Reducing the pathogen burden and promoting healing with polyhexanide in non-healing wounds: a prospective study

• **Objective:** Polyhexamethylene biguanide (PHMB) is a novel wound antiseptic solution that has a broad antimicrobial spectrum and wound healing promoting effect, with minimal side effects. The aim of present study was to demonstrate the efficacy of the PHMB on the bacterial burden of non-healing wounds, the reduction in wound size or closure of the wound in comparison to Ringer's lactate solution (RLS) after 21 days of wound dressing. A second objective was to investigate the differences in the C-reactive protein (CRP) levels and white blood cell (WBC) counts between the two groups.
• **Method:** In this prospective and open-cohort clinical study, patients who underwent cardiac surgery between July 2006 and August 2008 were included and placed in one of two groups, PHMB or RLS, by the block randomisation method. A wound care team, consisting of two surgeons, a wound care nurse and a microbiologist, was created. Classic moist dressings and wet compresses during dressing changes were applied at least once a day or as needed. During the 21 days of hospitalisation the team collected data on the wound healing status daily, on infection parameters every third day, and wound tissue for culture weekly.
• **Results:** There were 40 patients recruited of which 9 were excluded, leaving 31 in the intention-to-treat analysis. Of these 15 received PHMB and 16 received RLS. Overall 17 of 31 (66.7% PHMB group, 43.8% RLS group, p=0.181) patients were treated successfully by closure of surgically sutured wounds or healing by secondary intention, the wound tissue cultures of 19 of 31 patients (47.4% PHMB, 52.6% RLS, p=0.886) were negative, and wound size of all the patients were significantly reduced in clinical observations. Although the CRP levels were reduced significantly within group comparisons in both groups ($p<0.001$), it was significantly lower after 12 days ($p<0.05$) in the PHMB group compared with the RLS group.
• **Conclusion:** The results of this study emphasise that the successful treatment of chronic non-healing wounds require a multidisciplinary team approach under the control of a wound care specialist. Whatever the disinfectant used, consistency in the approach to treatment may be more important. We suggest that increasing the use of PHMB and adoption of this team approach in other cardiac centres or other populations may decrease the healing period, especially in chronic non-healing wounds.
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polyhexamethylene biguanide; antiseptics; wound healing

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Necrosis and wound infection usually inhibit wound healing.[1] For most patients, an underlying disease interferes with normal tissue repair, resulting in chronic wounds, which do not heal as expected. It has been shown that critical bacterial colonisation has a direct impact on wound healing.[2] Polyhexanide (polyhexamethylene biguanide, PHMB) is an antiseptic substance that plays a prominent role in wound treatment due to its efficient reduction of microbial loads and excellent cell and tissue tolerability. It has beneficial characteristics such as a broad antimicrobial spectrum, ability to bind to the organic matrix, and wound healing promoting effect.[3] An increasing number of articles on the subject of chronic wound antisepsis with PHMB can be found in the medical literature.[3–5] However, there are still very few published randomised clinical trials on the practical use of PHMB-containing wound antiseptics on non-healing wounds. Our aim is to determine the efficacy of PHMB in the treatment of the wound bacterial burden, and reduction in wound size or closure of the wound. A second objective was to investigate the differences in CRP levels and WBC count.

Material And Methods

Study design

We conducted a prospective open-cohort clinical study between July 2006 to August 2008 to assess the

rate of successful treatment of the wound bacterial burden, reduction of inflammation in the wound bed, and reduction in wound size. Patients who underwent cardiac surgery and had complications of a pressure ulcer (PU) or surgical site infection (SSI) with non-healing wound were included in the study. Written informed consent was obtained from all of the patients. In the study, the patients were classified into two groups; treatment with PHMB or treatment with Ringer lactate solution (RLS) group. The block randomisation method was used to allocate subjects.

Study population

The patients' inclusion criteria were having undergone coronary artery bypass graft surgery (CABG), aged 40–60 years. All the patients enrolled in the study were in the postsurgical and were taking antibiotic therapy empirically or according to culture results. The patients' exclusion criteria were body mass index (BMI) <18kg/m^2 or >34kg/m^2,[6] poor left or right ventricular function (ejection fraction <30%), patients with sepsis or multiorgan failure, death from any reason other than the wound complications during the study period, use of different disinfectants in the study arms and allergy to PHMB.

Wound care team

A wound care team, who were the part of nosocomial infection control committee, consisted of two surgeons, a wound care nurse and a microbiologist. The team examined the wound dressing of the patients, debridement of necrotic tissue, signs of infection in the wound and infection parameters in the blood. All chronic wound cases were diagnosed by attending team physicians and confirmed by the nosocomial infection control committee.

Case criteria

A chronic wound was defined according to the guidelines for assessment of wounds and evaluation of healing.[7] Wound infections were diagnosed on the basis of criteria developed by the Centers for Disease Prevention and Control (CDC). Infection at the wound site can include the following types:

- Superficial incisional (infection above the sternum or on extremities with no bone involvement)
- Deep incisional (infection involving the sternum or extremity under the deep fascia)
- Organ/space (site-specific infection, such as mediastinitis).

Signs and symptoms of wound infection include: the classical Celsian signs of inflammation, spontaneous separation of the incision edges to leave an open wound (the wound may need to be deliberately opened when there is a suspicion of a purulent collection), abscess or other evidence of infection found by direct examination during re-operation, or by histopathological or radiological examination.

After admission to the study, a researcher collected data on the participants' surgery, measurement of wound size, length of the epithelialised wound tissue (mean of the three different wound points), culture results, temperature, CRP level, WBC count, and antibiotics usage. The researcher continued to collect a daily wound healing status, data about infection parameters (CRP level and WBC count) every third day, and weekly wound tissue cultures throughout the hospitalisation period of 21 days. The team surgeons decided upon the frequency and the type of the wound debridement. Debridement was conducted once every week or as required. Antibiotics were administered based on the culture results and the antibiogram of the pathogen. In wound treatment, classic moist dressings and wet compresses during dressing changes were applied using RLS (RLS group; Neoflex, Turktıpsan Sag. Tic. A.S. Ankara, Turkey). No disinfectants were used in the control arm. In the PHMB group, 0.5% PHMB-containing disinfectant (Actolind, Abem Kimya Ltd. Sti. Istanbul, Turkey) was used for the preparation and irrigation of the wound, and in the moist dressing. The standard procedure included cleaning around the dressing, followed by opening of the dressing and cleaning around the wound site with moisturised gauze containing RLS or PHMB. A scalpel was used for cutting the necrotic tissues during wound debridement. The wound was washed with RLS or PHMB to remove any debris and exudate. Sterile gauze soaked with RLS or PHMB was used to wipe out the exudate on the wound. Finally, the wound was closed with moisturised gauze and compress containing RLS or PHMB. Wounds dressings were changed once a day in the morning or whenever dressing compress seemed contaminated with exudate. The study was conducted for 21 days as recommended for use of the PHMB solutions. After the study was terminated, moisturisation with RLS and wet gauze dressing was continued for all non-healed wound dressings. The primary end point was to obtain healthy and bloody granulation tissue on the wound site and negative bacterial cultures. The secondary end point of the study was closure of the surgical suture wounds or secondary intention.

Data analysis

Study data were evaluated using SPSS 21.0 for Windows (Lead Technologies, Chicago). The continuous variables were calculated as mean ± standard deviation (SD) and categorical variables were calculated as %. Repeated measure test and the Mann-Whitney U test were used for univariate continuous variables and the χ^2 test was used for categorical variables. In addition, Fisher's exact test was conducted whenever the χ^2 expected

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value of at least one cell was <5. Bonferroni post-Hoc tests were performed for within-group comparisons. The interaction between initial infection and patient characteristics (age, gender, diabetes mellitus, BMI and PU) were considered with logistic-regression model. p<0.05 was considered as statistically significant.

Result

A total of 40 patients were randomly assigned to each study group: 20 to the RLS group and 20 to the PHMB group. There were nine patients excluded from the per-protocol analysis: four underwent negative-pressure wound therapy, two dropped out of the study after 1 or 2 days due to an allergy to PHMB, and three died before completion of the 21-day follow-up. Therefore, 31 patients, 15 received PHMB (n=9 with PUs, n=6 for SSI) and 16 received RLS (n=9 with PUs, n=7 with SSI), were included in the per-protocol analyses.

Wound closure was successful in 17/31 (66.7% PHMB group, 43.8% RLS group, p=0.181) patients who were surgically sutured or healed by secondary intention. Wound tissue cultures in 19/31 patients (47.4% PHMB, 52.6% RLS, p=0.886) were negative, and wound size in all these patients were notably reduced in the clinical observations. The average wound closure time for all the patients was around 15 ± 4 days (15 ± 5 days in the PHMB group and 16 ± 3 days in the RLS group, p=0.462). The length of the new epithelial tissue was measured at the end of 21 days in patients who had not achieved complete closure. The average length of the epithelialised scar tissue was 6.4 ± 4.4mm. The length of the new scar tissue was 10.4 ± 4.09mm in the PHMB group, and 4.22 ± 2.81mm in the RLS group and the difference was statistically significant (p=0.015).

The patients groups were similar with respect to demographic characteristics, comorbidities, risk factors for infection, antimicrobial exposure, and the duration of and types of surgery (Table 1). The mean duration of the non-healed wound before treatment was 27 days (range: 7–63 days). All of the patients received systemic antibiotics after the initial cultures and antibiogram results were obtained. There were no significant differences in the type or number of antibiotics given to the two study groups.

At the baseline, the mean CRP values and WBC counts were not significantly different the between groups. Baseline CRP value was initially 95.3 ± 8.7 and was reduced to 7.8 ± 5.8 after 3 weeks of wound treatment with PHMB. Similarly, baseline CRP value was initially 88.2 ± 26.4 and was reduced to 18.3 ± 12.4 after 3 weeks of wound with RLS. The reduction of CRP was statistically significant within group comparison for both groups (p<0.001). During the study, CRP measurement was significantly lower after 12 days (p<0.05) in the PHMB group compared with the RLS group (Table 2).

The baseline WBC counts were 13.5 ± 5.9 versus 12.7 ± 5.4 (in PHMB and RLS groups respectively)

Table 1. Demographic and clinic information of the patients

Parameter	Group I (n=15)	Group II (16)	p*
Age (years)	64 ± 12	60 ± 10	0.276
Gender, Male (%)	40	43.8	0.561
BMI (Kg/m^2)	27.8 ± 5.2	28 ± 6.3	0.859
Obesity (%)	42.2	37.5	0.596
Diabetes mellitus (%)	60	56.3	0.561
Pressure ulcer (%)	60	56.3	0.561
COLD (%)	26.7	18.8	0.685
ICU stay (days)	13 ± 10	8 ± 11	0.033
Hospital stay (days)	56 ± 38	57 ± 46	0.514
Corticosteroid intake (%)	26.7	18.8	0.685
Cured During Study (%)	66.7	43.8	0.181
Superficial infection (%)	46.7	37.5	
Deep infection (%)	40	43.8	0.851
Organ spaced infection (%)	13.3	18.8	
Fewer in the first week of the study (˚C)	37.8 ± 0.8	37.7 ± 0.6	0.827

*p indicates the significance between groups comparison with independent samples T-test or Fisher's Exact Test and <0.05 is considered significant. BMI – Body Mass Index; COLD – chronic obstructive lung disease; ICU – intensive care unit; Group I patients were treated with polyhexamethylene biguanide; Group II patients were treated with Ringer's Lactate solution.

Table 2. Laboratory parameters of patients

	Parameter	Baseline	Day 3	Day 6	Day 9	Day 12	Day 15	Final	p*
CRP	Group I (n=15)	95.3±28.7	85.1±30.1	70.1±31.5	49.9±24.1[c]	31.8±18.2[d]	19.2±13.3[e]	**7.8±5.8[f]**	<0.001
	Group II (n=16)	88.2±26.4	92.3±28.7	82.3±13.2	62.8±18.3	48.4±17.3[d]	30.9±16.2[e]	**18.3±12.4[f]**	<0.001
p value for CRP between groups		0.45	0.55	0.32	0.41	0.16	0.36	**0.002**	
WBC	Group I (n=15)	13.5±5.9	19.4±8.2[a]	16.3±5.7	5.1±7.8	12.1±3.1	10.6±3.4	10.3±2.9	<0.001
	Group II (n=16)	12.7±5.4	21.8±6.9[a]	17.1±5.5	15.5±4.7	14.0±5.2	13.0±5.1	11.9±3.5	<0.001
p value for WBC between groups		0.707	0.277	0.843	0.313	0.527	0.192	0.173	

*p defines the inter group comparison which was found with repeated measure test; [a]Baseline versus day 3; [b]Baseline versus day 6; [c]Baseline versus day 9; [d]Baseline versus day12; [e]Baseline versus day;15 [f]Baseline-versus final day; Bonferroni Test which was significant at <0.05. Between groups comparison was found with independent samples T-test, and p<0.05 were considered significant.
CRP – C-reactive protein; WBC – white blood cell; Group I patients treated with polyhexamethylene biguanide; Group II Patients treated with Ringer's Lactate solution.

which increased to 19.4 ± 8.2 versus 21.8 ± 6.9 after the first week a significant increase in both groups (p<0.001). However after 3 weeks, the WBC count was reduced to the normal range 10.3 ± 2.9 versus 11.9 ± 3.5 in both of the in PHMB and RLS wound treatment groups, respectively (Table 2).

For the patients in the ITT population, the initial rate of wound infection was not significantly different between the groups. The infection rate of the wounds generally decreased in both groups, but the decrease was not statistically different between the PHMB and RLS groups (Table 3). To understand the relation between initial infection and patient characteristics, the data were included in a logistic-regression model which found none significant (p=0.587, p=0.595, p=0.152, p=0.918, p=0.793 for age, gender, diabetes mellitus, BMI and PU respectively).

Adverse events occurred in two patients in the PHMB group. The patients had pruritus, erythema, or both around the surgical wound that was judged to be related to the study drugs; however, no serious adverse events were associated with the study drugs. A total of three patients died; one them in the PHMB group who suffered from stroke and two in the RLS group who died from myocardial infraction (MI) and pulmonary emboli.

Discussion

In this prospective open-cohort clinical study, significant changes in plasma concentration of CRP and WBC count were observed after the first debridement. In the entire cohort, the CRP levels and WBC counts were observed to decrease over the 21-day with a significant peak in the CRP levels and WBC counts were detected in the first week. Although WBC counts did not show any significant changes between the groups over time, CRP levels were decreased significantly more after 12 days of PHMB treatment.

As reported previously by other researchers, the median WBC counts and CRP levels are usually higher in invasive bacterial infected wounds than non-infected wounds, but the values can vary widely.[5,7–9] Although the elevation of WBC count may not predict the presence of infection, elevation of both WBC count and CRP levels can indicate an inflamed and infected wound. CRP, an acute-phase protein, is secreted by the liver in response to a variety of inflammatory cytokines, particularly IL-6 and Tumor Necrosis Factor-α, after trauma, inflammation, or infection.[8] CRP acts as a pattern recognition molecule to activate the adaptive immune response via the innate immune system. CRP levels are of value in three clinical situations:

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Table 3. Infection Rates of the wounds which identified with weekly wound tissue cultures

Parameter		Group I (n=15)	Group II (16)	p*
Infection Rates identified with culture of the wounds (%)	1st culture	86.7	81.3	0.682
	2nd culture	53.3	56.3	0.870
	3rd culture	60.0	43.8	0.366
	4th culture	40.0	37.5	0.886

*p indicates the significance between groups comparison with χ^2 or Fisher's Exact Test; <0.05 is considered significant.

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- Monitoring the response to antibiotic treatment in patients with known bacterial infections
- Differentiation between active disease and infections in patients with systemic inflammatory diseases such as systemic lupus, rheumatoid arthritis, and ulcerative colitis
- Early detection of complications postoperatively.[9]

CRP values could be serially followed for diagnosing postoperative wound infections and inflammation of patients while antibiotics are being administered.[10] In the present study, all the patients had complications of postoperative non-healed wounds, or PUs and 26/31 were diagnosed with infection by wound tissue cultures. These patients' initial CPR values were 30 fold higher than the normal range and CRP was increased significantly from the initial value after the first week in both arms of the study. In this period of the treatment, all patients got a major debridement, which may be the main reason of the increase in CRP levels from the initial measurement. Abdel Hafez et al.,[11] reported that increases in CRP during the onset of sepsis and the release of IL-6, which is a proinflammatory mediator, induces the synthesis of hepatic acute-phase proteins including CRP. During the study period, CRP decreased to the lower values in all of the patients but decreased to normal ranges only in the patients whose wounds have healed completely. The decrease of the CRP value was significantly higher in the PHMB group when compared to the RLS group and the former therefore may be considered to be more effective in the management of bacterial burden. Moreover, wound bed inflammation was reduced with PHMB. It has been shown that PHMB shows good efficacy and tolerability in bacterial burden control for chronic wounds with different levels of microbial involvement.[12] Dissemond et al.,[13] have reported in an *in vitro* study that the anti-inflammatory effect of PHMB was through the inhibition of the formation of reactive oxygen species/reactive nitrogen species.[13] In the present study we concluded that the wound bed of the PHMB group had much more healthy granulation tissue when compared with the RLS group.

On the other hand, it would also be important to highlight that the process of wound management is not the domain of any particular discipline and requires a multidisciplinary team approach from hospital to home. It must also be incorporated into disease management and managed aftercare. In order to be successful, there must also be knowledgeable collaboration between wound care specialists (both physicians and nurses), dermatologists, paediatricians or geriatricians, surgeons, and primary care physicians. With team work, patient acuity could be documented accurately, risk factors weighted, existing wounds properly assessed and reassessed, plans of care developed, goals of care identified, care competently delivered, utilisation of services and products charted, and outcomes monitored and measured. In the present study, the multifaceted approach to the wound care management resulted in more successful positive outcomes and produced a faster healing response as has also been reported in the literature.[14]

Full-thickness skin wounds are preferably allowed secondary wound healing under controlled hydration dressings.[14] Secondary wound healing is a slow-running processes and the development of re epithelialisation can take 4–8 weeks.[7] Successful reepithelialisation in both groups shows the importance of a moisturised dressing and regular maintenance in the present study but length of epithelialised scar tissue was statistically significant and clinical examination of granulation tissue formation was notably in PHMB group. For successful completion of the healing process in addition to surgical debridement and systemic antibiotics, some antiseptics may be applied in wound dressing especially in chronic non-healing wounds. These antiseptics should be selected carefully, because cell membrane acting antiseptics have toxic effects on fibroblasts and impair the inflammation, epithelialisation, and synthesis of collagen.[1] Even at very low concentrations of povidone-iodine, which is toxic to phagocytes, has been shown to suppress lymphocytic response.[12] Dissemond et al.,[13] have reported in an *in vitro* study that the anti-inflammatory effect of PHMB was through the inhibition of the formation of reactive oxygen species/reactive nitrogen species. The results of this study may indicate that PHMB is more effective than RLS in providing regenerative events in the wound environment.

The limitations of the current study include its small size and restriction to patients who have undergone cardiac surgery. Several favourable results were obtained through observations in the study that were agreed upon by the all the members of the wound care team. Although consecutive photographs of these observations were taken, the data could not be demonstrated in a scientific manner in the manuscript.

Conclusion

We report a randomised prospective study that examines clinically relevant interventions and end points in a major complication that accompanies cardiac surgery. The results emphasise that the success of the chronic non-healing wound treatment requires a multidisciplinary team approach under the control of wound care specialists. Whatever the disinfectant used, regular practice and a consistent approach may be more important. We suggest that the use of PHMB and adoption of this team approach in other cardiac centres or other populations may increase the success of treatment and decrease the healing period, especially in chronic non-healing wounds. ■