Independent Peer-Reviewed
Published and Presented
Clinical Studies
Study 1: QuikClot® Interventional™ Published Clinical Study — International Journal of Cardiology (Oct 2010)
Forty (40) patients treated with QCI on a femoral approach demonstrated hemostasis was achieved in a mean time of 4.9 minutes and allowed for early ambulation at 4 hours without any incidence of re-bleeding or hematoma. The standard of care in the country where the study was performed is ambulation at 12 hours. QCI demonstrated advanced clotting time, with a shorter and painless hemostasis procedure along with early ambulation. The study concluded, “QuikClot® Interventional™ Hemostatic Bandage obtains prompt hemostasis and allows an early, safe ambulation following coronary diagnostic and interventional procedures by femoral approach.”

<table>
<thead>
<tr>
<th></th>
<th>QuikClot® Gauze (n=100)</th>
<th>Manual Compression (n=100)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Hemostasis Time (minutes)</td>
<td>5.4 ± 1.5</td>
<td>26.2 ± 15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cumulative Frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 minutes</td>
<td>83%</td>
<td>10%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 minutes</td>
<td>91%</td>
<td>30%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8 minutes</td>
<td>100%</td>
<td>38%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median Time to Ambulation (hours)</td>
<td>4</td>
<td>12</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Study 2: QuikClot® Interventional™ Published Clinical Study — European Society of Radiology (Apr 2011)
Two-hundred (200) patients treated with aspirin, clopidogrel, LMWH and warfarin regimens were either treated with QCI (n=100) or standard manual compression (n=100) following catheterization by femoral approach. In the QCI group, hemostasis was achieved in a mean time of 5.4 minutes and all patients ambulated by 4 hours. QCI demonstrated advanced clotting and much earlier ambulation times in comparison to standard of care (12 hours), and especially for patients treated with antiocoagulant therapies. See Figure 1.

Study 3: QuikClot® Radial™ Published Clinical Study — Journal of Interventional Cardiology (Feb 2011)
One-hundred and twenty (120) patients underwent cardiac catheterization via radial artery approach and were divided into 3 groups: Group 1 – QCI was applied for 15 minutes with manual pressure, Group 2 – Gauze (control) was applied for 15 minutes with manual pressure and Group 3 – Standard of Care with Gauze was applied for 2 hours with manual pressure. It should be noted that the study protocol was initially designed to consist of 150 patients (3 groups of 50), but the Gauze control (Group 2) was stopped at 20 patients since all patients bled profusely. See Figure 2.

The QCI product, with 15 minutes of applied manual pressure, achieved hemostasis 100% of the time with 0% incidence of radial artery occlusion. The study concluded, “…QCI pad utilization combined with short-time compression was significantly superior to conventional compression technique in reducing the risk of Radial Artery Occlusion (RAO) after percutaneous transradial coronary procedures. Furthermore, the study shows that early sheath removal and short-time compression [15 minutes versus the standard of care with 2 hour compression] with QCI is safe and effective.”

Study 4: QuikClot® Radial™ Presented Clinical Study — European Association of Percutaneous Cardiovascular Interventions Congress – PCR (May 2011)
Twenty-five (25) patients treated with aspirin and clopidogrel regimens underwent interventional cardiac catheterization via radial artery approach and were treated with QCR. The study demonstrated that QCR was safe and effective in favoring hemostasis after only 30 minutes of radial artery compression. All patients showed normal radial pulse following the use of QCR without any radial artery occlusion recorded.

Study 5: QuikClot® Interventional™ Post Market Surveillance
Eight-hundred and forty (840) patients treated with QCI demonstrated that QCI was successful in controlling bleeding within 5 minutes at the access sites in 98.2% of patients. The preliminary data (n=243) was published in Cath Lab Digest (Jan 2010).
Why Choose QuikClot®? "The Proven Clinical Difference"

Independent Peer-Reviewed Published and Presented Clinical Studies

**Study 6: QuikClot® Efficacy Published Pre-Clinical Study — Journal of Trauma (Sep 2009)**

This study evaluated the efficacy of 5 different hemostatic dressings, including QuikClot® Combat Gauze® (CG), HemCon® (HCs), Celox® (CXb), TraumaStat® (TS) and Placebo Gauze (PG) and found that QuikClot® Combat Gauze® was by far the most effective product. For example, at 110 minutes all animals treated with Celox® (CXb) and HemCon® (HCs) died, whereas all animals treated with QuikClot® Combat Gauze® survived. See Figure 3 and Figure 4.


**Study 7: QuikClot® Safety Published Pre-Clinical Study — Journal of Trauma (Feb 2010)**

This study evaluated the safety of QuikClot® Combat Gauze®, Kerlix gauze (control) and WoundStat in controlling external bleeding. The study found that in all animals treated with Kerlix gauze (control) or Combat Gauze®, the vessels remained patent and local and distal thrombosis was absent. The results show that vascular function was intact in wounds that were treated with Kerlix gauze (control) or Combat Gauze®, further proving that QuikClot® Combat Gauze® is as safe to use as Kerlix gauze (control).


---

**EFFECTIVE**
- Works fast - QuikClot® promotes clotting within minutes from application following arterial and venous bleeding even in severe settings
- 5 published independent clinical studies have proven the efficacy and safety of QuikClot® hemostatic agents
- QuikClot Combat Gauze® has been the #1 choice of hemostatic agent for the U.S. Armed Forces since 2008, and continues to be the only hemostatic product approved by the U.S. Military’s CoTCCC

**SAFE**
- No known contraindications and non-invasive
- Inert - no human or animal derived proteins - no thrombin, fibrinogen or shellfish by-products
- Kaolin, the active clotting agent, has been used safely in cosmetic products for many years
- Independent safety data has been published in the Journal of Trauma (Feb. 2010)

**EASY TO USE**
- Intuitive - simple-to-use dressing format
- Conforms readily to the wound
- Will not break down or fall apart under pressure

**COST EFFECTIVE**
- Safe and less expensive than protein-based products (i.e., thrombin)
- Stops bleeding rapidly, and may reduce the need for more expensive treatments
STUDY 1:

International Journal of Cardiology

A New Kaolin-Based Hemostatic Bandage Use After Coronary Diagnostic and Interventional Procedures

Daniela Trabattoni, Pamela Gatto, Antonio L. Bartorelli

October 2010
A new kaolin-based hemostatic bandage use after coronary diagnostic and interventional procedures

Daniela Trabattoni *, Pamela Gatto, Antonio L. Bartorelli

Department of Cardiovascular Sciences, Centro Cardiologico Monzino, IRCCS, University of Milan, Milan, Italy

**Abstract**

Background: Bleeding and vascular access site complications are an important cause of morbidity after percutaneous femoral procedures. Together with collagen-based and suture-based vascular closure devices, new hemostatic dressings have been developed to control heavy bleeding.

Aim of study: To evaluate safety and efficacy results of the first clinical QuikClot Interventional Hemostatic Bandage use for femoral artery closure after diagnostic or interventional procedures.

Methods: The first European safety study was performed at the Centro Cardiologico Monzino in Milan, Italy, on January 2010. Forty consecutive patients (75% male, mean age 68 ± 11 years) undergoing diagnostic angiography (62%) or PCI (38%) by femoral approach with a 6- (90%) or 7-Fr (10%) size introducer, received arterial sheath removal with the QuikClot Interventional gauze use. The mean ACT value at hemostasis time was 138 ± 24 s (range 95–186 s). Hemostasis was achieved in a mean time of 4.5 ± 0.5 min. Only one hemostasis failure occurred requiring prolonged mechanical compression. Neither major bleeding, re-bleeding nor hematoma occurred after early (4 h after procedure) ambulation.

Conclusions: QuikClot Interventional Bandage obtained prompt hemostasis and allowed for an early ambulation without clinical complications.

© 2010 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Vascular closure starts with a correct arterial access. Access site complications are the single greatest cause of morbidity after percutaneous femoral procedures. Bleeding complications, most notably pseudoaneurysm and hematoma, occur in 5% to 10% of patients and lead to surgical repair in 1.5% of them, approximately [1]. Major predictors for vascular closure device (VCD) failures and bleeding complications at the access site include age, gender, diabetes, renal failure, obesity and sheath insertion in a non-target puncture zone (femoral bifurcation, superficial femoral artery, collaterals – i.e. inferior epigastric artery, severe vascular calcifications or peripheral artery disease) [2,3]. According to the American College of Cardiology 2009 NCDR registry [4], VCD is still only used in a minority of patients (34.6% in diagnostic procedures, 37.6% in PCIs), in the U.S. No large randomized trials are yet available on VCDs compared to manual compression [5]; however, there is growing interest to reduce procedural complications.

In recent years, new hemostatic dressings have been developed to control heavy bleeding [6,7]. Previous in-vivo animal hemorrhage models found the kaolin-based gauze to be the most effective product among four new dressings tested. This device allowed the least amount of hemorrhage and the highest survival rate in tested animals [8]. Here we report safe and effective femoral puncture site closure with QuikClot Interventional Hemostatic Bandage use in the cath lab.

2. Device description

QuikClot Interventional Hemostatic Bandage (Z-Medica, Wallingford, CT) is a non-woven coated gauze (Fig. 1). Each dressing is a multiple-ply 1.5 in. x 1.5 in. x 0.5 in. rayon/polyester construction coated with kaolin. Kaolin is an aluminum silicate, a very potent coagulation initiator that acts as a surface activator. Its inert characteristics allow for no skin allergies at the site of application. The gauze is stable after opening the external aluminum envelop. It is absorbent and has good clotting ability. This advanced clotting gauze is a Food and Drug Administration (FDA) and CE cleared device.

3. Technical specifications

The method for QuikClot use is as follows: 1) apply a firm manual compression on the femoral artery with the QuikClot Intervention Bandage above the entry site while removing the arterial sheath; 2) maintain a firm compression for 5 min; 3) leave the QuikClot over the
access site and cover it with a non-compressive dressing; 4) check the groin at 15 min, 1, 4 and 12 h; and 5) allow the patient to ambulate four hours after hemostasis achievement.

4. Safety and effectiveness report

The first European safety study was performed at the Centro Cardiologico Monzino in Milan, Italy, after the Ethics Committee approval, on January 2010. Forty consecutive patients (75% male, mean age 68 ± 11 years) undergoing invasive diagnostic angiography (62%) or PCI (38%) by femoral approach with a 6- (90%) or 7-Fr (10%) size introducer, received arterial sheath removal at a mean time of 2.43 ± 4.0 h after procedure with the QuikClot Interventional gauze use, once ACT value was ≤ 180 s. The mean ACT value at hemostasis time was 138 ± 24 s (range 95–186 s). Patients were on aspirin (60%), aspirin+clopidogrel (27.5%), LMWH (2.5%) or aspirin+warfarin (5%). Effectiveness results are shown in Table 1.

Hemostasis failure occurred in 1 PCI case, thus hemostasis was achieved after prolonged mechanical compression with the occurrence of a minor (<5 cm) hematoma. Neither major bleeding, re-bleeding nor hematoma occurred after ambulation.

5. Discussion

Vascular closure device use for PCI may be associated with fewer vascular complications [9]. It is however known that anatomical issues (collaterals, severe vessel calcifications, peripheral artery disease, puncture at the bifurcation site, and previous multiple femoral artery stitches) may contraindicate VCD use. This preliminary clinical evaluation in the interventional cardiology field shows QuikClot Interventional Hemostatic Bandage is an easy to use and effective device that assists in achieving a short-term passive hemostasis. This availability of an alternative technique to standard manual or mechanical compression, with an advanced clotting time, allows for a shorter and painless hemostasis procedure as well as for an early ambulation time.

6. Conclusions

QuikClot Interventional Hemostatic Bandage obtains prompt hemostasis and allows an early, safe ambulation following coronary diagnostic and interventional procedures by femoral approach.

Acknowledgement

The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [10].

References


Table 1

<table>
<thead>
<tr>
<th>QuikClot gauze</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cumulative hemostasis time</td>
<td>4.9 ± 1.05 min</td>
</tr>
<tr>
<td>Diagnostic procedures</td>
<td>4.2 ± 0.9 min</td>
</tr>
<tr>
<td>Interventional procedures</td>
<td>5.3 ± 0.95 min</td>
</tr>
<tr>
<td>Cumulative frequencies</td>
<td>4 min 11</td>
</tr>
<tr>
<td>5 min 85</td>
<td></td>
</tr>
<tr>
<td>6 min 93</td>
<td></td>
</tr>
<tr>
<td>8 min 98</td>
<td></td>
</tr>
<tr>
<td>Ambulation time</td>
<td>4 h 100</td>
</tr>
</tbody>
</table>
STUDY 2:

European Radiology

A new kaolin-based haemostatic bandage compared with manual compression for bleeding control after percutaneous coronary procedures

Daniela Trabattoni, Piero Montorsi, Franco Fabbiocchi, Alessandro Lualdi and Pamela Gatto, et al.

April 2011
A new kaolin-based haemostatic bandage compared with manual compression for bleeding control after percutaneous coronary procedures

Daniela Trabattoni · Piero Montorsi · Franco Fabbriochi · Alessandro Lualdi · Pamela Gatto · Antonio L. Bartorelli

Received: 5 December 2010 / Revised: 15 January 2011 / Accepted: 21 February 2011 © European Society of Radiology 2011

Abstract

Objective Bleeding and vascular access site complications are an important cause of morbidity after percutaneous femoral procedures. New haemostatic dressings have been developed to control heavy bleeding. To evaluate the efficacy of a new kaolin-based haemostatic bandage for femoral artery closure after diagnostic or interventional procedures compared with manual compression.

Methods The first pilot European trial using this haemostatic bandage was performed at the in Milan, Italy. Two-hundred patients (71% male, mean age 66±11 years) undergoing angiography or PCI via a femoral approach were randomised to the haemostatic bandage (n=100) or manual compression (n=100) following sheath removal. The mean active clotting time (ACT) at haemostasis was 146±24 s (range 98–198 s). Haemostasis was achieved in 5.4±1.5 min with the bandage vs 25±15 min after manual compression, p<0.001. No haemostasis failure occurred in either group. No differences in oozing, minor and major haematoma and pseudoaneurysms were observed. All patients ambulated at 4 h. Major bleeding, re-bleeding or haematoma did not occur after early (4 h after the procedure) ambulation following use of the bandage.

Conclusions The haemostatic bandage obtained prompt and significantly shorter haemostasis than controls. This novel haemostatic device allowed for early ambulation without clinical complications.

Keywords Kaolin · Haemostasis · Vascular closure devices

Introduction

Vascular closure begins with a proper arterial puncture. Vascular access site complications and bleeding may occur in approximately 5% to 10% of patients and represent the single major cause of morbidity following percutaneous femoral procedures [1]. Among major vascular complications pseudoaneurysm, haematoma, arteriovenous fistula and retroperitoneal haemorrhage are mainly due to technical issues and inadequate bleeding control and may lead to surgical repair in 1.5% of cases [1, 2].

Vascular closure devices (VCD) have been studied extensively to achieve haemostasis at the vascular access site, to decrease the hospital stay, patient discomfort, costs and the rate of complications. During the early use of these devices the risk of complications exceeded that of manual compression [3, 4]. More recently reported experiences have shown VCD to have reduced the rate of vascular complications although in the USA they are still used in only a few patients (34.6% in diagnostic procedures, 37.6% in PCIs), according to the American College of Cardiology 2009 NCDR registry [5]. Consequently no large randomised trials are yet available on VCDs compared with manual compression [6]. In a few cases the anatomical characteristics of the femoral artery (severe vascular calcifications, peripheral artery disease,
vessel diameter <3 mm) and the site of the arterial sheath insertion (i.e. the femoral bifurcation, superficial femoral artery, collaterals – i.e. inferior epigastric artery) may translate into major predictors of VCD failures and bleeding and contraindicate a systematic VCD placement [7, 8].

In recent years, new haemostatic dressings have been developed to avoid collagen-based or suture-based haemostasis in specific vascular and patient settings and to control heavy bleeding [9, 10]. Previous in vivo animal haemorrhage models found a novel kaolin-based haemostatic gauze to be the most effective product among several new dressings. This device allowed the least amount of haemorrhage and the highest survival rate in animal tests [11]. After a preliminary clinical report regarding the safety and efficacy of femoral puncture site closure with QuikClot™ Interventional Hemostatic Bandage use in the catheterization laboratory [12], we now report the first European safety clinical study comparing standard manual compression to QCI, a novel kaolin-based haemostatic dressing.

The aim of our study was to assess the safety, efficacy and performance criteria of QCI compared with standard manual compression.

Materials and methods

Between January and March 2010, 200 patients undergoing coronary diagnostic (n=102) or interventional procedures (n=98) by the femoral approach at the Centro Cardiologico Monzino in Milan, Italy, were randomised in a 1:1 fashion to standard manual compression (M, n=100 patients) or to QuikClot™ use (QCI, n=100 patients) for achieving haemostasis. Patients with baseline INR >1.4 or who had had a previous arterial access at the same femoral site within 30 days were excluded. Only experienced personnel performed femoral artery access and closure with QCI or manual compression. The femoral arterial sheath was removed once the activated clotting time (ACT) was ≤180 s. Ambulation was allowed 4 h after haemostasis. Complete blood count were obtained at baseline and at 24 h in all cases.

Patients were evaluated at 15 min, 1 h, 4 h and 24 h after haemostasis achievement for vascular complications.

**Study enrollment**

If inclusion criteria were met, the patients were asked for written informed consent, as required by the institutional review board in accordance with the Declaration of Helsinki. The protocol was approved by the local Ethical Committee (approval number S131/609) and performed in compliance with good clinical practice.

**Definitions**

Major and minor bleeding were defined according to the Thrombolysis in Myocardial Infarction trial [13]. Major bleeding included ≥5 g/dl decrease in haemoglobin or >15% decrease in haematocrit; minor bleeding included gastrointestinal or genitourinary bleeding, bleeding with a decrease in haemoglobin ≥3 g/dl or >10% decrease in haematocrit or any absolute decrease in haemoglobin ≥4 g/dl or 12%, respectively. Oozing was any minimal bleeding of cutaneous or subcutaneous origin controlled with the application of light compression methods.

Time to haemostasis was defined as the time from the start of compression to the time at which no further compression was required to control bleeding at the arteriotomy site.

**Statistical methods**

Continuous data are expressed as mean value with standard deviation and compared using Student’s t-test. Qualitative data are presented as frequencies and/or percentages and compared using the Chi-squared test or Fisher’s exact test when cell values were <5.

All statistical analyses were performed using the software package SPSS version 17.0 (SPSS, Chicago, IL, USA).

**Device description**

QuikClot™ Interventional Hemostatic Bandage (QCI; Z-Medica, Wallingford, CT, USA) is a non-woven coated gauze impregnated with kaolin (Fig. 1). Each dressing is a multiple-ply 1.5 in.×1.5 in.×0.5 in. (381 mm×381 mm×
127 mm) rayon/polyester construction. Kaolin is an aluminium silicate, a very potent coagulation initiator that acts as a surface activator. Its inert characteristics eliminate the possibility of skin allergies at the site of application. The gauze is stable after opening the external aluminium envelope. It is absorbent and has a good clotting ability. This advanced clotting gauze is a Food and Drug Administration (FDA)- and CE (European Community)-cleared device.

Technical specifications

The method for QCI use was as follows:
1) Apply a firm manual pressure on the femoral artery using the QCI above the entry site while removing the arterial sheath;
2) Maintain firm compression for 5 min;
3) Leave QCI over the access site and cover it with a non-compressive dressing;
4) Allow the patient to ambulate 4 h after haemostasis is achieved.

Results

This first European comparative study enrolled 200 patients undergoing invasive diagnostic angiography or PCI via a femoral approach. The introducer size was 6-Fr in 90% and 7-Fr in the remaining 10% of cases in which a larger sheath size was required for complex coronary lesion treatment. Haemostasis following arterial sheath removal was performed with QCI (n=100) or with standard manual compression (n=100) at a mean time of 91±112 min after the procedure. Patients were well matched regarding clinical characteristics and antiplatelet therapy as shown in Table 1. Time intervals are summarised in Table 2. Mean time to haemostasis was significantly shorter with QCI compared with standard manual compression (5.4±1.5 vs. 25±15 min, p<0.001). Indeed, in the QCI arm, PCI patients required a slightly longer total compression time (6.0±2.0 min) compared with diagnostic patients (5.3±1.7 min; p<0.001). Oozing similarly occurred in 12% of QCI and 10% of manual compression cases (p=0.41) and was completely resolved with prolonged manual compression (mean additional compression time of 3.8±2.0 min) in both groups. This occurrence was more frequently observed in patients treated with PCI, under double antiplatelet treatment and with a higher activated clotting time (ACT) value at the time of sheath removal compared with diagnostic-alone patients (171±42 vs 137±24 s, respectively), thus requiring adjunctive compression at the arterial access site (26% in PCIs vs 17% diagnostics, respectively; p=0.08).

Table 1 Patients demographics

<table>
<thead>
<tr>
<th></th>
<th>QuikClot&lt;sup&gt;TM&lt;/sup&gt; (n=100)</th>
<th>Manual Compression (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>70</td>
<td>60</td>
<td>0.09</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>65.7±13</td>
<td>73.6±6.2</td>
<td>0.09</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>73.9±12</td>
<td>71.2±15</td>
<td>0.53</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>15</td>
<td>16</td>
<td>0.15</td>
</tr>
<tr>
<td>Renal Failure (%)</td>
<td>7</td>
<td>6</td>
<td>0.50</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>45</td>
<td>42</td>
<td>0.38</td>
</tr>
<tr>
<td>LMW Heparin (%)</td>
<td>4</td>
<td>2</td>
<td>0.34</td>
</tr>
<tr>
<td>Aspirin + Clopidogrel (%)</td>
<td>29</td>
<td>26</td>
<td>0.37</td>
</tr>
<tr>
<td>Aspirin (%)</td>
<td>60</td>
<td>60</td>
<td>0.11</td>
</tr>
<tr>
<td>Aspirin + Warfarin (%)</td>
<td>7</td>
<td>3</td>
<td>0.16</td>
</tr>
<tr>
<td>None (%)</td>
<td>-</td>
<td>9</td>
<td>0.50</td>
</tr>
</tbody>
</table>

Table 2 Haemostasis effectiveness

<table>
<thead>
<tr>
<th></th>
<th>QuikClot&lt;sup&gt;TM&lt;/sup&gt; (n=100)</th>
<th>Manual Compression (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACT at haemostasis time (sec)</td>
<td>145.4±29.6</td>
<td>140.2±20.4</td>
<td>0.67</td>
</tr>
<tr>
<td>Time to haemostasis (min)</td>
<td>87±137.8</td>
<td>94±126</td>
<td>0.21</td>
</tr>
<tr>
<td>Mean haemostasis time</td>
<td>5.4±1.5</td>
<td>26.2±15</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Cumulative Frequencies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>83%</td>
<td>10%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>6 min</td>
<td>91%</td>
<td>30%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>8 min</td>
<td>100%</td>
<td>38%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Median time to ambulation (hours)</td>
<td>4</td>
<td>4</td>
<td>NA</td>
</tr>
<tr>
<td>Delay in ambulation beyond 4 h (%)</td>
<td>8</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Table 3 Vascular complications

<table>
<thead>
<tr>
<th></th>
<th>QuikClot&lt;sup&gt;TM&lt;/sup&gt; (n=100)</th>
<th>Manual Compression (n=100)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major Bleeding (%)</td>
<td>1</td>
<td>1</td>
<td>0.50</td>
</tr>
<tr>
<td>Retroperitoneal (%)</td>
<td>1</td>
<td>1</td>
<td>0.50</td>
</tr>
<tr>
<td>Haematoma, &gt; 5 cm (%)</td>
<td>1*</td>
<td>2</td>
<td>0.37</td>
</tr>
<tr>
<td>Pseudoaneurysm (%)</td>
<td>1*</td>
<td>1</td>
<td>0.50</td>
</tr>
<tr>
<td>Total (n/patients)</td>
<td>2</td>
<td>4</td>
<td>0.23</td>
</tr>
</tbody>
</table>

*same patient
Occurrence of bleeding and vascular complications is described in Table 3. Major bleeding, re-bleeding and haematoma did not occur after ambulation.

**Discussion**

The incidence of vascular complications comparing VCD with manual compression after percutaneous coronary interventions was assessed in a previous observational study by Dangas et al. [3]. The use of a VCD was associated with a significantly higher rate of haematomas (9.3% vs. 5.1%, p<0.001), a greater decrease in haematocrit (5.2% vs 2.5%, p<0.001) and an increased need for surgical repair (2.5% vs. 1.5%, p=0.003). According to initial studies in the late 1990s, vascular complications were twice as common compared with manual compression and frequency of surgical repair of arterial damage, formation of haematoma and a drop in haematocrit were noted to be higher among the VCD cases [13]. However, the experience in VCD use gained by interventionalists and improvements in device design also allowed better results with reduced or similar vascular complications between device use and manual compression in more recently reported data [4, 14]. It is however known that anatomical issues (the presence of collateral vascularisation, peripheral artery disease, severe vessel calcifications, small vessel size, puncture at the bifurcation site, previous multiple femoral artery sutures) may contraindicate VCD deployment. Therefore, when a coronary interventional procedure is performed in these subsets with a specific contraindication to VCD deployment and heparin, bivalirudin or glycoprotein IIb-IIIa inhibitors administered, standard manual or mechanical compression need to be applied for a longer time after the end of procedure in order to reduce the excess bleeding and bed-rest is therefore prolonged. Furthermore, according to the literature, groin haematoma is expected in 5% to 23% of patients after manual compression, pseudoaneurysm in 0.5% to 9%, and arteriovenous fistula in 0.2% to 2% [4]. Patients with advanced age and/or peripheral vascular disease were shown to have a higher risk of major complications, approaching 5–10% [15]. Our first preliminary clinical evaluation in the interventional cardiology field showed QCI to be an easy to use and effective device compared with standard manual compression haemostasis were demonstrated to be successful. In the present study, major and minor bleeding associated with QCI or manual compression haemostasis were demonstrated to be similar. This preliminary comparative study confirmed that early haemostasis and ambulation may be possible even without a VCD, when adequate arterial compression is successfully achieved.

**Conclusions**

The kaolin-based QuikClot™ Interventional Hemostatic Bandage (QCI) obtains prompt haemostasis and allows early, safe ambulation following coronary diagnostic and interventional procedures via a femoral approach. QCI is a useful adjunctive tool in assisting manual or mechanical compression. This device may contribute to making the haemostatic process easier and shorter than with standard manual compression and could lead to a complete avoidance of intra- or extravascular device deployment. Further multicentre, randomised clinical evaluations comparing the safety and efficacy of QCI vs. VCD in PCI cases are warranted and currently underway and their results will gather further scientific evidence on QCI use for bleeding control at vascular access sites.

**References**

5. American College of Cardiology National Cardiovascular Data Registry, Cardiac Catheterization. Available at: http://www.ncdr.com/webNCDR/NCDRDocuments
STUDY 3:

Journal of Interventional Cardiology

Randomized Clinical Trial on Short-Time Compression with Kaolin-Filled Pad: A New Strategy to Avoid Early Bleeding and Subacute Radial Artery Occlusion after Percutaneous Coronary Intervention

Luigi Politi, Alessandro Aprile, Catia Paganelli, Andrea Amato, Giuseppe B. Zoccai, Fabio Sgura, Daniel Monopoli, Rosario Rossi, Maria G. Modena, and Guiseppe M. Sangiorgi

February 2011
RANDOMIZED TRIAL

Randomized Clinical Trial on Short-Time Compression with Kaolin-Filled Pad: A New Strategy to Avoid Early Bleeding and Subacute Radial Artery Occlusion after Percutaneous Coronary Intervention

LUIGI POLITI, M.D., ALESSANDRO APRILE, M.D., CATIA PAGANELLI, M.D., ANDREA AMATO, M.D., GIUSEPPE B. ZOCCAI, M.D., FABIO SGURA, M.D., DANIEL MONOPOLI, M.D., ROSARIO ROSSI, M.D., MARIA G. MODENA, M.D., and GIUSEPPE M. SANGIORGI, M.D.

From the Interventional Cardiology, Policlinico Hospital, University of Modena and Reggio Emilia, Modena, Italy

Background: Despite the increasing use of transradial techniques for cardiac percutaneous procedures, none of the strategies commonly utilized for hemostasis has been able to reduce the occurrence of radial artery occlusion (RAO). The aim of this study was to evaluate the occurrence of 24-hour RAO and the rate of bleeding of a novel hemostatic device for radial closure after percutaneous interventions, in adjunct to short-time compression.

Methods: Once the radial access was obtained, patients were randomized to 3 different strategies of radial closure: a short compression with the QuikClot\textsuperscript{®} Interventional\textsuperscript{™} pad (Z-Medica Corporation, Wallingford, CT, USA) (15 minutes, group 1), a short compression (15 minutes, group 2), and a conventional prolonged compression (2 hours, group 3) both without QuikClot\textsuperscript{®} utilization.

Results: Fifty patients in group 1, 20 in group 2, and 50 in group 3 were enrolled. The three groups were homogenous for baseline and procedural characteristics. None of patients in group 1 developed RAO, 1 (5%) occurred in group 2, and 5 (10%) in group 3 (P = 0.05). Active bleeding after compression removal occurred in 10 patients (20%) in group 1, 18 (90%) in group 2, and 1 (2%) in group 3 (P < 0.001). Among patients in group 1, at univariate analysis, the predictors of acute bleeding resulted in chronic therapy with clopidogrel (Odds Ratio 28.78, 95% Confidence Intervals 4.79–172.82, P < 0.001) and high levels of activated clotting time (ACT) at the time of sheath removal (OR 1.02, 95% CI 1.00–1.03, P = 0.009). At ROC analysis, the cutoff value of ACT for the risk of bleeding with a sensitivity of 80% and specificity of 75% was 287 seconds.

Conclusions: Early sheet removal and short-time compression with QuikClot\textsuperscript{®} Interventional\textsuperscript{™} can reduce the rate of RAO after diagnostic or interventional procedures especially in patients not on double antiplatelet therapy.

Introduction

Transradial access for percutaneous cardiovascular procedures provides a low rate of local complications and similar clinical results compared to other sites of access.\textsuperscript{1–3} Sheath removal after transradial catheterization is conventionally based on external compression achieved with a simple bandage applied to the entry site.\textsuperscript{4} Observational studies have shown that prolonged or aggressive compression techniques favor local complications such as radial artery occlusion (RAO).\textsuperscript{4,5} RAO is relatively infrequent (5%–10% of cases)\textsuperscript{6,7} and usually considered as a quiescent complication of transradial procedures because of the dual circulation of the palmar arch. Yet, its occurrence precludes future transradial access forcing the shift to the femoral access, which is responsible for more bleeding complications and prolonged hospitalization as well as greater discomfort for the patient. Therefore,
it seems reasonable to preserve radial patency by keeping compression time as short as possible. Dedicated hemostatic devices have been developed over the past few years, but the advantages in terms of efficacy of these tools compared to conventional manual compression technique have never been demonstrated. QuikClot® Interventional™ bandage (Z-Medica Corporation, Wallingford, CT, USA) is a novel hemostatic device, consisting of a pro-coagulant (Kaolin) agent-filled hydrophilic nonwoven pad that can be applied topically as an adjunct to manual compression and is indicated for the local management and control of surface bleeding from vascular access sites. When Kaolin comes into contact with blood in a wound, it rapidly absorbs the smaller water molecules from the blood. The larger platelets and clotting factor molecules remain in the wound in a highly concentrated form promoting a rapid natural clotting.

Randomization to Closure Techniques. After achieving the radial access and based on a computergenerated randomization list, patients were allocated into one of the following strategy groups. Group 1: the compression of radial artery was obtained with QuikClot® Interventional™ hemostatic pad applied directly on the skin with a folded gauze over the pad. All the dressing was then wrapped with adhesive tape and maintained for 15 minutes. After this period, the compression was removed leaving the QuikClot® pad for 2 hours secured with a Tegaderm™ (3M Company, St. Paul, MN, USA) adhesive bandage (Fig. 5). Group 2: hemostasis was obtained by direct compression of the radial artery with a folded conventional sterile gauze wrapped with tape and maintained for 15 minutes only (control group, similar to group 1 without the tested pad). After this period, the compression was removed leaving a sterile gauze secured with a Tegaderm™ adhesive bandage. Group 3: standard technique consisting of direct compression of the site of puncture with a folded conventional sterile gauze wrapped with tape and maintained for 2 hours (conventional strategy).

Radial Access. After sterile preparation and injection of 2% lidocaine at the puncture site, a 20-gauge needle was used to enter the radial artery 5 cm above the crease of the wrist using Seldinger’s technique. On the appearance of pulsatile flow, a Terumo® (Terumo Corporation, Tokyo, Japan) 0.018-inches guidewire was advanced into the radial artery lumen. A 6-Fr glide sheath was then advanced over the guidewire into the radial artery (Radifocus® Introducer II, Terumo Corporation, Tokyo, Japan). Two hundred micrograms of nitroglycerin or 5 mg of diltiazem were administered diluted in a 20-mL syringe intrararterially. In addition, a standard dose of 5,000 IU of unfractionated heparin was administered, followed by additional doses in case of coronary intervention to achieve and maintain activated clotting time (ACT) >250 seconds. The ACT was measured in all patients at the end of the procedure from the arterial introducer sheath using the Hemochron® (ITC, Edison, NJ, USA) system. All arterial sheaths were removed immediately after the diagnostic or interventional procedure irrespectively of the ACT value.

Methods

All consecutive patients undergoing transradial elective diagnostic or interventional coronary procedures between November 1, 2009 and January 31, 2010 at the Catheterization Laboratory of Modena University Hospital were considered to be enrolled in the study. The only exclusion criteria were an abnormal Allen’s test before puncture and failure to provide written informed consent. Once the radial access was obtained, patients were randomized into 3 arms corresponding to 3 different strategies of radial closure. Randomization was based on a computer-generated randomization list. Clinical variables were obtained by review of hospital charts and patient interview; any medication that could potentially affect bleeding was noted, and systolic and diastolic blood pressure were measured at the end of the procedure. Patients receiving chronic warfarin therapy before hospital admission were noted; in all cases, warfarin was stopped at least 3 days before the procedure until International Normalized Ratio (INR) was <1.5 and low molecular weight heparin (LMWH) was administered. The study protocol was approved by the institutional investigation committees and all patients signed informed consent.

End-points of the Study. The main end-point was subacute RAO; the secondary end-point was failure of the closure technique. Death, myocardial infarction, or major bleeding occurring in hospital was appraised as a secondary end-point.
Radial Artery Occlusion. Radial artery patency was assessed using the Barbeau’s test\(^1\) at 24 hours after the procedure. The investigator assessing the artery patency was blinded to the closure technique used. A pulse oximeter sensor was placed over the index fingertip to obtain a plethysmographic signal. Both radial and ulnar arteries were compressed to observe a loss of the plethysmographic signal. Next, the radial artery was released, and the return of the plethysmographic signal was observed. A return of the signal confirmed radial artery flow and, hence, patency. An absence of the return of the signal was interpreted as RAO. The ulnar artery was then released to observe the return of the signal, confirming proper functioning of the equipment. All findings of occlusion were confirmed ultrasonographically by another investigator. Efficacy was defined as a radial artery patency confirmed by the presence of plethysmographic signal and ultrasonographic wave 24 hours after the procedure.

Closure Technique Failure. After the scheduled time of compression (15 minutes for groups 1 and 2 and 2 hours for group 3), the entry site was revised for signs of active bleeding (acknowledged as failure of the closure strategy). In case of failure, the compression was restored using the conventional strategy that was maintained for additional 2 hours and observed thereafter until bleeding stop. Safety was defined as absence of bleeding from the radial access site after the scheduled time from sheath removal in the different groups.

Statistical Analysis. Data are expressed as percentages or mean ± standard deviation. Analysis was done by intention-to-treat. Comparison across groups were performed using Analysis of Variance (ANOVA) test for continuous variables and Pearson’s chi-square test or Fisher’s exact test where appropriate for categorical variables. Receiver operator characteristics (ROCs) analysis was used to test the value of ACT associated with greater sensitivity and specificity in predicting failure of closure technique. Logistic regression analysis was used to identify univariate predictors of outcomes. All tests were two-tailed and \(P < 0.05\) was used for statistical significance. The Statistical Package for Social Sciences, version 15.0, software (SPSS, Chicago, IL, USA) was used for analysis.

Results

Clinical and Procedural Data. Of the planned 150 patients (50 for each group), 120 patients were enrolled in the study and followed for end-points—50 in group 1, 20 in group 2, and 50 in group 3. For ethical reasons, the enrollment in group 2 was stopped after the 20th patient because of high rate of active bleeding after compression removal (18 out of 20 patients). Subsequently, the randomization was continued only for group 1 and 3 until the achievement of 50 patients per group. Mean age was 61.8 ± 13.2 years, 72.5% of the patients were male and 15% were diabetics. Interventional procedures were performed in 59.2% of cases. Right radial artery was used in 86.7% of cases, left radial artery in the remainder. The three groups were homogenous for baseline and procedural characteristics (Tables 1 and 2). No major adverse events such as death, myocardial infarction, or major bleeding occurred during the hospitalization in any of the randomization groups.

Radial Artery Occlusion. None of the patients enrolled in group 1 developed the main outcome variable. Among patients enrolled in group 2 RAO occurred in 1 case (5%) and among group 3 in 5 cases (10%) (\(P = 0.05\), Fig. 1). The predictors of RAO in group 3 were older age (OR 1.09, 95% CI 1.00–1.18, \(P = 0.049\)) and smaller amount of intraprocedural heparin infused (OR 0.70, 95% CI 0.49–0.99, \(P = 0.044\)) (Table 3), while no predictors could be appraised in group 2.

Failure of Closure Device. Active bleeding after compression removal occurred in 10 patients (20%) in group 1, 18 (90%) in group 2, and 1 (2%) in group 3 (\(P < 0.001\), Fig. 2). In all cases, hemostasis was achieved with a supplementary compression for 2 hours that did not produce any RAO in group 1. Among patients enrolled in group 1, at univariate analysis, the only predictors of acute bleeding resulted chronic therapy with clopidogrel (OR 28.78, 95% CI 4.79–172.82, \(P < 0.001\)) and high levels of ACT at the time of sheath removal (OR 1.02, 95% CI 1.00–1.03, \(P = 0.009\), Table 4). Among group 2 and group 3, none of the baseline clinical or procedural variables were associated with a significantly increased risk of active bleeding. At ROC analysis, when ACT was below 200 seconds none of the patients developed bleeding in group 1, while all patients with a value greater than 399 developed it. The cutoff value with a sensitivity of 80% and specificity of 75% was 287 seconds. The area under the curve was 0.761 (\(P < 0.001\), Fig. 3).

Clinical Follow-Up. A telephone contact at 6 months was initiated to all 6 patients (1 in group 2 and 5 in group 3) who have experienced RAO. They were
Table 1. Baseline Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group 1 N = 50</th>
<th>Group 2 N = 20</th>
<th>Group 3 N = 50</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>64.16 ± 11.53</td>
<td>61.30 ± 14.22</td>
<td>59.72 ± 14.23</td>
<td>0.241</td>
</tr>
<tr>
<td>Male sex</td>
<td>37 (74%)</td>
<td>14 (70%)</td>
<td>36 (72%)</td>
<td>0.939</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.87 ± 0.15</td>
<td>1.90 ± 0.18</td>
<td>1.93 ± 0.17</td>
<td>0.279</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>171.60 ± 8.29</td>
<td>171.20 ± 6.76</td>
<td>171.38 ± 6.69</td>
<td>0.977</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.42 ± 11.13</td>
<td>80.00 ± 14.96</td>
<td>82.02 ± 13.26</td>
<td>0.091</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>6 (12%)</td>
<td>4 (20%)</td>
<td>8 (16%)</td>
<td>0.676</td>
</tr>
<tr>
<td>Hypertension</td>
<td>29 (58%)</td>
<td>13 (65%)</td>
<td>32 (64%)</td>
<td>0.781</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>29 (58%)</td>
<td>11 (55%)</td>
<td>33 (66%)</td>
<td>0.602</td>
</tr>
<tr>
<td>Previous MI</td>
<td>14 (28%)</td>
<td>4 (20%)</td>
<td>12 (24%)</td>
<td>0.766</td>
</tr>
<tr>
<td>Previous radial puncture</td>
<td>3 (6%)</td>
<td>2 (10%)</td>
<td>4 (8%)</td>
<td>0.835</td>
</tr>
<tr>
<td>Chronic aspirin therapy</td>
<td>46 (92%)</td>
<td>19 (95%)</td>
<td>50 (100%)</td>
<td>0.132</td>
</tr>
<tr>
<td>Chronic clopidogrel therapy</td>
<td>10 (20%)</td>
<td>2 (10%)</td>
<td>11 (22%)</td>
<td>0.505</td>
</tr>
<tr>
<td>LMWH administration</td>
<td>8 (16%)</td>
<td>5 (25%)</td>
<td>5 (10%)</td>
<td>0.274</td>
</tr>
<tr>
<td>Warfarin before hospital admission</td>
<td>6 (12%)</td>
<td>3 (15%)</td>
<td>3 (6%)</td>
<td>0.435</td>
</tr>
</tbody>
</table>

BSA = body surface area; MI = myocardial infarction; LMWH = low molecular weight heparin.

Table 2. Procedural Characteristics

<table>
<thead>
<tr>
<th>Procedural Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Procedural time (minute)</td>
<td>74.20 ± 38.15</td>
<td>68.50 ± 36.63</td>
<td>66.10 ± 32.36</td>
<td>0.516</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>126.00 ± 27.77</td>
<td>122.95 ± 19.82</td>
<td>127.50 ± 21.18</td>
<td>0.733</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78.64 ± 15.15</td>
<td>78.25 ± 9.64</td>
<td>77.96 ± 11.62</td>
<td>0.966</td>
</tr>
<tr>
<td>Heparin (IU/10³)</td>
<td>6.91 ± 2.96</td>
<td>8.40 ± 3.40</td>
<td>7.74 ± 3.04</td>
<td>0.150</td>
</tr>
<tr>
<td>Interventional procedure</td>
<td>24 (48%)</td>
<td>12 (60%)</td>
<td>35 (70%)</td>
<td>0.081</td>
</tr>
<tr>
<td>Right radial access</td>
<td>46 (92%)</td>
<td>18 (90%)</td>
<td>40 (80%)</td>
<td>0.188</td>
</tr>
<tr>
<td>IIB/IIA glycoprotein inhibitors</td>
<td>3 (6%)</td>
<td>4 (20%)</td>
<td>7 (14%)</td>
<td>0.205</td>
</tr>
<tr>
<td>Activated clotting time at the end of procedure (second)</td>
<td>267.84 ± 71.99</td>
<td>266.10 ± 63.69</td>
<td>267.24 ± 55.82</td>
<td>0.995</td>
</tr>
</tbody>
</table>

all alive, no major cardiac events occurred, and they did not notice any discomfort at the arm corresponding to the site of puncture.

Discussion

Despite the widespread and increasing use of trans-radial techniques for cardiac and peripheral percutaneous procedures, none of the strategy or devices commonly used for hemostasis have been able to avoid the occurrence of RAO, likely because these methods need prolonged artery compression. In the present study, we utilized a new compression strategy in order to reduce the incidence of subacute RAO consisting in applying the QuikClot® pad on the radial access with a compression maintained for only 15 minutes, leaving thereafter only the dressing with a simple bandage. This trial tested the occurrence of 24-hour RAO and the rate of bleeding after compression removal comparing a short compression with the QuikClot® Interventional™ pad versus two other strategies, a short compression and a conventional prolonged compression both without QuikClot® utilization. The main result of the trial is that QuikClot® utilization prevents RAO (0 cases compared to 5% and 10%, respectively, in group 2 and 3, P = 0.05) with a relatively low risk of access site bleeding after its removal (bleeding occurred in 20% of cases in group 1 compared to 90% in group 2 and 2% in group 3, P < 0.001). The occurrence of subacute RAO in the conventional group is similar to that reported in the literature⁶,⁷ and much higher than in the other two groups. This finding underscores the importance of a short-duration compression to reduce this complication. The reason of higher rate of RAO in patients of group 3 than those of group 1 with long compression after bleeding may be simply related to higher number of cases (50 vs. 10). Moreover, we assume also that the higher levels of ACT and the more intense antiplatelets regimen in the 10 patients enrolled in group 1 may have reduced the risk of RAO. Although
SHORT-TIME RADIAL COMPRESSION WITH KAOLIN-FILLED PAD

20% of bleeding may seem high, it is worth noting that QuikClot® failure was associated with a chronic administration of double antiplatelet therapy and high levels of ACT after sheet removal. In particular at the univariate analysis, patients receiving clopidogrel were 28 times more likely to have early bleeding after compression removal, and ACT values greater than 287 seconds represented a strong predictor of bleeding with 80% sensitivity and 75% specificity. Nevertheless, in all patients who had QuikClot® failure, conventional compression was successfully performed with no further bleeding complications.

To date, there are no published studies reporting the use of QuikClot® InterventionalTM hemostatic bandage after percutaneous coronary or peripheral procedures associated to a short-time compression to obtain early closure of radial access without bleeding complications.

The QuikClot system consists of a soft, white, sterile, hydrophilic nonwoven pad that should be applied topically together with a 3M® TegadermTM adhesive bandage. The QuikClot system has been used over the past 6 years by United States troops in the battlefield for treating external compressible hemorrhages. QuikClot favors arterial closure by two mechanisms. First, it acts as a selective sponge by absorbing water and concentrating coagulation factors at the site of wound. Second, the pad is filled with Kaolin, a clay mineral that activates the XII factor of coagulation accelerating the coagulation cascade (Fig. 4). QuikClot Interventional is a pad simply applicable at the site of puncture that does not require a learning curve for the operator or cath lab personnel or a patient monitoring after removing at the end of 15 minutes compression. This may be relevant in terms of costs reduction because the other currently available compression tools (e.g., TRband, Terumo Corporation, Japan) usually require a specific training for nurses or cath lab operators and a

<table>
<thead>
<tr>
<th>Table 3. Univariate Predictors of Radial Artery Occlusion among Patients in Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Weight</td>
</tr>
<tr>
<td>Chronic clopidogrel therapy</td>
</tr>
<tr>
<td>LMWH</td>
</tr>
<tr>
<td>Interventional procedure</td>
</tr>
<tr>
<td>Procedural time</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>IIB/IIa glycoprotein inhibitor</td>
</tr>
<tr>
<td>Heparin (IU/103)</td>
</tr>
<tr>
<td>Activated clotting time</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

<table>
<thead>
<tr>
<th>Table 4. Univariate Predictors of Failure of Closure Technique among Group 1 Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR</strong></td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>BSA</td>
</tr>
<tr>
<td>Ongoing clopidogrel</td>
</tr>
<tr>
<td>LMWH</td>
</tr>
<tr>
<td>Warfarin before hospital admission</td>
</tr>
<tr>
<td>Interventional procedure</td>
</tr>
<tr>
<td>Procedural time (minute)</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
</tr>
<tr>
<td>IIB/IIa glycoprotein inhibitors</td>
</tr>
<tr>
<td>Heparin (IU/103)</td>
</tr>
<tr>
<td>Activated clotting time (second)</td>
</tr>
</tbody>
</table>
Figure 3. The blue line represents the ROC curve describing the sensibility and the specificity of ACT in predicting the device failure (active bleeding after 15 minutes removal) in group 1. As the area under the curve is 0.761 and the curve is on the left of neutral line (green), we can assume that ACT has a good accuracy in predicting the event.

Continuous monitoring for progressive compression decrease related to device deflation.

Clinical Implications

This study demonstrated that QuikClot® InterventionalTM is a useful option in the hands of interventional operators to obtain quick hemostasis of the radial artery with a reduced risk of RAO compared to conventional techniques. The present study suggests that the maximum benefit of this technique can be obtained especially in patients who are not on double antiplatelets therapy or who have lower values of ACT at the end of the procedure. Thus, waiting for lower ACT values before sheath removal is a reasonable option to reduce device failure.

Strengths and Limitations

To the best of our knowledge, this is the first randomized study to compare the QuikClot® InterventionalTM
for radial access closure in comparison to conventional techniques in a series of consecutive patients undergoing coronary angiography or angioplasty. Because this was a trial testing a new device and a new technique, the number of participants was limited to that necessary to test the main study end-point. However, the limited number of exclusion criteria enhances the generalizability of the results. We have not assessed chronic RAO by Barbeau’s test; therefore, it may be that some patients with RAO at 24 hours in group 3 would eventually recanalize at 30 days as previously shown. However, the advantage of QuikClot in group 1 remains evident as no patient developed RAO. Similarly to other small size trials with minor hazards, there was not an external independent safety monitoring board, but the study investigators monitored the study for the occurrence of adverse effects. Indeed, due to an unexpectedly high level of failure in one group, the study investigators decided to stop early one arm of the study for futility. Of note such complication was minor, and none of the patients enrolled in the trial developed major adverse events. Finally, there is an imbalance in interventional procedures with elective or bailout IIb/IIIaglycoprotein inhibitors-inhibitors administration among group 1 and 3. This is the result of a blind randomization performed before coronary angiography that could have produced an underestimation of RAO incidence in group 3 for the higher antiplatelets regimen respect to group 1.

**Conclusions**

The present randomized clinical trial demonstrated for the first time that a novel hemostatic technique based on QuikClot® Interventional™ pad utilization combined with short-time compression is significantly superior to conventional compression technique in reducing the risk of RAO after percutaneous transradial coronary procedures. Furthermore, the study shows...
that early sheet removal and short-time compression with QuikClot® Interventional™ is safe and effective especially in patients not on double antiplatelet therapy and can be performed routinely after diagnostic or interventional procedures. Despite QuikClot® Interventional™ application, early sheet removal with an ACT higher than 287 seconds or in patients taking chronic dual antiplatelet therapy should be cautious due to the risk of hemorrhage at the puncture site.

References

STUDY 4:
European Association of Percutaneous Cardiovascular Interventions Congress

Safety and efficacy of a new dedicated hemostatic kaolin pad in sealing vascular access in patients undergoing trans-radial coronary procedures: a pilot study

May 2011
Safety and efficacy of a new dedicated hemostatic kaolin pad in sealing vascular access in patients undergoing trans-radial coronary procedures: a pilot study

Background

Transradial access for percutaneous cardiovascular procedures provides a low rate of local complications and similar clinical results compared to other sites of access. None of the devices commonly used for hemostasis has been proved superior to manual compression for bleeding risk or able to avoid the occurrence of artery occlusion, likely because these methods need prolonged artery compression. QuikClot Radial (QCR) is a new hemostatic device consisting of a hydrophilic pad impregnated with kaolin, a pro-coagulant mineral, and accompanied by a compression bandage.

Methods

Between November and December 2010, 25 consecutive patients undergoing either interventional or diagnostic trans-radial cardiac catheterization were treated with QCR. At the end of the procedure the QCR was applied over the introducer and the catheter was then removed, leaving the pad in direct contact with the skin and the entry site. The compression bandage was then applied. After 30 minutes, the compression and QCR were completely removed and a simple medical bandage was applied. Patient’s vital signs and vascular access site were monitored for the following 12 hours.

Results

Twenty of the 25 (80%) patients were male, 36% were diabetics, mean age was 69±12 years. All patients were on chronic 100mg Aspirin and 24% also under 75mg Clopidogrel regimen. At the time of QCR removal there were no failures of the device and all patients reached complete hemostasis. No complications were recorded during the hospital stay and further intervention was not necessary to control bleeding. All patients showed a normal radial pulse following the use of QCR and no radial artery occlusion was recorded.

Conclusion

We demonstrated for the first time that QuikClot Radial is safe and effective in favoring hemostasis after 30 minutes only of radial artery compression following percutaneous cardiac procedures.
STUDY 5:

Cath Lab Digest

QuikClot® Interventional™ Hemostatic Bandage (QCI): A Novel Hemostatic Agent for Vascular Access

Mohan Pahari, Rachael Moliver, Denny Lo, David Pinkerton, Giacomo Basadonna

January 2010
QuikClot® Interventional™ Hemostatic Bandage (QCI): A Novel Hemostatic Agent for Vascular Access

Moham Tahari, MD, *Rachel Motzer, †Denny Lo, ‡David Pitterman, *Giuliano Basadonna, MD, PhD
*University of Massachusetts Medical School Department of Surgery, Worcester, Massachusetts; ‡Z-Medica Corporation, Wallingford, Connecticut

More than 1,000,000 percutaneous coronary intervention (PCI) procedures are performed in the U.S. every year and many more are performed annually worldwide. Even though hemostasis at the vascular access site has conventionally been achieved by manual compression followed by a period of recumbency, new devices have significantly increased the methods available to achieve hemostasis at the entry site. A number of complications are associated with puncture-related vascular access, including hemorrhage, thrombosis, embolization, and infection. Moreover, vascular complications occur in up to 7% of patients after PCI, including development of arteriovenous fistula, pseudoaneurysms, or large hematomas, and can require surgical repair and/or blood transfusions. Also, vascular complications at the femoral artery puncture site have been reported in 2.6-16.8% of patients after percutaneous transluminal coronary angioplasty (PTCA) or coronary stenting. Thus, there is a need for better and more effective methods to achieve hemostasis. For more than 30 years, manual compression has remained the gold standard for access site management; nevertheless, the development of interventional Cardiovascular procedures has called for large sheaths and anticoagulation. These new procedures led to a rising interest in the development of vascular closure devices (VCDs) to better manage bleeding at the access site. There are three main categories of VCDs: collagen-based, suture-based, and external methods such as pads, staples, and clips. VCDs have been demonstrated to reduce time to hemostasis, facilitate ambulation, and potentially decrease length of stay. The choice of a device usually depends on the availability of that particular device, operator preference, anticipation of repeat arterial access, site of the arteriovenous fistula, and the cost associated with the device. This study introduces a novel kaolin-based hemostatic device, the QuikClot® Interventional™ hemostatic bandage (QCI) (Z-Medica Corporation, Wallingford, CT), which is designed to control bleeding at the vascular access site.

Methods
Device description. The QuikClot Interventional hemostatic bandage (QCI) consists of a non-woven rayon and polyester gauze pad impregnated with kaolin, an inert mineral that does not contain animal or human protein. Contact between kaolin and blood initiates the clotting process by activating Factor XIIa. Factor XI and prekallikrein are then changed to their activated forms. QCI is FDA-cleared as an adjunct to manual compression and is indicated for the local management and control of surface bleeding from vascular access sites, percutaneous catheters, or tubular wiring introduced sheaths up to 12 F.

Figure 1. Arterial and venous vascular access in swine model.

Pre-clinical Evaluation of QuikClot® Interventional™ Hemostatic Bandage (QCI)

The animals were prepared and draped, consistent with standard protocol. The femoral artery, femoral vein, carotid artery, and jugular vein were accessed percutaneously using the Seldinger technique. Tissue diameters and introducer sheaths were used to produce wound tracks of 3 to 12 F in size. Each animal and each vessel was used for more than one access. Once the distal and the introducer were placed within the vessel, they were removed and the QCI pad was subsequently placed over the bleeding site at the groin. Manual compression was held for 2 minutes and then a 10 cm x 12 cm Tegaderm™ Transparent Dressing was applied over the pad for an additional 3 minutes. At 5 minutes, the Tegaderm and pad were both removed and the site was evaluated for bleeding and/or hematoma formation. Video recordings and photographs were taken throughout the study to accurately depict the observations and measurements recorded throughout the procedure. All animals had their vital signs recorded during the procedure with particular attention to blood pressure measurement. Animals were euthanized at the end of the procedure.

Human clinical data. Z-Medica Corporation, the manufacturer of QCI, has collected considerable clinical data on the use of this device as part of a post-market surveillance effort in fifteen institutions throughout the United States. Physicians and other health care providers were required to fill out a Use Report Form describing their overall clinical experience and outcome following each use. Data regarding 243 documented human uses are hereby reported.

Results
A total of 23 vascular access procedures in a swine model are reported. Wound type and vascular access characteristics are summarized in Figure 1. Blood pressure and bleeding status were recorded at 5 minutes after QuikClot Interventional hemostatic bandage (QCI) was placed over the wound. On average, animals were normotensive throughout the procedures (Table 1).

QCI successfully controlled surface bleeding within 5 minutes in all cases.
Table 1: Vascular Access in Swine Models

<table>
<thead>
<tr>
<th>Type of Access</th>
<th>Total Number of Vascular Access Procedures</th>
<th>Systolic Blood Pressure during Procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>25</td>
<td>101 ± 17.0</td>
</tr>
<tr>
<td>Venous</td>
<td>5</td>
<td>83 ± 8.1</td>
</tr>
<tr>
<td>Arterial/Venous</td>
<td>20</td>
<td>83 ± 8.1</td>
</tr>
</tbody>
</table>

Table 2: Outcome of Various Tissue Dilator Access in Swine Models

<table>
<thead>
<tr>
<th>Type of Access</th>
<th>Tissue Dilator Size</th>
<th>Percentage of bleeding controlled at 8 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>8 F</td>
<td>100%</td>
</tr>
<tr>
<td>Venous</td>
<td>8 F</td>
<td>100%</td>
</tr>
<tr>
<td>Arterial/Venous</td>
<td>12 F</td>
<td>100%</td>
</tr>
</tbody>
</table>

Table 3: Human Clinical Outcomes from Use Report Forms

<table>
<thead>
<tr>
<th>Type of Access</th>
<th>No. of Procedures</th>
<th>Success</th>
<th>Failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>238</td>
<td>231</td>
<td>7</td>
</tr>
<tr>
<td>Venous</td>
<td>5</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>243</td>
<td>226</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 2. Human clinical outcome

Clinical Evaluation of QuikCUT Interventional™ Hemostatic Bandage (QCI)

- Re-arrangement of Factor XI in space, making it more susceptible to become activated in a process overall defined as "contact activation". Kaolin improves clotting time by about 40s to 60s and has been tested extensively in stringent models of lethal vascular injury in vivo. In fact, QCI is directly derived from Combat Gauze™, which is currently the main hemostatic agent of choice for all branches of the U.S. military. The present study indicates that QCI is very effective in controlling bleeding following vascular access in both experimental animals and for routine clinical use.

In combination with bubble massage, QCI is successful in arresting hemorrhage in almost all instances following arterial and venous interventions for both diagnostic and interventional purposes, with no complications, even when large-size catheters were used. QCI is intuitive to use and does not contain human or animal protein, carrying virtually no risk of transmitting infection. It is safe and effective in controlling bleeding and could represent significant cost savings, given its reasonable price. Randomized prospective clinical trials are currently underway to confirm these preliminary results.

References


STUDY 6:

Journal of Trauma

Determination of Efficacy of New Hemostatic Dressings in a Model of Extremity Arterial Hemorrhage in Swine

Bijan S. Kheirabadi, Michael R. Scherer, J. Scot Estep, Michael A. Dubick, John B. Holcomb

September 2009
Determination of Efficacy of New Hemostatic Dressings in a Model of Extremity Arterial Hemorrhage in Swine

Bijan S. Kheirabadi, PhD, Michael R. Scherer, MA, J. Scot Estep, DVM, Michael A. Dubick, PhD, and John B. Holcomb, MD

Background: The HemCon (HC) bandage and QuickClot have been used over the past 6 years for treating external compressible hemorrhage in combat casualties. Previously, we tested three new hemostatic agents in granular/powder forms that were superior to these products. In this study, four new dressings (preselected) that are more suitable for battlefield application were evaluated. The efficacy and acute safety of the dressings were tested in our standard arterial hemorrhage model.

Methods: Anesthetized pigs (n = 38, 37 kg) were instrumented, and arterial blood was collected for hematological and coagulation assays. After splenectomy, the right femoral artery was isolated, injured (6 mm arteriotomy), and unrestricted bleeding allowed for 45 seconds. A hemostatic dressing (HC RTS [n = 6], Celox-D [CXb, n = 6], TraumaStat [TS, n = 10], Combat Gauze [CG, n = 10], or placebo gauze [PG, n = 6]) was then applied over the wound randomly and compressed for 2 minutes. Fluid resuscitation was administered and titrated to maintain a mean arterial pressure of 65 mm Hg. Animals were observed for 180 minutes or until death. Computed tomography angiography was performed on survivors and tissues were collected for histology.

Results: No differences were found in baseline blood measures, pretreatment blood loss or fluid infusion among groups. HCs and CXb testing discontinued after six unsuccessful tests, and the data were excluded. Stable hemostasis was achieved in two PG, two TS, and eight CG pigs in remaining groups resulting in stabilized mean arterial pressure and significantly different survival rates (20–80%, p = 0.03). CG secured hemostasis for 134.6 minutes ± 22.2 minutes, which was significantly longer than TS (35.7 ± 22.0 minutes, p < 0.05) but not different from PG (57.9 ± 36.2 minutes). The average survival time of CG-treated animals (167.3 ± 5.9 minutes) was also significantly longer (p < 0.05) than that of TS- (90.0 ± 15.3 minutes) or PG-treated (121 ± 19.3 minutes) pigs. Posttreatment blood loss was less in CG (37.4 ± 17.3 mL/kg) than that of the two other groups (TS = 79.8 ± 13.8 mL/kg and PG = 75.5 ± 23.8 mL/kg), but this difference was not significant. No significant rise in wound temperature (≥1°C) was recorded after treatment with dressings and computed tomography images showed no flow through the vessels. Histologic observations showed mild to moderate changes in treated vessels with no difference between CG and PG. In vitro analysis of blood treated with CG or PG (lesser extent) showed increased clotting rate and clot strength. TS treatment had no effect on blood clotting activity.

Conclusion: CG was the most effective dressing tested in this arterial hemorrhage model. The hemostatic property of CG is attributed to its raw material (nonwoven Rayon and polyester blend), kaolin coating, and the large surface area (3 inch × 4 yd) of this absorbent sponge. CG is now recommended as the first line of treatment for life-threatening hemorrhage on the battlefield, replacing HC.

Key Words: Combat gauze, TraumaStat, Celox D, HemCon, Hemorrhage control, Side effect, Swine.

(J Trauma. 2009;67: 450–460)

Uncorrected hemorrhage is the leading cause of death (50%) among combat casualties and is the second major cause of death in civilian trauma patients.1–4 Massive bleeding and trauma are major risk factors leading to the lethal triad of life-threatening coagulopathy, which include persistent hypothermia, metabolic acidosis, and inability to form clot and establish hemostasis.5,6 Hemorrhage also plays a significant role in late morbidity and mortality because of multiple organ failure that may be caused by prolonged hypotension, sepsis, and massive red cell and plasma product transfusion.7,8 A review of autopsies of 982 combat deaths in the current conflict by an expert panel showed that nearly 24% of the deaths could have potentially been prevented with prompt and effective treatment.9 Of these 24% victims, majority (85%) died of potentially preventable hemorrhage with one of three being compressible and two of three being noncompressible wounds. Although there is no hemostatic modality to treat noncompressible (internal) hemorrhage in the prehospital phase, since 2003 two new hemostatic products, QuickClot and HemCon (HC) bandage, have become available for treating compressible (external) hemorrhage in the battlefield in addition to tourniquets. Despite these advancements, some of the compressible hemorrhages could not be controlled promptly and eventually led to the death of soldiers. Thus, hemorrhage control and the search for more effective hemostatic modalities continue to have a high priority in the US Army Combat Casualty Care Research program.

As part of these efforts, we have recently identified three new hemostatic agents in granular/powder forms that were significantly more effective than the current hemostatic products used on the battlefield.10 These included WoundStat

DOI: 10.1097/TA.0b013e3181ac0c99

Submitted for publication February 9, 2009.
Accepted for publication April 15, 2009.
Copyright © 2009 by Lippincott Williams & Wilkins
From the Damage Control Resuscitation Division, US Army Institute of Surgical Research, Fort Sam Houston, Texas.
Presented at the 22nd Annual Meeting of the Eastern Association for the Surgery of Trauma, January 13–17, 2008, Lake Buena Vista, Florida.
The opinions or assertions expressed herein are the private views of the authors and are not to be construed as official or as reflecting the views of the US Department of the Army or the US Department of Defense.
None of the authors has any affiliation with the manufacturer of these products, and the authors have no potential conflicts of interest to declare.
Address for reprints: Bijan S. Kheirabadi, PhD, 3400 Rawley E., Chambers Ave., Building 3611, Fort Sam Houston, TX 78234; email: bijan.kheirabadi@us.army.mil.

The Journal of TRAUMA® Injury, Infection, and Critical Care • Volume 67, Number 3, September 2009

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.
(WS), a smectite mineral granules with potent hemostatic activity that forms highly adhesive clay material when mixed with blood; Super Quick Relief (SQR), a mineral-based hemostatic powder with which blood forms an artificial scab on the wound and seals injuries; and Celox (CX), a chitosan-based powder that seals bleeding sites by chemical and mechanical linkage with red blood cells in wounds. The hemostatic efficacy and acute safety of these products were tested against a groin arterial hemorrhage in swine that could not be controlled by standard gauze, QuikClot, or HC bandages (100% mortality). WS was found to be the most efficacious agent, followed by SQR and CX with 100%, 70%, and 60% survival rates, respectively. However, histologic examination of treated tissues (femoral artery and nerve) revealed significant thermal damage by SQR and presence of WS microscopic residues inside the treated arteries. The use of granular/powder agents are advantageous for treating complex irregular wounds because they can be spread and tightly packed in the wound, covering all the bleeding sites that may be missed when a small size inflexible dressings are used. On the other hand, handling and precise application of granular or powder agents to deep penetrating wounds with profuse bleeding are generally more difficult and inefficient than dressing, particularly in the field under extreme cold, insufficient light or windy conditions. For these reasons, we searched to find flexible and efficacious dressings that could have the advantages of granular agents but easily applied without the chance of spillage in any circumstances. We identified two new flexible hemostatic dressings namely Combat Gauze (CG) and TraumaStat (TS) plus Celox-D (CXb), dressing like packages of chitosan powder in small dissolvable bags, to test for their efficacy and tissue effects in our standard arterial hemorrhage model in swine. Two control dressings were also included in the study; one was a more advanced HC bandage (RTS, thinner and more flexible) and the other one was a placebo gauze (PG) that is used for production of CG but without the active clotting agent (kaolin).

**MATERIALS AND METHODS**

This study was approved by the Animal Care and Use Committee of the US Army Institute of Surgical Research. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals. Test Material

All the test materials have received Food and Drug Administration clearance as safe devices with indication for temporary external use to control moderate to severe bleeding in patients. The materials must be removed from wounds before definitive surgical repair. CG and TS dressings were initially tested in our pilot experiments with encouraging results using only a few pigs. Another dressing that was also tested was a textile dressing known as Staslon; however, because of its poor performance in our pilot test it was not included in this study. CX bags, which resemble small dressings, were included because of the success of CX powder in our previous study. The test materials were donated to our institute by the manufacturing companies through material transfer agreements. Brief descriptions of each test agent are as follows.

1. TS dressing is a unique nonwoven substrate comprised of porous polyethylene fibers filled with precipitated silica. The fibers are coated with a chitosan derivative for increased adhesiveness. The dressings used in this study were ~20 inch long, 4 inch wide, and 3 mm thick and produced by Ore-Medix, LLC Company (Lebanon, OR). They were folded into 4 inch × 4 inch packs and placed in the wound. The efficacy of TS was demonstrated in an earlier study using a groin laceration hemorrhage model in swine.

2. CG, a product of Z-Medica Corporation (Wallingford, CT) is a 4 yd long, 3 inch wide roll of nonwoven medical gauze impregnated with a contact (intrinsic) pathway activating clotting agent known as kaolin. The prototype of this dressing (X-Sponge) showed encouraging results in less severe hemorrhage models in swine when tested by our colleagues at the Naval Medical Research Center (personnel communication).

3. CXb, a product of SAM Medical (Portland, OR), is composed of small chitosan particles similar to regular CX powder but enclosed in four small transparent bags, which dissolve rapidly (<10 seconds) when they come in contact with blood. Unlike CX powder, the CXb were easy to apply to a wound with profuse bleeding and stayed in place without the risk of being washed away by blood loss.

4. HC RTS (HCs) dressing is an advanced version of chitosan dressing made by HemCon Inc. (Portland, OR), which is presoften, more flexible, and thinner than the original HC dressing.

5. PG, used as a control material, was identical to CG but was not coated with kaolin clotting agent. These surgical gauze rolls were also obtained from Z-Medica Corporation along with CG.

**In Vivo Methods**

Yorkshire cross-bred pigs (castrated males only) weighing 34 kg to 42 kg were purchased from Midwest Research Swine and used in this study. The original intent was to test each material in 10 pigs but for reasons described below, some materials were tested in only six animals. Before the surgery date, venous blood samples were collected from pigs and complete blood count (CBC) and standard coagulation parameters (prothrombin time, activated partial thromboplastin time, fibrinogen) were measured to ensure these values are within normal range before proceeding with experiments. Pigs were fasted for 12 hours to 18 hours before the surgery with free access to water. On the day of surgery, pigs were premedicated with buprenorphine (0.025 mg/kg, intramuscular [i.m.]) for analgesia and glycopyrrolate (0.01 mg/kg, i.m.) to reduce saliva secretion and block vagally mediated bradycardia during the surgical procedure. Animals were induced with an injection of tiletamine-zolazepam (Telazol, 4–6 mg/kg, i.m.) and anesthetized initially with 5% isoflurane in oxygen via face mask. They were then intubated and mechanically ventilated with 100% oxygen. The tidal volume and ventilation rate were adjusted to maintain an end tidal PCO2 of 40 mm Hg ± 2 mm Hg.
Anesthesia was maintained with 1% to 2% isoflurane added to oxygen gas by the ventilator. Maintenance fluid, lactated Ringer’s (LR), was administered at 5 mL·kg⁻¹·hr⁻¹ through a venous line placed in an ear vein.

**Surgical Procedures**

The right carotid artery was cannulated for blood draws and direct recording of blood pressure (systolic, diastolic, and mean) and heart rate throughout the experiment. A 9-mL blood sample was collected from the arterial line and mixed with 1 mL of Na citrate (3.2%) as anticoagulant and used for thrombelastography (TEG) assays, as described later. The right jugular vein was also catheterized for administering resuscitation fluid. A midline laparotomy was then performed, followed by a splenectomy to minimize blood changes that may occur as a result of autotransfusion from the pig’s contractile spleen. The blood loss from splenectomy was replaced by infusing LR at three times the weight of the spleen. A cystotomy was also performed to aid in the drainage of urine. The abdomen was then closed with suturing, and the skin was stapled. Preinjury (baseline) blood samples were collected from the arterial line for CBC, coagulation, and blood gas analysis.

To create a severe hemorrhage in the groin area, ~5 cm of femoral artery was dissected free from surrounding tissues, and the overlying abductor muscle was removed. Injury to the adjacent femoral vein and nerve was avoided. The vessel was then bathed with a few milliliters of 2% lidocaine to relax vasospasm and dilate the artery to its normal size. To measure wound temperature, a microelectrode was sutured to the muscle adjacent to the vessel but at least 1 inch away from the arteriotomy site, so that it would not interfere with the hemostatic treatment. Next, a 10-minute stabilization period was allowed (no manipulation) and baseline data including mean arterial pressure (MAP) and body temperature were recorded. A stable MAP of 60 mm Hg or higher was required before proceeding with the rest of the experiment. The maintenance fluid was discontinued at this point. The artery was clamped continuously at this point. Compression was stopped after 2 minutes and hemostasis observed for 3 minutes without removing the laparotomy gauze. If rebleeding occurred during this period, the laparotomy gauze was removed and the failed agent taken out and replaced with fresh material. The 2-minute compression was then repeated with a new laparotomy gauze. Wounds were treated at most twice with each product regardless of hemostatic outcome. Hemostasis was then observed for the next 3 hours with laparotomy gauze left in place. Any shed blood during this period was collected and measured as posttreatment blood loss.

After the infusion of Hextend, fluid administration was continued with LR (100 mL/min, maximum of 10 L) as needed to raise and maintain the MAP at 65 mm Hg throughout the experiment. The MAP of 65 mm Hg approximates systolic pressure of 90 mm Hg, which is in agreement with the level of permissive hypotensive resuscitation regimen. Animals were monitored up to 3 hours or until death as determined by end tidal PCO₂ <15 mm Hg and MAP <20 mm Hg. Final blood samples (arterial) were collected for hematological measurements before euthanizing the animals.

Surviving animals were CT scanned and images of arterial blood flow and vascular structures in their legs were obtained. Next, the treated legs of surviving pigs were flexed and stretched five times simulating walking condition to test the stability of the hemostasis provided by the test agents. At the conclusion of experiments, the product was removed from the wound to check the status of injury and the patency of the vessel. Animals were then killed and tissue samples including the injured artery, adjacent femoral vein, femoral nerve, and muscle tissues were collected for histologic examination. Histologic slides were prepared according to a standard procedure and stained with hematoxylin and eosin. The slides were coded and examined by a board certified veterinarian pathologist who was blinded to the treatment group. Once the examination of individual slides was completed, the codes were broken, and the results were categorized under each specific product. Control tissue samples (for histologic comparisons only) were collected from the contralateral leg of a few surviving pigs. The control arteries were isolated in the same manner, perforated with 6-mm puncher and bled for 45 seconds before harvesting for histology along with other tissues.

**In Vitro Methods**

TEG method was used to examine the hemostatic property of each test agent in vitro. The TEG machines (TEG Hemostasis Analyzer 5,000, Hemoscope, Niles, IL) were calibrated before use with quality control standards obtained from Hemoscope. To prepare the samples, 2-mm diameter circular pieces were cut out from each dressing using a punch biopsy instrument. Ten milligrams of each dressing pieces were placed in a small plastic vial and 2 mL of citrated blood, freshly collected from the arterial line, was added and capped. The vials were gently inverted eight times and 340-μL blood samples were taken and placed in TEG cups for analysis.
Calcium chloride (20 μL of 0.2 mol/L) was added to the cups before adding blood samples to overcome the anticoagulant effect. The coagulation effects of the new dressings were compared with standard kaolin vial, a known activator of the contact (intrinsic) clotting pathway. A recalcified blood sample with no treatment was also tested as control. Samples were tested in duplicate and tracing continued until 30 minutes after the clot reached maximum strength. The following variables were measured for each sample at 37°C: reaction time (R, minutes, the time that the initial fibrin formation is detected); clotting time (K, minutes, the speed of clot formation and is the time from the R time until a clot with a fixed firmness is formed); angle (α, degree, the kinetics of clot development); and maximum amplitude (MA, millimeter, the maximum strength or firmness of the developed clot). The velocity of clot formation was also calculated as the first derivative of the TEG tracings and maximum clotting velocity (Vmax, millimeter/minute) was determined.

**Data Analysis**

Data are expressed as mean ± standard of error of the mean (SEM) and analyzed by analysis of variance, Fisher’s exact, and Log rank for statistical comparisons. p values were adjusted according to False Discovery Rate method for bigroup comparison. The data with high variance were log transformed for analysis of variance. The nonparametric data were analyzed using Newman-Keuls multiple comparison test, and bigroup comparison was done using Dunnett’s test. A p < 0.05 was considered statistically significant.

**RESULTS**

**In Vivo**

No difference was found in baseline physiologic and hematological measurements among the treatment groups (Table 1).

**Hemostasis Achievement**

The incidence of initial hemostasis achievement (secure hemostasis for at least 3 minutes immediately after treatment) was relatively low ranging from 0% (HCs, CXb) to 30% (CG) with the dressings. In general, two treatments were required to produce hemostasis for all the products except for CG where hemostasis was achieved after the first dressing application in three experiments. The original intent was to test each new dressing in 10 pigs; however, because HCs and CXb failed to produce hemostasis in six consecutive experiments and all treated animals died of bleeding, further testing of these dressings was eliminated, and the related data were excluded from the data set for statistical analysis. The PG was tested only in six pigs because of limited supply.

CG produced immediate hemostasis in three pigs, and this lasted for the entire experiments. In five other animals, it stopped bleeding after 12 minutes to 26 minutes of slow hemorrhage and secured hemostasis for the rest of experiments. The simulated walking condition (movement of the legs) at the conclusion of experiments did not cause rebleeding in surviving animals. The average times that bleeding was controlled by the dressings (hemostasis time) and other hemostatic outcomes are shown in Table 2. The hemostasis time for CG-treated animals was significantly (p < 0.05) longer than TS group.

**MAP and Blood Loss**

The average MAP of each group, which reflects the hemostatic conditions of the pigs during the experiment, is shown in Figure 1. The baseline pressures (Table 1) and sharp decreases after injury and hemorrhage were not different among the groups. The MAP became significantly different (p < 0.05) 30 minutes after injury for the animals that were treated with CG as compared with those treated with TS. At 60 minutes postinjury, MAP of CG-treated animals was substantially higher than all other groups (p < 0.05).

<table>
<thead>
<tr>
<th>Measure</th>
<th>HemCon RTS (HCs), n = 6</th>
<th>Celox-D (CXb), n = 6</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>38.4 ± 1.1</td>
<td>37.4 ± 1.0</td>
<td>36.4 ± 1.0</td>
<td>34.9 ± 0.9</td>
<td>38.1 ± 0.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>37.7 ± 0.1</td>
<td>37.6 ± 0.1</td>
<td>37.5 ± 0.1</td>
<td>0.54</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>70.5 ± 2.4</td>
<td>69.0 ± 2.2</td>
<td>74.8 ± 2.2</td>
<td>76.8 ± 2.6</td>
<td>75.2 ± 2.4</td>
<td>0.20</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>10.0 ± 0.04</td>
<td>9.7 ± 0.3</td>
<td>10.1 ± 0.2</td>
<td>9.2 ± 0.2</td>
<td>9.8 ± 0.2</td>
<td>0.24</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>29.4 ± 1.1</td>
<td>28.1 ± 1.0</td>
<td>29.3 ± 0.6</td>
<td>26.7 ± 0.8</td>
<td>28.3 ± 0.5</td>
<td>0.14</td>
</tr>
<tr>
<td>PLT (1,000/μL)</td>
<td>363 ± 42</td>
<td>323 ± 30</td>
<td>410 ± 32</td>
<td>406 ± 69</td>
<td>396 ± 34</td>
<td>0.59</td>
</tr>
<tr>
<td>PT (s)</td>
<td>10.9 ± 0.2</td>
<td>11.5 ± 0.3</td>
<td>11.3 ± 0.3</td>
<td>11.4 ± 0.1</td>
<td>10.9 ± 0.3</td>
<td>0.32</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>17.2 ± 0.3</td>
<td>16.6 ± 0.3</td>
<td>16.6 ± 0.5</td>
<td>16.9 ± 0.6</td>
<td>17.1 ± 0.5</td>
<td>0.30</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>202 ± 10</td>
<td>200 ± 15</td>
<td>205 ± 22</td>
<td>215 ± 11</td>
<td>222 ± 6</td>
<td>0.35</td>
</tr>
<tr>
<td>pH</td>
<td>7.43 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>7.4 ± 0.0</td>
<td>0.51</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>2.25 ± 0.2</td>
<td>2.3 ± 0.4</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>6.0 ± 0.2</td>
<td>6.4 ± 0.7</td>
<td>4.5 ± 0.7</td>
<td>5.5 ± 0.7</td>
<td>5.3 ± 0.8</td>
<td>0.43</td>
</tr>
</tbody>
</table>

Data expressed as mean ± SEM and analyzed by one-way analysis of variance test. No significant difference was found among groups.

HGB, hemoglobin; PLT, platelets; PT, prothrombin time; aPTT, activated partial thromboplastin time.
was 19.5 mL/kg/H11006

30 minutes (p to a higher level than the pressure of the other animals at nonsurvival) treated with one type of dressing. The MAP of surviving pigs in the last 30 minutes of experiments that have returned to baseline levels.

The average pretreatment blood loss for all the animals was 19.5 mL/kg ± 1.1 mL/kg with no difference among groups (Table 2). The posttreatment blood loss ranged from 37.4 mL/kg for CG-treated to 113.8 mL/kg for CXb-treated animals (Table 2, Fig. 2). Although the average blood loss in CG group was nearly half of the TS or PG groups, this difference was not statistically significant because of high variability in collected data (CXb and HCs data were not considered).

The final values of CBC, coagulation and blood gas measurements at the conclusion of experiments are shown in Table 3. The hemoglobin, platelet counts, clotting times, and blood gas measures corresponded to the degree of blood loss and fluid replacement in each treatment group. In the case of CG, blood gas values (pH, lactate, and base excess) remained closer to baseline levels and along with hemoglobin, were significantly better than TS- or both TS- and PG-treated animals. No significant temperature increase was measured in the wounds as a result of treatment with any of the dressings (Table 2).

The CT images of surviving animals showed complete blockage of blood flow in femoral arteries at the treated site by all the dressings. Flow though collateral arteries, however, was not affected. A representative CT image of a CG-treated pig is shown in Figure 3.

**Survival**

Eighty percent of CG-, 33% of PG-, and 20% of TS-treated animals lived for the entire experiments (Table 2) with the final MAP of 64 mm Hg ± 2.3 mm Hg. The differences in survival rates among the three groups were significant (p = 0.03), but the difference between CG and TS or PG was not statistically significant (posttest bigroup comparison). The Kaplan-Meier analysis of survival time for all groups is shown in Figure 4. The average survival time of

---

**TABLE 2. Outcomes of Treating a Groin Arterial Hemorrhage With Different Hemostatic Dressings in Swine**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>HemCon* RTS (HCs), n = 6</th>
<th>Celox-D* (CXb), n = 6</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial hemostasis achieved† (no. application)</td>
<td>0/6 (12)</td>
<td>0/6 (12)</td>
<td>1/10 (20)</td>
<td>1/6 (12)</td>
<td>3/10 (17)</td>
<td>NS</td>
</tr>
<tr>
<td>Total time bleeding stopped (min)</td>
<td>3.0 ± 2.2</td>
<td>0.5 ± 0.04</td>
<td>35.7 ± 22.2</td>
<td>57.9 ± 36.2</td>
<td>134.6 ± 22.2†</td>
<td>0.02 (one-way ANOVA)</td>
</tr>
<tr>
<td>Pretreatment blood loss (mL/kg)</td>
<td>19.2 ± 1</td>
<td>21.8 ± 1.8</td>
<td>19.3 ± 1.2</td>
<td>19.3 ± 1.6</td>
<td>18.2 ± 0.7</td>
<td>NS</td>
</tr>
<tr>
<td>Posttreatment blood loss (mL/kg)</td>
<td>108.2 ± 7.5</td>
<td>113.8 ± 8.2</td>
<td>79.8 ± 13.8</td>
<td>75.5 ± 23.8</td>
<td>37.4 ± 17.3</td>
<td>NS</td>
</tr>
<tr>
<td>Total resuscitation fluid (mL/kg)</td>
<td>175.3 ± 24.8</td>
<td>189.1 ± 16.2</td>
<td>160.3 ± 14.4</td>
<td>186.2 ± 41.9</td>
<td>123.9 ± 27.2</td>
<td>NS</td>
</tr>
<tr>
<td>Survival rate</td>
<td>0/6</td>
<td>0/6</td>
<td>2/10</td>
<td>2/6</td>
<td>8/10</td>
<td>0.03 (χ²)</td>
</tr>
<tr>
<td>Survival time (min)</td>
<td>74.3 ± 10.5</td>
<td>74.2 ± 5.8</td>
<td>90.0 ± 15.3</td>
<td>121 ± 19.3</td>
<td>167.3 ± 5.9§</td>
<td>&lt;0.001 (logrank)</td>
</tr>
<tr>
<td>Peak wound temperature (°C)</td>
<td>36.8 ± 0.2</td>
<td>36.6 ± 0.2</td>
<td>36.6 ± 0.3</td>
<td>37.0 ± 0.1</td>
<td>35.7 ± 0.3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard error of the mean; NS = p > 0.1.

*Testing of HemCon bandage and Celox bag was stopped after six unsuccessful experiments. The related data were excluded from the data set for statistical analysis.

†Initial hemostasis was considered to occur when bleeding was stopped for at least 3 minutes after compression. ANOVA, analysis of variance; NS, not significant.

---

**Figure 1.** The average MAP of each group of pigs (survival and nonsurvival) treated with one type of dressing. The MAP of CG-treated animals increased in response to resuscitation to a higher level than the pressure of the other animals at 30 minutes (p < 0.05) and 60 minutes (p < 0.01) posttreatment time points. Note the MAP of surviving pigs in the last 30 minutes of experiments that have returned to baseline levels.

**Figure 2.** The pretreatment and posttreatment blood loss (mean ± SEM) of pigs treated with hemostatic dressings. No difference was found in initial hemorrhage (pretreatment blood loss) among groups. The posttreatment blood loss in CG was <50% of the volumes in the other groups (TS or PG), but this difference was not statistically significant (HCs and CXb data were not included).
CG-treated animals (167 minutes) was significantly longer than that of PG- (121 minutes) or TS-treated (90 minutes) pigs (p < 0.05) (Table 2).

Morphologic and Histologic Assessment

At conclusion of experiments, CG, PG, and TS dressings were easily removed from the wounds resulting in the rupture of the hemostatic clot and rebleeding at the injury site in surviving animals. No significant intraluminal clot was found in the distal or proximal ends of treated vessels after dressings were removed. Complete removal of chitosan particles (CXb) from the wound required more effort than other dressings and pieces of the bags (undissolved), and some dry chitosan material were often found in the wound.

The observed histologic changes were moderate injury to endothelial layers of treated arteries, minimal to mild changes in femoral vein and perivenous tissues, minimal to moderate focal necrosis on muscle surfaces, minimal to mild perineural inflammation, and moderate neutrophilic infiltration in all the tissues. Based on the histologic changes, the dressings were ranked by the veterinarian pathologist in the following order: HCs (least change) / TS / CG / CXb / PG (most change). The only possibly significant change in any of these tissues was the lack of endothelium in some treated segments of treated arteries (Fig. 5, arrows). The clinical implication of this damage cannot be determined without long-term survival studies. None of the described damages, however, were significant enough to disqualify the use of any of the dressings.

The Journal of TRAUMA® Injury, Infection, and Critical Care • Volume 67, Number 3, September 2009

Efficacy of New Hemostatic Dressings

© 2009 Lippincott Williams & Wilkins

455

TABLE 3. Final Hematological Measurements in the Operated Pigs

<table>
<thead>
<tr>
<th>Value</th>
<th>HemCon RTS* (HCs), n = 6</th>
<th>Celox-D* (CXb), n = 6</th>
<th>TraumaStat (TS), n = 10</th>
<th>Placebo Gauze (PG), n = 6</th>
<th>Combat Gauze (CG), n = 10</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGB (g/dL)</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.1</td>
<td>2.1 ± 0.6</td>
<td>2.7 ± 1.2</td>
<td>5.1 ± 0.8*</td>
<td>0.03</td>
</tr>
<tr>
<td>PLT (1,000/μL)</td>
<td>66.8 ± 10.5</td>
<td>63.7 ± 15.0</td>
<td>127 ± 35.9</td>
<td>121 ± 43.8</td>
<td>245 ± 51.4</td>
<td>0.1</td>
</tr>
<tr>
<td>PT (s)</td>
<td>29.5 ± 3.3</td>
<td>28.9 ± 2.6</td>
<td>26.9 ± 5.0</td>
<td>20.5 ± 2.9</td>
<td>17.5 ± 4.7</td>
<td>0.3</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>40.5 ± 3.9</td>
<td>39.1 ± 1.5</td>
<td>31.2 ± 3.9</td>
<td>32.1 ± 3.7</td>
<td>20.9 ± 2.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Fibrinogen† (mg/dL)</td>
<td>73.5 ± 2.0</td>
<td>120.4 ± 29.1</td>
<td>143 ± 32.4</td>
<td>123 ± 21.9</td>
<td>196.2 ± 10.9</td>
<td>0.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.5 ± 0.04</td>
<td>7.6 ± 0.04</td>
<td>7.6 ± 0.04</td>
<td>7.57 ± 0.04</td>
<td>7.45 ± 0.04†</td>
<td>0.04</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>16.1 ± 1.6</td>
<td>14.6 ± 0.4</td>
<td>11.6 ± 1.8</td>
<td>11.9 ± 3.3</td>
<td>2.4 ± 0.3†§</td>
<td>0.003</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>−2.65 ± −1.8</td>
<td>−2.1 ± 1.8</td>
<td>0.5 ± 1.4</td>
<td>0.1 ± 2.3</td>
<td>4.9 ± 1.6†</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard error of the mean. The data with large variance were transformed (log or squared) for statistical comparison by one-way analysis of variance. PT, prothrombin time; aPTT, activated prothromboplastin time.

* Testing of HemCon bandage (presoften) and Celox-D was stopped after six unsuccessful experiments. The data related to these products were excluded from the data set for statistical analysis.

† Fibrinogen concentration could not be measured in the final blood samples in 17 animals because of excessive hemodilution. The values represent the average fibrinogen concentration of the samples that were measured successfully in each group.

§ p < 0.05 versus TS (Newman-Keuls multiple comparison test).

© 2009 Lippincott Williams & Wilkins

455

Figure 3. A typical computed tomography image of the arterial blood flow in the hind legs of a surviving pig treated with CG 3 hours after surgery. Note the blockage of flow in femoral artery where it is compressed by the dressing (arrow). Limited collateral circulation is also seen.

Figure 4. Kaplan-Meier analysis of survival time of pigs treated with each dressing. The CG-treated animals lived significantly longer than PG- or TS-treated (p < 0.05) pigs.
The clotting profiles (TEG tracings) of blood samples treated with hemostatic dressings or kaolin are shown in Figure 6. The traces represent the average measurements of arterial blood samples collected from five pigs before surgery. Treatment of blood with HCs or TS dressing, both of which contain chitosan, either had no effect or decreased blood clotting activities as compared with untreated (recalci-
ified only) samples. On the other hand, treatment of blood with kaolin alone or kaolin containing gauze (CG) elicited strong response and shifted the clotting curves to the left side indicating faster (shorter R time and bigger angle) and stronger (larger MA) clot formation than untreated blood. To a lesser extent, exposure to regular gauze also stimulated faster and stronger clot formation than controls. The TEG measurements of different clotting parameters (R, K, [\alpha], MA, and Vmax) for each dressing and their analysis are summarized in Table 4.

DISCUSSION

In this study, the efficacies of three promising hemostatic dressings, CG, TS, CXb, and a new formulation of HC bandage (RTS) were examined and compared with a placebo-gauze equivalent to CG but without active agent (kaolin). All the dressings, including PG, were applied to the wound with the aid of a laparotomy sponge that was left in place for the entire experiment.

The results demonstrated that the HC RTS bandage, although thinner and more flexible (conformable), was less effective than the enhanced formulation of this dressing tested in our earlier study.\(^\text{10}\) The HC RTS bandages initially conformed and sealed the wound, capable of stopping low-pressure arterial hemorrhage, but once fluid was administered and blood pressure returned to baseline level, the adherence of dressing failed and rebleeding started and continued until animals exsanguinated. No secondary hemostasis occurred with these bandages. These results give further evidence that the hemostatic function of chitosan-based products is mediated by tissue adhesive properties and not by the clotting activities of these agents.\(^\text{14}\) This is also supported by the current TEG analysis of blood samples treated with the chitosan dressing that showed no change or slower clotting function than the untreated samples.

The other chitosan product, Celox-D was also found to be ineffective despite successful results with this product as free powder in our previous study\(^\text{10}\) and in another investigation.\(^\text{15}\) The packaging of free powder in small dissolvable bags improved handling and application of the product, but it interfered with the binding of chitosan particles with bleeding tissues. This occurred despite our best effort during application (massage the bags for few seconds) to assure that the bags were dissolved and free powder released in the wound before compression started. Because of six consecutive failures to obtain stable hemostasis with Celox-D or HC RTS, further testing of these products was discontinued.
Treatment with TS produced hemostasis only in two experiments; one developed immediately after application and the other 18 minutes after slow bleeding and both were stable for the duration of experiments. This large and relatively stiff dressing, made of chitosan-coated silica-filled polyethylene fibers, potentially has both hemostatic and adhesive properties. However, TEG analysis of blood samples treated with this dressing showed no increase in clotting activities. Perhaps changes that can increase flexibility and conformity of this dressing may improve the efficacy of this product.

CG was the most efficacious dressing tested in this study resulting in 80% (8 of 10) survival of the animals. The only other dressing that exhibited similar efficacy in this hemorrhage model (66% survival rate) was a prototype fibrin sealant bandage composed of lyophilized human fibrinogen and thrombin on an absorbable backing. However, unlike fibrin sealant or chitosan dressings (adhesive material), CG may not produce hemostasis immediately after application to the wound. In our experiments with CG, immediate hemostasis achieved only in three animals, whereas in five others stable hemostasis developed after a period of slow bleeding. Hemostasis did not occur in two experiments and animals died after massive hemorrhage. Because of this variability, the average blood loss of CG group was not significantly different from the other groups. This may suggest contradictory findings regarding survival benefit of CG if posttreatment blood losses were not different. The average posttreatment blood loss of the eight animals that survived with CG treatment was only 12 ± 4.1 mL/kg and was indeed significantly (p < 0.05) less than

<table>
<thead>
<tr>
<th>TEG Parameter</th>
<th>Untreated Control</th>
<th>HemCon RTS</th>
<th>Kaolin</th>
<th>TraumaStat</th>
<th>Placebo Gauze</th>
<th>Combat Gauze</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-time (min)</td>
<td>12.2 ± 1.3</td>
<td>15.8 ± 1.8</td>
<td>5.1 ± 0.3*</td>
<td>12.6 ± 1.1</td>
<td>8.4 ± 0.5</td>
<td>6.8 ± 0.4*</td>
</tr>
<tr>
<td>K-time (min)</td>
<td>3.7 ± 0.8</td>
<td>6.0 ± 1.1</td>
<td>0.8 ± 0.02*</td>
<td>3.5 ± 0.6</td>
<td>1.6 ± 0.2</td>
<td>1.2 ± 0.1*</td>
</tr>
<tr>
<td>Angle (°)</td>
<td>51.7 ± 4.4</td>
<td>37.1 ± 4.9*</td>
<td>80.2 ± 0.7*</td>
<td>50.3 ± 4.0</td>
<td>65.7 ± 2.2*</td>
<td>73.9 ± 1.8*</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>62.0 ± 1.2</td>
<td>57.2 ± 0.6*</td>
<td>67.2 ± 1.3*</td>
<td>59.3 ± 1.2</td>
<td>67.1 ± 0.9*</td>
<td>68.8 ± 1.3*</td>
</tr>
<tr>
<td>Vmax (mm/min)</td>
<td>11.3 ± 0.8</td>
<td>6.2 ± 1.0</td>
<td>29.8 ± 2.2*</td>
<td>7.5 ± 1.1</td>
<td>15.7 ± 1.6</td>
<td>22.1 ± 1.7*</td>
</tr>
</tbody>
</table>

Data expressed as mean ± standard error of the mean and analyzed by one-way analysis of variance test followed by Dunnett’s test for bigroup comparison.

* Values were significantly (p < 0.05) different than untreated controls.
the other groups. The discrepancy was caused by the large blood loss (139 ± 1.5 mL/kg) of the two animals that died because of unsuccessful treatment.

The hemostatic function of CG seems to be mediated by enhancing blood clotting activities and formation of hemostatic clots in conjunction with the gauze. This was evident at the conclusion of experiments when CG was gently removed from the wound. Hemostatic clots were often seen at the junction of the gauze and injury site which disrupted when the gauze was taken out resulting in immediate profuse bleeding. No intraluminal clot found in the treated vessels.

Because the hemostatic function of CG depends on blood clotting capacity, its efficacy may be affected by the coagulation status of patients and possibly less effective against coagulopathic bleeding than the tissue-sealant products. The TEG analysis of blood treated with this dressing showed the clotting activity of CG to be equivalent to the kaolin agent alone, causing significant increases in the speed and strength of clot formation. The physical characteristics and the type of gauze (50% Rayon and 50% Polyester) used for production of CG also contributed to the hemostatic function of CG. This was evident based on both the in vivo findings with the PG (CG without kaolin) that resulted in 33% survival (two of six) and the in vitro coagulation results (TEG analysis) of blood treated with PG that showed increased clotting rate and clot strength over untreated samples. The flexibility of CG, which is not changed by kaolin coating, and its large size (3.2 inch × 4 yd roll) are additional advantages that allow packing any type of wound with this material and covering the entire damaged tissues. The ease of application and familiarity with gauze material make this dressing a superior product to use in the field for self-application or buddy aid.

The experimental hemorrhage model used for evaluation of dressing was discussed in detail in our previous report. In general, it represents a severe wound in the groin area with partial laceration of femoral artery and high pressure bleeding that cannot be stopped with regular gauze and would be difficult to compress by tourniquet application. To the extent that was possible, all the adjutant factors, such as retraction and constriction of the injured vessel, hypotension, overlaying tissues, and prolonged compression that will aid spontaneous hemostasis, have been minimized or eliminated to test the true efficacy of each hemostatic product. Although the model provides a reproducible bleeding condition appropriate for laboratory testing, it is limited in that it may not represent a wound that trauma patients or military casualties suffer in the field. It should be recognized that there will be no single animal model that can replicate all types of wounds with different types of bleeding (arterial, venous, or both). Our focus in developing the model was to subject each hemostatic material to the most challenging and difficult bleeding condition which still can be treated with an effective modality. If the material proved to be successful under such condition, albeit may be artificial, it will most likely be effective against a majority of external bleedings.

The successful results with CG dressing were reported to a joint military committee (Tactical Combat Casualty Care Committee) responsible for developing new guidelines for treating wounded soldiers. Based on these results and similar findings by our colleagues at Naval Medical Research Center, the committee has recommended replacing HC bandage with the new dressing. The new Tactical Combat Casualty Care Committee guideline recommends using CG as the first line of treatment for life-threatening hemorrhage on external wounds that is not amendable to tourniquet placement.

In summary, CG was found to be the most effective product among four new dressings tested in this arterial hemorrhage model. This dressing allowed the least amount of hemorrhage (37.4 mL/kg) and resulted in the highest survival rate (80%) in tested animals. The chitosan-based dressings, TS, and CXb were significantly less effective. The efficacy of the CG is attributed to its raw material (special gauze) and the potent clotting agent (kaolin) that coats the dressing. The efficacy of this gauze will depend on normal coagulation function of the patient’s blood and therefore may be less effective in coagulopathic patients. The large size of CG and its flexibility offer additional advantages for packing complex wounds and covering multiple bleeding sites. It had no apparent side effect and did not initiate intravascular clotting during the course of this experiment. Histologic changes caused with this dressing are similar to those produced with regular gauze. This dressing is now recommended as the first line of treatment for life-threatening hemorrhage on combat wounds. We anticipate this dressing to be useful not only for treating combat casualties but also for civilian trauma patients with severe hemorrhage who are brought to hospital swiftly. Although application of CG may not stop the bleeding in these patients immediately, it will certainly reduce their blood loss and possibly the need for blood transfusion afterward.

ACKNOWLEDGMENTS

We thank Ms. Irasema Terrazas and Ashley Cox for their technical assistance in this study. We also acknowledge the staff of our Veterinary Support Division for their support and technical expertise in conducting these experiments.

REFERENCES

DISCUSSION

Dr. Mark Seamon (Philadelphia, Pennsylvania): Distinguished moderators, members, and guests, I want to thank the authors for the early submission of their manuscript and congratulate them on a very nice presentation. The authors studied the performance of a variety of new hemostatic dressings, including Hemcon, Celox, TraumaStat, Combat Gauze, and a Combat Gauze placebo after six millimeter femoral arteriotomies were made in their swine model.

After dressings were applied to thirty-eight pigs and data analyzed, Combat Gauze was found to be superior to the other measured dressings with respect to initial and eventual hemostasis rates, hemostatic time, and survival time. Furthermore, in vitro clotting analysis of the studied dressings revealed improved coagulation parameters in the Combat Gauze treated blood. Still, I have a few questions for the authors.

Please describe in more detail how the surgeons were blinded during these experiments. For example, how did you ensure that an equal amount of pressure was applied to each injured artery using each type of dressing?

Second, why was a goal MAP of sixty-five chosen, when Dr. Mattox has convincingly shown that pre-definitive surgical control resuscitation should be limited in our penetrating trauma victims?

Third, how do you explain some seemingly contradictory results? For example, how can there be a survival benefit in the Combat Gauze group without a difference detected in the post-treatment blood loss volume? I would have expected these two measured endpoints to be directly related.

Lastly, are your results applicable to civilian penetrating trauma? Initial hemostasis was achieved in three of ten pigs treated with Combat Gauze and eventual hemostasis was achieved in twelve to twenty-six minutes in five others. Furthermore, it took thirty to sixty minutes to detect a difference in either mean arterial pressure or survival time between Combat Gauze and the other measured dressings. How applicable is this to my city, where pre-hospital times average fifteen to twenty minutes for penetrating trauma victims?

I would like to congratulate the authors on yet another well-designed study that comes with a several-year history of related reports. Indeed, the authors contributions to this topic are significant, both for academic and practical reasons, as they continue to refine pre-hospital care for our troops injured on the battlefield. Lastly, I would like to thank the Eastern Association for the Surgery of Trauma for the privilege of discussing this important manuscript.

Dr. Bijan S. Kheirabadi (San Antonio, Texas): Thank you, Dr. Seamon, and thank you for the keen and insightful questions and I will try to answer them one by one. Regarding the randomization of the product, essentially the surgeon and assistant to the surgeon were kept blinded about what product was going to be tested on each animal, up to the point that the product was actually handed in. I was the one who placed all the products.

That randomization was done outside of the OR, ahead of the experiment, before even the study was started by an investigator not directly involved with the study. So I could not be biased in terms of the injury or isolation of the vessels and so forth. However, once the product was handed to me and I knew what it was the identity of product was released. In terms of how I make sure we apply equal pressure on each product from one animal to another, in the initial studies we actually used a Doppler to check whether we have blood flow on the distal part and the pressure was adjusted in such a way that the blood flow to the distal part of the injury was completely obstructed and vessel was occluded. There was no bleeding and no blood flow downstream.

After doing many of these experiments, we have now got enough experience that we exert almost equal pressure from one animal to another, essentially enough to stop the bleeding and occlude the blood vessels. Therefore, we’re not being biased in terms of how much pressure we put from one product to another.

Regarding a MAP of sixty-five mmHg, the sixty-five mmHg correspond to about ninety mmHg systolic pressure. On the battlefield, for the patients who are in shock, the recommendation is to give enough fluid to raise the blood pressure to such a degree that you can palpate radial pulse. Having a radial pulse corresponds to about ninety mmHg systolic pressure. The mean arterial pressure that we have selected corresponds to the pressure of the combat casualties that might be resuscitated on the field.

In addition, some casualties may have even higher than ninety mmHg systolic pressure with bleeding requiring hemostatic treatment. If we test the products below this pressure, we will have better success, but the point is that we want to make sure that the product works at a normal or slightly below normal systolic pressure. Testing at significantly below sixty five mmHg would not be appropriate for actual use later on in the soldiers that may have blood pressure substantially higher than sixty five mmHg.

Regarding the contradiction between the survival benefit and insignificant decrease of blood loss with Combat Gauze, there were ten animals in that group and in two
animals, Combat Gauze failed to produce hemostasis. In the other eight that the animals survived, the average blood loss was only twelve mL per kilogram. These data were significantly less than the blood losses seen with other products. Therefore, if you subtract the blood loss of two dead animals from the rest of the data (survival animals), you will find a strong correlation between the post-treatment blood loss and the survival rate. The reason it did not become significance was because of the death of two animals added significantly larger blood losses to the data base and increased the variability of the data set.

Finally, whether this will have any impact in civilian trauma, that’s true that we only obtained three immediate hemostasis and five hemostasis developed after twelve to twenty minutes after Combat Gauze applied. The point is that if we did not use the Combat Gauze in these situations to control bleeding, or even if you used regular gauze, the animals would have lost significantly more blood and most likely would have died. For that matter, using a Combat Gauze, which may cost only twenty dollars or so for the patient, may save him from losing significant amounts of blood and maybe save him from getting an additional transfusion when he gets to the hospital and maintain his blood pressure at a higher level. The fact that Combat Gauze did not produce hemostasis doesn’t mean that the animals were bleeding profusely. There were oozing and so there was no complete hemostasis, but in all of them, the bleeding was significantly reduced.

Dr. Lawrence Lottenberg (Gainesville, Florida): I’m somewhat troubled by the fact that about a year or two years ago the Army issued a statement that Hemcon was the product to be used and now, after what seems like a single swine study another product is being endorsed.

The problem that I see in my community is with the police and fire rescue groups. They pick up this information and they run with it. I would really appreciate it if there would be some cautioning about the use of this. What are the human data in the use of Combat Gauze from the war zone that would complement the animal study?

It’s very concerning, because the example that I can give you is we had a guy who had a stab wound to the epigastrium and it turned out to be a cardiac injury that was bleeding out and the guy came in with about a pound of Chitosan powder on his abdomen and chest. All of this is very concerning with such a preliminary study.

Dr. Bijan S. Kheirabadi (San Antonio, Texas): First of all, the reason of changing the tactical combat casualty from Chitosan to this new product, it wasn’t just based on this study. There was a concurrent study done by Navy scientist that reached to a similar conclusion. Again, that also was an animal study.

As you know, all of these products are recognized as device. They get FDA approval without going through large clinical trials and the fact that Chitosan dressing brought on and was recommended five years ago was that, at the time, that was no better product that we could find and chitosan dressing was found to be significantly better than gauze.

What we have seen in the past five years are significant improvement of the old product and new products that have come along. Do we have data that supports how these work in soldiers? In the few cases that they have been tested, the results were very positive. Combat Gauze is currently being sent to the field and so we really don’t have much data to comment on.

Based on the experimental data that I presented and the concurrent study with the Navy and other groups that are currently going on, everybody seems to be very happy with Combat Gauze, because it’s easy to use and it seems to be safe and more effective than what we have had in the past.

Dr. Andrew Kerwin (Jacksonville, Florida): That is correct. It is FDA approved.

Dr. Bijan S. Kheirabadi (San Antonio, Texas): This product, as well as all the other hemostatic products, are FDA approved as a device for external wound treatment. They don’t have indications for putting internally. If someone is using it for wrapping a liver, it’s off-label use and so therefore, this is not the indication that they got from the FDA.

Dr. Andrew Kerwin (Jacksonville, Florida): Do you have any clinical data then about using it in a liver injury, packing it and leaving it?

Dr. Bijan S. Kheirabadi (San Antonio, Texas): This product, as well as all the other hemostatic products, are FDA approved as a device for external wound treatment. They don’t have indications for putting internally. If someone is using it for wrapping a liver, it’s off-label use and so therefore, this is not the indication that they got from the FDA.

Dr. Andrew Kerwin (Jacksonville, Florida): Then, is there any data about using it in a liver injury, packing it and leaving it?

Dr. Andrew Kerwin (Jacksonville, Florida): That is correct. It is FDA approved.

Dr. Patrick Reilly (Philadelphia, Pennsylvania): One final question. Dr. Kheirabadi, again, just from a disclosure standpoint, any industry support in doing these studies?

Dr. Bijan S. Kheirabadi (San Antonio, Texas): Thanks for reminding me of this. No, there has been no funding from industry. The only thing we have asked from the industries is to donate their materials. Some materials were purchased, others were not commercially available and were donated by the companies, and in that way they participated in the studies. But in terms of funding, this study was solely funded by the U.S. Army Medical Research and Material Command. I would like to express my gratitude to the Eastern Association for the Surgery of Trauma for the privilege of presenting this manuscript.

© 2009 Lippincott Williams & Wilkins
STUDY 7:

Journal of Trauma

Safety Evaluation of New Hemostatic Agents, Smectite Granules, and Kaolin-Coated Gauze in a Vascular Injury Wound Model in Swine


February 2010
Safety Evaluation of New Hemostatic Agents, Smectite Granules, and Kaolin-Coated Gauze in a Vascular Injury Wound Model in Swine

Bijan S. Kheirabadi, PhD, James E. Mace, MD, Irasema B. Terrazas, MS, Chriselda G. Fedyk, MS, J. Scot Estep, DVM, Michael A. Dubick, PhD, and Lorne H. Blackbourne, MD

Background: In 2007, a potent procoagulant mineral called WoundStat (WS), consisting of smectite granules, received clearance from the Food and Drug Administration for marketing in the United States for temporary treatment of external hemorrhage. Previously, we found that microscopic WS particles remained in the injured vessels that were treated, despite seemingly adequate wound debridement. Thus, we investigated the thromboembolic risk of using WS when compared with kaolin-coated gauze, Combat Gauze (CG); or regular gauze, Kerlix (KK) to treat an external wound with vascular injuries in pigs.

Methods: The right common carotid artery and external jugular vein of pigs were isolated and sharply transected (50%). After 30 seconds of free bleeding, the neck wounds were packed with WS, CG, or KK and compressed until hemostasis was achieved (n = 8 per group). Wounds were debrided after 2 hours, and vascular injuries were primarily repaired with suture. Blood flow was restored after infusing 1 L of crystalloid (no heparin or aspirin) and the wounds were closed. Two hours later, computed tomographic angiography was performed, and the wounds were reopened to harvest the vessels. The brains and lungs were recovered for gross and microscopic examination after euthanasia.

Results: No differences were found in baseline measurements. Thrombelastography showed similar hypercoagulability of the final blood samples when compared with baselines in all groups. All vessels treated with KK or CG were patent and had no thrombus or blood clot in their lumen. In contrast, seven of eight carotid arteries and six of eight jugular veins treated with WS developed large occlusive red thrombi and had no flow. Small clots and WS residues were also found in the lungs of two pigs. Histologically, significant endothelial and transmural damage was seen in WS-treated vessels with luminal thrombi and embedded WS residues.

Conclusion: WS granules caused endothelial injury and significant transmural damage to the vessels that render them nonviable for primary surgical repair. The granules can enter systemic circulation and cause distal thrombosis in vital organs. More relevant in vitro and in vivo safety tests should be required for clearance of new hemostatic agents.

Key Words: WoundStat, Combat gauze, Hemorrhage control, Hemostatic agent, Vascular injury, Animal model, Swine.

Uncontrolled hemorrhage continues to be the number one cause of battlefield death. In 2003, two new Food and Drug Administration (FDA) approved hemostatic agents, QuikClot (QC) granules and the HemCon (HC) bandage, were deployed for treating compressible wounds to control hemorrhage refractory to tourniquet and gauze application. Despite a few positive anecdotal reports, other reports and personal communication with combat medics implied limited use or avoidance of these agents in the field because of either painful side effects (thermal injury with QC) or poor efficacy in controlling severe bleeding. Earlier experimental studies have also cast doubt about the efficacy of these agents in more challenging arterial hemorrhage.

We recently reported two experimental studies in search of identifying novel and more effective topical hemostatic products than those previously deployed. These studies examined the efficacy and acute safety (tissue reaction) of four new hemostatic granules/powders and four new hemostatic dressings in comparison to QC and HC dressings. The test products were preselected among numerous new hemostatic products based on initial pilot testing. The selected products were tested in a lethal femoral artery hemorrhage model in pigs that could not be stopped by gauze or tourniquet application. Based on blood loss and survival results, WoundStat (WS; smectite granules) was found to be the most effective hemostatic with 100% survival rate followed closely by Combat Gauze (CG; kaolin-coated surgical gauze) with 80% survival rate with no significant difference in efficacy between the two products.

Both products were significantly more effective than the recent HC bandage and QC beads (QC ACS+), with 10% and 16% survival rates, respectively. Neither WS nor CG is biodegradable and must be removed from wounds before surgical repair and closure of the wounds. In our previous studies, the removal of CG was an easy procedure, but cleaning WS clay and removing all particles required extensive and meticulous debridement. Microscopic residues of WS, however, were seen in the majority of treated vessels, suggesting it had the potential to be the source for thrombosis if blood flow was restored. Traces of kaolin were also detected in one specimen.

This study was therefore designed to investigate the potential thrombogenicity of WS and CG when they are used to control external bleeding due to major vascular injury. For this purpose, a new wound model was developed in pigs that involved a neck injury with partial transection of the carotid artery and the jugular vein. Histologic changes and thrombosis in the injured vessels were observed after 2 hours of free bleeding. Histologic examination revealed significant endothelial injury and transmural damage in all WS-treated vessels. Significant thrombi were found in the majority of WS-treated vessels, suggesting it had the potential to be the source for thrombosis if blood flow was restored. Traces of kaolin were also detected in one specimen.

This study was therefore designed to investigate the potential thrombogenicity of WS and CG when they are used to control external bleeding due to major vascular injury. For this purpose, a new wound model was developed in pigs that involved a neck injury with partial transection of the carotid artery and the jugular vein. Histologic changes and thrombosis in the injured vessels were observed after 2 hours of free bleeding. Histologic examination revealed significant endothelial injury and transmural damage in all WS-treated vessels. Significant thrombi were found in the majority of WS-treated vessels, suggesting it had the potential to be the source for thrombosis if blood flow was restored. Traces of kaolin were also detected in one specimen.
sis occurrence were examined in the treated vessels after surgical repair and 2-hour blood reflow. In addition, the distal organs (lung and brain) in which the residue may reside were examined for evidence of thromboembolism. Regular gauze (Kerlix, [KX]) was used as a control agent.

**MATERIALS AND METHODS**

This study was approved by the Animal Care and Use Committee of the U.S. Army Institute of Surgical Research. All animals received care and were used in strict compliance with the Guide for the Care and Use of Laboratory Animals. WS and CG were purchased from commercial sources. Both products are approved by the U.S. FDA and are available for purchase without the need for prescription. These agents are indicated for temporary treatment of external wounds to control moderate-to-severe bleeding in patients.

Yorkshire cross-bred male pigs (n = 24) weighing 34 kg to 42 kg were purchased from Midwest Research Swine (Gibbon, MN) and used in this study. Before the surgery date, venous blood samples were collected from femoral veins (percutaneous catheter) and complete blood count and standard clotting tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], and fibrinogen) were performed to ensure that these measures were within the normal range and met our inclusion criteria. After at least 1 week of acclimation, pigs were fasted for 12 hours to 18 hours before the surgery with free access to water. On the day of surgery, animals were premedicated, anesthetized, and ventilated as described previously. Anesthesia was maintained with 1% to 2% isoflurane in 100% oxygen gas administered by the automatic respirator. Maintenance fluid, lactated Ringer’s (LR), was infused at 5 mL/kg/h through an 18-gauge catheter placed in an ear vein.

**Surgical Procedures**

A 5-cm incision was made in an inner thigh muscle, and a superficial branch of the femoral artery was isolated above the knee. The artery was cannulated with a gel-filled thin cannula attached to a small sensor device (TL 11M2-C70-PCT; Data Sciences International, St. Paul, MN), which was temporarily implanted in the subcutaneous groin. Vital signs (heart rate and systolic, diastolic, and mean arterial pressures) received by the sensor were transmitted remotely to a computer system (via a receiver plate) and displayed and recorded throughout the experiment. The left femoral vein was also cannulated for blood sampling and fluid (Hextend and LR) administration.

To create the injury and hemorrhage, a 10-cm incision was made in the lateral ventral region of the neck, and the underlying tissues were dissected to expose the vessels. Segments (~5 cm long) of the common carotid artery and the external jugular vein were isolated, and lateral branches were cauterized and divided with minimum trauma to the vessels. For clamping purposes, umbilical tape loops were placed loosely around the vessels and passed through small plastic tubing. After allowing 10 minutes of stabilization (with no surgical manipulation), baseline blood samples were collected, and the vessels were occluded by pulling the umbilical tapes through the tubing and then marked for transection.

With the use of an iris scissor, vessels were partially transected (~50% of their circumference). The vascular loops were then released, and free bleeding was allowed for 30 seconds (pretreatment blood loss). Animals were randomized and wounds were packed either with two packages of WS or CG, or one roll of (KX) gauze (n = 8 per group). The surgeons were blinded to the identity of the hemostatic agent until treatment started. Two packages of WS or CG were required to fill the relatively large wound space. The agents and the control (KX) were then covered with a laparotomy sponge and manually compressed for as long as needed until hemostasis was achieved. Compression, however, was interrupted after 2, 5, 15, 30, 45, 60, 90, and 120 minutes to check for hemostasis; and the laparotomy sponge was replaced if it had absorbed a significant amount of blood. These sponges were collected, and the absorbed blood was measured and recorded as posttreatment blood loss. At the start of compression, 500 mL of colloid fluid (Hextend) was also administered intravenously (IV; 50 mL/min) to compensate for pretreatment blood loss and to raise and maintain the pigs’ mean arterial pressure at 60 mm Hg to 65 mm Hg.

Two hours after treatment, the hemostatic materials were taken out, the vessels were occluded again to prevent rebleeding, and the wounds were debrided according to standard clinical procedure using 1 L (CG and KX) to 2 L (WS) of saline with a bulb syringe in pulsatile fashion to flush and clean the wound thoroughly. No visible WS was left in the wound. Next, the loops were momentarily released to allow free bleeding, and the vessels were flushed with saline to remove any hemosat residue or clots in the lumens. The vascular injuries were then sutured (primary repair) using a monofilament nylon suture (7-0 Prolene). All of the anastomoses were performed by one investigator only (B.S.K.). During anastomosis, 1 L of LR fluid was administered IV to produce a mild hemodilution. Blood flow was restored first in the artery and then in the vein after the repair of each vessel and administration of LR. No anticoagulant or platelet inhibitor was given to any of the pigs during clamping, anastomosis, or the reflow period. The neck wounds were then closed in layers by suturing (2-0 Vicryl), and the pigs were monitored for an additional 2 hours under anesthesia.

After the monitoring period, final blood samples were collected for laboratory tests; and the pigs were transferred to the imaging room. Computed tomography (CT) angiography was performed, and images of the blood flow through the arteries and veins of the neck were taken seconds after infusion of a contrast agent (100 mL of Omnipaque, 300 mg/mL, IV). The neck wounds were then reopened, blood flow (or lack of it) through the repaired vessels was examined; and vessels were ligated and, along with the vagus nerve, collected for histology. At this time, the pigs were injected with 10 mL of heparin (1000 U/mL, IV) and then euthanized by an injection of sodium pentobarbital (4.5 mg/kg, IV). The purpose of the heparin injection at this point was to prevent postmortem blood clotting in the organs and tissues. During necropsy, the entire lung was harvested, sliced in 1-cm to 1.5-cm thickness, and carefully examined for any residues and blood clots in the vessels. Four tissue sections were examined for evidence of thromboembolism. Regular gauze (Kerlix, [KX]) was used as a control agent.
samples from the upper and the lower lobes of the right and left lungs were collected for histology. Brains were harvested from the skull in intact form and placed in fixative solution for 48 hours. After partial hardening (fixation), the organs were sliced and observed for the presence of blood clots or foreign materials. Tissue samples were collected from three regions of the brain for histologic analysis. The necropsy procedure, tissue sampling, and examination of histologic slides were done by a board-certified veterinarian pathologist (J.S.E.) who was initially blinded to the treatment of the samples.

The blood samples collected during the experiments (baseline and final) were analyzed for blood gases, complete blood count, and plasma coagulation tests (PT, aPTT, and fibrinogen). In addition, whole-blood coagulation assays in response to tissue factor were also performed by using thrombelastography (TEG). Briefly, the TEG machines (TEG Hemostasis Analyzer 5000; Hemoscope, Niles, IL) were calibrated before use and set at 37°C. Ten microliters of 1:200 diluted tissue factor (Innovin; Dade Behring, Marburg, Germany), 2 µL of corn trypsin inhibitor (3.8 mg/mL; Hematologic Technologies, Essex, VT), and 340 µL of fresh venous blood sample (drawn within 2 minutes) were placed sequentially in each TEG cup, and coagulation tracing was recorded. Each sample was tested in quadruplicate, and tracing continued until 30 minutes after the clot reached maximum strength. The TEG parameters measured include reaction time (R-time, the time that the initial fibrin formation is detected); clotting time (K-time, the time from the R-time until a firm clot is formed); angle (α, the kinetics of clot formation); maximum amplitude (the maximum strength or firmness of the developed clot); and clot lysis (LY 30) measured at 30 minutes after the clot reached maximum strength and calculated as the percentage reduction of the area under the TEG graphs.

**Data Analysis**

Data are expressed as the mean ± SEM and analyzed by *t* test and analysis of variance for parametric data. The nonparametric data were analyzed by using the Newman-Keuls multiple comparison test, and the bigroup comparison was done by using Dunnett’s test. A *p* < 0.05 was considered statistically significant.

**RESULTS**

The baseline hemodynamic and hematological parameters measured before vascular injuries were within normal ranges and not different among treatment groups (Table 1). These parameters were also measured at the conclusion of the experiments (Table 2). Although some measures such as core temperature and pH remained essentially unchanged, others were affected as a result of the injury and hemorrhage. Hemoglobin (22%), platelet count (22.5%), and fibrinogen (13%) were significantly reduced (*p* < 0.05), whereas the standard clotting times (PT and aPTT) were essentially unaffected. No differences were found in the final blood measurements among the treatment groups.

TEG assays of whole blood suggested development of a hypercoagulable state after injury and repair of the blood vessels when compared with baselines (Fig. 1). On average, R-time and K-time were reduced by 36% and 42%, respectively; and α angle was increased by 24%. The final clot strength (maximum amplitude) and the percentage fibrinolysis for the first 30 minutes (LY 30), however, were unchanged. The increase in coagulation rate was similar among treatment groups (Table 3).

The average pretreatment blood loss, a measure of uniformity of injury and bleeding response, was ~9 mL/kg with no differences among the groups (Table 4). Posttreatment blood loss, however, was significantly less in animals treated with CG and WS (*p* < 0.001) than in controls (KX), with no difference between the two test agents (Table 4). The total compression time to achieve hemostasis with each

---

**TABLE 1. Baseline Physiological and Hematological Measurements of the Operated Pigs**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Kerlix (n = 8)</th>
<th>Combat Gauze (n = 8)</th>
<th>WoundStat (n = 8)</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>37.0 ± 1.0</td>
<td>37.1 ± 0.9</td>
<td>36.6 ± 0.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>37.6 ± 0.2</td>
<td>37.5 ± 0.1</td>
<td>37.7 ± 0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>68.6 ± 2.3</td>
<td>66.0 ± 2.2</td>
<td>64.8 ± 2.1</td>
<td>0.5</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>9.4 ± 0.2</td>
<td>9.2 ± 0.2</td>
<td>9.4 ± 0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>28.1 ± 0.7</td>
<td>27.4 ± 0.6</td>
<td>28.4 ± 0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>PLT (1,000/µL)</td>
<td>373 ± 35</td>
<td>391 ± 39</td>
<td>334 ± 26</td>
<td>0.5</td>
</tr>
<tr>
<td>PT (s)</td>
<td>10.9 ± 0.2</td>
<td>10.9 ± 0.1</td>
<td>11.1 ± 0.1</td>
<td>0.4</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>15.8 ± 0.2</td>
<td>15.4 ± 0.1</td>
<td>16.2 ± 0.3</td>
<td>0.5</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>230 ± 20</td>
<td>239 ± 17</td>
<td>248 ± 19</td>
<td>0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.4 ± 0.01</td>
<td>7.4 ± 0.00</td>
<td>7.4 ± 0.00</td>
<td>0.4</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.6 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>1.5 ± 0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>6.2 ± 0.5</td>
<td>6.9 ± 0.6</td>
<td>6.3 ± 0.6</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM and analyzed by one-way ANOVA test. No significant difference was found among the groups.  
HGB, hemoglobin; HCT, hematocrit; PLT, platelet.

**TABLE 2. Final Hematological Measurements in the Operated Pigs**

<table>
<thead>
<tr>
<th>Measure</th>
<th>Kerlix (n = 8)</th>
<th>Combat Gauze (n = 8)</th>
<th>WoundStat (n = 8)</th>
<th>Overall p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temp (°C)</td>
<td>38.3 ± 0.1</td>
<td>38.3 ± 0.1</td>
<td>38.5 ± 0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>47.8 ± 1.4</td>
<td>48.8 ± 1.6</td>
<td>48.5 ± 1.4</td>
<td>0.9</td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>7.2 ± 0.2</td>
<td>7.2 ± 0.2</td>
<td>7.4 ± 0.2</td>
<td>0.9</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>21.3 ± 0.6</td>
<td>21.7 ± 0.7</td>
<td>22.0 ± 0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>PLT (1,000/µL)</td>
<td>287 ± 28</td>
<td>298 ± 23</td>
<td>266 ± 25</td>
<td>0.7</td>
</tr>
<tr>
<td>PT (s)</td>
<td>113.0 ± 2.0</td>
<td>112.0 ± 2.2</td>
<td>114.0 ± 3.3</td>
<td>0.8</td>
</tr>
<tr>
<td>aPTT (s)</td>
<td>167.0 ± 0.5</td>
<td>161.0 ± 0.5</td>
<td>166.0 ± 0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>7.4 ± 0.01</td>
<td>7.4 ± 0.01</td>
<td>7.4 ± 0.01</td>
<td>0.7</td>
</tr>
<tr>
<td>pH</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Base excess (mM)</td>
<td>8.6 ± 0.5</td>
<td>8.9 ± 0.7</td>
<td>8.6 ± 0.6</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM and analyzed by one-way analysis of variance test. No significant difference was found among groups.  
HGB, hemoglobin; HCT, hematocrit; PLT, platelet.
The assessment of blood flow by CT images (Figs. 2 and 3) was confirmed by direct observation of the vessels when the wounds were reopened. The results showed that all the vessels treated with KX or CG were somewhat constricted but patent with no apparent difference between the two groups. No significant thrombus or blood clot was found in the lumen or on the suture line of these vessels after recovery (Fig. 4). In contrast, seven of eight carotid arteries treated with WS were occluded with thrombus and had no blood flow when examined at 2 hours postrepair. Similarly, six of the eight jugular veins treated with WS developed large red clots with no blood flow through the vessels. A red thrombus layer covering the entire inner wall was also seen in a patent vein (Fig. 4, right lower panel).

Gross examination of the whole brain shortly after recovery and brain slices after fixation did not show any abnormal findings in all the pigs. Inspection of the lungs, however, revealed a blood clot (2–3 cm long and 2–3 mm thick) in a lower lobe of one lung and some residue similar to WS particles in the lung of another WS-treated pig. No gross abnormalities were detected in the lung of KX- or CG-treated animals.

The histologic changes of CG- and KX-treated vessels were equivalent in almost every way with minimal diffuse

---

**TABLE 3.** Thrombelastography (TEG) Analysis Blood Drawn From the Pigs Before Vascular Injury (Baseline) and 2 h After Repair and Reflow of the Vessels

<table>
<thead>
<tr>
<th>TEG Parameter</th>
<th>Baseline</th>
<th>Kerlix</th>
<th>Combat Gauze</th>
<th>WoundStat</th>
<th>p (Among Groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R-time (min)</td>
<td>6.9 ± 0.3</td>
<td>4.7 ± 0.3*</td>
<td>4.0 ± 0.3*</td>
<td>4.7 ± 0.4*</td>
<td>0.5</td>
</tr>
<tr>
<td>K-time (min)</td>
<td>3.8 ± 0.3</td>
<td>2.3 ± 0.2*</td>
<td>2.0 ± 0.2*</td>
<td>2.3 ± 0.2*</td>
<td>0.5</td>
</tr>
<tr>
<td>Angle (°)</td>
<td>49.7 ± 1.6</td>
<td>61.0 ± 1.3*</td>
<td>64.2 ± 2.6*</td>
<td>60.1 ± 2.0*</td>
<td>0.4</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>72.7 ± 0.5</td>
<td>70.3 ± 0.9</td>
<td>71.5 ± 1.2</td>
<td>72.4 ± 1.0</td>
<td>0.8</td>
</tr>
<tr>
<td>LY 30 (%)</td>
<td>0.8 ± 0.2</td>
<td>0.8 ± 0.3</td>
<td>1.0 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>0.7</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM and analyzed by t test (comparison with baseline) and one-way analysis of variance test.

* Values were significantly (p < 0.05) different from the respective baseline.

Baseline values represent the average of three groups.

MA, maximum amplitude.

---

**TABLE 4.** Bleeding Outcomes Following Injury and Treatment With Different Agents

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Kerlix</th>
<th>Combat Gauze</th>
<th>WoundStat</th>
<th>p (Among Groups)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood loss pretreatment (mL/kg)</td>
<td>9.3 ± 0.8</td>
<td>8.4 ± 0.7</td>
<td>9.6 ± 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Blood loss posttreatment (mL/kg)</td>
<td>7.3 ± 0.7</td>
<td>3.9 ± 0.8*</td>
<td>2.9 ± 0.2*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Compression time (min)</td>
<td>54.4 ± 12.6</td>
<td>13.9 ± 8.9*</td>
<td>9.1 ± 3.6†</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM and analyzed by one-way analysis of variance (ANOVA) with posttest comparison of all pair groups using Newman-Keuls test.

* p < 0.01 vs. Kerlix controls.

† p < 0.05 vs. Kerlix controls.

No differences were found between CG and WS.
endothelial blebbing and no intraluminal thrombus. Pigs from the KX group had a high incidence of microthrombi in their lung (six of nine), and one animal had a small thrombus in a vessel of the brain. These microthrombi in capillaries are commonly formed in hemorrhage trauma models and considered clinically insignificant. The prevalence of microthrombi in this group may have been due to the larger blood loss and much longer compression time on the neck area proximal to lung to achieve hemostasis with regular gauze. In contrast, significant endothelial and transmural injury across the carotid arterial walls with large intraluminal thrombi were found in WS-treated vessels (Fig. 5). In the veins, WS caused significant delamination of the outer adventitia and necrosis of associated nuclei and inflammatory cells (Fig. 6). Within most of the luminal thrombi (eight of eight veins and six of eight arteries), gray granular materials were visible under polarizing light that was confirmed to be WS. The WS residues were also found in several areas of the lung in one pig. A large piece of the residue was associated with an arterial thrombus in one lung (Fig. 7).

**DISCUSSION**

This study evaluated the short-term safety of using two new hemostatic agents to control external bleeding with major vascular injuries in pigs. The materials were applied to a neck wound with both arterial and venous injuries for 2 hours and subsequently removed. After surgical repair and 2 hours of blood reflow, the structure and function of the treated vessels were carefully examined. In addition, end organs distal to the vessels were inspected for evidence of embolization. The results showed essentially no difference in vascular function of the wounds that were treated with regular gauze (controls) or CG. The treatment of the wounds...
with WS, however, resulted in severe endothelial injury, significant transmural damage, massive thrombosis, and complete occlusion of the injured vessels following blood reflow. Despite our best effort for complete debridement, small microscopic residues of WS remained in the wound and in the lumen of vessels, providing additional sources for the development of thrombosis. Embolized WS particles associated with thrombi were also found in the lungs of two animals. Although the damaged blood vessels can be replaced with viable grafts, the likely chronic inflammation and potential endothelial necrosis caused by embolized WS in distal organs could be a major problem.

WS is composed of smectite granules, an aluminum phyllosilicate mineral with high water absorbency, which concentrates the clotting factors in blood. The negative charges of the smectite granules also activate the clotting cascade and promote clot formation. The potent clotting activity of this agent was apparent when the WS-treated blood was analyzed by the TEG method.9 The main hemostatic mechanism of WS, however, appears to be due to

Figure 4. Views of representative CG- or WS-treated arteries (a) and veins (v) immediately after recovery from the pigs.

Figure 5. Composite micrographs of carotid arteries treated with KX (A), CG (B), or WS (C and D). Damaged endothelium, intraluminal blood clots, WS residue, and necrosis of smooth muscle cells are evident in the WS-treated vessels.
the strong tissue-sealant properties of this mineral. Once the WS granules are mixed with blood, pliable clay is formed that on compression binds tightly to underlying bleeding tissues and provides immediate hemostasis in the wound.\textsuperscript{7}

CG is a combination of special surgical gauze (50% Rayon and 50% polyester) with another aluminum phyllosilicate mineral, kaolin. Kaolin is a strong contact pathway activator agent initiating rapid clot formation in a wound. A substantial amount of kaolin powder (~10% of total weight) is incorporated into each CG; however, the product is indistinguishable from regular gauze because of the soft and fine nature of the white kaolin powder. CG has all the advantages of normal gauze (flexibility, large coverage, ease of application, and ease of removal) plus increased hemostatic function. The strong clot formed within CG adheres the material to the bleeding site and stops the bleeding after some initial blood loss. The adherence of CG to the vessel injury was noted during debridement when the last layers of CG were removed from the wound.

In our previous studies examining the efficacy of WS and CG, we found microscopic traces of WS particles in the lumen of nearly all the treated arteries (9 of 10) despite adequate debridement and saline flushes of wounds before

\textbf{Figure 6.} Composite micrographs of jugular veins treated with KX (A), CG (B), or WS (C and D). Large blood clots associated with WS residues are seen in the lumen of the vein (C). Necrosis of smooth muscle cells and delamination of outer adventitia are also apparent in the vessel (D).

\textbf{Figure 7.} Embolized WS residues and associated arterial thrombosis in the lung. The hematoxylin and eosin stained tissue as seen under normal light (A) and under polarized light (B), which identifies the WS residue clearly.
vascular tissues. Full thickness burn as well as tissue the thermal damage of QC on hepatocyte, nerve, muscle and to achieve hemostasis in moderate-to-severe bleeding." The emergency use for external temporary traumatic wound treatment are printed on the package itself. The intended use is "emer-
gency that they do not allow performing a controlled study with reproducible and quantifiable outcomes. Nonetheless, the findings in this porcine model point out serious side effects of both products.

Both WS and CG have received FDA clearance (510[k] Premarket Notification) for marketing in the United States for temporary treatment of external wounds with moderate-to-severe bleeding. To obtain FDA clearance, WS was demonstrated to be essentially equivalent, if not superior, to QC, another previously approved hemostatic agent. The original QC was made of zeolite granules with high water absorbency which, by concentrating clotting factors in blood, promoted clot formation and produced hemostasis. The water absorption reaction, however, was exothermic, causing high temperature and occasionally significant thermal injuries that required skin grafting. Earlier animal studies clearly showed the thermal damage of QC on hepatocyte, nerve, muscle and vascular tissues. Full thickness burn as well as tissue necrosis and large abscesses were also found in the groin wounds of the pigs treated with QC 4 weeks after recovery. A few years after marketing, the QC manufacturing company (Z-Medica) recognized this serious side effect and modified the chemical structure of zeolite granules to minimize heat generation (cool formulation). In addition, Z-Medica has placed the new QC in small water-permeable bags to facilitate application and removal of granules and to eliminate the risk of emolization.

According to the manufacturer, WS was subjected to cytotoxicity (fibroblast cell culture), systemic toxicity, and intracutaneous irritation tests according to the International Organization for Standardization (ISO) guidelines and was found to be safe for intended use. However, none of these standard tests had exposed WS to endothelial cells or blood vessels to determine the true effect of WS on these targeted tissues. The risk of embolism also could not have been determined by these tests. Although the ISO tests may be sufficient for safety clearance of some products that are applied externally (over the skin or on a superficial wound), more specific safety tests should be required for approval of hemostatic agents that may be placed in deep penetrating wounds with potential internalization. Relevant information may be obtained by conducting an animal study with a typical wound that is treated with the test agent to assess the short- and long-term effects of the materials on the exposed tissues and the overall recovery and health status of treated subjects. It worth mentioning that WS, like other topical hemostatic products, has no package insert and all the information regarding indication, directions for use, and safety warnings are printed on the package itself. The intended use is "emergency use for external temporary traumatic wound treatment to achieve hemostasis in moderate-to-severe bleeding." The only safety warning printed on the package is "do not use in eyes or swallow."

The WS tissue damages, including endothelial degeneration and myofibril necrosis, may result from the toxicity of this mineral toward these sensitive cells. Murphy et al. have shown the significant cytotoxicity of aluminum phyllosilicate clay minerals, including montmorillonite (the main smectite mineral), bentonite (a smectite mineral), and kaolinite, on human umbilical vein endothelial cells. Incubation of 0.1 mg/mL of montmorillonite with human umbilical vein endothelial cells caused 100% cell lysis after 24 hours. The kaolinite also had a toxic effect on the cells but to a lesser extent. The lack of toxicity of kaolin in CG, as noted in this study, may be attributed to the fact that only a small amount of kaolin powder is incorporated into each roll of CG and that perhaps most of the mineral remains in the gauze when it is applied to the wound (indirect exposure). On the other hand, a large quantity of WS-smectite (150 g) is poured into the wound directly, exposing the tissues to the mineral at the highest concentration and maximizing the toxicity effect. In some primary neuronal cultures, addition of bentonite or montmorillonite at 0.1 mg/mL concentration caused complete cell lysis within 60 minutes. Interestingly, however, these minerals had no harmful effect on other cell lines such as oligodendroglia or neuroblastoma. This finding suggests that WS cytotoxicity may not have been detected when it was tested on the fibroblast cell culture as guided by ISO tests for device assessment.

Our initial study, which revealed the potential thrombogenicity of WS, was conducted in a porcine model with femoral artery injury and focused on determining the efficacy of the product. That model was not used for this study, in favor of developing a new model for testing the safety of the new hemostatic agents. The new model involved injuries to both an artery and a vein in the neck area. The expectation was that the risk of thromboembolism might be higher in the low-pressure venous circulation than in the arterial vessels and therefore injuries to both vessel types should be included. The model also allowed tracing and detecting the potential emboli in the distal end organs (lung and brain) of the treated vessels. Such follow-up would have been much more difficult and may be inconclusive if the extremity injury model was used. Heparin treatment was avoided during surgical repair and blood reflow, so that it would not mask thrombosis occurring in the damaged vessels. In addition, surgery either with a low dose of heparin or without heparin was consistent with standard vascular surgery practices in combat support hospitals.

The main limitation of this study is that the model does not mimic a relevant battlefield wound. On the other hand, such wounds by their nature are so complex and heterogeneous that they do not allow performing a controlled study with reproducible and quantifiable outcomes. Nonetheless, the findings in this porcine model point out serious side effects of WS and should send strong warning to the care providers who may use this product as the last resort for control of severe hemorrhage.

In summary, the safety of two new and extremely effective hemostatic agents was tested in a surgical model that was able to reveal local tissue damage and embolization.
to distal organs as a result of treatment with the agents. Although CG produced changes that were not different from regular gauze, WS treatment caused severe endothelial injury and significant transmural damage that rendered the vessels nonviable for primary surgical repair. The WS residues were also emobilized in the venous circulation and were trapped in the lung with associated thrombosis. If used in a wound, it may be necessary to replace the injured vessels with interposition grafts to avoid thrombotic complications. The present findings also suggest that more stringent safety tests should be performed before approval of hematicostatic products that are indicated for treating moderate-to-severe bleeding.

ACKNOWLEDGMENTS

We thank the staff of our Veterinary Support Division for their support and assistance in conducting these experiments.

REFERENCES


DISCUSSION

Dr. Charles A. Adams (Providence, Rhode Island): Thank you, Doctor Spain. I had the pleasure of just hosting a lunch symposium with Doctor Croce and he really is on his good behavior today. It was a pleasure.

Doctor Sise, members and guests of the association, Doctor Kheirabadi and his colleagues at the ISR, Fort Sam Houston, have continued their work evaluating topical adjuncts for the control of external hemorrhage.

They utilized a porcine model, as you can see, with combined venous and arterial injury which is an improvement on their previous models, I believe. And he explained the rationale behind that.

Following a brief period of uncontrolled hemorrhage they then tried these various products, specifically they used: Kaolin coated gauze and then they used the WoundStat which is the smectite containing product.

I was not familiar with smectite but it’s actually an aluminum phyllosilicate and it absorbs like most of these adjuncts a lot of the plasma leaving the clotting factors in high concentration.

What they showed is that the QuickClot – correction, the WoundStat formulation resulted in hemostasis quite rapidly.

They monitored a whole host of parameters looking at blood loss, hematocrit, hemodynamics and they also used thromboelastograms which he didn’t show you in the presentation which was really nice in the manuscript.

After hemostasis was achieved they then underwent CT scanning with a CT angio which I thought was also impressive and they showed that there was a great deal of thrombosis.

And as you can see in his graphics they actually show that there was a significant amount of WoundStat retained in the wound. Following, these animals were sacrificed and tissues were taken for histology.

While WoundStat was the most effective at controlling hemorrhage it was also associated with the highest degree of thrombosis. And as he showed in his histologic data it really promoted a lot of intense degradation of the endothelium as well as localized damage inside the wound.

I have four questions. Actually I had a couple more but I’ll trim it down in the interest of time. He mentioned that he performed bulb irrigation with one to two liters to try and remove that and that’s a fairly relevant clinical situation.

But I was curious if you had tried any type of motorized mechanical pulse irrigation, sym pulse, lavage, such as that, because those are also clinically used quite frequently to, as an adjunct for debridement?

Although the model is consistent with a real-life clinical scenario, primary repair is probably not as common as interposition graph with either venous interposition graph or PTFE, especially when dealing with gunshot wounds where debridement of the artery is necessary otherwise there is a higher incidence of thrombosis.

I was curious, since the WoundStat remains in the wound have you done any of those venous interposition graph? And if you have, what has been the effect on long-term vascular patency with that WoundStat still present.
in the wound? If you haven’t done those experiments, that might be something interesting to try next.

Next, WoundStat’s major effect on hemostasis is the fact that this stuff really becomes a clay and your data was quite eloquent showing that it was very effective at obtaining hemostasis.

But since it’s like a granule that needs to be poured in, I was wondering if you have had any work or if you have considered trying to reformulate it with a non-adherent barrier, some type of interface, so that it didn’t really incorporate in the wound; and that might be one of the ways to get rid of it so that it’s not retained in the wound.

Again, there is also many products commercially available that have a similar process where there is a non-adherent barrier to keep it from adhering.

And, finally, as you pointed out, the data submitted to the FDA might be inadequate. Work by Murphy several years ago did indeed show that WoundStat was cytotoxic to endothelial cells and, as you pointed out, the FDA testing was done on fibroblast as well as oligodendro sites.

So in light of that fact and in light of your findings of your results, do you think that this product should be recalled from the market if it has such dramatic negative effects on local vascular repairs?

In conclusion, I applaud the authors for their continued hard work on this clinically relevant topic.

And I’d like to thank the association for the privilege of reviewing this as well as for the privilege for myself to become educated on products that I frequently don’t use since these, as I can see, are probably best used in the pre-hospital setting as well as in combat situations. Thank you.

Dr. Bijan S. Kheirabadi (Fort Sam Houston, Texas): Thank you, Doctor Adams, for insightful questions, comments. As far as whether we have used motorized pumps for debridement, indeed we have done an earlier study in our institute comparing the two debridement methods.

Actually, we found that using a syringe to flush with the pulsatory fashion removed the material better and caused less tissue damage than using a high pressure motorized device.

As far as why did we use primary anastomosis and have we considered to do a interposition graft in this study, we have not done in these repair procedures. However, we have done additional experiments in which vessels were not injured but the wound were packed with WoundStat to see how the vessels amy be affected.

These vessels did not develop occlusive thrombosis but the toxicity of WoundStat was still present on the outer (adventitia) and muscular layers of the vessels. Therefore, the damage is not limited to endothelial cells.

Even if the vessel was grafted, the area of the vessel that came in contact with the WoundStat would have developed some kind of tissue necrosis in the long-term.

Whether the product has been considered to have some kind of barrier between the mineral and the tissue, I think this approach is being persuade by the company.

For instance, the QuickClot originally was marketed in granular form which produced a great deal of heat and tissue damage in wounds. Later on it was put in some kind of permeable bags that prevented its direct contact with the tissue and yet maintained hemostasis.

I think the same kind of modification is being considered by the company if they can maintain the same efficacies by putting the material in a small bag. And, finally, we have shared these information with FDA and they are aware these animal studies and the findings. We have also plan to meet with FDA officials to discuss these results and present our concerns. What would be the FDA final decision regarding the WoundStat approval is not known at this time.

The company is also aware of these findings and they are working their way of decreasing some of these side effects.

Again, thank the association for the privilege of the podium.