Endoscopic application of highly mucoadhesive drug loadable powder for acute severe GI bleeding in a swine model

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Background and Aim

Although endoscopic hemostasis is highly effective, it can be challenging depending on the location and severity of gastrointestinal bleeding. Several hemostatic powders have been recently developed allowing straightforward procedures, but these can be improved. We have developed a new hemostatic powder (UI-EWD) that can be used endoscopically. The aims of this study were to investigate 1) the hemostatic ability of UI-EWD powder in a porcine model of acute severe bleeding, and 2) its persistence after gelation at the ulcer base. In addition, we tested 3) the ulcer healing effects of drug-loaded UI-EWD in the rat.

Methods

Material

UI-EWD consists of oxidized dextran and succinic acid modified amino acid in a ratio of 3:1. (Fig 1A,1B). These raw materials are immediately converted into high adhesive gel by Schiff base cross-linking reaction when contacting the water. However, the raw material of UI-EWD (Fig 1C) clogs the catheter due to high water absorption capacity. In addition, the fine powder consistency of the raw material results in scattering when sprayed using a delivery catheter. Therefore, we modified the raw material suitably for water absorption and particle size for endoscopic use by coating the particles (Fig 1C, 1D).

Fig. 1
Composition of UI-EWD

Characteristics of UI-EWD
1. Adhesive polymer.
2. Biodegradable & Biocompatible
3. Better adhesive than commercial glue
4. Reaction of Amine (-NH2) and Aldehyde (-CHO)
5. UI-EWD can load drugs containing amide group

Adhesion forces of UI-EWD gel

Adhesion forces of UI-EWD gel were measured by TXA™ Texture Analyzer (Yeonjin Corporation, Seoul, South Korea) under the following conditions: pre-loads 10 gf, descent rate 0.5 mm/sec, maximum load 600 gf, and three replications. Gelation of UI-EWD powders and commercial hemostatic powders was induced by adding 250 µl of water to 50 mg of UI-EWD powder and two commercial hemostatic powders (Commercial A and B).

Animals

Eight male mini-pigs weighing 35 kg to 40 kg (Medi Kinetics, Pyeongtaek, South Korea) were used for this study. The experimental protocol followed the guidelines of the Experimental Animal Research Committee of Inha University (protocol number; INHA-130822-234, Incheon, South Korea).

Induction of ESD-induced bleeding gastric ulcers in mini-pigs

Heparin (200 IU/kg) was intravenously injected before endoscopy. The endoscope was inserted into the animals in a lateral decubitus position. We created two gastric ulcers in each animal. The target areas were marked with an argon plasma coagulator, and isotonic saline was injected into the submucosal layer. Endoscopic submucosal dissection (ESD) was performed to make approximately 2 cm sized artificial gastric ulcers. To maximize bleeding, hemostasis was not performed during ESD

Evaluation of hemostatic efficacy and gel persistence on the ulcer base in mini-pigs

Three animals were assigned to the control group and five animals to the test group. Normal saline was sprayed on the ESD site in the control group. In the test group, three to six grams of UI-EWD was applied to the ESD-induced ulcer via the delivery catheter using Alto shooter™ (Kaigen Co., Ltd, Osaka, Japan). After the procedure, the mini-pigs were placed in individual cages for recovery. The mini-pigs were starved for another 24 h after ESD. Serial endoscopy was performed at 6, 18, 42, and 72 hours after the procedure to evaluate the re-bleeding rate and gel persistence on the ESD site. At the end of the observation period, the pigs were euthanized, with immediate necropsy and histopathologic evaluation.

Fig. 2 Research Plan Summary

Mucosal healing effect of drug loading UI-EWD in acetic acid induced gastric ulcer rat model

The therapeutic effect of ecabet sodium-loaded UI-EWD (UI-DLW) was evaluated in acetic acid-induced gastric ulcer (AAU) model in rats The rats with AAU were randomly divided into 3 groups; control, UI-EWD and UI-DLW group. Control group was not treated anything, and UI-EWD group was treated with 30mg of UI-EWD and UI-DLW group was treated with 30mg of UI-EWD containing 16 mg of ecabet sodium. Rats were sacrificed 3 days after the ulcer treatment, and the gastric tissues were observed grossly and histologically. Ulcer areas were calculated using by Image J program.

Results

Adhesion forces of UI-EWD gel

Table 1. Adhesion forces of UI-EWD gel

Products	Adhesive forces (gf)	%
UI-EWD	53.7	200
Commercial A	26.9	100
Commercial B	19.1	70

Average adhesive force of UI-EWD was 53.7 gf, commercial A was measured at 26.9 gf and commercial B at 19.1 gf. UI-EWD tested at over double the adhesive force of the two commercial products.

The initial and permanent hemostatic effect of UI-EWD in mini-pig model

Table 2. Bleeding rate in ESD-induced gastric ulcer mini-pig model

Time (h)	Control (n=6)	UI-EWD (n=10)
0	100 % (6/6)	0 % (0/10)
6	50 % (3/6)	10 % (1*/10)
24	0 % (0/6)	0 % (0/10)
42	17 % (1/6)	0 % (0/10)
72	17 % (1/6)	0 % (0/10)

No procedure-related adverse events were observed in any pigs. Bleeding was induced successfully due to heparinization before ESD. UI-EWD powder was precisely sprayed to the active bleeding focus via shooter without delivery catheter clogging. UI-EWD spraying achieved immediately hemostasis in all ESD lesions (Table 2). The ESD sites were observed via endoscope at 6, 18, 24, 42, and 72 h after treatment. In the control group, hemorrhage was observed in 50% of ESD site (3/6) at 6 hours after ESD. No further bleeding was observed in any lesions at 18 hours after procedure. However, delayed bleeding was observed in one lesion at 42 hours and 72 hours after procedure. In the UI-EWD group, bleeding was observed in one lesion at 6 hours after ESD. It may have been caused by the early separation of UI-EWD gel in the ESD site. However, no further bleeding was observed at 18, 42 and 72 hours after ESD (Table 2).

Gel persistence

With the exception of one lesion, the UI-EWD gel was attached to the lesion at 6 hours after spray. UI-EWD gel was attached to the lesions 80% at 18 hours and 50% at 42 hours after spray (Table 3) (Fig 3). All gels had detached by 72 hours after spray.

Table 3. The persistence rate of UI-EWD gel on ESD-induced gastric ulcer in mini-pigs

Time (hour)	0	6	18	42	72
UI-EWD (site=10)	100 % (10/10)	90 % (9/10)	80 % (8/10)	50 % (5/10)	0 % (0/10)

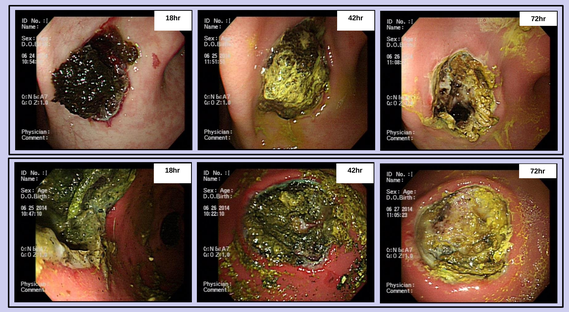
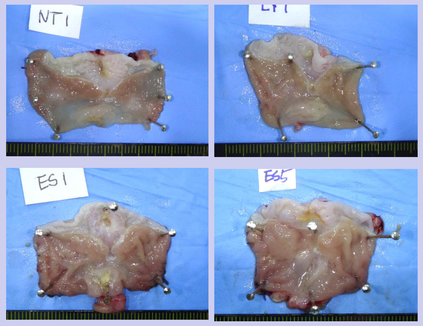


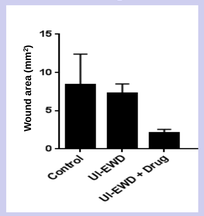
Fig. 3 Gel persistence on the ulcer base over time

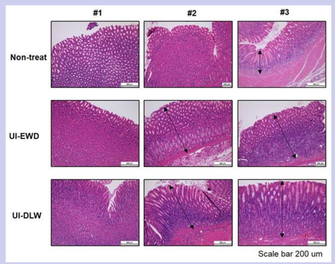
Preliminary Test of Drug Loading Capacity

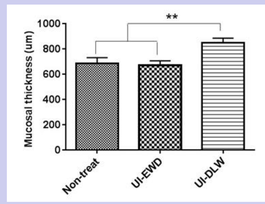
Three days after spray



- Animal: SD rat
- number: 5
- Powder: 30 mg
- Drug: Ecabet sodium 3mg







In rat model, the ulcer areas of drug-loaded UI-EWD group were decreased approximately 3.3-folds compared with those of non-treated and UI-EWD group on day 3 after ulcer induction. In addition, mucosal thickness of drug-loaded UI-EWD group was increased approximately1.3-folds more than that of non-treated and UI-EWD group.

Conclusions

- UI-EWD powder is effective in a porcine model of acute severe gastric bleeding due to high adhesiveness and persistent attachment time
- Mucosal regeneration is accelerated by loading ecabet sodium in the UI-EWD
- Therefore, UI-EWD can be a promising candidate for endoscopic treatment of acute gastric ulcer bleeding

This research was financially supported by the Ministry of Trade, Industry and Energy(MOTIE) and Korea Institute for Advancement of Technology(KIAT) through the International Cooperative R&D program.