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Evaluation of the topical hemostatic efficacy and safety of TISSEEL VH S/D fibrin sealant compared with currently licensed TISSEEL VH in patients undergoing cardiac surgery: a phase 3, randomized, double-blind clinical study

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Aim. **TISSEEL VH is the only commercially available fibrin sealant indicated as an adjunct to conventional methods of hemostasis during cardiac surgery. A next generation fibrin sealant (TISSEEL VH S/D) has been developed in frozen, ready-to-use form with an added virus inactivation step (solvent/detergent [S/D] treatment) to provide added safety and convenience to the currently licensed product. This study was performed to compare efficacy and safety of the two products.**

Methods. **Phase 3, prospective, randomized, double-blind, multicenter study to compare TISSEEL VH S/D to TISSEEL VH during cardiac surgery. The primary efficacy endpoint was the proportion of patients who achieved hemostasis at the primary treatment site within 5 min, and maintained hemostasis until surgical closure.**

Results. **The proportion of patients who achieved hemostasis at the primary treatment site within 5 min, and maintained hemostasis until surgical closure was 88.2% for TISSEEL VH S/D and 89.6% for TISSEEL VH in the intent-to-treat population. The difference in proportions, TISSEEL VH S/D minus TISSEEL VH, was –1.4% with a standard error of 3.70%. The lower 97.5% confidence bound of this difference was –8.6%, which is above the predefined noninferiority margin of 15%. Therefore, TISSEEL VH S/D is at least as efficacious as TISSEEL VH. The safety profile of TISSEEL VH S/D was very**

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similar to that of currently licensed TISSEEL VH as assessed by the safety endpoints.

***Conclusion*. TISSEEL VH S/D is safe and effective for use as an adjunct to hemostasis in patients undergoing cardiac surgery.**

KEY WORDS: Fibrin tissue adhesive – Hemostasis - Cardiac surgery.

Despite recent technical improvements, cardiac surgery is still an intervention associated with significant blood loss. Complications arise not only out of the invasiveness of the procedure, but because patients undergoing such interventions are treated with drugs interfering with coagulation and platelet function. In addition, cardiopulmonary bypass (CPB) requires aggressive systemic anticoagulation, and results in increased fibrinolytic activity.[1-8] Proven surgical methods of achieving hemostasis include sutures, cautery, clamps, and clips, which may be used alone or in combination with conventional topical hemostatic agents such as collagen or thrombin.[9] Skilled cardiothoracic surgeons employing these conventional methods of hemostasis are still confronted with a certain percentage of patients in whom bleeding is difficult to control.[10, 11]

Fibrin sealants have been shown to be effective hemostatic agents in a variety of different surgical procedures.[12-17] However, at the time of writing, the only commercially available fibrin sealant indicated as an adjunct to conventional methods of hemostasis during cardiac surgery is TISSEEL VH (Baxter Healthcare Corporation). TISSEEL VH has been shown to be an effective adjunct in patients undergoing CPB, as well as in the treatment of splenic injuries and closure of colostomies. Although the safety profile of TISSEEL VH has been excellent since US licensure in 1998, a next generation fibrin sealant (TISSEEL VH S/D) has been developed with an additional state-of-the-art virus inactivation step incorporated into the manufacturing process to further increase the margin of safety for this plasma-derived product. TISSEEL VH S/D contains the same active components as TISSEEL VH; however, it is manufactured using a two-step process of virus inactivation that includes a solvent/detergent (S/D) treatment in addition to the vapor heat (VH) treatment employed during the manufacture of TISSEEL VH. VH treatment is highly effective against a broad spectrum of viruses, including nonenveloped viruses such as hepatitis A virus (HAV).[18] S/D treatment is an extremely effective inactivation technology for lipid-enveloped RNA and DNA viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV).[19-23]

The main active ingredients in both TISSEEL VH and TISSEEL VH S/D are fibrinogen, thrombin, and aprotinin. The components of these fibrin sealants are mixed immediately prior to application, and the thrombin cleaves the fibrinogen to yield fibrin that forms a physiological clot at the site of application. The aprotinin acts as a fibrinolysis inhibitor that increases the life of the clot. TISSEEL VH is supplied as a lyophilized product, whereas TISSEEL VH S/D is provided as a frozen liquid (when marketed, the product will also be available in lyophilized form). This frozen liquid formulation is supplied in two separate prefilled syringes contained in a preassembled delivery device. The frozen product formulation provides greater convenience and handling since it eliminates the reconstitution and device assembly required with the lyophilized product.

This clinical study compared the hemostatic efficacy and safety of TISSEEL VH S/D and TISSEEL VH in patients undergoing cardiac surgeries requiring CPB and median sternotomy.

Materials and methods

Patients

All patients provided informed consent prior to entry into this clinical study, which was administered according to the principles set forth in the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines.

Patients ≥18 years of age undergoing cardiac surgery requiring CPB and median sternotomy were treated under double-blind conditions with either TISSEEL VH S/D or TISSEEL VH as randomized. Patients were only eligible for treatment if hemostasis was not achieved using conventional surgical methods following discontinuation of CPB and heparin reversal.

Interventions

INVESTIGATIONAL PRODUCT DESCRIPTION AND PREPARATION

TISSEEL VH S/D is comprised of two components: sealer protein solution and thrombin solution. The sealer protein solution and thrombin solution are derived from human plasma. In the final form, the sealer protein solution contains 72-110 mg/mL fibrinogen (human)

TABLE I.—*Pack size and thawing conditions.*

Pack size	Thawing conditions (33 - 37°C)		
	Sterile water bath	Nonsterile water bath	Incubator
	(Continuous heating not required; both peel bags removed from product)	(Continuous heating required; product contained in both peel bags)	(Continuous heating required; product contained in both peel bags)
2 mL	5 min	30 min	40 min
4 mL	5 min	40 min	85 min
10 mL	12 min	80 min	105 min

and 3 000 KIU/mL aprotinin (bovine) and the thrombin solution contains 500 IU/mL of thrombin (human) and 40 mmol/mL of calcium chloride.

TISSEEL VH S/D was provided in two double sterile easy to open peel bags. The frozen product was completely thawed prior to use according to the protocol. The product label provides three alternative thawing methods for TISSEEL VH S/D (shown below). For all three methods the temperature of the water/incubator must be 33-37°C. The thawing time depends on pack size (Table I).

TREATMENT OF BLEEDING SITES

All patients meeting the preoperative eligibility criteria were randomized on the day of surgery and received study product in a blinded fashion. The investigator selected a bleeding site that met the eligibility criteria for the primary treatment site. Bleeding areas eligible for designation as the primary treatment site included: surface of the heart; anastomoses; mammary artery pedicle or harvest site; surface of the suprasternal notch; surface of the thymus; and other sites in the cardiac cavity, as deemed appropriate by the surgeon. Bleeding sites not eligible for designation as the primary treatment site included: margins of the sternotomy wound; areas of brisk and massive arterial bleeding; and areas previously treated with any topical hemostatic agent (*e.g.* thrombin, bone wax, Surgicel®, Oxycel®, Gelfoam®, Avitene®, Ultrafoam®, FloSeal®) during the operation.

The time required to achieve hemostasis was monitored after application of TISSEEL VH S/D or TISSEEL VH to the primary treatment site. Hemostasis was defined as the point at which the surgeon visually determined that bleeding at the treated site had been controlled and did not require revisitation to control bleeding by any other means prior to sternal closure. The actual mean volume of TISSEEL VH S/D applied to all bleeding sites was 5.4±3 mL (range: 0.5 to 12.0 mL). Similarly, the mean volume of TISSEEL VH applied to all bleeding sites was 5.1±3.1 mL (range: 0.2 to 12.0 mL).

Study objectives

The primary efficacy endpoint of this study was the proportion of patients who, after application of either TISSEEL VH S/D or TISSEEL VH, achieved hemostasis at the primary treatment site within 5 min of treatment and maintained hemostasis at the primary treatment site until closure of the surgical wound. Secondary efficacy endpoints included: time to hemostasis at the primary treatment site following treatment with investigational product; amount, type, and frequency of blood products received intraoperatively through 48 h postoperative; incidence of rebleeding at the primary treatment site following determination of hemostasis and prior to closure of the surgical wound; volume of postoperative chest tube drainage at 0, 2, 4, 6, 8, 10, 12, 16, 20, 24 and 48 h or at time of removal, whichever occurred first; incidence of resternotomy for bleeding; incidence of mortality within 30 days postoperative, or at any time during hospitalization; and time to hospital discharge.

The primary safety endpoint was the proportion of patients with one or more adverse experiences (AEs) deemed possibly or probably related to treatment with investigational product. Secondary safety endpoints included: incidence of postoperative mediastinitis; amount, type and frequency of blood products received 48 h postoperative through 30 days postoperative; evaluation of seroconversion for parvovirus B19 (PV B19) in all patients one month after treatment; and evaluation of seroconversion for HAV, HBV, HCV and HIV-1/HIV-2 in all patients for 6 months after treatment.

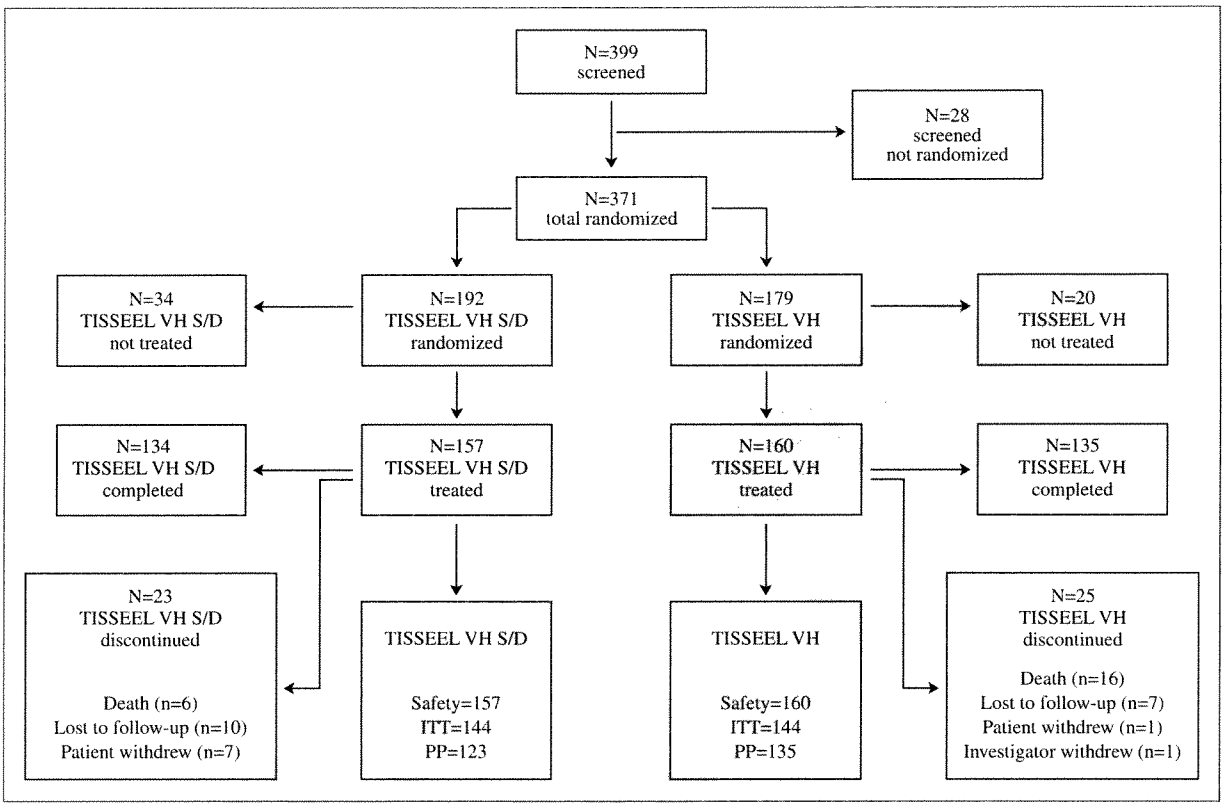

Figure 1.—Patient disposition.

Statistical analysis

For the primary efficacy analysis, the one-sided 97.5% confidence interval on the difference in the proportion of patients treated with TISSEEL VH S/D or TISSEEL VH who achieved the primary endpoint was calculated with a lower confidence bound of 15%. The secondary efficacy variables were evaluated using a two-sided hypothesis testing scheme, where the null hypothesis was one of equality and the alternative hypothesis represented a difference. Mann-Whitney tests between TISSEEL VH S/D and TISSEEL VH were performed for all quantitative secondary efficacy variables. Fisher's exact tests were performed to analyze differences in incidence rates between TISSEEL VH S/D and TISSEEL VH for all qualitative secondary efficacy variables.

For the primary safety analysis, the proportion of patients in each treatment group with one or more AEs deemed possibly or probably related to the treatment was compared using Fisher's exact test. The secondary safety variable, amount and type of blood products received 48 h to 30 days postoperative, was evaluated using the Mann-Whitney test between TISSEEL VH S/D and TISSEEL VH. All other secondary safety variables were analyzed using Fisher's exact test for differences in incidence rates between TISSEEL VH S/D and TISSEEL VH. The alpha was 0.05 for all statistical tests performed to assess safety and efficacy in this study.

A prospectively planned bias analysis was also performed to monitor the effects of prognostic factors on the primary efficacy outcome. Logistic regression was performed on the primary endpoint outcomes for both intent-to treat (ITT) and per protocol (PP) populations. The interaction term looked at the ratio of hemostasis success rates between TISSEEL VH-treated patients and TISSEEL VH S/D-treated patients

TABLE II.—*Demographic and disease characteristics at baseline for intent to treat patients.*

Characteristic	TISSEEL VH (N=144)	TISSEEL VH S/D (N=144)	P-value
Gender [N(%)]			0.5914a
Female	40 (27.8%)	35 (24.3%)	
Male	104 (72.2%)	109 (75.7%)	
Age (years)			0.0521b
Mean (SD)	66.2 (11)	63.4 (12.9)	
Median	67.0	64.0	
Range	38-94	19–88	
Weight (kg)			0.3320b
Mean (SD)	84.8 (19.2)	87 (19.6)	
Median	79.9	85.2	
Range	38.6 - 135.6	40–164	
Current cardiac surgery [N(%)]			0.7239c
Other	0 (0%)	1 (0.7%)	
Reoperative valve	11 (7.6%)	9 (6.3%)	
Reoperative valve and other	2 (1.4%)	2 (1.4%)	
Primary CABG	67 (46.5%)	65 (45.1%)	
Primary CABG and other	4 (2.8%)	4 (2.8%)	
Reoperative CABG	16 (11.1%)	23 (16%)	
Reoperative CABG and other	3 (2.1%)	0 (0%)	
Reoperative CABG and reoperative valve	10 (6.9%)	8 (5.6%)	
Reoperative CABG , reoperative valve and other	1 (0.7%)	0 (0%)	
Primary valve	15 (10.4%)	18 (12.5%)	
Primary valve and primary CABG	15 (10.4%)	14 (9.7%)	

a=Fisher's exact test; b=*t*-test; c=χ^2 test; N=number; SD=standard deviation; CABG=coronary artery bypass graft.

TABLE III.—*Description of primary treatment site.*

Description	Intent to treat patients		
	TISSEEL VH (N=144)	TISSEEL VH S/D (N=144)	P-value (Fisher's exact test)
Bleeding site area			0.3632
Surface of heart	42 (29.2%)	36 (25%)	
Anastomosis (distal then proximal)	37 (25.7%)	25 (17.4%)	
Mammary artery bed	9 (6.3%)	13 (9.0%)	
Surface of suprasternal notch	6 (4.2%)	9 (6.3%)	
Surface of thymus	1 (0.7%)	2 (1.4%)	
Other sites in cardiac cavity	49 (34%)	59 (41%)	
Type of bleeding			0.0223
Localized	76 (52.8%)	96 (66.7%)	
Diffuse	68 (47.2%)	48 (33.3%)	
Severity of bleeding			0.9275
Minimal	31 (21.5%)	28 (19.4%)	
Mild	70 (48.6%)	71 (49.3%)	
Moderate	43 (29.9%)	45 (31.3%)	
Bleeding source			0.6569
Arterial	70 (48.6%)	62 (43.1%)	
Venous	28 (19.4%)	31 (21.5%)	
Mixed	46 (31.9%)	51 (35.4%)	
Application device			0.4414
Duploject with tissomat	47 (32.6%)	40 (27.8%)	
Duploject with application needle	97 (67.4%)	104 (72.2%)	

and asked if these ratios were different among the categories of the other independent factor.

Results

Patient disposition and demographics

A total of 399 patients were screened for entry into this study, with 371 randomized and 317 actually treated with one of the two investigational products: TISSEEL VH S/D or TISSEEL VH (Figure 1). The ITT population consisted of all randomized patients with an observed primary endpoint. A total of 29 patients, designated as test patients for the purpose of ensuring adequate blinding procedures at each clinical study site, were excluded from the ITT analysis. The PP population consisted of all treated patients who met all inclusion/exclusion criteria, were randomized and treated correctly, and adhered to study procedures. The safety population comprised all treated patients (test and nontest patients), and these patients were analyzed according to the treatment that they actually received (Figure 1).

There were no statistically significant differences between the 2 treatment populations for any of the demographic or disease characteristics measured for the ITT population (Table II) or the PP population (data not shown). Likewise, there was no statistically significant difference between TISSEEL VH S/D and TISSEEL VH for the primary treatment sites selected by the investigators in terms of bleeding site areas, severity of bleeding, bleeding source, or application device in the ITT population (Table III) or PP population (data not shown). However, analysis of the type of bleeding (localized or diffuse) revealed that a larger proportion of TISSEEL VH S/D patients had localized bleeding at the primary treatment site, compared with TISSEEL VH (ITT, P=0.0223). This prognostic factor was investigated, along with other factors, in a bias analysis of the primary efficacy results.

Primary efficacy results

For the primary analysis population (ITT), the proportion of patients who achieved hemostasis at the pri-

TABLE IV.—*Primary efficacy endpoint for intent-to-treat and per protocol patients.*

Endpoint	Intent to treat	Per protocol
Proportion achieved hemostasis [n/N (%)]		
TISSEEL VH S/D	127/144 (88.2%)	108/123 (87.8%)
TISSEEL VH	129/144 (89.6%)	122/135 (90.4%)
Difference in proportions, TISSEEL VH S/D minus TISSEEL VH	-1.4%	-2.6%
Standard error of the difference	3.7%	3.89%
Lower 97.5% one-sided confidence interval on the difference in proportions	-8.6%	-10.2%

mary treatment site within 5 min, and maintained hemostasis until surgical closure was 88.2% for TISSEEL VH S/D patients and 89.6% for TISSEEL VH patients (Table IV). The difference in proportions, TISSEEL VH S/D minus TISSEEL VH, was –1.4% with a standard error of 3.70%. The lower 97.5% confidence bound of this difference for the ITT population was –8.6%, which is above the predefined noninferiority margin of -15%. Analysis of the PP population revealed a similar result (Table IV). Therefore, TISSEEL VH S/D is noninferior to TISSEEL VH for the proportion of patients achieving hemostasis at the primary treatment site within 5 min and maintaining hemostasis until closure of the surgical wound in both the ITT and PP populations.

Analysis of the primary treatment site at baseline revealed that a larger proportion of patients had localized bleeding in the TISSEEL VH S/D group compared to the TISSEEL VH group (ITT, P=0.0223; PP, P=0.0322). To analyze the impact of this prognostic factor, and others, a prospectively planned bias analysis was conducted to measure the effect on the primary efficacy outcome. No statistically significant differences between the 2 treatment groups were found for bleeding type (localized *versus* diffuse; data not shown). Likewise, no significant differences were found for any of the other prognostic factors tested: blind maintained in the operating room, application of nonstudy hemostatic agents, bleeding site, bleeding source, bleeding severity, application device, i.v. antifibrinolytic agent used intraoperatively, gender, race, and age (data not shown).

Analysis of hemostasis at additional bleeding sites

After primary site treatment, investigators were given the option to treat additional eligible bleeding sites with the assigned investigational product. For the ITT population, hemostasis was achieved in 95.1% of the 155 additional bleeding sites treated with TISSEEL VH S/D and 94.4% of the 134 additional bleeding sites treated with TISSEEL VH. A similar result was recorded for the PP population (data not shown).

Secondary efficacy endpoint results

For the ITT population, there were no differences between TISSEEL VH S/D and TISSEEL VH for any of the secondary efficacy endpoints, except median length of rebleeding episodes (Table V). For median length of rebleeding episodes, a difference between TISSEEL VH S/D (2.33 min) and TISSEEL VH (0.89 min) was observed (P=0.0344). However, the incidence of rebleeding was too low in the ITT population to allow a clinically relevant assessment; 2.1% of the 144 TISSEEL VH S/D patients rebled and 6.9% of the 144 TISSEEL VH patients rebled. Therefore, TISSEEL VH S/D did not differ clinically from TISSEEL VH for any of the secondary efficacy endpoints measured in the ITT population.

In the PP population, there were no differences between TISSEEL VH S/D and TISSEEL VH for any of the secondary efficacy endpoints except for a difference between TISSEEL VH S/D (247.7 mL) and TISSEEL VH (390.0 mL) for mean volume of blood products received up to 48 h postoperative for packed red blood cells (P=0.0199). A difference was also observed in the PP population for the incidence of mortality within 30 days after surgery: 1.6% (2/123) for TISSEEL VH S/D and 7.4% (10/135) for TISSEEL VH (P=0.0366).

Safety results

PRIMARY SAFETY ENDPOINT RESULTS

There was no significant difference (P=0.4480) between the number of patients with at least 1 AE deemed possibly or probably related to investigational product for TISSEEL VH S/D-treated patients (1.3%) compared to TISSEEL VH-treated patients (3.1%). In addition, there was no significant difference between the 2 treatment groups in terms of out-of-range laboratory values and clinically significant AEs. Therefore, the primary safety objective was met for this clinical study.

SECONDARY SAFETY ENDPOINT RESULTS

There were no differences between TISSEEL VH S/D and TISSEEL VH in terms of any of the secondary safe-

TABLE V.—*Summary of secondary efficacy endpoints for intent-to-treat patients.*

Endpoint	TISSEEL VH		TISSEEL VH S/D		P-value
	No.	Summary statistics	No.	Summary statistics	
Time to hemostasis in minutes [Median (Min-Max)]	144	0.88 (0.00 - 22.7)	142	0.67 (0.00 - 12.8)	0.2443a
Rebleeding after hemostasis was Achieved [n (%)]	144	10 (6.9%)	144	3 (2.1%)	0.0850b
Time to re-bleeding in minutes [Median (Min-Max)]	10	2.57 (0.33-5.1)	3	2.95 (1.58 - 6.9)	0.5536a
Length of re-bleeding episode in minutes [Median (Min-Max)]	10	0.89 (0.22 – 2)	3	2.33 (1.88 - 2.4)	0.0344a
Blood products (mL) received to 48 h postoperative [Mean (SD)]					
Cryoprecipitate	144	3.1 (26.6)	144	2.5 (23.7)	1.0000a
Fresh frozen plasma	144	61.5 (204.8)	144	66.4 (217.7)	0.8601a
Platelet concentrate	144	49.9 (131.2)	144	55.8 (147.1)	0.9603a
Packed red blood cells	144	390.3 (561.6)	144	286.2 (525.6)	0.0613a
Washed packed red blood cells	144	0 (0)	144	4.5 (39.2)	0.1580a
Albumin heta starch	144	92.7 (224.5)	143	92.3 (236)	0.6745a
Frequency of receiving blood products [n (%)]	144	90 (62.5%)	144	79 (54.9%)	0.2314b
Total volume of postoperative chest tube drainage (mL) [Median (Min, Max)]	143	815 (74, 4032)	144	865 (200, 10120)	0.6141a
Incidence of resternotomy for bleeding [n (%)]	142	6 (4.2%)	144	5 (3.5%)	0.7688b
Incidence of mortality within 30 days postoperative [n (%)]	144	10 (6.9%)	144	4 (2.8%)	0.1688b
Time to hospital discharge (days) [Median (Min-Max)]	144	6 (0-50)	144	7 (1 - 43)	0.7130c

[a]: Wilcoxon rank sum test; [b]: Fisher's exact test; [c]: Log rank test; N: number; SD: standard deviation.

ty endpoints. Analysis of PV B19 seroconversion 1 month after surgery revealed a 0% incidence of confirmed seroconversion in both the TISSEEL VH S/D-treated patients and the TISSEEL VH-treated patients. Analysis of HAV, HBV, HCV and HIV-1/-2 six months after surgery revealed a 0% incidence of confirmed seroconversion in both the TISSEEL VH S/D-treated patients and the TISSEEL VH-treated patients. None of the 144 serious adverse experiences (SAEs) reported during the entire period of the study were considered related to TISSEEL VH S/D by the investigator, including 16 cases of death. The high number of unrelated SAEs is not unexpected in a population undergoing cardiac surgery. Of the 195 SAEs (including 6 cases of death) reported in TISSEEL VH-treated patients, 1 SAE (thrombocytopenia) was deemed possibly related to the investigational product by the investigator; however, a panel of three independent hematology experts considered the probability of TISSEEL VH being the cause of the thrombocytopenia as negligible (< 0.1%). Further investigations revealed that the most likely cause was severe dilutional and consumptive thrombocytopenia. In fact, thrombocytopenia is an expected complication in some patients after cardiac surgery involving CPB regardless of the use of fibrin sealant.[24]

Six nonserious AEs were considered related to investigational product by the investigator. All 6 related nonserious AEs were increases in D-dimer levels: 2 cases in TISSEEL VH S/D-treated patients and 4 cases in TISSEEL VH-treated patients. Regardless of causality, the most frequent AEs in the TISSEEL VH S/D-treated patients were pyrexia (15.3%), pleural effusion (8.9%), thrombocytopenia (8.9%), atrial fibrillation (8.3%), cardiac failure congestive (8.3%), respiratory failure (8.3%), anaemia (7%), and renal failure acute (6.4%). Again, the type and frequency of these AEs would be expected in this population regardless of the use of TISSEEL VH S/D.

Discussion

The data collected and analyzed during this study demonstrate that double virus inactivated TISSEEL VH S/D is at least as efficacious and safe as the currently licensed TISSEEL VH for use as an adjunct to hemostasis in surgeries involving CPB and median sternotomy.

TISSEEL VH S/D was found to be noninferior to TISSEEL VH as defined by the primary efficacy end-

point in both the ITT and PP populations analyzed. The success rate in the ITT population was 88.2% for TISSEEL VH S/D and 89.6% for TISSEEL VH at the primary treatment site. Prognostic factors were found not to have affected the outcome of the primary efficacy endpoint. For the additional bleeding sites treated, the hemostasis success rate was higher than that in the primary treatment site: 95.1% for TISSEEL VH S/D and 94.4% for TISSEEL VH (ITT). However, the investigators selected a higher proportion of moderate (*versus* mild or minimal) bleeding sites for the primary treatment site, compared with the additional bleeding sites treated.

Analysis of the secondary efficacy endpoint data in the ITT population indicates that TISSEEL VH S/D did not differ clinically from TISSEEL VH for any of the endpoints measured. Some differences were observed between the ITT and PP populations. Unlike the results of the ITT analysis, PP analysis revealed a higher mean volume of packed red blood cells received by TISSEEL VH patients (within 48 h of surgery) compared with TISSEEL VH S/D patients. This difference may be explained by the lower mean values for hematocrit and hemoglobin in the TISSEEL VH patients at baseline (data not shown), increasing the probability of blood transfusion requirement in the perioperative period for this patient group. In addition, a lower incidence of mortality within 30 days after surgery was suggested by the PP population analysis for the TISSEEL VH S/D patients. However, the lack of a statistically significant difference in mortality for the ITT population, coupled with the lack of a difference in the primary efficacy and safety outcomes means a clinically significant effect on mortality is unlikely.

There were no SAEs related to TISSEEL VH S/D. The 6 nonserious AEs reported as being related to investigational product were all increases in D-dimer levels: 2 cases in TISSEEL VH S/D-treated patients and 4 cases in TISSEEL VH-treated patients. D-dimer is a degradation product of fibrin that results from the dissolution of fibrin clots *in vivo*. Significant increases in D-dimer levels have been reported up to one month after CPB surgery in the absence of fibrin sealant treatment.[25, 26] Consistent with these reports, a strong increase in D-dimer levels was observed in this study population after surgery. An increase in D-dimer levels may also be expected after the use of fibrin sealants as a result of the degradation of the investigational product by the normal fibrinolysis mechanisms. Degradation of the investigational product is expected within 2 weeks.[27-29] Regardless of the causality of the increases in D-dimer levels, no correlation with any negative clinical outcome was observed.

The safety profile of TISSEEL VH S/D was very similar to that of TISSEEL VH as assessed by the primary safety endpoint. Therefore, the primary safety objective was met for this study. Additionally, there were no differences between TISSEEL VH S/D and TISSEEL VH in terms of any of the secondary safety endpoints, including a 0% incidence of confirmed seroconversion for HAV, HBV, HCV, HIV-1/-2, and PV B19. The S/D treatment, coupled with the VH step, provides TISSEEL VH S/D with the highest level of protection currently available for commercially prepared fibrin sealants.

In addition to the described efficacy and safety of TISSEEL VH S/D, the provision of this next generation fibrin sealant in a frozen form provides added convenience in terms of preparation. The frozen presentation circumvents the reconstitution steps previously required with lyophilized products. TISSEEL VH S/D can be taken directly from the freezer and thawed within as little as 5 min, depending on the volume being utilized (see *Methods* for detailed thawing procedures).

Conclusions

Overall, the outcomes of this study support TISSEEL VH S/D as a clinically proven, next generation fibrin sealant that should expand the current surgical armamentarium.

References

1. Borst HG, Haverich A. Fibrin sealant in cardiovascular surgery. Proceedings of an international workshop, Hannover, November 14, 1981. Thorac Cardiovasc Surg 1981;30:195-241.
2. Borst HG, Haverich A, Walterbusch G, Maatz W. Fibrin adhesive: an important hemostatic adjunct in cardiovascular operations. J Thorac Cardiovasc Surg 1982;84:548-53.
3. Haverich A, Walterbusch G, Borst HG. The use of fibrin glue for sealing vascular prostheses of high porosity. Thorac Cardiovasc Surg 1981;29:252-4.
4. Koveker G. Clinical application of fibrin glue in cardiovascular surgery. Thorac Cardiovasc Surg 1982;30:228-9.
5. Salmenpera MT, Levy JH, Harker LA. Hemostasis and cardiopulmonary bypass. In: Mora C editor. Cardiopulmonary bypass: principles and techniques of extracorporeal circulation. New York: Springer-Verlag; 1995. p. 88-113.
6. Eisses MJ, Seidel K, Aldea GS, Chandler WL. Reducing hemostatic activation during cardiopulmonary bypass: a combined approach. Anesth Analg 2004;98:1208-16.
7. Eisses MJ, Velan T, Aldea GS, Chandler WL. Strategies to reduce

hemostatic activation during cardiopulmonary bypass. Thromb Res 2006;117:689-703.
8. Koster A, Huebler S, Merkle F, Hentschel T, Grundel M, Krabatsch T *et al.* Heparin-level-based anticoagulation management during cardiopulmonary bypass: a pilot investigation on the effects of a half-dose aprotinin protocol on postoperative blood loss and hemostatic activation and inflammatory response. Anesth Analg 2004;98:285-90.
9. McCarthy PM. Fibrin glue in cardiothoracic surgery. Transfus Med Rev 1993;7:173-9.
10. Salzman EW, Weinstein MJ, Weintraub RM, Ware JA, Thurer RL, Robertson L *et al.* Treatment with desmopressin acetate to reduce blood loss after cardiac surgery. A double-blind randomized trial. N Engl J Med 1986;314:1402-6.
11. Kieser TM, Maitland A, Parsons L, Davison L, Woodman RC. Reduced postoperative bleeding following use of TISSEEL fibrin sealant in 300 patients undergoing open heart surgery. In: Cardiovascular Surgery Thoracic Surgery. Springer-Verlag (Berlin); 1994. p. 43-54.
12. Spotnitz WD, Prabhu R. Fibrin sealant tissue adhesive—review and update. J Long Term Eff Med Implants 2005;15:245-70.
13. Spotnitz WD, Welker RL. Clinical uses of fibrin sealant. In: Mintz PD editor. Transfusion therapy: clinical principles and practice. Bethesda: American Association of Blood Banks Press; 1999. p.199-222.
14. Spotnitz WD. As originally published in 1990: four years' experience with fibrin sealant in thoracic and cardiovascular surgery. Updated in 1998. Ann Thorac Surg 1998;65:592-3.
15. Mintz PD, Mayers L, Avery N, Flanagan HL, Burks SG, Spotnitz WD. Fibrin sealant: clinical use and the development of the University of Virginia Tissue Adhesive Center. Ann Clin Lab Sci 2001;31:108-18.
16. Rousou J, Levitsky S, Gonzalez-Lavin L, Cosgrove D, Magilligan D, Weldon C *et al.* Randomized clinical trial of fibrin sealant in patients undergoing resternotomy or reoperation after cardiac operations. A multicenter study. J Thorac Cardiovasc Surg 1989;97:194-203.
17. Koveker G, de Vivie ER, Hellberg KD. Clinical experience with fibrin glue in cardiac surgery. Thorac Cardiovasc Surg 1981;29:287-9.
18. Barrett PN, Meyer H, Wachtel I, Eibl J, Dorner F. Determination of the inactivation kinetics of hepatitis A virus in human plasma products using a simple TCID50 assay. J Med Virol 1996;49:1-6.
19. Eriksson B, Westman L, Jernberg M. Virus validation of plasma-derived products produced by Pharmacia, with particular reference to immunoglobulins. Blood Coagul Fibrinolysis 1994;5 Suppl 3:S37-44.
20. Horowitz B, Bonomo R, Prince AM, Chin SN, Brotman B, Shulman RW. Solvent/detergent-treated plasma: a virus-inactivated substitute for fresh frozen plasma. Blood 1992;79:826-31.
21. Horowitz B, Prince AM, Hamman J, Watklevicz C. Viral safety of solvent/detergent-treated blood products. Blood Coagul Fibrinolysis 1994;5 Suppl 3:S21-8.
22. Roberts P, Sims G. Use of vegetable-derived tween 80 for virus inactivation by solvent/detergent treatment. Biologicals 1999;27:263-4.
23. Kreil TR, Berting A, Kistner O, Kindermann J. West Nile virus and the safety of plasma derivatives: verification of high safety margins, and the validity of predictions based on model virus data. Transfusion 2003;43:1023-8.
24. Levy JH, Tanaka KA, Steiner ME. Evaluation and management of bleeding during cardiac surgery. Curr Hematol Rep 2005;4:368-72.
25. Parolari A, Colli S, Mussoni L, Eligini S, Naliato M, Wang X *et al.* Coagulation and fibrinolytic markers in a two-month follow-up of coronary bypass surgery. J Thorac Cardiovasc Surg 2003;125:336-43.
26. Mannucci L, Gerometta PS, Mussoni L, Antona C, Parolari A, Salvi L *et al.* One month follow-up of haemostatic variables in patients undergoing aortocoronary bypass surgery. Effect of aprotinin. Thromb Haemost 1995;73:356-61.
27. Pfluger H. Lysis and absorption of fibrin sealant (Tissuccol/Tisseel). In: Schlag G, Redl H editors. Vol. 1: Fibrin sealant in operative medicine. Springer-Verlag (Berlin); 1986.p.39-50.
28. Adant JP, Detroz B, D'Silva M, Natowitz L, Ledoux M, Pestiaux B *et al.* Skin grafting with fibrin glue in burns. Eur J Plast Surg 1993;16:292-7.
29. Koltai JL, Gerhard A. Intraperitoneal application of fibrinogen gluing in the rat for adhesions prophylaxis. Prog Pediatr Surg 1990;25:71-80.