Bacteremia and endotoxemia after endoscopic submucosal dissection for gastric neoplasia: pilot study

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Abstract

Background Because the invasive procedure of endoscopic submucosal dissection (ESD) entails a large mucosal defect which is left open, with extensive submucosal exposure to the indigenous bacterial flora, the procedure may have a substantial risk for bacteremia. Our aim was to examine gastric ESD-related bacteremia and endotoxemia in gastric neoplasia patients.

Methods In patients who underwent ESD for superficial gastric neoplasia, blood cultures and plasma endotoxin measurements were done before, immediately after, and on day 2 after ESD. Clinically manifest infections and inflammatory markers, including C-reactive protein (CRP) and white blood cells, were monitored.

Results Fifty patients (aged 69 ± 8 years; mean $\pm$ SD) were enrolled. The diameter of the resected specimens was 38 ± 18 mm and the procedure time of ESD was 66 ± 53 min. Two percent (2/100) of blood cultures after ESD were positive, with findings as follows:

Propionibacterium species immediately after ESD, and *Enterobacter aerogenes* on day 2 after ESD, but no clinically manifest infection was observed. In 30% of the enrolled patients, CRP on day 2 after ESD had increased to levels higher than 1.0 mg/l. Plasma endotoxin levels, immediately after and on day 2 after ESD were correlated with CRP levels on day 2 after ESD.

Conclusions In spite of the invasive procedure with massive submucosal exposure to the indigenous bacterial flora, gastric ESD has a low risk for bacteremia. Gastric ESD-related endotoxemia may be linked to inflammatory reactions such as those shown by the increase of CRP or fever observed after ESD.

Keywords Bacteremia · Endotoxemia · Endoscopic submucosal dissection (ESD) · Gastric neoplasia

Introduction

The issue of antibiotic prophylaxis in gastrointestinal endoscopy has evoked considerable controversy in recent years. Although patients at high risk of endocarditis were traditionally recommended to receive antibiotics for all endoscopic procedures [1–3], in recent guidelines antibiotic prophylaxis is no longer recommended for the prevention of infective endocarditis [4, 5]. The reasons for this dramatic change are: (1) cases of infective endocarditis associated with endoscopic procedures are anecdotal, and no data demonstrate a conclusive link between endoscopic procedures and the development of infective endocarditis, and (2) no data exist that demonstrate that antibiotic prophylaxis prevents infective endocarditis after endoscopic procedures. Although antibiotic prophylaxis solely to prevent infective endocarditis is no longer recommended, in

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the recent guidelines of the American Society for Gastrointestinal Endoscopy (ASGE) [6] and the new British guidelines [7] antibiotic prophylaxis to prevent infections other than infective endocarditis is indicated for some endoscopic procedures, such as percutaneous endoscopic gastrostomy or fine needle aspiration of cystic lesions along the gastrointestinal tract.

Endoscopic submucosal dissection (ESD) [8–10] is a novel, promising endoscopic technique for gastrointestinal neoplasia because it achieves higher rates of radical en-bloc resection in comparison to conventional endoscopic mucosal resection (EMR) [11–13]. However, the ESD-induced mucosal defect is usually left open, without endoscopic closure, and extensive submucosal exposure to indigenous bacterial flora in the gastrointestinal tract may cause bacteremia and/or endotoxemia. Indeed, fever or an increase of inflammatory markers such as C-reactive protein (CRP) and white blood cells (WBCs) is often observed after ESD. Because the rates of bacteremia or endotoxemia associated with ESD are not well demonstrated to date, no guidelines mention the indication for antibiotic prophylaxis for this new invasive endoscopic therapy. Therefore, we conducted the present study to elucidate the status of ESD-related bacteremia and endotoxemia, using a new *Limulus* amoebocyte lysate (LAL) test that can measure plasma endotoxin levels with high sensitivity [14].

Patients and methods

Patients referred for gastric ESD between January and October 2008 were considered for enrollment in this study. The following exclusion criteria were applied: (1) patients who had cardiac lesions regarded to be at high risk for the development of infective endocarditis, as demonstrated by the ASGE guidelines [6]; (2) patients who had received antibiotics for any reason within the previous 7 days; (3) patients who had possible signs of any infection at the time of the procedure; and (4) patients who had chronic inflammatory diseases such as rheumatic arthritis or inflammatory bowel diseases.

The indication for ESD was gastric adenoma (category 4.1 of the revised Vienna classification) [14] larger than 2 cm in diameter, and gastric carcinoma that was diagnosed by preoperative endoscopy as intramucosal (category 4.4 of the revised Vienna classification) [14]. If the eligible carcinoma had ulceration, the indication was restricted to that smaller than 3 cm in diameter, and if not, those of any size were indicated for the procedure. ESD was done as follows. Using magnifying endoscopy combined with narrow band imaging (GIF-H260Z and EVIS 260 Spectrum; Olympus Medical Systems, Tokyo, Japan), circumferential markings were achieved by brief bursts of cautery

with the tip of a Hook knife (KD-620LR; Olympus Medical Systems), a few millimeters away from the margin of the target lesion. Then, 0.5% sodium hyaluronate (Mucoup®; Seikagaku Industry, Tokyo, Japan) in 10% glycerine solution with 0.025% epinephrine and 0.05% indigo carmine were injected submucosally. A circumferential incision was made a few millimeters outside the marked spots with a needle knife (KD-1L-1; Olympus Medical Systems) and/or the Hook knife, using the Drycut and/or Swiftcoag mode of Erbotom Vio (ERBE Elektromedizin, Tübingen, Germany). Submucosal dissection was then performed with the Hook knife using the Drycut and/or Swiftcoag mode. The ulcer created in this way after resection was carefully examined, and any visible vessels and adherent clots were coagulated using a hemostatic forceps (HDB2418W-W; Pentax, Tokyo, Japan) in the Soft coag mode for preventing post-ESD hemorrhage. ESD specimens were pinned flat onto a corkboard with adequate tension and immersed in formalin. The maximal diameters of the resected specimens were measured on the corkboard. If two or more lesions were resected simultaneously, the sum of the diameters of these specimens was tentatively used as the diameter. All endoscopic procedures were recorded on a mini-digital video. The length of the procedure time for ESD was calculated from the video recording. The elapsed time from the beginning of the circumferential marking to the end of the prophylactic coagulation after ESD was used as the procedure time for ESD. Patients had nothing per os from the day of the ESD procedure to day 3 after the ESD. The basic allowance of fluid was dripped intravenously, but no antibiotic was administered if no signs of clinically manifest infections were observed. CRP and peripheral blood counts were examined in the morning of day 2 after ESD for assessing inflammatory reaction and anemia. Patients started taking standard liquid meals from day 3 after ESD and rice gruel from day 4 after ESD. During the hospitalization, with an average stay of 7 days, infectious and hemorrhagic complications were carefully monitored.

Three sets of blood cultures were obtained, and processed by a standard technique [15, 16], before ESD, immediately after ESD, and in the morning of day 2 after ESD. In addition, three sets of plasma were simultaneously obtained for endotoxin measurement. To prevent bacterial contamination, we collected blood without placing any intravenous cannula. The skin site for the sampling was initially cleaned with 70% isopropyl alcohol, followed by air-drying for 30 s. The area was then cleaned with 10% povidone-iodine for 60 s and was allowed to air-dry for another 60 s. Thirty-four milliliters of blood were collected; 30 ml for blood cultures and 4 ml for plasma endotoxin measurement. The 30 ml for blood cultures was equally distributed into commercially available aerobic and

anaerobic blood culture bottles (BD BACTEC™; Becton, Dickinson, Franklin Lakes, NJ, USA). All samples were incubated for 5 days. For samples with positive blood cultures, the laboratory performed species identification studies and determined antibiotic susceptibilities. The 4 ml for plasma endotoxin measurement was equally distributed into two endotoxin-free plastic tubes containing sodium heparin and the tubes were immediately immersed in ice. One of the tubes was centrifuged at 4200g for 40 s, and platelet-rich plasma was collected with sterilized equipment. The other tube was centrifuged at 10,000g for 2 min, and platelet-poor plasma was collected.

Plasma endotoxin levels were measured by endotoxin scattering photometry (ESP) [17]. Briefly, 0.1 ml of plasma was diluted with 0.9 ml of distilled water containing 0.02% (v/v) Triton X-100, and heated for 10 min at 70°C. After cooling on ice for 5 min, an aliquot of the plasma mixed with LAL lyophilized powder (LAL ES of Endotoxin Single Test Wako; Wako Pure Chemical Industries, Tokyo, Japan) was moved to a photometric cuvette for laser scattering photometry (model PA-200 or PA-20, Analytical Software, version 3; Kowa, Nagoya, Japan). Because this assay system directly detects the appearance of the clotting enzyme product induced by endotoxin in the LAL test reaction mixture, it can measure low levels of endotoxin with high accuracy. Standard curves were calculated at every assay using a standard endotoxin (titer of endotoxin: 1 pg equivalent to 0.007 EU; Wako Pure Chemical Industries) dissolved in distilled water (100 ng/ml) and diluted with endotoxin-free, conditioned human plasma for calibration (concentration 0.1–100 pg/ml).

The study was approved by the ethics committee of the Jikei University Hospital and was conducted in accordance with the revised Helsinki Declaration (1989), and informed consent was obtained from all patients. The statistical significance of differences was tested using Pearson's correlation coefficient to evaluate the relationship between the CRP level and the endotoxin level (STATA 11; StataCorp, College Station, TX, USA), and a significant difference was considered to exist for $P < 0.05$. Quantitative data are summarized as means $\pm$ standard deviation (SD).

Results

During the study period, 133 eligible patients underwent ESD. Eighty-four were excluded from the study because they did not agree to participate in it; 51 were assigned to the study with written informed consent. One of these 51 patients suffered from a common cold just before ESD and was withdrawn from the study. Finally, 50 patients (42 men, 8 women; mean age, 69 ± 8 years; range 50–88 years)

underwent blood cultures in accordance with the study schedule. Twenty-nine of the 50 patients additionally underwent the endotoxin test after the setting of the measurement was established (Fig. 1).

In 2 of the 50 patients, two gastric neoplasias were simultaneously resected by ESD. All target lesions were resected in an en-bloc fashion, and no perforation or major bleeding after ESD was observed. The characteristics of the patients, lesions, and procedures are summarized in Table 1. Inflammatory reactions such as fever or increases of CRP and WBCs were observed in some of these patients (Table 1), but no clinically manifest infections such as shivering or a high spike fever were observed.

Blood cultures were positive in 5 of 150 samples in total; 3 before ESD, 1 immediately after ESD, and 1 on day 2 after ESD. Three cultures before ESD grew *Bacillus* sp. ($n = 2$) and *Corynebacterium jeikeium* ($n = 1$), which are normal skin flora and were considered contaminants [18, 19]. One blood culture immediately after ESD grew *Propionibacterium* species; this culture sample was from a 56-year-old male, who had a small gastric cancer and presented no infectious symptoms or signs during or after ESD. One blood culture that grew *Enterobacter aerogenes* on day 2 after ESD was considered true positive. The patient positive for *Enterobacter aerogenes* was an 88-year-old female, who had superficial gastric cancer of type IIc, with a 30×40 mm diameter ESD specimen. Her ESD procedure time was 85 min in total, but she had no clinically manifest infection and no signs of sepsis such as high spike fever or shivering.

In 25 patients who underwent plasma endotoxin measurement, platelet-rich plasma endotoxin levels (pg/ml) before, immediately after, and on day 2 after ESD were 1.6 ± 2.9 , 3.3 ± 5.6 , and 3.0 ± 6.1 , respectively. Platelet-poor plasma endotoxin levels (pg/ml) before, immediately after, and on day 2 after ESD were 8.1 ± 22.4 , 8.1 ± 24.4 , and 12.7 ± 49.8 . No significant relationships between endotoxin levels and clinical factors including age, gender, diameter of lesion, diameter of ESD specimen, length of ESD procedure time, body temperature, and WBC count were observed. In contrast, CRP levels on day 2 after ESD were significantly related to the platelet-rich plasma endotoxin levels immediately after ESD and on day 2 after ESD. However, CRP levels were not related to the platelet-poor plasma endotoxin levels (Fig. 1).

Discussion

The present study showed two (4%) of 50 patients with positive blood cultures after ESD; one culture showed *Propionibacterium* species immediately after ESD, and the other showed *Enterobacter aerogenes* on day 2 after ESD.

Correlation between platelet-rich plasma endotoxin levels and CRP levels

Correlation between platelet-poor plasma endotoxin levels and CRP levels

Fig. 1 Correlation between platelet-rich plasma (PRP) endotoxin levels and C-reactive protein (CRP) levels and correlation between platelet-poor plasma (PPP) endotoxin levels and CRP levels. 2nd ESD day day 2 after endoscopic submucosal dissection

Although *Propionibacterium* species can cause endocarditis [20], they are members of the normal flora of the skin, and a positive culture for *Propionibacterium* species is generally considered as contamination. In contrast, *Enterobacter aerogenes* is generally found in the human

gastrointestinal tract, including the stomach. Patients with gastric cancer were found to harbor bacterial species in the stomach more often than normal controls did [21], probably due to the differences in gastric acidity. The blood culture showing *Enterobacter aerogenes* in one (2%) of the

Table 1 Characteristics of patients, lesions, and procedures

	All patients who underwent blood cultures (<i>n</i> = 50)	Patients who also underwent endotoxin measurement (<i>n</i> = 25)
Age (years, mean ± SD)	69 ± 8	69 ± 8
Gender	M 42 F 8	M 22 F 3
Complications related to ESD (perforation or major bleeding)	0	0
Diameter of resected specimen (mm, mean ± SD)	38 ± 18	39 ± 21
Procedure time (min, mean ± SD)	66 ± 53	69 ± 59
BT after ESD		
BT <37°C	44 (88%)	23 (92%)
37°C ≤ BT < 37.5°C	3 (6%)	1 (4%)
37.5°C ≤ BT < 38.0°C	1 (2%)	0 (0%)
38°C ≤ BT	2 (4%)	1 (4%)
ESD endoscopic submucosal dissection, CRP C-reactive protein, BT body temperature, 2nd ESD day day 2 after ESD	Increase of CRP (>1.0 mg/l) on 2nd ESD day	5 (20%)
	Increase of WBC (>10,000/mm ³) on 2nd ESD day	10 (40%)

50 patients in our study was considered to be true positive. The patient positive for *Enterobacter aerogenes* presented no clinically manifest infections, suggesting a transient bacteremia.

In ESD, because the large ESD-induced mucosal defect is left open, with extensive submucosal exposure to the indigenous bacterial flora, this procedure may have a substantial risk for bacteremia, with a higher rate in comparison to conventional EMR. However, in the present study, we have demonstrated that gastric ESD induced a 2.0% rate of bacteremia, with no clinically manifest infection. The low rate of bacteremia associated with gastric ESD is comparable to the rate associated with conventional EMR for gastric neoplasias [22]. Min et al. [23] have also demonstrated that colonic EMR, or even colonic ESD (small numbers of 10 ESD cases) is considered a low-risk procedure for bacteremia. These findings, taken together with the results of our study, lead us to consider that the rates of bacteremia related to the radical endoscopic procedure of ESD are as low as those of conventional endoscopic procedures, other than variceal sclerotherapy or esophageal bougienage. However, inflammatory symptoms or signs after gastric ESD are observed more often than has been supposed from the low rate of ESD-related bacteremia. In the present study, indeed, 4 and 12% of patients had fever of more than 38 and 37°, respectively, and 30% of patients showed increases of CRP >1.0 mg/ml.

We speculated that ESD related-endotoxemia may be one of the possible causes of inducing the inflammatory reactions, and tested the hypothesis. Using a highly sensitive endotoxin assay system, we have shown that platelet-rich plasma endotoxin levels immediately after and on day 2 after ESD were correlated with CRP levels on day 2 after

ESD. The data suggest that ESD-related endotoxemia is one of the causes of ESD-related inflammatory reactions.

In the present study, CRP levels on day 2 after ESD were not correlated with platelet-poor plasma endotoxin levels. Platelet-rich plasma contains platelets and many WBCs, but platelet-poor plasma contains no WBCs. Because WBCs englobe endotoxin that has acutely penetrated into the circulating blood [24], the endotoxin yield from platelet-rich plasma may directly reflect the acute levels of endotoxin that may have penetrated into the circulation from the stomach via an ESD-induced mucosal defect. However, the reason for the discrepancy between our findings for platelet-rich plasma and platelet-poor plasma is still not known. Because the present study has a limitation in that plasma endotoxin levels were analyzed in only half of the enrolled patients, further study with a large sample size may elucidate the reason for this discrepancy.

The present study was not designed directly to elucidate the necessity for prophylactic antibiotic administration in ESD, and a further study to be done in a randomized comparative fashion is required for that purpose. However, we suppose that prophylactic administration of antibiotics may not be necessary in patients who undergo ESD, because the rate of ESD-related bacteremia is as low as the rate of bacteremia induced by conventional endoscopy.

In conclusion, gastric ESD has a relatively low risk of bacteremia, and gastric ESD-related endotoxemia may cause inflammatory reactions such as the increase of CRP and fever, which are often observed after gastric ESD.

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Conflict of interest Drs. Masayuki Kato, Mitsuru Kaise, Toru Obata, Jin Yonezawa, Hirobumi Toyozumi, Noboru Yoshimura,

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