Novel Infectious Agent-Free Hemostatic Material (TDM-621) in Cardiovascular Surgery

Hiroshi Masuhara, MD,1 Takeshiro Fujii, MD,2 Yoshinori Watanabe, MD,2 Nobuya Koyama, MD,2 and Keiichi Tokuhiro, MD1

Subjects: Currently, hemostatic materials made from human blood components and animal-derived collagen is used for controlling operative hemorrhage in the cardiovascular surgery field. In this study, we focused on an entirely synthetic self-assembling peptide (development code: TDM-621) that gels when in contact with blood or other bodily fluids and stops bleeding upon contact with a wound site. We investigated its usefulness as a hemostatic material in animal and clinical studies.

Methods: Before we began the clinical study, we demonstrated the hemostasis efficacy and safety of TDM-621 in animal experimental models. Twenty-five patients (22 men, 3 women) were enrolled in the clinical study, and the following procedures were performed: 1) coronary artery bypass graft (CABG) (n = 9), 2) abdominal aortic graft replacement (n = 4), and 3) peripheral artery bypass (n = 12). The TDM-621 material was applied to a total of 33 vascular anastomotic graft sites (some patients received material at more than one site). Both hemostatic efficacy and safety were examined.

Results: A total of 33 anastomotic graft sites in 25 patients were evaluated, and the averaged primary and secondary efficacy rate was 94.5%. No postoperative bleeding or adverse events (including serious adverse events) with a causal relationship to treatment were observed.

Conclusion: This study indicated that TDM-621 is a more effective and reliable hemostat than commonly-used general hemostatic agents and, therefore, will be very useful in several cardiovascular surgery applications.

Keywords: hemostatic materials, synthetic self-assembling peptide, collagen-like fibrous network

Introduction

Hemostatic materials made from animal-derived collagen and human blood components are commonly administered locally to bleeding sites during surgery. These collagen and blood component products are primarily composed of tissue-building proteins and possess excellent biocompatibility. However, use of these products poses serious clinical issues in that human blood component-derived substances may contain infectious viruses and prions, while animal-derived substances may trigger anaphylactic shock in response to foreign proteins or cause infection by diseases such as bovine spongiform encephalopathy (BSE). To eliminate such risks, we have developed a novel hemostatic material known as TDM-621. This material leverages the gel-forming properties of...
an aqueous peptide solution that is widely used in basic research for the clinical development of cell cultures and regenerative medicine and that forms a colorless peptide hydrogel at neutral pH (Fig. 1). The component peptide in TDM-621 is four repeats of alternating hydrophilic natural amino acids (aspartic acid negatively charged, and arginine positively charged) and hydrophobic amino acids (alanine) (Fig. 2). The peptides form a gel with a collagen-like fibrous network under physiological conditions (i.e., pH around 7 in the presence of salts such as Na+ and K+).

The hemostatic action of TDM-621 is as follows. The acidic aqueous peptide solution is neutralized by contact with blood or other bodily fluids, causing the beta-structure of peptides in the aqueous solution to rapidly form fibers that create a hydrogel. This peptide hydrogel covers the bleeding point to physically occlude the vascular adventitia of the broken vessel and cause blood coagulation in the vascular intima, resulting in hemostasis. The efficacy of hemostasis using these properties was first demonstrated in an animal experiment by Ellis-Behnke, et al.1) We have undertaken the clinical development of TDM-621 as an absorbent topical hemostatic material, while focusing on its physicochemical properties and biocompatibility. To evaluate the safety of TDM-621, we designed a mouse intravascular administration model. After the animal experiments proved safety and efficacy, we also investigated the human clinical hemostatic efficacy of TDM-621 on blood oozing in cardiovascular surgery.

Animal Study

Subjects

We designed the present study based on the results of an efficacy validation study of an approved hemostatic material. The oozing needle hole hemorrhage model of prosthetic vascular graft implantation in a beagle dog was designed to mimic the oozing needle hole hemorrhage from anastomotic sites at the autologous vascular.

Materials and Methods

All animals have received humane care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Research (ILAR), published by the National Academies Press (1996 version).

A Rabbit abdominal aortic puncture bleeding model

Laparotomy was performed to expose approximately 10 cm of the abdominal aorta of each rabbit. Heparin sodium 500 IU was administered intravenously; then, the bleeding model was created by puncturing the abdominal...
aorta using an injection needle (23–26G). Following confirmation of bleeding, peripheral and central blood flow was stopped with hemostatic clamps and TDM-621 was immediately applied to the wound site. Blood flow was allowed to resume after 1–2 min, and the puncture site was visually inspected for bleeding. Rabbit abdominal aorta used in the present study was fixed in formalin, and vascular cross sections of both TDM-621-treated and untreated sites (control) were used to make pathology specimens that were then observed under a microscope.

**Beagle abdominal aortic graft replacement model**

Two male beagles with body weights of 13.1 kg and 11.4 kg, respectively, were used for this model. The abdominal aorta, exposed via laparotomy and performed under general anesthesia and heparin sodium, was intravenously infused at 1000 IU. After confirmation that the active coagulation time (ACT) had exceeded 200s, the abdominal aorta was clamped, and an end-to-end graft replacement procedure was performed. Exudative hemorrhage (blood oozing) from the graft anastomosis and needle hole were observed. TDM-621 was applied to the needle hole in order to evaluate the hemostatic efficacy.

**Mouse intravenous administration model**

TDM-621 forms a gel as soon as it comes in contact with blood from a bleeding point, the possibility of gel entering the blood vessel cannot be ruled out. There is also an undeniable risk of gelatinized TDM-621 entering the blood stream as a result of erroneous intravascular administration. To evaluate these risks, we carried out tests on mice and rabbits simulating accidental IV administration of TDM-621.

**Results**

In the anticoagulant-administered rabbit abdominal aorta puncture bleeding model, total hemostasis was observed in all animals following treatment with a ≥2% peptide concentration of TDM-621, except in 1 animal treated with a 2% peptide concentration. The structureless and eosinophilic gelatinized TDM-621 was observed at the vessel puncture site and surface, and was found to have formed a coating on the tissue surface that physically occluded the puncture (Fig. 3).

In a beagle abdominal aorta graft replacement model, oozing bleeding from anastomosis site was stopped, and about 1 min after applying approximately 2 mL of 2.5% TDM-621, hemostasis of the anastomotic oozing was confirmed, and the needle-hole oozing was also arrested. The abdominal aorta puncture bleeding model was prepared by piercing the artificial vascular graft with the same-sized 26-G injection needle used on the rabbits, and blood spurting was consequently observed. Peripheral and central blood flow was stopped, and approximately 1 mL of 2.5% TDM-621 was applied. Blood flow was allowed to resume after about 1-min hemostasis was confirmed at the wound site. This procedure was repeated 3 times at 3 different sites on the graft. Postoperative observation was done until post 3 days.

In the intravenous administration model, in mice, after
injecting the gelatinized TDM-621 in suspension, at doses deemed likely to have adverse biological effects, mouse deaths were observed up to a 40-fold dilution. While causes were not determined, we suspect death was likely attributable to pulmonary embolism. The precise cause of death is unclear, since an autopsy was not done. However, the following abnormal behaviors indicate pulmonary infarction: reduction of spontaneous behavior, squatting position, and accelerated respiration. Additional experiments to confirm the cause of death have been planned. Moreover, no deaths were observed at the 80-fold dilution, although abnormal findings (inactivity and tachypnea) were seen. At the 160-fold dilution, no abnormalities were observed. We have also previously administered 0.2 mL of TDM-621 in a 160-fold diluted suspension to guinea pigs and did not observe any abnormal findings in any of the animals. Extrapolating these results to humans, if we assume that the subject is an adult with a body weight (BW) of 60 kg and a total blood volume (TBV) of 4.6 L, based on the test mouse BW of approximately 40 g and TBV of approximately 3 mL, the amount of TDM-621 gel that would have to enter the blood stream, in order to cause death, similar to that in the mouse experiment, would be a 4- to 40-fold dilution of approximately 770 mL administered intravenously, or approximately 19.3-193 mL of TDM-621, a much larger volume than what would have been administered during surgery. Taken together, these studies confirm the efficacy and safety of TDM-621 as a hemostat in several critical animal models.

Clinical Study

Subjects

We conducted the clinical study for the purpose of evaluating the efficacy and safety of TDM-621 as a hemostat in human cardiovascular surgery.

Patients and Methods

Study protocols were approved by the Institutional Review Board of Toho University Medical Center Sakura Hospital and Omori Hospital. We obtained informed consent from all patients. The study targeted 33 application sites in 25 patients (22 men, 3 women) who satisfied the above criteria and underwent CABG or vascular surgery for abdominal aortic aneurysm (AAA) or arteriosclerosis obliterans (ASO) between January, 2010 and April, 2011.

Exclusion criteria were the following

1) Individuals with a past medical history of hypersensitivity to peptide drugs or protein preparations
2) Individuals with serious complications other than diseases indicated for surgery that may hinder the study procedures
3) Individuals who were unable to discontinue drugs that may affect the evaluation of the efficacy or safety of TDM-621, such as blood-clotting drugs (blood coagulation accelerators; i.e., hemocoagulase) and antifibrinolytic agents (drugs with antifibrinolytic action; epsilon aminocaproic acid, tranexamic acid, aprotinin preparations etc.)
4) Individuals with Child's classification of B or C
5) Other individuals deemed unsuitable for the study by the investigator (or sub-investigator)

Procedures

All procedures were performed while the patient was under general anesthesia. CABG was performed without cardiopulmonary bypass. Heparin sodium was administered at 200 IU/kg during the procedure, and protamine sulfate, after the procedure for achieving the target ACT of 200 s. Prosthetic vascular graft replacement surgery to treat AAA was performed with a woven Dacron graft (J-graft; Japan Lifeline, Tokyo, Japan). Graft bypass surgery or autologous vein patch plasty to treat ASO was performed with an ePTFE ringed Gore-Tex vascular graft (WL Gore & Associates; Flagstaff, AZ, USA) and saphenous vein, respectively. Heparin sodium was administered at 5000 IU during procedures, but protamine sulfate was essentially not used after the procedure.

Target hemostasis sites

Target sites were vessel-to-vessel anastomotic sites in CABG and the graft anastomotic site and autologous vein patch plasty site in procedures to treat AAA and ASO. Types of bleeding targeted for hemostasis were 1) blood oozing that typically would be arrested with fibrin and collagen hemostatic materials, and 2) blood oozing during typical hemostatic treatment such as ligation, clips, and coagulation that were ineffective or could not be performed. In the case of copious blood spurting or gushing, hemostatic treatments were generally performed with ligation, clips, or coagulation; these types of bleeding were not subject to hemostasis treatment using TDM-621.

Method of application

After the removal of as much anastomotic blood as
possible with gauze, TDM-621 was evenly applied and smeared into each of the target sites before the administration of protamine sulfate. Specifically, approximately 1 mL of TDM-621 was applied to coronary anastomotic sites, approximately 2 mL was applied to aortic anastomotic sites, and approximately 1 mL was applied to other peripheral vascular anastomotic sites.

**Evaluation**

The primary endpoint of TDM-621 treatment was intraoperative hemostatic efficacy, determined as follows. Complete, partial, minor, and no response efficacy levels were defined as follows: complete response (CR), total hemostasis of the target site observed; partial response (PR), temporary total hemostasis confirmed, but permanent total hemostasis only observed after reapplying TDM-621 to application sites due to intraoperative secondary bleeding requiring treatment; minor response (MR), temporary total hemostasis confirmed, but permanent total hemostasis only observed after using a procedure other than TDM-621 due to intraoperative secondary bleeding from application sites requiring treatment; and no response (NR), bleeding from target sites not reduced and hemostasis not achieved.

The secondary endpoint was post-operative after bleeding, determined as follows: CR, no after-bleeding observed during post-operative examination; PR, after-bleeding from TDM-621 application sites inferred from the post-operative examination; however, hemostasis was deemed possible without the need for reoperation; and NR, after-bleeding requiring reoperation observed during the post-operative examination and deemed to have originated from TDM-621 application sites.

**Adverse events (abnormal findings including adverse reactions)**

Adverse events that occurred during the study period were examined in terms of symptoms, severity, duration, treatment, course and outcome, and association with the study drug (as well as the rationale for determining any association) and recorded on the case report form.

**Results**

Subjects comprised 25 patients (23 men, 2 women) with an age range of 54–80 years. Of these patients, 9 underwent CABG, 4 underwent AAA surgery, and 12 underwent surgery for ASO. TDM-621 was used on 33 sites, specifically at areas of the internal thoracic artery-coronary artery anastomosis (n = 1), saphenous vein-coronary artery anastomosis (n = 4), ascending aorta-saphenous vein anastomosis (n = 4), graft anastomosis (n = 15), and autologous vein patch plasty (n = 9). Mean area of the application was 3.03 cm² (range, 0.25–10 cm²). Mean amount of TDM-621 applied was 1.5 mL (range, 0.5–3 mL). The efficacy rate was 87.9% for the primary endpoint (hemostatic efficacy) and 100% for the secondary endpoint (occurrence of post-operative after bleeding) (Table 1). For heparin treatment, the efficacy rate was 85.2% (23/27), and hemostasis time was 153.6 ± 38.7 s (mean ± S.E.). For the protamine treatment, the efficacy rate was 100% (6/6), and the hemostasis time was 195.0 ± 130.1 s (mean ± SE). No adverse events (including serious adverse events) having a causal relationship to the treatment equipment were observed.

**Discussion**

Hemostatic procedures during surgery affect patient progress. In cardiovascular surgery in particular, the anticoagulant heparin is used on virtually all patients so coagulation function is significantly reduced. Ensuring good hemostasis is, therefore, of paramount importance.

The present study targeted blood oozing, which is not suited to additional suturing and is typically arrested using fibrin and collagen hemostatic materials. Indicated sites were those where the bleeding was small and

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<th>Application site</th>
<th>n</th>
<th>Primary endpoint Number of efficacy site</th>
<th>Secondary endpoint Number of efficacy site</th>
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</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>29 (87.9%)</td>
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Usefulness of Hemostat (TDM-621) in Cardiovascular Surgery


relatively low intensity, where multiple minor bleeding sites not suited to additional suturing were present, or where bleeding was seen from graft suture needle holes. The usefulness of fibrin glue in the field of cardiovascular surgery has already been demonstrated.\textsuperscript{2,3}) However, use of fibrin glue poses serious clinical issues in that human blood component-derived substances may contain viruses and prions that are infectious, while animal-derived substances may trigger anaphylactic shock in response to foreign proteins or infection by diseases such as BSE. We confirmed the biological safety of TDM-621 after obtaining negative results in all tests. Furthermore, TDM-621 poses no risk of infection, since it is made entirely of chemically-synthesized peptides.

The group of self-assembling peptides to which TDM-621 belongs has been developed over the past decade as novel peptide biomaterials. Based on results of electron microscopy, the structure of self-assembling peptide hydrogel is understood to be a network of fibers with a diameter of around 10–20 nm and pores with a diameter of about 50–200 nm that resembles the mesh structure of natural collagen. The component peptide in TDM-621 is a sequence of arginine-alanine-aspartate-alanine (RADA) repeated 4 times and was discovered while searching for a self-assembling peptide. The peptides form a gel with a collagen-like fibrous network under physiological conditions (i.e., pH around 7 in the presence of salts such as Na\textsuperscript{+} and K\textsuperscript{+}) (\textbf{Fig. 4}).

These peptides possess an alternating structure of the hydrophobic amino acid alanine (A) and the hydrophilic amino acids arginine (R) and aspartate (D), in which the respective positive and negative charges determine the relative position of the adjoining molecules, and self-assembly is thought to be completed by hydrophobic bonding between neutral amino acid side chains and hydrogen bonding between peptide backbones. External chemical environments that promote the self-assembly of TDM-621 include a pH in the vicinity of the isoelectric point (around pH 7) and the presence of a low-concentration (about several millimoles) of univalent alkali metal ions (Na\textsuperscript{+}, K\textsuperscript{+}).

Cell culture experiments have demonstrated that TDM-621’s main constituent peptide (CH\textsubscript{3}CO-(Arg-Ala-Asp-Ala)\textsubscript{4}-NH\textsubscript{2}) does not exhibit bioactivity by acting on the signal transduction system of living organisms.\textsuperscript{4} A search of databases (namely the European Molecular Biology Laboratory (EMBL) and Kyoto University’s GenomeNet Database Resources) containing protein sequence motifs for all amino acid sequences in which the main constituent peptide can be generated by cleavage did not reveal any sequences indicating a high degree of homology with known motifs. Furthermore, once a gel is formed, these peptides resist degradation even when exposed to digestive enzymes such as trypsin, α-chymotrypsin, papain, protease K and pronase\textsuperscript{5}) and are, therefore, expected to be used during cell transplantation in the field of cell therapy.

An aqueous peptide solution with the same components as TDM-621 is currently being marketed around the world as a research reagent by Becton, Dickinson and Company (USA). This solution is used as a cell culture scaffold in basic research on regenerative medicine, and

\textbf{Fig. 4} TDM-621 peptide nanofiber and electron microscopy.
no reports to date have identified serious adverse effects in cultured cells or laboratory animals such as toxicity or carcinogenicity.6, 7) Unlike hemostatic agents such as oxidized cellulose or starch-based absorbent topical preparations that stem blood flow by the formation of clots,8,9) the hemostatic action of TDM-621 is realized by modification of physical properties upon a change in pH to seal off the bleeding point in the same manner as an approved collagen-based absorbent topical hemostatic agent and physiological tissue adhesive (fibrin glue).

TDM-621 exhibited good hemostatic efficacy in our preliminary animal experiments using a rabbit abdominal aorta puncture bleeding model and a beagle abdominal aorta graft replacement model. Although the present results cannot necessarily be directly extrapolated to humans, our evaluation of the risks of TDM-621 gel-induced intravascular embolism using the mice i.v. administration model indicates that a single syringe dose of TDM-621, 5 mL or less, is unlikely to cause the onset of pulmonary embolism or other adverse events resulting in death, even in the case of a mistaken administration, directly into a blood vessel.

TDM-621 is manufactured by preparing peptides consisting of chemically synthesized amino acids using solid-phase synthesis, dissolving the peptides in water for injection, filtering the solution with a bacterial filter (0.2 mm), and filling the resulting filtrate in a sterile manner into a syringe. As such, manufacture is completed without using any animal-derived materials, eliminating any risk of infection by biological materials.

TDM-621 is a clear, colorless liquid and its hemostatic effects can be visually confirmed during use. The drug is provided in a pre-filled syringe so, unlike fibrin glue, there is no need to prepare any liquids. As it is made from peptides, TDM-621 can be completely broken down and so does not remain in the body as a foreign material and potential source of infection. In short, TDM-621 possesses a number of benefits. Clinical trials of TDM-621 are yet to commence in Japan and overseas, so data on clinical performance are not currently available. Given the inherent difficulty of numerically expressing hemostatic efficacy, our clinical evaluation of TDM-621 was implemented based on a determination of ‘excellent’, ‘good’, or ‘unsatisfactory’, as suggested by Stark, et al.3) Previous studies on hemostatic materials approved in Japan have reported total hemostasis rates on oozing bleeding of 23.1%–100%,10–15) which we think is comparable to TDM-621’s clinical usefulness of 87.9%, despite the limited population size in our study. Assuming there is no difference between the treatment results, use of the infection-free hemostatic material that does not include animal-derived collagen or human blood components would be preferable. Biological viral infections such as iatrogenic hepatitis from infected blood products16) and iatrogenic Creutzfeldt-Jakob disease (CJD) from artificial dura mater or corneal transplant17,18) have occurred in Japan and have become social problems. In the case of products derived from biological sources, the presence of unknown viruses cannot be completely ruled out. TDM-621, on the other hand, is entirely synthetic and so appears to represent a useful, safe hemostatic material that poses absolutely no risk of viral infection.

**Conclusions**

We conducted animal experiments and a human clinical study to investigate the hemostatic efficacy and safety of TDM-621, a novel infectious and agent-free hemostatic material. In animal experiments, we confirmed the hemostatic effects in a rabbit abdominal aortic puncture bleeding model and a beagle AA graft replacement model. We also confirmed the safety of the intravenous administration of very low concentrations of TDM-621 in a mouse model. In the clinical study, TDM-621 was applied to 33 sites in 25 patients and exhibited an efficacy and safety rate of 87.9% (29/33), and no differences in the efficacy of TDM-621 in heparin- and protamine-treated individuals were observed. No postoperative bleeding or other adverse events (including serious adverse events) with a causal relationship to treatment were observed. Based on these findings, we consider TDM-621 to be a safe and useful hemostatic material that demonstrates excellent local hemostatic efficacy on oozing bleeding during cardiovascular surgery.

**Disclosure Statement**

For the purposes of the present study, we received samples of TDM-621 from 3-D Matrix, Ltd.

**References**


2) Borst HG, Haverich A, Walterbusch G, et al. Fibrin adhesive: an important hemostatic adjunct in cardio-