Treatmet of Acute Necrotizing Fasciitis Using Negative Pressure Wound Therapy and Adjunctive NeutroPhase Irrigation Under the Foam

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Abstract: Necrotizing fasciitis is a complication of a bacterial infection that activates the immune system in perifascial planes. This case report highlights initial diagnostic failures that delay early treatment, which causes profoundly negative consequences. Antimicrobial control with abolition of the inciting bacteria does not neutralize the subsequent endopathologic ravages. A new therapeutic technique, which combines negative pressure wound therapy (NPWT) and a pure hypochlorous acid solution 0.01% (NeutroPhase, NovaBay Pharmaceuticals Inc, Emeryville, CA) along with debridement and antibiotics is described in this study. It is believed that the combination of neutralization of the toxins produced by bacteria with NeutroPhase along with the NPWT action of removing exudates is effective in saving the patient.

Key words: negative pressure, necrotizing fasciitis, hypochlorous acid, debridement, tissue repair

Necrotizing fasciitis, commonly known as flesh-eating disease, is an infection of the deeper layers of skin and subcutaneous tissues which easily spreads across the fascial planes under the subcutaneous tissue. Necrotizing fasciitis is a quickly progressing and severe disease of sudden onset. It is best treated immediately with high doses of intravenous antibiotics. Type I necrotizing fasciitis is classified as a polymicrobial infection, whereas type II is classified as a monomicrobial infection. Many types of bacteria can cause necrotizing fasciitis (eg, Group A Streptococcus [GAS], Streptococcus pyogenes, Group B Streptococcus [GBS], Streptococcus agalactiae, Staphylococcus aureus, Vibrio vulnificus, Clostridium perfringens, and Bacteroides fragilis). Such infections are more likely to occur in people with compromised immune systems.1 Historically, GAS made up most cases of type II infections. However, since as early as 2001, another serious form of monomicrobial necrotizing fasciitis has been observed with increasing frequency.1 In these cases, the bacterium causing it is methicillin-resistant Staphylococcus aureus.
ylococcus aureus (MRSA). Group B *Streptococcus* has recently emerged as a causative agent of necrotizing fasciitis.³

Due to its subtle symptoms, the early diagnosis of necrotizing fasciitis can be difficult, unless triggered by toxic shock syndrome or organ failure, which present with acute symptoms. When the onset occurs, early diagnosis is what is needed to minimize the morbidity and mortality rate of 10%-70%, which occurs even in younger patients. The toxicity caused by GAS or Staphylococcal superantigen release results in nonspecific T-cell activation and massive cytokine release, and can dramatically change the patient’s situation from acute to critical, requiring surgical incision and drainage.

While the virulence factors that enable GBS to cause necrotizing fasciitis have not yet been established, horizontal transfer of DNA encoding virulence factors is common among different strains of GAS and a similar process may have occurred between Group A and Group B streptococci, conferring the virulent strain of GBS.³

Pure hypochlorous acid (HOCl) is a naturally occurring, well-known, broad-spectrum, fast-acting antimicrobial agent produced as part of the innate immune system’s response to infection during oxidative burst by neutrophils and monocytes.⁴ Pure HOCl has been shown to have activity against both gram-positive and gram-negative bacteria without toxicity to human cells, and to control the tissue bioburden in a rodent model of infected granulating wound without inhibiting the wound healing process.⁵,⁶ The active species in all hypochlorite solutions is undisassociated HOCl. This is because pure HOCl, unlike diluted bleach/Dakin solution, is an uncharged molecule and can penetrate microbial cell walls and spores with ease. NeuroPhase (NovaBay Pharmaceuticals Inc, Emeryville, CA) is pure 0.01% hypochlorous acid (ie, > 97% relative molar distribution of active chlorine species as HOCl) in a 0.9% saline solution at pH 4-5. It is believed that neutralization of the superantigens occurs with frequent irrigation of pure HOCl under the negative pressure wound therapy (NPWT) foam and removal of exudates, helping in the management of necrotizing fasciitis.

**Keypoints**

- Many types of bacteria can cause necrotizing fasciitis (eg, Group A *Streptococcus* [GAS], *Streptococcus pyogenes*, Group B *Streptococcus* [GBS], *Streptococcus agalactiae*, *Staphylococcus aureus*, *Vibrio vulnificus*, *Clostridium perfringens*, *Bacteroides fragilis*).
- Due to its subtle symptoms, the early diagnosis of necrotizing fasciitis can be difficult, unless triggered by toxic shock syndrome or organ failure, which present with acute symptoms.

**Materials and Methods**

A combination of pure hypochlorous acid solution 0.01% as the irrigation solution and NPWT was used to treat a patient with acute necrotizing fasciitis. A line diagram of the equipment is shown on Figure 1. Before treatment, the wound area was cleansed, the wound debrided, and the skin dried. A foam dressing (V.A.C. GranuFoam, KCI, San Antonio, TX) was sized and placed in the wound. A separate inflow tube (ie, an intravenous extension with a port) was placed on and through the foam. The adhesive drape was attached and placed over the entire area, including the foam. The area around the tubing was sealed with a skin barrier ointment (Stomadhesive Paste, ConvaTec, Skillman, NJ). The NPWT device was then turned on and adjusted...
from 50-125 mm Hg suction. The irrigation solution (5 mL) was instilled via syringe through the inlet-port into the wound bed with the vacuum turned on (Figure 2).

Activity of the irrigation solution against alpha-hemolysin toxin of *S. aureus* was tested by cytotoxicity assay. Human lung epithelial cells (A549, ATCC CCL 185) were grown in F12K cell culture medium supplemented with 10% fetal bovine serum (Life/Invitrogen, Carlsbad, CA) and 100 IU/mL penicillin/100 μg/mL streptomycin (Mediatech Inc, a Corning subsidiary, Manassas, VA). Assays were performed using F12K medium with different concentrations of purified toxin by measuring the reduction of MTS tetrazolium compound into formazan that is soluble in cell culture medium. On the day before the assay, 5000 cells/well were seeded in a flat-bottom 96-well plate. One ug/mL alpha-hemolysin toxin (Sigma Aldrich, St. Louis, MO) from *S. aureus* was incubated with a series of irrigation solution dilutions for 1 hour. Excess irrigation solution was inactivated by adding an equal volume of 20 mM methionine for 1 hour at room temperature and added to the cells for 16 hours at 37°C with 5% CO2. At the end of the experiment, cell viability was determined with an assay (CellTiter 96 AQueous One Solution Cell Proliferation Assay [MTS], Promega, Madison, WI).

Inactivation of streptokinase was determined by clot formation enzymatic assay (Sigma Aldrich, St. Louis, MO), whereby thrombin converts the soluble fibrinogen into soluble fibrin. In the presence of streptokinase enzyme from *S. pyogenes*, the insoluble fibrin is converted into soluble fibrin fragments. Several hours before the study, streptokinase (175 U/mL) was incubated with the irrigation solution for 1 hour, followed by inactivation of excess irrigation solu-

**Figure 2. Method of treatment.**

A. A standard silicone IV extension tube, fitted at one end with a standard valve, is inserted into a hydrophobic black foam cut to fit the wound.

B. Before treatment, the wound area is cleansed and the wound debrided, then the skin is dried. The black foam with the tube is placed into the wound and secured with transparent, thin film adhesive dressing.

C. The wound is covered with a clean, transparent, thin film adhesive dressing. The area around the tubing is sealed with a skin barrier ointment.

D. A hole is made in the transparent dressing to create access to the sponge.

E. A track pad is secured with the transparent dressing and the suction port is connected with a tube leading to the vacuum pump for NPWT. The entire system is placed under a mild vacuum (50 mm to 125 mm Hg PSI) to check for any air leaks.

F. Irrigation solution (5 mL) is instilled via syringe through the inlet-port into the wound bed with the vacuum on.
tion by adding an equal volume of 20 mM methionine for 1 hour at room temperature. In a separate glass tube, fibrinogen solution containing borate buffer, gelatin diluent, and plasminogen were mixed by swirling, and then equilibrated at 37°C for 3 minutes. Irrigation solution and methionine-treated streptokinase was added to the solution, mixed by swirling, and equilibrated at 37°C for 1 minute prior to the addition of thrombin. The solution was mixed by swirling and equilibrated at 37°C for approximately 2-3 minutes to allow for clot formation. A 4 mm glass bead was added to the top of the reaction mixture, and the time for the glass bead to touch the bottom of the tube was observed.

Results

A healthy female, 51 years of age with no comorbidities, presented to the authors’ emergency room with a history of a minor abrasion on her left elbow from a fall in a parking lot 3 days earlier. She complained of fever and her left arm had swelling and tenderness. She went into sudden shock with an unobtainable blood pressure. The patient was immediately transferred to the coronary care unit (CCU) for resuscitation with norepinephrine bitartrate acutely for shock. Her arm swelling was mild but progressed in the next 2-3 days along with a white blood cell elevation to 12 000 and a d-dimer of 2206 with mild renal failure and anemia. She responded well initially after restoration of a normal blood pressure. The diagnosis of cellulitis was made from culturing Group B Streptococcus with a positive gram stain on a single culture from a left elbow aspiration. No further cultures or gram stain smears were positive throughout the entire admission. The patient showed enough improvement in the CCU that continued care was ordered and a surgical consultation deemed unnecessary. Six days postadmission, the patient experienced sudden skin blistering, swelling, and pain in her left arm with a white blood cell count rising to 16 000. A surgical consultation 8 days postadmission resulted in an emergency subfascial incision and drainage, performed with a longitudinal incision in the left upper arm. On postoperative day 1, an instill type NPWT was placed on the upper arm wound using black polyurethane foam. Deep irrigation using HOCI was made by instilling 10 mL every 4 hours while leaving the vacuum on. Six days later, another area in the forearm became symptomatic and required an emergency forearm incision and drainage after which a dual instill NPWT port was installed, adding a line that extended blindly into the involved area around the elbow. This was allowed to drain into the forearm NPWT foam without direct surgical exposure and drainage. Here the untouched subcutaneous area was irrigated only with HOCI every 4 hours. The patient’s condition slowly improved. All cultures of the wound, nares, blood, and urine were negative for acid-fast bacillus, fungus, and bacteria. The surgical wounds performed in the forearm and upper arm were treated with dual instill catheters with a single NPWT device while injecting with separate sites for subcutaneous irrigation using an intravenous extension port into the undermined area by the elbow. This area had not been exposed for direct drainage but just irrigated with HOCI and again all recultures were negative (Figure 3). The patient’s clinical condition continued to improve and she was discharged home, where her spouse continued using the same technique of instillation, 3 times every 24 hours, with a home NPWT device. The NPWT was changed every 2-3 days in the authors’ Wound Care Clinic. Upon discharge, the patient’s antibiotics were changed from intravenous vancomycin, clindamycin, and ceftazidime through her peripherally inserted central catheter line to oral ceftazidime. After complete healing at home, the patient developed clinical Clostridium difficile colitis, which responded to oral vancomycin and metronidazole.

A cytotoxicity assay was performed using human lung epithelial cells (A549) to evaluate the effectiveness of the irrigation solution to inactivate bacterial toxins. Serial dilutions of the irrigation solution were incubated with 1 ug/mL alpha-hemolysin for 1 hour. Excess irrigation solution was neutralized with 20 mM methionine. The impact of the irrigation solution on alpha-hemolysin was evaluated by measuring the reduction of MTS tetrazolium compound. It was determined that 0.1 ug/mL irrigation solution (1000 dilution) completely inactivated alpha-hemolysin toxin.

Inactivation of streptokinase by the irrigation solution was determined using an enzymatic assay. Serial dilutions of the irrigation solution were incubated with 175 U/mL of streptokinase for 1 hour. Excess irrigation solution was neutralized with 20 mM methionine for 1 hour. In this clot assay, 0.001 ug/mL of the irrigation solution (100 000 dilution) completely inactivated streptokinase, causing the fibrin to remain insoluble in solution, and the glass bead to remain at the top of the reaction mixture.

Discussion

S. aureus and S. pyogenes express several types of superantigens that corrupt the normal humoral immune response, resulting in immunosuppression. 

Examples of staphylococcal superantigens include toxic shock syndrome toxin (TSST-1) and enterotoxins, of which there are 6 antigenic types (SEA, B, C, D, E, and G). Toxic shock syndrome toxin is a pyrogenic superantigen known to cause profound disturbances in the homeostasis of the immune system.
including massive proliferation of T-cells and uncontrolled release of proinflammatory cytokines. Toxic shock syndrome toxin, as well as other superantigens, binds nonspecifically to major histocompatibility complex class II in the antigen-presenting cells and T-cell receptors bearing specific Vβ elements on the receptors. The structural stability of TSST-1 is critical in maintaining its function as a superantigen. Group A *Streptococcus* also produces a number of superantigens and hemolytic exotoxins including streptolysins O and S. The virulence factors that enable GBS to cause necrotizing fasciitis have not yet been identified, but transfer of the genes encoding virulence factors from GAS is implicated.

Superantigens are highly resistant to acid, heat, and protease digestion. Therefore, once secreted, they can cause pathogenic response even after all the bacteria have been killed. This pathogenic response can result in amputations or in toxic shock and death. Inactivation of superantigens by the chosen irrigation solution would result in attenuating the virulence of the pathogen and mitigation of the course of infection.

Hypochlorous acid has beneficial effects in addition to its antimicrobial activity resulting in disruption of biofilms, penetration of microbial cells, spore walls and amoeba cysts, and promoting wound repair with tissue regeneration. This case illustrates and justifies the use of HOCl in necrotizing fasciitis, creating healable fresh wounds. The mechanism of action of HOCl and N-chloramines against pathogens is by the rapid oxidative modification of extracellular and intracellular components such as thiols, aromatic groups, and amines resulting in bacterial dysfunction and death. Iron sulfur proteins are inactivated extremely rapidly, followed in decreasing order by beta-carotene, nucleotides, porphyrins, and heme proteins. Enzymes containing essential cysteines are inactivated with an effectiveness that roughly parallels the
nucleophilic reactivities of their sulphydryl groups. It was shown in this work that the pure hypochlorous acid solution 0.01% used as an irrigation solution rapidly inactivates S. aureus and S. pyogenes toxins even when diluted 1000-100 000 fold. Reaction with amino compounds produces chloramines, which retain the oxidizing ability of HOCl, and their analogs were shown to inactivate virulence factors (ie, secretory aspartyl proteinases of C. albicans and C. dubliniensis as well as gliotoxin of A. fumigatus). Notably, the elbow area that was not surgically debridged, and treated with the irrigation solution alone, healed rapidly and without scarring. Presumably, the neutralization of the toxins and superantigens paralleled or exceeded the results obtainable by incision and drainage therapy (Figure 3).

Conclusions
Necrotizing fasciitis is a serious infection of the deeper layers of skin, subcutaneous tissue, and fascia. The toxicity caused by Group B Streptococcus superantigen release changed the patient’s situation from acute to critical, requiring surgical incision and drainage. It is evident that the combination of pure hypochlorous acid solution 0.01% as an irrigation solution and NPWT played an important role in the recovery of this patient by rapidly killing bacteria and presumably inactivating toxins and superantigens. The authors have also demonstrated that the irrigation solution inactivates bacterial toxins in vitro.

References