Nonsteroidal anti-inflammatory drugs may affect cytokine response and benefit healing of combat-related extremity wounds

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Background. *After adequate operative debridement and antimicrobial therapies, combat-related extremity wounds that either heal or fail are both associated with a distinct inflammatory response. Short-term use of nonsteroidal anti-inflammatory drugs in postoperative pain management may affect this response and, by consequence, the healing potential of these wounds. We investigated whether patients treated with nonsteroidal anti-inflammatory drugs had a distinct inflammatory response; different rates of critical colonization, defined as* $>10^5$ *colony forming units on quantitative bacteriology; and healing potential.*
Methods. *We retrospectively reviewed the records of 73 patients with combat-related extremity wounds. Patients were separated into 2 groups: those who received nonsteroidal anti-inflammatory drugs during the debridement period (nonsteroidal anti-inflammatory drugs group,* N = *17) and those who did not (control group;* N = *56). Serum and wound tissue samples collected during each operative debridement were measured for 32 known cytokines and tested for quantitative bacteriology, respectively. We compared cytokine concentrations between groups and then designed a logistic regression model to identify variables associated with successful wound healing, while controlling for known confounders.*
Results. *Despite similar demographics and wound characteristics, the nonsteroidal anti-inflammatory drugs group had significant lesser concentrations of inflammatory cytokines, interleukin-2, interleukin-6, interleukin-8, and monocyte chemoattractant protein-1. On multivariate analysis, nonsteroidal anti-inflammatory drug treatment emerged as a predictor of successful wound healing after controlling for known confounders such as wound size, tobacco use, Acute Physiology and Chronic Health Evaluation II score, and critical colonization.*
Conclusion. *Treatment with nonsteroidal anti-inflammatory drugs for postoperative pain management after major combat-related extremity trauma is associated with lesser concentrations of inflammatory cytokines and may contribute to a more favorable inflammatory response leading to successful wound healing. (Surgery 2017;161:1164-73.)*

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Modern combat-related extremity injuries are most often due to explosive blasts. The resulting wounds contain massive zones of injury and range from soft tissue injuries, to comminuted open fractures, to multiple traumatic amputations. The treatment of these polytraumatized individuals involves a series of operative debridements, performed every 24–72 hours, and is followed by eventual delayed primary closure, coverage with various cutaneous or myocutaneous flaps, and liberal use of split thickness skin grafts,[1,2] depending on the extent of the wound(s).

Systemically, patients exhibit a massive inflammatory response that persists throughout the debridement phase. The polytraumatic nature[3,4]

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and extent of injuries in these patients may contribute to this persistent inflammatory response. The patterns and magnitude of inflammation is associated with various untoward outcomes such as venous thromboembolism,[5] heterotopic ossification, and wound failure[6] in combat casualties. These associations highlight not only the severity of wounds, but also the role of the dysregulated and potentially exaggerated systemic inflammatory response in influencing each wound's local inflammatory response. As such, it is possible that modulating the systemic inflammatory response would have an effect on the local response in terms of modified, and even improved, wound healing.

During the debridement phase, nonsteroidal anti-inflammatory drugs (NSAIDs) may be used as part of a comprehensive pain management regimen. As with other patient populations,[7-9] combat-injured casualties are prescribed NSAIDs to help control their postinjury pain. These drugs are known to inhibit cyclooxygenase (COX) enzymes and, by consequence, affect the production of prostaglandins and thromboxanes, which participate in the inflammatory response. On a more fundamental level, NSAIDs may interact with transcriptional factors and affect the production of cytokines,[10-14] specifically by inhibiting the level of interleukin (IL)-6 and tumor necrosis factor-α, mediators known to play a role in angiogenesis,[15,16] tissue repair,[17] and wound healing.[18]

After injury, the innate inflammatory response involves the balanced production and release of cytokines, which are required to initiate and control the inflammatory response and ultimately promote wound healing. Failure of appropriate organization of this response, whether insufficient in patients who are immunosuppressed or hyperinflammatory, as observed in severely injured combat casualties,[19,20] may be associated with local wound complications such as critical colonization (CC), defined as growth of at least 10^5 colony forming units (CFU) per gram of tissue samples in culture,[21] or frank wound failure (WF).[6,20] In the present study involving combat casualties, we investigate whether NSAIDs used during the debridement phase are associated with measurable changes in the systemic inflammatory response and whether NSAID use is associated with successful wound healing.

METHODS

Study population. We prospectively reviewed collected clinical, laboratory, and treatment-related variables from 73 adult combat casualties enrolled in an institutional review board–approved study and treated at our institution between 2007 and 2012. As part of a continuum of care, these patients were treated previously at US military medical facilities abroad and transferred to the Walter Reed National Military Medical Center (WRNMMC) 3–6 days after injury. Throughout the patients' treatment, standardized clinical practice guidelines were utilized as part of the Department of Defense Joint Trauma System. Casualties were evacuated and underwent their first operative debridement and damage control operation based on the "golden hour" mandate by the Secretary of Defense Robert M. Gates.[22,23] These patients would then move along the continuum of care until they arrived at WRNMMC. At each facility they would undergo further operative debridement or definitive operation as appropriate. Patients who did not receive NSAIDs were transferred to the WRNMMC 3–6 days after injury (average of 4.5 days), and patients who received NSAIDs arrived at the WRNMMC 3.5–6 days after injury (average of 4.0 days; Table I).

This study was performed in compliance with the Federal Health Insurance Portability and Accountability Act and informed consent was obtained at the time of treatment from all participating patients. The informed consent included study participation involving the collection of samples and demographic data, follow-up treatment outcomes, and an authorization for future data analysis and publications.

Demographics and clinical data, including mechanism of injury, wound characteristics, and days to wound closure, were collected for each patient. A detailed review was performed of each patient's operative notes, hospital course, and wound culture data. Regardless of the mechanism of injury, patients treated with NSAIDs comprised the study group (NSAIDs group; $N = 17$), whereas those who were not prescribed these drugs comprised the control group ($N = 56$). We compared age, body mass index (BMI), tobacco use, duration of treatment until definitive wound closure, Injury Severity Score (ISS), Acute Physiology and Chronic Health Evaluation II (APACHE II) score, critical colonization (bacterial growth $\geq 10^5$ CFU per gram of tissue) of wounds, wound surface area, wound size, systemic cytokine profile, and wound healing outcome between groups. Wound failure was defined as wound dehiscence, spontaneous partial or complete wound disruption after primary closure or loss of >90% of a skin graft. Wounds that did not require a return to

Table I. Demographics, NSAIDs treatment, and outcomes

Demographics median (IQR) or %	*Control (N = 56)*	*NSAID (N = 17)*
Age (y)	22 (20–25)	22 (21–25)
BMI (kg/m^2)	25 (23–26.7)	25.6 (24.3–27.3)
Previous tobacco use (%)	22 (39)	5 (29)
Wound mechanism		
Crush injury (%)	—	1 (6)
Gunshot wound (%)	8 (14)	1 (6)
Explosive blast (%)	48 (86)	15 (88)
Extremity wounds	86	30
Soft-tissue injuries (%)	23 (27)	8 (27)
Open fractures (%)	20 (23)	3 (10)
Total amputations (%)	43 (50)	19 (63)
Transhumeral amputation (%)	1 (2)	—
Transradial amputation (%)	2 (5)	—
Partial hand (%)	1 (2)	2 (11)
Hip disarticulation (%)	1 (2)	—
Transfemoral amputation (%)	13 (30)	10 (53)
Knee disarticulation (%)	4 (9)	1 (5)
Transtibial amputation (%)	21 (49)	6 (32)
ISS	16 (9–25)	18 (12.5–20.5)
APACHE II on admission	4 (3–6)	4 (2.5–8)
APACHE II at definitive closure	4 (3–7)	4 (2–6.5)
Admission time (d)	4.5 (3–6)	4 (3.5–6)
Wound size on admission (cm^2)	174 (130.2–262.6)	222.5 (118.7–389.3)
Wounded surface on admission (cm^2)	223.8 (133.6–489.1)	426 (373.5–575.7)
NSAID started (days from injury)	—	7 (4.5–13.5)
NSAID treatment (d)	—	4 (1–7.5)
Wounds with CC (%)	36 (42)	13 (43)
Patients with CC (%)	19 (34)	7 (41)
Definitive closure time (d, %)	10 (8–12.5)	10 (8–17.5)
Wounds which failed (%)	22 (26)	4 (13)
Patients with wound failure (WF, %)	14 (25)	3 (18)
Wounds which failed with CC (%)	10 (12)	2 (7)
Patients with WF and CC (%)	6 (11)	2 (12)

Distribution of patients in the control and NSAIDs group according to age, BMI, previous tobacco use, wound mechanism, wound type, amputation level, ISS, APACHE II on admission and at definitive closure, admission time, wound size on admission, wounded surface area on admission, NSAID starting time in days from injury, NSAIDs treatment duration, wounds with CC, patients with CC, definitive closure time, wounds which failed, patients with WF, wounds which failed with CC, and patients with CC and WF. Median and interquartile range (IQR) values are shown for age, BMI, ISS, APACHE II, admission time, wound size, wound surface area on admission, NSAIDs starting time in days from injury, NSAIDs treatment duration, definitive closure time, and percent of total (%) values are shown for previous tobacco use, wound mechanism, extremity wound types, wounds with CC, patients with CC definitive closure time, wounds which failed, patients with WF, wounds which failed with CC, and patients with CC and WF.

the operating room at 30 days were regarded as healed.[19]

Sample collection. Sample collection was performed as previously described[19] and is summarized here. Peripheral venous blood (~8 mL) was collected in the operating room prior to each operative debridement. Samples were immediately separated using a refrigerated centrifuge at 2,500 *g* at 40°C for 10 minutes. After centrifugation, supernatants of serum samples were transferred to Cryo-Loc polypropylene tubes and labeled (Lake Charles Manufacturing, Lake Charles, LA). Samples were then flash-frozen in liquid nitrogen and stored at −80°C until batched analysis. Pictures were obtained before each debridement procedure and analyzed using the PictZar Pro digital planimetry software (PictZar, Elmwood Park, NJ) to measure wound surface area.

Cytokine analysis. Serum samples were filtered using a 0.65 μm filter (Millipore, Billerica, MA) prior to analysis with a Human Cytokine 30-plex panel supplemented with a custom Human 2-plex panel (Invitrogen; Cat. No LH6003 and LCP0002) for 32 cytokines: IL-1α, IL-1β, IL-1ra, IL-2, IL-2R, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-15, IL-17, granulocyte-macrophage colony-stimulating factor, granulocyte-colony stimulating factor, interferon-γ, interferon-α, tumor necrosis

factor-α, epidermal growth factor, bfibroblast growth factors, hepatocyte growth factor, vascular endothelial growth factor, Eotaxin, monocyte chemoattractant protein (MCP)-1, macrophage inflammatory protein-1α, macrophage inflammatory protein-1β, regulated on activation, normal T cell expressed and secreted, monokine induced by gamma interferon, and interferon gamma-induced protein-10. To perform these assays, we used a multiplex analysis platform (Luminex 100 System, Luminex, Austin, TX) and analyzed the results using the software BeadView (Upstate V1.0.4.23259, Millipore, Billerica, MA).

Quantitative bacteriology. A wound tissue biopsy sample ($\sim$1 cm^3) was collected during each debridement from the center of the wound. These samples were weighed and stored in a sterile 15-mL conical vial at 4°C for subsequent culture. As described previously,[24] tissue samples were transferred to a sterile disposable tissue grinder and diluted 1:10 (wt/vol) in fastidious broth and homogenized, reaching a final concentration of 0.1 gram of tissue per milliliter. Next, samples were inoculated on sheep's blood agar and MacConkey agar plates in triplicate. These plates were incubated overnight at 37°C and, after incubation, colonies were counted. The total number of CFUs per gram of tissue was determined and a phenotypic identification of colonies performed using an automated bacterial identification system (Phoenix, Becton Dickinson, Sparks, MD). Bacterial growth $\geq 10^5$ CFU per gram of tissue was defined as CC.

Statistical analysis. A power analysis of the study population was performed to provide a 2-tailed comparison of 2 independent means considering a ratio of 0.30 as the incidence of patients receiving NSAIDs, $\alpha = 0.05$, a proposed effect size of 0.8, and power of at least 0.8. The analysis verified that a minimum of 55 patients would be required for the control group and 17 patients would be needed for the NSAIDs group to obtain an actual power of at least 0.81. Fifty-six patients in the control group and 17 patients in the NSAIDs group were evaluated. In addition, we analyzed the difference in proportions of successful healing considering all debridement operations in all wounds of both groups (NSAIDs = 117/125 and control = 193/252) with an effect size of 0.498, considering $\alpha = 0.05$. We calculated a power of 0.995 for a 2-tailed comparison.

Next, we analyzed continuous variables using an unpaired t test with Welch's correction or the Kruskal-Wallis, followed by Dunn's multiple comparison, depending on the results of a Kolmogorov-Smirnov test. This approach demonstrated whether the data followed a normal distribution. The P values were adjusted using the false discovery rate controlled with a Benjamini-Yekutieli adjustment. Furthermore, the relationship of wound size, previous tobacco use, ISS, APACHE II, NSAIDs treatment, days of debridement, development of CC, and the level of serum cytokines with a successful healing outcome were evaluated in a linear logistic regression model that considered P values, the odds ratio of each variable, and 5-fold cross-validation. The level of all cytokines associated with NSAID use were evaluated further based on time from injury by the permutational multivariate analysis of variance using distance matrices (nonparametric multivariate analysis of variance) test, as described previously.[25] Statistical analyses were performed using the RStudio software (version 0.98.1103, RStudio, Inc, Boston, MA). The normality test and the edition of all figures were performed using the GraphPad Prism 4.0 software (GraphPad Software, Inc., La Jolla, CA). Serum analysis and outcomes were considered per patient, as opposed to per wound, unless stated otherwise.

RESULTS

During the 6-year study period, the 73 combat casualties comprising our study population all had the following clinical characteristics: ISS $\geq$8, a combat-related extremity wound requiring at least 2 debridement operations, and wound treatment with negative pressure wound therapy. Overall, the median age, BMI, ISS, and admission APACHE II score for our study group was 22 (21–25), 25.6 (24.3–27.3), 18 (12.5–20.5), and 4 (2.5–8), respectively. Among these basic demographics and injury characteristics, including wound size, ISS, APACHE II, and time to wound closure, there was no statistically significant difference between the study groups.

Overall, CC was not statistically different between groups and occurred in 35% of the study population. For the control group, CC was present in 36 wounds (41%) of 19 patients (34%) and in 13 wounds (43%) of 7 patients in the NSAIDs group (N= 17). Therefore, casualties prescribed NSAIDs had similar odds of developing CC compared with controls (OR = 1.3; confidence interval, 0.4–4.1). Among patients who used NSAIDs and developed CC, 3 had positive cultures identified with *Acinetobacter baumannii* and 3 had the following single organisms isolated: *Enterococcus faecium*, *Achromobacter sp.*, and *Escherichia coli*. In the control group, 4 patients who

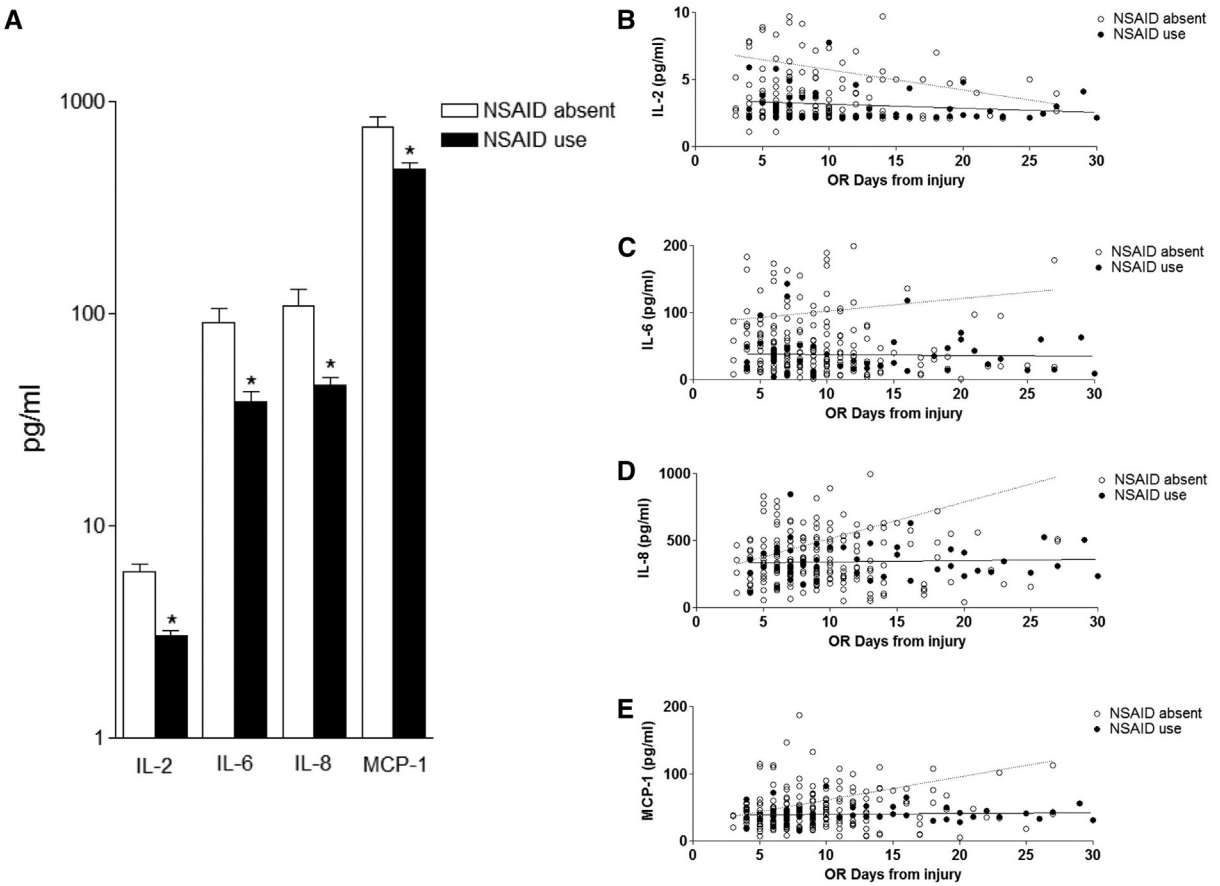

Fig 1. Serum cytokine level of patients associated with NSAIDs treatment. (*A*) Serum level of cytokines in the absence of NSAIDs and during the use of these drugs. (*B–E*) Level of cytokines depicted in *A*: IL-2, 6, 8, and MCP-1, respectively, of all patients in the absence and presence of NSAID use. Linear regression was calculated to illustrate the progression of the level of these cytokines from injury to definitive wound closure. All outliers were included in these regression calculations, although some may not be shown. *A statistical significant difference ($P < .05$) as indicated.

developed CC had positive cultures identified with *A baumannii* and 4 tested positive for a single organism, which included *Staphylococcus aureus*, *Achromobacter sp.*, *E faecium*, and *Enterobacter cloacae*. The remaining patients in both groups who developed CC had multiple organisms isolated in culture.

Patients used NSAIDs for approximately 4 days (range, 1–7.5 days), starting at a median of 7 days (range, 4.5–13.5 days) after injury. Dosing of these drugs varied with each patient's condition and pain status. Within the NSAIDs group, 9 patients used cyclooxygenase nonspecific inhibitors (COX NSI), and 7 patients used Celecoxib 100 mg or 200 mg (Celebrex, Pfizer Inc, Mission, KS), a cyclooxigenase-2 specific inhibitor (COX-2 SI). In addition, 1 patient was prescribed both a COX NSI and Celecoxib. Among the COX NSI, 5 patients were given acetylsalicylic acid 325 mg, 3 patients were administered ketorolac 15 mg or 30 mg (Toradol, Roche Inc, Nutley, NJ), 2 patients used ibuprofen 600 mg or 800 mg (Motrin, Johnson & Johnson, New Brunswick, NJ), and 1 patient was provided indomethacin 25 mg (Glenmark Pharmaceuticals Inc, Mahwah, NJ; Table I).

Samples obtained during all debridement operations performed before primary closure of each wound were studied. When compared with the control group, we found that patients using NSAIDs had significantly lesser serum levels of IL-2, IL-6, IL-8, and MCP-1 (Fig 1, *A*). Linear regression illustrated how patients using NSAIDs maintained stability of the levels of these cytokines throughout operative debridement until primary closure of wounds was achieved (Fig 1, *B–E*). In addition to the linear regression, a nonparametric multivariate analysis of variance, which was used as a confirmatory test, showed the level of these same cytokines was greater in the absence of NSAID treatment ($P = .02$) based on time from injury.

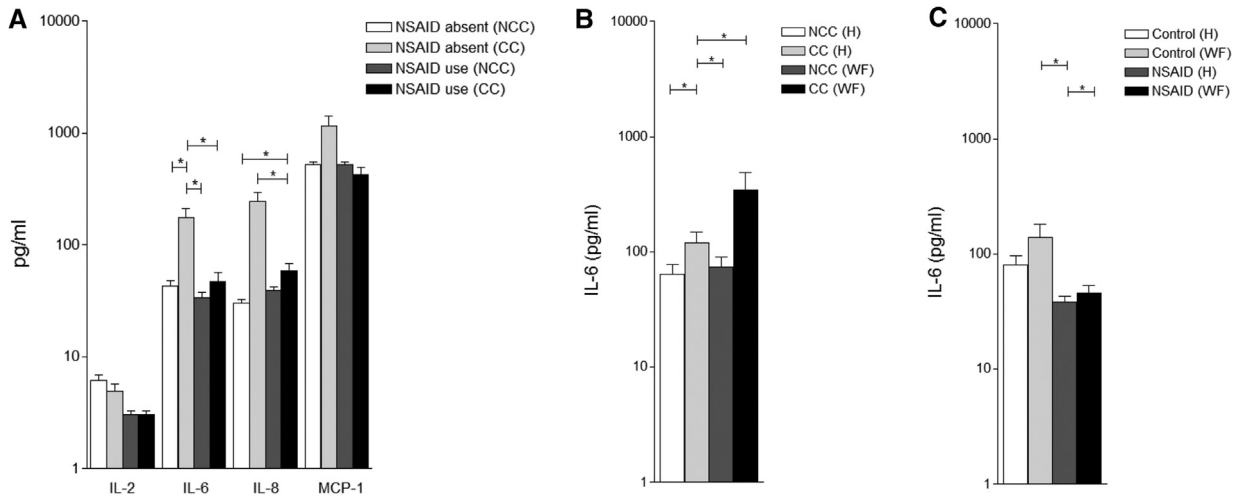

Fig 2. The key role of IL-6. (*A*) Serum level of cytokines in the absence of NSAIDs and during the use of these drugs considering separately patients who were not critically colonized and those who developed CC. (*B*) Serum level of IL-6 in patients whose wounds healed successfully (H) and in those with WF considering separately patients who were not critically colonized and those who developed CC. (*C*) Serum level of IL-6 in patients in the control group and of those in the NSAIDs group shown separately considering those whose wounds healed successfully (H) and those with WF. *A statistical significant difference ($P < .05$) as indicated.

The critical role of IL-6 in the development of CC and WF is depicted in Fig 2. Samples obtained during all debridement operations until wound closure revealed that patients with CC in the control group had significantly greater levels of IL-6 (and IL-8) compared with patients in the NSAIDs group with CC (Fig 2, *A*). For all patients who developed both CC and WF, a significantly greater level of serum IL-6 was observed compared with casualties without CC or WF (Fig 2, *B*). Additionally, when compared with the control group, the NSAIDs-treated patients with successful wound healing had significant lesser levels of IL-6 (Fig 2, *C*).

We developed a logistic regression model to identify predictors of successful wound healing in our study population, while controlling for known confounders. Regression analysis demonstrated an association of smaller wound size, absent tobacco use, lesser APACHE II, presence of NSAIDs treatment, greater levels of serum IL-2, and lesser levels of serum IL-6 with successful wound healing (Table II).

Table II. Logistic regression model of successful wound healing outcome

Successful wound healing outcome	*Odds ratio (95% CI)*	P *value*
Wound size	0.9 (0.9–0.9)	<.01*
Previous tobacco use	0.4 (0.2–0.8)	.01*
ISS	1.0 (0.9–1.0)	.15
APACHE II	0.8 (0.7–0.9)	<.01*
NSAID treatment	6.5 (2.7–17.7)	<.01*
Critical colonization	1.8 (0.8–3.9)	.10
Treatment time	1.0 (0.9–1.2)	.07
IL-2	1.1 (1.0–1.2)	.01*
IL-6	0.9 (0.9–0.9)	.02*
IL-8	1.0 (0.9–1.0)	.57
MCP-1	1.0 (0.9–1.0)	.41

*A statistical significant difference ($P < .05$), as indicated.
The occurrence of successful wound healing was evaluated using the following predictors: wound size, previous tobacco use, ISS, APACHE II, NSAIDs treatment, development of CC, operative treatment time from admission to definitive wound closure, and the level of cytokines associated with the use of NSAIDs. Odds ratio, 95% confidence interval, and *P* value of each predictable variable are shown.

DISCUSSION

We investigated the effect of NSAIDs prescribed for perioperative pain management on wound outcomes and the systemic inflammatory response in patients with combat-related extremity wounds. These drugs were prescribed for each patient depending on his or her pain status following the standard of care. The effects of NSAID use and possible association with successful wound healing were evaluated as these drugs were prescribed. The evaluation also considered several variables as possible sources of selection bias. Those variables, described in Table I, include wound size and wounded surface area upon admission, number of wounds with critical colonization, admission time, and definitive time of wound closure. None

of these variables had significant differences when comparing patients who received NSAIDs to those who did not.

In general, proper wound healing after injury requires a balanced release of cytokines regulating the inflammatory response. The underlying mechanism of an imbalanced and dysregulated response on wound healing is poorly understood. To this end, we have demonstrated previously that CC and WF are associated with distinct cytokine responses, and when associated with combat-related extremity wounds, may pose considerable challenges in the treatment of these patients.[20,21] However, clarifying the process involved in the normal regulation of this response may be invaluable in preventing and treating wound complications.

NSAIDs are known to be associated with the inhibition of inflammatory cytokines.[10,12] However, the effects of NSAIDs on the systemic levels of cytokines during the treatment of traumatic extremity wounds have not been extensively characterized.[26] In the present study, we observed that patients treated with NSAIDs had lesser concentrations of serum inflammatory cytokines and chemokines, which may work to dampen or organize this hyperinflammatory response and favors a lesser rate of wound failure. In addition to the effects on the inflammatory profile, it also is possible that the inhibition of the COX enzyme by NSAIDs may promote increasing leukocyte phagocytic uptake and reactive oxygen intermediate-mediated killing of bacteria,[27] which may further contribute to successful wound healing, particularly in combination with antibiotic treatment. However, we did not observe a significant difference in the rate of CC between groups, and our understanding of the potential physiologic role of NSAIDs on mitigating local complications and wound infections remains unclear.[28]

The present study demonstrated NSAID treatment may be associated with lesser and perhaps more stable systemic levels of IL-2, IL-6, IL-8, and MCP-1. Among these cytokines, IL-2 is known to participate in the proliferation and differentiation of T-cells,[29] activation of natural killer cells,[30] and induction of immunoglobulin production by B-cells,[31] a process also regulated by IL-6.[32] IL-2 also plays an important role in wound healing.[33] Therefore, the regulatory effects of NSAIDs may influence both cellular and humoral immune responses by these 2 cytokines. Interestingly, we have shown previously that lesser levels of wound effluent IL-2 are associated with wound dehiscence,[19] which favors our current findings that increased serum IL-2 levels are associated with successful healing. Although the NSAIDs group had lesser levels of IL-2 compared with the control group, greater IL-2 levels were found to be markers for success; thus, it seems that a balanced expression of IL-2 may be beneficial for proper healing.

The effects of IL-6 are described as having both anti- and proinflammatory activity depending on cell type and phase of the response. An important mechanism regulated by IL-6 is the pool of neutrophils in inflammatory sites, previously attracted by chemokines. Neutrophils and monocytes are attracted to these sites by different groups of chemokine signals. Among these signals, IL-8 favors neutrophils and MCP-1 favors monocytes. IL-6 inhibits IL-8-based signals and enhances MCP-1-based signals producing a switch in favor of attracting monocytes to inflammatory sites. Additionally, IL-6 is known to skew monocyte differentiation toward macrophages,[34] which are more stationary phagocytes and contribute in the clearing of cell debris and bacteria in inflammatory sites. In the current study, CC patients had increased levels of IL-6 in the absence of NSAID treatment, but not during NSAID treatment. Patients critically colonized also had a greater level of IL-8 in the absence of NSAIDs. Both IL-6 and IL-8 are thought to be associated with sepsis,[35] although the level of these cytokines in CC during NSAID treatment is not well characterized. Furthermore, patients with WF and CC had greater levels of IL-6, which was lesser in NSAID-treated patients with successful healing. Therefore, among the cytokines affected by NSAID treatment, IL-6 may play a key role in association with wound healing in these patients. It is important to acknowledge that despite similarly occurring proportions of CC and identified bacteria in both groups, even if most of the wounds had not being critically colonized, it still is possible that different bacteria may promote different cytokine response[36-38] and thus establish differing local wound microenvironments.

The current study also evaluated the association of independent variables in successfully predicting wound healing in a logistic regression model. Among these variables, smaller wound size, no previous tobacco use, lesser APACHE II, greater IL-2, lesser IL-6, and NSAID treatment were found to be associated with successful healing. Of these variables, NSAID treatment, the only modifiable variable, had the highest odds ratio in association with wound healing and may represent an important variable to predict successful healing in our study group. It also should be acknowledged that, although not statistically significant, the

development of CC was inversely related to wound healing in this study population. This may be due to the relatively few patients who developed CC.

Several limitations to this work deserve mention. These patients represent a very specific cohort of predominately blast victims. As we described previously,[19,20,24] the inflammatory response in patients with combat-related extremity wounds is very pronounced. It is important to note that some patients have been exposed to extensive and complex injuries involving traumatic amputations of multiple extremities, as well as pelvic and abdominal trauma. Furthermore, the subsequent series of debridement operations, combined with the development of critical colonization in certain cases, could prolong or intensify this after-injury inflammatory response, thus providing an additional challenge to proper healing.

Furthermore, it is unknown whether the results observed in this study apply to other populations, including civilians, presenting with less severe injuries; however, this investigation is currently underway by our group and collaborating civilian universities.

In addition, our relatively small sample size may have over- or understated physiologic relationships[39]; therefore, we reported our findings based on the patient distribution according to prescription of NSAIDs. This distribution was shown to be independent of variables such as age, BMI, previous tobacco use, overall wound dimensions, admission time in days from injury, ISS, and APACHE II scores. Also, due to the sample size, we were not able to account for the differences between specific types of NSAID medications, the dosing, timing, or treatment duration and potential effects on wound healing. Other confounding variables such as the type of wound closure, antibiotic choice, treatment duration, and concomitant injuries limiting NSAIDs use could not be controlled. Finally, prospective corroboration of these findings is underway by our group in a randomized clinical trial designed to evaluate the effects of COX2 inhibition on the healing of combat-related extremity wounds.

In the present study, NSAID treatment may affect the level of systemic cytokines in patients with major combat-related extremity trauma. In addition to other variables such as wound bioburden, patient treatment based in subsequent debridement operations may further prolong or maintain the inflammatory response in such a way that the inhibitory effects of NSAID use in the level of inflammatory cytokines may be associated with favorable healing outcomes, a subject worthy of additional study. Additional studies are necessary to investigate the use of these drugs in the regulation of the immune response after injury to clarify possible beneficial effects of the use of these drugs in wound healing after major extremity trauma. These studies should include a larger group of patients while controlling for wound size and injury severity, if possible, and use a randomized design to further investigate the role of NSAIDS in preventing postoperative complications such as wound failure.

CONFLICT OF INTEREST DISCLOSURES

None reported. The views expressed in this manuscript are those of the authors and do not reflect the official policy of the Department of the Army, Department of the Navy, the Department of Defense or the United States Government. This effort was supported (in part) by the US Navy Bureau of Medicine and Surgery under the Medical Development Program and Office of Naval Research work unit number (604110HP.3740.001.A1506 and 604771N.0933.001.A0604). We are military service members or employee of the US government. This work was prepared as part of our official duties. Title 17 U.S.C. 105 provides the "copyright protection under this title is not available for any work of the United States Government." Title 17 U.S.C. 101 defines a US government work as a work prepared by a military service member or employee of the US government as part of that person's official duties. This study protocol was approved by the Naval Medical Research Center (NMRC) and Walter Reed National Military Medical Center Institutional Review Board in compliance with all applicable Federal regulations governing the protection of human subjects. The NMRC/WRNMMC institutional review board approved protocol number is NMRC.2005.0012/352334 and the protocol title is "The use of the vacuum assisted wound closure device in treating extremity wounds." We certify that all individuals who qualify as authors have been listed; each has participated in the conception and design of this work, the analysis of data (when applicable), the writing of the document, and the approval of the submission of this version; that the document represents valid work; that if we used information derived from another source, we obtained all necessary approvals to use it and made appropriate acknowledgments in the document; and that each takes public responsibility for it.

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