A randomized controlled pilot study of epsilon-aminocaproic acid as a topical hemostatic agent for postoperative bleeding in the sheep model of chronic sinusitis

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A randomized controlled pilot study of epsilon-
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ABSTRACT

Background: Significant bleeding with blood clot formation in the postoperative period may predispose to the formation of postoperative adhesions. A topical hemostatic agent would potentially improve postoperative comfort and lessen adhesion formation. This pilot study was performed to evaluate the effects of a novel topical hemostatic agent AMICAR (ε-aminoacapric acid; Xanodyne Pharmaca, Inc., Florence, KY) on postoperative bleeding after endoscopic sinus surgery (ESS).

Methods: In a prospective randomized controlled pilot study full thickness mucosal injuries were created on the lateral nasal wall, ethmoidal and the maxillary ostium on both sides of 10 sheep. Eosinophilic chronic sinusitis was confirmed both by endoscopy and by biopsy before full thickness injuries. The topical hemostatic agent (AMICAR) was sprayed onto a randomly selected side with or without mucocoadhesive methyl cellulose (5 sheep in each group). The control side received a spray of saline of equal volume. The degree of bleeding in the surgical field was graded using a visual analog scale until total hemostasis was achieved. The grades at 2-minute intervals as well as the time to achieve total hemostasis were recorded.

Results: Eosinophilia of the epithelium did not correlate with the severity of bleeding (p < 0.05). There was a statistically significant (reduction in the grade of bleeding in the lateral nasal wall both superior and inferior to the middle turbinates which was used compared with normal saline (p = 0.004 and p = 0.003, respectively) but when AMICAR was used in combination with methyl cellulose this significance was lost (p = 0.076 and p = 0.502).

Conclusion: AMICAR may be considered for use either during or after sinus surgery to reduce bleeding.


Key words: Adhesion, AMICAR, aminoacapric acid, bleeding, blood clot, controlled, ESS, hemostasis, randomized, topical hemostatic agent

Endoscopic sinus surgery (ESS) has become the standard technique for the management of chronic rhinosinusitis (CRS). To achieve an optimal outcome after ESS the surgeon needs to minimize bleeding and thereby minimize adhesion formation with collapse of the middle meatus and the failure of the surgery. This is best achieved by a combination of preserving the mucous membrane and prevention of a postoperative fibrin clot. Prevention of significant postoperative hemorrhage will prevent significant clot formation in the middle meatus.

Adhesions are formed as a result of an inflammatory response caused by trauma and hemorrhage. If blood clots are not removed by fibrinolysis or absorption, the fibrin deposits can initiate an inflammatory response leading to proliferating fibroblasts, scar tissue formation, and adhesions between adjacent surfaces.1

The role of the blood clot in wound healing and scar formation has become clear with in vivo and in vitro studies. Initially, the clot provides plasma fibronectin and numerous platelet-derived cytokines and growth factors to activate migrating cells initiating inflammation and proliferation. This, in turn, leads to collagen synthesis. In addition, the clot acts as a scaffold for migrating cells and provides an initial framework for the deposition of matrix. Thus, if there is a blood clot within the nasal cavity after endoscopic surgery, it may lead to enhanced inflammatory and proliferative phases resulting in scarring and adhesion formation. Therefore, if blood clot formation is prevented, there will be less inflammation and less scarring, which prevents adhesion formation.

Although nasal packs have been used to create space between the middle meatus and lateral nasal wall, none are hemostatic other than by the pressure they apply to the mucosal surface. In addition, when these packs are removed significant hemorrhage may occur.2 Recently, a hemostatic dissolvable gel made up of a gelatin matrix and thrombin (FloSeal; Fusion Medical Technologies, Inc, Mountain View, CA) has been shown to effectively reduce bleeding after ESS.3 However, although this gel has been effective, it has been shown that it increases the likelihood of adhesion formation in the middle meatus and has therefore had limited uptake among rhinologists.3

Among the compounds used in the past to minimize postoperative hemorrhage is the synthetic derivative of the amino acid lysine, 6-aminoheptanoic acid (aminoacapric acid or AMICAR; Xanodyne Pharmaca, Inc., Florence, KY). This amino acid reversibly binds to the kringle regions of plasminogen resulting in a marked conformational change in the molecule.

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This significantly limits the ability of plasminogen to be activated to plasmin. If added to a surface wound, AMICAR inhibits the fibrinolytic system by binding to plasminogen. This prevents the breakdown of minute clots and platelet plugs formed at the cut ends of vessels, which leads to a lower rate of bleeding and prevents the accumulation of blood and fibrin degradation products within the wound cavity. This may lead to reduced rates of inflammation and matrix deposition that in turn may prevent scarring and adhesion formation. Furthermore, no side effects were observed when AMICAR was used topically.\(^6\)

AMICAR is an aqueous solution that can be sprayed onto a surface wound and therefore is a good candidate for a topical hemostatic agent. As the solution is washed off by bleeding, the contact of AMICAR with the mucosal surface may be prolonged by addition of a mucoadhesive agent. To study the effects of AMICAR on postoperative bleeding, a suitable animal model is needed. In our department we have established a sheep model of chronic sinusitis\(^7\) for the study of manipulations of the nose and sinus cavity in the presence of chronic eosinophilic rhinosinusitis. The following study was conducted to evaluate the effects of topically applied AMICAR on bleeding of the nasal mucosa of sheep after ESS.

**METHOD**

Ethics approval for the study was obtained from The Queen Elizabeth Hospital Research Ethics Committee. Ten sheep with endoscopic evidence of eosinophilic CRS were included in the study. All sheep in the study underwent the standardization procedure of total middle turbinatectomy.\(^8\)

Four to 6 weeks after the turbinatectomies full thickness mucosal injuries were performed over the lateral nasal wall both superior and inferior to the middle turbinate attachment, circumferentially over the maxillary ostium and the lateral surface of the ethmoturbinals and adjacent lateral nasal wall using a microdebrider fitted with a 4-mm cutting blade (Medtronic, Jacksonville, FL).

The side to receive the hemostatic agent was randomly selected using a random number table. In sheep 1–5, a total of 6 mL of AMICAR (\(\epsilon\)-aminocaproic acid, 250 mg/mL i.v. preparation; Xanodyne Pharmaceul, Inc.) was sprayed as a mist immediately after injury. Approximately 3 mL was sprayed over the lateral nasal wall and 3 mL over the maxillary ostium and the ethmoturbinal. In sheep 6–10, a total of 5 mL of AMICAR mixed with 5 mL of mucoadhesive methyl cellulose (0.6% in 6% phosphate-buffered saline, pH 6.8) was sprayed on the test side. Methyl cellulose is viscous and lipophilic and has the ability to adhere to mucosal surfaces. This was used in an attempt to enhance contact between AMICAR and the mucosal surface and to prolong the time of contact. To serve as a control, saline was sprayed on the same injuries on the contralateral side.

The degree of bleeding in the four sites was graded and recorded immediately after the injury and at 2-minute intervals until hemostasis was achieved according to a visual analog scale. The grading was 1–10, 1 being no bleeding and 10 being bleeding that completely obscured the surgical field. Values from 2 to 9 were assigned to varying degrees of bleeding between the aforementioned extremes. The total time to achieve complete hemostasis was recorded for each wound on each side. The animals were observed in the laboratory for the following 3 days.

The nasal cavities of the sheep were endoscopically examined on the day after this procedure, under sedation using 0.1–0.2 mL of xylazine (20 mg/mL), and the appearance of the injured surface, any bleeding, or crusts were recorded. This was mainly to ensure that hemostasis was achieved and no bleeding continued from the denuded surfaces of the nasal cavities. The grades of surgical field bleeding for each side at all time points between the time of injury until total hemostasis for normal saline was compared with that for AMICAR with or without methyl cellulose using the Wilcoxon matched pairs signed-rank test (W).

**RESULTS**

The summary of the degree of surgical field bleeding of a composite of all time points for each site is presented in Table 1. The surgical field bleeding was assigned a grade according to the visual analog scale at each time point as described in the Methods section. The grades thus obtained were compared between AMICAR and saline or AMICAR + methyl cellulose and saline for each site and at corresponding time points using the Wilcoxon matched pairs signed-rank test. Thus, the W value obtained for each site was used to calculate a p value to evaluate statistical significance of the difference between the treatment and the control side. There was a statistically significant reduction in the grade of bleeding in the lateral nasal wall both superior and inferior to the middle turbinate attachment when AMICAR alone was used, compared with normal saline; however, when AMICAR was used in combination with methyl cellulose this significance was lost. Furthermore, there was a trend toward a reduction in the grade

<table>
<thead>
<tr>
<th>Surgical Site</th>
<th>AMICAR vs NS</th>
<th>AMICAR + MC vs NS</th>
<th>AMICAR + MC vs NS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>W ( p )</td>
<td>W ( p )</td>
<td>W ( p )</td>
</tr>
<tr>
<td>Ethmoturbinals</td>
<td>89.5 0.10</td>
<td>8.0 0.183</td>
<td>151 0.704</td>
</tr>
<tr>
<td>Maxillary ostium</td>
<td>80 0.268</td>
<td>11.5 0.062</td>
<td>137.5 0.341</td>
</tr>
<tr>
<td>Superior lateral nasal wall</td>
<td>111 0.004</td>
<td>7 0.076</td>
<td>199 0.166</td>
</tr>
<tr>
<td>Inferior lateral nasal wall</td>
<td>342 0.002</td>
<td>86.5 0.502</td>
<td>780 0.050</td>
</tr>
</tbody>
</table>

\(\text{NS} = \) normal saline; \(\text{MC} = \) methyl cellulose; \(W = \) Wilcoxon number.
Table 2  Mean time (min) to achieve complete hemostasis

<table>
<thead>
<tr>
<th>Treatment Site</th>
<th>Normal Saline (n = 10)</th>
<th>AMICAR (n = 5)</th>
<th>AMICAR + MC (n = 5)</th>
<th>AMICAR ± MC (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethmoturbinals</td>
<td>4.8</td>
<td>4.9</td>
<td>3.9</td>
<td>4.4</td>
</tr>
<tr>
<td>Maxillary ostium</td>
<td>5.4</td>
<td>6.4</td>
<td>4.8</td>
<td>5.6</td>
</tr>
<tr>
<td>LNW (superior)</td>
<td>5.6</td>
<td>4.8</td>
<td>4.4</td>
<td>4.6</td>
</tr>
<tr>
<td>LNW (inferior)</td>
<td>9.2</td>
<td>10.4</td>
<td>6.8</td>
<td>8.75</td>
</tr>
</tbody>
</table>

NW = lateral nasal wall; AMICAR = amino caproic acid, MC = methyl cellulose.

of the surgical field bleeding with the use of normal saline in comparison with AMICAR with methyl cellulose. When data for all sheep were pooled for each site (n = 10) using AMICAR with or without methyl cellulose, only the lateral nasal wall inferior to the attachment of the middle turbinate showed any significant reduction in the grade of bleeding compared with normal saline.

The mean time (minutes) to achieve complete hemostasis for the four injured sites with normal saline, AMICAR, and AMICAR with methyl cellulose are presented in Table 2. The results of a paired two-tailed Student’s t-test for the four sites, with normal saline versus AMICAR, with or without methyl cellulose are presented in Table 3. There was no correlation between initial degree of bleeding and time to achieve hemostasis in each site.

The results of a paired two-tailed Student's t-test for pooled data for all four sites are shown in Table 4. There was no significant effect on the time to achieve complete hemostasis with the use of AMICAR on any of the four sites examined. However, there was a trend (p = 0.065) toward a shorter time to achieve hemostasis with the use of AMICAR alone when data for all sites were pooled.

DISCUSSION

In this study AMICAR applied to the surgical wound after surgery resulted in a significant reduction in the grade of bleeding seen on the lateral nasal wall compared with the side that received the normal saline spray. This effect was not seen when AMICAR was combined with the mucoadhesive methyl cellulose. AMICAR alone may be of benefit in reducing bleeding after sinus surgery. However, the time to achieve complete hemostasis was longer with AMICAR alone compared with normal saline. With the use of methyl cellulose, the time to achieve complete hemostasis was shorter with AMICAR compared with normal saline. If the AMICAR data is combined then there was a shorter time to achieve hemostasis than with saline alone. Why the grade of bleeding at various time points was significantly better with the AMICAR alone group but the time to complete hemostasis was longer is not clear and may be related to the small numbers in each arm of this pilot study.

During this study it was noted that the AMICAR was easily washed away by the bleeding and therefore seemed to have a limited time of contact with the wounded area. In an attempt to prolong the contact time and thereby improve hemostasis AMICAR was mixed with mucosalhesive methyl cellulose for the second half of the study. The desired effect was for AMICAR and methyl cellulose to bind at a molecular level and for the methyl cellulose to bind to the injured mucosa. However, the combination did not have the desired effect, with no difference in bleeding between the combination (AMICAR with methylcellulose) and the control side that has been sprayed with normal saline. In fact, in the region of the maxillary ostium the normal saline performed better than the AMICAR mixed with methyl cellulose. However, the time to complete hemostasis was shorter in the AMICAR with methy
cellulose group than in the saline group.

Although we were unsure exactly why this occurred, we surmise that the method of mixing used may not have been effective, because chemical bonding between the two molecules is required in order for the hydrophobic methyl cellulose to act as a vehicle of hydrophilic AMICAR. Second, the dissociated methyl cellulose may have bound to the mucosal surface and prevented AMICAR from coming into contact with the injured surface. Another possibility is that once the mucosa is injured a hydrophilic or aqueous substance may bind to the surface better than a hydrophobic substance, methyl cellulose.

Table 3  Paired two-tailed t-test of time to achieve complete hemostasis, for each surgical site—normal saline vs AMICAR ± MC (n = 10) -

<table>
<thead>
<tr>
<th>Site</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethmoturbinals</td>
<td>0.4</td>
<td>0.7</td>
</tr>
<tr>
<td>Maxillary ostium</td>
<td>0.48</td>
<td>0.64</td>
</tr>
<tr>
<td>LNW (superior)</td>
<td>1.41</td>
<td>0.19</td>
</tr>
<tr>
<td>LNW (inferior)</td>
<td>0.63</td>
<td>0.55</td>
</tr>
</tbody>
</table>

LNW = lateral nasal wall superior to middle turbinate attachment; LNW (inferior) = lateral nasal wall inferior to middle turbinate attachment.

Table 4  Paired two-tailed t-test for time to achieve total hemostasis each treatment group (data for all sites pooled)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>t-Statistic</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal saline vs AMICAR</td>
<td>1.96</td>
<td>0.065</td>
</tr>
<tr>
<td>Normal saline vs AMICAR + MC</td>
<td>-0.14</td>
<td>0.89</td>
</tr>
<tr>
<td>Normal saline vs AMICAR ± MC</td>
<td>1.68</td>
<td>0.19</td>
</tr>
</tbody>
</table>

MC = methyl cellulose.
Furthermore, the concentration of AMICAR of 10–32.5 μg/mL is required to achieve antifibrinolytic effects. Although these concentrations have been achieved within the aqueous humor with topical application over the eye, no studies have been done to calculate the tissue concentrations resulting from local application over mucus membranes. In order for the molecule to remain on the surface for a sufficient time in a clinically relevant concentration, a tissue or clot permeation enhancer may be required as its vehicle. On the corneal surface carboxypolymethylene was shown to be the most effective vehicle, which resulted in a duration of action of 6 hours, when applied with AMICAR.

The use of AMICAR with or without the mucoadhesive agent did not significantly affect the time to achieve complete hemostasis, although it was shorter in this group. However, there was a trend toward a shorter time to achieve hemostasis when AMICAR was used when data for all sites were pooled. In some previous studies done using other hemostatic agents within the human nasal cavity after ESS, the time to achieve total hemostasis was not measured. Instead, the need for additional packing was used as a measure of effective hemostasis and general observation after the application of the substance or on postoperative visits were used to determine the extent of bleeding. Gall et al. used a five-point visual analog scale to determine the severity of bleeding in the operative site before and after the application of FloSeal, and documented the time to complete cessation of bleeding.

Furthermore, in this study the control side received equal volumes of normal saline spray on the injured surface of the nasal cavity. This provided some degree of irrigation and may have aided in hemostasis. If the control side was left untreated and did not receive saline spray there may have been a more significant difference between the treatment side and control side in terms of degree of hemostasis and time to achieve total hemostasis.

CONCLUSIONS

In this randomized controlled pilot study AMICAR showed a tendency to reduce the time to achieve total hemostasis for all sites and, on its own, achieved a significant reduction in the grade of surgical bleeding on the lateral nasal wall. However, when AMICAR was mixed with methyl cellulose in an attempt to improve adherence, this effect was lost.

ACKNOWLEDGMENTS

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REFERENCES