A prospective evaluation of methylene blue and gentian violet dressing for management of chronic wounds with local infection

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Abstract

The objective of this prospective, non-randomised study was to evaluate the performance of an antibacterial foam dressing containing methylene blue and gentian violet (Hydrofera Blue Classic dressing®) for the management of chronic wounds with local infection. Patients in this study were ≥18 years of age (n = 29), and each had at least one chronic wound ≥1 cm² in size that showed signs of localised infection or critical colonisation but with good potential for healing based on clinical assessment. To all of these wounds, the dressing was applied and changed three times per week over the 4-week study period. The primary endpoints of the study were: (i) changes in wound surface area measurement, (ii) changes in Pressure Ulcer Scale for Healing (PUSH) scores, (iii) changes in percent surface area of devitalised tissue (i.e., yellow slough or other necrotic tissue) and (iv) changes in clinical signs associated with localised wound infection/critical colonisation. Participants were evaluated at presentation (week 0 = baseline), week 2 and at week 4 (end of the study). The 29 patients completed the study, and at week 4, the following wound improvements were observed: (i) baseline mean wound surface area was significantly reduced by 42.5%, from 21.4 cm² at week 4 (P = 0.005); (ii) baseline mean PUSH score decreased significantly from 13.3 at week 4 (P = 0.001); (iii) baseline mean wound coverage by devitalised tissue (%) was significantly reduced, from 52.6% at week 4 (P = 0.001) and (iv) the mean UPPER and LOWER wound infection scores were reduced from 3.6 at baseline to 0.9 at week 4 (75%; P < 0.001). These results indicate that the Hydrofera Blue Classic dressing was effective at managing these chronic wounds and helped them progress onto a healing trajectory.

Introduction

Chronic wounds are increasingly common because of an ageing population and a growing prevalence of chronic conditions, such as diabetes and vascular diseases (1). However, these wounds are becoming more difficult to treat, contributing to high morbidity and placing a significant burden on health care systems worldwide (2, 3). For example, chronic wound care now consumes 2–3% of health care budgets in developed countries (3). In Canada, these wounds have been recognised as an important health issue, particularly among the elderly and those with diabetes (4). Non-healing wounds affect an estimated 2% of the US population alone, at an annual cost of care exceeding $25 billion (1, 5, 6).

Key Messages

• localised wound infection is common but difficult to treat
• methylene blue and gentian violet dressing promote wound healing and reduce signs associated with wound infection
• the dressing was effective in promote desloughing of the wound bed

Wound healing can stall for a number of reasons, including an elevated bioburden (7). It has been demonstrated that when bacterial growth reaches a critical threshold, bacterial toxins can cause tissue damage in the superficial wound compartment,
delaying the healing process. This phenomenon is referred to in the literature as critical colonisation, increased bacterial burden, superficial infection or local infection. Early treatment of local infection may prevent the formation of biofilms and the invasion of bacteria into deeper tissue. Topical antimicrobial dressings are warranted when signs and symptoms of local infection are present (7). Biofilms are complex and organised microbial communities; they are suspected to be responsible for the prolonged and excess inflammatory state in chronic wounds (8).

Unfortunately, systemic antibiotics have been – and in some cases continue to be – prescribed for the treatment of chronic wounds. In a retrospective analysis of the records of 185,000 patients who presented to family medical practitioners in Wales, 60% of patients with chronic wounds received at least one systemic antibiotic during one year (9). The data showed a strong association between the occurrence of chronic wounds and the prescribing of systemic antibiotics in primary healthcare (9).

Although systemic antibiotics are effective for treating overt, deep wound infection and to decrease the possibility of systemic infection, they are largely ineffective for wounds in which the species of bacteria have not been identified (10) – their widespread overuse is a key factor contributing to the emergence of multi-drug resistant bacteria (11). Topical antibiotic creams or ointments are also frequently prescribed to control bioburden. However, many topical antibiotics have a narrow antimicrobial spectrum, may not assist in moisture balance or autolytic debridement, do not support a sustained release of an antibacterial agent over time and can result in episodes of contact sensitivity and reaction (12–14).

Hence, select topical antibacterial agents are increasingly recommended for the prompt management of localised bacterial burden in the superficial wound compartment as based on the ability of these technologies to promote broad spectrum action, to control bioburden and to reduce the incidence of resistance (15). Innovations in antibacterial technology have led to the development of many modern, advanced wound dressings that incorporate antibacterial agents, such as silver, iodine, polyhexamethylene biguanide (PHMB), honey and gentian violet and methylene blue. Nevertheless, no single antibacterial ingredient is superior; each plays an important role in the antibacterial toolbox for managing an increased bacterial burden in wounds (16). A dressing that contains the antibacterial agents gentian violet and methylene blue (Hydrofera Blue® Classic, Hollister Wound Care, Libertyville, IL) was recently evaluated for use in Canada. Gentian violet and methylene blue (GV/MB) antibacterial foam dressings are made by preferentially binding these two antibacterials to open-cell polyvinyl alcohol (PVA) foam. These GV/MB PVA dressings are highly absorbent and non-cytotoxic, and they have demonstrated antibacterial activity against a broad spectrum of yeast and bacteria commonly found in wounds, including methicillin-resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa (17). Furthermore, the GV/MB antibacterial PVA foam dressings can be cut to size to fill deep wounds or placed directly on top of a shallow wound to enable a contact interface between the dressing material and wound tissue.

Several small animal studies and clinical case series have suggested favourable outcomes after using this particular type of dressing (18–20). Here, a prospective study was undertaken to measure changes in wound size, percent coverage of devitalised tissue and clinical signs associated with an increased bacterial bioburden in chronic wounds treated by GV/MB dressings.

**Methods**

A total of 29 human participants completed a 4-week prospective study to evaluate the GV/MB antibacterial dressing for managing chronic wounds that indicated an increased bacterial burden. Patients over 18 years of age with at least one chronic wound ≥1 cm² in size that had signs of localised infection or critical colonisation with good potential for healing, based on clinical assessment, were eligible to participate in the study. Eligible patients were recruited from participating hospitals in Canada. Wounds persisting for 2 weeks or longer were considered chronic, and wounds of all aetiologies were eligible, including pressure ulcers, venous leg ulcers, arterial leg ulcers, mixed leg ulcers, surgical wounds and diabetic foot ulcers. Those patients receiving systemic antibiotic treatment at the time of recruitment were excluded from the study; however, patients who received systemic antibiotic treatment initiated during the study period were not excluded. Patients with a history of allergy/hypersensitivity to methylene blue or gentian violet were also excluded. The study protocol is conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in the approval by the institution’s human research review committee. Participants were provided with a detailed explanation of the study protocol, and they were informed of their right to withdraw from the study at any time.

**Wound care**

All participants received wound care according to best practices and local standard of care (e.g. compression for venous leg ulcer, pressure redistribution measures for pressure ulcers). All wounds were cleansed with sterile water or saline. The GV/MB dressings were hydrated prior to their application and then applied as the contact layer for all wounds and covered with a secondary dressing according to institutional policies. Dressings were changed at least three times per week during the 4-week study period.

**Instrument/measurement**

The primary endpoints of the study were: (i) changes in wound surface area measurement over time, (ii) changes in PUSH scores over time, (iii) changes in percent surface area of devitalised tissue (yellow slough or other necrotic tissue) over time and (iv) changes over time in clinical signs associated with localised wound infection or critical colonisation. All participants were evaluated at presentation (week 0 = baseline), week 2 and week 4 (end of the study).
**Wound size measurement**

Wound surface area was calculated by multiplying the longest length and width of wound dimensions perpendicular to each other.

**Pressure ulcer scale for healing**

The pressure ulcer scale for healing (PUSH) tool was developed by the National Pressure Ulcer Advisory Panel (21). It was used to measure wound healing and the changes in wound status at baseline and at 4 weeks for all monitored wounds. The PUSH tool was selected to measure changes in wound status for this series because of its reported accuracy in predicting healing outcomes. Although originally developed for pressure ulcer evaluation, the PUSH tool has since been used to study the healing of venous leg ulcers (22) and diabetic foot ulcers (23). In the latter, 18 participants were followed over a 13-week period, and their total PUSH scores predicted total healing times (23). Criterion validity has been demonstrated by a significant reduction of PUSH scores, indicating an improvement in wound status and prediction of wound closure (24–26).

The tool consists of three assessment parameters: size (score of 0–10 based on a surface area estimation), amount of exudate (score of 0–3 representing none, light and moderate to heavy exudation, respectively) and wound surface appearance according to the types of tissue present (score of 0–4 corresponding to a closed wound, epithelial tissue, granulation, slough and necrotic tissue, respectively). The parameter subscores were summed (range: 0–17).

**Evaluation of clinical signs of infection**

A standardised UPPER and LOWER mnemonic for a wound infection checklist (16) was used to evaluate clinical signs of infection (Table 1). This UPPER/LOWER list of criteria was validated in a previous study evaluating silver alginate powder for the treatment of 34 patients with critically colonised chronic wounds (27). The checklist incorporates a total of 10 signs and symptoms associated with critical colonisation (upper compartment) and deep infection (lower compartment). UPPER refers to: Unhealthy tissue, Pain, Poor healing, Exudate and Reek, while LOWER refers to: Larger in size, Osseous tissue, Warmth, Edema and Redness (16). The research team evaluated each criterion separately; a score of 1 was assigned to the presence of an individual sign or symptom, yielding a maximum score of 10 on this checklist.

**Table 1 Clinical signs and symptoms of wound infection: UPPER and LOWER wound compartments (Adapted from Woo et al., 2014) (16)**

| Signs and symptoms | Definition | Present/absent |
|--------------------|------------|----------------|
| **UPPER wound compartment infection: Signs and symptoms related to local infection because of bacterial damage in the upper wound compartment** | | |
| U: unhealthy tissue | Increased surface area on wound bed covered by devitalised tissue and unhealthy granulation | Yes/No |
| | tissue (thin and friable, bleeds easily, dark red, dull or dusky discoloration, over-granulation, pocketing and bridging) | |
| P: pain | New or increased pain | Yes/No |
| P: poor healing | Stall ed wound healing with no significant change in wound size or volume (approximately 10% in the last 7 days) | Yes/No |
| E: exudate | Increased volume of exudate, change of consistency: viscous and thick exudate | Yes/No |
| R: reek | Presence of foul odour | Yes/No |
| **LOWER wound compartment infection: signs and symptoms of wound infection related to bacterial damage in the lower or deeper wound compartment** | | |
| L: larger in size | Sudden or unexplained increase in wound size or new areas of satellite breakdown | Yes/No |
| O: osseous tissue | Wound that probes to bone or deep structures; crepitus may be present | Yes/No |
| W: warmth | Increased periwound temperature of more than 3°C compared to areas distant from the wound | Yes/No |
| E: oedema | Increased oedema or induration around the wound | Yes/No |
| R: redness | Redness of >2 cm beyond the wound margin | Yes/No |
| | Total score /10 | |

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Results
A total of 29 participants with 29 wounds completed the study. Patient demographics are summarised in Table 2. After 4 weeks of treatment with the GV/MB dressing, the mean wound surface area was reduced by 42.5%, and the mean PUSH score decreased by 19.5% (Table 3). A post hoc power analysis with the program G*Power revealed that on the basis of the mean matched pairs comparison effect size observed in the present study ($d = 0.54$), a sample size of 29 was sufficient to detect a significant effect with a power of $0.88$ and an alpha of $0.05$. All wounds exhibited unhealthy tissues (yellow slough or other necrotic tissue) at baseline. Mean percent wound surface area covered with devitalised tissue was reduced substantially, by four-fifths, as was the mean UPPER and LOWER wound infection score (Table 3). In summary, reductions in mean surface area, mean PUSH score, mean wound surface area covered with devitalised tissue and mean infection score were all significant (paired t-tests) over the 4-week study period (Table 3). None of the wounds exhibited signs and symptoms associated with wound infection in the lower compartment that necessitated systemic treatment during the study. No adverse events were reported.

Discussion
The results from our prospective study revealed a significant reduction in mean wound surface area, PUSH score, infection score and percent-devitalised tissue coverage during the 4 weeks of GV/MB antibacterial PVA foam dressing on the chronic wounds. The investigators observed an improvement in the wound surface character of chronic wounds in this series with the use of the GV/MB dressing. None of the 29 wounds deteriorated during use of this dressing. All chronic wound bases contained devitalised tissue at baseline that improved at 4 weeks of GV/MB antibacterial PVA foam dressing on the wound surface. The test products

Although the mechanistic action of the GV/MB antibacterial PVA foam dressing is not fully known, it is understood that the mechanical properties of the foam facilitate the wicking of wound exudate (containing bacterial bioburden) away from the wound surface into the dressing itself. Inside the foam dressing, it has been posited that the two antibacterials bound to the foam create a microenvironment that inhibits the metabolism of microorganisms by altering the oxidation/reduction potential inside the dressing to a state not conducive to bacterial growth or attachment (29–32). We believe that the combination of antibacterial properties, autolytic debridement effects and absorption capabilities of the GV/MB dressing contributed to its effectiveness in promoting wound healing in this series. Sibbald and colleagues (2014) suggested that the antibacterial nature of GV/MB foam may assist host resistance to minimise further bacterial damage and facilitate the proliferative stage of healing (33). This may, in part, account for the reduction in slough, infection score and wound size documented in this study. In addition, a large absorption capacity is an inherent benefit of the micro-porous structure of the PVA foam (34), and absorption of excess drainage is an important factor for reducing a wound’s bioburden.

Moisture in a wound enhances the natural autolytic process to remove devitalised tissue and facilitates the transport of essential growth factors during epithelialisation (35). During the study, we observed a moist, non-macerated wound bed at dressing changes; dressings were moist upon removal, leading us to presume that an adequate moisture balance and autolytic debridement were facilitated by usage of the GV/MB antibacterial PVA foam dressings.

Autolytic debridement with the use of GV/MB dressings has been previously described in clinical cases (19,20) and most recently in an animal study (18). The latter evaluated the effectiveness of GV/MB antibacterial foam dressing, collagenase ointment, collagenase ointment plus GV/MB antibacterial foam dressing, medical-grade honey and moist dressing control in debriding eschar in porcine wounds. The test products

Table 2  Patient demographics (n = 29)

| Characteristics                  | Dressings used prior to the GV/MB PVA dressings |
|----------------------------------|-----------------------------------------------|
| Female 10 (34-5%)/male 19 (65-5%) | Honey                                          |
| Mean age in years = 60.2 (SD = 15.06; range = 22–80) | Silver alginate                               |
| Wounds = 29                      | PHMB gauze                                     |
| Pressure ulcers = 18 (62-1%)     | Povidone iodine dressing                       |
| Surgical-trauma = 7 (24-1%)     | Foam                                           |
| Venous leg ulcers = 4 (13-8%)    | Saline gauze dressing                          |

GV/MB PVA, gentian violet and methylene blue polyvinyl alcohol; PHMB, polyhexamethylene biguanide; SD, standard deviation.

Table 3  Study results

| Assessment                        | Week 0 mean (SD) | Week 4 mean (SD) | Mean Δ week 4–0 (SD) | t value (df) |
|-----------------------------------|------------------|------------------|----------------------|--------------|
| Wound surface area (cm$^2$)       | 21.4 (27.6)      | 12.3 (18.7)      | 9.1 (16.0)           | 3.07 (28)*   |
| PUSH scores                       | 13.3 (2.2)       | 10.7 (2.3)       | 2.6 (1.4)            | 9.76 (28)*   |
| % devitalised tissue              | 52.6 (32.8)      | 11.4 (17.7)      | 41.2 (27.4)          | 8.09 (28)*   |
| Infection score                   | 3.6 (1.1)        | 0.9 (0.9)        | 2.7 (1.5)            | 9.88 (28)*   |

*P < 0.05.
Δ, change; df, degrees of freedom; SD, standard deviation.
were applied to 20 eschar wounds on each of three pigs using a split-back study design. On day 14, a significantly greater reduction in eschar was reported for treatment by the GV/MB antibacterial foam dressing alone and also in conjunction with the collagenase ointment when compared with the other dressings (18).

To date, our study is the largest reported case series of chronic wounds as treated by GV/MB dressings. Outcomes with the use of GV/MB foam dressings were previously described in one small case series (20) and in a review (19). The former consisted of a retrospective analysis of patients with 15 chronic venous leg or diabetic foot ulcers as treated with GV/MB PVA antibacterial foam dressings, which the authors reported were safe for managing the ulcers (20). Improvements in surface critical colonisation and pain score at the end of the study period were noted in some patients, especially in those patients with diabetic foot ulcers. Decreased wound size was observed in eight of 14 (57%) patients at week 4. Finally, autolytic debridement was observed in some wounds, with the presence of slough observed on the surface of the removed dressing (20).

**Case study #1**

A 49-year-old female with a medical history of severe peripheral vascular disease, bilateral amputations and type 2 diabetes mellitus was admitted for rehabilitation following a cerebral vascular accident. She had a small wound remaining on her left amputation site that had been present for 2 years, but it had not changed in size for about 6 months. Previous dressings included silver alginate and a foam cover, both changed three times per week.

On admission, the wound measured 1.5 × 1.4 cm (Figure 1A). There was neither odour nor pain, and the drainage was moderate. There was 1+ (≤2 mm) pitting oedema to the limb and surrounding the wound. The GV/MB antibacterial dressings were initiated, covered with a foam cover dressing and changed three times per week. At 2 weeks, the wound size decreased by 35%, and drainage and oedema also decreased (Figure 1B). The patient reported neither odour nor pain. At 4 weeks, the wound size had decreased by 77%, and there was a significant decrease in the amount of oedema and drainage (Figure 1C). The pain score remained constant at zero of 10, and there was no discernible odour.

**Case study #2**

An 80-year-old female was admitted for rehabilitation following a femoral-popliteal arterial bypass and an amputation of the left great toe and second toe (4 weeks postoperative). Her medical history includes peripheral vascular disease, congestive heart failure and type 2 diabetes mellitus. Dressing regimen prior to her admission was 0.9% saline-soaked gauze applied twice a day.

On admission, her amputation site measured 6.3 × 4.5 cm with heavy drainage and a faint odour (Figure 2A). Self-reported pain was eight of 10, and there was a slight increase in temperature around the wound (3°F). The GV/MB antibacterial PVA dressings were applied onto the wound, covered by a superabsorbent dressing and changed every 3 days.

After 2 weeks, there was an 18% reduction in wound size (Figure 2B); pain was scored lower, at four of 10; and the drainage and surrounding oedema were decreased. There was no odour, and the temperature was normal. This patient was discharged home after the initial 2-week treatment as she was progressing well in her therapy. The Hydrofera Blue Classic dressing was provided to her on discharge, but investigators were unable to follow-up at the 4-week endpoint.

**Case study #3**

A 72-year-old male with quadriplegia (C6–C7 Spinal Cord Injury), traumatic amputation of the left arm, left below-the-knee amputation, restrictive lung disease, hypothyroidism, osteoarthritis and a seizure disorder sustained a Stage 4 pressure ulcer on his right ischial tuberosity. At the start of the study, the ulcer measured 7 × 5 × 4 cm with 1.8 cm undermining to 6 o’clock (Figure 3A). Drainage was purulent, and a foul odour was present. There was a 3°F increase in temperature around the wound.

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**Figure 1** Case 1 at baseline (A), week 2 (B) and week 4 (C).
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Previous dressings included honey alginate, silver alginate and PHMB gauze packing with absorbent cover dressings. However, given the difficulty of pressure redistribution this patient experienced, some of these dressings likely exacerbated the pressure at the wound base. For example, the gauze packing and calcium alginate dressings appeared bunched at the wound base, where they could have increased localised pressure.

A GV/MB antibacterial PVA dressing was moistened with 0.9% sodium chloride and lightly packed into the wound. A superabsorbent dressing was used as a cover dressing for the initial 2-week period. At 2 weeks, there was already a notable decrease in drainage (Figure 3B), and the cover dressing was changed to a hydrocolloid dressing to accommodate whirlpool baths, which the patient felt was an important aspect for his quality of life. At 4 weeks, the wound size decreased by 50% with no undermining, and the edges were advancing (Figure 3C). The wound was moisture balanced with dressing changes three times per week. The wound base no longer showed signs of pressure injury and was on a healing trajectory.

Limitations
The results of the present study share all of the limitations of a descriptive study design, including lack of a control group and vulnerability to patient and treatment selection biases. Large, randomised controlled studies would be beneficial to determine wound environments that respond best to GV/MB antibacterial PVA foam dressings.

Conclusion
In this prospective study, the GV/MB dressings applied were effective in managing these challenging chronic wounds and helped them progress towards healing. There was a significant change in the mean wound surface area, mean PUSH score, mean surface area containing devitalised tissue and mean infection score over the 4-week study period. All chronic wound bases were covered with unhealthy tissue that improved by week 4. Evidence from this case series indicates that devitalised tissue (e.g. slough) was removed by the GV/MB dressing. This dressing was well tolerated by patients, and no adverse events were reported or observed.

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