Published in final edited form as:

Gastrointest Endosc. 2008 September ; 68(3): 548–553. doi:10.1016/j.gie.2008.03.1087.

Creation of an effective and reproducible nonsurvival porcine model that simulates actively bleeding peptic ulcers

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Abstract

Background: Efforts to develop improved endoscopic therapeutic methods for upper GI bleeding require an effective animal model.

Objective: To develop a nonsurvival porcine model that simulates acute peptic ulcer bleeding.

Design: Prospective animal (porcine) study.

Setting: Animal laboratory.

Interventions: A surgical seromyotomy was created along the external surface of the greater curvature of the stomach in anesthetized pigs. A submucosal plane was developed and the gastropiploic bundle, in continuity, was placed adjacent to the mucosa, and the seromuscular tissues were re-approximated over the vascular bundle. By using EGD, a needle-knife with electrocautery was then used to incise the mucosal tissue overlying the vascular bundle. Standard endoscopic methods for bleeding control were then tested in this animal model.

Main Outcome Measurements: To evaluate whether successful bleeding that simulates submucosal arterial bleeding from peptic ulcer disease could be achieved in a porcine animal model.

Results: Successful simulation of active peptic ulcer bleeding was achieved with this nonsurvival porcine model in a total of 5 sequential pigs. Other porcine models for bleeding were tested and found to be unsatisfactory. Hemoclips and combination injection-thermal therapy were used to stop bleeding over Doppler-positive areas, with subsequent endoscopic nonimaging Doppler US probe examination of the ulcer bed revealing a negative Doppler signal.

Limitations: This was an animal laboratory study. Further human studies would be ideal once any future endoscopic interventions are proven to be safe in animals.

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DISCLOSURE

The authors report that there are no disclosures relevant to this publication. This research was supported by the American Society for Gastrointestinal Endoscopy Sivak Endoscopy Research Award, Research & Outcomes & Effectiveness Grant, 2006, given to V. K. Chen. A. Chak is supported by a K24 Mid-Career Award in Patient Oriented Research, DK002800.

Conclusions: This active bleeding ulcer model can be used to develop future endoscopic therapies and for training purposes.

Upper gastrointestinal bleeding (UGIB) results in 300,000 hospitalizations annually in the United States, with estimated health care costs exceeding 2.5 billion dollars.^{1,2} The majority of these admissions are attributed to peptic ulcer hemorrhage. Despite advances that enable urgent endoscopic diagnosis and intervention, the mortality rate from UGIB remains at 5% to 10%, unchanged over the last 50 years.^{3–5} Although current endoscopic strategies result in a significant reduction in recurrent bleeding, the rate of rebleeding in clinical practice remains as high as 21% for peptic ulcers with “visible vessels.”⁶ Improved methods for the treatment of peptic ulcer hemorrhage are clearly required.

Recurrent hemorrhage is responsible for the major morbidity and mortality of UGIB and is related to the presence of submucosal arterial vessels.^{4,7} Improvements in endoscopic therapy intended to treat acute UGIB can be facilitated with an effective animal model that simulates actively bleeding submucosal arterial vessels in peptic ulcer bleeding. An animal model would facilitate safe testing of novel endoscopic therapies prior to human testing. Additionally, an animal model for acute UGIB would also have merit in training endoscopists on hemostasis techniques.

Prior peptic ulcer models, mainly in dogs, have involved mechanically created gastric ulcers or incision of mesenteric arteries.^{8–13} The last decade has seen an increased reluctance by animal research regulatory boards to permit canine research. Also, most of these ulcer models required a survival surgery to create the ulcer defect, followed by a separate endoscopic procedure at a later date once the ulcer developed. These 2-stage models are more expensive, and the animals may experience pain while awaiting ulcer development. Two-stage animal ulcer models may not represent the optimal model. There is a paucity of non-survival porcine models that simulate acute peptic ulcer bleeding because of an inability to identify submucosal vessels.^{14–17} The aim of our study is to develop a nonsurvival porcine model that simulates acute UGIB.

METHODS

All animal experiments were completed in accordance with preauthorized consent obtained from Case Western Reserve University Institutional Animal Care and Use Committee. Five healthy female domestic farm pigs, weighing 25 kg (± 5 kg), were obtained from a local vendor (Pine-view Farms, Valley City, Ohio). Veterinary personnel inspected all of the animals to ensure baseline health. Animals were removed from all bedding 72 hours prior to the procedure, and not permitted food 24 hours prior to surgery. Animals were allowed to drink water ad libitum.

All animals were sedated with 8 mg/kg tiletamine hydrogen chloride and zolazepam (Telazol, Fort Dodge Animal Health, Fort Dodge, Iowa) injected intramuscularly, and intubated with a 6F endotracheal tube via direct laryngoscopy. Peripheral intravenous access was obtained by using a 21-gauge catheter placed on the dorsal ear vein. Supplemental anesthesia, if required, was dosed with intravenous 8 to 10 mg/kg thiopental sodium (Pentothal, Abbot Laboratories, North Chicago, Ill). Isoflurane 0.5% to 2% inhaled

(Aerrane, Baxter, Deerfield, Ill) was administered via the endotracheal tube. Cardiac and pulse oximetry ensured normal physiologic response to anesthetic agents. Animals were mechanically ventilated at 15 to 20 mL/kg of tidal volume with 100% oxygen at 12 respirations per minute. Intravenous fluids were given if the animals became hypotensive during our experiments.

For all models, a small McBurney's incision was made to allow passage of a laparoscope into the peritoneal cavity. Simultaneous EGD enabled intraluminal visualization of submucosal vessels and gastroepiploic plexus from laparoscopic transillumination. Arterial flow within these submucosal vessels was confirmed by using a non-imaging, 20-MHz pulsed-wave endoscopic Doppler probe (Vascular Technology, Inc, Nashua, NH) placed with light to moderate pressure onto the intraluminal surface at 0 to 1.5-mm penetration depth settings¹⁸ (Fig. 1).

Endoscopic luminal models of bleeding ulcers

Repeated endoscopic biopsy specimens were obtained with a 4.4-mm standard fenestrated biopsy forceps (Olympus America, Inc, Melville, NY) over the Doppler-positive areas in an attempt to rupture the underlying vessel. This sequence was repeated with a standard oval polypectomy snare (Olympus, 25 mm) to "cold-guillotine" the gastric mucosa over laparoscopically transilluminated submucosal vessels. The sequence was repeated by using a modified needle-knife, which was created by stripping insulation off the ends of a 450-cm Jagwire (Boston Scientific Corporation, Natick, Mass) and attaching one end to electrocautery (DRE Inc, Louisville, Ky). All of the above were repeated after administration of a single bolus dose of (18 units/kg) of intravenous nonfractionated heparin without satisfactory bleeding at the treatment site.

The initial models were expanded by using endoscopic multiband ligators (Wilson-Cook Medical Inc, Bloomington, Ind) to create a pseudo-polyp of suctioned mucosa overlying submucosal arterial vessels identified by laparoscopic transillumination. The pseudo-polyp was then resected with a cold standard oval polypectomy snare to create a large ulcer base. We also repeated the process after administering nonfractionated heparin intravenously.

Combined surgical-endoscopic serosalluminal model of bleeding ulcer

A 5-cm linear seromyotomy was created with electro-cautery along the external surface of the greater curvature of the stomach just parallel to the gastroepiploic vessels (Fig. 2). Next, the gastroepiploic vascular bundle was carefully dissected from the omentum and serosal surface of the stomach, and perforating branches were ligated with 4-0 silk ties (Fig. 3). Care was taken to avoid contact of the electrocautery with the gastroepiploic vessels. The gastroepiploic vascular bundle, with an outer diameter of approximately 3 mm, was handled carefully with Silastic vascular loops (Quest Medical Inc, Allen, Tex).

A submucosal space was developed and the gastroepiploic bundle, in continuity, was placed adjacent to the gastric mucosa. A 5-mm gastric mucosal defect was created with a scalpel (Fig. 4). The isolated vascular bundle was then placed over the gastric mucosal defect and the seromuscular tissues were then loosely re-approximated over the vascular bundle and

secured with interrupted 2–0 silk sutures (Fig. 5). The average resultant “depth” of vessels was 2 to 3 mm below the gastric mucosa.

A GIF-140 Olympus diagnostic gastroscope was inserted through the mouth of the pig and advanced into the gastric lumen. The site and course of the submucosal vessel was identified with an endoscopic Doppler US probe. The laparotomy was then closed. The previously described modified Jagwire needle-knife was then used to incise the underlying submucosal vascular structures with “pure-cut” electrocautery. Intravenous heparin was not used for this final model.

RESULTS

Aside from minor bleeding from the mucosal edges, no major hemorrhage resulted from the strictly luminal models. Repeated endoscopic biopsies of the gastric mucosa over Doppler-positive areas failed to incise a vessel. Neither repeated resection of mucosa with a cold snare nor incision with a modified needle-knife of the gastric mucosa over submucosal vessels, which were only about 2 mm below the surface of the stomach, resulted in appreciable bleeding, even to the point of perforating the pig stomach. Attempts were not made to recreate this model in the duodenal bulb, due to the “long” porcine stomach and anatomical differences from humans that place the porcine pylorus adjacent to the esophagus, requiring a “retroflexion” position for endoscopic intubation. All of the strictly luminal methods were repeated after administration of a single bolus dose of (18 U/kg) of intravenous non-fractionated heparin and still failed to produce satisfactory bleeding.

Several problems were encountered when attempting this bleeding porcine ulcer model. Electrocautery contact with tissue in close vicinity to the gastroepiploic plexus resulted in vasospasm of the artery and undesired clotting of the vessel. Excessive manipulation or tension on the gastroepiploic plexus also resulted in inadvertent clotting. Careful surgical dissection and handling of the artery plexus with silastic vascular loops was required to prevent occlusion of the vascular plexus.

The body of the stomach along the greater curvature proved to be the ideal place to create the “ulcer site.” Other more proximal locations were more difficult to visualize and localize because they could only be easily imaged in the retroflexed position. The retroflexed endoscope position is more cumbersome because the pig stomach is not fixed, and axial rotation or twisting of the pig stomach can cause disorientation. Simultaneous EGD and laparoscopic transillumination of the gastroepiploic plexus helped in planning the ulcer site.

A combined surgical approach to the gastroepiploic vascular plexus on the serosal side and an endoscopic approach to the luminal surface were required in this porcine model to produce satisfactory arterial bleeding. Oozing of blood to active arterial spurting was observed and depended on the size of the vessel used and the size of the intraluminal needle-knife incision. Active peptic ulcer bleeding was simulated (Fig. 6). Intravenous heparin was not required to produce active arterial bleeding in this combined successful ulcer model. This active peptic ulcer bleeding model was successfully and easily reproduced in a total of 5 sequential pigs.

Of note, the arterial spurting was controlled with placement of a “Resolution” clip (Boston Scientific Co) over the Doppler-positive area. Endoscopic Doppler US probe examination of the ulcer bed after placement of hemoclips revealed a negative Doppler signal. On a separate occasion, a standard endoscopic sclerotherapy needle was used to inject of a total of 10 mL of 1:100,000 dilution of epinephrine into the subsurface tissue to create a mucosal bleb. Thermal coagulation was then performed with a 10F heat probe unit (Olympus Optical Co, Ltd, Tokyo, Japan) lightly applied to the index site with settings of 20J $\times$ 5 pulses. Combination epinephrine injection and heater probe coagulation also produced satisfactory hemostasis of the ulcer model.

A fasting period of 24 hours was used prior to surgery for all of the animals. Although in several animals, some food was observed in the stomach at the time of endoscopy, the relatively small amount of fluid could be easily suctioned and did not hamper our experiments.

DISCUSSION

Porcine models are ideal because they avoid societal concerns associated with other animals such as dogs. Additionally, the cost and time benefits of a 1-stage nonsurvival animal model compared to the previously described 2-stage models are substantial. Prior to successfully creating the combined surgical-endoscopic porcine bleeding ulcer model, several other methods to create hemorrhage were attempted, and failed to produce satisfactory bleeding. These initial methods were restricted to the luminal surface and failed because gastroepiploic vessels of sufficient caliber are on the serosal surface of the pig stomach. The failed models were described as an aid to other investigators. Surgical manipulation of the serosal vessels is required in the pig to place large vessels close to the mucosal surface. The successful nonsurvival gastroepiploic ulcer model described in this study represents an easily reproducible animal model that can be used for testing endoscopic innovations, and for training.

Hu et al^{16,17} previously described a porcine ulcer model that used a divided short gastric artery sutured into the lumen of the stomach through a mucosal defect. An inflatable plastic cuff was placed around the base of the artery; cuff deflation led to massive bleeding. The pigs were anticoagulated with an intravenous bolus of 100 to 300 U/kg of intravenous heparin. These porcine ulcer models were created by using an end-to-side configuration (end of blood vessel to side of stomach), with a completely transected short gastric artery. In contrast, our ulcer model used a surgically isolated but intact gastroepiploic plexus with a side-to-side configuration (side of blood vessel to side of stomach) that is similar to peptic ulcer hemorrhage in humans. Massive bleeding was initiated with a needle-knife incision of the ulcer bed rather than by deflating an arterial cuff. Our model did not permit binary control of arterial blood flow, but simulated a bleeding submucosal vessel. No heparin was used in our successful bleeding gastric ulcer model.

There are several important technical aspects in the creation of our successful porcine bleeding ulcer model that merit discussion. When selecting the initial surgical seromyotomy site, it is important to choose a site close to and parallel to the main gastroepiploic vascular

bundle, to avoid excessive tension when completing the model. It is also important to choose a site easily accessible intraluminally by the endoscope. When isolating the gastroepiploic vessels, care should be taken to avoid direct contact of the electrocautery with the vessels, as vasospasm and undesired occlusion can result. In addition, the main gastroepiploic artery is relatively fragile and clots easily. Silastic vascular loops are helpful in manipulating the vascular bundle. Another important technical aspect is that the seromuscular tissues should be loosely re-approximated over the isolated gastroepiploic artery to avoid compression and occlusion of the vessel. The gastroepiploic pulse can be palpated following completion of the tunneling to ensure maintenance of arterial flow through the vessel. Before closing the laparotomy, the endoscope should be advanced into the stomach to ensure localization of the surgically created ulcer site.

The body of the stomach along the greater curvature proved to be the ideal place to create the “ulcer site.” Other more proximal locations were more difficult to visualize and localize because they could only be easily imaged in the retroflexed position. The retroflexed endoscope position is more cumbersome because the pig stomach is not fixed, and axial rotation or twisting of the pig stomach can cause disorientation. However, it is important to note that bleeding sites in the retroflexed position can be important to simulate in order to obtain competence in treating humans, in whom bleeding locations may present in challenging locations.

Another consideration is creating 2 or more “bleeding ulcer sites” at different locations. This would provide versatility in testing different treatment modalities or allow for multiple practice locations. Although this may be a possibility in some cases, the authors have experienced limitations in that shock from blood loss often limits the creation of multiple “bleeding” sites.

Of note, a 1-stage nonsurvival model was chosen by the authors due to ethical and cost concerns of a survival model. However, a survival model could potentially allow for the evaluation of “rebleeding” and “healing.” This concept could be an area of future investigations.

Importantly, non-living “ex-vitro” organ models, such as the “compactEASIE,” have been reported in the medical literature. Many of these models have shown benefits in facilitating learning of endoscopic procedures by fellows.^{19,20} Different endoscopic hemostasis techniques and equipment have even been compared using these ex-vitro organ models.^{21,22} For the purpose of training workshops, ex-vitro models have some advantages over live models, such as lower costs, reduced ethical concerns, and easier access for multiple hemostasis sites per specimen. However, there are certain training situations in nonhemostasis related techniques, such as for endoscopic US, which have not been simulated with ex-vivo tissue. A prior study showed benefit of live porcine models for training in endoscopic US.²³ Within such endoscopic training courses that use live animals, our live porcine bleeding model could be incorporated to provide similar benefits to that shown with ex-vivo model training, and are potentially more realistic. Our live porcine model would also be useful in the development, testing, and comparison of new endoscopic devices and techniques.

Our study describes lessons learned from failed ulcer models and successful combined surgical-endoscopic techniques for the creation of a nonsurvival porcine model that simulates active peptic ulcer bleeding. This animal model of an actively bleeding submucosal vessel, akin to a bleeding gastric ulcer, can be used to study future endoscopic therapies and may also be useful for endoscopic training in hemostasis techniques.

Abbreviations:

U	unit
UGIB	upper gastrointestinal bleeding

REFERENCES

1. Kozak LJ, Owings MF, Hall MJ. National Hospital Discharge Survey: 2002 annual summary with detailed diagnosis and procedure data. *Vital Health Stat* 2005;13:1–199.
2. Kozak LJ, Owings MF, Hall MJ. National Hospital Discharge Survey: 2001 annual summary with detailed diagnosis and procedure data. *Vital Health Stat* 2004;13:1–198.
3. Vreeburg EM, Snel P, de Bruijne JW, et al. Acute upper gastrointestinal bleeding in the Amsterdam area: incidence, diagnosis, and clinical outcome. *Am J Gastroenterol* 1997;92:236–43. [PubMed: 9040198]
4. Rockall TA, Logan RF, Devlin HB, et al. Risk assessment after acute upper gastrointestinal haemorrhage. *Gut* 1996;38:316–21. [PubMed: 8675081]
5. Czernichow P, Hochain P, Noursbaum JB, et al. Epidemiology and course of acute upper gastrointestinal haemorrhage in four French geographical areas. *Eur J Gastroenterol Hepatol* 2000;12:175–81. [PubMed: 10741931]
6. Laine L, Peterson WL. Bleeding peptic ulcer. *N Engl J Med* 1994;331: 717–27. [PubMed: 8058080]
7. Rockall TA, Logan RF, Devlin HB, et al. Incidence of and mortality from acute upper gastrointestinal haemorrhage in the United Kingdom. Steering Committee and members of the National Audit of Acute Upper Gastrointestinal Haemorrhage. *BMJ* 1995;311:222–6. [PubMed: 7627034]
8. Protell RL, Silverstein FE, Piercey J, et al. A reproducible animal model of acute bleeding ulcer—the “ulcer maker.” *Gastroenterology* 1976;71: 961–4. [PubMed: 992279]
9. Johnston JH, Jensen DM, Auth D. Experimental comparison of endoscopic yttrium-aluminum-garnet laser, electrosurgery, and heater probe for canine gut arterial coagulation. Importance of compression and avoidance of erosion. *Gastroenterology* 1987;92:1101–8. [PubMed: 3493938]
10. Dennis MB, Silverstein FE, Gilbert DA, et al. Evaluation of Nd:YAG photocoagulation using a new experimental ulcer model with a single bleeding artery. *Gastroenterology* 1981;80:1522–7. [PubMed: 7194830]
11. Randall GM, Jensen DM, Hirabayashi K, et al. Controlled study of different sclerosing agents for coagulation of canine gut arteries. *Gastroenterology* 1989;96:1274–81. [PubMed: 2784774]
12. Deng XY, Zheng ZT. [An animal model of peptic ulcer induced by destruction of dopaminergic neurons]. *Zhonghua Nei Ke Za Zhi* 1994;33: 313–6. [PubMed: 7835142]
13. Pinkas H, McAllister E, Norman J, et al. Prolonged evaluation of epinephrine and normal saline solution injections in an acute ulcer model with a single bleeding artery. *Gastrointest Endosc* 1995;42: 51–5. [PubMed: 7557177]
14. Mall AS, Hickman R, Terblanche J, et al. The pig as an ulcer model. *Gastroenterology* 1997;113:366–7.
15. Terblanche J, van Hoorn-Hickman R. The prevention of peptic ulceration by highly selective vagotomy in a new peptic ulcer experimental model: the bile duct-ligated pig. *Surgery* 1978;84:206–11. [PubMed: 684612]

16. Hu B, Chung SC, Sun LC, et al. Developing an animal model of massive ulcer bleeding for assessing endoscopic hemostatic devices. *Endoscopy* 2005;37:847–51.
17. Chiu PW, Hu B, Lau JY, et al. Endoscopic application of massively bleeding peptic ulcer by using the Eagle Claw VII device: a feasibility study in a porcine model. *Gastrointest Endosc* 2006;63:681–5. [PubMed: 16564872]
18. Wong RC. Endoscopic Doppler US probe for acute peptic ulcer hemorrhage. *Gastrointest Endosc* 2004;60:804–12. [PubMed: 15557966]
19. Hochberger J, Matthes K, Maiss J, et al. Training with the compactEASIE biologic endoscopy simulator significantly improves hemostatic technical skill of gastroenterology fellows: a randomized controlled comparison with clinical endoscopy training alone. *Gastrointest Endosc* 2005;61:204–15. [PubMed: 15729227]
20. Maiss J, Wiesnet J, Proeschel A, et al. Objective benefit of a 1-day training course in endoscopic hemostasis using the “compactEASIE” endoscopy simulator. *Endoscopy* 2005;37:552–8. [PubMed: 15933929]
21. Maiss J, Baumbach C, Zopf Y, et al. Hemodynamic efficacy of the new resolution clip device in comparison with high-volume injection therapy in spurting bleeding: a prospective experimental trial using the compactEASIE simulator. *Endoscopy* 2006;38:808–12. [PubMed: 17001570]
22. Maiss J, Dumser C, Zopf Y, et al. “Hemodynamic efficacy” of two endoscopic clip devices used in the treatment of bleeding vessels, tested in an experimental setting using the compact Erlangen Active Simulator for Interventional Endoscopy (compactEASIE) training model. *Endoscopy* 2006;38:575–80. [PubMed: 16802269]
23. Bhutani MS, Wong RF, Hoffman BJ. Training facilities in gastrointestinal endoscopy: an animal model as an aid to learning endoscopic ultrasound. *Endoscopy* 2006;38:932–4. [PubMed: 16981113]

Capsule Summary**What is already known on this topic**

- Peptic ulcer models, mainly in dogs, have required a 2-stage procedure to simulate active bleeding of submucosal arterial vessels in peptic ulcer disease, an expensive process that may cause the animal pain while the ulcer develops.

What this study adds to our knowledge

- Successful simulation of active peptic ulcer bleeding was achieved in 5 sequential pig models by developing a submucosal plane, placing the gastroepiploic bundle adjacent to the mucosa, and re-approximating the seromuscular tissues over the vascular bundle.

Figure 1.
Endoscopic view of Doppler-positive submucosal vessels identified by simultaneous laparoscopic transillumination.

Figure 2.
Seromyotomy along the external surface of greater curvature of the stomach.

Figure 3.
Isolated gastroepiploic vascular bundle.

Figure 4.
A 5-mm-long gastric mucosal defect.

Figure 5.
Re-approximated seromuscular tissue over isolated gastroepiploic vascular bundle.

Figure 6.
Actively bleeding gastroepiploic ulcer model.