Carbohydrate-derived fulvic acid is a highly promising topical agent to enhance healing of wounds infected with drug-resistant pathogens

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BACKGROUND: This work was intended as a proof-of-principle study to help establish carbohydrate-derived fulvic acid (CHD-FA) as a safe and effective agent that can be deployed to prevent the onset of drug-resistant bacterial and fungal infections in military and civilian personnel experiencing traumatic wound.

METHODS: Minimum inhibitory concentrations for CHD-FA were established on a total of 500 clinical isolates representing wound-associated drug-sensitive and drug-resistant bacterial and fungal pathogens. The efficacy of early use of CHD-FA to enhance healing of wounds infected with methicillin-resistant *Staphylococcus aureus* or *Pseudomonas aeruginosa* was evaluated in an in vivo rat model.

RESULTS: CHD-FA showed strong activity against a variety of bacterial and fungal pathogens with minimum inhibitory concentration values equal or less than 0.5%. Compared with infected but untreated wounds, improved wound healing upon CHD-FA treatment was observed in both infection models, demonstrated by wound surface area measurement, histopathologic examination, and expression profiling of wound healing genes. Up-regulation of proinflammatory cytokine interleukin 6 (IL-6) at Day 3 after infection was significantly dampened at Days 6 and 10 in the CHD-FA–treated wounds in both infection models, displaying an improved and accelerated wound healing.

CONCLUSION: CHD-FA is a promising topical remedy for drug-resistant wound infections. It accelerated the healing process of wounds infected with methicillin-resistant *S. aureus* and multidrug-resistant *P. aeruginosa* in rats, which is linked to both its antimicrobial and anti-inflammatory properties. (*J Trauma Acute Care Surg*. 2015;79: S121–S129.

KEY WORDS: CHD-FA; wound infections; cutaneous wound; rat model.

Combat-related injuries are frequently accompanied by significant tissue damage resulting in a variety of wounds that place injured soldiers at high risk for bacterial and fungal infections. The overall incidence of wound infections is estimated between 5.5% and 30% and can reach 40% for critically ill patients.[1–4] Injured combatants require timely administration of topical antimicrobial drugs to prevent infections resulting in serious complications and death. However, currently used topical drugs possess limited utility because of toxicity, incomplete pathogen coverage, inadequate wound bed penetration, and importantly, the growing problem with multidrug-resistant (MDR) organisms, such as *Pseudomonas* and *Acinetobacter* strains.[3,5] Fulvic acid, which is part of the humic substances formed during the decay of plant and animal residues in the environment, has been reported to have highly stable and unusual broad-based antimicrobial properties against both bacteria and fungi as well as anti-inflammatory properties.[6,7] Carbohydrate-derived fulvic acid (CHD-FA) is a pure form of fulvic acid, free of heavy metals and environmental pollutants, produced by a patented process to GMP standards (PA107470/GB).

Our goals of this preliminary proof-of-principle study were to confirm the wide-spectrum antimicrobial properties of CHD-FA and to evaluate the efficacy of topical application of CHD-FA on wounds in the early stage of methicillin-resistant *Staphylococcus aureus* (MRSA) or *Pseudomonas aeruginosa* infections in an in vivo rat model.

MATERIALS AND METHODS

In Vitro Susceptibility Testing

CHD-FA was supplied as a 4.6% solution by Fulhold, Ltd., Ebene Mews, Mauritius. Minimum inhibitory concentrations (MIC_{50} and MIC_{90}) for CHD-FA were established on a total of 500 clinical isolates representing wound-associated gram-negative bacteria (e.g., carbapenem-resistant *Escherichia coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, MDR *Acinetobacter baumannii*, *Enterobacter* species), gram-positive bacteria (e.g., MRSA), and fungi (e.g., azole-resistant *Aspergillus fumigatus*). Bacterial isolates were obtained from Dr. Barry Kreiswirth (PHRI, Rutgers, New Jersey Medical School). Fungal isolates

Submitted: October 30, 2014, Revised: February 26, 2015, Accepted: March 13, 2015.

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Part of this study was presented at 2014 MHSRS (Military Health Systems Research Symposium).

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DOI: 10.1097/TA.0000000000000737

were stocked in the Perlin laboratory serving as the Pfizer Echinocandin Resistance Reference Center. These isolates were derived largely from bloodstream, soft tissue, burn, wound, pustules, and respiratory fluid specimens. The clinical isolates represent a wide array of resistance profiles, especially multidrug resistance phenotypes, molecular types, and resistance mechanisms. All clinical isolates were subcultured onto semisolid rich medium to prepare fresh bacteria for the susceptibility testing. A highly standardized broth-based in vitro susceptibility assay following the Clinical and Laboratory Standards Institute (CLSI) protocol M07-A9 was used to determine the CHD-FA MIC values for all the bacterial strains,[8] while the CLSI protocol M38-A2 was used for all *Aspergillus* strains,[9] and the CLSI protocol M27-A3 was used for all *Candida* strains.[10] As specified in the guidance documents, quality control antibiotic-susceptible gram-negative and gram-positive bacterial strains from the American Type and Culture Collection and control antibiotics ciprofloxacin and doxycycline were used to ensure testing parameters conforming to the CLSI methodology.

In Vivo Animal Model

Induction of Wounds in Rats

Male 6-week to 8-week-old Sprague-Dawley rats (approximately 200 g) were used for this study. All experimental procedures were performed in accordance with National Research Council guidelines and approved by the Rutgers University Research Institutional Animal Care and Use Committee. Rats were anesthetized by intraperitoneal injection of 100-mg/kg ketamine +10-mg/kg xylazine. The dorsal side of the rats were shaved with electrical clippers and chemically depilated. The exposed skin was wiped with betadine. Using an 8-mm-diameter disposable biopsy punch, two symmetrical wounds were created on the dorsum of each rat. Sterile polyurethane rings were placed over the fresh wounds and attached with adhesive and also with four nylon microfilament sutures. Wounds were covered with a Tegaderm visible adhesive dressing, and rats were rehydrated with saline administered via subcutaneous injection. The analgesic buprenorphine (0.05 mg/kg) was administered twice daily for 2 days to minimize pain during surgical recovery. Rats were weighed daily, and wounds were observed daily for 10 days.

Wound Infection and Topical Treatment

MRSA strain Xen31 and *P. aeruginosa* strain Xen5 were used to establish wound infection, respectively. Once created, wounds were inoculated with 50-μL bacterial cell suspension at 1×10^8 colony-forming units (CFU) for MRSA or 1×10^7 CFU for *P. aeruginosa*. Infected rats were randomized into four treatment groups as follows: 4.6% CHD-FA, 0.46% CHD-FA, antibiotic control (20-μg/mL vancomycin for MRSA, and 20-μg/mL Colistin for *P. aeruginosa*), and untreated (sham) control. A total of 11 rats were used for MRSA trial, 4 rats for each CHD-FA group, 2 rats for untreated control, and 1 for antibiotic control. In *P. aeruginosa*–infected trial, 16 rats were divided as 5 rats per CHD-FA group and 3 rats per control group. All treatments were applied daily in 25-μL volumes, starting at 4 hours after inoculation. To monitor wound healing process, digital measurements of wound surface area were taken daily using a Nikon 4x Stereomicroscope with FS-1 digital camera every day throughout the 10-day experiment. To assess cellular response as well as histopathologic changes upon treatment, one rat from each group was sacrificed at Day 3 and experiment end point of Day 10. A Day 6 time point was added in the *P. aeruginosa* trial. At each time point, wounds were aseptically removed and split into three parts for bacterial burden counts, histopathologic examination, and expression profiling of wound healing genes.

Determination of Wound Healing Rate

Wound healing rate was calculated as the relative percentage of wound surface area at a specified time point compared with their respective Day 0 time point:

Wound healing rate = day N surface area / Day 0 surface area × 100%.

Assessment of Microbial Burden in Wound Sites

At each time point, skin covering the entire wound area from each sacrificed rat was aseptically removed, homogenized in 10-mL phosphate buffered saline, and plated onto brain-heart infusion or Luria Broth agar plates for microbial burden enumeration.

Histopathologic Analysis of the Wound

Samples for histopathologic examination were sent and processed in the pathology laboratory at Cornell University College of Veterinary Medicine in New York. Each wound was fixed in 10% neutral buffered formalin, paraffin embedded, sectioned, and stained with hematoxylin and eosin. The wounds were microscopically evaluated for the presence of neutrophils and macrophages to assess inflammation, the initiation of healing by the presence of fibroblasts and indicators of angiogenesis, and lastly, epithelialization to complete the remodeling of the wound site. A detailed point scoring system from 0 to 3 is used for histologic analysis. Scoring is based on cellularity, fibroblast proliferation, collagen deposition, granulation tissue formation, and epithelial travel in the wound.

Expression Profiling of Wound Healing Genes

Wounds collected for expression profiling were placed in RNAlater (Ambion, Austin, TX) solution and stored at −80°C until RNA extraction. Total RNA was extracted from the wound tissue by using the RNeasy Fibrous Tissue Mini Kit (Qiagen), according to the manufacturer's instructions. RNA was then subjected to expression profiling of 84 wound healing gene targets using Wound Healing RT^2 Profiler PCR Array (PARN-121Z; Qiagen). Real-time PCR array data were analyzed using the RT^2 profiler PCR array data analysis online software (Qiagen).

Statistical Analysis

Analysis of variance (ANOVA) was performed in GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA) to determine difference between various groups. Significance of expression fold change and fold regulation was tested by Student's *t* test in the PCR array data analysis online software (Qiagen). A $p < 0.05$ was considered statistically significant.

RESULTS

In Vitro Susceptibility

CHD-FA shows potent antimicrobial efficacy against a wide range of bacterial and fungal organisms with MIC_{50} and MIC_{90} values for clinical isolates of drug-sensitive and drug-resistant bacteria equal to or less than 0.125% CHD-FA, while the values for clinical isolates of drug-resistant fungi were less than or equal to 0.5% (Table 1). Consistent MIC values were observed from testing on four different batches of CHD-FA against a panel of representative MDR bacterial and fungal pathogens (data not shown). In general, CHD-FA showed strong activity against a variety of bacterial and fungal pathogens associated with wound infections.

In Vivo Efficacy on Cutaneous Wounds in Rats

To assess the wound healing efficacy of CHD-FA within a MDR pathogen infection scenario, we created an open wound model in rats and infected the wounds with MRSA and MDR *P. aeruginosa*, respectively. For each trial, wound healing rate, bacterial burden, histopathologic examination of wounds, and expression profiling of wound healing genes were observed and analyzed as follows.

Wound Healing Rate and Bacterial Burden

In MRSA-infected rats, both wound images and surface area measurement demonstrated improved healing from both CHD-FA concentration groups from Day 3 through Day 6, compared with the untreated sham control (Fig. 1*A* and *B*). However, because of the small animal numbers at each treatment group, the statistical significance of such differences was not achieved.

In the *P. aeruginosa*–infected wound treatment trial, three rats (two from 4.6% CHD-FA group and one from sham control) became moribund by Day 1 at the infection dose of 1×10^7 CFU. The remaining rats did display some signs of stress and presence of infection (e.g., slightly ruffled fur and mild recumbency). The study was permitted to continue as the rats were administered analgesia from the wound preparation and they maintained their weight throughout the trial. At Day 6, the last rat remaining in the sham control group became systemically infected; thereafter, no untreated rat was available for wound healing evaluation throughout the rest of the experiment. Even though wound surface area measurements were somewhat erratic because of the unexpected fatality in the early stage of infection as well as to scab formation and the prevalence of purulent discharge in all groups, wound healing in survived rats were significantly better in the CHD-FA–treated group compared with the Colistin treatment control group (Fig. 2*A* and *B*).

Bacterial burden in the wound sites were also assessed for both animal trials. However, the burden reduction from CHD-FA–treated groups in MRSA-infected rats was not significantly different from that observed in the untreated controls, neither was the vancomycin-treated control. Similarly, bacterial burdens in the wounds infected with *P. aeruginosa* were more than 10^7 CFU for all treatments groups. No significant burden reduction difference was observed at any time points between various treatment groups.

Histopathologic Evaluation of the Wound

For MRSA-infected rats, histopathologic evaluation of the CHD-FA and untreated wounds samples from Day 3 showed

TABLE 1. Average MIC_{50} and MIC_{90} Values of CHD-FA at 24 Hours and 48 Hours for All Bacterial and Fungal Strains

Organism	No. Isolates	MIC 24 h (%)			MIC 48 h (%)		
		MIC_{50} 24 h	MIC_{90} 24 h	Range*	MIC_{50} 48 h	MIC_{90} 48 h	Range*
Enterobacter cloacae	50	0.125	0.125	0.06–0.125	0.125	0.125	0.06–0.125
Enterobacter aerogenes	24	0.125	0.125	0.06–0.125	0.125	0.125	0.06–0.125
E. coli	50	0.125	0.125	0.06–0.125	0.125	0.125	0.06–0.125
K. pneumoniae	50	0.125	0.125	0.125–0.25	0.125	0.125	0.125–0.25
P. aeruginosa	50	0.06	0.125	0.06–0.125	0.125	0.125	0.06–0.125
Enterococcus faecium	50	0.06	0.06	0.03–0.125	0.125	0.125	0.03–0.125
MRSA	50	0.125	0.125	0.06–0.125	0.125	0.125	0.06–0.125
MSSA	50	0.125	0.125	0.06–0.125	0.125	0.125	0.06–0.125
Streptococcus pyogenes	50	0.06	0.06	0.06	0.06	0.06	0.06
A. fumigatus	12	0.5	0.5	0.125–0.5	0.5	0.5	0.125–0.5
Aspergillus flavus	12	0.5	0.5	0.5	0.5	0.5	0.5
Aspergillus terreus	5	0.125	0.125	0.125–0.5	0.5	0.5	0.125–0.5
Aspergillus niger	10	0.5	0.5	0.5	0.5	0.5	0.5
Candida albicans	24	0.5	0.5	0.125–0.5	0.5	0.5	0.125–0.5
Fusarium solani	5	0.125	0.125	0.125–0.5	0.125	0.125	0.125–0.5
Absidia corymbifera	5	0.5	0.5	0.125–0.5	0.5	0.5	0.125–0.5
Rhizopus oryzae	3	0.5	0.5	0.5	0.5	0.5	0.5
Penicillium marneffei	2	0.25	0.25	0.25	0.25	0.25	0.25
Penicillium chrysogenum	2	0.25	0.25	0.25	0.25	0.25	0.25

*MIC range of all isolates.
MSSA, methicillin-sensitive *S. aureus*.

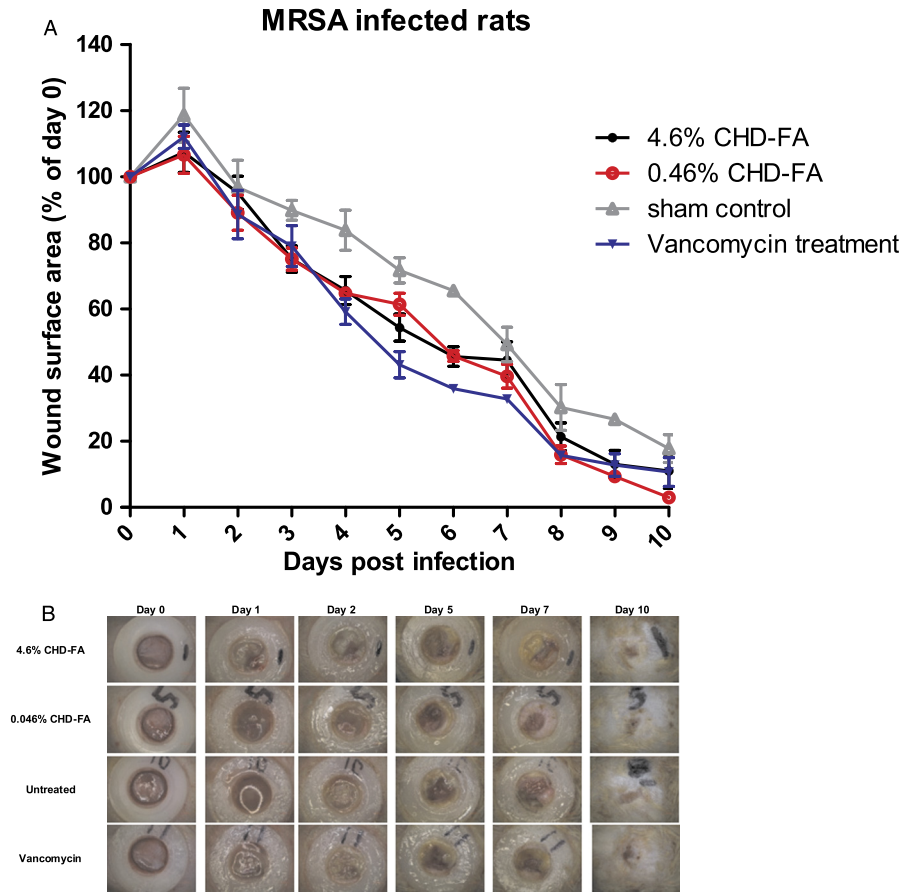

Figure 1. *A*, Wound surface area measurement in rats infected with 1×10^8-CFU MRSA under different treatment. *B*, Representative wound images taken over the 10-day experiment period from each treatment group.

higher scores in both macrophage and fibroblast categories in the 4.6% CHD-FA–treated wound (Fig. 3*A*). This is indicative of earlier wound healing and remodeling. On Day 10, epithelialization for the wounds treated with CHD-FA was better than the untreated wound and even the vancomycin-treated wound. Conversely, the neutrophils score was higher in the untreated wound. The decrease in cellular inflammation and increase in epithelialization suggested that wound healing was significantly improved with CHD-FA treatment.

A similar trend was observed from study looking at *P. aeruginosa* infections. Data (Fig. 3*B*) showed that the infection and inflammation in the wounds treated with CHD-FA were better controlled relative to untreated and Colistin-treated groups, as indicated by lower neutrophils scores as early as Day 3. Tissue remodeling was also at a more advanced stage for CHD-FA–treated wounds at the end point of experiment compared with the Colistin control group.

Expression Profiling of Wound Healing Gene Targets

To better understand the nature of the enhanced wound regeneration observed in response to CHD-FA, we examined a large panel of genes implicated in this wound remodeling process.

MRSA-Infected Rat Wound Model

Of the 84 key genes central to wound healing, there was a marked increase of fivefold or greater in 15 genes, namely, CCL12, CCL7, CSF2, CSF3, CXCL1, CXCL11, CXCL3, CXCL5, IL-10, IL-1b, IL-6, MMP9, PTGS2, SERPINE1, and TNF, and 4 genes, namely, ACTC1, CDH1, ITGB6, and TGFA, had decreased expression by fivefold or greater in infected wounds, compared with baseline normal skin (skin punctures collected from wound creation served as baseline control). At Day 3, wounds from both 4.6% CHD-FA treatment and sham control group shared similar expression profiling, except the expression of CSF2 (granulocyte-macrophage-colony stimulating factor, GM-CSF), CXCL3 (inflammatory chemokine), IL-10 (anti-inflammatory factor), and PTGS2 (signal transduction) was up-regulated by an additional threefold in CHD-FA–treated wound compared with the sham control (Fig. 4*A*), indicating more balanced inflammation response and progression toward accelerated wound healing in the CHD-FA–treated wounds. By Day 10, while the expression of most genes returned to baseline level in the CHD-FA–treated wound, two prominent genes IL-6 and MMP9, of which prolonged overexpression lead to unfavorable healing outcome, were still overexpressed in the untreated wound (Fig. 4*B*).

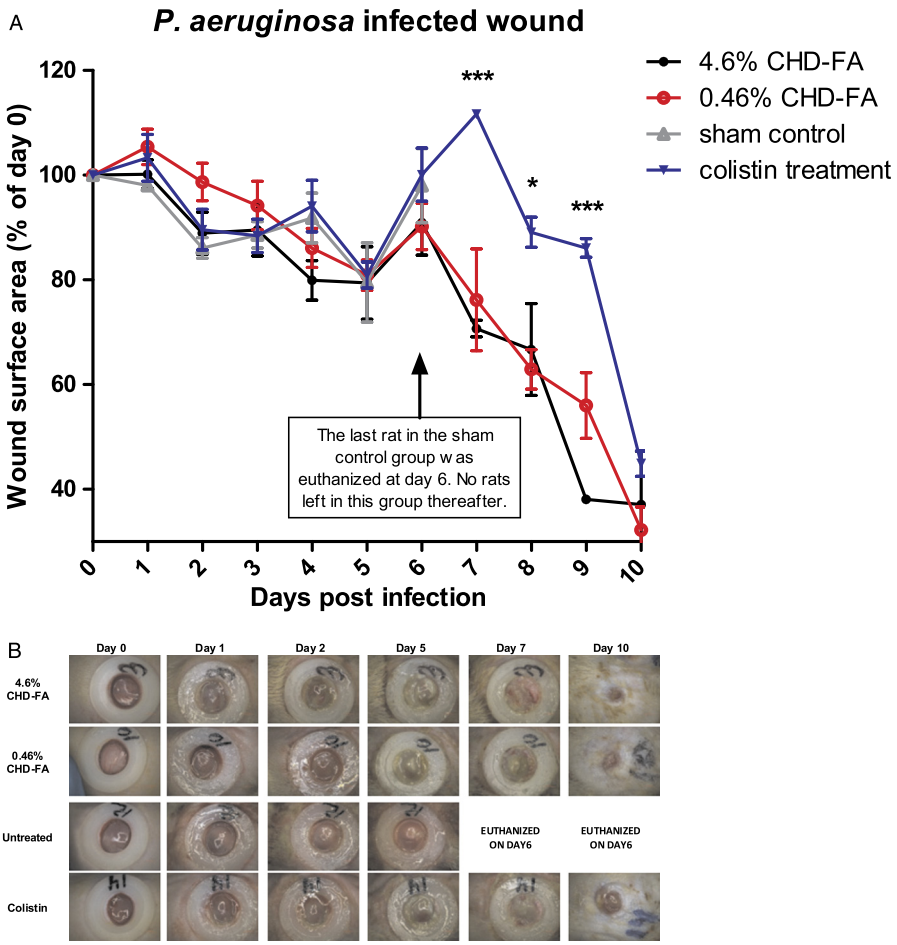

Figure 2. *A*, Wound surface area measurement in rats infected with 1×10^7-CFU *P. aeruginosa* under different treatment. *$p < 0.05$, ***$p < 0.001$: comparison between CHD-FA groups with Colistin control. *B*, Representative wound images taken over the 10-day experiment period from each treatment group.

P. aeruginosa–Infected Rat Wound Model

The expression profile of *P. aeruginosa*–infected wounds at Day 3 was very similar to MRSA-infected wounds but displayed more drastic expression fold changes. Genes that showed significant overexpression were nearly identical with those described earlier for MRSA-infected wounds at the same time point. Whereas, more genes with decreased expression levels were found in *P. aeruginosa*–infected wounds compared with those infected with MRSA. These genes are mainly involved in extracellular matrix component (COL14a1, COL1a1, and COL1a2) and cellular adhesion (CDH1 and ITGB6), indicating more profound tissue damage caused by *P. aeruginosa* (Fig. 5*A* and *B*). As expected, the expression changes at Day 3 were generally greater in the CHD-FA–treated wound than in the sham control. Namely, growth factor genes CSF2 (GM-CSF) and CSF3 (granulocyte-colony stimulating factor, G-CSF), chemokine CXCL1 and CXCL3, anti-inflammatory factor IL-10, and signal transduction genes PTGS2 and SERPINE1 all had an additional threefold or higher up-regulation in the CHD-FA–treated wound compared with the untreated control. In addition to the anti-inflammatory nature of IL-10 and the confirmed influence of CSF2 and CSF3 on migration and proliferation of endothelial cells, CXCL1 and CXCL3 are also known to induce endothelial cell proliferation in vitro and angiogenesis in vivo.[11,12] Taken together, greater overexpression of these genes in the CHD-FA–treated wound relative to the untreated control indicated an accelerated wound healing process induced by CHD-FA. Furthermore, expression profiling at Day 6 revealed contrasting behavior between CHD-FA–treated and untreated wounds (Fig. 5). At Day 6, most genes except IL-10 were swiftly returning back to baseline expression level in CHD-FA–treated wound but gradually increasing expression in the sham control. The rapid restoration of differentially regulated wound healing gene expression in the CHD-FA–treated wounds demonstrated a faster cellular response and improved tissue repair exerted by CHD-FA. In particular, the expression level of IL-6 was gradually increased during the first 6 days of the experiment, and the increase reached to 2,485-fold by Day 6 in the untreated wound. In contrast, in the CHD-FA–treated wound, IL-6 was overexpressed by 791-fold at Day 3, whereas the up-regulation was rapidly and significantly dampened at Day 6.

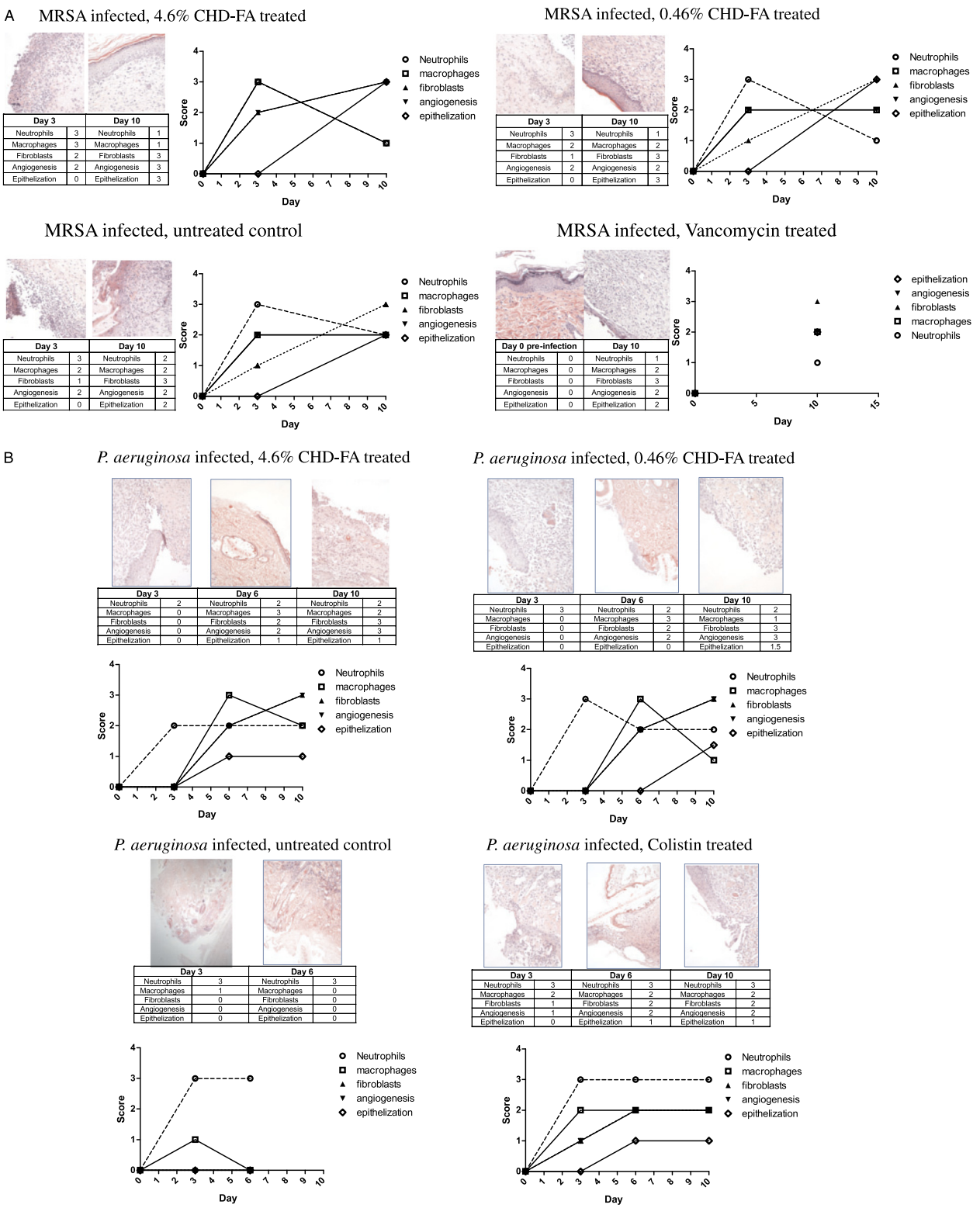

Figure 3. Histopathologic analysis of wounds from rats infected with 10^8-CFU MRSA and treated with 4.6% CHD-FA, 0.46% CHD-FA, sham control, and 20-μg/mL vancomycin, respectively (*A*); wounds from rats infected with 10^7–CFU *P. aeruginosa* and treated with 4.6% CHD-FA, 0.46% CHD-FA, sham control, and 20-μg/mL Colistin, respectively. Wound samples were scored based on the abundance as well as morphologic characteristics of neutrophils, macrophages, fibroblasts, angiogenesis, and epithelialization. Briefly, the presence of neutrophils and macrophages provides evidence of inflammation, fibroblasts and angiogenesis scores measure the proliferation phase, and the extent of epithelialization corresponds to the remodeling of the wound site.

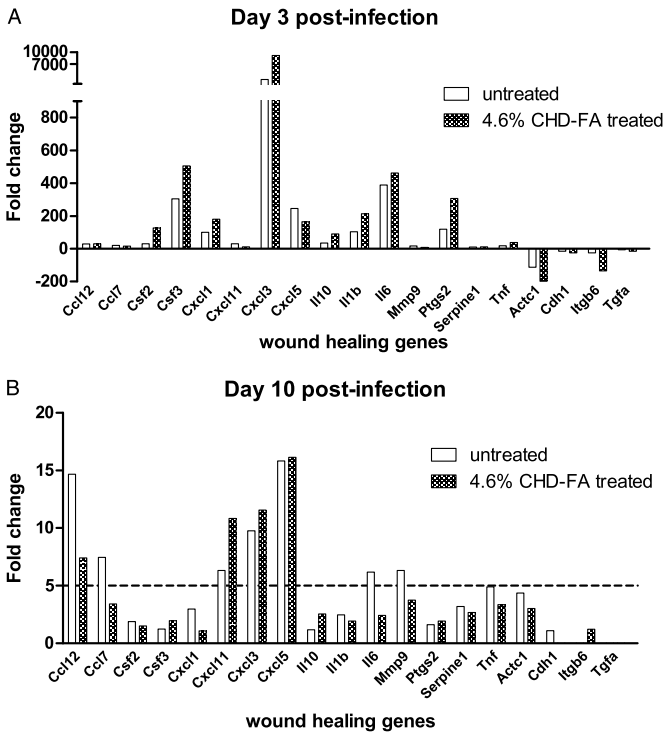

Figure 4. Prominent wound healing gene expression level changes in wounds infected with 1×10^8–CFU MRSA, treated with 4.6% CHD-FA, or sham control, respectively, at Day 3 after infection (*A*) and Day 10 after infection (*B*).

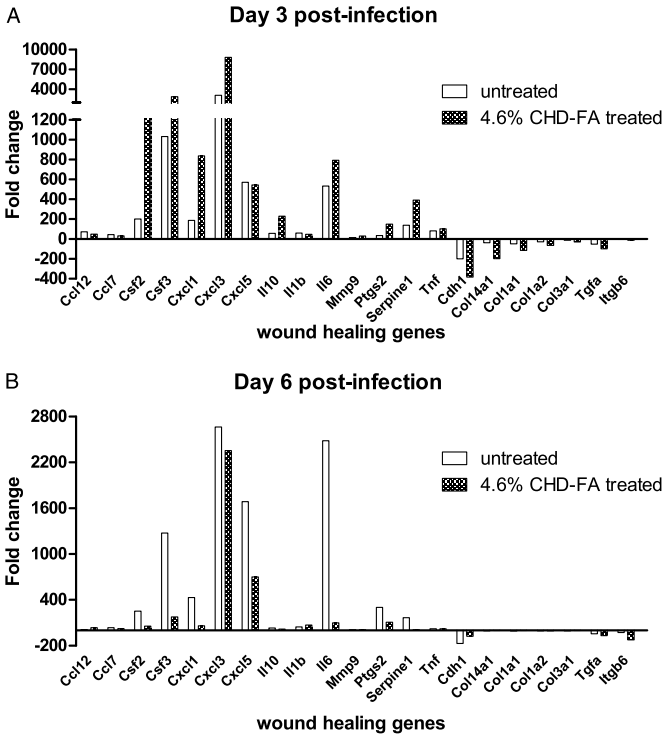

Figure 5. Prominent wound healing gene expression level changes in wounds infected with 1×10^7-CFU *P. aeruginosa*, treated with 4.6% CHD-FA, or sham control, respectively, at Day 3 after infection (*A*) and Day 10 after infection (*B*).

DISCUSSION

Wound healing is a complex, dynamic process composed of four distinct yet overlapping phases as follows: hemostasis, inflammation, proliferation, and remodeling.[13,14] These phases are well coordinated by a cascade of external and internal stimuli such as growth factors and cytokines in a well-orchestrated manner, resulting in regeneration and restoration of the damaged skin.[15,16] To comprehensively evaluate the potency of CHD-FA to promote healing of traumatic wounds in the early stage of drug-resistant bacterial infections, we have applied a multidimensional data analysis strategy from in vitro MIC testing to in vivo wound size measurement, histopathology, and gene expression profiling. Robust antimicrobial potency of CHD-FA was demonstrated by consistent MIC data in a large collection of MDR bacteria and fungi. Furthermore, in vivo studies also displayed promoted healing upon early use of CHD-FA on infected wounds, although the composite results are less straightforward than MIC values.

MRSA-infected wounds treated with CHD-FA showed an improved wound size reduction relative to the sham control from Day 3 to Day 6. This observation corresponded well with the histopathologic examination, which presented both more advanced proliferation phase at the early stage of infection, more reduced inflammation, and better tissue remodeling at the end point of the experiment upon the use of CHD-FA. We did not observe any remarkable bacterial burden reduction induced by CHD-FA treatment in this MRSA-infected rat model, which seemed to be linked with the fact that a bacterial biofilm mixed with scab formed over the partial wound area, resulting in a complex that was largely refractory to drug action. The drug seemed to penetrate the wound bed at the periphery of the biofilm-scab complex restricting emergence of free cells, but the tight complex remained intact and rather drug impermeant, which prevented us from observing a significant bacteria burden reduction.

Similarly, CHD-FA also enhanced wound healing in the *P. aeruginosa*–infected rats. Although the unexpected morbidity caused by the highly virulent MDR *P. aeruginosa* strain resulted in no rat available for the sham control group after Day 6, histopathologic data strongly supported the fact that wound healing was accelerated by CHD-FA treatment. Moreover, the healing rate in the later stage (from Day 6 to Day 10) of CHD-FA–treated wounds was even faster than that of the Colistin-treated control. Bacterial burden in the wound sites remained stable throughout the entire experiment or even slightly increased for some of the animals, mainly because of the high infection dose. Survived animals may have developed transient systemic infections during the first 6 days of the experiment and slowly recovered thereafter.

Direct observation of wound healing through wound closure and bioburden measurement was prone to many confounding factors such as inoculation dose, scab and biofilm forming, and pathogen virulence, and thus can obscure underlying tissue remodeling. Expression profiling of wound healing genes allowed us to gain clearer insights into the cellular and molecular responses of the infected wound in the presence of different topical therapies. By using a pathway-specific PCR array technology, a panel of genes was found to have pronounced differential expression over the course of wound healing in both MRSA and *P. aeruginosa*–infected rats. It is noticeable that CHD-FA–treated wounds were more primed to proceed toward wound healing in both infection models. For instance, IL-10, one of the most important anti-inflammatory cytokines in which its overexpression promotes wound healing,[11,17] was threefold more highly expressed than the untreated control at Day 3, suggesting that CHD-FA–treated wounds were more likely to progress into the next stage of healing by lessening the severity of inflammation. Therefore, by Day 10 when most genes restored to baseline expression, there were still four genes (inflammatory chemokine CCL12 and CCL7, proinflammatory cytokine IL-6, and matrix metalloproteinase MMP9) highly expressed in the sham control, compared with the CHD-FA–treated wound. Given that both prolonged overexpression of IL-6 and MMP9 are associated with impaired wound healing,[18–21] such data were in line with histopathologic results and provided further evidence of enhanced wound healing effect of CHD-FA. More convincingly, the same dynamic trend was again observed in the *P. aeruginosa*–infected rat model, where Day 6 wounds demonstrated a completely reversed expression profile relative to that obtained from Day 3. Most overexpressed genes in the CHD-FA group were found to be at a much greater level at Day 3 compared with the untreated control, and their expression swiftly restored toward baseline at Day 6 when the same panel of genes in the sham control was still slowly rising. In particular, the key biomarker of impaired wound healing IL-6[18] was constantly overexpressed through Day 3 to Day 6 in the untreated wound. In contrast, the significantly reduced (25-fold) expression of IL-6 in the CHD-FA–treated wound manifested accelerated and better controlled wound healing.

In summary, CHD-FA is a highly promising topical remedy to promote healing of wounds infected with MRSA and *P. aeruginosa*. With more data acquired from ongoing animal experiment tested with other drug-resistant bacterial and fungal species, further evaluation of this product in human clinical trial is warranted to determine efficacy of topical CHD-FA in preventing wound infections and promoting healing in injured military and civilian personnel.

AUTHORSHIP

S.P. and D.S.P. conceived of this study. Y.Z., S.P., and D.S.P. designed the experiments. S.L. provided CHD-FA and participated in project progress discussion. P.P., G.D., E.G., Y.Z., and M.H.L. performed the experiment and data acquisition. M.S., Y.Z., P.P., G.D., M.H.L., and M.S. analyzed the data. Y.Z., S.P., and D.S.P. drafted the manuscript. All authors participated in critical revision and approval of the final manuscript.

DISCLOSURE

D.S.P. is the principal investigator of this project, and this work is funded by US DMRDP with award number W81XWH-12-2-0076. S.L. is the chief scientific officer of Fulhold, Ltd. All other authors declare that they do not have any conflicts of interest.

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