The Effectiveness of a Fibrinogen-Thrombin-Collagen-based Hemostatic Agent in an Experimental Arterial Bleeding Model

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The hemostyptic agent used in this study is a recently developed material that consists of a collagen fleece containing fibrinogen, thrombin, and aprotinin integrated into its surface (FTCH) with excellent topical hemostyptic properties. The potential use of this substance for cardiovascular surgery was evaluated in a canine arterial bleeding model, which allowed comparison of the new agent with previously used pure collagen (CHF) as well as study of the hemostyptic under elevated blood pressure conditions. The results revealed that FTCH induced reliable hemostasis in 10-mm injuries of the canine hypogastric artery up to a systolic blood pressure of 260 mmHg, whereas bleeding control by CHF alone was impossible. To assess the long-term reliability of FTCH, the dogs were re-explored at intervals of 14 and 31 days after operation. At relaparotomy, the arteries were patent and there was no evidence of recurrent bleeding, thrombosis formation, or aneurysmatic changes. Histologic examinations showed well-healed vascular lesions covered by cell-depleted collagen tissue and a partially resorped hemostyptic. FTCH will not replace adequate surgical techniques but could be useful as a quickly available and easily applicable hemostatic means in otherwise uncontrollable diffuse or acute bleeding in cardiovascular surgery.

COMMONLY USED HEMOSTATIC agents in surgery arrest bleeding either by artificial clot formation or by producing a mechanical matrix that facilitates clotting when applied directly to denuded or bleeding surfaces. These agents include absorbable gelatin sponge, oxidized cellulose, and thrombin. They are applied to control oozing from minute vessels and will not effectively combat bleeding from parenchymal injuries or major arteries and veins.[1-3] This and possible complications caused by implanted foreign materials has spurred the development of resorbable topical hemostatic agents that directly enhance the normal coagulation process.

Purified collagen as a hemostatic agent was introduced into surgery in 1969. Many reports[3-9] that describe its physical, biological, and medical aspects show that the hemostatic efficacy of collagen surpasses that of any other previously used agent.

Neither collagen powder nor collagen fleece, however, resulted in satisfactory hemostatis when applied to hemorrhaging parenchymal injuries or major bleeding in cardiovascular surgery. Despite promising experimental results[3,4,8] they did not become routine hemostatic adjuncts in these situations because of their lack of adhesion to severely bleeding surfaces and their insufficient hemostatic potency.

An important improvement in the field of local bleeding control was reported by Spängler et al. in 1975.[10] They used highly concentrated human fibrinogen in combination with bovine thrombin to achieve hemostasis and a sufficient gluing effect on various parenchymal injuries. The additional use of a collagen fleece as a carrier for this fibrin tissue adhesive (FS) seemed to improve its hemostatic efficacy.[11-13] FS and heterologous collagen has been used clinically in Europe for over 6 years and is a highly effective and reliable means of achieving hemostasis in cardiovascular, urological, thoracic, and general surgery.[14-19] Its application, however, is expensive, complicated, and time consuming. Preparation and preheating of its components take at least 15 minutes, and a specially made double-barrelled syringe is necessary, permitting a simultaneous mixture and application of FS, either directly to the oozing side or, more commonly and more effectively, to the collagen fleece. The FS–collagen combination is then applied to the bleeding surface.

The answer to this complicated application procedure was the development of a hemostatic FS–collagen-based agent in a form that had the components of FS: fibrinogen,

thrombin, and aprotinin, integrated into the surface of the collagen fleece. Only recently have biochemical manipulations been proven successful in producing large sheets of this fibrinogen-thrombin-collagen-hemostat (FTCH) (HORMON-CHEMIE, Munich, West Germany).

Collagen powder and collagen as a fleece (CHF) have long been used as a topical hemostatic agent around arterial anastomoses.[3,4] Subjectively, FTCH seemed superior to other topical means used to secure hemostasis. Therefore, the following experiments were done to assess the usefulness and reliability of FTCH in cardiovascular surgery by seeking the answers to the following key questions: (1) could the hemostatic effectiveness of FTCH be documented objectively? (2) could its superiority to CHF be demonstrated? (3) is the effectiveness retained in the presence of high systemic blood pressure? and (4) what morphologic and histologic changes are induced by FTCH at various intervals after surgery?

Materials and Methods

Materials

FTCH (9.5 × 5-cm sponges) consists of equine collagen and contains 2.5–3.5 mg/cm^2 lyophilized human fibrinogen, 0.5–1.0 NIH U/cm^2 of bovine thrombin (1 U.S. NIH unit is comparable to 1 International Unit of the First International Standard), and 1.6 μg/cm^2 of the antifibrinolytic agent aprotinin. CHF*, which was used as a control, represents the same material without clotting factors and aprotinin.

Animal Experiments

Nine adult mongrel dogs, weighing 21–31 kg were used for the study. A morphine-phenothiazine-isoflurane combination was applied as anesthesia, and after midline laparotomy the retroperitoneum was opened and both external iliac arteries were mobilized and exposed. The right or left artery was clamped distally and proximally and opened with a 10-mm longitudinal incision. The defect was wrapped carefully with a 2-cm broad patch of FTCH in a circular fashion until the defect was covered with a double layer of FTCH, showing a 5-mm bilateral overlap.

First the distal and then the proximal occluding clamp were removed after 5 minutes. The stability of the adhesive cover was tested by a pharmacologically induced increase in blood pressure achieved by means of a perfusion system administering 0.36 mg/min of adrenalin and 0.2 mg/kg of atropin. The initial arterial systolic pressure was mea-

sured by a catheter lodged in the femoral artery. Pressure was recorded automatically.

After the application and testing of FTCH, a 1-cm incision in the contralateral external iliac artery was covered with CHF, using the same technique as described above for FTCH. This served as a control. The stability of this cover was also tested by subsequently increasing the blood pressure. If no bleeding occurred after 2 hours, the abdomen was closed.

Trial Groups

The nine mongrel dogs were divided at random into two groups: the animals in group 1 (N = 6) were re-explored after 14 days, and those in group 2 (N = 3) were re-explored after 30 days.

Postoperative Surveillance

Red blood cell count, hemoglobin concentration, and packed cell volume were determined at 2-day intervals. Daily abdominal palpation and control of the femoral artery pulse were done to ensure clinical exclusion of bleeding or vessel obliteration.

Histologic and Microbiologic Examination

During re-exploration after 14 and 30 days, respectively, specimens of the covered areas were taken in all cases for gross and histologic examination. Microbiologic smears were taken in all cases under sterile conditions to exclude microbial contamination.

Results

Arteriotomy Repair

After application of FTCH and removal of the occluding clamps at a systolic pressure of 100 ± 8 mmHg, hemostasis was achieved in all animals in groups 1 and 2 (the vessels were primarily patent). After an increase in systolic blood pressure to 200 ± 13 mmHg, there were no signs of bleeding. In one animal from group 1 the hemostatic separated at a peak blood pressure of 280 mmHg. At a pressure of 260 mmHg, hemmorrhages were scattered diffusely over the entire surface of the collagen fleece. The arterial incision was covered again after the blood pressure in this animal had been lowered. The stability of the cover was tested again at an arterial systolic pressure of 215 mmHg.

All experimental vascular injuries, which were covered with CHF, had to be repaired with a primary vessel suture. In five dogs in group 1 and in all dogs in group 2, CHF separated immediately after the vessel clamps had been opened at systolic pressure of 100 mmHg, and in one dog in group 1 at 170 mmHg (Table 1).

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* Introduced to the market under the name Tachotop® by HORMON-CHEMIE, Munich, West Germany.

TABLE 1. *Hemostatic Effectiveness of CHF versus FTCH in the Presence of High Systemic Blood Pressures*

	Maximum Systolic Blood Pressure (mmHg)	
	CHF-treated	FTCH-treated
Group 1		
1	<100*	205
2	<100*	215
3	<100*	190
4	<100*	175
5	<100*	280*
6	170*	200
Group 2		
7	<100*	210
8	<100*	190
9	<100*	200

* Separation of the hemostyptic and strong hemorrhage.

Postoperative Controls

The femoral arterial pulse could be palpated in all cases distally to the repaired vascular injury. There was no evidence of postoperative bleeding on abdominal palpation. Red blood cell count, hemoglobin concentration, and packed cell volume in both groups also provided no evidence of hemorrhage.

Gross Findings

At re-exploration, there was no gross evidence of hematomas or recurrent bleeding. Gross tissue reaction was minimal and limited to perivascular adhesions strictly localized to the area covered with the hemostatic. No intra-abdominal abscesses or signs of peritonitis could be found. The arteries remained patent and were not obliterated or narrowed by thrombosis formation. In all cases there was evidence of the arteriotomy in form of a groove-like retraction of the intima. No aneurysmal changes could be found.

Histologic Appearance

There was no histologic evidence of recurrent bleeding, thrombosis formation in the vessel lumen, or aneurysm formation.

All specimens that were taken after an interval of 14 days (group 1) showed an unspecific, fibrovascular granulation tissue containing fibroblasts, neutrophils, lymphocytes, and a small number of giant cells typical of foreign-body reaction. There were microscopic remnants of the hemostatic visible as rough, fibrous, collagen-like material.

Studies after 4 weeks (group 2) showed progressive resorption of FTCH. The amount of hemostatic still visible was greatly reduced and partially replaced by a cell-de-

pleted collagen tissue. The granulation tissue appeared to be of a more mature type containing fewer leucocytes, more fibroblasts, and no giant cells.

Neither group showed any remnants of the fibrinogen contained in FTCH. There was no histologic or microbiologic evidence of infection.

Discussion

In this canine arterial bleeding model, FTCH proved to be a highly successful topical hemostyptic agent in the arrest of major arterial hemorrhage. It proved superior in this setting over a widely used commercially available agent CHF. Due to the severity of the bleeding model it may be assumed that other hemostatic materials such as oxidized cellulose cloth or gelatine sponge will be even less efficacious than CHF.

The superior hemostatic ability of FTCH may be attributable to the compound's mechanism of action: when the hemostatic is brought into close contact with a wet or bleeding surface, thrombin located on the collagen fleece splits the fibrin-peptides A and B from fibrinogen molecules on the surface of FTCH, converting it to monomeric fibrinogen, which undergoes spontaneous polymerization to fibrin strands connected by noncovalent bonds. Under the influence of clotting factor XIIIa, peptide bonds between adjacent fibrin-polymers are formed, reinforcing a network of fibrin strands that are responsible for the high adhering properties of FTCH to bleeding surfaces.[20] The collagen fleece mainly acts by inducing aggregation and release reaction of platelets, stimulating clotting factor XII[1,4,7,9] (Fig. 1) and prevents lift-off of the clotting factors integrated into its structures.

It is from its physical form that FTCH derives its major advantage over the combined use of FS and collagen fleece: easier handling characteristics and probably lower costs. On the other hand, positive aspects of the FS–collagen compound system, as described by others,[10,12,13,17] are retained: high hemostyptic potency and absolute tissue compatibility despite the use of heterologous materials such as bovine thrombin or equine collagen. Tissue necrosis or extensive fibrosis did not occur, and immune reactions are not induced by both systems.[5,11,21]

A possible disadvantage of using fibrinogen made from human plasma in a hemostyptic preparation is that the transfer of hepatitis of HTLV-III virus cannot be ruled out completely. Plasma donors are carefully selected and tested for the absence of hepatitis B surface (HBs) antigenemia, HTLV III antibody, and for normal glutamate pyruvate transaminase (GPT) levels. Donors with HBs antigenemia, HTLV III antibodies, or elevated GPT levels are excluded from the plasmapheresis program.

The fibrinogen used in the FTCH is heat-treated in aqueous solution at a temperature of 60 C over 20 hours as a virus-inactivating measure. Additional safety is gained
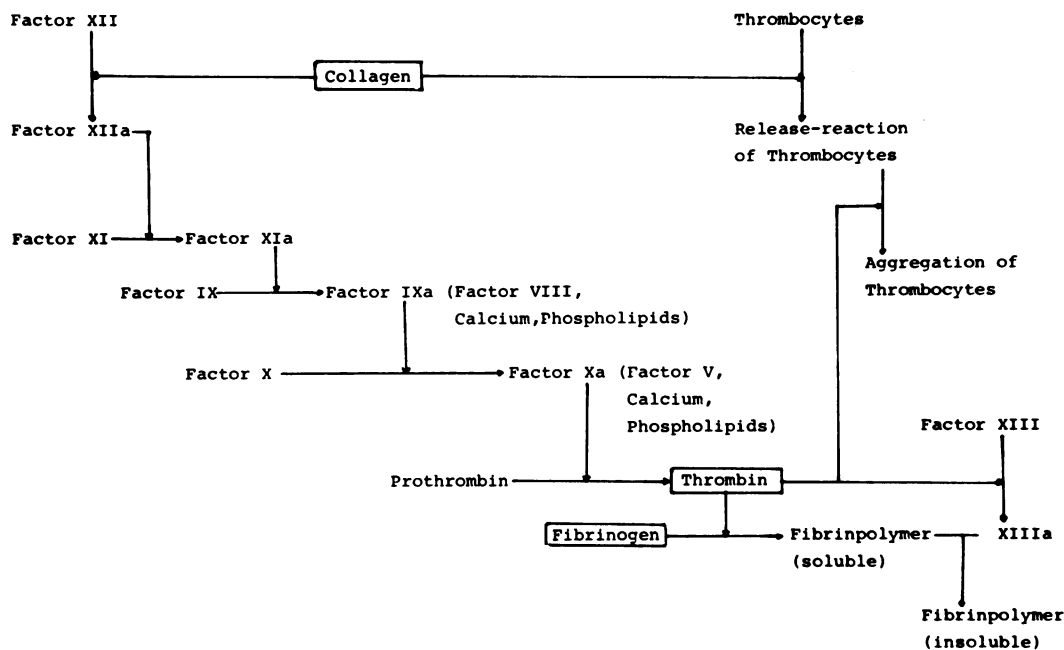
FIG. 1. Possible mechanisms of action of FTCH during the normal coagulation process.

by gamma ray sterilization of the whole compound. Although the risk of hepatitis or AIDS transmission is possible, it is probably minimal. Fibrinogen as a hemostyptic agent has been used clinically in Europe for over 6 years and has been applied more than 500,000 times without a single case of hepatitis or AIDS transmission.

We do not believe that FTCH will replace adequate surgical techniques but it will probably find its place as a valuable, quickly available, and easily applicable hemostatic means in selected cases of otherwise uncontrollable diffuse or acute bleeding in vascular surgery. It is currently undergoing extensive clinical testing and shows increasingly promising results.

References

1. Hausmann P, Mergard U, Koehnlein H. Zur Wirkung verschiedener Haemostyptika. Fortschr Med 1974; 92:579–583.
2. Koehnlein HE, Mergard U. Experimentelle Testung lokaler Hämostyptika. Chirurg 1972; 43:378–387.
3. Silverstein E, Keown K, Chvapil M. Collagen fibers as a fleece hemostatic agent. J Trauma 1980; 20:688–694.
4. Abbot M, Austen G. The effectiveness and mechanism of collagen-induced hemostasis. Surgery 1975; 78:723–729.
5. Gay S. The immunology of collagen. Monogr Pathol 1983; 24:120–125.
6. Hait M, Battista A, Stark L. Microcristalline collagen as a biologic dressing, vascular prosthesis and hemostatic agent. Surg Forum 1969; 20:51–57.
7. Katthagen B, Hellstern P. In vitro studies on the effect of dissolved and undissolved heterologous collagen sponge on human thrombocytes. Z Orthop 1984; 122:677–682.
8. Morgenstern L. Microcrystalline collagen used in experimental splenic injuries. Arch Surg 1974; 109:44–47.
9. Zucker MB, Borelli I. Platelet clumping by connective-tissue suspension and by collagen. Proc Soc Exp Biol Med 1962; 109:779–784.
10. Spängler H, Holle I, Moritz E, et al. Experimentelle Untersuchungen und erste klinische Erfahrungen über die lokale Blutstillung mittels hochkonzentriertem Fibrin. Tagungsbericht der Österr Gesellschatt f Chirurgie 1975; 4:605–608.
11. Braun F, Holle W, Knapp W, et al. Immunologische und histologische Untersuchungen bei der Gewebeklebung mit heterologen, hochkonzentriertem Fibrinogen. Wien Klin Wochenschr 1975; 87:815–819.
12. Jakob H, Campbell C, Stemberger A, et al. Combined application of heterologous collagen and fibrin sealant for liver injuries. J Surg Res 1984; 36:571–576.
13. Spängler H, Braun F, Holle I, et al. Die lokale Anwendung von Fibrinogen und Kollagen zur Blutstillung in der Herzchirurgie. Wien Med Wochenschr 1976; 7:86–89.
14. Borst HG, Haverich A, Walterbusch G, Maatz W. Fibrin adhesive: an important hemostatic adjunct in cardiovascular operations. J Thorac Cardiovasc Surg 1982; 84:548.
15. Haverich A, Walterbusch G, Borst HG. The use of fibrin glue for sealing vascular prostheses of high porosity. Thorac Cardiovasc Surg 1981; 29:252–256.
16. Koeveker G. Clinical application of fibrin glue in cardiovascular surgery. Thorac Cardiovasc Surg 1982; 30:228–229.
17. Rousou JA, Engelmann RM, Bryer RH. Fibrin glue: an effective hemostatic agent for nonsuturable intraoperative bleeding. Ann Thorac Surg 1984; 38:409–410.
18. Scheele J. Clinical experiences with fibrin adhesion in parenchymatous organs in the epigastric region. Akt Chir 1982; 17:153–161.
19. Scheele J, Gentsch HH, Matteson E. Splenic repair by fibrin tissue adhesive and collagen fleece. Surgery 1984; 95:6–13.
20. Stemberger A, Bluemel G. Fibrinogen-fibrin conversion and inhibition of fibrinolysis. Thorac Cardiovasc Surg 1982; 30:209–214.
21. Heine W, Braun A, Edinger D. Gewebliche Abwehrreaktionen auf das Fibrinklebesystem. Fibrinkleber in Orthopädie und Traumatologie, 4. Heidelberger Orthopädie-Symposium. New York: Georg Thieme Verlag Stuttgart, 1982.