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Severe upper gastrointestinal bleeding is halted by endoscopically delivered self-propelling thrombin powder: A porcine pilot study

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ABSTRACT

Background and study aims Hemostatic powders have emerged recently to treat upper gastrointestinal bleeding (UGIB). Previously, we developed a novel self-propelling thrombin powder (SPTP) that effectively manages external pulsatile arterial bleed without compression, by effervescing and carrying thrombin into the wound. Here, we tested if SPTP, sprayed endoscopically, can manage severe UGIB in a live porcine model.

Materials and methods Anesthetized pigs underwent laparotomy to insert the gastroepiploic vascular bundles into the stomach lumen via a gastrotomy. Bleeding was initiated endoscopically in the stomach by needle knife. SPTP was delivered to the site of bleeding from a CO_2-powered spray device using a 7 FR catheter. Successful primary hemostasis, time to hemostasis, and the mass of SPTP delivered were measured.

Results Hemostasis was achieved at all bleeding sites using SPTP. Mean time to hemostasis was 4.2 ± 0.9 minutes (mean ± standard error of the mean, n = 12). The average mass of SPTP delivered was 2.4 ± 0.6 g.

Conclusions In this pilot study, SPTP successfully stopped 12 cases of severe UGIB, demonstrating early promise asa novel hemostatic powder.

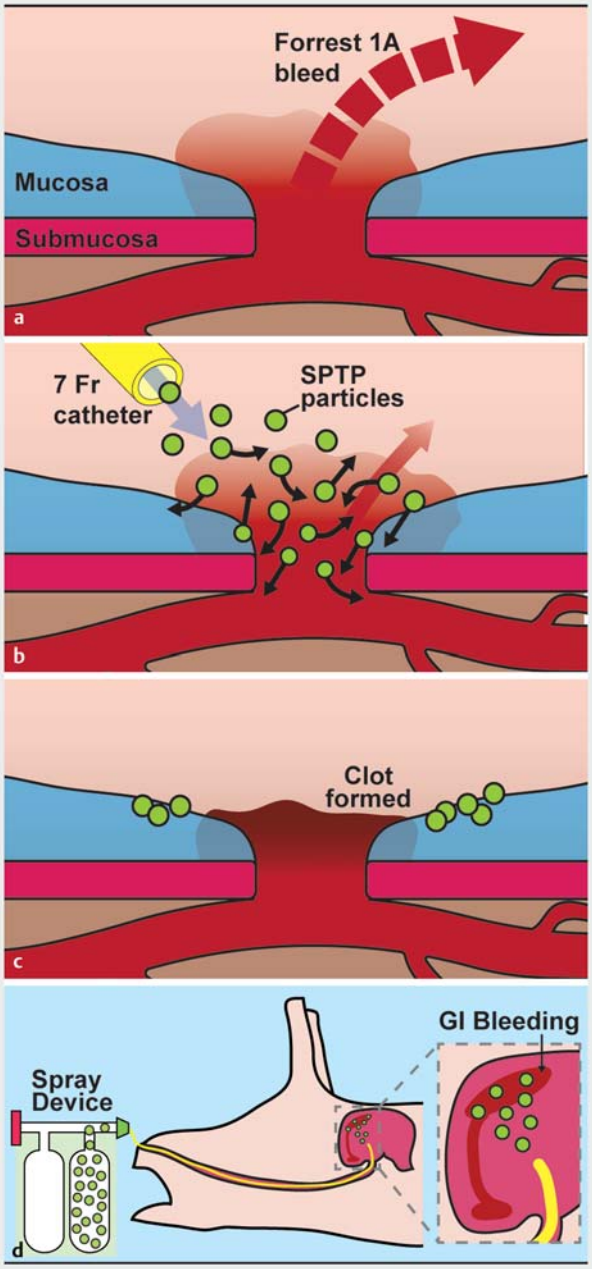
Introduction

Upper gastrointestinal bleeding (UGIB) affects up to 150 per 100,000 adults per year, with approximately 5 % to 30 % of cases leading to death [1]. While epinephrine injections and thermal and mechanical modalities have become the mainstay in managing GI bleeding, they are limited by the precision and skill of the operator and the accessibility of the surface of the bleed [2]. To address these limitations, clay and polysaccharide based hemostatic powders have been developed to rapidly stop active bleeds endoscopically [3, 4].

Current marketed powders do not reliably achieve lasting hemostasis in UGIB. Hemostatic powders based on clay or polysaccharides, often obscure the field of vision when applied [5, 6] and, do not deliver pharmacologically active hemostatic therapeutics to the bleed, such as hemostatic enzymes or antifibrinolytic drugs. Rather, they function by covering the wound and presenting a coagulant surface, in addition to absorbing water which concentrates blood cells on the wound surface to aid hemostasis [7–9]. In severe bleeds, powders can be washed away by the brisk outward flow of blood [5] or quickly become oversaturated before hemostasis is achieved. Rapid hemostasis requires a high concentration of procoagulants at the wound site; however, achieving effective contact between therapeutic agents and the damaged vessels is particularly difficult in severe UGIB or Forrest Class 1A bleeds because it is characterized by spurting arterial blood flow.

To address these limitations, we hypothesized that self-propelling thrombin powder (SPTP), an agent that is effective at halting hemorrhage from large arterial bleeds without compression [10], could be formulated to spray through an endoscope and halt severe UGIB. SPTP consists of porous calcium carbonate microparticles loaded with thrombin and mixed with an organic acid. Upon contact with blood, SPTP actively transports hemostatic agents throughout blood by effervescence, penetrating deep into wounds to halt hemorrhage (▶ **Fig. 1**). In previous studies, our group has shown that SPTP halted multiple lethal femoral artery and carotid hemorrhages in pigs and sheep, respectively [10–13].

Here, we conducted a single-arm, non-recovery study to evaluate the ability of SPTP to halt bleeds equivalent to Forrest Class 1A and IB bleeds in a live porcine model of UGIB.



▶ **Fig. 1** Schematic of the application of SPTP in UGIB. **a** Severe, pulsatile UGIB emerging through mucous and submucosal layers of gastrointestinal tract. **b** SPTP particles applied to the bleed though a catheter and those particles propelling thrombin deep into the bleed. **c** Hemostasis achieved and residual particles temporarily left around the bleed. **d** Schematic showing application of SPTP transesophageally by EGD in a porcine model of UGIB.

Materials and methods

SPTP preparation

SPTP for UGIB was prepared using previously described methods [11]. $CaCO_3$ microparticles (3 µm, American Elements, Los Angeles, California, United States) were loaded with human α-thrombin (Haematologic Technologies, Essex Junction, Vermont, United States) and excipients in 6.75-mL cold glycine-buffered solution. The suspension was frozen by liquid nitrogen and lyophilized at –40 °C and < 50 mTorr until dried. The dried $CaCO_3$/thrombin powder particles were adjusted to diameters < 100 µm and mixed mechanically with a proprietary organic acid ground to the same particle size. SPTP was prepared with thrombin concentrations ranging from 333 NIH units/g to 1000 NIH units/g. A total of 15 g of powder was loaded into the spray device and was available for each bleed. The actual dose of thrombin delivered, and the actual amount of powder used are reported in ▶ **Table 1**.

▶ **Table 1** Forrest classification, time to hemostasis, total mass of powder, and dose of thrombin delivered for each bleed.

Pig #	Bleed #	Forrest classification	Time to hemostasis (min)	Mass of powder delivered (g)	Dose of thrombin (NIH units)
1	1	1B	5.7	1.0	1000
	2[1]	1A	3.8	6.0	5990
2	3	1A	4.6	1.7	1650
	4	1A	2.4	1.2	1200
3	5	1A	2.0	1.4	450
	6	1B	1.0	1.5	490
4	7	1B	8.0	1.5	500
	8	1B	7.0	2.5	850
	9	1A	1.1	1.1	370
5	10	1A	11.0	6.7	2230
	11	1B	3.2	2.7	900
	12	1B	1.2	1.8	600

[1] This bleed is shown in ▶ **Fig. 2**.

Animal model and care

This study was approved by the University of British Columbia Animal Care Committee (Protocol #A18–0348) and performed according to the guidelines of the Canadian Council on Animal Care. The model partially replicated the laparotomy-based model by Giday et al. that creates severe Forrest 1A bleeds [3].

Female Yorkshire pigs (40 to 50 kg) received ketamine (20 to 30 mg/kg) and midazolam (0.1 to 1 mg/kg) by intramuscular (IM) injection. Animals were anesthetized by inhalation of 4 % isoflurane followed by intubation and mechanical ventilation for the duration of the procedure. Isoflurane anesthesia (1 % to 3 %) was maintained in combination with propofol (2 to 7 mg/kg/h) and midazolam (0.4 to 0.7 mg/kg/h) when required. Buprenorphine (0.01 to 0.05 mg/kg) was administered by IM injection for analgesia. Heart rate, electrocardiogram, blood pressure, peripheral capillary oxygen saturation, carbon dioxide, temperature, appearance of the skin mucous membrane, and jaw tone and reflexes all were monitored and maintained throughout anesthesia and procedures.

In five pigs, a sterile laparotomy was performed by a general surgeon. The gastroepiploic arteriovenous bundle was exposed from the stomach and pushed through a 1-cm gastrotomy, at two to three sites, into the inner lumen of the stomach. The gastrotomies were then closed in a standard fashion, leaving the vessels exposed in the greater curvature in the body of the stomach with the abdomen left open.

Upper endoscopy was performed post-laparotomy. The gastroepiploic vessels inserted into the stomach were incised with a needle knife endoscopically (MicroKnife XL; Boston Scientific, Marlborough, Massachusetts, United States) to initiate bleeding equivalent to a Forrest Class 1 arterial bleed. The specific bleed type was noted. SPTP was delivered through a 7 Fr catheter and a prototype spray device using compressed carbon dioxide. Powder was sprayed ad libitum onto the bleeding site until the bleeding slowed (▶ **Video 1**).

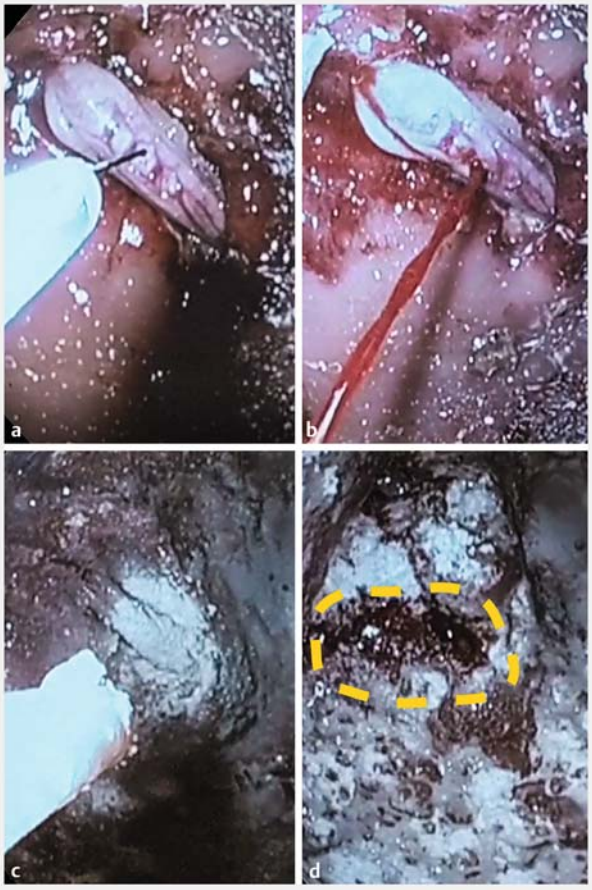
Hemostasis was measured as a stable, lack of visible outward flow of blood from the wound. Each bleed was observed for up to 10 minutes after initial hemostasis occurred. This procedure was repeated for each vessel inserted into the stomach lumen starting with the distal vessel to maintain the integrity of blood flow for upstream incisions.

The primary endpoint was successful hemostasis. Secondary endpoints included time to hemostasis, measured starting from the first application of SPTP, and total dose of propelled thrombin applied to achieve hemostasis. Total dose of thrombin applied was reverse calculated with knowledge of the powder's thrombin concentration, and the mass of powder applied. Animals in this study were not recovered and were euthanized immediately after observation that the final bleed had achieved hemostasis.

Results

Experiments were conducted on five pigs over five sessions (▶ **Table 1**). The procedures were imaged using a diagnostic video gastroscope (▶ **Fig. 2**). ▶ **Video 1** shows that SPTP does not produce a cloud of powder and the visual field remains clear. Hemostasis was achieved in all 12 bleeds in 4.2 ± 0.9 minutes (mean ± standard error of the mean) with 2.4 ± 0.6 g of powder applied (▶ **Table 2**). The average dose of thrombin delivered to these bleeds was 1350 ± 450 NIH units. This dose of thrombin is less than most topically-applied surgical thrombin products that are currently available.

Of the 12 bleeds, six were equivalent to high-pressure Forrest Class 1A bleeds, and hemostasis was achieved in 4.1 ± 1.5 minutes with 3.0 ± 1.1 g of powder. The remaining six bleeds were equivalent to Forrest Class 1B bleeds, which were stopped



▶ **Fig. 2** Representative endoscopy photos showing how bleeding was initiated, SPTP applied and bleeding stopped. **a** Identification of the gastroepiploic AV bundle in the stomach lumen and puncture with an endoscopic needle knife. **b** Initiation and classification of pulsatile Forrest Class 1A bleed. **c** Application of SPTP ad libitum via catheter, and significantly reduced bleeding 1 min post-application. **d** Robust hemostasis 3.8 min post-application, which persisted until sacrifice.

VIDEO

▶ **Video 1** Endoscopic application of SPTP to a spurting bleed listed as Bleed #2 followed by application of SPTP to a high flowrate arterial bleed listed as Bleed #9. Videos are sped up 1.5×.

in 4.3 ± 1.2 minutes with 1.8 ± 0.3 g of powder. The dose of thrombin applied was slightly more in the Forrest 1A bleeds, with 1990 ± 850 NIH units delivered compared to 720 ± 90 NIH units delivered in Forrest 1B bleeds.

Discussion

Hemostatic powders are an emerging technology in the management of UGIB. They are easier to use and quickly cover large surface areas. Powders do not require the technical expertise of more conventional methods, including injection, mechanical clips, and thermal devices. However, the currently available hemostatic powders have poor ability to manage severe UGIB or Forrest Class 1A bleeds [14–16], suggesting that their use should be limited to being a bridging therapy to more definitive treatment [17, 18]. Rebleeding from these hemostatic powders can occur in 27 % to 49 % of cases within 7 days, which necessitates reapplication and prolongs the treatment time [7, 15, 17, 19–21]. For example, in 296 cases of non-variceal UGIB, a 27 % rebleeding rate occurred, with the majority occurring within 3 days of endoscopy [19]. Of these cases, spurting bleeds were a common cause of the powder failing. In a separate study comparing EndoClot (EndoClot Plus Inc, Santa Clara, California, United States) and Hemospray as primary treatments, there was an overall rebleeding rate of 22 %, and there were no differences in rebleeding or hemostatic efficacy between them [20].

The pilot study here was an initial step to validation of a new technology that could be more effective than current sprayable powders, but easier to use than clips and thermal devices. We evaluated the short-term hemostatic efficacy of SPTP in a live porcine model of UGIB similar to that used in the development of Hemospray. The severity of the bleeds created in this model have been described as requiring urgent endoscopic treatment [22] and were expected to be fatal for the animals if left untreated. No direct comparisons were made to approved hemostatic powders, and this is a next step in the development of SPTP.

SPTP's mechanism of action (MOA) differs from available clay- and polysaccharide-based hemostatic powders. SPTP delivers therapeutics to the wound and dissolves rapidly, while other powders form a mechanical barrier to prevent outward flow that requires a high volume of powder. In a study assessing Hemospray in a similar porcine model, six bleeds (50 % Forrest 1A) required an average of 24.3 g of Hemospray (range 10 to 50 g) to stop bleeding; our study required 10 times less powder, which highlights that SPTP has a different MOA [23]. Similarly, at least 4 g of EndoClot was required to stop slowly oozing, ulcerated Forrest 1B bleeds, which is twice as much powder needed for SPTP to stop Forrest 1B bleeds reported here [4]. A large volume of powder also makes the bleed site difficult to visualize due to clouding [5] and caking, and must be irrigated to visualize the wound. Visualizing the wound after powder application is important for identifying risks of rebleeding.

SPTP also offers advantages over an endoscopically sprayed thrombin solution. By effervescing and increasing the transport of thrombin into the wound, SPTP increases thrombin efficacy [11]. SPTP also delivers Ca^{2+} to the wound to further enhance hemostasis. In addition, the gas-liquid interfaces of the bubbles

▶ **Table 2** Summary of results in each group.

Forrest classification [n]	All [12]	1A [6]	1B [6]	*P* value[1]
Time to hemostasis (min ± SEM)	4.2 ± 0.9	4.1 ± 1.5	4.3 ± 1.2	0.919
Mass of powder delivered (g ± SEM)	2.4 ± 0.6	3.0 ± 1.1	1.8 ± 0.3	0.324
Dose of thrombin (NIH units ± SEM)	1350 ± 450	1990 ± 850	720 ± 90	0.200

SEM, standard error of the mean.
[1] *P* values are for 1A vs 1B bleeds.

generated from the effervescence of SPTP further localize coagulation proteins to augment hemostasis [24, 25]. In our previously published studies, SPTP increased thrombin efficacy, reduced bleeding, and improved survival in multiple animal models, including endoscopic sinus surgery in sheep and junctional hemorrhage without compression in swine [10, 11, 13]. SPTP performed better than currently marketed hemostatic agents for surgery and trauma in all models, even without compression, by increasing the transport of thrombin in wounds [10, 13]. This may be particularly useful for gastrointestinal bleeds that are difficult to access, or when there are large volumes of blood for which it is not possible to use epinephrine injections or clip placement. SPTP also has potential for use in managing tumor bleeding, for which conventional endoscopic treatments are often unsuitable [26, 27], to provide lasting hemostasis before more definitive treatment. Delivering thrombin to gastrointestinal bleeds may be especially valuable for forming and maintaining a stable clot in the gastrointestinal tract, because high concentrations of tissue plasminogen activator in gastrointestinal tissue contribute to fibrinolysis and rebleeding [28, 29]. Halting of severe arterial bleeding in the gastrointestinal tract has not been demonstrated with current hemostatic powders. The data from the present study data suggest that SPTP is a promising new agent for accomplishing this. It is composed of safe materials that have been used clinically for many years. No toxicity was observed, and there were no instances of thromboembolism when SPTP was used in other models of severe arterial bleeding [13]; therefore, thromboembolism would not be expected in the gastrointestinal tract, although future studies should be completed to verify this.

This study had a number of limitations. It was a single-arm pilot in which the primary objective was successful hemostasis. Future studies are required in which SPTP is applied and compared to a control group that receives no powder. These studies would be able to establish therapeutic significance of SPTP. No conclusions can be drawn about the effectiveness of SPTP in comparison to currently available hemostatic powders. In addition, because this was a non-recovery experiment, we could not assess whether hemostasis would persist long-term or assess indicators of thromboembolism, although we expect SPTP to be safe. Further studies are required to compare SPTP safety and efficacy with other interventions for UGIB, such as other hemostatic powders.

Conclusions

Hemostatic powders are an innovative concept in managing bleeding in the gastrointestinal tract but are not yet an established first-line therapy. Our novel hemostatic powder uses a different mechanism of action and delivers active drugs to the bleeding site. We have previously shown its efficacy in animal models of large, arterial, non-compressible hemorrhage; now, in this pilot study using a live porcine model of UGIB, we have shown that it successfully stopped bleeding in 12 cases, demonstrating early promise as a novel gastrointestinal hemostatic powder.

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Competing interests

Drs. Baylis and Kastrup are inventors on patents and intellectual property related to the self-propelling particle technology. Mr. Ali-Mohamad and Mr. Cau, and Drs. Baylis, Donnellan, and Kastrup are involved in commercialization activities related to the selfpropelling particle technology.

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