Linezolid Effects on Bacterial Toxin Production and Host Immune Response: Review of the Evidence

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ABSTRACT

BACKGROUND: Linezolid is active against a broad range of gram-positive pathogens and has the potential to also affect production of bacterial toxins and host immune function.

OBJECTIVE: To assess the evidence for direct effects of linezolid on bacterial toxin synthesis and modulation of host immune responses.

METHODS: Literature searches were performed of the PubMed and OVID databases. Reviews and non–English language articles were excluded. Articles with information on the effect of linezolid on bacterial toxin synthesis and immune responses were selected for further review, and data were summarized.

RESULTS: Substantial in vitro evidence supports effects of linezolid on bacterial toxin production; however, the strength of the evidence and the nature of the effects are mixed. In the case of Staphylococcus aureus, repeated observations support the inhibition of production of certain staphylococcal toxins (Panton-Valentine leukocidin, protein A, and α- and β-hemolysin) by linezolid, whereas only solitary reports indicate inhibition (toxic shock syndrome toxin-1, coagulase, autolysins, and enterotoxins A and B) or stimulation (phenol-soluble modulins) of toxin production by linezolid. In the case of Streptococcus pyogenes, there are solitary reports of linezolid inhibition (protein M, deoxyribonuclease, and streptococcal pyrogenic exotoxins A, B, and F) or stimulation (immunogenic secreted protein 2 and streptococcal inhibitor of complement-mediated lysis) of toxin production, whereas published evidence for effects on streptolysin O production is conflicting. In vitro data are limited, but suggest that linezolid might also have indirect effects on host cytokine expression through inhibition of bacterial production of toxins. In vivo data from preclinical animal studies and a single clinical study in humans are limited and equivocal insofar as a potential role for linezolid in modulating the host inflammatory response; this is due in part to the difficulty in isolating antimicrobial effects and toxin synthesis inhibitory effects of linezolid from any secondary effects on host inflammatory response.

CONCLUSIONS: Available evidence supports the possibility that linezolid can inhibit, and in some cases stimulate, toxin production in clinically relevant patho-
INTRODUCTION

Bacterial toxins are important mediators of pneumonia, sepsis, and septic shock, in part because of their effects on components of the host immune system. Different classes of antibacterial agents may have different effects on the production and release of bacterial toxins and on the subsequent immune response. Antimicrobial agents that disrupt bacterial cell wall synthesis (β-lactams) lead to bacterial death and the release of pathogen-associated molecular patterns such as lipopolysaccharide (LPS), lipoprotein, or DNA. The release of pathogen-assisted molecular patterns may induce more robust host immune responses. In contrast, antimicrobial agents that inhibit the microbial ribosome system (eg, protein synthesis inhibitors such as lincomycins and oxazolidinones) or DNA gyrase (eg, fluoroquinolones) suppress the synthesis of bacterial toxins, and may have secondary effects on dampening toxin-induced host inflammatory responses. These antimicrobial agents are often used in clinical practice to treat toxin-mediated infections (eg, toxic shock syndrome [TSS] and pneumonia). Nevertheless, it remains to be determined whether the effects of bacterial protein synthesis inhibitors on either bacterial toxin production or immunomodulation have any effect on treatment outcomes.

Linezolid is an oxazolidinone antibiotic indicated for treatment of complicated skin and skin structure infections caused by susceptible pathogens (eg, methicillin-resistant and methicillin-susceptible strains of Staphylococcus aureus [MRSA and MSSA, respectively], Streptococcus pyogenes, or Streptococcus agalactiae), including diabetes-related foot infections without concomitant osteomyelitis, and nosocomial pneumonia caused by MRSA or MSSA or Streptococcus pneumoniae. Linezolid binds to the bacterial ribosome and prevents the formation of the 70S initiation complex, thereby inhibiting protein synthesis. Linezolid is bacteriostatic against enterococci and staphylococci and is bactericidal for most streptococci strains. In addition to this activity, linezolid has been reported to inhibit bacterial toxin production and, therefore, has the potential to indirectly modulate host immune function during resolution of infection. Herein, we review the evidence for linezolid-mediated effects on bacterial toxin production and the host immune responses in the published peer-reviewed literature and provide perspectives on the clinical relevance of these findings.

METHODS

A retrospective literature search of the PubMed database was performed from August through December 2011, without any year limitations, using the key words “linezolid” or “protein synthesis inhibitor antibiotic” and each of the following: “animal,” “chemokine,” “C-reactive protein,” “cytokine,” “immune,” “inflammation,” “lipo-
polysaccharide,” “neutrophil,” “Panton-Valentine leukocidin” (PVL), “phenol-soluble modulins” (PSM), “Staphylococcus,” “Streptococcus,” and “toxin.” Reviews and non–English language articles were excluded, and the remaining articles were screened manually for relevance (ie, whether the article included data on linezolid-mediated effects on bacterial toxin production and/or the immune system). Search results were confirmed by performing another search of the OVID database using the same key words noted above. All selected references were categorized as in vitro, in vivo animal, or in vivo human. Key data were summarized and critically reviewed.

RESULTS

Literature Selection

The PubMed search yielded 48,976 articles, 841 of which were reviewed and assessed for relevance to this review. Results from search term combinations that yielded more than 500 articles were discarded as not sufficiently specific. Articles with relevant information from the remaining results were reviewed and summarized. The confirmatory search of the OVID database yielded no additional articles for inclusion in this review.

Linezolid Effect on Staphylococcal Toxins

Seven articles were found that provided evidence of linezolid-mediated effects on toxins produced by S. aureus, and these are summarized below. A brief summary of the toxins and the results from each study are also given in Table I.

Panton-Valentine Leukocidin

PVL is a toxin that is characteristically produced by invasive community-associated MRSA (CA-MRSA) strains that cause diseases of the lung, bone, and soft tissues (Table I).13–17 In 2 articles, Dumitrescu et al9,10 examined the in vitro effect of linezolid and other antibiotics on PVL production by laboratory and clinical strains of PVL-positive MSSA and CA-MRSA. Subinhibitory concentrations of oxacillin (one-eighth to one-half the minimum inhibitory concentrations (MIC) increased PVL production 2- to 6.5-fold in the MSSA and MRSA strains tested. Exposure to subinhibitory concentrations of vancomycin had no significant effect on PVL production. In contrast, subinhibitory concentrations of clindamycin, fusidic acid, rifampicin, or linezolid inhibited PVL production.9,10 Furthermore, the increased PVL production induced by subinhibitory concentrations of oxacillin was inhibited by co-incubation with rifampicin, clindamycin, or linezolid (at one-eighth, one-fourth, and one-half their MIC, respectively).9

Stevens et al11 compared the effects of nafcillin (at up to ~one-third of its MIC) with those of vancomycin, linezolid, and clindamycin (at 5-fold their MIC) on the growth and expression of PVL mRNA and protein in a PVL-positive CA-MRSA strain (Table I). Compared with untreated control cultures, nafcillin added during log-phase growth had little or no effect on growth through the late log phase into the stationary phase (up to 34 hours); however, it prolonged expression of PVL mRNA and increased expression of detectable PVL protein. Vancomycin transiently inhibited both growth and PVL mRNA expression; however, PVL protein levels were not
Table I. Effect of linezolid on *Staphylococcus aureus* toxin synthesis.

<table>
<thead>
<tr>
<th>Toxin and Effect</th>
<th>Reference</th>
<th>S aureus Strain</th>
<th>Linezolid Concentration (MIC) and Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVL: Pore-forming leukotoxin associated with primary skin and soft tissue infections and severe necrotizing pneumonia</td>
<td>Dumitrescu et al,\textsuperscript{10} 2007</td>
<td>PVL-positive MSSA, PVL-positive CA-MRSA</td>
<td>0.125 to 1 × MIC; significantly reduced production of PVL by all strains</td>
</tr>
<tr>
<td></td>
<td>Dumitrescu et al,\textsuperscript{9} 2008</td>
<td>PVL-positive MSSA</td>
<td>0.25 to 0.5 × MIC; significantly reduced PVL production (including oxacillin-induced expression)</td>
</tr>
<tr>
<td></td>
<td>Stevens et al,\textsuperscript{11} 2007</td>
<td>PVL-positive CA-MRSA</td>
<td>5 × MIC; significantly reduced expression of PVL protein but not mRNA</td>
</tr>
<tr>
<td>TSST-1: Superantigen; induces TSS</td>
<td>Stevens et al,\textsuperscript{12} 2006</td>
<td>MSSA</td>
<td>0.25 to 5 × MIC; significantly reduced TSST-1 production</td>
</tr>
<tr>
<td>PSM: Activates innate immune system; associated with severe skin infections</td>
<td>Joo et al,\textsuperscript{19} 2010</td>
<td>CA-MRSA, HA-MRSA</td>
<td>0.1 to 0.2 × MIC; significantly increased PSM production in both strains</td>
</tr>
<tr>
<td>Protein A: Inhibits opsonophagocytosis</td>
<td>Gemmell and Ford,\textsuperscript{21} 2002</td>
<td>S aureus strain expressing virulence factors</td>
<td>0.5 × MIC; significantly reduced protein A activity</td>
</tr>
<tr>
<td></td>
<td>Bernardo et al,\textsuperscript{22} 2004</td>
<td>MSSA</td>
<td>0.125 to 0.9 × MIC; significantly reduced protein A secretion</td>
</tr>
<tr>
<td>Coagulase: Inhibits phagocytosis</td>
<td>Gemmell and Ford,\textsuperscript{21} 2002</td>
<td>S aureus strain expressing virulence factors</td>
<td>0.125 to 0.5 × MIC; significantly reduced coagulase activity</td>
</tr>
<tr>
<td></td>
<td>Gemmell and Ford,\textsuperscript{21} 2002</td>
<td>S aureus strain expressing virulence factors</td>
<td>0.125 to 0.5 × MIC; significantly reduced α- and β-hemolysin activity</td>
</tr>
<tr>
<td>α- and β-Hemolysins: Cause pore formation and host cell death</td>
<td>Bernardo et al,\textsuperscript{22} 2004</td>
<td>MSSA</td>
<td>0.125 to 0.9 × MIC; significantly reduced α- and β-hemolysin secretion</td>
</tr>
<tr>
<td>Autolysins: Promote release of proinflammatory peptides</td>
<td>Bernardo et al,\textsuperscript{22} 2004</td>
<td>MSSA</td>
<td>0.125 to 0.9 × MIC; significantly reduced autolysin family protein secretion</td>
</tr>
<tr>
<td>Enterotoxin A and B: Superantigens; induce T-cell activation</td>
<td>Bernardo et al,\textsuperscript{22} 2004</td>
<td>MSSA</td>
<td>0.125 to 0.9 × MIC; significantly reduced enterotoxin A and B secretion</td>
</tr>
</tbody>
</table>

CA-MRSA = community-associated methicillin-resistant *S aureus*; HA-MRSA = hospital-acquired methicillin-resistant *S aureus*; MIC = minimum inhibitory concentration; MSSA = methicillin-susceptible *S aureus*; PSM = phenol-soluble modulins; PVL = Panton-Valentine leukocidin; TSS = toxic shock syndrome; TSST-1 = toxic shock syndrome toxin-1.
different from untreated control cultures at any time. In addition, linezolid and clindamycin markedly suppressed PVL protein expression for at least 24 hours despite the presence of significant levels of PVL mRNA.

Toxic Shock Syndrome Toxin-1

One case report was found in which staphylococcal TSS in a 56-year-old man was initially and successfully treated using linezolid for 48 hours, then switched to clindamycin when bacterial susceptibilities were confirmed. The investigators cultured the patient’s *S aureus* isolate and tested the effects of various antibiotics on growth and toxic shock syndrome toxin-1 (TSST-1) production (Table I). When antibiotics were added to the culture at concentrations 5 times their MIC, bacterial growth was inhibited by nafcillin, clindamycin, or linezolid, but not vancomycin. Maximal production of TSST-1 occurred between 8 and 24 hours in untreated cultures and was not inhibited by either vancomycin or nafcillin. In contrast, both clindamycin and linezolid completely suppressed TSST-1 production. At a concentration of one-fourth its MIC, when bacterial numbers were still high, linezolid continued to significantly suppress TSST-1 production.

Phenol-Soluble Modulins

PSMs are secreted virulence factors that elicit proinflammatory immune responses and mediate neutrophil lysis. Joo et al assessed the effect on PSM production of a range of antibiotics at concentrations that caused only minimal effects on bacterial growth (Table I). These investigators tested one strain each of CA-MRSA and hospital-acquired MRSA (HA-MRSA). At subinhibitory concentrations in the CA-MRSA strain, oxacillin inhibited the productions of PSM, whereas subinhibitory concentrations of antibiotics that target protein synthesis (erythromycin, tetracycline, clindamycin, and linezolid) all increased PSM production. In the HA-MRSA strain, oxacillin had no effect on PSM production, and the PSM-inducing effects of erythromycin, tetracycline, clindamycin, and linezolid were less pronounced than in CA-MRSA. Because PSM expression is controlled by the accessory gene regulator (agr) locus (a quorum-sensing system that upregulates secretory protein expression and downregulates surface protein expression during the transition from log phase to stationary growth), the investigators also tested the activity of agr by examining expression of RNAIII and psm-operon transcripts. The effects of oxacillin, tetracycline, and clindamycin on agr activity mirrored their effects on PSM production, strongly suggesting the involvement of agr in those effects.

Other Staphylococcal Virulence Factors

Gemmell and Ford tested the effect of subinhibitory concentrations of linezolid on the production of a range of toxins in *S aureus* including α- and δ-hemolysins, which lyse rabbit and human red blood cells; coagulases, which clot rabbit plasma; and protein A, which affects susceptibility to opsonophagocytosis (Table I). At concentrations from one-eighth to one-half MIC, linezolid inhibited the activity of coagulase and α- and δ-hemolysins in *S aureus*. At one-half MIC, linezolid signifi-
cantly increased susceptibility to phagocytosis, suggesting decreased production of protein A.

Bernardo et al\textsuperscript{22} used protein assays to examine the effect of subinhibitory concentrations of linezolid on the secretion of proteins by MSSA (Table I). With 1- and 2-dimensional gel electrophoresis techniques, concentrations of linezolid from 12.5% to 90% of its MIC added during log-phase growth reduced the secretion of high-molecular-weight proteins. Spectroscopic techniques were applied to identify selected proteins that had been reduced. Among those were protein A, $\alpha$- and $\delta$-hemolysins, and autolysin family proteins, which promote release of proinflammatory peptides. Western blot analysis was used to show that staphylococcal enterotoxins A (SEA) and B, which cause gastritis and T-cell proliferation, were also reduced. To test the effects of linezolid on the proinflammatory activity of toxins secreted from MSSA, supernatants of bacterial cultures incubated with concentrations of linezolid from 12.5% to 90% MIC were added to murine splenic or peritoneal macrophages to examine the effect on production of tumor necrosis factor-$\alpha$ (TNF-$\alpha$), a proinflammatory mediator produced by activated macrophages. Culture supernatants of \textit{S aureus} treated with linezolid inhibited TNF-$\alpha$ production in macrophages, whereas linezolid alone (no \textit{S aureus} culture supernatant) did not affect TNF-$\alpha$ production by the macrophages. These data suggest that linezolid has no direct effect on macrophages but that its suppression of TNF-$\alpha$ production by macrophages was due to an indirect effect on bacterial production of toxins that can induce a proinflammatory response in these cells.

\section*{Streptococcal Toxins}

Eight articles were found that provided evidence of linezolid-mediated effects on toxins produced by \textit{S pyogenes}; these are summarized below. A brief summary of the toxins and the results from each study are also given in Table II.

In addition to their research on \textit{S aureus} toxins, Gemmell and Ford\textsuperscript{21} also tested the effects of subinhibitory concentrations of linezolid on the activity of toxins produced by \textit{S pyogenes}, including protein M, streptolysin O (SLO), and deoxyribonuclease (DNase) (Table II). As also used by these investigators in their analysis of \textit{S aureus} toxins, the assays used in this analysis were indirect and included cell lysis (SLO), DNA agar dissolution (DNase), or opsonophagocytosis (protein M). At concentrations from one eighth to one half its MIC, linezolid inhibited the activities of SLO and DNase and increased susceptibility to opsonophagocytosis.

Tanaka et al\textsuperscript{23} used 1- and 2-dimensional gel electrophoresis to analyze the effects of subinhibitory concentrations of a range of antibiotics that included benzylpenicillin, linezolid, and clindamycin on the production of exoproteins by \textit{S pyogenes} (Table II). In supernatants from late stationary-phase cultures, the exoprotein profile from benzylpenicillin-treated cultures was not remarkably different from untreated controls. In cultures treated with protein synthesis inhibitors including clindamycin, linezolid, kanamycin, tetracycline, chloramphenicol, and erythromycin, the exoprotein profile demonstrated reduced production of streptococcal pyrogenic exotoxin (Spe) B (reduced between one half to less than one eighth), and most of those agents
reduced Spe F as well (reduced by one half to one fourth except by erythromycin). However, levels of several exoproteins were increased by this class of antibiotics. For example, SLO was increased by 2-fold to more than 8-fold by all of those agents.

Table II. Effect of linezolid on *Streptococcus pyogenes* toxin synthesis.

<table>
<thead>
<tr>
<th>Toxin and Effect</th>
<th>Reference</th>
<th>S pyogenes Strain</th>
<th>Linezolid Concentration (MIC) and Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein M: Inhibits opsonophagocytosis</td>
<td>Gemmell and Ford, 2002</td>
<td><em>S pyogenes</em> strain expressing virulence factors</td>
<td>0.125 to 0.5 × MIC; significantly reduced protein M activity</td>
</tr>
<tr>
<td></td>
<td>Gemmell and Ford, 2002</td>
<td><em>S pyogenes</em> strain expressing virulence factors</td>
<td>0.125 to 0.5 × MIC; significantly reduced streptolysin O activity</td>
</tr>
<tr>
<td>Streptolysin O: Causes pore formation and host cell death</td>
<td>Tanaka et al, 2005</td>
<td><em>S pyogenes</em> M1 (clinical isolate)</td>
<td>MIC not defined; linezolid used at 0.25 μg/mL; increased streptolysin O protein secretion</td>
</tr>
<tr>
<td>DNase: Hydrolyzes DNA</td>
<td>Gemmell and Ford, 2002</td>
<td><em>S pyogenes</em> strain expressing virulence factors</td>
<td>0.125 to 0.5 × MIC; significantly reduced DNase activity</td>
</tr>
<tr>
<td></td>
<td>Tanaka et al, 2005</td>
<td><em>S pyogenes</em> M1 (clinical isolate)</td>
<td>MIC not defined; linezolid used at 0.25 μg/mL; reduced Spe B and F protein secretion</td>
</tr>
<tr>
<td>Spe A, B, and F: Stimulate immune system, cause fever</td>
<td>Coyle et al, 2003</td>
<td>Spe A–producing <em>S pyogenes</em></td>
<td>MIC not defined; linezolid used at 600 mg q12h; reduced Spe A secretion, either alone or in combination with penicillin or clindamycin</td>
</tr>
<tr>
<td>SIC: Inhibits innate immune system</td>
<td>Tanaka et al, 2005</td>
<td><em>S pyogenes</em> M1 (clinical isolate)</td>
<td>MIC not defined; linezolid used at 0.25 μg/mL; increased SIC protein secretion</td>
</tr>
<tr>
<td>Isp2: Immune system activator</td>
<td>Tanaka et al, 2005</td>
<td><em>S pyogenes</em> M1 (clinical isolate)</td>
<td>MIC not defined; linezolid used at 0.25 μg/mL; increased Isp2 protein secretion</td>
</tr>
</tbody>
</table>

DNase = deoxyribonuclease; Isp2 = immunogenic secreted protein 2; MIC = minimum inhibitory concentration; SIC = streptococcal inhibitor of complement-mediated lysis; Spe = streptococcal pyrogenic exotoxin.
except kanamycin, whereas streptococcal inhibitor of complement-mediated lysis, which inhibits the innate immune system, and immunogenic secreted protein 2, an immune system activator, were increased by all protein synthesis inhibitors (4-fold to more than 8-fold, and 2-fold to more than 8-fold, respectively). The induction of toxin production was unique to protein synthesis inhibitors; these effects were not observed with other classes of antibiotics including peptidoglycan synthesis inhibitors, DNA replication inhibitors, or RNA polymerase inhibitors. Clindamycin-induced increases in SLO and other genes were also accompanied by increases in the corresponding mRNAs, and were observed in 5 different strains of *S pyogenes* that cause toxic shock–like syndrome.

Coyle et al.24 used an in vitro pharmacodynamic model to compare the effects of penicillin (4,000,000 U q4h), clindamycin (900 mg q8h), and linezolid (600 mg q12h), alone or in combination, on the growth and production of Spe A in 2 *S pyogenes* isolates (Table II). In both strains, 99% killing by penicillin alone was reached within 4 to 6 hours; penicillin-containing combinations required 8 to 24 hours, and clindamycin and linezolid, alone or in combination, required 24 hours to achieve that end point. However, Spe A production was increased by 1 hour after treatment with penicillin alone, whereas it was decreased using all other regimens that contained clindamycin and/or linezolid.

**In Vitro Studies With Host Immune Cells**

Eight articles were found that examined the effects on linezolid on toxin-induced cytokine release, and these studies are summarized below.

The uptake and intracellular activity of linezolid has been tested in vitro in phagocytic cells (polymorphonuclear leukocytes [PMNs]) and, to a lesser extent, non-phagocytic cells. Pascual et al.25 demonstrated that, at concentrations up to 40 \( \mu g/mL \), linezolid was rapidly taken up by human PMNs and McCoy cells, and peak intracellular concentrations slightly exceeded those outside the cells. The uptake was unaffected by temperature or cell viability, which suggests that it occurred via a passive mechanism. Ballesta et al.26 also found that concentrations of linezolid as high as 20 \( \mu g/mL \) did not significantly affect phagocytosis by PMNs of strains of MSSA, MRSA, and *Enterococcus faecalis* (both vancomycin-resistant and vancomycin-susceptible strains). In addition, preincubation of PMNs with linezolid did not affect their production of superoxide or hydrogen peroxide radicals, which are integral to the bactericidal mechanism of PMNs. Naess et al.27 demonstrated that, at concentrations between 10 and 160 \( \mu g/mL \), linezolid did not significantly affect chemotaxis, phagocytosis, or respiratory burst of PMNs. Moreover, linezolid had no effect on TNF-\( \alpha \) production by peritoneal macrophages.22

Because TSST-1 causes overproduction of proinflammatory cytokines such as TNF-\( \alpha \), interferon-\( \gamma \) (IFN-\( \gamma \)), and interleukin (IL)--2,28 Kushiya et al.29 examined the effects of a range of antibiotics on the production of these cytokines by peripheral blood mononuclear cells (PBMC) stimulated with 10 ng/mL TSST-1. Of the antibiotics tested (all at 10 and 40 \( \mu g/mL \)), only some of the macrolides and related agents
suppressed TSST-1–induced cytokine production at either of the tested concentrations, and linezolid had no significant effect.

More recently, Pichereau et al.\(^{30}\) have tested the effects of a range of antibiotics including linezolid, clindamycin, azithromycin, vancomycin, trimethoprim–sulfamethoxazole, tigecycline, and daptomycin on cytokine production elicited by various staphylococcal toxins in PBMCs. At a concentration of 100 ng/mL, PVL, TSST-1, SEA, and α-hemolysin each induced, to varying extents, production of TNF-α, IL-1β, IL-6, IFN-γ, and IL-8. For example, PVL tended to be most effective in stimulating production of TNF-α and IL-8, whereas SEA and TSST-1 tended to be more effective at stimulating production of IL-6, IFN-γ, and IL-1β (differences between the amount of stimulation for each cytokine not statistically significant). When antibiotics were added at concentrations corresponding to their peak serum concentrations, linezolid (25 μg/mL) was overall the most effective antibiotic for reducing TNF-α and IL-8 elicited by PVL, TSST-1, SEA, and α-hemolysin (\(P < 0.05\) compared with no antibiotic). Trimethoprim–sulfamethoxazole (1 and 34 μg/mL) was most effective for reduction of IL-1β elicited by TSST-1, SEA, and α-hemolysin. Tigecycline (1 μg/mL) was most effective for reduction of IL-6 and IFN-γ by all 4 toxins. All other tested antibiotics had inconsistent and variable effects on toxin production at peak serum concentrations. At even higher concentrations (≥50 μg/mL), all antibiotics tended to reduce cytokine production. The concentration dependence of the effects of each antibiotic for reducing TNF-α and IFN-γ was also tested at concentrations between 5 and 100 μg/mL. Concentration-dependent suppression of cytokine release was most evident for trimethoprim–sulfamethoxazole, tigecycline, and clindamycin, and concentration dependence was also significant for linezolid and azithromycin; however, no such relationship was found for the other tested antibiotics.

Garcia-Roca et al.\(^{31}\) also tested the effects on PBMC of linezolid and erythromycin at concentrations between 1 and 30 μg/mL, although those researchers used LPS, a gram-negative bacterial endotoxin (100 ng/mL), to induce inflammatory cytokine release by these cells. At 10 and 30 μg/mL, the 2 drugs exhibited a modest reduction in LPS-induced PBMC release of proinflammatory cytokines including TNF-α, IL-1β, IL-1 receptor antagonist (IL-1ra), and IL-6.

Two studies have tested the effects of linezolid on LPS-stimulated whole blood from healthy volunteers. Takahashi et al.\(^{32}\) found that in blood samples stimulated with 10 μg/mL LPS, levels of TNF-α were reduced by linezolid added at concentrations between 2 and 15 μg/mL, whereas reductions in levels of IFN-γ were found at concentrations between 4 and 15 μg/mL. Levels of IL-10 and monocyte chemoattractant protein-1 were not affected by linezolid at any of those concentrations.\(^{32}\) Levels of LPS were unaffected by linezolid, thereby excluding the possibility that linezolid might have acted to reduce LPS availability. Lambers et al.\(^{33}\) tested the effects of linezolid (13 μg/mL) on both mRNA and protein levels for TNF-α, IL-6, and IL-8, and mRNA levels only for IL-1β, in whole blood stimulated with LPS. Those researchers argued that previous studies had used LPS levels that were far higher than what might be expected in patients with gram-negative sepsis, and,
therefore, used 50 pg/mL LPS in their study. Compared with baseline, levels of mRNA for all of these cytokines were increased up to several thousand-fold by LPS stimulation. Linezolid treatment significantly suppressed TNF-α, IL-6, IL-8, and IL-1β mRNA expression after 2 and 4 hours. However, there was little effect of linezolid treatment on supernatant cytokine protein levels compared with the LPS-only controls (reduction in IL-6 protein secretion at 2 hours but not at 4 hours).

In Vivo Studies in Preclinical Animal Models

Mouse Model of Pulmonary Infection

Six articles were found that examined the immunomodulatory effects on linezolid in animal models, and these are summarized below.

Yanagihara et al. compared the effects of linezolid, vancomycin, and teicoplanin in a murine model of hematogenous pulmonary infection with MRSA or vancomycin intermediate S. aureus (VISA; vancomycin MIC, 8–16 μg/mL). All antibiotics were delivered via intraperitoneal injection twice a day at a dose of 100 mg/kg body weight per day, beginning 24 hours after inoculation. Compared with treatment with either vancomycin or teicoplanin, treatment with linezolid significantly improved survival in VISA-infected mice. Lung tissues of mice infected with VISA treated with linezolid showed less histopathologic inflammatory damage than did those treated with vancomycin or teicoplanin. Bacterial numbers detected in lung homogenates were significantly reduced compared with vancomycin in the MRSA-infected mice.

In a separate study, Yanagihara et al. compared the effects of linezolid and vancomycin (100 mg/kg/d IP twice daily for each) on a PVL-positive MRSA strain in the same mouse pulmonary infection model. Again, compared with vancomycin, linezolid significantly reduced the number of viable bacteria and the amount of histopathologic inflammatory damage. Compared with control, levels of TNF-α, IL-1β, and macrophage inflammatory protein-2 were significantly reduced by each antibiotic. Levels of each cytokine were numerically lower in the linezolid group compared with the vancomycin group; however, the differences were not statistically significant.

In a more recent study, Akinnusi et al. compared the effects of linezolid (80 mg/kg IV q12h) and vancomycin (110 mg/kg IV q12h) on pulmonary innate immune system responses in a mouse model of MRSA infection. In that study, no significant differences were found between linezolid and vancomycin insofar as eradication of the pathogens. Furthermore, no differences were found in various measures of inflammation that included histologic damage, numbers of leukocytes in bronchoalveolar lavage (BAL) fluid, concentrations of cytokines (IL-6 and monocyte chemotactic protein-5), matrix metalloproteinase-9 and myeloperoxidase activity (inflammatory mediators) in BAL fluid, and the rate of apoptosis in neutrophils isolated from BAL fluid.

Piglet Model of Ventilator-Associated Pneumonia

Luna et al. compared the effects of linezolid (300 mg q8h), vancomycin (500 mg q6h), and teicoplanin (200 mg q12h for 3 doses, then once daily) in mechanically
ventilated piglets infected with MRSA. Treatment with linezolid resulted in significantly enhanced survival when compared with no treatment; however, no difference was noted when compared with treatment with vancomycin or teicoplanin. Compared with untreated controls, the percentage of animals with MRSA-negative blood cultures or lung fluids was significantly reduced in the linezolid group but not in the other treatment groups. Serum concentrations of C-reactive protein (a marker of systemic inflammation), TNF-α, and IL-6, and lung fluid levels of TNF-α and IL-6 were lower in treated compared with untreated animals; however, no differences between treatment groups were observed.

In a recent study, Martinez-Olondris et al. compared the effects of linezolid (15 mg/kg q12h) versus vancomycin provided either twice daily (10 mg/kg) or as a continuous infusion (1 g; initial bolus of 250 mg over 60 minutes) in mechanically ventilated piglets infected with PVL-negative MRSA. Compared with untreated controls after 96 hours, all treatments significantly reduced MRSA-positive cultures in BAL specimens, whereas only linezolid and twice-daily vancomycin significantly reduced MRSA-positive cultures from postmortem lung tissues. In histopathology samples, all treatments significantly reduced the severity and extent of inflammation; however, severe inflammation was significantly reduced only in linezolid-treated animals. No significant differences between groups were observed for serum concentrations of proinflammatory cytokines (TNF-α, IL-6, and IL-8). The ratio of concentrations between lung tissue and serum reflected significantly greater tissue penetration of linezolid when compared with either vancomycin treatment group.

**Rat Uterine Model of Intraperitoneal Adhesion**

Inflammation is often associated with abnormal healing and scar tissue formation. In one study, in vivo immunomodulatory effects of linezolid were assessed in the absence of an administered pathogen. Aytan et al. tested the ability of orally administered linezolid to prevent intraperitoneal adhesions induced by cauterizing injury in the uterine horn of the rat. Adhesions are commonly caused by injury such as surgical trauma, and their formation involves an influx of inflammatory cells and inhibition of fibrinolysis. In initial dose–response studies using doses between 5 and 150 mg/kg/d, a dose of 100 mg/kg/d was found to be the minimum effective dose to significantly reduce adhesion formation. Treatment was more effective when linezolid treatment was initiated 3 days before surgery and continued for 14 days after surgery, compared with either presurgical treatment alone or restriction of the postsurgical treatment period to 7 days. Although no pathogen was administered, the protective effect of linezolid in this model could be due to its antimicrobial property.

**Clinical Study in Humans**

One study was found that examined the short-term effect of linezolid treatment on levels of IL-1ra, IL-6, and transforming growth factor-β1 in inflammatory periapical lesions in patients in whom endodontic treatment had failed. In total, 22 patients
were assigned to receive either placebo (n = 11) or oral linezolid (n = 11) at 600 mg twice daily for 5 days before the operation to remove the tooth. Compared with placebo-treated controls, levels of IL-1ra, but not IL-6 and transforming growth factor-β1, in linezolid-treated patients were significantly reduced. It should be noted that in 50% of patients (5 of 10) treated with linezolid for 5 days before the operation to remove the tooth, no bacteria were grown from the periapical lesions, compared with 11% of patients (1 of 9) in the placebo control group, which indicated that linezolid treatment exerted an antimicrobial effect.

**DISCUSSION**

A growing number of in vitro studies support the theory that linezolid may modulate the production of bacterial toxins. For example, suppression of PVL production by linezolid has been reported by independent laboratories and has been observed at subinhibitory concentrations, thus providing strong support for this phenomenon.9–11 The evidence supporting linezolid-mediated suppression of protein A and of α- and δ-hemolysins has also been confirmed in independent studies.21,22 However, evidence suggesting effects of linezolid on production of other toxins produced by either *S. aureus* or *S. pyogenes* is less conclusive. For example, for *S. aureus*, only single studies have provided evidence of linezolid-mediated suppression of TSST-1,12 coagulase,21 autolysins,22 streptococcal enterotoxins A and B,22 or linezolid-mediated increases in PSM production.19 The study that reported PSM induction by linezolid also observed a similar effect with clindamycin, which seems to contradict a previous study that reported little or no effect of subinhibitory concentrations of clindamycin on agr activity in an *S. aureus* strain.42 For *S. pyogenes*, only single studies have reported linezolid-mediated suppression of protein M,21 DNase,21 and Spe A, B, and F,23,24 or linezolid-mediated increases in streptococcal inhibitor of complement-mediated lysis and immunogenic secreted protein 2.23 Although one study suggested that SLO protein secretion was increased by linezolid,23 another study suggested that its activity was actually decreased.21 It is not clear whether this indicates that linezolid induces secretion of inactive SLO protein or whether there might have been different effects on the bacterial strains used in each study. Further studies will be needed to confirm some of these observations and to resolve the seemingly contradictory findings in other studies. Therefore, no firm conclusions can be drawn until those issues are adequately addressed.

Studies of linezolid effects on immune function suggest that linezolid is unlikely to have any effects on phagocytes. Contradictory results have been reported insofar as the effect of linezolid on cytokine release elicited by TSST-1.29,30 The differences between these studies may be attributable to the differences in the tested concentrations of this toxin (10 vs 100 ng/mL). To date, only a single study has provided evidence for linezolid-mediated suppression of cytokine release induced by PVL, SEA, and α-hemolysin.30 Three studies have provided evidence for linezolid-mediated suppression of LPS-induced cytokine expression by immune cells,31–33 although the magnitude of the reported effects was substantially different be-
tween studies. It is not clear to what extent the differences between these various studies are related to the difference between the nature and/or concentrations of agents used to induce cytokine release and/or other methodologic differences. Moreover, it is difficult to interpret the relevance of linezolid effects on cytokine production induced by LPS, a gram-negative bacterial toxin.

In vivo data from preclinical animal studies and a single clinical study in humans are limited and equivocal insofar as a potential role for linezolid in modulating the host inflammatory response. Two each of the studies in mice and in piglets were designed to test the antibacterial efficacy of linezolid; thus, it is difficult to differentiate possible direct immunomodulatory effects from those that might have occurred indirectly as a result of efficacious antimicrobial treatment. Moreover, the only study in mice that focused more carefully on immunomodulatory activity also reported comparable efficacy of linezolid and vancomycin, which directly conflicts with the previous studies. It is not clear whether that difference was due to differences in the bacterial strain, drug dosages, or other details of the experiments. The study of linezolid-mediated inhibition of adhesion formation in the rat had been designed to test effects of linezolid unrelated to infection; thus, it did not include an inoculum. However, the possibility that the efficacy of linezolid in that model might be related to control of postsurgical infection was not directly addressed. It is noteworthy that the minimum effective dose was similar to that used for antimicrobial therapy in the murine pulmonary infection model. Future studies of linezolid-mediated immunomodulation should test the effects of this antibiotic either at subinhibitory dosages in an animal infection model or in a noninfective animal model of inflammation. In either case, inclusion of an unrelated antimicrobial agent as a control would also aid in interpretation of the results.

The precise mechanism(s) underlying the clinical efficacy of any antimicrobial agent is an important factor in directing appropriate treatment decisions. Nevertheless, given these limitations in preclinical studies, it is not surprising that evidence for immunomodulatory effects in clinical studies are almost nonexistent. Only one small clinical study has provided equivocal evidence that linezolid has the potential to affect cytokine levels within infected tissues. However, linezolid also reduced the numbers of active pathogens in those patients, again raising the possibility that the immunomodulatory effects resulted from efficacious antimicrobial treatment rather than any direct effect of linezolid on immune function. It seems likely that more compelling evidence from preclinical studies may be needed to design clinical studies that are appropriate to address the possible benefits directly associated with linezolid effects on bacterial toxin production and/or immunomodulation.

CONCLUSION

A growing body of evidence supports the possibility that linezolid affects (increases or decreases) toxin production in clinically relevant pathogens such as S. aureus and streptococci; however, the results of these experiments seem to depend on the bacterial strain used, the replication stage of the bacteria (early vs
stationary phase), and the animal species and the disease model used in the in vivo experiments. Data suggest that linezolid is passively taken up by PMNs and even at high concentrations does not inhibit PMN function (phagocytosis, chemotaxis, or respiratory burst). Data on the direct effect of linezolid on host immune responses to microbial antigens, including gram-negative LPS, are limited and equivocal. The only study in humans that examined the immunomodulatory effect of linezolid was in patients with periradicular lesions of the tooth, and the observed effect of linezolid could have been due in part to inhibition of bacterial replication. More research will be needed to confirm and extend these studies. Research will also be needed to explore the potential mechanisms, extent of secondary immunomodulatory effects, and potential clinical relevance of those findings for linezolid.

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CONFLICT OF INTEREST
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