Influence of gastric endoscopic submucosal dissection on serum opsonic activity measured by chemiluminescence

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This study aimed to elucidate whether changes in serum opsonic activity measured by lucigenin-dependent chemiluminescence and luminol-dependent chemiluminescence are useful for estimating physical stress during the perioperative period of gastric endoscopic submucosal dissection. Serum opsonic activity in the peripheral blood of 87 patients was examined in the morning of the day of endoscopic submucosal dissection, the next day, and at 4 days after endoscopic submucosal dissection. Peak height and area under the curve for lucigenin-dependent chemiluminescence were 106.1 ± 22.7% and 102.0 ± 24.7% on the day of endoscopic submucosal dissection, which increased significantly to 113.6 ± 29.4% and 111.0 ± 29.1% on the next day (both p<0.01), and 112.4 ± 27.0% and 110.0 ± 28.1% at 4 days after endoscopic submucosal dissection (both p<0.01), respectively. In contrast, significant changes were not observed in peak height and area under the curve for luminol-dependent chemiluminescence during the perioperative period of endoscopic submucosal dissection. This difference suggests that serum opsonic activity during the perioperative period of gastric endoscopic submucosal dissection is associated with the production of substances with lower oxidizing potential.

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Materials and Methods

Patients. Patients who underwent ESD in Hiroki University Hospital from January 2016 to February 2017 were prospectively entered into the study. During the study period, 110 consecutive patients were enrolled. We excluded patients who had a history of liver cirrhosis, were undergoing artificial dialysis, had other malignancies, and/or were using immunomodulatory drugs because SOA would be modulated under these conditions. Finally, 87 patients (mean age: 73.5 ± 7.6 years, 59 male patients) were studied (Fig. 1). ESD was performed using a conventional single channel endoscope (GIF-Q260J, GIF-H260 or H290; Olympus, Tokyo, Japan) with hood. The ESD procedure was performed using a water jet short needle-knife with a small ball tip (Flush Knife BT-S; DK2620J, Fujinon, Tokyo, Japan), a water jet hook knife (Hook Knife J; KD-625, Olympus, Tokyo, Japan), and a high frequency generator with an automatically controlled system, ICC200 or VIO300D (both supplied by RFBE, Tübingen, Germany). In all patients, we initially used pethidine hydrochloride

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110 patients with gastric cancer underwent ESD

Did not consent to participate 14 cases  
Chronic renal failure 3 cases  
Had other malignant tumor 4 cases  
Withdrew consent 2 cases  
Excluded 23 patients

Enrolled in the study 87 patients

Fig. 1. Flowchart of this study.

(25–100 mg/patient), together with midazolam or diazepam. All ESD procedures were performed by board-certified endoscopists of the Japan Gastroenterological Endoscopy Society.

Blood samples were obtained in the morning on the day of ESD, the next day, and 4 days after ESD, under fasting over 12 h and non-smoking condition over 24 h, while resting in bed. Blood sampling was performed from the intermediate antecubital vein using a vacuum blood collection tube. Serum samples were separated by centrifugation at 3,000 rpm for 10 min, after allowing the blood to clot for 30 min at room temperature. Serum samples were stored at −80°C until analysis. The number and differential counts of white blood cells (WBC) was analyzed by an XE-5000 (Sysmex, Kobe, Japan). The serum level of C-reactive protein (CRP) was measured by a JCA-BM6070 (EOL Ltd., Tokyo, Japan).

Complications (C3 and C4), and 50% hemolytic complement activity (CH50) were also measured by turbidimetric immunoassay. 

Table 1. Patient characteristics

| Parameter                              | Total patients | Age (years)a | 73.5 ± 7.6 (53–86) |
|----------------------------------------|----------------|--------------|---------------------|
| Sex                                    | Male:Female    | 59:28        |                     |
| Patients with multiple lesion b        | 34 (39%)       |              |                     |
| Histopathological diagnosis of the total 127 lesions b | 90 (70.0%) |              |                     |
| Indication lesion                      | 27 (21.2%)     |              |                     |
| No-indication lesion                   | 10 (8.0%)      |              |                     |
| Total resection area (cm²) a           | 12.0 ± 7.6 (2.7–41.2) |          |                     |
| Operation time (min) b                 | 123.8 ± 54.2 (42–249) |          |                     |
| Complications b                        |                |              |                     |
| Bleeding                               | 3 (4.6%)       |              |                     |
| Perforation                            | 1 (1.1%)       |              |                     |
| Fever (>38°C)                          | 7 (8.0%)       |              |                     |

Data are presented as mean ± SD (range). aData are presented as a percentage of positive case (percentage).
The resection area was computed by approximation as an ellipse with the length and breadth of the resection specimen. In the 27 patients with multiple lesions, the total resection area of all lesions was computed. Postoperative complications were as follows: perforation in 1 patient (1.1%), postoperative bleeding in 4 (4.6%), and fever >38°C in 7 (8.0%). All patients were treated successfully, and none required additional surgical treatment.

Serial changes in peripheral leukocytes, neutrophil count and CRP. WBC and the number of neutrophils increased significantly on the next day and at 4 days after ESD compared to those on the day of ESD (p < 0.001, Table 2). The increase in WBC and neutrophils was smaller at 4 days after ESD than on the next day of ESD. CRP increased significantly on the next day of ESD (p < 0.001), and further increase was observed at 4 days after ESD (p < 0.001).

Perioperative changes in the serum. Table 3 shows the changes in the serum levels of immunoglobulins and complements. The IgG level decreased significantly on the next day and at 4 days after ESD compared with that on the day of ESD (p < 0.001). However, no significant changes were observed in the IgA level. The IgM level decreased significantly on the next day (p < 0.01), but recovered at 4 days after ESD.

The C3 and C4 levels increased significantly on the next day of ESD (p < 0.05, p < 0.001, respectively) and at 4 days after ESD (p < 0.001, respectively). The CH50 level increased significantly at 4 days after ESD (p < 0.001), although there were no differences between the day of ESD and the next day of ESD.

Perioperative changes in SOA. Figure 2 shows the PH and AUC of LgCL. PH of LgCL was 106.1 ± 22.7% on the day of ESD and increased to 113.6 ± 29.4% on the next day (p < 0.01), and to 112.4 ± 27.0% at 4 days after ESD (p < 0.01). The AUC of IgG was 102.0 ± 24.7% on the day of ESD, and a significant increase was observed on the next day (111.0 ± 29.1%; p < 0.01) and at 4 days after ESD (110.0 ± 28.1%; p < 0.01). Both PH and AUC tended to decrease at 4 days after ESD compared to those on the next day of ESD. However, as shown in Fig. 3, significant changes were not observed in PH and AUC of LmCL during the perioperative period. The PH and AUC of Lm CL were 98.9 ± 20.6% and 100.7 ± 17.8% on the day of ESD, 103.8 ± 19.3% and 102.3 ± 22.5% on the next day, and 102.3 ± 22.3% and 103.7 ± 19.1% at 4 days after ESD, respectively.

We also examined the influence of total resection area, operation time, and histological type on the change of SOA (Table 4). Total resection area and operation time was divided into two groups according to their mean values (mean total resection area: 12.0 cm², mean operation time: 123.8 min). The histological type of the tumor was also divided into two groups: tub1 or other types. In all groups, AUC measured by LgCL increased both on the next day and at 4 days after ESD compared with that on the day of ESD. However, AUC was not significantly different in all groups.

Discussion

In this study, SOA to stimulate neutrophil ROS production was increased during the perioperative period of ESD for gastric cancer. SOA was highest on the next day of ESD and continued to increase even at 4 days after ESD. Therefore, the increase in SOA after ESD might not only be caused by the physical stress of the procedure but also by gastric mucosal inflammation, including post-ESD ulceration.

In previous studies, changes in SOA were assessed using LgCL and LmCL. Neutrophils produce O$_2^-$ mediated by NADPH oxidase activated by phagocytosis, and O$_2^-$ is rapidly converted to hydrogen peroxide (H$_2$O$_2$) either spontaneously or by superoxide dismutase. On the other hand, neutrophil azurophilic granules contain large quantities of MPO, which is discharged by the process of degranulation and reacts with H$_2$O$_2$ and Cl$^-$ to produce HOCl. These ROS produced by neutrophils have different toxicities. HOCl has a significantly higher oxidizing potential than its precursors O$_2^-$ and H$_2$O$_2$ (15-18) LgCL reflects the production of O$_2^-$, whereas LmCL is recognized to reflect total ROS production including HOCl. SOA measured by LgCL was reported to be increased by intense exercise and training including rugby sevens matches, (30) long-term judo training, (31) and intense sumo training. (28)

With respect to SOA measured by LmCL, no significant changes were observed in the perioperative period of spinal surgery, which is consistent with the results observed in our study. (32) From the results obtained by LgCL and LmCL in this study, the change in SOA during the perioperative period of gastric ESD may enhance production of substances with lower oxidizing potential, such as O$_2^-$, rather than those with higher oxidizing potential.

In the present study, the number of neutrophils increased significantly on the next day of ESD and at 4 days after ESD. The increase in the number of circulating neutrophils in parallel to physical stress after the surgical operation has been well demonstrated (32-36) These studies showed 2.64- to 4.48-fold increase in peripheral neutrophil counts on the day after gastrectomy. (35-37,38) On the other hand, the present study showed a 2.41-fold increase

| Table 2. Changes in WBC, neutrophils and CRP |
|----------------------------------------------|
| The day of ESD | The next day of ESD | 4 days after ESD |
|----------------|---------------------|-----------------|
| WBC (x10$^3$) | 5.18 ± 1.325        | 8.940 ± 2.285*  |
| Neutrophils (x10$^3$) | 2.797 ± 99         | 6.735 ± 145*   |
| CRP (mg/dl)    | 0.202 ± 0.617       | 1.102 ± 1.268* |

Data are presented as mean ± SD. *p<0.001, vs the day of ESD. WBC, white blood cells; CRP, C-reactive protein; ESD, endoscopic submucosal dissection.

| Table 3. Changes in the levels of immunoglobulin and complements |
|---------------------------------------------------------------|
| The day of ESD | The next day of ESD | 4 days after ESD |
|----------------|---------------------|-----------------|
| IgG (mg/dl)    | 1,214.5 ± 319.1     | 1,168.3 ± 300.6*** |
| IgA (mg/dl)    | 258.6 ± 123.2       | 255.6 ± 130.8    |
| IgM (mg/dl)    | 70.5 ± 31.9         | 68.7 ± 31.5**    |
| C3 (mg/dl)     | 101.2 ± 21.8        | 103.2 ± 22.1*    |
| C4 (mg/dl)     | 25.6 ± 7.1          | 27.1 ± 7.6***    |
| CH50 (U/ml)    | 55.6 ± 16.3         | 56.5 ± 11.8      |

Data are presented as mean ± SD. *p<0.05, **p<0.01, ***p<0.001, vs the day of ESD. ESD, endoscopic submucosal dissection.
Fig. 2. Changes in PH and AUC of LgCL. Results on the next day of ESD and at 4 days after ESD are compared with those on the day of ESD. Results are expressed by percentages of the values obtained from standard serum as 100%. PH, peak height; AUC, area under the curve; LgCL, lucigenin-dependent chemiluminescence; ESD, endoscopic submucosal dissection. *p<0.01.

Fig. 3. Changes in PH and AUC of LmCL. Results on the next day of ESD and at 4 days after ESD are compared with those on the day of ESD. Results are expressed by percentages of the values obtained from standard serum as 100%. PH, peak height; AUC, area under the curve; LmCL, luminol-dependent chemiluminescence; ESD, endoscopic submucosal dissection.
in peripheral neutrophil counts after gastric ESD. Therefore, the increase in inflammatory cytokines to accelerate neutrophil migration induced by the physical stress of ESD and gastric mucosal inflammation would be lower than that induced by gastric surgery. Immunoglobulins and complements play an important role in SOA. In this study, during the perioperative period of gastric ESD, serum levels of IgG, IgA, and IgM tended to decrease, whereas those of C3, C4, and CH 50 increased significantly. With respect to the changes in neutrophil counts, immunoglobulins seemed to be consumed by the inflammatory response, whereas complements were increased due to nonspecific immunity. Our previous study on metabolic changes in patients during the ESD perioperative period also indicated that the energy requirements increased slightly on the next day of gastric ESD. However, in the present study, total resection area, operation time, and tumor histological type were all not associated with the change in SOA. Therefore, in terms of the physical stress of ESD, the inflammatory response after the procedure could be more relevant than procedural characteristics itself as the size of the resection area and operation time.

This study has some limitations. First, this study was performed at a single institute. However, our ESD procedure was performed according to the standard methods approved by the health insurance system of Japan. Therefore, it is likely that similar results would be obtained even when multicenter studies are performed. Second, no control group was included in this study to assess the physical stress associated with surgery. However, it is difficult to perform surgery for small gastric cancers at an early stage. Finally, the physical stress assessed in this study cannot be entirely attributed to physical stress due to the ESD procedure. During the perioperative period, other factors such as fasting and sedation would also be associated with the observed physical stress. In conclusion, SOA measured by CL can be used to estimate the physical stress of ESD safely and easily. Therefore, this method could be an option that allows the study of physical stress of new techniques with lower invasiveness in numerous clinical fields. The difference between LgCL and LmCL suggests that SOA during the perioperative period of gastric ESD is associated with the production of substances with lower oxidizing potential. The results support the recognition that ESD a less invasive procedure.

**Author Contributions**

TA, DC and TS wrote the manuscript and interpreted the data. TA, KS, KA, KM, NA, SS and SH performed data management and analysis. DC conceived the study design. TT, HK, HH, MS, HS, TM, SN, and SF played a role in reviewing and revising the manuscript. All authors approved the final draft of the manuscript.

**Abbreviations**

AUC area under the curve
CH50 50% hemolytic complement activity
CL chemiluminescence
CRP C-reactive protein
ESD endoscopic submucosal dissection
HBSS Hanks’ Balanced Salt Solution
HOCI hypochlorous acid
H2O2 hydrogen peroxide
LgCL lucigenin-dependent chemiluminescence
LmCL luminol-dependent chemiluminescence
MPO myeloperoxidase
OZ opsonized zymosan
O2•− superoxide anions
PH peak height
ROS reactive oxygen species
SD standard deviation
SOA serum opsonic activity
WBC white blood cells

**Conflict of Interest**

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