Application of Hemocoagulase Bothrops Atrox in the submucosal injection for endoscopic submucosal dissection: a preliminary trial

Yiting Liu, Rui Li, Chenhuan Tan, Yifan Ma, Ji Feng, Qingpeng Xu and Jianing Sun

Objective This study aimed to evaluate the efficacy and safety of using Hemocoagulase Bothrops Atrox in the submucosal injection solution for endoscopic submucosal dissection (ESD).
Methods A total of 120 patients with superficial neoplastic lesions of the esophagus, stomach, and colon receiving ESD were randomly divided into two groups: The epinephrine group used epinephrine-containing submucosal fluid cushion for ESD, while the hemocoagulase group used Hemocoagulase Bothrops Atrox-containing submucosal fluid cushion for ESD. The preoperative, intraoperative, and postoperative clinical parameters and postoperative adverse events of the two groups were recorded, and comparative analysis within and between groups was performed.
Results There was no significant difference in the demographic and clinical characteristics between the hemocoagulase and epinephrine group (all $P > 0.05$). ESD surgery was completed in all patients. The hemocoagulase group had significantly shorter surgery time ($P = 0.003$) and less number of intraoperative bleeding ($P = 0.010$) than the epinephrine group. However, there was no significant difference in the incidences of postoperative delayed hemorrhage, and adverse events between the two groups (all $P > 0.05$). Multivariate linear regression demonstrated that the epinephrine group had significantly more number of intraoperative bleeding (B: 0.98, 95% confidence interval: 0.04–1.93) as compared with the hemocoagulase group.
Conclusion Compared with epinephrine, using Hemocoagulase Bothrops Atrox in the submucosal injection for ESD surgery can significantly reduce the number of intraoperative bleeding, shorten the operation time, and did not elevate the incidence of adverse events. Eur J Gastroenterol Hepatol 33: e681–e685

Introduction

Endoscopic submucosal dissection (ESD) is a procedure for en-bloc resection of neoplastic lesions in the gastrointestinal tract, which has gradually become the preferred treatment for early cancer and precancerous lesions of the gastrointestinal tract [1]. Common complications associated with ESD include hemorrhage, perforation, coagulation syndrome, and postoperative stenosis [2].

During ESD, submucosal injection is essential, which can form a submucosal fluid cushion to separate the mucosal layer from the muscularis propria, in turn elevating the lesion and reduce the risk of thermal injury, perforation, and hemorrhage [3]. Hemostatic agents commonly added to the submucosal injection can further reduce the risk of hemorrhage [4]. At present, epinephrine is widely used in submucosal injection solutions for ESD due to its vasoconstriction effect [3]. After epinephrine injection, however, patients may develop the symptoms, such as palpitation, headaches, and elevated blood pressure and heart rate, and even potential heart diseases [5].

Hemocoagulase is a thrombin-like enzyme purified from snake venoms that have been widely used in the prevention and treatment of surgical hemorrhage [6–8]. Hemocoagulase can accelerate the formation of fibrin monomers and hastens fibrin clot formation at the site of hemorrhage to achieve the physiological hemostatic effect [9]. Accumulating evidence has suggested that hemocoagulase can significantly reduce hemorrhage in abdominal, urological, thyroid, and orthopedic surgeries [10–13]. Hemocoagulase Bothrops Atrox for Injection (brand name: baquting) is a high-purity enzymatic hemostatic agent isolated from Bothrops atrox venom [14], which has been used in orthopedics and urological surgeries [15,16].

However, Hemocoagulase Bothrops Atrox has not been applied in ESD. We hypothesized that Hemocoagulase Bothrops Atrox may be used as the hemostatic agent in submucosal injection solution. In this study, we conduct a prospective randomized controlled trial to evaluate the efficacy and safety of using Hemocoagulase Bothrops Atrox in submucosal injection solution for ESD.

Methods

Study subjects and grouping

Patients with gastrointestinal superficial tumors undergoing ESD surgery in the Gastroenterology Department of

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Department of Gastroenterology, the First Affiliated Hospital of Soochow University, Suzhou, China

Correspondence to Rui Li, MD, Department of Gastroenterology, the First Affiliated Hospital of Soochow University, NO. 899 Pinghai Road, Gusu District, Suzhou 215000, China
Tel: +86 13771725877; e-mail: lrhcsz@163.com

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our hospital between October 2018 and November 2019 were enrolled. Inclusion criteria were as follows: (1) aged from 18 to 75 years; (2) patients with superficial neoplastic lesions of the esophagus, stomach, and colon undergoing elective ESD surgery; (3) cardiopulmonary function meeting the indications for ESD surgery and without disorders of the coagulation system; (4) preoperative haemoglobin, platelet, prothrombin time, international normalized ratio, and activated partial thromboplastin time meeting the ESD indications. The exclusion criteria included are as follows: (1) patients with mental and neurological diseases, severe heart disease, lung disease, liver disease, kidney disease, immune, blood system disease, or other serious diseases that affect survival (such as tumors and AIDS); (2) coagulation disorders (including thrombocytopenia, hemophilia, von Willebrand disease) or those were using anticoagulants or antiplatelet drugs that cannot be stopped; (3) with the risk of thrombosis (such as long-term bed rest and history of venous thrombosis); (4) allergic to hemocoagulase or similar products; (5) planning to become pregnant, pregnant women, and breastfeeding women; (6) participating in other clinical trials at 3 months before surgery or during the trial; (7) patients were judged to be unfit to participate in the trial due to other reasons.

The eligible participants were randomly divided into two groups according to the order of enrollment. The epinephrine group: intraoperative use of submucosal injection solution containing (indigo carmine 2 ml, 1:100 000 dilute epinephrine saline 200 ml); The hemocoagulase group: intraoperative use of submucosal injection solution containing (indigo carmine 2 ml, Hemocoagulase Bothrops Atrox 20U, saline 200 ml). This study was registered in the Chinese Clinical Trial Register (ChiCTR, registration no. CHi CTR1800016341, http://www.chictr.org.cn/showproj.aspx?proj=27003). This study was conducted in accordance with the Declaration of Helsinki and approved by the institutional review board of our hospital. Written informed consent was obtained from the patient.

Endoscopic submucosal dissection

All operations were completed by a surgeon who has completed more than 150 cases of ESD surgeries. All patients were checked for complete blood routine, blood coagulation, liver and kidney function, and electrocardiogram before the operation. The vital signs, ASA classification, and combined medication were recorded. Under general anesthesia, an argon knife (German ERBE high-frequency electrosurgical device and APC300 argon-ion coagulator) was used to mark the outer edge of the lesion at 0.5 cm (output power 15 W, interval 2 mm). The multi-point submucosal injection was performed outside the marked point on the edge of the lesion using the NM-200L-0423 injection needle. After the lesion was fully lifted, a dual knife (KD-650L, KD-650U) was used to cut the mucosa around the lesion along the edge of the lesion, and then the Dual knife or IT knife (KD-611L) was used to peel off the lesion with the help of a transparent cap. During the peeling process, if the submucosal injection was absorbed, multiple submucosal injections were performed to keep the lesion separated from the muscle layer. During or after the peeling, bleeding on the wound was directly electrocoagulated with a Dual knife or IT knife, or a hot biopsy forceps can be used to clamp the bleeding point and electrocoagulation for hemostasis. After the dissection was completed, the exposed blood vessels on the wound surface were all treated with APC cauterization (German ERBE high-frequency resection device and APC300 argon-ion coagulator) or titanium clamps for preventive hemostasis treatment.

Definitions of hemorrhage

The definitions of hemorrhage events were as follows [17]: intraoperative acute hemorrhage was defined as intraoperative wound bleeding or jet bleeding lasting for within 1 min, which can be successfully stopped by an endoscope. Intraoperative acute massive bleeding was defined as intraoperative active oozing or jet bleeding, which was difficult to stop under endoscopy, and interruption of surgery and/or blood transfusion was needed. Postoperative delayed hemorrhage was defined as postoperative bleeding that requires endoscopic hemostasis. Postoperative delayed massive hemorrhage was defined as the hemoglobin level on one day after surgery decreased by ≥2 g/dl than before surgery.

Postoperative observation and management

Three days after the operation, the vital signs were monitored, the blood routine was reviewed. The postoperative delayed hemorrhage, perforation, and adverse events (allergic, elevated blood pressure, palpitations, dizziness, vomiting) were recorded, and the patients were given accordingly medication treatments. No serious adverse reactions occurred in all patients.

Outcomes measurements

The efficacy outcomes included the number of intraoperative bleeding, operation time, and the incidence of postoperative delayed hemorrhage. Safety indicators include adverse events, vital signs before and after surgery.

Table 1. Patients demographic and clinical characteristics

Parameters	Hemocoagulase group (n = 60)	Epinephrine group (n = 60)	All (n = 120)	*P*-value
Age (year)	60.06 ± 10.84	62.14 ± 10.64	61.10 ± 10.75	0.284
Sex				0.346
Male	40 (66.67%)	35 (58.33%)	75 (62.50%)	
Female	20 (33.33%)	25 (41.67%)	45 (37.50%)	
Lesion site				0.088
Stomach	23 (38.33%)	28 (46.67%)	51 (42.50%)	
Esophagus	31 (51.67%)	20 (33.33%)	51 (42.50%)	
Rectum/colon	6 (10.00%)	12 (20.00%)	18 (15.00%)	
Lesion area (cm^2)	3.69 ± 3.75	5.77 ± 10.83	4.73 ± 8.13	0.172
Pathological type				0.854
Elevated	33 (55.00%)	34 (56.67%)	67 (55.83%)	
Flat/depressed	27 (45.00%)	26 (43.33%)	53 (44.17%)	
Ulcer				0.142
No	51 (85.00%)	56 (93.33%)	107 (89.17%)	
Yes	9 (15.00%)	4 (6.67%)	13 (10.83%)	
Heart rate (bpm)				
Preoperative	73.13 ± 7.11	73.42 ± 11.82	73.28 ± 9.71	0.620
Postoperative	77.77 ± 6.97*	78.15 ± 7.88*	77.96 ± 7.41*	0.773
SBP (mmHg)				
Preoperative	129.93 ± 12.63	128.15 ± 11.84	129.04 ± 12.22	0.345
Postoperative	128.98 ± 13.07	129.07 ± 13.09	129.03 ± 13.02	0.803
DBP (mmHg)				
Preoperative	80.93 ± 7.87	77.77 ± 9.05	79.35 ± 8.59	0.051
Postoperative	76.47 ± 9.16*	77.80 ± 8.82	77.13 ± 8.98*	0.441

*$P < 0.05$ compared to preoperative result.

Table 2. Surgical outcomes

Parameters	Hemocoagulase group (n = 60)	Epinephrine group (n = 60)	All (n = 120)	*P*-value
Surgery time (mins)	62.73 ± 37.99	78.92 ± 37.46	70.83 ± 38.43	0.003
Number of intraoperative bleeding	2.67 ± 2.18	3.76 ± 3.06	3.21 ± 2.70	0.010
Postoperative delayed hemorrhage				0.559
No	58 (96.67%)	59 (98.33%)	117 (97.50%)	
Yes	2 (3.33%)	1 (1.67%)	3 (2.50%)	
Adverse reactions				0.537
Allergy	0	0	0	
Headache	2 (3.33%)	1 (1.67%)	3 (2.50%)	
Faint	1 (1.67%)	3 (5.00%)	4 (3.33%)	
Vomit	3 (5.00%)	4 (6.67%)	7 (5.83%)	

Statistical analysis

Continuous variables were reported with mean ± SD and were compared using Student's independent *t*-test or Mann–Whitney U test (if normality was not assumed). The comparisons between pre- and post-operative heart rate, SBP, and DBP were using Student's paired *t*-test. Categorical variables were presented as numbers and percentages and were compared using the Chi-square test or Fisher's exact test (if the expected value ≤ 5 was found). Univariate and multivariate linear regression was used to investigate the association between group variable and the number of intraoperative bleeding while covariates were adjusted. The covariates, which were significant in univariate results, would be entered into a multivariate model. A $P < 0.05$ would be recognized as reaching the significance of each test, two-tailed. All analyses were performed using IBM SPSS Version 25 (SPSS Statistics V25, IBM Corporation, Somers, New York).

Results

Patients' demographic and clinical characteristics

A total of 120 patients (male/female = 75/45) with superficial neoplastic lesions undergoing ESD were enrolled and randomized into the hemocoagulase group and the epinephrine group (n = 60 for each group). The superficial neoplastic lesions mainly located in the stomach (n = 51, 42.50%), esophagus (n = 51, 42.50%), and rectum/colon (n = 18, 15.00%).

As shown in Table 1, there was no significant difference in the demographic and clinical characteristics between the hemocoagulase and epinephrine group (all $P > 0.05$), indicating the comparativeness between the two groups.

As for intragroup comparisons, the heart rate was significantly elevated after ESD surgery in both groups (all $P < 0.05$). The DBP was significantly reduced after ESD surgery in the hemocoagulase group ($P < 0.05$).

Surgical outcomes

ESD surgery was completed in all patients of both groups. The surgical outcomes were compared in Table 2. The hemocoagulase group had significantly shorter surgery time ($P = 0.003$) and less number of intraoperative bleeding ($P = 0.010$) as compared with the epinephrine group.

Table 3. The association between independent variables to number of intraoperative bleeding

	Univariate		Multivariate	
Parameters	B (95% CI)	*P*-value	B (95% CI)	*P*-value
Group				
Hemocoagulase	ref.	ND	ref.	ND
Epinephrine	1.10 (0.13–2.06)	0.026	0.98 (0.04–1.93)	0.042
Sex				
Male	ref.	ND	ref.	ND
Female	−1.12 (−2.12 to −0.13)	0.027	−1.12 (−2.09 to −0.15)	0.023
Age (year)	0.05 (0.00–0.09)	0.037	0.02 (−0.02 to 0.07)	0.358
Lesion site				
Stomach	ref.	ND		
Esophagus	0.53 (−0.53 to 1.59)	0.326		
Rectum/colon	−0.77 (−2.23 to 0.70)	0.301		
Lesion area (cm^2)	0.00 (−0.06 to 0.06)	0.921		
Pathological type				
Elevated	ref.	ND	ref.	ND
Flat/depressed	0.98 (0.01–1.96)	0.048	0.67 (−0.34 to 1.67)	0.192
Ulcer				
No	ref.	ND		
Yes	−1.01 (−2.58 to 0.55)	0.203		
Preoperative				
Heart rate	−0.02 (−0.07 to 0.03)	0.439		
SBP	0.01 (−0.04 to 0.05)	0.797		
DBP	−0.06 (−0.12 to −0.01)	0.027	−0.05 (−0.11 to 0.00)	0.052

CI, confidence interval; ND, no data.

However, there was no significant difference in the incidences of postoperative delayed hemorrhage, and adverse events between the two groups (all $P > 0.05$). No intraoperative acute massive bleeding occurred in this study. No serious adverse events occurred in all patients.

The independent variables associated with the number of intraoperative bleeding

To determine the independent variables associated with the number of intraoperative bleeding, univariate, and multivariate linear regression models were adopted. As shown in Table 3, group and gender were independent variables associated with the number of intraoperative bleeding (both $P < 0.05$). The epinephrine group had significantly more number of intraoperative bleeding [B: 0.98, 95% confidence interval (CI): 0.04–1.93] as compared with the hemocoagulase group. Female patients had a comparatively lower number of intraoperative bleeding than male patients (B: −1.12, 95% CI: −2.09 to −0.15).

Figure 1 shows the results of subgroup analysis stratified by gender within both groups. It seems that sex effectiveness was more significant in the hemocoagulase group ($P = 0.021$).

The univariate and multivariate analyses of stomach lesion subgroup were performed. In the univariate analysis, significance differences were found in group factor, age, and pathological type. It seems that patients in epinephrine group, with higher age, and with flat/depressed pathological type were more likely to have more times of intraoperative bleeding (all $P < 0.05$, Table 4). However, in the multivariate model, pathological type was the only significant factor (B: 1.29, 95% CI: 0.05–2.53, Table 4).

Discussion

ESD has been widely used in the treatment of early gastrointestinal cancer and submucosal tumors. Compared

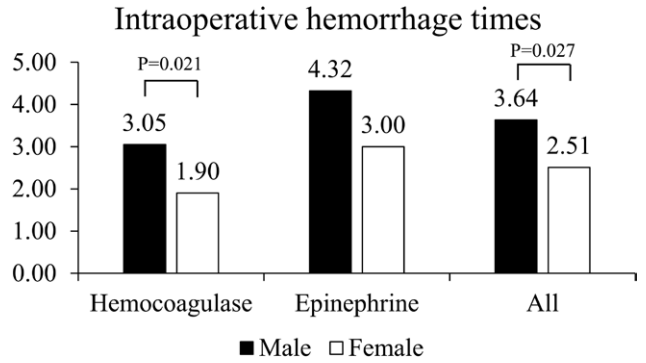

Fig. 1. The differences of number of intraoperative bleeding between male and female patients in each group.

to conventional endoscopic mucosal resection (EMR), ESD can achieve en-bloc resection of the lesion regardless of the lesion size, providing more precise pathological staging and the opportunity of curative resection of the tumor [17]. However, ESD surgery is a highly technical demand procedure associated with high incidences of complications, such as hemorrhage and perforation. The most common complication of ESD is hemorrhage [18]. A submucosal injection is a crucial step of ESD, which lefts the lesion and effectively separates the lesion from the muscularis propria, promotes the en-bloc resection of the lesion, and reduces the risk of perforation and hemorrhage [19,20]. The ideal submucosal injection solutions should meet the following requirements: (1) providing a sufficiently thick submucosal fluid cushion; (2) It can be maintained under the mucosa for a long time to reduce the number of submucosal injections; (3) It is cheap and easy to obtain, preserve, and inject; (4) Reducing the incidence of adverse events of ESD, such as hemorrhage and perforation; (5) Ensuring the integrity of the excised specimens to obtain precise pathological results. Currently, the commonly used submucosal injections are physiological saline, indigo carmine, glycerin, hypertonic glucose water, hyaluronic acid, hydroxypropyl methylcellulose, fibrinogen mixture, succinic gelatin, and hydroxyethyl starch [3]. The saline (0.9%) is currently the most used submucosal injections but is required multiple injections during the ESD procedure due to quick absorption by the surrounding tissues. Glycerol fructose is a hypertonic solution (10% glycerol and 5% fructose) and is a more effective submucosal injection than saline [21]. However, during the ESD operation, the glycerol fructose may generate ionized smoke due to electrocoagulation, which interferes with the surgical field of vision [22]. Hypertonic glucose solution can maintain submucosal bulge better than normal saline because of its hypertonicity. However, Fujishiro *et al.* [23] have reported that the hypertonic glucose solution exceeding a concentration of 15% may cause histopathological damage, which has an adverse effect on the subsequent pathological assessment and the healing of ulcers. Hyaluronic acid is recognized as an ideal submucosal injection with high viscosity and water-storage capacity. Nevertheless, it is relatively expensive and may increase the risk of residual tumor growth [24]. Fibrinogen mixture has a high viscosity that can reduce the number of injections and shorten the operation time during the ESD operation as compared with the normal saline [25]. But it is relatively expensive [26]. All above submucosal injections do not contain hemostatic components, and their function mainly focuses on maintaining the submucosal bulge.

Table 4. The independent variables associated with the number of intraoperative bleeding in patients with stomach lesions

	Univariate		Multivariate	
Parameters	B (95% CI)	*P*-value	B (95% CI)	*P*-value
Group				
Hemocoagulase	ref.	ND	ref.	ND
Epinephrine group	1.39 (0.18–2.61)	0.026	0.89 (–0.32 to 2.10)	0.146
Sex				
Male	ref.	-		
Female	–0.92 (–2.19 to 0.36)	0.155		
Age (year)	0.07 (0.01–0.13)	0.028	0.04 (–0.02 to 0.10)	0.182
Lesion area (cm^2)	0.03 (–0.14 to 0.20)	0.755		
Pathological type				
Elevated	ref.	ND	ref.	ND
Flat/depressed	1.71 (0.49–2.93)	0.007	1.29 (0.05–2.53)	0.042
Ulcer				
No	ref.	ND		
Yes	–0.80 (–2.45 to 0.85)	0.334		
Preoperative				
Heart rate	–0.01 (–0.07 to 0.04)	0.662		
SBP	–0.02 (–0.07 to 0.03)	0.490		
DBP	–0.02 (–0.09 to 0.06)	0.675		

CI, confidence interval; ND, no data.

At present, there is no consensus on the composition of submucosal injection in ESD. In China, the most commonly used submucosal injection solution is normal saline with a small amount of epinephrine and indigo carmine, which can reduce hemorrhage and detect the range and depth of peeling of the submucosal layer, basically meeting the needs of ESD treatment [3]. However, epinephrine treatment may cause palpitation, headaches, elevated blood pressure, and heart rate, in turn inducing potential heart diseases [5,27]. The hemostatic mechanism of Hemocoagulase Bothrops Atrox is to promote physiological hemostasis at the hemorrhage site, with the best efficacy on capillary below 0.3 mm in diameter [1,20]. In this study, we evaluated the efficacy and safety of using Hemocoagulase Bothrops Atrox in submucosal injection solution for ESD. Our results showed that the ESD surgery was completed in all patients of both groups, indicating that both epinephrine and Hemocoagulase Bothrops Atrox-containing submucosal injection solution can meet the requirements for ESD surgery. In China, Hemocoagulase Bothrops Atrox is currently widely used in the departments of internal medicine, surgery, obstetrics and gynecology, stomatology, otolaryngology, which is relatively easy to obtain. The cost of Epinephrine and Hemocoagulase Bothrops Atrox is about 5 CYN/unit and 60 CYN/unit. Although Hemocoagulase Bothrops Atrox is relatively expensive, it is fully reimbursed by medical insurance in China, which is favorable for patients with medical insurance.

We also found that the hemocoagulase group had significantly shorter surgery time than the epinephrine group. This phenomenon is related with reducing number of intraoperative bleeding in the hemocoagulase group. Therefore, the hemostatic effect of Hemocoagulase Bothrops Atrox simultaneously reduced the number of intraoperative bleeding and the surgery time during the ESD operation. These findings suggested that as an enzymatic hemostatic agent in the submucosal injection

solution, Hemocoagulase Bothrops Atrox can effectively reduce the incidence of hemorrhage after vascular injury, thereby improving the efficiency of ESD surgery.

Both epinephrine and hemocoagulase added to the submucosal fluid cushion for ESD are hemostatic drugs. Once bleeding occurs during mucosal dissection, these two drugs would exert a hemostatic effect and directly reduce intraoperative bleeding. Reducing intraoperative bleeding is of great significance for ESD operation. Therefore, intraoperative bleeding was chosen as a primary outcome measure while delayed postoperative bleeding was chosen as a second outcome measure. In this study, the incidence of postoperative delayed hemorrhage was low and similar between the two groups. A larger trial is needed to further confirm these issues. Local epinephrine injection has been reported to induce myocardial infarction in two elderly patients receiving ESD [5]. However, in this study, no patients developed elevated blood pressure or arrhythmia after epinephrine injection. Our results showed that the difference in adverse events between the two groups was not statistically significant, and no new adverse events occurred. These findings suggested that using hemocoagulase and epinephrine as the hemostatic agents in the submucosal injection for ESD surgery are both relatively safe. Using hemocoagulase in the submucosal injection for ESD surgery is an option for patients with a history of hypertension or heart disease.

There are still some limitations to this study. First, the sample size was relatively small. In addition, different lesions on the gastrointestinal tract may cause heterogeneity and bias in the study subjects of this study. Furthermore, since delayed bleeding after ESD is one of the common complications of ESD, this study was limited as delayed postoperative bleeding was not used as a primary outcome measurement. In the future, a large well-designed prospective trial should be conducted to further validate the findings of this study.

In summary, our study suggested that the use of Hemocoagulase Bothrops Atrox in the submucosal injection solution for ESD can effectively reduce the number of intraoperative bleeding, shorten the operation time, without increasing the adverse events.

Acknowledgements

None.

Conflicts of interest

There are no conflicts of interest.

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