Comparison of a New Fibrin Sealant With Standard Topical Hemostatic Agents

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Background: Bleeding following liver resection continues to be a significant morbidity of the procedure. Fibrin sealants represent an improvement over conventional topical hemostatic agents, because they contain components that actively form clot. However, most available agents contain nonhuman protein, which represents an immunologic risk.

Hypothesis: An investigational surgical fibrin sealant (Crosseal; American Red Cross, Washington, DC) composed of human clottable proteins and human thrombin is more effective than standard topical hemostatic agents in reducing the time required to achieve hemostasis after liver resection.

Design: Prospective, randomized, controlled trial.

Setting: Fifteen major referral centers in the United States and the United Kingdom.

Methods: After liver resection using standard surgical techniques, 121 patients seen between May 1999 and May 2000 were randomized to treatment with a 2-component fibrin sealant (n=58) or to standard topical hemostatic agents, used singly or in combination (n=63). Up to 10 mL of Crosseal was administered by a spray applicator, as recommended by the manufacturer, whereas agents used in the control group were applied according to their instructions for use.

Main Outcome Measures: The primary outcome measured was time to hemostasis. Secondary outcomes measured included blood loss between application of the hemostatic agent and closure of the abdomen, duration of postoperative biliary drainage, and the occurrence of complications, defined a priori as reoperation for any reason, development of abdominal fluid collections, or bilious appearance of drained fluid for at least 1 day postoperatively.

Results: The mean time to hemostasis was 282 seconds with Crosseal, compared with 468 seconds with standard agents (2-sided; \( P = 0.06 \)), for the 116 efficacy-evaluable patients. Hemostasis was achieved within 10 minutes in 53 patients (91.4%) treated with the study fibrin sealant and in 44 control patients (69.8%) (2-sided; \( P = 0.003 \)). Intraoperative blood loss was similar in the 2 groups. In the Crosseal group, the percentage of patients developing postoperative complications was 17.2%, compared with 36.5% in the control group (2-sided; \( P = 0.2 \)).

Conclusions: Compared with the use of standard topical hemostatic agents, Crosseal fibrin sealant significantly reduced the time to achieve hemostasis following liver resection. Patients treated with the new fibrin sealant also experienced significantly fewer postoperative complications.

Arch Surg. 2004;139:1148-1154

Advances in surgical technique have reduced the occurrence of postoperative complications following liver resection. However, operative blood loss remains a major problem affecting the prognosis of patients undergoing liver resection. During the past 2 to 3 decades, surgical techniques to facilitate hemostasis during liver resection have been developed that may lead to improved outcomes. These techniques include diathermy, argon beam coagulation, suture ligation (an outdated technique), hepatic inflow occlusion (Pringle maneuver), and total vascular exclusion.

Topical agents have also been developed as adjunctive measures to promote hemostasis. These include microfibrillar collagen, bovine collagen–based composite mixed with autologous plasma, and fibrin sealants. The use of adjunctive treatment to facilitate hemostasis can be particularly important in hepatic surgery. Because many postoperative complications after liver resection are due to bleeding and bile leakage, effective seal-
ing of vessels and biliary radicals may lead to a reduction in postoperative complications.

Topical fibrin sealants have been used in Europe for more than 2 decades to promote hemostasis during a variety of surgical procedures. The first fibrin sealant marketed in the United States was Tisseel (Baxter Healthcare, Deerfield, Ill), which was approved by the US Food and Drug Administration (FDA) in February 2000. Cross-seal fibrin sealant (human), marketed as Quixil outside the United States, received FDA approval in March 2003 Cross-seal. Crosseal is a new-generation, virally inactivated surgical sealant, formulated from a concentrate of human clotting proteins called biological active component (BAC), and a highly purified preparation of human α-thrombin (1000 IU/mL). Fibrinogen is the active agent in BAC. Unlike fibrin sealants containing bovine protein, inactivin as an antifibrinolytic ingredient or bovine thrombin, Crosseal contains no animal protein. Anaphylactic reactions to bovine-derived components, which appear to be the subject of an increasing number of reports, are thus avoided.

The multicenter study described here constitutes the FDA-monitored phase 3 clinical trial of Crosseal and was designed to meet regulatory guidelines for approval of fibrin sealants. The study was conducted to determine the effect of Crosseal on the time to achieve hemostasis following liver resection and on postoperative complications.

METHODS

PATIENTS

The study was conducted at 15 medical centers, 11 in the United States and 4 in the United Kingdom. Adult patients (>18 years) were candidates for entry if they required liver resection for any reason except trauma and underwent no major surgical intervention beyond the liver. Following liver resection and control of bleeding from discrete vessels, patients were eligible for the entry only if they had generalized oozing from the cut surface of the liver and the surgeon intended to use a topical hemostatic agent. Patients were excluded from the study if they had a coagulation disorder other than that resulting from liver disease, previous contact with bovine thrombin, participation in another clinical trial, known intolerance to blood products, or pregnancy. The institutional review board at each US and UK center approved the study. All patients provided written informed consent prior to study participation.

RANDOMIZATION

The study was single-blind, as the nature of the compared treatments made it impossible for the surgeon to be blinded. Insofar as possible, all staff, including data analysts, were blinded to treatment allocation. Treatment allocation employed a sealed envelope system. Both Cross-seal fibrin sealant and the selected control treatment were prepared for use in each case. Randomization envelopes were not opened until immediately prior to treatment, when the surgeon had determined that all discrete bleeding points had been controlled and that use of a topical hemostatic agent was necessary. Randomization was by patient, stratified by surgeon within each clinical center, using the method of randomly permuted blocks. A variable and un-

HEMOSTATIC INTERVENTIONS

Patients were randomly assigned to be treated with either Cross-seal fibrin sealant or a standard FDA-approved topical hemostatic agent (control) within 1 minute from the time gauze was applied to the cut surface of the liver.

Cross-seal fibrin sealant is provided in frozen form and can be thawed and stored in the refrigerator before use. When needed, the components are easily loaded into 2 syringes (1 for BAC and 1 for thrombin) incorporated into a patented needle-free device that mixes them, and the syringes are attached to a spray device. For patients randomized to the study group, up to 10 mL of Cross-seal was sprayed in short bursts (0.1-0.2 mL) onto the cut surface of the liver to form a thin, even layer.

Commercially available hemostatic agents used for the control group were Actifoam (formerly made by Davol, Cranston, RI; discontinued in May 1999), Avitene (CR Bard, Murray Hill, NJ), Gelfoam (Pharmacia & Upjohn, Kalamazoo, Mich), Oxsycel (formerly made by Deseret, Sandy, Utah; now offered by Parke-Davis, Detroit, Mich), Surgicel and Surgicel Nu-Knit (Ethicon, Somerville, NJ), and Thrombinar (formerly made by Armour Pharmaceuticals, Tarrytown, NY; now offered by King Pharmaceuticals, Bristol, Tenn, under the brand name Thrombin-JMI). These agents, used singly or in combination, were applied according to the package insert and the surgeon's standard of care, with standard of care taking precedence.

When the surgeon believed hemostasis was achieved, the liver surface was observed for 1 minute to confirm hemostasis. If bleeding from discrete vessels was seen at any time during the observation period, further attempts were made to achieve hemostasis (ie, gentle pressure, suture, or electrocautery) and another 1-minute observation period began. This process was repeated at 1-minute intervals until hemostasis was achieved. For control products applied with patches or gauze, the evaluation was made by the surgeon's carefully lifting a corner of the product to check for bleeding. If bleeding recurred, the process of reevaluation was recommenced.

OUTCOME MEASURES

The primary efficacy end point, time to hemostasis, was defined as the time between application of the product to the surface of the liver (after resection was completed and all discrete bleeding points were controlled by suture or cauterezation) and the time when there was no further evidence of bleeding after direct observation for 1 minute. In addition, Kaplan-Meier assessment of the number of patients achieving hemostasis at 10 minutes was determined.

Secondary efficacy measures included the volume of blood loss between the time of initial application of the hemostatic agent and closure of the abdomen, the duration of postoperative bilious drainage between drain insertion and removal, and the occurrence of abdominal fluid collections. Several procedures were used to measure these secondary outcomes. One procedure estimated intraoperative blood loss by weighing the gauze or sponge pads that were used after the initial application of the hemostatic agent (subtracting the dry weights of the swabs and pads and assuming that 1 mL of blood weighs 1 g), and including the net suction volume after subtraction of rinse fluids. To permit differentiation between intraoperative blood loss before and after application of the hemostatic agent, the study site coordinator ensured that all swabs and pads were removed and that the suction bottles were removed and that the suction bottles were removed and that the suction bottles...
An additional test for hepatitis C was conducted 6 months after hepatitis A, B, and C at baseline and 3 months after surgery. Viral markers for human immunodeficiency virus (HIV) and event reporting up to 6 weeks after surgery and by testing for agent(s) used in each case. Safety was evaluated by adverse events recorded. The investigator to be clinically relevant. All adverse events occurred as adverse events if they were considered by the study, whether or not the condition was considered re-occur, the results of these tests to the manufacturer, whose agents were documented separately.

Another procedure assessed the duration of postoperative biliary drainage by recording and summing the periods after the operation during which the drainage fluid was characterized (qualitatively) as having a bilious appearance. The participating centers employed either computed tomography (CT) or ultrasonography to identify ascites, bilomas, and other abdominal fluid collections. The centers submitted the results of these tests to the manufacturer, whose medical officer conducted a blinded review.

Other measures used to assess efficacy were the percentage of patients experiencing complications, defined as reappearance for any reason, diagnosis of abdominal collections, and bilious appearance of drainage fluid for at least 1 day. Other data recorded are listed in Table 1.

An adverse event was defined as any undesirable change, either clinical or in laboratory values, that occurred during the study, whether or not the condition was considered related to the investigational agent. Out-of-range laboratory values were reported as adverse events if they were considered by the investigator to be clinically relevant. All adverse events occurring from the time of consent to 6 weeks after surgery were recorded.

Investigators rated the maximum intensity of each adverse event as mild, moderate, or severe according to the definitions in Table 2. Investigators used the definitions in Table 3 to assess the relationship of adverse events to the hemostatic agent(s) used in each case. Safety was evaluated by adverse event reporting up to 6 weeks after surgery and by testing for viral markers for human immunodeficiency virus (HIV) and hepatitis A, B, and C at baseline and 3 months after surgery. An additional test for hepatitis C was conducted 6 months after surgery.

Table 1. Other Data Recorded

<table>
<thead>
<tr>
<th>Data Recorded</th>
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<tr>
<td>Total intraoperative blood loss (based on the net weight of the gauze and sponge pads and the net suction volumes used during the entire procedure).</td>
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<tr>
<td>Postoperative fluid loss (based on the volume of drainage fluid collected).</td>
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<tr>
<td>Number of units of hemoglobin-containing blood products (whole blood or packed red blood cells) transfused during the postoperative period (ie, between closure of the abdomen and discharge from the hospital).</td>
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<td>Preoperative hemoglobin levels compared with hemoglobin levels 1 day postoperatively.</td>
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<td>Minimum hemoglobin level in the postoperative period.</td>
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<td>Total time of operative procedure.</td>
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<tr>
<td>Duration of drainage (defined as the elapsed time between insertion of the drain and last recorded emptying time during hospitalization).</td>
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Table 2. Classification of Intensity of Adverse Events

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<thead>
<tr>
<th>Intensity</th>
<th>Description</th>
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<tbody>
<tr>
<td>Mild</td>
<td>Awareness of signs or symptoms, but no disruption of usual activity.</td>
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<tr>
<td>Moderate</td>
<td>Event sufficient to affect usual activity (disturbing).</td>
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<tr>
<td>Severe</td>
<td>Inability to work or perform usual activities (unacceptable).</td>
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Table 3. Definitions of Relation of Adverse Event to Hemostatic Agent

<table>
<thead>
<tr>
<th>Relation</th>
<th>Description</th>
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<tr>
<td>Unrelated</td>
<td>Clearly and incontrovertibly due only to extraneous causes and does not meet criteria listed under unlikely, possible, or probable.</td>
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<tr>
<td>Unlikely related</td>
<td>Does not follow a reasonable temporal sequence from administration. May have been produced by the patient’s clinical state or by environmental factors or other therapies administered.</td>
</tr>
<tr>
<td>Possibly related</td>
<td>Follows a reasonable temporal sequence from administration. May have been produced by the patient’s clinical state or by environmental factors or other therapies administered.</td>
</tr>
<tr>
<td>Probably related</td>
<td>Clear-cut temporal association with improvement on cessation of test drug or reduction in dose. Reappears upon rechallenge. Follows a known pattern of response to test drug.</td>
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STATISTICAL ANALYSIS

The results of phase I and II studies and interviews with liver surgeons indicated that hemostasis was achieved within 10 minutes after application of Crosselect, and often within 1 to 5 minutes. Interviews with liver surgeons also indicated that the time to hemostasis after application of various other hemostatic agents was 5 to 15 minutes. This information was utilized to determine the required sample size for demonstration of a statistically significant reduction in time to hemostasis with Crosselect. Although it was estimated that efficacy might be established after treating 40 patients, it would be necessary to recruit 120 patients to provide a sufficiently large safety database.

Time to hemostasis was subjected to analysis of covariance using log-transformed data. The terms included in the model, prespecified in the statistical analysis plan, were: treatment, surgeon, surgeon × treatment interaction, preoperative platelet count, type of liver resection, and whether argon beam coagulation was used. Analyses were done using both the efficacy-eligible and all-patients-randomized (ie, intention-to-treat [ITT]) populations.

A 95% confidence interval (CI) was derived for the percentage reduction in time to hemostasis associated with the use of the fibrin sealant. This was converted to a difference in absolute time by reference to the unadjusted mean time to hemostasis observed in the control group. The individual times to hemostasis were shown graphically as cumulative distributions using a Kaplan-Meier plot.

Other efficacy variables such as intraoperative blood loss and postoperative fluid loss were also subjected to analysis of covariance using log-transformed data. The percentage of patients with abdominal collections of fluid was analyzed using a stratified exact permutation test. The time for which the drain- age fluid contained bile (or, separately, blood) was analyzed using an exact permutation test. The percentage of patients exhibiting complications was analyzed using the Fisher exact test with a mid-P adjustment.

All patients enrolled in the study were included in the safety analysis. Specific adverse events that occurred in at least 5% of patients in either group were formally tested for a difference between treatment groups using the Fisher exact test.

RESULTS

DEMOGRAPHICS

Demographic characteristics of the 121 patients enrolled in the study are shown in Table 4. The first
In patients receiving Crosseal, 1 kit with a mean dose of 7.9 mL was used, compared with 3 units of control product(s), either as multiple units of the same product or combinations of more than 1 product, to achieve hemostasis. The mean time to hemostasis among the efficacy-evaluable patients (Table 6) was significantly shorter in the Crosseal group than in the control group (282 vs 468 seconds) (2-sided; \( P = .006 \)). The treatment effect corresponded to an estimated 22.4% reduction in time to hemostasis (95% CI, 7.1%–35.1%), equivalent to a time reduction of 104.4 seconds (95% CI, 33–164.4 seconds). The cumulative distributions of the 2 groups were similar up to approximately 3 minutes but showed a marked divergence thereafter, with a higher percentage of control patients still bleeding.

For the all-patients-randomized group (Table 6), the mean time to hemostasis was also shorter in the Crosseal group than in the control group (318 vs 462 seconds) (2-sided; \( P = .02 \)). The mean times to hemostasis in both treatment groups varied according to study site. When adjusted by study site, the mean time to hemostasis was reduced by 21.1% (95% CI, 5.1%–34.4%) (2-
sided; \( P = .01 \) in the Crosseal group, equivalent to a time reduction of 97.2 seconds (95% CI, 23.4-159 seconds). The times to hemostasis for the 2 treatment groups are displayed as Kaplan-Meier plots in Figure 2. After the initial 3 minutes of observation, fewer patients in the Crosseal group were still bleeding.

The percentage of patients achieving hemostasis by time interval is shown in Figure 3. The percentage of patients achieving hemostasis within 10 minutes was significantly higher in the Crosseal group (91.4%) than in the control group (69.8%) (2-sided; \( P = .003 \)).

### SECONDARY OUTCOMES

The percentage of patients with reoperation for any reason, a diagnosis of abdominal fluid collections, or bilious drainage for at least 1 day was lower in the Crosseal group than in the control group (17.2% vs 36.5%) (2-sided; \( P = .02 \)). When these complications were analyzed individually, the percentage of patients with abdominal collections was significantly lower in the Crosseal group than in the control group (3.4% vs 14.3%) (2-sided; \( P = .05 \)). No significant differences between treatment and control groups were seen in intraoperative blood loss, duration of postoperative bilious drainage, percentage of patients with bile loss, volume of drainage fluid, and duration of drainage, although differences favored the Crosseal group. Patients in the Crosseal group were discharged a mean of 1.5 days earlier than those in the control group (mean hospital stay, 9 days vs 10.5 days), but the difference was not significant. The median duration of hospitalization was 7 days for each treatment group.

### ADVERSE EVENTS

Although adverse events were reported in nearly all patients, the most frequently reported events were consistent with the type of surgery and the patient’s underlying medical condition. With the exception of 2 adverse events (dyspepsia and gallbladder disorders), no differences in the type or frequency of adverse events were shown between the 2 treatment groups. The percentages of patients with dyspepsia (6.9% vs 20.6%) and gallbladder disorders (3.4% vs 17.5%) after treatment were significantly lower in the Crosseal group than in the control group (2-sided; \( P = .05 \)). Eleven patients in each treatment group had adverse events that were considered severe. There were 6 deaths in the control group (metastatic colon cancer with liver failure, liver failure, hepatic coma secondary to recurrent hepatoma, multiple organ failure, cholangiocarcinoma with obstructive jaundice, and gastrointestinal hemorrhage) compared with 1 in the Crosseal group (multiple organ failure and bile duct tumor 3 weeks after surgery) (2-sided; \( P = .07 \)); the deaths were considered unrelated to treatment.

None of the adverse events in the study were assessed as probably related to treatment. Two events (gallbladder disorder and hematoma) occurring in the same control-group patient were considered possibly related to treatment. Nine patients in the Crosseal group had 33 adverse events that were considered unlikely to be re-
The primary objective of this study was to determine whether application of Crosseal fibrin sealant (human) resulted in a statistically significant improvement in time to hemostasis. Crosseal significantly reduced the time to hemostasis after liver resection in both the efficacy-evaluable group and the ITT group. Individual surgeons must determine for themselves whether a shorter mean time to hemostasis of 97 seconds translates to a clinically meaningful hemostatic advantage. A shorter time to hemostasis was evident even though the methods of hemostasis after resection and the choice of control agents varied by surgeon. Crosseal was compared with a total of 7 different control agents, including varieties of collagen, cellulose, gelatin, and thrombin. This nonhomogeneous control group reflects the current state of the art with respect to surgical hemostasis using topical hemostatic agents.

Like the study described by Chapman and colleagues, this study was designed to compare hemostatic agents by identifying the percentage of patients achieving hemostasis within a predetermined period. The percentage of patients who achieved hemostasis within 10 minutes in this study was 91.4% in the Crosseal group compared with 69.8% in the control group.

The patient enrollment criteria for this study were designed to reduce variability and provide a controlled setting, which would allow the generation of safety and efficacy data appropriate for FDA review. Additionally, the spraying of the fibrin sealant was limited to the end of the operation, when all major vessel bleeding was controlled and only diffuse parenchymal oozing remained. This design helped maintain the blinded nature of the study insofar as possible. However, there are many occasions in hepatic surgery when bleeding can occur early during a procedure, sometimes precipitating a critical situation. Crosseal may prove of great value when used as a hemostatic adjunct during these early stages of surgery.

Secondary outcome measures such as the reduction of complications (which were more clinically relevant) also showed differences in favor of Crosseal that were statistically significant. In addition, patients in the Crosseal group had less bile loss and a shorter duration of bile leakage. This finding supports the theory that Crosseal acts as a sealant as well as a hemostat, forming a barrier that prevents blood and bile from leaking while the wound heals naturally.

All adverse events reported in the Crosseal group in this study were thought to be either unrelated or unlikely to be related to treatment. For the majority of adverse events, no differences in frequency were seen between the treatment groups. “Dyspepsia,” a nonspecific descriptor used in the clinical research report, was 1 of a number of potential gastrointestinal adverse events including flatulence, nausea, hiccup, and gastrointestinal disorder not otherwise specified. The relationship to study agents and the clinical significance, if any, of the difference in rate of occurrence of dyspepsia compared with that of other gastrointestinal adverse events is difficult to assess. Similarly, the exact relationship of the nonspecific descriptor “gallbladder disorder” to study agents and its clinical significance is difficult to assess, particularly among this group of patients who had undergone hepatic surgery and who may have experienced postoperative bile leakage.

Abdominal ultrasound or CT was not performed on every patient. Only patients with clinical findings suggestive of an abdominal fluid collection underwent imaging studies, which were necessarily requested in an unblinded manner, and asymptomatic bilomas may have gone undetected. These asymptomatic collections, however, are not a clinically relevant end point.

The manufacture of Crosseal includes processing steps designed to reduce the risk of viral transmission. Both BAC and thrombin undergo discrete viral inactivation/removal steps. Both are subjected to the robust and safe solvent detergent treatment. In addition, BAC is pasteurized, and thrombin undergoes nanofiltration.

In conclusion, results of this multicenter, randomized, controlled trial demonstrated that Crosseal was superior to standard-treatment hemostatic agents in reducing the time required to achieve hemostasis following liver resection and reduced the number of complications following surgery. No safety concerns were associated with the use of Crosseal.

Accepted for Publication: March 16, 2004.

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Acknowledgments: Additional study sites were the DuMont UCLA Transplant Center, Los Angeles, Calif; University of Nebraska Medical Center, Omaha, Neb; and University of Medicine and Dentistry of New Jersey—New Jersey Medical School, Newark, NJ.
REFERENCES


