRSA-infected animals, and MPO activity decreased gradually but did not reach baseline values measured in uninfected mice. Linezolid treatment resulted in significantly lower MPO levels in lungs 8 and 48 hours after infection, compared with levels in control and vancomycin-treated mice (Figure 5*A*).

To confirm the MPO results, neutrophil attraction and accumulation were directly assessed in MRSA-infected lungs by Ly6G immunohistochemical analysis (neutrophil-specific antigen). Ly6G staining on lung tissue slides showed a smaller degree of neutrophil infiltration after 8 hours of infection in linezolid-treated mice as compared to control and vancomycin-treated animals (Figure 5*B*). Quantitative analysis of Ly6G-stained paraffin sections by use of the SIOX algorithm strongly confirmed these results, with a dramatic decrease in the Ly6G-positive area observed in linezolid-treated mice (Figure 5*C*). No difference in terms of neutrophil recruitment was observed between the untreated and vancomycin-treated groups.

Linezolid Prevents the Development of Endothelial Lesions in Lung

A rapid increase of the total lung weight that was not influenced by vancomycin treatment was observed in MRSA-infected mice during pneumonia (Figure 6*A*). On the contrary, the lung weight of animals that received linezolid treatment was lower than that for untreated and vancomycin-treated mice. Furthermore, FITC-albumin has been used to determine albumin flux from the pulmonary circulation into the alveolar space [23]. The increase of endothelial permeability is a broad indicator of lung injury and, in murine models, of edema [25]. An increase in endothelial permeability was observed in all groups after 24 hours of infection, suggesting the onset of endothelial lesion development (Figure 6*B*). Interestingly, endothelial permeability measured in vancomycin-treated mice was higher than in the control and linezolid groups ($P < .001$). At 48 hours, the endothelial permeability in the linezolid group was significantly less than that in the untreated animals, suggesting a protective effect on the evolution of pulmonary damages.

DISCUSSION

Using an acute experimental model of MRSA-induced pneumonia, we investigated the in vivo activity and potent immunomodulative effects of 2 antistaphylococcal drugs, linezolid and vancomycin. Our results indicate that (1) both drugs showed similar antibacterial activity in the infected lung after 48 hours of treatment, (2) linezolid and vancomycin were able to modulate the immune response to *S. aureus* infection by modifying the production of proinflammatory cytokines and chemokines, and (3) linezolid dampened neutrophil-mediated inflammation in the first stage of MRSA pneumonia and had a protective effect against all of the surrogate markers of lung damage.

The incidence of *S. aureus* continues to increase in United States, and the pathogen is often involved in hospital-acquired and community-acquired pneumonia (CAP) [26]. The empirical use of vancomycin for the presumed treatment of gram-positive nosocomial pneumonia led to the emergence of *S. aureus* strains exhibiting a decreased susceptibility for vancomycin. More recently, a number of studies reported a positive correlation between increased MICs (≥1.5 mg/L) and poor outcomes [27, 28]. Clinical studies call into question the use of vancomycin as first-line therapy; recent randomized, double-blind, controlled trials demonstrated a greater clinical efficacy and a survival advantage of linezolid, compared with vancomycin, for the treatment of MRSA nosocomial pneumonia [29, 30].

By use of similar plasma exposure and comparable percentage of time above the MIC, the major pharmacodynamic index correlating with in vivo efficacy for time-dependent antibiotics [31], we found that linezolid and vancomycin displayed a similar antibacterial activity in infected lung tissues, leading to a 1-$\log_{10}$ CFU decrease after 48 hours of treatment. Consistent with these data, several experimental studies showed no significant difference between both antibacterial agents against MRSA [32, 33] and no superiority of linezolid over the gold standard, vancomycin [33, 34]. Of interest, no advantage of the bactericidal mechanism of action of vancomycin was demonstrated in both experimental and clinical studies, suggesting a poor clinical relevance of the necessity of bactericidal drugs in the management of MRSA pneumonia. Studies highlighted adverse effects encountered with bactericidal drugs, such as the release of large amounts of pathogen-associated molecular patterns

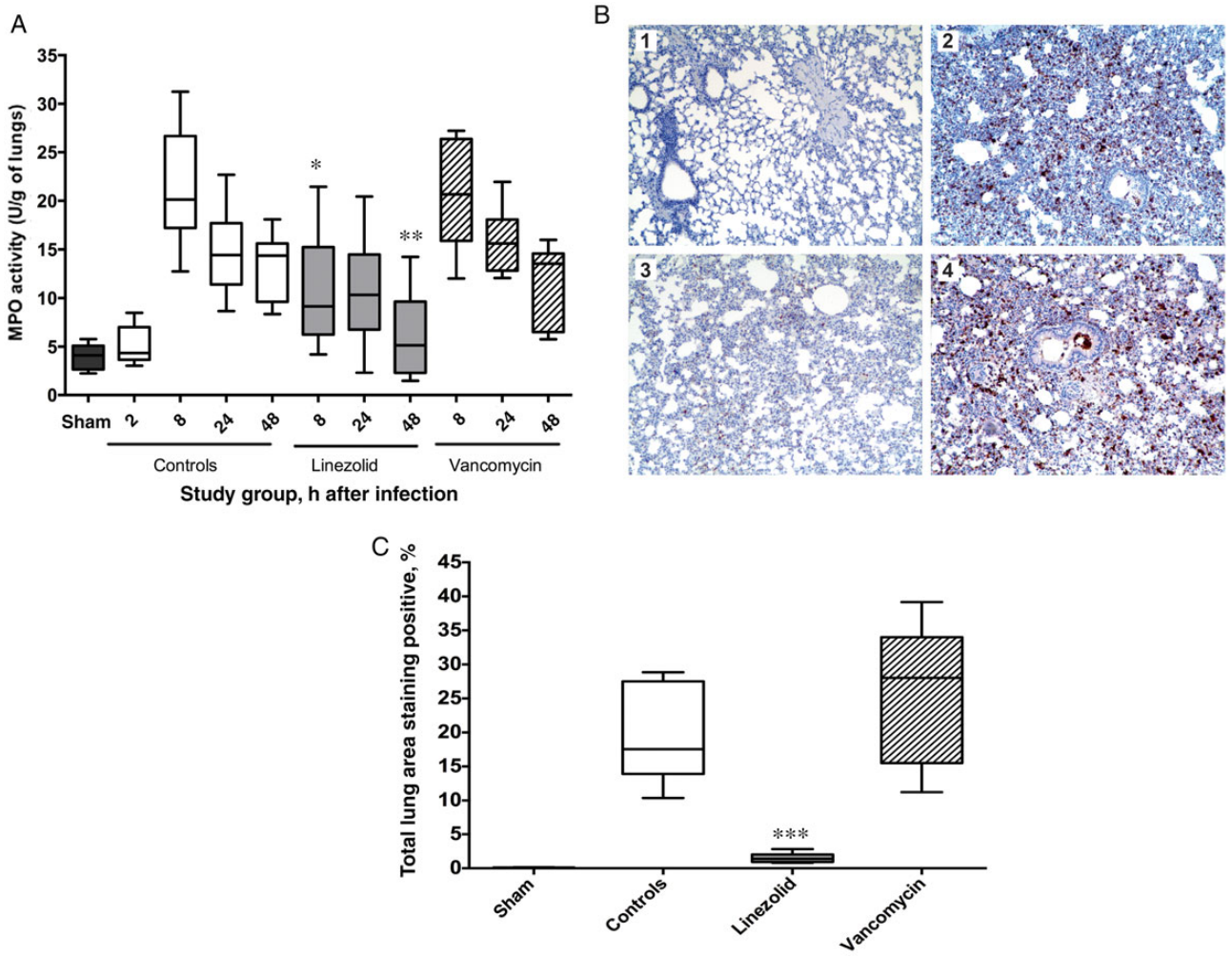

Figure 5. Neutrophil-mediated inflammation is decreased in linezolid-treated animals. *A*, Myeloperoxidase activity in infected lung tissues after 2, 8, 24, and 48 hours of infection. Four groups of mice were studied: sham-treated (noninfected, nontreated) mice (Sham), untreated (control) mice (C), linezolid-treated mice, and vancomycin-treated mice (VAN). Boxes represent median (interquartile range [IQR]). Data are representative of 3 independent experiments (6 mice/group), $*P < .001$ vs C (24 hours after infection) and VAN (8 hours after infection); $**P < .05$ vs C (48 hours after infection) and VAN (48 hours after infection). *B*, Ly6G immunohistochemical analysis involving Ly6G staining of paraffin sections of mouse lungs obtained 8 hours of infection from uninfected and untreated animals (panel 1), methicillin-resistant *Staphylococcus aureus* (MRSA)–exposed and untreated animals (panel 2), MRSA-exposed and linezolid-treated animals (panel 3), and MRSA-exposed and vancomycin-treated animals (panel 4). *C*, Simple interactive object extraction analysis of Ly6G-stained paraffin sections of mouse lungs 8 hours after infection. Four groups of mice were studied: Sham mice, control mice, linezolid-treated mice, and vancomycin-treated mice. Boxes represent median (IQR). Data are representative of 2 independent experiments. Six fields per specimen (2 animals/group) were analyzed. $***P < .01$ vs all other groups.

(including bacterial lipoproteins and lipoteichoic acids) recognized by Toll-like receptors and the overproduction of reactive oxygen species in mammalian cells [35–37]. Consequently, an excessive inflammatory reaction could be observed as a response to the bacterial lysis resulting from the action of bactericidal antibiotics [9]. A network of cytokine signals plays an essential role in the modulation of the inflammatory response and the clearance of the pathogen. Giving the context of acute pneumonia, we focused on proinflammatory cytokines (TNF-α and IL-1β) known as major inducers of the inflammatory response [38]. TNF-α acts as a general marker of inflammation in pneumonia through its involvement in systemic response and local injury [39, 40]. Eight hours after the bacterial challenge, high levels of TNF-α were measured in lung homogenates for infected animals, followed by a rapid decrease observed at 24 and 48 hours after infection. However, little is known about the role of antibacterial agents on cytokine production and timing. Our data showed that antibacterial treatment strongly modulates the secretion of TNF-α during MRSA infection. Of interest, linezolid treatment altered the production of TNF-α over time in infected mice by significantly decreasing the cytokine lung concentration at 8 and 24 hours after infection as

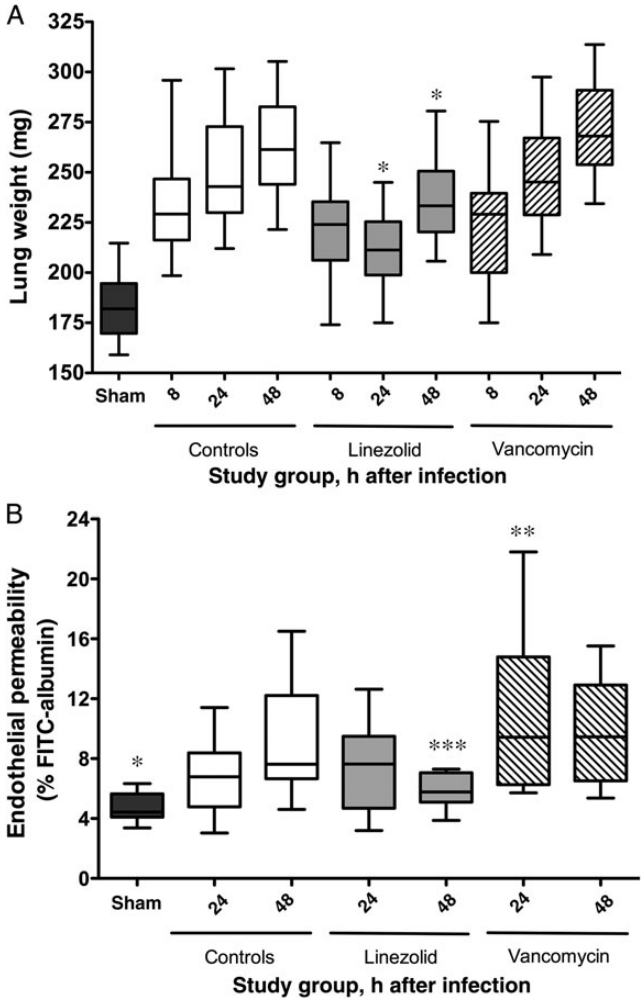

Figure 6. Linezolid therapy dampens inflammatory lung lesions and edema. *A*, Total lung weight of untreated and treated animals. Four groups of mice were studied: sham-treated (noninfected, nontreated) mice (Sham), untreated (control) mice (C), linezolid-treated mice, and vancomycin-treated mice (VAN). Boxes represent median (interquartile range [IQR]). $^{**}P < .05$ vs C and VAN groups at 24 and 48 hours after infection. *B*, Vascular permeability assessed by measuring fluorescein isothiocyanate (FITC)–albumin in lung homogenates of infected mice. Four groups of mice were studied: Sham mice, control mice (C), linezolid-treated mice (LZD), and vancomycin-treated mice (VAN). Boxes represent median (IQR). Data are representative of 3 independent experiments (6 mice/group). $^{*}P < .05$ vs C (24 and 48 hours after infection), LZD (24 hours after infection), VAN (24 and 48 hours after infection); $^{**}P < .001$ vs C (24 hours after infection) and LZD (24 hours after infection); $^{***}P < .05$ vs C (48 hours after infection) and VAN (48 hours after infection).

compared to the TNF-α levels observed in control animals. Surprisingly, 7- and 40-fold greater TNF-α levels were detected in the vancomycin group at 24 hours, compared with levels in control and linezolid-treated animals, respectively. In addition, the vancomycin group showed the highest TNF-α levels after 48 hours of infection. A clinical study by Lee et al [39] demonstrated that the concentrations of TNF-α in bronchoalveolar lavage and serum specimens were higher among nonsurvivors than among survivors with CAP. Other cytokines considered as proinflammatory were investigated in the present study. Circulating levels of IL-1β are usually elevated in patients with pneumonia [41]. If TNF-α may be a marker of severity of pneumonia, IL-1β seems to be associated with the severity of infection [41]. Both linezolid and vancomycin were able to decrease the early peak in the IL-1β level, but a more pronounced effect was observed with linezolid therapy.

Neutrophils recruited at the infected site after migration and infiltration steps are the major source of proinflammatory cytokines (TNF-α and IL-1β) and are involved in the loss of epithelial integrity [42]. Acting as a chemokine, MIP-2 induces neutrophil activation, chemotaxis, exocytosis, and the respiratory burst [43]. Although no difference was observed at 24 and 48 hours between treated and untreated animals, both antistaphylococcal drugs decreased the early production of MIP-2, with a more pronounced effect of linezolid as compared to vancomycin ($P < .05$). Ly6G staining confirmed a smaller degree of neutrophil infiltration after 8 hours of infection in linezolid-treated mice but not after vancomycin administration. Using the same experimental model, Akinnussi et al concluded that linezolid may not display an advantage over vancomycin in modulating the pulmonary innate immune response [32]. The authors did not observe any difference between both drugs in term of cytokine (ie, interleukin 6 and monocyte chemotactic protein 5 [MCP-5]) levels, MPO activity, neutrophil apoptosis, and phagocytosis of apoptotic neutrophils. In the present work, we focused on the early stage of infection (8 hours after infection), which was not investigated in the study by Akinnussi et al. Taken together, these data emphasized the importance of the kinetic of the immune response when assessing the immunomodulatory effects of antibacterial agents.

Under the action of cytokines and chemokines (interleukin 8, TNF-α, and IFN-γ) [44], neutrophils activate in « primed neutrophils », which means a special ability of the cells to trigger a respiratory burst response (10–20-fold increase) [45]. Although primed neutrophils show an advantage for the clearance of the infection, overwhelming activation of neutrophils is known to elicit tissue damage [46]. To go further, we investigated the impact of both MRSA infection and antibiotic treatment on the development of edema and endothelial lesions. Determination of endothelial permeability is considered to be a broad indicator of lung injury in murine models [23]. Treatment with linezolid exerts a protective effect on the development of pulmonary edema, as suggested by the inhibition of the MRSA-induced increase in lung weight and endothelial permeability at 24 and 48 hours after infection. Glycopeptide therapy worsens the apparition of lung damages, as suggested by the early increment of the endothelial permeability after only 24 hours of infection. Taken together, the surrogate markers of lung damage suggest that the reduction in the development of endothelial lesions and

pulmonary edema during linezolid therapy could be the result of a dramatic decrease in neutrophil recruitment and associated tissue damage.

There is increasing evidence that an adequate cytokine balance plays a crucial role in determining outcomes in hospitalized patients with pneumonia [38]. In this context, if the balance cannot be maintained in an appropriate manner, deleterious effects can increase the susceptibility to and influence the severity of pneumonia. It is likely that active antibacterial agents such as linezolid and vancomycin play a crucial role in this complex phenomenon.

One limitation of the present study is that other factors not evaluated here could be involved and may contribute to worse outcomes. Many studies demonstrated that antimicrobial agents might induce and enhance toxin production, as well as induce/suppress virulence factors. Gemmell et al observed in vitro a reduction in virulence factor expression, using sub-MICs of linezolid and *S. aureus* [47]. More recently, a study by Otto et al demonstrated that linezolid dramatically reduced the expression of Panton-Valentine leukocidin (a β-pore-forming toxin) and protein A, whereas vancomycin seemed to have no significant effects [14]. Using a rabbit model of MRSA necrotizing pneumonia, Diep et al showed that early treatment with linezolid (but not vancomycin) was associated with suppression of Panton-Valentine leukocidin and α-toxin production in the lung [15]. Finally, neither clinical scoring for assessing symptoms nor mortality could be used in these experiments.

In conclusion, despite similar antibacterial activity in MRSA-infected lungs, linezolid and vancomycin showed distinct profiles in the modulation of the lung inflammatory status. The data presented here suggest that linezolid, a protein synthesis inhibitor exhibiting a bacteriostatic mode of action, could be superior to vancomycin by attenuating an excessive inflammatory reaction and protecting the lung of MRSA-associated damages.

Notes

Acknowledgments. We thank the Cellular and Tissular Imaging Core Facility of Nantes University (MicroPICell) for assistance in histological analysis.

Disclaimer. The funding sources had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Financial support. This work was supported by Pfizer (ASPIRE investigator award to K. A.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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