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Therapeutic Efficacy of "Nubiotics" against Burn Wound Infection by *Pseudomonas aeruginosa*

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"Nubiotics" are a novel class of proprietary protonated nucleic acid-based drugs shown to have potent in vitro antibacterial activities against a number of gram-positive and gram-negative bacteria. These nubiotics are evaluated here for their in vivo therapeutic efficacy for the treatment of burn wound infection caused by *Pseudomonas aeruginosa*. To achieve this, a burn wound infection model was established in mice by using a highly pathogenic burn wound clinical isolate of *P. aeruginosa*. Lethal doses of the bacteria were determined for two routes of infection (subcutaneous and topical), representing systemic and local forms of infection, respectively. Using this infection model, treatment with nubiotics by various routes of drug administration was evaluated and optimized. A total of 12 nubiotics and their analogues were tested and of these, Nu-2, -3, -4, and -5 were found to be extremely efficacious in the postexposure treatment of burn wound infection (60 to 100% survival rates versus 0% for untreated control [$P < 0.05$]). These nubiotics were effective when given either systemically by intravenous and/or subcutaneous administration or given locally to the affected site in the skin by topical application. Treatment by these two routes resulted in almost 100% survival rates and complete eradication of the bacteria from infection sites in the livers, spleens, and blood. These nubiotics were found to be as effective as intravenously administered ciprofloxacin, a potent and broad-spectrum fluoroquinolone. These results suggest that nubiotics may be a promising and effective approach for the treatment of burn wound infection caused by *P. aeruginosa*.

Nearly 10 million patients with traumatic wounds are treated annually in the United States (5). Infections of the skin and skin structures in these patients frequently occur in surgical wounds, burns, and other exposed tissues (14). These infections are responsible for significant human mortality and morbidity and often result in prolonged hospital stays and/or increased health care costs (7). These infections are caused by both gram-negative and gram-positive microorganisms, especially *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (1, 2, 6). *P. aeruginosa* is an opportunistic pathogen found along with other *Pseudomonas* spp. as part of the normal flora of the human skin (11). When the host is immunocompromised, as in the case of a thermal burn or surgical wound, this opportunistic bacterium can quickly colonize and infect the burn and wound sites. Since *P. aeruginosa* can rapidly disseminate from the burn wound sites into distant organs via the bloodstream and can produce a number of virulence factors that induce endotoxic shock, the clinical outcome in these patients can lead to sepsis which is often fatal. In case studies of burn patients who developed *P. aeruginosa* septicemia, the mortality rate was >75% (9, 16). Antibiotics that are administered orally, with the exceptions of the fluoroquinolones, are generally ineffective against most serious skin and soft tissue infections by *P. aeruginosa* (6). Effective therapy of burn wound infections requires the additional expense of parenterally administered broad-spectrum antibiotics. The introduction of fluoroquinolones such as ciprofloxacin and ofloxacin offers a promising effective therapy of these types of infections caused by gram-negative burn wound pathogens (6, 8) but may not be effective against gram-positive infections. In addition, the numbers of nosocomial and community-acquired strains of bacteria which have developed drug resistance to fluoroquinolones have increased rapidly in recent years (13). Therefore, there is an important and compelling need to develop novel and effective classes of antibiotic to combat these drug-resistant wound and burn isolates.

"Nubiotics" are a novel class of antibiotics that could offer an exciting approach for the therapy for a wide range of clinically relevant bacterial infections, including burn and wound infections. Nubiotics are proprietary DNA- and RNA-based antimicrobial agents developed by Oligos Etc., Inc. (Wilsonville, Oreg.). Although the exact mechanisms of actions for these nubiotics are not fully elucidated, they are believed to be entirely different from that of conventional antibiotics. This new generation of antibiotics has been shown to demonstrate strong in vitro activity against a number of skin and wound pathogens (M. B. Perri, R. Dale, and M. J. Zervous, Abstr. 39th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1811, 1997) and may therefore offer a promising and broad-spectrum therapeutic approach for burn and wound infections.

The objective of the present study was to evaluate the in vivo efficacy of nubiotics in the experimental treatment of burn wound infections by *P. aeruginosa*. We use a murine burn wound infection model with a highly pathogenic strain of *P. aeruginosa* that could cause both a systemic and local forms of infection. With this model, the therapeutic efficacy of 12 different nubiotics was evaluated.

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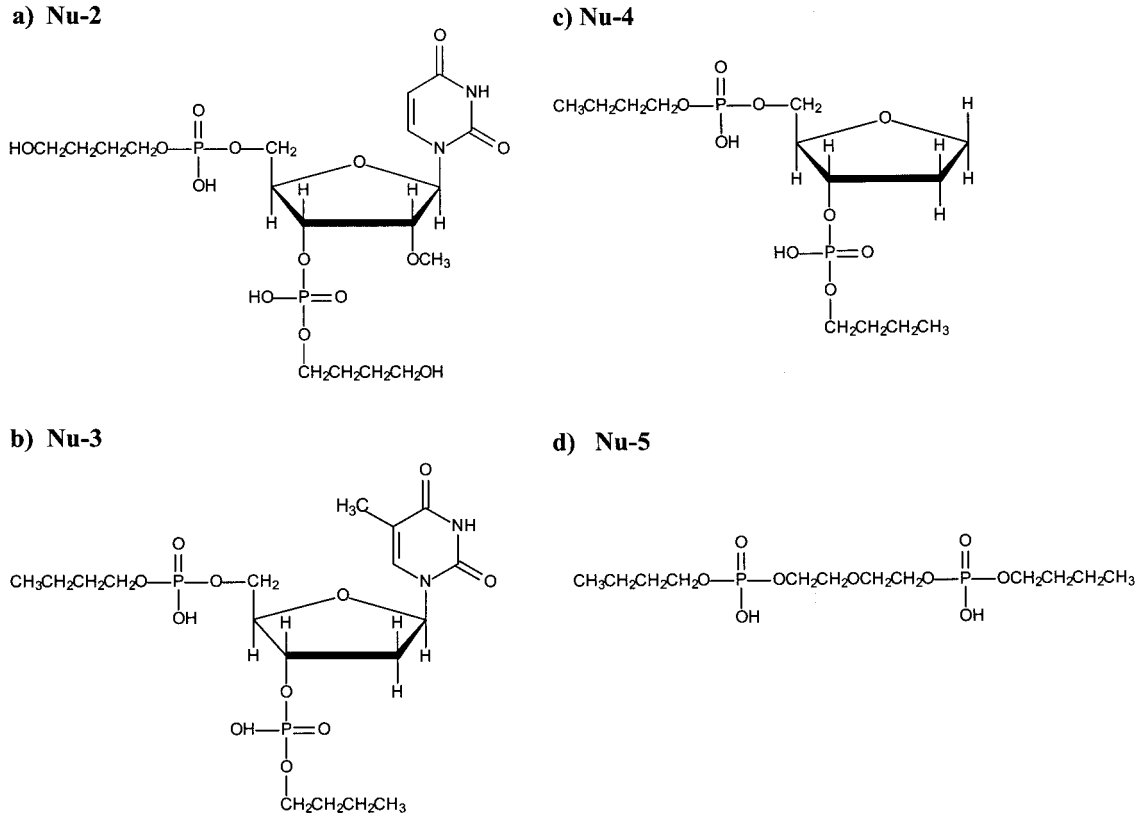

FIG. 1. Chemical structures of Nu-2 (a), Nu-3 (b), Nu-4 (c), and Nu-5 (d).

MATERIALS AND METHODS

Animals. Six-week-old BALB/c female mice were obtained from the mouse breeding colony at Defense R&D Canada–Suffield (DRDC Suffield), with original breeding pairs purchased from Charles River Canada, Ltd. (St. Constant, Quebec, Canada). The use of animals described in the present study was approved by DRDC Suffield Animal Care Committee. Care and handling of animals described in the present study followed guidelines set out by the Canadian Council on Animal Care.

Bacteria. *P. aeruginosa* (Strain Utah 4) was generously provided by Merle Olsen, Biofilm Research Lab, University of Calgary. This strain was isolated from a patient who developed a burn wound infection by *P. aeruginosa*. The bacterium was initially cultured on the Trypticase soy broth (TSB), divided into aliquots, and frozen at 70°C. Prior to use, aliquots were thawed and diluted serially in sterile phosphate-buffered saline (PBS) just prior to administration into animals. To ensure viability and virulence, aliquots of the bacteria were periodically reamplified in TSB and colonies determined on Trypticase soy agar (TSA) plates.

Susceptibility tests. The antibacterial activities of nubiotics and ciprofloxacin were determined by broth dilution method with TSB. The overnight broth culture of *P. aeruginosa* was diluted in TSB to ca. 2×10^6 CFU/ml, and 0.5 ml of the bacterial culture was added to bacterial culture tubes containing 0.5-ml graded concentrations of the nubiotics in TSB. Bacterial broth not containing the antibacterial agent was inoculated as a control for organism viability (growth control). After 24 h of incubation at 37°C, aliquots of 100 μl of the suspension were plated on TSA plates, and the CFU were counted after 48 h of incubation at 37°C. The MIC was defined as the lowest drug concentration that inhibited at least 99% of bacteria compared to the growth control. By this method the MIC of nubiotic Nu-2 for *P. aeruginosa* was determined to be 3.29 mg/ml. The MIC_{90} of ciprofloxacin against *P. aeruginosa* has been reported to be 0.5 μg/ml (6). The in vitro susceptibilities of a number of multidrug-resistant bacteria, including *P. aeruginosa*, to nubiotic Nu-2 have been described elsewhere (Perri et al., 39th ICAAC).

Nubiotics. Nubiotics used in the present study were synthesized and purified by Oligos Etc., Inc. The chemical structures of nubiotics Nu-2, Nu-3, Nu-4, and Nu-5 are shown in Fig. 1. The sequences of the other nubiotics are as follows: Nu-1, 5′-ACG CGC CAT TGG-3′ butanol; AUG, 2′O-methyl-AUG-3′ butanol; G, 5′ butanol-2′O-methyl G-3′ butanol; GGG, 5′ buthanol-2′O-methyl GGG-3′ butanol; UUU, 5′ butanol-2′O-methyl-UUU-3′ butanol; UMP, 5′OH-uridine-PO_4; 114.6, 5′ butanol-2′O-methyl-CAT TGG-3′ butanol; and 114.9, 5′ butanol-2′O-methyl-CGC CAT TGG-3′ butanol.

Preparation of liposome-encapsulated nubiotics. The liposomes for the encapsulation of Nu-2 and Nu-3 were small unilamellar vesicles prepared by using phosphatidylcholine and cholesterol at a 55:45 molar ratio. The liposomes were prepared by a modification of the remote drug-loading method (12) with the following changes: 400 mM in place of 200 mM ammonium sulfate was used for drug loading. The preformed liposomes were extruded 10 times under high pressure through two polycarbonate 200-nm filters. Free, unecapsulated nubiotics were removed by ultracentrifugation at $125{,}000 \times g$ for 1 h. Entrapment rates were determined by measuring the total amount of free nubiotics in the supernatants. The differences in the total nubiotic added to the liposome preparations and the amounts in the wash supernatants after centrifugation were considered the amount of nubiotics associated with the liposomes.

Burn wound infection. To establish the lethal doses of the bacteria for the systemic burn wound infection, groups of mice were anesthetized with ketamine-xylazine mixture (50 mg/kg each, given intramuscularly), their backs were then shaved by using a clipper, razor, and shaving cream. To induce burn in the backs of these animals, a brass bar (10 by 10 by 100 mm) was heated in boiling water for 15 min. The end of the heated bar was then applied on the shaved back of the mice for 45 s. After a waiting period of 30 min, 50 μl of the bacterial inoculum (containing 10^7 to 10^{11} CFU of total bacteria) was then applied subcutaneously into the sites of the burn on the animal's back. The mice were then allowed to recover and were monitored daily for symptoms and deaths. For establishment of a topical infection, the mice were preprimed with cyclophosphamide (200 mg/kg of body weight) given intraperitoneally. Three days later, the mice were shaved, and burns were induced as described above. The inoculum containing the same numbers of bacteria was then topically applied (100 μl) evenly on the sites of the burn, and a custom made "mouse jacket" was then put on the infection site, for at least 2 h. These mice were then monitored daily for symptoms and deaths. At day 7 postinfection, the number of animals which survived the infection was recorded. The surviving animals were then euthanized by cervical dislocation,

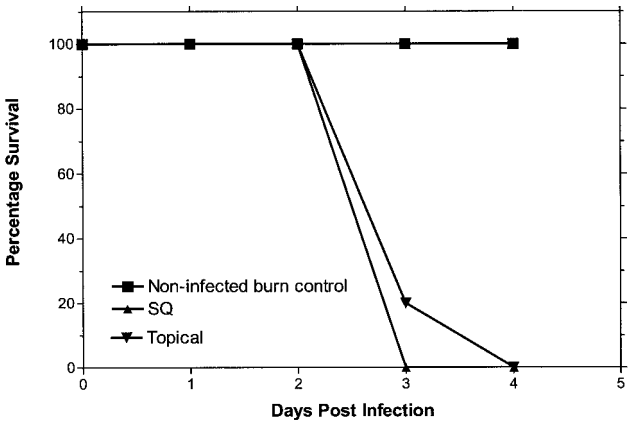

FIG. 2. Survival curves of mice for burn wounds infected with 5 LD_{50}s of a burn wound clinical isolate of *P. aeruginosa* (Utah 4 strain). The infection was established by using either the subcutaneous (SQ) or topical route of administration.

and the blood, livers, spleens, and skin were aseptically harvested and assayed for bacterial counts as described below. Animals that succumbed to the infection were noted. Blood samples, livers, and spleens were aseptically harvested close to the time of death and were assayed for bacteriological counts.

Treatment of burn wound infection. To determine the effectiveness of various nubiotics for the treatment of burn wound infection, mice were subcutaneously or topically infected with five 50% lethal doses (LD_{50}s) of *P. aeruginosa* as described above. Mice were then treated in the following manner. For treatment of systemic infection (infection by subcutaneous injection of the bacteria), mice were treated at 2 and 8 h postinfection on day 1 and twice daily on days 2 and 3. Treatment with various nubiotics and nubiotics in liposomes was administered subcutaneously, intravenously, and/or topically. For topical treatment of burn wound infection, mice were treated by using the same schedule as for the intravenous and subcutaneous treatment. The concentrations of the nubiotics were 19.7 to 21.1 mg/ml for subcutaneous (200-μl) and intravenous (100-μl) administrations and 105.8 mg/ml for topical administration (50 μl). For the treatment of systemic burn wound infection with ciprofloxacin, ciprofloxacin was administered at a drug concentration of 20 mg/ml at 2 and 8 h postinfection on day 1 and twice daily on days 2 and 3 by the subcutaneous and intravenous routes (200 and 100 μl, respectively).

Bacterial determination of organ homogenates. To determine the bacterial load in the blood and organs of experimental animals, blood, spleens, livers, and the burnt skins were aseptically removed. The blood (100 μl) was serially diluted in sterile PBS, and 100 μl of the diluted blood was plated for growth in TSA plates. For the organs, samples were homogenized in 2 ml (spleens and skins) or 5 ml (livers) of sterile PBS by using a hand-held tissue grinder. The tissue homogenates were serially diluted in sterile PBS and were then plated for growth in TSA. The inoculated plates were incubated at 37°C overnight. The number of CFU was then determined.

Statistics. The survival rates of control and treated mice were compared by using the Fisher exact test (two tailed). These tests were performed by using GraphPAD Prism software program (version 2.0; GraphPAD Software, Inc., San Diego, Calif.). Differences were considered statistically significant at a P value of <0.05.

RESULTS

Establishment of burn wound infection. Burn wound infection in mice can be established by subcutaneous or topical administration of the bacteria to the sites of the burn. LD_{50} values were determined by the method of Reed and Muench (4) and were found to be ca. 4×10^8 and 2×10^9 CFU, respectively, for the subcutaneous and topical routes of infection. For all treatment studies, five LD_{50}s of the bacteria were used. The survival pattern of the mice infected with five LD_{50}s of the bacteria administered by these two routes of infection was similar (Fig. 2). Both routes of administration resulted in eventual death of all mice in the test groups by day 3 or 4 postinfection. All control animals that received equivalent doses of bacteria by either subcutaneous or topical administration without the burn were asymptomatic and found to be completely resistant to the infection. In the mice that received the burn and infection, the LD_{50} of the bacteria administered topically was ~5-fold higher than that of bacteria administered by the subcutaneous route. Unless otherwise stated, all treatment studies described below were carried out by using the subcutaneous route of infection. This route of administration was chosen for subsequent studies since it does not require pretreatment of the mice with cyclophosphamide at 3 days prior to infection, and it causes a more systemic infection.

Optimization of routes of administration. To determine the most effective therapeutic route(s) of administration for the nubiotics, mice subcutaneously infected as described earlier were treated with nubiotic Nu-2 by the subcutaneous and/or intravenous routes (Fig. 3). Treatment by both the subcutaneous and the intravenous routes was found to be the most effective, resulting in a 100% survival rate ($P < 0.01$ versus control). When treatment was administered by the intravenous or subcutaneous route alone, the efficacies were 40% ($P > 0.05$ versus control) and 80% ($P < 0.05$ versus control), respectively. These results indicate that both subcutaneous and intravenous administrations of the nubiotics are required to achieve optimal therapeutic efficacy against systemic burn wound infection.

Comparative efficacy of various nubiotics. Throughout the study, a total of 12 nubiotics were tested against burn wound infection. These nubiotics included Nu-1, Nu-2, Nu-3, Nu-4, Nu-5, AUG, G, GGG, UUU, 114.6, 114.9, and a control compound, UMP. The comparative efficacies of the nubiotics are summarized in Table 1. Nubiotics differ greatly in their therapeutic effectiveness against burn wound infection, ranging in the present study from 0% (UMP and 114.9) to 93% (Nu-2). In all, Nu-2 was the most efficacious, with 93% (28 of 30) of the mice responded to treatment ($P < 0.0001$ compared to con-

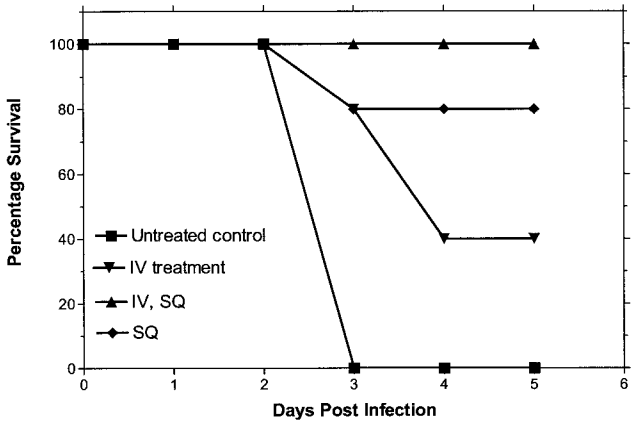

FIG. 3. Therapeutic efficacy of nubiotic Nu-2 against LD_{50} of *P. aeruginosa* given subcutaneously to mice. Treatment with nubiotic Nu-2 was administered subcutaneously (SQ), intravenously (IV), or both. The treatment was given to the mice at 2 and 8 h on day 1 and twice daily on days 2 and 3. The concentrations of the nubiotics were 19.7 to 21.1 mg/ml for subcutaneous (200-μl) and intravenous (100-μl) administrations.

TABLE 1. Therapeutic efficacy of various nubiotics for therapy of systemic burn wound infection by *P. aeruginosa*

Nubiotic treatment	No. of survivors/ total no. of animals	% Survival	P^a
None (PBS control)	1/45	2	
Nu-1	2/5	40	<0.05
Nu-2	28/30	93	<0.05
AUG	3/5	60	<0.05
G	3/5	60	<0.05
UUU	2/5	40	<0.05
GGG	3/5	60	<0.05
UMP	0/5	0	>0.05
114.6	2/5	40	<0.05
114.9	0/5	0	>0.05

[a] Versus infected controls.

trols). Nubiotics AUG, G, and GGG were moderately efficacious (60% effectiveness), whereas Nu-1, UUU, and 114.6 were marginally effective (40% effectiveness). The control compound UMP and nubiotic 114.9 did not appear to shown any therapeutic activity in the present study. Lower doses of nubiotics Nu-2 to -4 (11.8 mg/ml) were also tested. All Nu analogues were found to be effective at 12.9 mg/ml, with therapeutic efficacies ranging from 60% for Nu-2, to 80% for Nu-3 and Nu-5, and to 100% for Nu-4 (results not shown).

Comparison of Nu-2 and ciprofloxacin. The efficacy of nubiotic Nu-2 for the treatment of burn wound infection was compared to that of ciprofloxacin, a potent fluoroquinolone that has been shown to be efficacious in the treatment of burn wound infection. Nubiotic Nu-2 was found to be as effective as ciprofloxacin for the treatment of systemic form of burn wound infection, with both therapeutic agents resulting in 100% survival rates (Table 2). Microbiological comparisons of the CFU in livers, spleens, skin, and blood of both treated groups reveal no significant difference in the abilities of the drugs to eradicate these organisms from these infection sites (Table 3).

Topical use of nubiotics for burn wound infection. To determine whether burn wound infection could be effectively treated by nubiotics administered topically, mice were infected by topically applying the bacteria into the burn sites on the animals' backs and treated in the same manner (Tables 4 and 5). Nubiotic Nu-2 administered topically was found to be very effective in the treatment of local burn wound infection, resulting in 90% survival rate, whereas all untreated control infected topically succumbed to the infection. The effectiveness of nubiotic administered topically was found to be dependent on the drug concentration. Decreasing the concentration of the nubiotic Nu-2 from 105.8 to 19.7 mg/ml resulted in a sharp decrease in survival rates from 90 to 40%. When the blood, spleens, livers, and skins of three mice that were treated with Nu-2 (105.8 mg/ml) were analyzed and compared to that of untreated controls, it was found that all blood, spleen, and two of three liver samples from the treated group were devoid of any detectable CFU, in contrast to the untreated group which harbored high numbers of bacteria in these tissues.

TABLE 2. Comparison of in vivo efficacies of Nu-2 and ciprofloxacin for treatment of systemic burn wound infection

Antibiotic treatment	No. of survivors/ total no. of animals	% Survival	P^a
None (untreated control)	0/5	0	
Ciprofloxacin	5/5	100	<0.05
Nu-2	5/5	100	<0.05

[a] Versus control.

TABLE 3. Microbiological analyses of blood and tissues from mice treated with nubiotic Nu-2 and ciprofloxacin

Antibiotic treatment	Avg CFU[a] in:			
	Blood	Liver	Spleen	Skin
None (untreated control)	2.3×10^6	2.1×10^7	3×10^7	ND
Ciprofloxacin	1.8×10^5	NG	NG	3×10^6
Nu-2	1.4×10^3	NG	NG	2×10^6

[a] NG, no growth; ND, not determined.

Effect of HPMC on efficacy of Nu-2. To determine the effect of hydroxy propyl methylcellulose (HPMC) on the absorption of Nu-2 into the burn wound infection site, the efficacy of topically applied Nu-2 formulated in various concentrations of HPMC (0.5 to 2.0% [wt/vol]) was evaluated. HPMC is a drug-coating agent with sustained release property (3). In the present study, Nu-2 without HPMC and Nu-2 in 0.5 to 2.0% HPMC were found to be effective for the treatment of topical form of burn wound infection. Topical treatment with Nu-2 without HPMC resulted in an 80% survival rate. The addition of 0.5% HPMC resulted in increased survival rates ranging from 80% to 100%. Increasing the HPMC from 0.5 to 1 and 2% decreased the efficacy from 100 to 80% survival. These results suggest HPMC did not affect the therapeutic efficacy of the nubiotics.

Treatment of burn wound infection by liposome-encapsulated nubiotic. To determine the therapeutic efficacy of liposome-encapsulated nubiotics for burn wound infection, nubiotics Nu-2 and Nu-3 were encapsulated in small unilamellar vesicles. Treatment studies with the liposome-encapsulated nubiotics were carried out by using the subcutaneous and intravenous routes of administration (Table 6). Treatment with liposome-encapsulated nubiotic by the subcutaneous route was found to be completely protective against the topical form of the burn wound infection (Table 6). Treatment of the systemic burn wound infection with intravenously injected liposome-encapsulated Nu-3 resulted in 60% survival rate, whereas all PBS control mice died ($P < 0.05$). Liposome-encapsulated Nu-2 administered subcutaneously into the burn infected sites was shown to be therapeutically effective, providing a 100%

TABLE 4. Efficacy of topically applied nubiotics for treatment of burn wound infection induced topically: survival rates

Nubiotic treatment	Nubiotic concn (mg/ml)	No. of survivors/ total no. of animals	% Survival	P^a
None (untreated control [PBS])		0/10	0	
Nu-2	105.8	9/10	90	<0.05
Nu-2	19.7	2/5	40	>0.05
Nu-1	105.8	2/5	40	>0.05

[a] Versus control.

TABLE 5. Efficacy of topically applied nubiotics for treatment of burn wound infection induced topically: microbiological quantitation of tissues

Treatment group and mouse no.	Avg CFU[a] in:			
	Blood	Livers	Spleens	Skin
PBS control				
1	ND	5.5×10^7	NG	ND
2	ND	1.0×10^6	1.9×10^8	ND
3	ND	2.4×10^8	5.6×10^9	ND
Nu-2 (105.8 mg/ml)				
1	NG	NG	NG	3.9×10^8
2	NG	NG	2×10^5	4.2×10^7
3	NG	NG	NG	2.1×10^9

[a] See Table 3, footnote *a*.

survival rate, whereas all PBS control infected mice died from the infection (Table 6).

DISCUSSION

It is estimated that potential mortality from burn wound infections, even with aggressive antibiotic therapy, may approach or exceed 50% (16). Burns, wounds, and other exposed tissues are particularly susceptible to microbial contamination and infections (10, 14, 15). Wound and burn pathogens can often colonize and multiply in the exposed tissues and, once established, these bacteria may penetrate the blood capillaries of the affected tissues and thus may lead to bacteremia. Moreover, these bacteria are known to release virulence factors that can lead to endotoxic shock. Infections from burns or wounds, once reaching the bacteremia and/or endotoxic shock phase, are generally beyond treatment by conventional antibiotic therapy. Therefore, it is imperative that effective antibiotic treatment be administered at an early stage of infection to be efficacious.

The treatment studies shown in the present study suggest that nubiotics may offer a promising and novel means for the treatment of burn wound infections. Although the mechanisms of action by which these nubiotics work have not been fully elucidated, the preliminary results presented here suggest that the in vivo potency of the Nu-2 nubiotics for treatment of burn wound infections is comparable to that of ciprofloxacin given intravenously. Treatment of mice with nubiotics Nu-2 resulted in close to 100% survival rate and in almost complete eradication of the bacteria from the spleens, livers, and blood of infected animals. It is generally thought that oligonucleotide-based nubiotics exert their bactericidal activities through mechanisms of action that are believed to be different from conventional antibiotics. Nubiotics are believed to be proton donors, and the hydrogen ions induce bacterial cell death by membrane depolarization, although this remains to be elucidated. Nubiotics may therefore provide an effective therapeutic approach for burn wound infections caused by bacteria that are resistant to conventional antibiotics. In addition, since nubiotics have been shown to have excellent in vitro activity against a number of burn and wound pathogens, including *P. aeruginosa* and *S. aureus* (Perri et al., 39th ICAAC) and *E. coli*, nubiotics have the potential to be used as broad-spectrum therapy against these pathogens found in burns and wounds. It is interesting that, in the present study, nubiotics administered topically were found to be as effective as those administered systemically for the treatment of burn wound infection, as measured by increased survival rates and eradicating bacteria from blood, livers, and spleens. These results suggest that topically applied nubiotics are effective in preventing the local infection of the burned skin from spreading systemically to vital organs, such as livers and spleens. This is especially important in view of the fact that infection in sites other than the burn wound, principally in the lungs, remains the most common cause of death in burn patients (15). This may represent a noninvasive means to administer the nubiotics since it does not require intravenous, subcutaneous, or invasive means of drug administration and thus may increase the level of patient compliance.

It is proposed that the efficacy of nubiotics for the treatment of burn wound infections could be further enhanced by encapsulating the oligonucleotides in liposomes. Liposomes are excellent drug delivery systems for the controlled release of antibiotics into the sites of infection (17). Although liposome-encapsulated antibiotics can be effectively administered intravenously or by aerosol inhalation, they are well suited for topical administration on skin, burn wound, or other exposed soft tissues. Liposomes are made from phospholipid components which are excellent skin moisturizers, and they have been used in a number of skin cream formulations by the cosmetic industry. When liposomes are applied topically, they may interact with the cell membranes of exposed tissues. This may lead to the formation of a semipermeable lipid film that blankets the skin, and therefore protects the burn wound tissues from further bacterial contamination. In addition, the liposomes can protect the oligonucleotides from nuclease degradation in vivo; the entrapped oligonucleotides may be released from the liposomes into the burn wound tissues in a gradual and sustained manner, thereby making it possible to achieve high, therapeutic levels in the sites of infection. It has been postulated that liposomes enhance the efficacy of oligonucleotides by reducing the number of doses required and in eradicating the bacterial pathogens from the exposed tissues and infected livers and spleens. Research on the development of liposome formulations for nubiotics is currently under way. The results described here suggest that liposome-encapsulated nubiotics, when injected intravenously or subcutaneously into the burn wound site, is effective in the treatment of burn wound infection by *P. aeruginosa*.

TABLE 6. Liposome-encapsulated nubiotic for treatment of topical burn wound infection by *P. aeruginosa*

Treatment group	No. of survivors/ total no. of animals	% Survival
Infection by topical route, treatment by topical route		
PBS control	0/4	0
Liposomal Nu-2	4/4	100
Infection by subcutaneous route, treatment by intravenous route		
PBS	0/5	0
Free unencapsulated Nu-3	0/5	0
Liposome-encapsulated Nu-3	3/5	60

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