A Novel Surfactant Nanoemulsion with Broad-Spectrum Sporicidal Activity against *Bacillus* Species

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Two nontoxic, antimicrobial nanoemulsions, BCTP and BCTP 401, have been developed. These emulsions are composed of detergents and oils in 80% water. BCTP diluted up to 1 : 1000 inactivated >90% of *Bacillus anthracis* spores in 4 h and was also sporicidal against three other *Bacillus* species. This sporicidal activity is due to disruption of the spore coat after initiation of germination without complete outgrowth. BCTP 401 diluted 1 : 1000 had greater activity than BCTP against *Bacillus* spores and had an onset of action of <30 min. Mixing BCTP or BCTP 401 with *Bacillus cereus* prior to subcutaneous injection in mice reduced the resulting skin lesion by 99%. Wound irrigation with BCTP 1 h after spore inoculation yielded a 98% reduction in skin lesion size, and mortality was reduced 3-fold. These nanoemulsion formulas are stable, easily dispersed, nonirritant, and nontoxic compared with other available sporidical agents.

Bacteria of the *Bacillus* genus form stable spores that are resistant to harsh conditions and extreme temperatures. Contamination of farmlands with *Bacillus anthracis* leads to a fatal disease in domestic, agricultural, and wild animals [1]. Human infection by *B. anthracis* usually results from contact with infected animals or infected animal products [2]. Human clinical symptoms include a pulmonary form that has a rapid onset and is frequently fatal. The gastrointestinal and cutaneous forms of anthrax, although less rapid, can also result in fatalities unless treated aggressively [3, 4]. *B. anthracis* infection in humans is no longer common, because of effective animal control that includes vaccines, antibiotics, and appropriate disposal of infected livestock. However, animal anthrax still represents a significant problem because of contamination of farmland. Although a vaccine is available [5] and can be used for the prevention of anthrax, genetic mixing of different strains can render it ineffective [6]. The potential consequences of the use of *B. anthracis* spores as a biologic weapon were demonstrated by the accidental release of *B. anthracis* from a military microbiology laboratory in the former Soviet Union. Seventy-seven cases of human anthrax, including 66 deaths, were attributed to the accident. Some infections occurred as far as 4 km from the laboratory [7]. Genetic analysis of infected persons revealed the presence of either multiple strains or genetically altered *B. anthracis* [8].

Other members of the *Bacillus* genus are also reported to be etiologic agents for many human diseases. *B. cereus* is a common pathogen. It is involved in foodborne diseases because its spores can survive cooking procedures. Local sepsis and wound and systemic infections have also been attributed to *B. cereus* [9].

Disinfectants and biocides (e.g., sodium hypochlorite, formaldehyde, and phenols) that are highly effective against *Bacillus* spores are not well suited for decontamination of the environment, equipment, or exposed persons because of toxicity that leads to tissue necrosis and severe pulmonary injury after inhalation of volatile fumes. The corrosive nature of these compounds also renders them unsuitable for decontamination of sensitive equipment [10–15].

Concerns about these issues have stimulated interest in new types of biocidal agents that can safely decontaminate *Bacillus* spores. We have investigated the sporidical properties of two antimicrobial lipid emulsions. Nanoemulsions are produced by mixing a lipid-oil “discontinuous” phase with an aqueous “continuous” phase under high shear forces. The result is an oil droplet of ~400–800 μm in diameter that is able to fuse with...
Figure 1. Sporicidal activity of BCTP against 4 different *Bacillus* species compared with that of BCTP 401 against 2 *Bacillus* species. BCTP showed significant sporicidal activity after 4 h of treatment against *Bacillus cereus*, *B. circulans*, and *B. megaterium* spores but not against *B. subtilis* spores. BCTP 401 showed more effective killing against *B. cereus* in 4 h and also had sporicidal activity against *B. subtilis* that was resistant to BCTP. Bleach diluted 1:100 was used as positive control and was comparable to BCTP or BCTP 401 at same dilutions.

and subsequently disrupt the membrane of a variety of different pathogens [16]. BCTP is a nanoemulsion made of soybean oil, Triton X-100 detergent, and tri-n-butyl phosphate in 20% water. BCTP 401 is a mixture of this emulsion and a liposome. P10 is made of glycerol monostearate, refined soya sterols, and the cationic compound cetylpyridinium chloride. These two compounds have antimicrobial activity against enveloped viruses and bacteria through membrane disruption (unpublished data). In the current studies, we examined the ability of these emulsions to inactivate different *Bacillus* spores.

**Materials and Methods**

**Surfactant lipid preparations.** BCTP is a water-in-oil nanoemulsion, in which the oil phase is made from soybean oil, tri-n-butyl phosphate, and Triton X-100. Stock solutions contained 80% lipid components and 20% water. Three different preparations of BCTP, 2, 8, and 16 months old, were tested for their stability. BCTP 401 was prepared by mixing equal volumes of BCTP with P10, the latter being a liposome-like compound. P10 is made of glycerol monostearate, refined soya sterols, Tween 60, soybean oil, a cationic ion halogen-containing cetylpyridinium chloride, and peppermint oil. The average size of these nanoemulsions is in the range of...
Figure 2. Comparison of sporicidal activity of 3 different preparations of BCTP aged 2, 8, and 16 months. Preparations have equivalent sporicidal activity, showing that BCTP is stable for up to 16 months.

400–800 nm, as determined by laser light scatter (LS230; Coulter, Hialeah, FL). These surfactant lipid preparations were stable after boiling for 1 h or exposure to 1 N nitric acid or 1 N sodium hydroxide for 2 h. This treatment resulted in a <20% reduction in the emulsion mean particle size [16]. These solutions were stored at room temperature and were diluted before each experiment to the working dilution. All dilutions herein are in reference to the stock solution.

Spore preparation. For induction of spore formation, B. cereus (ATCC 14579), B. circulans (ATCC 4513), B. megaterium (ATCC 14581), and B. subtilis (ATCC 11774) were grown for 1 week at 37°C on nutrient agar with 0.1% yeast extract and 5 mg/L MnSO₄. The plates were scraped, and the bacteria and spores were suspended in sterile 50% ethanol and incubated at 22°C for 2 h with agitation to lyse the remaining vegetative bacteria. The suspension was centrifuged at 2500 g for 20 min, and the pellet was washed twice in cold distilled water. The spore pellet was resuspended in trypticase soy broth (TSB) and used immediately for experiments. B. anthracis spores, Ames and Vollum 1B strains, were supplied by Bruce Ivins (US Army Medical Research Institute of Infectious Diseases [USAMRIID], Fort Detrick, Frederick, MD) and were prepared as described elsewhere [5]. Four other strains of B. anthracis were provided by Martin Hugh-Jones (Louisiana State University, Baton Rouge). These strains (from South Africa; Mozambique; Bison, Canada; and Del Rio, TX) represent isolates with high allelic dissimilarity.

In vitro sporicidal assays. For assessment of sporicidal activity on solid medium, trypticase soy agar (TSA) was autoclaved and cooled to 55°C. BCTP was added to the TSA at a 1 : 100 final dilution and continuously stirred while the plates were poured. The spore preparations were serially diluted (10-fold), and 10-μL aliquots were plated in duplicate (highest inoculum, 10⁶ spores/plate). Plates were incubated for 48 h aerobically at 37°C and evaluated for growth.

For assessment of sporicidal activity in liquid medium, spores were resuspended in TSB. Next, 1 mL of spore suspension containing 2 × 10⁶ spores (final concentration, 10⁶ spores/mL) was mixed with 1 mL of BCTP or BCTP 401 (at 2× final concentration in distilled water) in a test tube. The tubes were incubated in a tube rotator at 37°C for 4 h. Treatment of B. anthracis was done at 37°C, which promotes spore germination, and at 22°C, which does not promote spore germination [5]. After treatment, the suspensions were diluted 10-fold in distilled water. Duplicate aliquots from each dilution were then streaked on TSA and incubated overnight at 37°C; then colonies were counted. Sporicidal activity expressed as percentage of killing was calculated as follows: \( \frac{\text{cfu(posttreatment)} - \text{cfu(initial)}}{\text{cfu(initial)}} \times 100 \).

The experiments were repeated at least 3 times, and the mean and SE of the percentage of killing were calculated by use of StatView software (Abacus Concepts, Berkeley, CA). Analysis of variance tables and paired t test were used when applicable.

Electron microscopy. B. cereus spores were treated with BCTP at a final dilution of 1 : 100 in TSB by means of Erlenmeyer flasks in a 37°C shaker incubator. The spore-BCTP mixture was washed with saline and centrifuged at 2500 g for 20 min, and the supernatant was discarded. The pellet was fixed in 4% glutaraldehyde
Figure 3. Time course of nanoemulsion sporicidal activity against *Bacillus cereus*. Incubation with BCTP diluted 1:100 resulted in 95% killing in 4 h. Incubation with BCTP 401 diluted 1:1000 resulted in 95% killing in only 30 min. Difference in killing between BCTP diluted 1:100 and BCTP 401 diluted 1:1000 up to 4-h point was significant (*P* < .05).

in 0.1 M cacodylate (pH 7.3). Spore pellets were processed for transmission electron microscopy, and thin sections were examined after staining with uranyl acetate and lead citrate.

**Germination inhibitors or enhancers.** *B. cereus* spores (final concentration, 10⁶ spores/mL) were suspended in TSB with either the germination inhibitor D-alanine (final concentration, 10 mM) or the germination enhancer L-alanine (final concentration, 5 mM) [17–19]. This suspension was then immediately mixed with BCTP (final dilution, 1:100) and incubated for variable intervals. Then the mixtures were serially diluted, plated, and incubated overnight. The next day, growth on the plates was counted, and the percentage of sporicidal activity was calculated.

**In vivo toxicity testing.** Mice were exposed to various concentrations of the different emulsions by means of different routes of administration. The highest concentrations that produced no gross or histopathologic lesions in mice were reported. Exposures included subcutaneous or intramuscular injection of 100 μL, open wound irrigation with 2 mL of the emulsions, and intranasal instillation of 25 μL/naris. The emulsions are relatively viscous when not diluted, so toxicity testing in the nares was conducted at the
Results

In vitro sporicidal activity. To assess the sporicidal activity of BCTP, spores from four species of Bacillus genus (B. cereus, B. circulans, B. megaterium, and B. subtilis) were tested. BCTP at a 1 : 100 dilution showed 97% sporicidal activity against B. cereus and B. megaterium in 4 h (figure 1). B. circulans was less sensitive to BCTP, showing only an 83% reduction in spore count, whereas B. subtilis appeared resistant to BCTP in 4 h. The other nanoemulsion, BCTP 401, was more efficient in killing the Bacillus spores. At a 1 : 1000 dilution, it showed 99% killing of B. cereus spores in 4 h (compared with 50% with a 1 : 1000 dilution of BCTP). BCTP 401 at a 1 : 1000 dilution resulted in 96% killing of B. subtilis spores in 4 h, in contrast to its resistance to BCTP. Bleach diluted 1 : 100 (i.e., 0.0525% sodium hypochlorite) showed 98% sporicidal activity against B. cereus in 4 h. There was no significant difference in sporicidal activity against B. cereus between BCTP diluted 1 : 100, BCTP 401 diluted 1 : 1000, and bleach diluted 1 : 100 (P = .23).

Testing the stability of BCTP. Three different preparations of BCTP, stored for 2, 8, and 16 months at room temperature, were evaluated simultaneously for sporicidal activity against B. cereus to determine the stability of the emulsions. BCTP was diluted 1 : 10 and 1 : 100 for the experiments (figure 2), and there was no significant difference in the sporicidal activity of the preparations (P = .94 and .77).

B. cereus sporicidal time course. An 8-h experiment was done to analyze the time course of the sporicidal activity of BCTP (diluted 1 : 100) and BCTP 401 (diluted 1 : 1000) against B. cereus. Incubation of a 1 : 100 dilution of BCTP with B. cereus spores resulted in a 77% killing in the number of viable spores at 1 h and a 95% reduction after 4 h. Again, BCTP 401 diluted 1 : 1000 was more effective than BCTP diluted 1 : 100 and resulted in an ~95% reduction in count in 30 min (figure 3). The improvement in killing between BCTP 401 diluted 1 : 1000 and BCTP diluted 1 : 100 was statistically significant up to the 4-h time point (P < .05).

Sporicidal activity of BCTP against B. anthracis. After initial in vitro experiments, the sporicidal activity of BCTP was tested against two virulent strains of B. anthracis (Ames and Vollum 1B). We found that BCTP at a 1 : 100 final dilution incorporated into growth medium completely inhibited the growth of 1 × 10^3 B. anthracis spores. Sporicidal assays in fluid media, after 4 h of incubation with BCTP at dilutions up to 1 : 1000 with either the Ames or the Vollum 1B spores, resulted in 91% sporicidal activity when the mixtures were incubated at 22°C and 96% sporicidal activity when the mixtures were incubated at 37°C (table 1). Because BCTP 401 was effective at higher dilutions and against more species of Bacillus spores than BCTP, it was tested against

### Table 1. Sporicidal activity of BCTP against 2 different strains of Bacillus anthracis spores as determined by colony reduction assay (% killing).

<table>
<thead>
<tr>
<th>B. anthracis strain</th>
<th>Ames</th>
<th>Vollum 1B</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCTP dilution</td>
<td>22°C</td>
<td>37°C</td>
</tr>
<tr>
<td>1 : 10</td>
<td>91.05 ± 0.45</td>
<td>96.35 ± 0.65</td>
</tr>
<tr>
<td>1 : 100</td>
<td>92.90 ± 0.20</td>
<td>97.15 ± 0.55</td>
</tr>
<tr>
<td>1 : 1000</td>
<td>93.00 ± 0.60</td>
<td>96.75 ± 0.25</td>
</tr>
</tbody>
</table>

#### NOTE. BCTP at dilutions of up to 1 : 1000 effectively killed ≥91% of both strains in 4 h at either 22°C or 37°C, conditions that differed markedly in extent of spore germination. More killing was achieved at 37°C because of killing of few germinating spores. Sporicidal activity was consistent at spore concentrations of up to 10^4/mL. Data are mean % ± SE.

### Table 2. Sporicidal activity of BCTP 401 against 4 different strains of Bacillus anthracis representing different clinical isolates.

<table>
<thead>
<tr>
<th>Source of B. anthracis strain</th>
<th>South Africa</th>
<th>Bison, Canada</th>
<th>Mozambique</th>
<th>Del Rio, Texas</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCTP 401 dilution</td>
<td>1 : 10</td>
<td>1 : 100</td>
<td>1 : 1000</td>
<td>1 : 10000</td>
</tr>
<tr>
<td>81.55 ± 0.27</td>
<td>85.90 ± 4.26</td>
<td>41.86 ± 6.98</td>
<td>38.01 ± 9.25</td>
<td></td>
</tr>
<tr>
<td>83.96 ± 1.60</td>
<td>91.08 ± 1.44</td>
<td>96.51 ± 0.23</td>
<td>91.27 ± 1.88</td>
<td></td>
</tr>
<tr>
<td>98.42 ± 0.24</td>
<td>92.16 ± 0.75</td>
<td>99.92 ± 0.02</td>
<td>85.96 ± 1.71</td>
<td></td>
</tr>
<tr>
<td>79.68 ± 2.67</td>
<td>94.13 ± 0.36</td>
<td>95.74 ± 1.01</td>
<td>97.09 ± 0.51</td>
<td></td>
</tr>
<tr>
<td>52.41 ± 2.67</td>
<td>80.33 ± 4.59</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

#### NOTE. Spores were treated with BCTP 401 at different dilutions at 22°C to reduce germination. There was no significant killing by dilutions of emulsion (in medium) <1 : 100. Maximum sporicidal effect was observed between 1 : 1000 and 1 : 5000 dilutions. Data are mean % ± SE. ND, not determined.
Figure 4. Electron micrographs of *Bacillus cereus* spores before (top) and after (bottom) treatment with BCTP. Note uniform density in cortex and well-defined spore coat before treatment with BCTP. Spores after 4 h of BCTP treatment show disruption in both spore coat and cortex, with loss of core components.

4 different strains of *B. anthracis* at dilutions of up to 1:10,000 at 22°C to prevent germination. BCTP 401 showed peak sporicidal activity between ~1:1000 and ~1:5000 dilutions (table 2). It was less efficient at concentrations >1:100.

*Electron microscopic examination of the spores.* We used *B. cereus* because it is the most closely related to *B. anthracis*. Transmission electron microscopic examination of *B. cereus* spores treated with BCTP diluted 1:100 in TSB for 4 h revealed physical damage to the *B. cereus* spores, including extensive disruption of the spore coat and cortex with distortion and loss of density in the core (figure 4).

*Germination stimulation and inhibition.* To investigate the effect of initiation of germination on the sporicidal effect of BCTP on *Bacillus* spores, the germination inhibitor, D-alanine [17, 18], and germination enhancer, L-alanine [19, 20], were incubated with the spores and BCTP for up to 1 h. Percentage of killing was calculated at different time points. The sporicidal effect of BCTP was delayed in the presence of 10 mM D-alanine.
and accelerated in the presence of 5 mM L-alanine (figure 5). All of the individual time points showed a significant difference in killing between the three treatments ($P < .002$).

**In vivo toxicity testing.** CD-1 mice injected with BCTP diluted 1:10 in saline did not exhibit signs of distress or inflammatory reaction, either grossly or histologically (figure 6A, 6B). Identical results were obtained when the toxicity of BCTP 401 was tested in mice subcutaneously. Intramuscular injection of the BCTP or BCTP 401 diluted 1:10 did not have any toxic effects in the form of inflammatory reaction, edema, or necrosis in mice. Open wound irrigation with 2 mL of the emulsions did not result in any pathologic damage. Intranasal instillation of the emulsion was less tolerable because of its viscosity; however, there was no injury from BCTP diluted 1:50 and BCTP 401 diluted 1:25. Oral administration of 10% BCTP (4 mL/kg of body weight daily) in rats for 1 week did not result in any gross or pathologic changes, and the rats maintained normal weight gain during this period (data not shown). In these tests, pathologic examination of local tissues and internal organs was done, and no abnormalities were detected.

**In vivo sporicidal activity.** *B. cereus* infection in experimental animals had been previously used as a model system...
Figure 6. Gross and histologic photographs of animals injected subcutaneously with different combinations of BCTP and *Bacillus cereus* spores. 

A, B, animals injected with BCTP alone at dilution of 1:10. There was no gross tissue damage, and histology showed no inflammation.

C, D, animals injected with $4 \times 10^7$ *B. cereus* spores alone subcutaneously. Large necrotic area resulted, with average area of $1.68 \pm 0.35$ cm$^2$. Histologic examination of this area showed essentially complete tissue necrosis of epidermis and dermis, including subcutaneous fat and muscle.

E, F, mice injected with $4 \times 10^7$ *Bacillus* spores that had been immediately premixed with BCTP nanoemulsion at final dilution of 1:10. These animals showed minimal skin lesions, with average area of $0.02 \pm 0.01$ cm$^2$ ($\sim$98% reduction from lesions resulting from untreated infection with spores; $P < .002$). Histology of F indicated some inflammation; however, most cellular structures in epidermis and dermis were intact. All histopathology is shown at $\times4$ magnification.
for the study of anthrax and causes an illness similar to experimental anthrax [2, 9, 21–24]. Two animal models of cutaneous B. cereus disease were developed to assess the in vivo sporicial activity of BCTP. A suspension of $4 \times 10^7$ B. cereus spores was mixed with saline or with BCTP at a final dilution of 1:10 and then immediately injected subcutaneously into the backs of CD-1 mice. Mice that were infected subcutaneously with B. cereus spores without BCTP developed severe edema in 6–8 h. This was followed by a gray, necrotic area surrounding the injection site at 18–24 h, with severe sloughing of the skin present by 48 h, leaving a dry, red-colored lesion (figure 6C, 6D). CD-1 mice injected with B. cereus spores premixed with BCTP never developed such a necrotic lesion, and edema and inflammation were minimal (figure 6E, 6F). The size of the necrotic lesion in BCTP-treated mice was $\approx 98\%$ smaller than the necrotic lesion size in untreated mice (from $1.62 \pm 0.35 \text{ cm}^2$ to $0.02 \pm 0.01 \text{ cm}^2$; $P < .002$). Similar results were observed with BCTP 401 diluted 1:10.

In additional studies, a 1-cm skin wound was infected with $2.5 \times 10^7$ B. cereus spores and then closed (figure 7A, 7B). For some of the animals 1 h later, the wounds were irrigated with either BCTP diluted 1:10 or saline to simulate postexposure decontamination. Irrigation of experimentally infected wounds with saline did not result in any apparent benefit (figure 7C, 7D). BCTP irrigation of wounds infected with B. cereus spores showed substantial benefit, resulting in a consistent $98\%$ reduction in the lesion size (from $4.84 \pm 0.48 \text{ cm}^2$ to $0.06 \pm 0.03 \text{ cm}^2$; $P < .001$; figure 7E, 7F). This reduction in lesion size was accompanied by a 3-fold reduction in mortality (from $60\%$ to $20\%$) compared with that in experimental animals receiving either no treatment or saline irrigation. Similar results were observed with BCTP 401 diluted 1:10.

Discussion

In these studies, we demonstrated that BCTP and its derivative BCTP 401 have effective sporicial activity against a variety of Bacillus spores, including B. anthracis. BCTP diluted 1:10 has a sporicial activity against B. cereus, B. circulans, and B. megaterium, whereas 1:1000 is effective against B. anthracis in 4 h. BCTP 401, a BCTP-P10 mixture, appears to have a more rapid and broader sporidal activity than BCTP. BCTP 401 diluted 1:1000 killed $95\%$ of B. cereus spores in 30 min at 37°C, compared with a $70\%$ reduction achieved by BCTP diluted 1:100. BCTP 401 diluted 1:1000 was also effective in 4 h against B. subtilis spores that were resistant to BCTP for up to 24 h. BCTP 401 did not show effective sporidal activity against B. anthracis at dilutions of <1:100, contrary to the original BCTP, which showed killing at dilutions between 1:10 and 1:1000. The fact that BCTP 401 requires dilution to be effective against B. anthracis spores suggests that BCTP 401 needs dispersion by water to minimize its aggregation and to facilitate direct contact with spores.

Comparison of the sporidal activity of BCTP against B. anthracis at 22°C, a temperature that does not promote spore germination, and at 37°C, at which germination occurs (as confirmed by microscopic examination), indicates that complete spore germination (i.e., outgrowth) is not necessary for the bactericidal activity of the emulsion. The small difference observed between the sporidal activity at 37°C and 22°C may represent the killing of additional organisms from a few germinating spores. Sporicidal activity was also confirmed in water, a condition unsuitable for B. anthracis spore germination (data not shown). The sporidal effect seems to start almost immediately and occurs within 30 min of incubation with the emulsion. Factors facilitating germination resulted in acceleration of the sporidal activity of BCTP. Inhibition of the initiation of germination with D-alanine delayed BCTP's sporidal activity. On the basis of these observations, we hypothesize that the sporidal action of these emulsions occurs through initiation of germination before complete reversion to the vegetative form, leaving the spore susceptible to disruption by the emulsion. The initiation of germination could be mediated by the action of the emulsion or its components, but the emulsion appears necessary, as spores do not initiate germination in its absence. The results of the electron microscopy studies show disruption of the spore coat and cortex with disintegration of the core contents after BCTP treatment. However, the exact mechanism of killing is unclear and requires future investigation. Sporicidal activity appears to be mediated by both the Triton X-100 and tri-n-butyl phosphate components, because nanoemulsions lacking either component are inactive in vitro (data not shown). This unique sporidal action of the emulsions, which is similar in efficiency to that of 1% bleach, is interesting because Bacillus spores are generally resistant to most disinfectants, including many commonly used detergents [15].

Animal studies demonstrated the protective and therapeutic effect of BCTP in vivo. B. cereus infection in experimental animals has been used previously as a model system for the study of anthrax [21, 22, 25]. The disease induced in animals experimentally infected with B. cereus is in many respects similar to anthrax [9, 23]. In this study, we demonstrated that mixing BCTP with B. cereus spores before injecting the spores into mice prevented the pathologic effect of B. cereus. We also demonstrated that BCTP treatment of simulated wounds contaminated with B. cereus spores markedly reduced the risk of infection and mortality in mice. Because the emulsion appeared to lose sporidal activity when diluted past 1:100, higher concentrations of the emulsions (1:10) were used for the in vivo studies to make sure they remained effective after dilution with body fluids. Other experiments show that testing BCTP 401 in mice under similar conditions demonstrated similar effects. These results suggest that decontamination of spores prior to or after exposure can effectively reduce the morbidity and mortality from B. cereus infection. This appeared to be a valuable
Figure 7. Gross and histologic photographs of animals with experimental wounds infected with Bacillus cereus spores. A, B, mice with experimental wounds infected with $2.5 \times 10^7$ B. cereus spores but not treated. Histologic examination indicated extensive necrosis and marked inflammatory response. C, D, mice with wounds that were infected with $2.5 \times 10^7$ B. cereus spores and irrigated 1 h later with saline. By 48 h, large necrotic areas surrounded wounds, with average area of $4.86 \pm 0.48 \text{ cm}^2$. In addition, 60% of animals in this group died as result of infection. Histologic examination of these lesions indicated total necrosis of dermis and subdermis and large numbers of vegetative Bacillus organisms. E, F, mice with wounds infected with $2.5 \times 10^7$ B. cereus spores and irrigated 1 h later with 1 : 10 dilution of BCTP. There were small areas of necrosis adjacent to wounds ($0.06 \pm 0.03 \text{ cm}^2$), 98% reduction compared with animals receiving spores and saline irrigation ($P < .001$). In addition, only 20% of animals died from these wounds. Histologic examination of these lesions showed no evidence of vegetative Bacillus organisms and minimal disruption of epidermis. All histopathology is shown at $\times 4$ magnification.
application, because unlike other sporicidal agents, BCTP or BCTP 401 did not demonstrate any toxic effects, grossly or by histopathologic examination of the mice [26]. Other tests in mice showed that these emulsions are nontoxic if administered intramuscularly, intranasally, or orally, providing other potential sites for treatment.

BCTP and its derivative BCTP 401 appear to have great potential as environmental decontamination agents or for treatment of exposed persons in either a military operation or a terrorist attack. The inactivation of a broad range of pathogens, including vegetative bacteria, enveloped viruses [27] (unpublished data), and bacterial spores, combined with low toxicity in experimental animals, seems to make it suitable for use as a general decontamination agent that can be deployed even before a specific pathogen is identified. The nanoemulsions can be rapidly produced in large quantities and are stable for many months unless frozen, which causes separation of the oil and lipid phases. Undiluted, they have the texture of a semisolid cream and can be applied topically by hand or mixed with water. Diluted, they have a consistency and appearance similar to skim milk and can be sprayed to decontaminate surfaces or potentially interact with aerosolized spores before inhalation. These properties provide a flexibility that will be useful for a broad range of decontamination applications. Further studies are warranted to determine the exact mechanism of the sporicidal effect of BCTP and its derivatives, and this may lead to further improvement in formulations.

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