Doxycycline Is Anti-Inflammatory and Inhibits Staphylococcal Exotoxin-Induced Cytokines and Chemokines

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Proinflammatory cytokines mediate the toxic effect of superantigenic staphylococcal exotoxins (SE). Doxycycline inhibited SE-stimulated T-cell proliferation and production of cytokines and chemokines by human peripheral blood mononuclear cells. These results suggest that the antibiotic doxycycline has anti-inflammatory effects and is therapeutically useful for mitigating the pathogenic effects of SE.

Staphylococcal toxic shock syndrome toxin 1 (TSST-1) and the structurally related exotoxins are bacterial exotoxins that bind directly to major histocompatibility complex class II molecules on antigen-presenting cells (1, 5, 8, 18, 23) and activate T cells expressing specific Vβ elements (7). These toxins are called superantigens because of their ability to polyclonally stimulate large populations of T cells (1, 4, 7, 14). Thus, staphylococcal exotoxins (SE) are potent activators of the immune system and cause a variety of diseases in humans, including food poisoning, toxic shock, and autoimmune diseases (1, 2, 6, 12, 14, 22). Their interactions with cells of the immune system result in massive production of proinflammatory cytokines and chemokines (1, 4, 15, 17). The cytokines tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), and gamma interferon (IFN-γ) are key mediators in superantigen-induced toxic shock (1, 21). Both TNF-α and IL-1 have potent immunostimulating activities and act synergistically with IFN-γ to enhance immune reactions and promote tissue injury (16). Consequently, these cytokines are pathogenic at high concentrations in vivo and are responsible for fever and toxic shock induced by SE (13, 14, 18, 19).

Doxycycline is a broad-spectrum antibiotic widely used for infections caused by both gram-negative and gram-positive microorganisms. It acts as a bacteriostatic agent and is highly effective against many microorganisms, including Staphylococcus aureus, Streptococcus pyogenes, Bacillus anthracis, and Yersinia pestis. Doxycycline belongs to the tetracycline antibiotic family, the members of which have been shown to have other immune effects and are therapeutically useful for mitigating the pathogenic effects of SE. Doxycycline is anti-inflammatory and inhibits staphylococcal exotoxin-induced cytokines and chemokines by human peripheral blood mononuclear cells. These results suggest that the antibiotic doxycycline has anti-inflammatory effects and is therapeutically useful for mitigating the pathogenic effects of SE.

Purified SEB and TSST-1 were obtained from Toxin Technology (Sarasota, Fla.). The endotoxin content of these preparations was <1 ng of endotoxin/mg of protein, as determined by the Limulus amoeboocyte lysate assay (BioWhittaker, Walkersville, Md.). Human recombinant TNF-α (hTNF-α), antibodies against hTNF-α, peroxidasie-conjugated anti-rabbit immunoglobulin G, and peroxidase-conjugated anti-goat immunoglobulin G were obtained from Boehhringer Mannheim (Indianapolis, Ind.). Recombinant monocyte chemotactic protein 1 (MCP-1), macrophage inflammatory protein 1α (MIP-1α), MIP-1β, and antibodies against hIL-1β, hIL-6, hMIP-1α, and MIP-1β were purchased from R&D Systems (Minneapolis, Minn.). Human rIL-1β was kindly provided by J. Oppenheim (National Cancer Institute, Frederick, Md.). Human recombinant IFN-γ (rIFN-γ) and rIL-6 were obtained from Collaborative Research (Boston, Mass.). Antibodies against hIL-1β and MCP-1 were obtained from Pharmingen (San Diego, Calif.). Doxycycline was purchased from Sigma (St. Louis, Mo.) and dissolved in phosphate-buffered saline, pH 7.4. All other reagents were also from Sigma.

Human PBMC were isolated by Ficoll-Hypaque density gradient centrifugation of heparinized blood from normal human donors. PBMC (10⁶/ml) were cultured at 37°C in 24-well plates containing RPMI 1640 medium and 10% heat-inactivated fetal bovine serum. Cells were incubated with either SEB (200 ng/ml) or TSST-1 (200 ng/ml) for 16 h, and the supernatants were harvested and analyzed for IL-1β, TNF-α, IL-6, IFN-γ, MCP-1, MIP-1α, and MIP-1β. Cytokines and chemokines were measured by an enzyme-linked immunosorbent assay with cytokine- or chemokine-specific antibodies in accordance with the manufacturer’s instructions (15, 17). Human recombinant cytokines and chemokines (20 to 1,000 pg/ml) were used as standards for calibration on each plate. The detection limit of each assay was 20 pg/ml. The cytokine and chemokine data were expressed as the mean reading ± the standard deviation (SD) of duplicate samples. Doxycycline, when present, was added simultaneously with the stimulating agent. Cytotoxicity

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Proinflammatory cytokines mediate the toxic effect of superantigenic staphylococcal exotoxins (SE). Doxycycline inhibited SE-stimulated T-cell proliferation and production of cytokines and chemokines by human peripheral blood mononuclear cells. These results suggest that the antibiotic doxycycline has anti-inflammatory effects and is therapeutically useful for mitigating the pathogenic effects of SE.
amount of releasable LDH (100%) was obtained by lysing cells with 1% Triton X-100. T-cell proliferation was assayed with PBMC (10^5/well), which were plated in triplicate with SEB or TSST-1 (200 ng/ml), with or without doxycycline, for 48 h at 37°C in 96-well microtiter plates. Cells were harvested onto glass fiber filters, and incorporated \(^{3}H\)thymidine was measured by liquid scintillation. All data were analyzed for significant differences by Student’s t test with Stata (Stata Corp., College Station, Tex.). Differences between doxycycline-treated and untreated control groups were considered significant if \(P < 0.05\).

On the basis of the report that doxycycline blocked lipopolysaccharide-induced IL-1 in epithelial cells and prevented lethal endotoxemia in vivo (20, 25), we tested the hypothesis that this antibiotic might have direct effects on SE-induced cytokines. As shown in Fig. 1, doxycycline dose dependently inhibited the production of the cytokines IL-1β, IL-6, TNF-α, and IFN-γ and the chemokines MCP-1, MIP-1α, and MIP-1β by PBMC incubated with SEB. Similar dose-dependent reduction of cytokines and chemokines by doxycycline was also observed for TSST-1-stimulated PBMC (data not shown). The inhibitory effect of doxycycline on SEB- or TSST-1-mediated cytokines and chemokines obtained with PBMC from seven normal donors is summarized in Fig. 2. Production of MCP-1 and IFN-γ was completely blocked by 50 μM doxycycline. This concen-

![Figure 1](image1.png)

**FIG. 1.** Dose-response inhibition of IL-1β and IL-6 (A), TNF-α and IFN-γ (B), and MCP-1, MIP-1α, and MIP-1β (C) production by PBMC stimulated with 200 ng of SEB per ml in the presence of various concentrations of doxycycline. Values represent the mean ± SD of duplicate samples from three experiments.

was measured by the release of lactate dehydrogenase (LDH) from the cytosol into culture supernatant. LDH was quantitated by using a colorimetric cytotoxicity assay kit (Boehringer Mannheim) as instructed by the manufacturer. The maximum
tation of doxycycline reduced IL-1β, IL-6, TNF-α, MIP-1α, and MIP-1β to 15 to 22%, 37 to 41%, 21 to 25%, 10 to 15%, and 59 to 61% of that of untreated, SEB- or TSST-stimulated cells, respectively. TNF-β, when present, was also inhibited to 25% of that of untreated, SEB-stimulated cells. Doxycycline was not cytotoxic to PBMC at this concentration as measured by the exclusion of trypan blue and the lack of lactate dehydrogenase release from treated cells. Complete inhibition of these cytokines and chemokines was observed at high doses of doxycycline (>0.1 mM). Similar dose-response inhibition by doxycycline was observed at lower concentrations of SEB (1 and 10 ng/ml) (data not shown).

Because superantigens also cause T-cell proliferation, the effect of doxycycline on SE-induced T-cell proliferation was investigated. Figure 3 shows that doxycycline inhibited SEB- and TSST-1-stimulated T-cell proliferation in a dose-dependent manner, achieving 98% inhibition at 0.05 mM.

This study demonstrated that doxycycline effectively inhibited superantigen-mediated production of cytokines and chemokines by human PBMC in vitro. T-cell proliferation induced by staphylococcal superantigens was also suppressed completely. Downregulation of proinflammatory cytokines and chemokines by doxycycline in SEB- and TSST-1-stimulated PBMC suggested that doxycycline may affect the pathophysiology of toxic shock. These findings extend the observations of other investigators of the immunomodulatory effects of doxycycline in addition to its antimicrobial activities.

Multiple molecular mechanisms, both transcriptional and posttranscriptional, may be involved in the anti-inflammatory effects of doxycycline (11, 26). The suppression of proinflammatory cytokines may involve the downregulation of the PKC pathway by doxycycline, as suggested by a study of its effects on granuloma formation (26). The reported inhibitory dose of doxycycline (10 to 15 μM) that reduces collagenase, gelatinase, and other metalloproteinases in vitro (10, 11) is comparable to that used in this study and is severalfold higher than that observed in human serum after oral dosing of 200 mg daily (10, 24). However, clinical studies indicate that this dose was sufficient in reducing the collagenase and gelatinase activities in human osteoarthritic cartilage extracts ex vivo (24). A subantimicrobial dose of doxycycline (20 mg twice daily) has been shown to inhibit gingival fluid collagenase activity (9). In addition, in vivo studies of experimental endotoxemia also found doxycycline and other tetracyclines efficacious in downregulating inflammatory cytokines and preventing shock (20).

In conclusion, the results presented here indicate that doxycycline down-regulates proinflammatory cytokines and chemokines, thus suggesting its potential utility for treating superantigen-induced toxic shock. In a clinical setting when the host is exposed to multiple biological agents, including both bacteria and bacterial exotoxins, the use of doxycycline offers an additional advantage of providing both antimicrobial and anti-inflammatory effects.

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REFERENCES