A new fibrin sealant derived from snake venom candidate to treat chronic venous ulcers

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Introduction

- Venous ulcer is a health problem of Western countries

- Venous ulcers treatment remains a challenge

- Among its alternative therapies, there is the commercial fibrin sealant
Introduction

- Since 1993, the Center for the Study of Venoms and Venomous Animals (CEVAP) of São Paulo State University (UNESP), Brazil, have been developing a new fibrin sealant composed of fibrinogen extracted from large animals and an enzyme derived from snake venom.
Objective

This study assessed the safety and most appropriate dose of the new fibrin sealant for treating chronic venous ulcers.
Methods

- Phase I/II clinical trial was supported by the Clinic of Chronic Ulcers of the Dermatology Service at the Botucatu Medical School, UNESP, Brazil, where the participants were selected.

- The present study was approved by the Research Ethics Committee and consent was obtained from each person.

- Patients with chronic venous ulcer which met the inclusion and exclusion criteria were selected.
Methods

- **Inclusion Criteria**
  
  a) Sign the informed consent;
  
  b) Age above 18 years, of both sexes;
  
  c) Chronic venous disease with chronic venous ulcers;
  
  d) One or more ulcers the minimum evolution time is 6 weeks and a maximum of 5 years;
  
  e) The sum of areas of ulcers of both members between 5 and 60 cm²;
  
  f) At least one ulcer whose larger area is above 5 cm²;
  
  g) Do not use these drugs within the last two weeks prior to screening: i. venotonics; ii. pentoxifylline; iii. Fibrinolytic;
  
  h) Available to attend once a week for treatment.
Methods

Exclusion Criteria

a) Ulcers in the lower limbs of other etiologies (hematologic, neoplastic, infectious causes, etc.);
b) Use of anticoagulants;
c) Infected ulcers;
d) Ulcers with critical colonization;
e) Necrosis in the ulcer bed;
f) Ulcer with devitalized tissue covering all its bed;
g) Venous ulcers associated with peripheral arterial disease;
h) Patients be unable or unwilling to remain with compressive treatment of lower limb for seven days;
i) Suspected or confirmed pregnancy;
j) Coagulation values outside the normal range;
k) Female of childbearing age, not using contraception
Methods

- Investigational product: new fibrin sealant

- It is composed of a serine protease extracted from *Crotalus durissus terrificus* venom, a cryoprecipitate rich in fibrinogen extracted from buffaloes and the diluent calcium chloride.

- The active ingredient of the final fibrin sealant mimics the step of the coagulation cascade, using a rich cryoprecipitate fibrinogen and an enzyme like-thrombin, also known as serine protease, extracted from snake venom. The enzyme acts on fibrinogen molecule transforming it into fibrin monomers in the presence of calcium to polymerize to form a stable clot adhesive effect and hemostatic sealant.
Methods

- Each dose of the drug for topical use are packaged and distributed in three bottles (Figure 1), namely diluent bottle (white label): 0.6 ml containing calcium chloride; Fraction 1 bottle (red label) containing 0.4 ml serine protease extracted from snake venom; Bottle Fraction 2 bottle (black label) containing 1 ml of fibrinogen cryoprecipitate extracted from buffaloes. All bottles were kept frozen in a freezer at -4 °F until the time of use.

Figure 1: Fibrin sealant: sealant study - use only in clinical trial
Methods

Upon use, the components were mixed in the previously set proportions to generate a stable clot with a dense fibrin network.

Dense fibrin network formed by the fibrin sealant. (4,000x magnification).

Methods

- The application consisted of defrost at room temperature all bottles during 10 minutes (Figure 2)

Figure 2: The new fibrin sealant preparation
The patients were treated with the fibrin sealant, essential fatty acid and Unna's boot during 12 weeks (*Figure 3*). The dressing remained for seven days.

*Figure 3:* A - Application of the product on the ulcer and formation of colorless gel sealant aspect occurred about 2 to 5 minutes; B - gauze embedded with essential fatty acids, in sufficient quantity for making cover all ulcerated surface; C - Placed the Unna's boot is bandaging half and half, from foot dorsum to just below the knee.
Methods

- The following variables were evaluated:
  - primarily, local and systemic adverse events related or not to the product;
  - secondly, determination of the safe dose for the maximum coverage of 60 cm\(^2\); and, finally, assessment of the healing process.
Methods

- **Statistical Analysis**
  - The ulcers areas and pain intensity were compared in two stages (V1 - first visit, and V8 – last visit) using the Wilcoxon test.

  - Adverse events were compared in V1 and V8 using the McNemar test.

  - The total area of ulcers was compared in V1 and V8 by Wilcoxon test.

  - Laboratory results were compared in V1 and V8 by McNemar test.

  - Differences were considered statistically significant when $p < 0.05$.

  - The analysis was performed using SPSS V15.0 software
Results

- Ten participants were studied, 9 females and 1 male, with ages ranging from 50 to 83 years.

- The main results are in table 1.

- Eighteen ulcers were initially diagnosed, their time of appearance ranged from 2 to 60 months and the initial area from 1.5 to 59.6 cm² (Table 1).

- The total dose of sealant used ranged from 6 to 22.8, with an average of 12.8 doses per patient.
Table 1 - Distribution of participants and their ulcers, gender, age (years), ulcer duration (months), the initial and final areas (cm²) and the presence of adverse events.

<table>
<thead>
<tr>
<th>Patient number/number of ulcers</th>
<th>Gender</th>
<th>Age (years)</th>
<th>Ulcer duration (months)</th>
<th>Initial area (cm²)</th>
<th>Final area (cm²)</th>
<th>Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>2/1</td>
<td>M</td>
<td>67</td>
<td>36</td>
<td>12,85</td>
<td>7,3</td>
<td>critical colonization/ myiasis (NR); opening new ulcer (NR)</td>
</tr>
<tr>
<td>2/2</td>
<td></td>
<td></td>
<td>36</td>
<td>14,1</td>
<td>8,2</td>
<td>critical colonization/ myiasis (NR)</td>
</tr>
<tr>
<td>2/3</td>
<td></td>
<td>12</td>
<td>1,35</td>
<td>healing (V3)</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>2/4</td>
<td></td>
<td>2</td>
<td>1,5</td>
<td>healing (V5)</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>3/1</td>
<td>F</td>
<td>70</td>
<td>24</td>
<td>17,1</td>
<td>healing (V6)</td>
<td>critical colonization (NR)</td>
</tr>
<tr>
<td>4/1</td>
<td>F</td>
<td>75</td>
<td>36</td>
<td>25,4</td>
<td>52</td>
<td>Increasing the area (NR); opening new ulcer (NR); pain (NR)</td>
</tr>
<tr>
<td>5/1</td>
<td>F</td>
<td>74</td>
<td>36</td>
<td>5,05</td>
<td>14,8 (union of ulcers 1 and 2)</td>
<td>opening new ulcer (NR)</td>
</tr>
<tr>
<td>5/2</td>
<td></td>
<td>36</td>
<td>7,01</td>
<td></td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>6/1</td>
<td>F</td>
<td>66</td>
<td>2</td>
<td>3,35</td>
<td>4,15</td>
<td>pain (PR); opening new ulcer (NR)</td>
</tr>
<tr>
<td>6/2</td>
<td></td>
<td>12</td>
<td>21,3</td>
<td>6,25</td>
<td>pain (PR)</td>
<td>pain (PR)</td>
</tr>
<tr>
<td>6/3</td>
<td></td>
<td>6</td>
<td>1,95</td>
<td>healing (V7)</td>
<td>pain (PR)</td>
<td>pain (PR)</td>
</tr>
<tr>
<td>6/4</td>
<td></td>
<td>12</td>
<td>6,9</td>
<td>healing (V8)</td>
<td>pain (PR)</td>
<td>pain (PR)</td>
</tr>
<tr>
<td>7/1</td>
<td>F</td>
<td>83</td>
<td>6</td>
<td>8,2</td>
<td>1,1 (V7)</td>
<td>myiasis (NR)</td>
</tr>
<tr>
<td>8/1</td>
<td>F</td>
<td>50</td>
<td>13</td>
<td>6,95</td>
<td>healing (V6)</td>
<td>absent</td>
</tr>
<tr>
<td>8/2</td>
<td></td>
<td>4</td>
<td>0,3</td>
<td>healing (V3)</td>
<td>absent</td>
<td>absent</td>
</tr>
<tr>
<td>9/1</td>
<td>F</td>
<td>74</td>
<td>60</td>
<td>11,9</td>
<td>12,6 (V6)</td>
<td>pain (PR)</td>
</tr>
<tr>
<td>10/1</td>
<td>F</td>
<td>50</td>
<td>18</td>
<td>12,8</td>
<td>11,1 (V5 - withdraw)</td>
<td>local infection (NR), opening new ulcer (NR)</td>
</tr>
<tr>
<td>11/1</td>
<td>F</td>
<td>72</td>
<td>48</td>
<td>59,6</td>
<td>48,8 (V5)</td>
<td>myiasis (NR)</td>
</tr>
</tbody>
</table>

M= male; F = female; V3, V5, V6, V7 e V8= visits 3, 5, 6, 7 and 8; NR= not related to the product; PR= probably related to the product;
Results

- The appearance of a new ulcer occurred in 5 patients (50%).
- Local pain was reported by 3 patients, 3 cases of myiasis, 2 cases of critical colonization, and one patient was discontinued due to a local infection.
- Four ulcers showed an increase in their areas (22.2%).
- The development of new ulcers, as well as the augmentation of 4 of them, was related to the placement of Unna’s boot.
- Systemic adverse events occurred in 2 patients and they were not related to the product.
- All adverse events were mild, with no severe events.
- At the end of the process, we had 7 healed ulcers (38.8%) and 7 presented a decrease of their initial areas (33.3%), totalizing 72.1% of the ulcers with significant improvement.
Results

The following images demonstrate the evolution of four participating participants.

**Figure 4:** Participant 3 - Ulcer two years ago. Healing in last assessment (V8)

**Figure 5:** Participant 6 - Initial Area = 33.5 cm² and area on the last assessment (V8) = 15.1 cm²
Figure 5: Participant 8 - Ulcer 1 year ago. Healing the assessment 4 (V4)

Figure 6: Participant 11 - Ulcer 4 years ago. Initial area $59.6 \, \text{cm}^2$, Visit 5 - area $48.8 \, \text{cm}^2$
The new fibrin sealant is a safe and clinically promising candidate for treating this type of ulcers. A multicenter clinical trial, phase II/III, with a larger number of participants will be performed to prove the efficacy of the product.


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Fibrin sealant Team
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