Negative Pressure Wound Therapy-associated Tissue Trauma and Pain: A Controlled In vivo Study Comparing Foam and Gauze Dressing Removal by Immunohistochemistry for Substance P and Calcitonin Gene-related Peptide in the Wound Edge

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Abstract
Pain upon negative pressure wound therapy (NPWT) dressing removal has been reported and is believed to be associated with the observation that granulation tissue grows into foam. Wound tissue damage upon removal of the foam may cause the reported pain. Calcitonin gene-related peptide (CGRP) and substance P are neuropeptides that cause inflammation and signal pain and are known to be released when tissue trauma occurs. The aim of this controlled in vivo study was to compare the expression of CGRP and substance P in the wound bed in control wounds and following NPWT and foam or gauze dressing removal. Eight pigs with two wounds each were treated with open-pore structure polyurethane foam or AMD gauze and NPWT of 0 (control) or -80 mm Hg for 72 hours. Following removal of the wound filler, the expression of CGRP and substance P was measured, using arbitrary units, in sections of biopsies from the wound bed using immunofluorescence techniques. Substance P and CGRP were more abundant in the wound edge following the removal of foam than of gauze dressings and least abundant in control wounds. The immunofluorescence staining of the wound edge for CGRP was 52 ± 3 au after the removal of gauze and 97 ± 5 au after the removal of foam (P <0.001). For substance P, the staining was 55 ± 3 au after gauze removal and 95 ± 4 au after foam removal (P <0.001). CGRP and substance P staining was primarily located to nerves and leukocytes. The increase in CGRP and substance P immunofluorescence was especially prominent in the dermis but also was seen in subcutaneous and muscle tissue. Using gauze may be one way of reducing NPWT dressing change-related pain. New wound fillers designed to optimize granulation tissue formation and minimize pain issues presumably will be developed in the near future.

Keywords: animal model, controlled study, negative pressure wound therapy, pain, dressing

Index: Ostomy Wound Management 2011;57(12):30–35

Potential Conflicts of Interest: This study was supported by the Swedish Medical Research Council, Lund University Faculty of Medicine, the Swedish Research Grant for Clinical Research, Lund University Hospital Research Grants, the Swedish Medical Association, the Royal Physiographic Society in Lund, the Ake Wiberg Foundation, the Anders Otto Swärd Foundation/Ulrika Eklund Foundation, the Magn Bergvall Foundation, the Crafoord Foundation, the Anna-Lisa and Sven-Erik Nilsson Foundation, the Jeansson Foundation, the Swedish Heart-Lung Foundation, Anna and Edvin Berger’s Foundation, the Märtå Lundqvist Foundation, Lars Hierta’s Memorial Foundation, and Prospera (Forth Worth, TX).
The most commonly used wound filler materials for negative pressure wound therapy (NPWT) are open-pore polyurethane foam or cotton gauze (eg, Kerlix, AMD gauze, Coviden, Mansfeld, MA) dressings.

Recent preclinical study results have shown that granulation tissue grows into the most commonly used foam dressing used with negative pressure wound therapy (NPWT), which may cause tissue trauma and pain upon removal.

Researchers examined the presence and amount of neuropeptides that signal pain in tissues from porcine wounds after treatment with gauze or foam dressing with (-80 mm Hg) or without (0 mm Hg) NPWT.

Both neuropeptides were most abundant in tissues from foam-dressed than gauze-dressed wounds, with the lowest number of peptides observed in control wound tissues.

These results suggest that NPWT is painful and that open-pore foam causes more pain than gauze dressings.

Ostomy Wound Management 2011;57(x):30–35

Key Points

- Recent preclinical study results have shown that granulation tissue grows into the most commonly used foam dressing used with negative pressure wound therapy (NPWT), which may cause tissue trauma and pain upon removal.
- Researchers examined the presence and amount of neuropeptides that signal pain in tissues from porcine wounds after treatment with gauze or foam dressing with (-80 mm Hg) or without (0 mm Hg) NPWT.
- Both neuropeptides were most abundant in tissues from foam-dressed than gauze-dressed wounds, with the lowest number of peptides observed in control wound tissues.
- These results suggest that NPWT is painful and that open-pore foam causes more pain than gauze dressings.

Morphine or the application of local anesthetics. However, the latter are known to be cytotoxic and may interfere with tissue regeneration.

Several clinical problems are associated with dressing changes when using foam in NPWT. No controlled study has yet been performed to examine the degree of tissue trauma and pain associated with foam compared to gauze dressing removal during NPWT.

Calcitonin gene-related peptide (CGRP) and substance P are released from sensory nerve endings and inflammatory cells when local tissue trauma and injury occur. Both are known to signal pain; they also initiate a local inflammatory reaction.

The aim of this controlled, in vivo study was to examine the expression of CGRP and substance P in the wound bed following NPWT and foam or gauze dressings.

Material and Methods

Porcine peripheral wounds were treated with NPWT at -80 mm Hg for 72 hours, and the effects of foam and gauze on the wound bed were compared by examining sections of biopsies from the wound bed after hematoxylin-eosin and immunofluorescence staining for CGRP and Substance P.

Animals. Eight healthy domestic pigs of both genders (mean body weight of 70 kg), were fasted overnight and provided free access to water. The experimental protocol for this study was approved by the Ethics Committee for Animal Research, Lund University, Sweden. All animals received humane care in compliance with the European Convention on Animal Care.

Anesthesia and surgical procedure. Premedication was provided using an intramuscular injection of xylazine (Rompun® vet. 20 mg/mL; Bayer AG, Leverkusen, Germany; 2 mg/kg)
mixed with ketamine (Ketaminol® vet. 100 mg/mL; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg). Two peripheral veins in the pigs’ ears were cannulated for induction and maintenance of anesthesia and for fluid administration. Anesthesia was maintained with a continuous infusion of ketamine (0.4–0.6 mg/kg/hour). Complete neuromuscular blockade was achieved with a continuous infusion of pancuronium bromide (Pavulon; N.V. Organon, Oss, The Netherlands; 0.3–0.5 mg/kg/hour). To compensate for fluid loss, a continuous infusion of Ringer’s acetate at a rate of 200 mL/kg/hour was provided for the first 24 hours, followed by 110 mL/hour for the remainder of the experiment. The animals received total parenteral nutrition (Kabiven; Fresenius Kabi AB, Uppsala, Sweden). Antibiotics were given once daily as intravenous bolus injections (Streptocillin® vet. 250 mg/mL + 200 mg/mL; Boehringer Ingelheim Vetmedica, Malmö, Sweden; 10 mL).

The animals were orally intubated with cuffed endotracheal tubes. Mechanical ventilation was established with a

**Figure 1.** CGRP-stained sections of biopsies from the wound bed after 72 hours of treatment with NPWT at 0 (control) or -80 mmHg in a porcine peripheral wound. Control samples were taken from fresh tissue. The black area in the right of the immunofluorescence images shows where the wound filler was located. The diagram shows the CGRP staining intensity in the wound edge expressed as arbitrary units (au). Insets show an example of an area used for comparative quantitative analysis.

**Figure 2.** Substance P-stained sections of biopsies from the wound bed after 72 hours of treatment with NPWT at 0 (control) or -80 mm Hg in a porcine peripheral wound. Control samples were taken from fresh tissue. The black area in the right of the images shows where the wound filler was located. The diagram shows the staining intensity of substance P in the wound edge, expressed as arbitrary units (au). Insets show an example of an area used for comparative quantitative analysis.
were created on each pig's back. Kerlix, AMD gauze (Covidien, Mansfield, MA) or open-pore structure (400–600 micropore size) polyurethane foam (V.A.C. Therapy® black GranuFoam®, KCI, San Antonio, TX) was used as wound filler. The gauze was soaked with saline. The gauze used is impregnated with polyhexamethylene biguanide (PHMB) to provide antimicrobial control. Wound filler, estimated at surgery, was approximately one-and-a-half times wound volume to allow volume reduction during negative pressure application. This process was the same for both the gauze and foam dressing used; the two different wound fillers were compared in each pig.

A drainage tube was inserted into the wound filler and connected to a vacuum source (Prospera PRO-III®, Prospera Technologies LLC, Fort Worth, TX). The wound was sealed with a transparent adhesive drape, which overlapped the wound margins by 10 cm. The wounds were sealed for NPWT and either left at atmospheric pressure (0 mm Hg) or treated with NPWT at -80 mm Hg. The level of negative pressure was continuous — ie, left at a constant pressure for the entire duration of the therapy. After 72 hours, the adhesive drape covering the wound was cut, using a scalpel, along the borderline between the tissue and the wound filler. The cut was superficial enough to only slice the thin drape and not affect the underlying tissue structures or dressings, which was confirmed by inspection of the wound and the wound filler. After the drain was removed, the wound filler was attached to a force measurement device and a motor-driven traction device set to withdraw the wound filler at a constant speed of 4 mm/second.

Histology. For the control group, histology samples were taken from fresh tissue. For the treatment group, a strip of the wound filling material (1 cm x 1 cm x 2 cm) was sewn onto the bottom of each wound. After NPWT was completed and the wound filler removed, the strip and the underlying wound bed tissue were excised with a scalpel. Both control and treatment group tissue then was treated in 4% paraformaldehyde, dehydrated, embedded in paraffin, and left over night. Sectioning was performed with a rotary microtome HM 355 (ThermoFisher Scientific, MA).

Hematoxylin-eosin staining was performed for morphological analysis. Slides with 4 µm-thick tissue sections were deparaffinized for 2 x 4 minutes in xylene, 2 x 3 minutes in 99.5% ethanol, and 3 minutes in tap water. The slides were stained for 12 minutes in Mayer's hematoxylin (Histolab AB, Rockville, MD) and then treated/rinsed with tap water for 8 minutes, erythrosin (1.5 g in 500 mL water) for 6 minutes, tap water for 3 minutes, 99.5% ethanol for 2 x 3 minutes, and xylene for 4 minutes.

For immunostaining, the sections were blocked in incubation buffer (5% goat serum in PBS with 1% bovine serum albumin and 0.25% Triton X100) for 1 hour at room temperature.

Siemens-Elema ventilator (Siemens-Elema AB, Solna, Sweden) in the volume-controlled mode (65% nitrous oxide, 35% oxygen). Ventilation settings were identical for all animals (respiratory rate, 15 breaths/minute; minute ventilation, 12 L/minute). A positive end-expiratory pressure of 5 cm water was applied. A Foley catheter was inserted into the urinary bladder through a suprapubic cystostomy. After the experiments were completed, the animals were euthanized with a lethal dose (60 mmol) of intravenous potassium chloride.

Wound preparation. Two circular wounds, extending 1.5 cm below the wound surface into the subcutaneous tissue,
and incubated overnight at +4°C with 1:400 rabbit anti-CGRP (B47-1, Santa Cruz Biotechnology Inc, CA) or 1:400 rabbit antisuicide P (B45-1, Santa Cruz Biotechnology Inc.) diluted in incubation buffer. Slides were washed three times for 10 minutes in PBS with 0.25% Triton X100, followed by incubation for 2 hours at room temperature with 1:400 goat anti-rabbit IgG FITC (Cayman Chemical, MI) diluted in PBS with 0.25% Triton X100. The slides were washed three times for 10 minutes in PBS with 0.25% Triton X100 and mounted with Vectashield mounting medium (Vector Laboratories Inc., CA). Microscopy was performed using a Zeiss Axiophoto microscope equipped with an AxioCam camera (Carl Zeiss, Oberkochen, Germany). The morphology of the biopsy sections was examined, and the intensity of CGRP and substance P staining was quantified and expressed as arbitrary units (au).

Calculations and statistics. The differences in staining intensity were calculated and analyzed using GraphPad 5.0 software (San Diego, CA). Statistical analysis was performed using the Mann-Whitney test when comparing two groups, and the Kruskal-Wallis test with Dunn's post-test for multiple comparisons when comparing three groups or more. Results are presented as means ± the standard error of the mean (SEM) (n = 8).

Results

CGRP and substance P staining. After NPWT, the intensity of staining for substance P and CGRP was higher in the wound edge in foam-dressed compared to gauze-dressed wounds (see Figures 1 and 2). The immunofluorescence staining of the wound edge for CGRP was 52 ± 3 au after the removal of gauze and 97 ± 5 au after the removal of foam (P < 0.001). For substance P, the staining was 55 ± 3 au after gauze removal and 95 ± 4 au after foam removal (P < 0.001). The least intense staining was observed in both the foam and gauze control wounds that were sealed as for NPWT but not subjected to negative pressure (see Figures 1 and 2).

Tissue morphology. Both CGRP and substance P staining was observed in the wound edge; the staining was most intense in the superficial tissue (75 ± 5 au for CGRP and 78 ± 6 au for substance P) that had been exposed to the forces of the negative pressure and in direct contact with the wound filler and least intense in deeper tissue (23 ± 2 au for CGRP and 31 ± 3 au for substance P). CGRP and substance P staining was especially intense in the dermis, but was also present in the subcutaneous and muscle tissue. Slides stained for CGRP and substance P were compared to adjacent slides stained with hematoxylin-eosin. Staining for both CGRP and substance P were primarily observed located to nerves and leukocytes (see Figures 3 and 4).

Discussion

The results of the present study show that substance P and CGRP are more abundant in the wound edge of porcine wounds after the removal of foam than the removal of gauze after NPWT. This is in line with clinical observations that dressing changes are more painful when NPWT has been carried out using foam than when using gauze.

A literature review by Krasner et al.2 addressed the application and removal of foam as a source of procedural pain. It was suggested that patients who experienced pain during dressing changes be given pain relief 30 to 60 minutes before the procedure, following the World Health Organization's three-step analgesic ladder, proceeding from nonopioids to opioids. In many cases, it was necessary to administer opioids systemically before changing dressing to ensure an adequate and acceptable (to the patient) level of pain relief. Attempts also have been made to reduce pain by instilling a 1% lidocaine solution through the drain to reach the surface of the wound; the effects were evaluated in a clinical, double-blind, prospective, randomized study.13 However, we believe that administration of local anesthetics is undesirable because they may be cytotoxic, as shown in in vitro studies.13

Previously, foam was used as a wound filler in almost all patients treated with NPWT. Today, NPWT that utilizes gauze-type dressings are widely available.

The effects of gauze and foam on the wound bed have been shown to differ substantially in porcine wound models.5,6 The granulation tissue may adhere to the foam or actually grow into the pores of the foam, but in vivo studies of porcine wounds treated with NPWT show no ingrowth into gauze.1,3 On a cellular level, ingrowth has been examined in histological sections of the wound bed treated with overlapping gauze or foam wound filler.5 Preclinical in vivo studies show that more force is needed to remove foam than to remove gauze after NPWT.

The pain during NPWT dressing changes may be due to the disruption of the granulation tissue in the wound bed resulting from foam removal.2,9,11 Per clinician observation, it has been suggested that dressing changes when using gauzes may result in less pain than when using foam. Another strategy may be to use foam of a smaller pore size, such as the polyvinyl alcohol (PVA) white foam dressing, but data examining tissue ingrowth for this type of foam have not been published. It is difficult to conduct comparative studies in the clinical situation because the pain assessment tools (eg, visual analog scales or the Faces scale) are subjective measures and are affected by the individual patient's pain threshold. Furthermore, each wound is different, and this will affect the level of pain experienced — eg, changing the dressing on a diabetic foot ulcer may be relatively painless as a result of neuropathy as quantified using the above-mentioned pain assessment tools.

The present study aimed to quantify the tissue trauma and pain resulting from the removal of foam and gauze wound fillers. As described in previous literature reviews,6 CGRP and substance P are released from sensory nerve endings and inflammatory cells upon local tissue trauma and injury. These neuropeptides are known to signal pain and also to initiate a local inflammatory reaction.6 The results of the present study...
show that the use of foam in NPWT leads to greater expression of substance P and CGRP in the wound edge than when using gauze. This is in line with clinical observations that NPWT dressing removal causes pain and that patients treated with NPWT using foam experience more pain during dressing changes than patients treated with NPWT using gauze.17

Multiple strategies often are required to manage pain adequately in patients treated with NPWT. The obvious method is to provide the patient with adequate medication to alleviate pain. However, it is also important to consider the cause of the pain and to try and avoid or minimize it. This may reduce the need for strong medication. Other strategies also should be considered. When the clinician anticipates complication, a wound contact layer can be placed under the wound filler as described in previous clinical case series.18,19 A recent preclinical in vivo study in pigs20 showed that the use of a nonadherent wound contact layer prevents the growth of tissue into foam, thus facilitating dressing changes, although this may slightly reduce the formation of granulation tissue. The degree of wound bed adhesion may be reduced by increasing the frequency of dressing changes, thus reducing the pain during dressing changes. Another frequently employed strategy is to instill saline into the wound, through the wound filler (e.g., via the drain) 15 to 30 minutes before gently removing the dressing.

NPWT using foam is known to create thick granulation tissue in the wound bed; gauze creates a thinner but stable granulation tissue in pig wounds.2,5 These differences are believed to be the result of differences in the mechanical shear forces caused by the foam or gauze on the wound bed. The results from the present study show a greater release of CGRP and substance P after treatment of foam than gauze. This may reflect the presence of a greater mechanical force causing deformation of the wound bed under foam than under gauze.

Study Limitations

One limitation of the present study is that it is was performed in pigs. It would be of clinical relevance to perform a similar study in humans. One option would be to collect biopsies from wounds treated with NPWT using foam or gauze and examine these using immunohistochemistry for CGRP and substance P. Another limitation of the present study is that it was only performed with one type of gauze (i.e., the clinically most commonly used Kerlix AMD gauze). It is important to note that nearly all gauze used in NPWT has been a particular type of cotton gauze (Kerlix AMD) to facilitate impregnation with polyhexamethylene biguanide (PHMB) to provide antimicrobial control. The observations from this study may not apply to all types of gauze.

Conclusion

CGRP and substance P are neuropeptides released as a result of tissue trauma and are known to signal pain and initiate a local inflammatory response. In the present study, it was shown that the expression of CGRP and substance P in the wound edge was more pronounced in wounds treated with NPWT than control wounds and more pronounced after removing foam compared to gauze dressings when used in NPWT. This may confirm previous reports that granulation tissue grows into foam but not into gauze and the clinical observation that dressing removal after NPWT using foam is more painful than dressing removal after NPWT using gauze. The use of gauze may be one way of reducing the problem of pain during dressing changes in NPWT.

References

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