Thieme

Intraoperative bleeding model for swine gastric endoscopic submucosal dissection via heparinization

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ABSTRACT

Background and study aims: Live swine have a high degree of coagulation and aggregation and using them for training about how to manage intraoperative bleeding during endoscopic submucosal dissection (ESD) is unsatisfactory. This study aimed to identify the appropriate heparin dose in an intraoperative bleeding model and validate its applicability.

Methods: First, we explored the dose of heparin required for a swine bleeding model in which the activated clotting time reached and maintained the upper limit of measurement (1500 s) after 10 minutes. Second, we compared intraoperative bleeding and hematoma frequency during ESD for 2-cm lesions between the heparinized bleeding model and control groups. Intraoperative bleeding was classified according to the Forrest classification.

Results: The combination of a bolus (300 U/kg), continuous infusion (300 U/kg/h), and a bolus dose (150 U/kg) of heparin 10 minutes after the first infusion was identified as the dose for the bleeding model. Five ESDs were performed in each heparinized bleeding model and the control group. The median number of intraoperative bleeds was significantly higher in the heparinized model than in the control group (5 interquartile range [IQR] 4–7 vs. 3 [IQR 0–4, P = 0.028). All of the intraoperative bleeding events oozing (Forrest Ib) rather than spurting (Forrest Ia). The median number of hematomas was significantly higher in the heparinized model group than in the control group (3 [IQR 1–4] vs. 0 [IQR 0–1], P = 0.023).

Conclusions: High doses of heparin significantly increased intraoperative bleeding and hematoma during swine ESD.

Introduction

Endoscopic submucosal dissection (ESD) is an effective treatment for superficial gastrointestinal tumors. This procedure is technically challenging and requires considerable training to be performed safely and efficiently. Training using not only dry-lab simulators and ex vivo models but also virtual simulation has been reported to be effective [1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13]. However, these models cannot simulate "intraoperative bleeding", and therefore, are not considered optimal for novice training.

On the other hand, a living swine model provides a more realistic environment resembling clinical ESD conditions [14]. It potentially can be useful in the final stage of training. However, swine blood coagulates and aggregates more quickly and thus the animals experience less severe bleeding than humans [15, 16, 17]. As a result, it is not possible to practice hemostasis for spurting bleeding during a procedure on living swing or have the operative field obstructed by oozing in such a model. Developing a living swine model in which intraoperative bleeding frequently occurs during ESD is necessary.

We previously conducted a study to create a bleeding living swine model for endoscopic training, which revealed that activated clotting time (ACT) and mucosal bleeding time by biopsy gradually increased in proportion to heparin bolus dose [17]. However, it is unclear whether heparin administration would similarly induce intraoperative bleeding in ESD, which requires a long procedure time and an electrosurgical knife. This study aimed to identify the appropriate heparin dose for a bleeding model of ESD and to validate the availability of this model.

Methods

Study design

This in vivo study was conducted in two steps using a living swine stomach. Step 1 was identification of the dose of unfractionated heparin that reduces the coagulation function in swine enough, and several combinations of bolus and continuous infusion of heparin were administered to swine and the kinetics of the coagulation function were observed for each. Step 2 was validation of the availability of the intraoperative bleeding model created by heparinization at the doses identified in Step 1 and the degree of intraoperative bleeding in gastric ESD was compared between the heparinized and non-heparinized (control) models.

The experimental protocol for this study was approved by the Institutional Animal Care and Ethical Review Board (IVTeC Co., Ltd. Animal Welfare Committee) (approval number: IVT22–96). This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals.

Animals

All procedures were conducted in a standard manner under general anesthesia in 3-month-old female swine (average weight, 35 kg). Each swine underwent pretreatment and was humanely euthanized upon completion of the experiment.

Device and equipment

ESD was performed using a therapeutic endoscope with water-jet function (GIF-H290T; Olympus Medical Systems, Tokyo, Japan). A transparent hood (D-201–11804; Olympus Medical Systems, Tokyo, Japan) was attached to the tip of the endoscope. Mucosal incision and submucosal dissection was performed using a 2.0-mm DualKnife J (Olympus Medical Systems, Tokyo, Japan). For submucosal injection, the saline solution was mixed with a small amount of indigo carmine. We used VIO 3 (ERBE Elektromedizin, Tübingen, Germany) in EndoCUT I mode (effect 1.0, duration2.0, interval2.0) for mucosal incisions and submucosal dissection.

Procedure

Step 1: Identification of unfractionated heparin dosage

A model in which intraoperative bleeding can occur as frequently as possible and which can be produced easily and quickly is desirable. The previous study showed that the higher the ACT, the longer the bleeding time [17]. Because it is difficult to control the ACT precisely by injecting heparin into living swine (**Supplement 1**), we focused on determining the heparin dose at which the ACT reached and maintained the upper limit of measurement (1500 s) after 10 minutes. Several heparin doses were administered using the following procedure: 1) insertion of the catheter into the femoral vein in order to take blood sample and administration heparin; 2) measurement of the ACT of the swine as a pre-value; 3) administration of bolus and continuous infusion of unfractionated heparin; and 4) measurement of ACT every 10 minutes.

Step 2: Evaluation of degree of intraoperative bleeding during ESD

First, ESD was performed for 2-cm lesions in a pre-heparinized control swine. Subsequently, the same swine were heparinized at the doses identified in Step 1, and ESD was performed for 2-cm lesions. The stomach is divided into upper, middle, and lower regions. The lesions were created with no differences in location between the heparinized and non-heparinized models. Experts with experience perform more than 100 ESD experiences undertook this procedure.

Measured outcomes of Step 2

The primary outcome was the number of intraoperative bleeding events. Secondary outcomes were lesion location, resection time, en bloc resection rate, intraoperative perforation rate, and number of hematomas. Intraoperative bleeding was classified according to the Forrest classification [18]. Hematoma was defined as one observed in the submucosal layer during ESD (▶ **Fig. 1**).

Statistical analysis

Fisher's exact test was used to analyze categorical data. Quantitative data were compared using Student's *t*-test. Statistical significance was set at $P < 0.05$. All statistical analyses were per-

formed using JMP software (version 17.0.0; SAS Institute, Cary, North Carolina, United States).

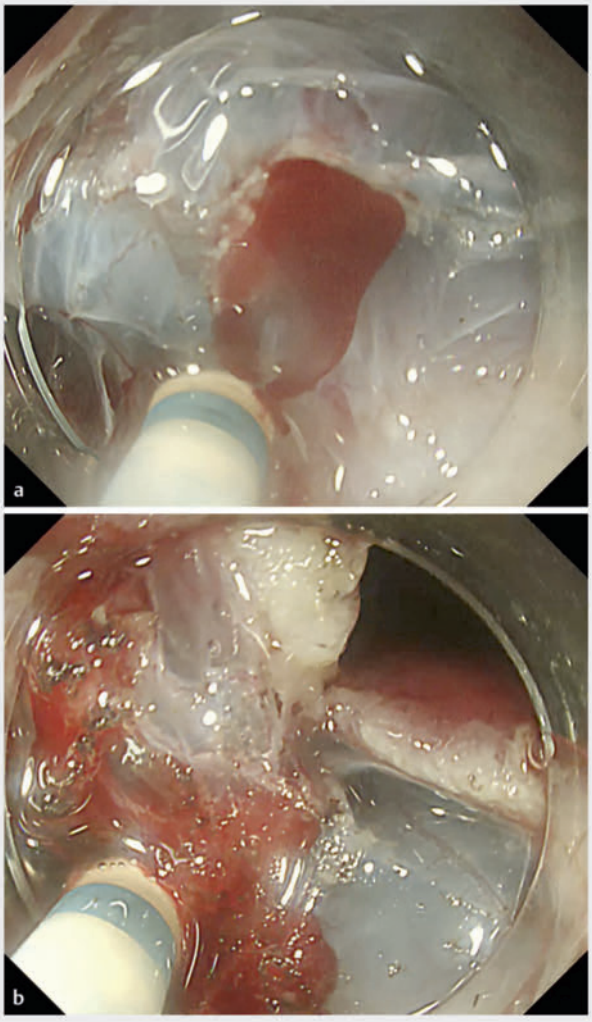
Results

Step 1: Identification of unfractionated heparin dosage

Four patterns of doses of heparin were administered to individual swine. ▶ **Fig. 2** presents the results. The combination of a bolus (100 U/kg) and continuous infusion (50 U/kg/h) and the combination of a bolus (200 U/kg) and continuous infusion (100 U/kg/h) of unfractionated heparin did not increase the ACT to 1500 s, even after 30 minutes. The combination of a bolus (300 U/kg) and continuous infusion (300 U/kg/h) increased the ACT to 1500 s 10 minutes after infusion. However, the ACT temporarily decreased to 958 s 20 minutes after starting the infusion and was maintained for 1500 s 30 minutes after infusion. In addition, by adding a bolus dose (150 U/kg) 10 minutes after dosing, the ACT reached 1500 s after 10 minutes of administration, which was maintained for 2 hours.

Step 2: Evaluation of degree of intraoperative bleeding during ESD

In two swine, a total of 10 ESDs were performed for each of the control and heparinized models developed in Step 1 (dose shown in ▶ **Fig. 2d**). During the procedure, the vital signs of these swine remained stable. ▶ **Fig. 3** demonstrates the primary outcomes. The median number of intraoperative bleeds was significantly higher in the heparinized model than in the control group (5; interquartile range [IQR] 4–7) vs. 3; IQR 0–4; $P = 0.028$). All intraoperative bleeding events were oozing (Forrest Ib) and not spurting (Forrest Ia). ▶ **Table 1** and ▶ **Fig. 4** outline the secondary outcomes. En bloc resection without perforation was achieved in all cases. The median number of hematomas was significantly higher in the heparinized model group than in the control group (3 [IQR, 1–4] vs. 0 [IQR, 0–1], $P = 0.023$).



▶ **Fig. 1** Intraoperative bleeding and hematoma during ESD. **a** Intraoperative bleeding. **b** Hematoma in the submucosa.

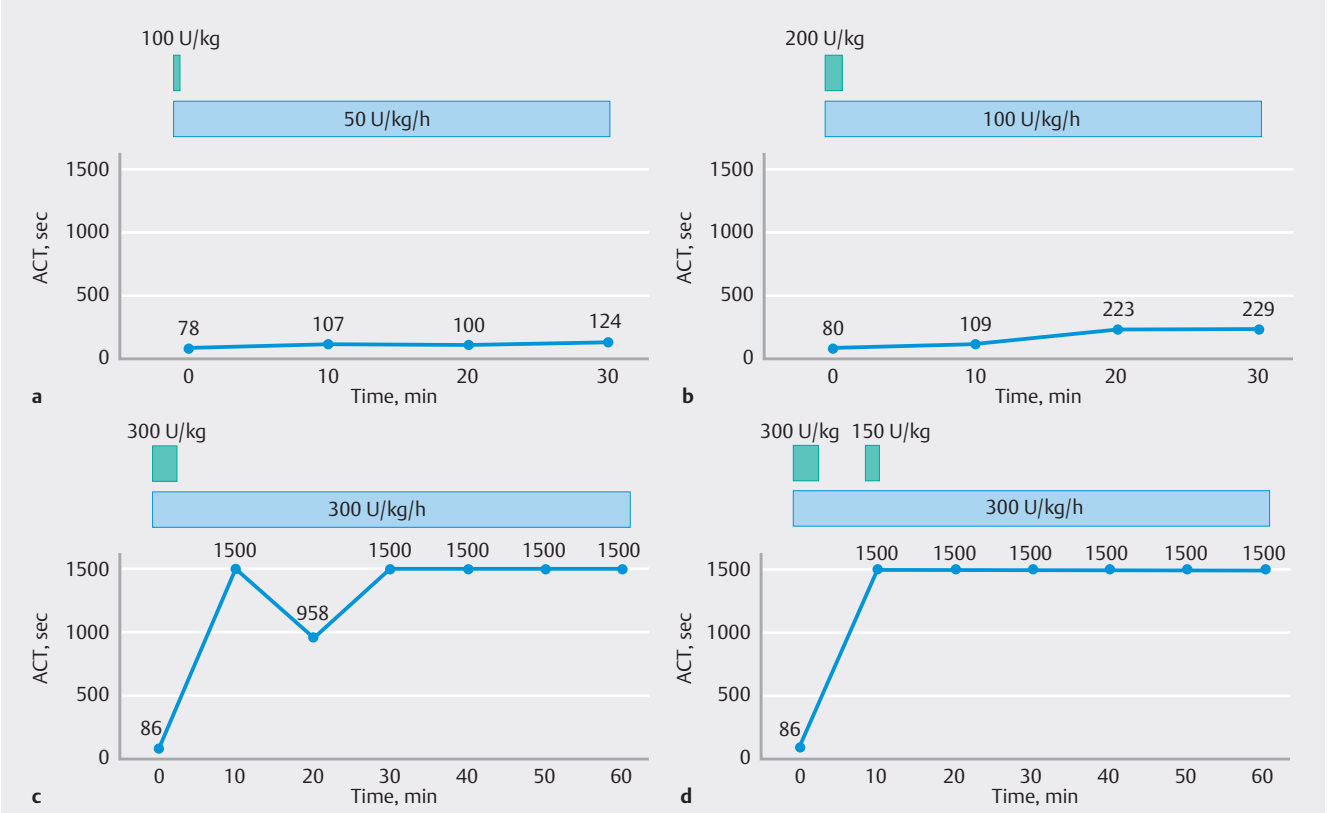
Discussion

This study successfully revealed that the combination of a bolus (300 U/kg), continuous infusion (300 U/kg/h), and a bolus dose (150 U/kg) administered 10 minutes after the first infusion immediately reached and maintained ACT at 1500 s. This dosage is considered to reduce the coagulation function in swine as much as possible within a measurable range. Furthermore, intraoperative bleeding during gastric ESD was significantly higher in the heparinized group than in the control group. To the best of our knowledge, this is the first study to develop and evaluate an intraoperative bleeding swine model for ESD.

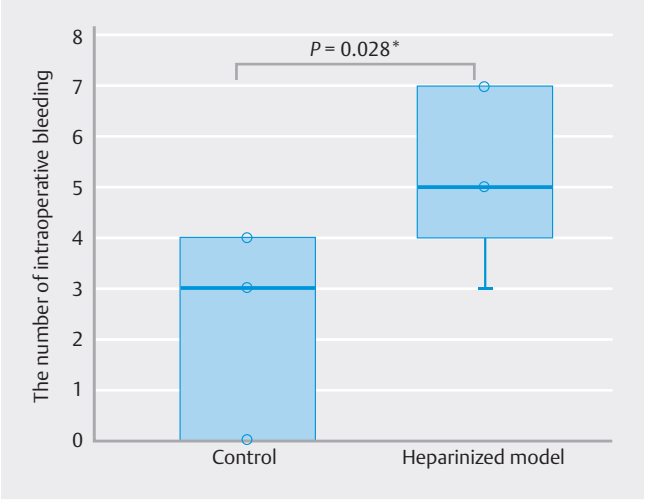
Although swine is a suitable training model due to its similarity to human gastrointestinal anatomy, inappropriate experiment models can waste animal life [15, 19]. It is important to optimize models for the purpose. The functions of coagulation and aggregation in swine are well known, with mean platelet counts higher in swine than in humans (350; standard deviation [SD] 150 vs. 265; SD 135 $\times 10^6$/mL) [16]. Interventions are required to induce reproducible bleeding in live swine.

Although a previous study reported on a bleeding model created by implantation of an artery into the stomach, creating such a model is time-consuming and requires expertise [19, 20, 21]. A bleeding model can be easily created using antithrombotic drugs. Antithrombotic drugs are of two main types: antiplatelet agents and anticoagulants. The combination of antiplatelet agents and heparin can reproducibly cause active bleeding from the mucosal defect by snare resection [15, 22]. However, antiplatelet agents are oral medicines that require administration several days in advance, which is also time-consuming and logistically challenging. Heparin is a major anticoagulant available as an injection and has an immediate effect. Thus, we considered the bleeding model created using only heparin as the easiest to generalize.

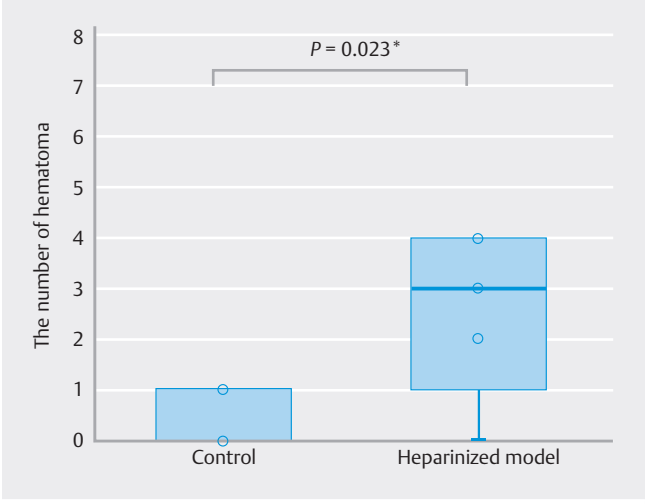
ACT is a method for easy and immediate monitoring of the anticoagulation effects of heparin. It measures the time required for clotting to occur by activating the intrinsic pathway of the coagulation cascade [23]. In this study, we first attempted



▶ **Fig. 2** Kinetics of the coagulation function (ACT over time). **a** The combination of bolus (100 U/kg) and continuous infusion (50 U/kg/h) of unfractionated heparin. **b** The combination of bolus (200 U/kg) and continuous infusion (100 U/kg/h) of unfractionated heparin. **c** The combination of bolus (300 U/kg) and continuous infusion (300 U/kg/h) of unfractionated heparin. **d** The combination of bolus (300 U/kg) and continuous infusion (300 U/kg/h), and a bolus dose (150 U/kg) of unfractionated heparin 10 minutes after the first infusion.



▶ **Fig. 3** Comparison of the number of intraoperative bleeds between the heparinized model and control.



▶ **Fig. 4** Comparison of the number of intraoperative bleeds and hematoma between the heparinized model and control

▶ **Table 1** Characteristics and treatment outcomes of the enrolled cases. ESD, endoscopic submucosal dissection; IQR, interquartile range.

Values		Control n = 5	Heparinized model n = 5	*P* value
Location	Upper, N (%)	2 (40)	2 (40)	> 0.99
	Middle, N (%)	2 (40)	2 (40)	
	Lower, N (%)	1 (20)	1 (20)	
Resection time, min	Median [IQR]	12 [5.5–28]	12 [8–16]	0.49
En bloc resection	yes, N (%)	5 (100)	5 (100)	> 0.99
Intraoperative perforation	yes, N (%)	0 (0)	0 (0)	> 0.99

to identify the optimal heparin dose. Although it was difficult to control the ACT precisely by injecting heparin into living swine, our aim was not controlling the ACT precisely but creating a model easily and quickly in which intraoperative bleeding frequently occurs. Thus, we focused on determining the heparin dose that immediately reaches and maintains the ACT at 1500 s, the upper limit of measurement. The combination of a bolus (300 U/kg) and continuous infusion (300 U/kg/h) allowed the ACT to reach 1500 s 10 minutes after the first administration. However, the ACT decreased 20 minutes after the first administration. This is because heparin is only effective for 20 to 30 minutes [17]. Ten minutes after the first administration, an additional heparin bolus administration made it possible to maintain the ACT at 1500 s for at least 2 hours.

Second, we demonstrated that the frequencies of intraoperative bleeding and hematoma during gastric ESD were significantly higher in the heparinized bleeding swine model than in the control group. This is because the action of heparin may have manifested submucosal bleeding, causing electrocautery to normally stop at that moment. This model, even when using only heparin, allows us to experience hemostasis and situations in which bleeding causes a poor endoscopic view and difficulty in identifying the resection line in the submucosa due to hematoma. This high-fidelity swine model will serve not only for ESD training but also for development of various instruments and technology evaluations.

In the clinical setting, both oozing and spurting bleeding occasionally occur, particularly during gastric ESD. However, in this model, not all bleeding was spurting (Forrest Ia), but rather, oozing (Forrest Ib). Thus, trainees cannot experience hemostasis of spurting bleeding in this model. Spurting bleeding was rarely observed in a previously reported model in which a combination of heparin and antiplatelet agents was used, although large submucosal arterioles were observed on ulcer histology [15, 22]. This may result from not only the high function of coagulation and aggregation but also the high rate of vasoconstriction in response to injury in swine [15]. Under current circumstances, a surgical approach is needed to create spurting bleeding in swine [15, 19, 20, 21].

This study has several limitations. First, the sample size was small. Second, the endoscopists were not blinded. There was a possibility that the endoscopists could intentionally cause bleeding during ESD. Third, it is unclear whether a higher dose of the heparin than that identified in our study is effective or not. Fourth, we used only the cut mode as an electrical surgical unit setting to increase intraoperative bleeding frequency, which is inconsistent with a clinical setting. Fifth, we did not simulate hemostasis for spurting bleeding in our models. Fourth, intraoperative bleeding was evaluated only for gastric ESD. The increase in frequency of bleeding in other organs remains uncertain. Sixth, the procedure was performed by experts and the usefulness of the bleeding model for actual training was not evaluated. Further studies with larger sample sizes are warranted to investigate the efficacy of the training model in novice endoscopists.

Conclusions

In conclusion, high doses of heparin increased intraoperative bleeding during swine ESD, making the ESD situation more similar to that in clinical settings.

Conflict of Interest

The authors declare that they have no conflict of interest.

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