round channel of the knife clamp; this jet produces straight, narrow water flow that is easily targeted.

There is concern that the water-irrigation function of the endosurgical knife may replace that of an endoscope. As shown in this study, we consider that the 2 mechanisms of water irrigation from the endoscope and knife are very useful for facilitating a less stressful and less complicated ESD procedure. We recommend loading water into the water-jet supplier of the endoscope and normal saline or Glyceol into that of the knife. The former should be mainly used to wash out the blood or mucus of the target area in order to keep endoscope view clean. The latter can also be used to keep the endoscope view clean, but its main uses are to precisely identify the bleeding points from a small vessel during the procedure and provide an additional submucosal fluid cushion without changing the device. The alternative use of conductive and nonconductive fluids may provide an avenue for further research.

In summary, the technical outcomes of ESD completed with the second-generation endosurgical knife known as the splash-needle were not less successful than those of the first-generation knives. This novel knife unquestionably has several functional advantages that enable a step forward in ESD. Further accumulation of knowledge and cases verifying the usefulness of the knife and a study comparing the splash-needle with the first-generation endosurgical knives are warranted.

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ORIGINAL ARTICLE

Gut and Liver, Vol. 2, No. 2, September 2008, pp. 95-98

Submucosal Injection of Normal Saline can Prevent Unexpected Deep Thermal Injury of Argon Plasma Coagulation in the *in vivo* Porcine Stomach

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Background/Aims: There have been several reports of thermal injury induced by argon plasma coagulation (APC) in animal models, but no follow-up studies have revealed the actual thermal injury. **Methods:** APC was performed on the stomachs of two living minipigs with and without prior submucosal injection of normal saline. The power and argon gas flow were set to 60 watts and 2 L/min, respectively, and pulse durations of 5, 10, and 20 seconds were used. One of the minipigs was killed immediately thereafter and the other was killed 1 week later. **Results:** The minipig killed immediately showed only subtle differences between noninjected and injected injuries under all the conditions, and the usefulness of prior submucosal injection was not obvious. However, the minipig killed 1 week later had a deep ulcer extending to the deeper muscle layer at the noninjected site where APC had been applied for 20 seconds, whereas tissue injury of the injected site was limited to the submucosal layer. **Conclusions:** Unexpected tissue damage can occur even using a short-duration APC. Prior submucosal injection for APC might be a safer alternative technique, especially in a thinner and narrower gut wall. **(Gut and Liver 2008;2:95-98)**

Key Words: Argon; Submucosa; Injection; Animal model; Thermal

INTRODUCTION

The safety of argon plasma coagulation (APC) has been reported in clinical practice and the rate of severe complications such as perforation and stricture was less than 1%.[1-7] Previous study using the fresh resected porcine models shows that tissue damage caused by APC may be limited up to the submucosal layer under the condition generally used,[8,9] and prior submucosal injection may be further safe to prevent deep tissue destruction.[9-11] However, the obtained results are only the facts of the resected materials or immediate euthanasia in an *in vivo* study. We sometimes experience unexpected deep ulceration or stenosis after APC at a follow-up endoscopic examination.[3,6,12,13] In this study, we investigate tissue damage on the stomach of a living minipig to confirm the safety of APC and the usefulness of prior submucosal injection in a living body, including a follow-up case.

MATERIALS AND METHODS

1. Preparation of study animals

Two living minipigs (Sus scrofa; Miniature Swine) were used for this study, which were provided from Chugai Research Institute for Medical Science, Inc., Shizuoka, Japan, and the use for research purpose was fully approved by the institution.

2. Endoscopic procedures

Endoscopic procedures were performed on the minipigs under general anesthesia and mechanical ventilation after overnight fasting. The stomachs were sufficiently inflated

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with air after the residual food and mucus on the mucosal surface were washed out with tap water that was splashed from the instrumental channel of an endoscope. After these preparations, the application of APC over the mucosa was performed on the body of the fully-insufflated stomachs with or without prior submucosal injection of normal saline, at the setting of the power of 60W and the argon gas flow of 2 L/min. The pulse duration was changed as 5, 10, and 20 seconds. The unit used for application of APC was the standard APC equipment consisting of a high-frequency generator (Erbotom ICC 200), an automatically regulated argon source (APC 300), and a flexible APC applicator, 2.3 mm in diameter. All of them were products of ERBE Elektromedizin, Tubingen, Germany. A flexible APC applicator was inserted into the stomach through the endoscopic channel. Two milliliters of normal saline per site were injected into the submucosal layer for the testing of prior submucosal injection, by using a 23-gauge injection needle. Although it was difficult to apply APC at the same condition, we tried to keep a separation distance of an applicator and tissue approximately 2 mm and the angle 10 to 30 degree. After the coagulation was performed, one minipig was killed without delay and the other was killed after observation for one week.

3. Pathological analysis

The resected stomachs were cut on the points of coagulation and fixed with formalin and embedded in paraffin. A histological section was made from each block and stained with hematoxylin and eosin and examined about tissue damage microscopically.

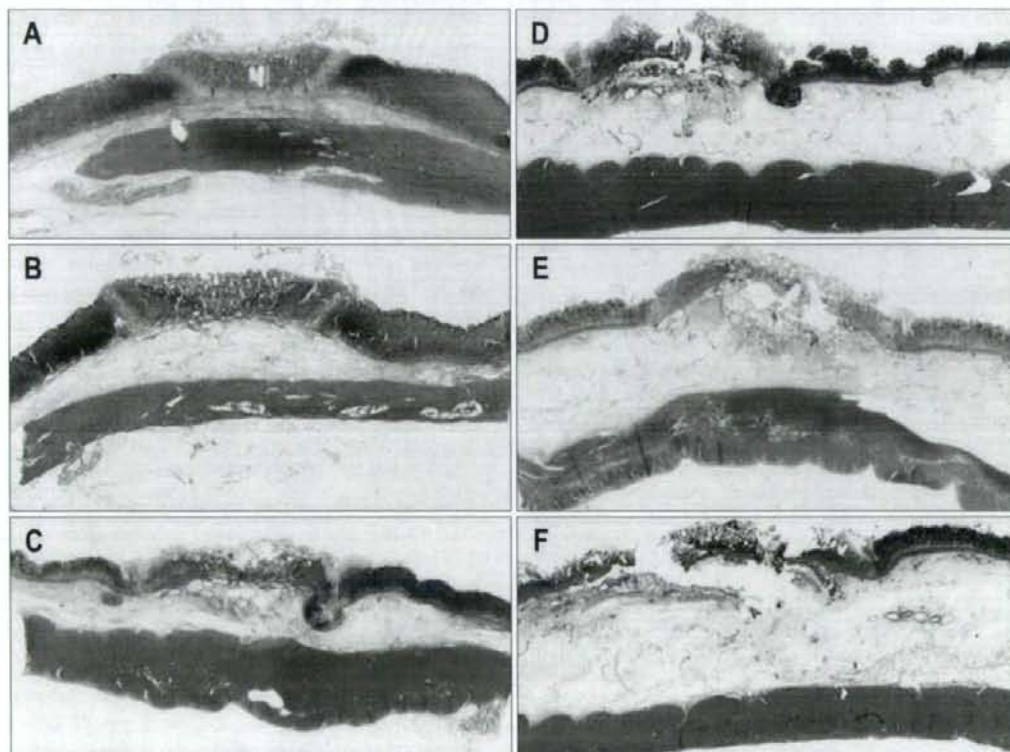

Fig. 1. Tissue injury in a minipig killed immediately after argon plasma coagulation (APC). (A) APC (5 seconds) without prior submucosal injection. (B) APC (5 seconds) after submucosal injection. (C) APC (10 seconds) without prior submucosal injection. (D) APC (10 seconds) after submucosal injection. (E) APC (20 seconds) without prior submucosal injection. (F) APC (20 seconds) after submucosal injection. Tissue coagulation was limited to the deeper submucosal layer under all the conditions. The thermal effects tended to do deeper with a longer pulse duration and no prior saline injection. With prior saline injection, the increased thickness of the submucosal layer might prevent injury to the deeper submucosal layer.

RESULTS

1. Tissue damage of immediate euthanasia

A minipig killed without delay after APC application revealed that tissue damage without injection was extended deeper as the applied time increased and tissue damage with injection was limited to the shallower submucosal layer regardless of the applied time. However, the difference between noninjected area and injected area was subtle and the usefulness of prior submucosal injection was not revealed significantly (Fig. 1).

2. Tissue damage of delayed euthanasia

A minipig killed after one week's follow-up revealed that granulomatous and fibrotic changes existed in the submucosal layer of the artificial ulcers at the pulse durations of 5 seconds and 10 seconds, regardless of submucosal injection. The difference between noninjected injury and injected injury, or between pulse durations of 5 seconds and 10 seconds was not obvious, and injury of the proper muscle layer was not observed in any of those conditions. On the contrary, the noninjected injury of the pulse duration of 20 seconds made the deep ulceration extended to the deeper proper muscle layer, whereas injected injury of the same duration did not extend to the proper muscle layer (Fig. 2).

DISCUSSION

This study may give us two important messages. First, unexpected deep tissue destruction may possibly occur and follow-up study is necessary to find the true damaged area in a living body. Second, prior submucosal injection of normal saline may be useful to prevent deep tissue destruction in a living body and the resected stomach.

The reason why the discrepancy of tissue damage exists between the area with and without follow-up may be the limitations of histology in detectability of thermal damage. One of the characteristics of APC is reported that it uniformly creates deep zones of shrinking, desiccation, coagulation and devitalization, in turn, from the applied surface.[14] However, histological investigation may

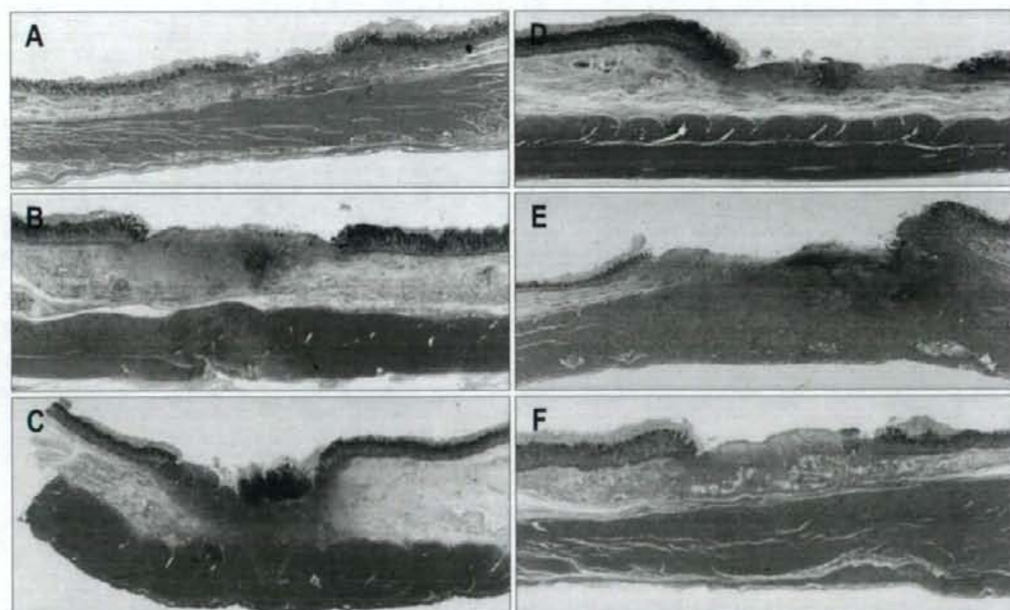

Fig. 2. Tissue damage in a minipig killed 1 week after APC. (A) APC (5 seconds) without prior submucosal injection. (B) APC (5 seconds) after submucosal injection. (C) APC (10 seconds) without prior submucosal injection. (D) APC (10 seconds) after submucosal injection. (E) APC (20 seconds) without prior submucosal injection. (F) APC (20 seconds) after submucosal injection. Under all the conditions except for 20 seconds of APC without prior submucosal injection, granulomatous and fibrotic changes were evident in the submucosal layer of the artificial ulcer, although the actual muscle layer appeared intact. Deep ulceration that destroyed the muscle layer was evident for 20 seconds of APC without submucosal injection.

not detect the zone of devitalization, which means that tissue damage is underestimated than the true one. Follow-up study is necessary to find the true damaged area. In this study, 20 seconds' application of APC resulted in damage limited to the submucosal layer when examined soon after application, but created a deep ulcer with destruction of the proper muscle layer when examined after one week. This result suggests that we have to be aware of late perforation, which may occur a few days to a few weeks after APC application.

Previous study using a resected stomach revealed the usefulness of prior submucosal injection.[9] The present study using a living minipig also supports it. When APC was applied after submucosal injection, tissue damages were limited to up to submucosal layer regardless of time duration. Furthermore, the follow-up study for one week also revealed the same results. In practice, we sometimes experience an unexpected extension of tissue destruction, which may be affected by inevitable various factors; e.g. host factors (mucosal thickness, blood flow, inflammation, etc), technical factors (the extension of the gastric wall by inflated air, the applied angle, the distance between an applicator and tissue, etc).[3,6,12,13] Therefore submucosal injection of normal saline may be essential to get the sufficient results at any encountered situation, without the fear of extensive damage up to the proper muscle layer. Since tissue damage up to submucosal layer is sufficient for treating most of lesions with APC, submucosal injection can become the standard preparation prior to APC application in humans. One recent case series of colonic angiodysplasia showed the safety and efficacy.[15] Further prospective study, including a large number of patients with and without prior submucosal injection will be warranted to show the clinical impact.

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DISCLOSURE

Koji Kashimura is a member of the Product Research Department, Kamakura Research Laboratories, Chugai Pharmaceutical Co., LTD., Kamakura, Kanagawa, Japan; Toyokazu Matsuura is a member of the Chugai Research Institute for Medical Science, INC., Gotenba, Shizuoka, Japan. No funding is related with this work.

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CASE REPORT

Gut and Liver, Vol. 2, No. 3, December 2008, pp. 209-212

Submerging Endoscopic Submucosal Dissection Leads to Successful En Bloc Resection of Colonic Laterally Spreading Tumor with Submucosal Fat

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A 72-year-old female with a colonic laterally spreading tumor (LST) was referred to our department. A total colonoscopy revealed a large nongranular LST, 30 mm in diameter, in the ascending colon. Detailed examination with chromoendoscopy confirmed that the lesion was an intramucosal tumor, and endoscopic submucosal dissection (ESD) was performed. After a circumferential incision around the lifted lesion with a submucosal fluid cushion, diffuse adipose tissue was observed in the submucosal layer beneath the lesion. The endoscopic view was blurred when dissecting the submucosal layer due to fat adhering to the lens. Since this made it difficult to continue the procedures, we infused water into the lumen and kept the endoscope tip immersed in the collected water. The resulting improved view made it possible to complete all procedures without withdrawing the endoscope to wipe the lens. The lesion was successfully resected en bloc without complications. The pathological examination indicated the curative resection of a tubulovillous adenoma. We propose that a submerged ESD could also be an effective procedure for colonic neoplasms with submucosal fat by avoiding blurring of the endoscopic view. **(Gut and Liver 2008;2:209-212)**

Key Words: Colonoscopy; Colonic neoplasms; Submucosa; Resection; Adipose tissue

INTRODUCTION

Endoscopic submucosal dissection (ESD) is a developing therapeutic procedure for neoplasms in early stage of the gastrointestinal tract. The promising clinical outcomes have been reported, but ESD for colorectal neoplasms is performed at a very limited number of institutions even in Japan.[1] One of the major reasons is that there are still some technical difficulties in terms of manipulability of the scope, especially at the proximal colon, including injection, mucosal cutting and submucosal dissection. In dissecting of the submucosal layer, we sometimes encounter unexpected submucosal fat; that is the adipose tissue. In case of lesions with submucosal fat, blurring of view caused by adhesive fat on lens make it difficult to continue procedures smoothly. We present a case of colonic laterally spreading tumor (LST) with submucosal fat, managed with submerging ESD successfully without withdrawing of the endoscope.

CASE REPORT

A 72-year-old obese female with fecal occult blood was referred to our department for total colonoscopy, which revealed a nongranular type LST (LST-NG) at the ascending colon, approximately 30 mm in diameter. Chromoendoscopy revealed type III L pit on the surface. Because LST-NG with III L pit had some possibilities of cancerous lesion, endoscopic submucosal dissection was explained as the treatment procedure and informed consent was obtained for the patient and her family.

A single-channel thin endoscope (PCF-Q240AI; Olympus Co, Tokyo, Japan) with a transparent attachment (D-201-12704; Olympus Co) and a high-frequency automated electrosurgical generator (VIO300D; ERBE Elektromedizin GmbH, Tubingen, Germany) were equipped for the ESD.

A mixture of 1% hyaluronate (Suvenyl; Chugai Pharma-

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ceutical Co, Tokyo, Japan) and 10% glycerin, 5% fructose plus 0.9% saline solution (Glyceol; Chugai Pharmaceutical Co., Tokyo, Japan) was used as the submucosal injection solution. The two solutions were premixed in a ratio of 1:7.

A circumferential incision of mucosa all around the lesion was made by a flex knife (KD-630L; Olympus Co) after a sufficient volume of the submucosal injection. The oral incision of the mucosa was performed, followed by the anal incision at the setting of Endocut I mode (effect 1, duration 3 and interval 3). Submucosal dissection was also performed by a flex knife sequentially at the setting of swift coagulation mode (output 40W, effect 4). To control any visible bleeding, hemostatic forceps (HDB2422W; Pentax Corporation, Tokyo, Japan) were used at the setting of soft coagulation mode (output 50W, effect 5). The details of ESD procedure with a flex knife were described by elsewhere.[2]

During the submucosal dissection, diffuse submucosal fat was encountered beneath the lesion, and adhesive fat on lens blurred the endoscopic view. Since the lesion was located in the ascending colon, it was quite time-consuming to withdraw the endoscope and wipe the lens repetitively. As a sequence, the lumen of the ascending colon was filled with tap water to keep the endoscope tip immersed in water collects. Suctioning the luminal extra air and changing position were useful for submerging procedure. The improved view through the filling water made it possible to identify the submucosal layer and blood vessels clearly. The bleeding was recognized as a spurting of blood into transparent water, identified with relative ease. The lesion was resected in an en bloc fashion (Fig. 1). After removal of the lesion, submerging observation showed a whole image of the artificial mucosal defect without any bleedings or any perforations (Fig. 2). Any visible vessels within the artificial mucosal defect were treated with hemostatic forceps. Thus, we achieved procedures completely and safely. The total ESD procedure time was approximately 60 minutes. The patient recovered well and discharged on the 6th postoperative day. The resected specimen measured 38×25 mm with the tumor occupying an area of 27 mm in longest diameter. The histological assessment showed a tubulovillous adenoma with moderate atypia. Both the vertical and horizontal margins were free of tumor.

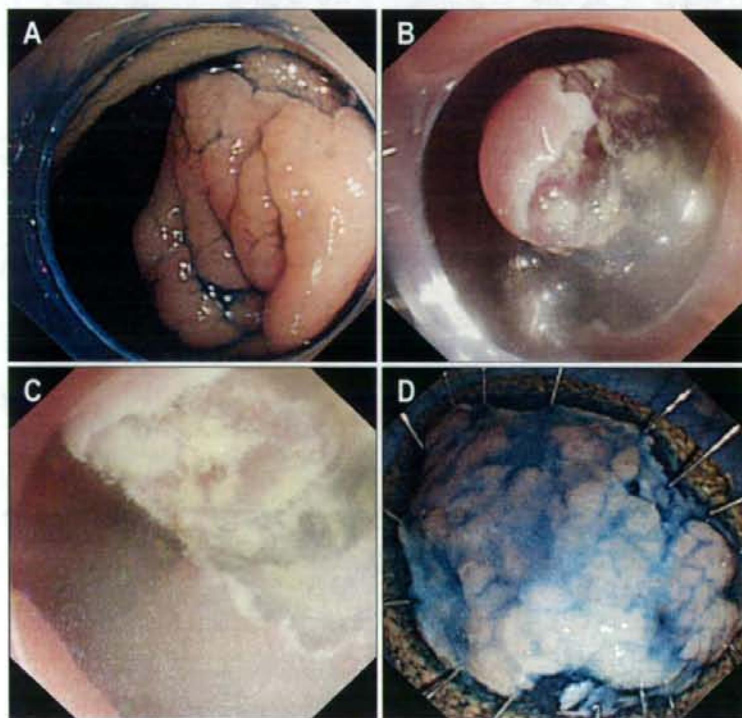

Fig. 1. Submerged endoscopic submucosal dissection. (A) Colonoscopy revealed a nongranular laterally spreading tumor in the ascending colon that had a flat surface and was approximately 30 mm in diameter. (B) After circumferential incision, the resected piece was separated using a flex knife. Fat adhering to the lens blurred the view during this process. (C) The submerged observation markedly improved the view, with precise identification of the submucosal layer enabling us to achieve the procedures. (D) The lesion was resected en bloc. The pathological examination revealed it to be tubulovillous adenoma with moderate atypia and curative excision.

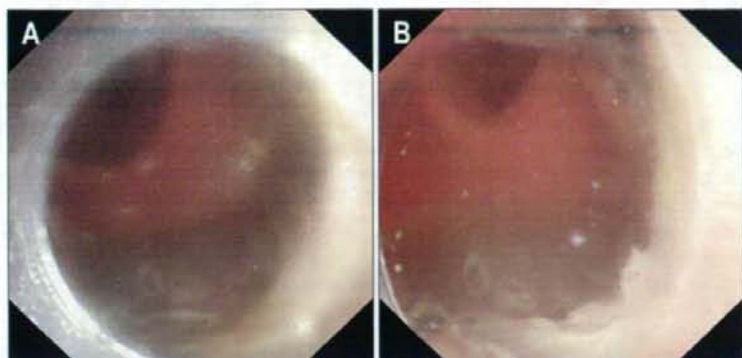

Fig. 2. Submerged observation of the artificial mucosal defect. (A) After resecting the lesion, the endoscopic view was blurred by adhering fat. (B) Submerged observation provided a complete image of the artificial mucosal defect without any bleeding or perforation.

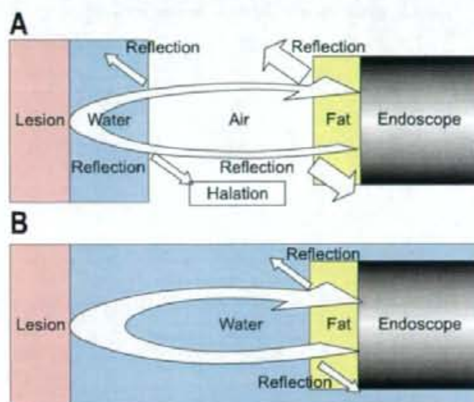

Fig. 3. Scheme of the reflection boundaries in the light pathway. (A) The adhering fat provokes reflections at the boundary with air. The reflectance ratio is higher than that at the boundary of water and air. (B) Filling water removes the boundary between water and air. Reflections occur only at the boundary with water and fat, for which the reflectance ratio is lower.

DISCUSSION

The adipose tissue in the submucosal layer is one of factors making therapeutic endoscopy more difficult due to blurring of view. On computed tomography studies, the adipose tissue in the submucosal layer is reported to be observed more frequently at the proximal colon than at distal colon.[3] Keeping the clear view is essential for therapeutic endoscopy, especially for ESD, but the submucosal fat may disturb the clear endoscopic view. Although the most reliable solution to this problem is wiping the lens after withdrawing the endoscope, at the proximal colon, repetitive cannulation and withdrawing of endoscope is troublesome and painful for patients. In this case, we tried submerging ESD and achieved all the procedures of ESD successfully with improved view through the filled water.

The endoscope illuminates the luminal structures with light guides on the tip, and detects the reflected light on the lesion with its charge coupled device (CCD) on the tip. The adhesive fat on lens not only reduces light intensity due to its turbidity, but also provokes reflection in several boundary lines in the light pathway (Fig. 3A). The reflectance ratio of light is defined with the difference in refractive index of two substances neighboring. The refractive index of air is lower than that of water and that of fat, and the effect of reflection at the boundary line of fat and air is higher than that of fat and water. In the submerging ESD, filling water between the endoscope and the lesion can reduce total reflectance ratio of the light pathway (Fig. 3B). In addition, water on the surface of the lesion can cause the diffuse reflection. This type of reflection can be observed as halations on the video monitor of endoscopy system. In the submerging ESD, filling water also cancels the boundary line with air and reduces diffuse reflection on the surface of the lesion (Fig. 3B). These effects might improve the view and enable us to achieve the procedures more smoothly.

A colonoscopic technique with water filling into the lumen is reported in several papers, especially about insertion technique.[4] In this insertion technique, complete removal of air is reported to be necessary. They also mention improvement of endoscopic view in the water. However, there are no reports of usefulness of transparent view for therapeutic endoscopy, including ESD.

Of course, filled water can affect transmission of electric current changes generated by high-frequency automated electrosurgical generator. In our case, we equipped the advanced generator with automatically controlled sys-

tem, which enabled stabilization of cutting and coagulation effects under various conditions.[5] Some generators in lower versions may not be expected to have similar effects in submerging ESD.

ESD is still in a developing stage for colonic neoplasms, although promising for large lesions or lesions with submucosal fibrosis. To complete the procedures safely and smoothly, keeping clear view is essential. We propose the submerging ESD can be an effective procedure to avoid blurred view caused by submucosal fat, especially at the proximal colon.

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