Aim: Use of skin grafting is a widely method used in the treatment of painful and very secreting chronic leg ulcers. Their management in the post-operative period can be altered by bacterial infections. To prevent infections silver is considered the best antiseptic. In this study silver impregnated dressings were tested to evaluate their effectiveness.

Method: 14 subjects (9 females and 5 males) underwent to skin grafting (8 homologous cryopreserved skin/5 acellular skin/3 dermal substitutes). In each patient a non adherent gauze and one Ag-dressing* were applied. A multi-layer bandaging was applied. Systemic antibiotic therapy started in the postoperative period and was administered for 7 +/- 2 days. The dressing change was performed every week evaluating secretion, pain and wound area.

Results / Discussion: The data collected showed that in 12 subjects secretion was reduced to consensus clinical improvement of the treated lesion, while in 1 case there was a bacterial contamination and 1 case super infection. Secretion decreased of 73% after 4 weeks; pain was present only in 2 patients. Wound areas decreased in 12 patients of 84% (in 3 patients closure) after 8 weeks.

Conclusion: The study showed that the application of silver impregnated dressing allows to effectively manage the lesions treated (even large) avoiding 85% of cases the onset of infection that would determine definitely the failure of surgery.

* Idrofiber
EVALUATION OF ANTIBACTERIAL L AND CYTOTOXIC POTENTIAL OF ARGEMONE MEXICANA LINN: A PERSPECTIVE FOR TREATMENT OF INFECTED WOUND

Maria Gabriella Silva Araujo¹, MACEIO, Brazil, Regina Veríssimo¹, MACEIO, Brazil, Andressa Leticia Lopes da Silva¹, MACEIO, Brazil, Joice Fragoso da Silva Oliveira¹, MACEIO, Brazil, Thais Honorio Lins Bernardo¹, MACEIO, Brazil, Maria Lysete de Assis Bastos¹, MACEIO, Brazil, Joao Xavier Araujo-Junior¹, MACEIO, Brazil

¹Federal University of Alagoas; Esenfar

Wednesday, May 13, 2015
E-poster Session: Antimicrobials 2

Aim: This is an in vitro experimental research that analysed the antibacterial potential and cytotoxicity of vegetal species Mexican Poppy Linn with a perspective for the treatment of infected wounds.

Method: The gross ethanolic extract was obtained after the solution concentration on a rotary evaporators and the fractionation of the extract was performed by chromatography in classical liquid column. The antibacterial activity was measured using a Minimum Inhibitory Concentration (MIC) of the adapted protocol from the National Committee for Clinical Laboratory Standards [NCCLS], where the gross ethanolic extract and fractions were tested front the major infection-causing bacteria in wounds and to evaluate the cytotoxicity of the samples was performed the Methyl Thiazolyl Tetrazolium (MTT) method based on mitochondrial activity of the cells by the MTT reduction.

Results / Discussion: The cytotoxicity of the samples was observed in the two tested concentrations. Among these, the chloroform fraction in the 1000μg concentration and the methanolic fraction at concentration of 100μg not present cytotoxicity, as 87% and 90,2%, respectively, of the cells remained viable at the end of testing. The antibacterial activity was observed with the methanolic fraction that proved moderately active front strains of Staphylococcus aureus and Staphylococcus epidermidis.

Conclusion: This study may be useful to guide new and more systematic and deepened investigations about the chemical components of this species to serve as subsidies for the future development of herbal medicine with the prospect in the treatment of infected wounds.
[EP332] EFFECT OF MEDICAL HONEY ON BIOBURDEN IN CHRONICALLY INFECTED VENOUS LEG ULCERS

Chun Yang1, New York, United States; Selena Goss1, New York, United States; Sean Alcantara1, New York, United States; Qingping Yang2, Gainesville, United States; Daniel Gibson2, Gainesville, United States; Gregory Schultz2, Gainesville, United States; John Lantis1, New York, United States

1Mount Sinai St. Luke's Hospital and Mount Sinai Roosevelt Hospital; Surgery
2University of Florida; Institute for Wound Research

Wednesday, May 13, 2015

E-poster Session: Antimicrobials 2

Aim: Chronic venous leg ulcers (VLUs) affect millions of people in the United States, resulting in enormous burden to those affected. VLUs are often critically colonized, preventing the natural course of healing. Studies have demonstrated that topical medical grade honey may improve wound healing by decreasing overall bioburden. The goal of this study is to study the effects of topical honey in vivo on planktonic bacteria and biofilm in chronic VLUs.

Method: A 4-week, prospective study of 20 patients was conducted to study the effect of topical honey on the reduction in bioburden of chronically infected VLUs. Patients were treated with weekly sharp wound debridement and topical medical honey with multi-layer compression wrap. Serial wound scrapings and biopsies were performed to test for quantitative biofilm and planktonic bacteria.

Results / Discussion: Overall wound size decreased by 21% at 4 weeks (P<0.05). Topical medical honey reduced planktonic bacterial count by 30% (P<0.05) within 1 week. In contrast, sharp debridement did not have a significant effect on planktonic bacterial count. Overall biofilm concentration did not change with either medical honey or sharp debridement. Medical grade honey is gaining popularity in the treatment of chronic wounds. Topical medical honey provides a moist wound environment, has antimicrobial properties, and may promote wound healing. Our study demonstrates that while topical medical honey is effective at reducing planktonic bacterial count, it does not appear to have debridement activity necessary for the reduction of biofilm.

Conclusion: Further investigation into the antimicrobial action of medical honey is necessary.
Aim: Chronic venous leg ulcers (VLUs) affect millions of people in the United States and cause an enormous burden on those affected. A dysregulation of cytokines in favor of pro-inflammatory mediators has been shown to impede wound healing. Topical medical grade honey may promote healing by reducing pro-inflammatory cytokines in the local wound environment. The goal of our study is to measure the effect of topical honey on TNF-α and IL-1 cytokines in VLUs.

Method: A 4-week, prospective study of 20 patients was conducted to study the effect of topical honey on the local concentration of TNF-α and IL-1 in chronically infected VLUs. Patients were treated with weekly wound debridement and topical medical honey dressing with multi-layer compression wrap. Weekly measurements of cytokine concentration were obtained from superficial wound fluid.

Results / Discussion: Overall wound size decreased by 21% at 4 weeks (P<0.05). A 52% (P<0/05) reduction in TNF-α concentration was seen after one week of treatment with topical honey. Sharp debridement of wounds did not produce a significant difference in TNF-α concentration. However, at the end of 4 weeks no significant change in TNF-α concentration was seen. No significant change in IL-1 concentration was seen at any point with topical honey. The process of wound healing involves a complex interplay between pro-inflammatory and anti-inflammatory cytokines. While IL-1 concentrations were not affected, topical medical honey use in chronic VLUs produced a significant but transient decrease in TNF-α concentrations. It is unclear whether change affects overall wound healing.

Conclusion: Further investigation into the association of local wound cytokine, inflammation, and closure is necessary.
Aim: To determine the safety and preliminary clinical efficacy of the novel dressing in critically-colonised wounds which have ‘stalled’ in a non-healing state. Existing silver dressings have been used in these indications, occasionally with reports of adverse effects. This novel dressing relies upon an enhanced activity silver oxy-salt used at a lower concentration than other dressings.

Method: The study was conducted according to a protocol with Ethics Committee approval. Twenty patients with leg or foot ulcers were enrolled and treated for up to 8 weeks. The principles of wound bed preparation were followed throughout (TIME Sibbald et al 2011). Dressings were changed three times per week. Patients were assessed for pain (VAS), critical colonisation/healing, and adverse reactions.

Results / Discussion: Pain was reported in 17/20 wounds at enrollment (mean VAS score 2.6). This had reduced to a mean 1.9 in 8 weeks. One patient healed and 16/20 ulcers reduced in size of which 7/20 reduced by over 50%. No adverse dressing-related events were noted in the course of the study.

Conclusion: The Ag oxy-salt dressing is clinically effective and safe in VLU and foot ulcer patients. Further clinical studies are now required to explore these findings in these, and other, pathologies.

Aim: To determine the effect of a high oxidation state silver dressing activity (Ag oxy-salt) against biofilms grown in vitro.

Method: Anti-biofilm activity was measured by determining the minimum biocidal concentrations at 4h, minimum biofilm inhibition concentration and minimum biofilm eradication concentrations (MBEC) of biofilm populations of *S. aureus*, *E. coli*, and *P. aeruginosa* at 24h. Organisms were grown in the Calgary Biofilm Device using peptone or simulated wound fluid media. Silver and copper salts were used as control. The MBEC assay and confocal microscopy vital staining techniques were used to determine effect.

Results / Discussion: The MBC 4h/MBEC 24h Ag oxy-salt for *P. aeruginosa* was 36/56µM and, for AgNO₃, 58/1250µM. The corresponding values for *E. coli* are 16µM/42µM and 8µM/38µM; and for *S. aureus* 250µM/1260µM

Conclusion:

1) Ag oxy-salts (Ag²⁺/³⁺) have anti-biofilm activity at lower concentrations than AgNO₃ (Ag⁺) in all the organisms tested.

2) *S. aureus* appears to tolerate tested metals at higher concentrations than the other two organisms tested.


Richard James White\textsuperscript{1}, Worcester, United Kingdom

\textsuperscript{1}University of Worcester; Institute of Health & Society

Wednesday, May 13, 2015

E-poster Session: Antimicrobials 2

\textbf{Aim:} To explore the potential efficacy advantages of using Ag\(^{2+}\) and Ag\(^{3+}\) (as derived from Ag\(_7\)NO\(_{11}\) high oxidation state silver (HOSS: Rodijk et al 2011). Current silver-containing dressings are either silver salts, which produce Ag\(^{+}\) in solution, or nano-silver which is Ag\(^0\) \(\rightarrow\) Ag\(^{+}\) in solution. HOSS has a higher redox potential than Ag\(^+\).

\textbf{Method:} Antibacterial activity against planktonic cultures of wound pathogens was measured using the method of Cavanagh \textit{et al}. Eradication of established biofilms \textit{in vitro} using the Calgary system. A clinical study on stalled ulcers of various aetiologies, monitored by the BWAT score (Bates-Jensen 2001) for outcomes.

\textbf{Results / Discussion:} The oxidation reduction potential of the HOSS is \(>665\text{mV}\) indicating that E. coli survival is less than 10s. Both dressings were effective in their bactericidal activity against all of the pathogens tested. The novel dressing achieved a \(4\times\log_{10}\) reduction in 4h or less for all organisms, and eradicated mature biofilms. The clinical study involved 50 patients. The mean BWAT at start was 35, decreasing to 15 \(R^2 = 82.9\%\).

\textbf{Conclusion:} The novel dressing is highly effective against a range of pathogens \textit{in vitro}, and against mature biofilms. Clinical results on ulcer patients show positive progression towards healing. The incorporation of HOSS into a wound dressing represents a promising development which merits further study and clinical usage.


Aim: To explore the potential efficacy advantages of Ag2+ and Ag3+ (as derived from Ag7NO11 high oxidation state silver: Rodijk et al 2011) when used in dressings for wound bioburden control. A nano-silver dressing was used as control.

Method: Antibacterial activity against planktonic cultures of known wound pathogens was measured using the method of Cavanagh et al.

Results / Discussion:

<table>
<thead>
<tr>
<th>Organism</th>
<th>Growth at t=0.</th>
<th>Time to 4xlog10 reduction: Exsalt</th>
<th>Time to 4xlog10 reduction: Acticoat.</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>6.5 x log10</td>
<td>4h</td>
<td>4h</td>
</tr>
<tr>
<td>E. coli</td>
<td>6.4 x log10</td>
<td>1h</td>
<td>1h</td>
</tr>
<tr>
<td>VRE</td>
<td>6.2 x log10</td>
<td>3.5h</td>
<td>3h</td>
</tr>
<tr>
<td>P aeruginosa</td>
<td>5.6 x log10</td>
<td>~20mins</td>
<td>~20mins</td>
</tr>
<tr>
<td>A. baumanii</td>
<td>5.5 x log10</td>
<td>~45mins</td>
<td>~50mins</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>5.8 x log10</td>
<td>1.5h</td>
<td>3.5h</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>6.5 x log10</td>
<td>3h</td>
<td>4h</td>
</tr>
</tbody>
</table>

Both dressings were effective in their bactericidal activity against all of the pathogens tested. The novel dressing achieved a 4 xlog10 reduction in 4h or less for all organisms.

Conclusion: The novel dressing is highly effective against a range of pathogens in vitro.


Aim: Research suggests up to 60% of chronic wounds contain bacterial biofilms, which can delay healing\textsuperscript{1,2}. Chronic wounds with suspected biofilm should be managed with a biofilm-based approach, combining cleansing/irrigation with debridement and topical antimicrobial agents to disrupt and remove biofilm and prevent reformation\textsuperscript{3}.

Mature biofilms are extremely resistant to biocidal penetration\textsuperscript{2}, rendering antimicrobials largely ineffective\textsuperscript{4}. A wound cleansing solution containing Hypochlorous acid (HOCl)* has rapid-acting, broad-spectrum antimicrobial activity\textsuperscript{5} with potential anti-biofilm capabilities.

The biofilm removal activity of the HOCl Solution was assessed in-vitro using the Minimum Biofilm Eradication Concentration microtitre plate assay.

Method: In-vitro testing was performed at an external accredited microbiology laboratory against Staphylococcus aureus (SA) and Pseduomonas aeruginosa (PA) biofilms. Established 72 hour biofilms were transferred to challenge plates containing HOCl solution and controls of either Phosphate Buffered saline (PBS)(PA) or PBS+1% Tryptone Soya Broth (TSB)(SA). Biofilms were exposed to the HOCl solution for either 3, 5 or 10 minutes or 24 hours. Serial dilutions and drop plates were performed on the recovered media to quantify recovered viable microorganisms.

Results / Discussion: No viable organisms were recovered after 3 minutes of treatment with HOCl solution, representing a 5.68±0.28 and 5.69±0.26 log reduction in viable bacteria compared with PBS and PBS+1% TSB treated controls respectively. This was also the case for all longer treatment periods.

Results suggest the HOCl solution is able to rapidly penetrate and kill PA and SA bacteria within biofilm communities.

Conclusion: Preliminary in-vitro testing suggests the HOCl solution has effective anti-biofilm activity, potentially providing a simple option for addressing cleansing and antimicrobial aspects of biofilm-based wound care to facilitate chronic wound healing.

*Woundox Irrigation Solution, Martindale Pharma, UK