Author's response to reviews

Title: Usefulness and safety of 0.4 percent sodium hyaluronate solution as a submucosal fluid cushion for endoscopic resection of colorectal mucosal neoplasms: A prospective multi-center open-label trial

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Author's response to reviews: see over
Dear Dr. Zauner,

Thank you for your kind review. I was pleased to learn that you found this case interesting.

Point-by-point responses to the comments are as follows.

【Referee 1】

・ The authors should clarify the final concentration of the 0.4% SH, since epinephrine and indigo carmine were added to the solution at the discretion of the endoscopist. They should at least be able to give a range of the final concentration of SH (or an estimate).

(Response)
We added following explanation to the results and discussion section.
Fourteen patients (34.1%: 14/41) had indigo carmine added to 0.4% SH, and the range of the amount used was from 0.2 mL to 1.0 mL. Fourteen patients (34.1%: 14/41) had epinephrine added to 0.4% SH, and the range of the amount used was from 0.1 mL to 1.0 mL. The final concentration of SH was from 0.38% to 0.4%. In this study, we allowed epinephrine and indigo carmine to be added to submucosal injection solutions. We did not investigate the potential effect of volumes of indigo carmine and epinephrine because we used such small amount of these solutions. Therefore, we believe that additional indigo carmine and epinephrine had an insignificant effect on the final concentration of SH.

・ Figure 1: What happened to the 6 incomplete resections described in figure 1 but not in the result section?

(Response)
We added following explanation to the results section.
Six patients were judged as incomplete resection. Two patients did not have safe vertical and lateral margin because of multifragment resections (Although one more patient had multifragment resection, the patient was judged to have had a complete resection because all the neoplasm was contained in one fragment). The margin of the fragment was positive for tumor or the lateral margin of the lesion could not be evaluated histologically because of the effects of the electrosurgical current or mechanical damage in 4 patients.

・ Figure 2 does not add additional information. The numbers are already presented in table 4.

(Response)
We removed Figure 2 as suggested.
· Table 1 and Table 6 are difficult to comprehend (even with the table description). The tables should be better presented. Table 6: What does variable (n=41) stand for. Since only 40 patients were evaluated, (1 patient was excluded due to exclusion criteria as presented in figure 1)

(Response)

As suggested, the table descriptions were revised. The outcome measures of one patient were excluded because this patient had violated one of the exclusion criteria 1 day after endoscopic resection. However, the patient’s data were included in adverse event analysis because the patient used 0.4% SH in his endoscopic resection. As you suggested, we revised from “variable (n=41)” to “All patient = 41” at Table 6. In addition, the table descriptions were revised too.
1) The study has a number of substantial limitations which may significantly reduce the scientific and technical value of the overall investigation conducted by the Japanese colleagues.

(Response)

The study is a prospective open-label study. Although the study had only a single-arm and a relatively small sample size, the findings of the current study shed light on the usefulness of HA as an aid for surgical operation when compared with available historical control data and reported findings from studies making use of other agents as a surgical aid.

2) The first limitation regards the size of the sample size. The authors considered that “the reported rate of en bloc complete resection with normal saline as the submucosal injection solution is 72.9-85%. Hence, when the rate of en bloc complete resection for the 0.4% SH was set at 90%, of 35 patients was required in order to bound the hypothesized rate of resection with 95% confidence interval”. For this statement the authors cited references 16-18 which are very old and really not fitting the topic of the rate of en-bloc resection. It's very easy to find more recent data with higher rate of en-bloc resection for lesions smaller than 20 mm. This means that the sample size should be calculated on different basis which may require much higher number of patients.

(Response)

Our extensive literature review with PubMed, Web of Science, and Medline did not show recent research findings that reported higher rate of normal saline en-bloc resection for lesions smaller than 20 mm than the rates (72.9-85%) we used as a basis for sample size calculation. (However, we found that a research article titled “Effectiveness of Glycerol as a Submucosal injection for EMR” (Gastrointestinal Endoscopy 2005, Vol 61, No. 6) reports the en-bloc resection rate of 66.7% for laterally spreading tumors with size less than 20 mm, which is lower than 72.9-85%.) This suggests that even though the rates of 72.9-85% for en-bloc resection with normal saline were reported in journals about 10 years ago, those rates should still hold some validity. In light of this, the rates should still serve as a useful basis for sample size calculation.

3) The second crucial point is the size of the lesions. The authors have included lesions between 5 and 20 mm which normally are very easily removed en-bloc with the technique of standard polypectomy. For these lesions injection of HA is not really required at all and the rate of en-bloc resection is not influenced by the injected substance rather than the small size. This bias prevent the correct evaluation of one of the major outcome of the study: the en-bloc resection.

(Response)

This clinical trial was conducted on the patients with colorectal mucosal neoplasm to evaluate the use of 0.4% sodium hyaluronate solution in EMR or ESD. The other purpose of this study was
ensuring the same results as those from the Gastric study* so that its results could become part of the application to Ministry of Health, Labor, and Welfare of Japan for the manufacture of the said medical device. Therefore, this study was conducted with the same study design, sites, and study periods as those for the Gastric study*. As mentioned in Methods section in the manuscript, the guideline of the Japanese Society of Colorectal Cancer Research stated that the size of colorectal neoplasm that can be resected en bloc with reasonable effort is 20 mm or less. Moreover, neoplasms of more than 20 mm in size are frequently resected in multiple pieces in typical operations. Therefore, we defined the size of limit to be less than 20 mm.

While small tumors are certainly easier for resection than large tumors, successful en-bloc resection or complete resection are not guaranteed for the tumors with size less than 20mm when the technique of standard polypectomy is used. The article “Effectiveness of Glycerol as a Submucosal injection for EMR” (Gastrointestinal Endoscopy 2005, Vol 61, No. 6) reports the normal saline en-bloc resection rate of 66.7% (46/69) and the complete resection rate of 34.8% (24/69) for laterally spreading tumors smaller than 20mm. Our data shows that HA can achieve a rate of complete resection (62.5%, 5/8 for laterally spreading tumor smaller than 20mm), which is much higher than the rate of 34.8% for the historical placebo data reported in Gastrointestinal Endoscopy 2005 article.


4) Despite the large number of involved centers and small size of the lesions different technique of mucosal resection and dissection have been used by the authors. This limits again the statistical analysis making impossible correlations between the HA injection and the different subgroups of patients selected for the specific mucosal resection technique adopted.

(Response)

By establishing and limiting the size of neoplasms to be resected en bloc in this study, we aimed to control one potential source of bias that varying neoplasm sizes may make resections considerably different in difficulty levels of the operation. In addition, all techniques of mucosal resection and dissection involved submucosal fluid cushion during endoscopic resection. Therefore, we think that there is negligible difference among those techniques used as far as the evaluation of the effectiveness of the SH injection in en bloc resections is concerned.

5) The criteria adopted to evaluate the usefulness of HA injection are quite subjective and not in line with previous studies conducted to evaluate the usefulness of different solutions for submucosal injection.

(Response)

As there were no published clinical outcome measures that could indicate the performance of injection solutions, we defined an outcome measure using complete en bloc resection that was an
indicator of the degree of completion endoscopic resection and the counting of additional injections. However, the counting of additional injections was a measurement that an operating surgeon could have a subjective influence on. Therefore, we addressed it in the Discussion section as a limitation. Yamamoto et al had already published the result of the use of 0.4% SH using the same outcome measures.

Reference

6) The lesion morphology is not specified by the authors. This should be clearly indicated.

(Response)
This study was designed on the assumption that we selected patients who had the need of endoscopic resection. According to the guidelines for the Japanese Society for Cancer of the Colon and Rectum, the indications for endoscopic resection are defined as an intramucosal cancer or a smaller extent of submucosal invasion cancer. Since the morphology is not needed for their diagnosis, we did not specify any criteria on morphology in this study.

7) Despite the small size of the lesion, the rate of complication is high as compared with previous series of EMR for colonic lesions. This should be commented by the authors who should also discuss the role of HA in preventing complications.

(Response)
Adverse events were assessed in all 41 patients who underwent endoscopic resection. In this study, adverse events were seen in 19 of the 41 patients (46.3%). This appears to be a high rate of adverse events, because we collected all minor adverse events that were recorded in the medical records in strict compliance with GCP (Good Clinical Practice). The major complications were bleeding during endoscopic resection (7.3% (3/41)) and postoperative bleeding (9.7% (4/41)) (Table 6). Those adverse event rates in our study are slightly higher than the adverse event rates reported by Uraoka et al.: the bleeding during endoscopic resection (0.9% for glycerol and 7.0% for saline) and the postoperative bleeding (6.4% for glycerol and 4.4% for saline). The slightly elevated bleeding rates in our study are thought to be unrelated to the use of SH and to be comparable to the bleeding rates reported in Uraoka et al.’s study using glycerol and saline. Nonetheless, it should be kept in mind that because unexpected bleeding may occur during colorectal endoscopic resection and such bleeding is very hard to predict in advance, one should pay careful attention to the possibility of bleeding during endoscopic resection using 0.4% SH as well. We followed your suggestion and modified the discussion and conclusion section accordingly.

8) The conclusions of the study are not supported by the study design,
methodology and results.

(Response)
Since some outcome measurements had elements of subjective evaluations, the study had some limitations. Therefore, we addressed them in the Discussion section as a limitation of the study. However, because the usefulness rate we investigated also took account of the counting of additional injection solutions, our findings indicated the feasibility of 0.4% SH in colorectal endoscopic resection.

9) Economic impact of the use of HA for colonic EMR should be discussed

(Response)
This issue is addressed in the Discussion section.
1. Please explain the logistics of the study. It is unclear to me a) how patients were recruited to the study, b) at which time patients were informed about the experimental nature of the procedure. On page 6 the authors state that informed consent was obtained from 44 patients. Had all these patients already undergone a previous diagnostic colonoscopy? Usually when one sees a neoplastic lesion during colonoscopy, the lesion will be taken out in the same session. How was such a situation treated in the study?

(Response)
   a) Informed consent was obtained from 44 patients. Two patients withdrew informed consent before endoscopic resection. One patient didn’t have a target neoplasm that was defined in our clinical protocol. Therefore, the final patients who underwent endoscopic resection using 0.4% SH were 41 patients. We added this explanation to the figure legend.
   b) Forty-four patients underwent confirmation colonoscopy within 4 weeks of the due date of endoscopic resection. If a patient had already undergone colonoscopy within 4 weeks before informed consent, the data for that colonoscopy was used as part of the study data to avoid another colonoscopy in consideration for the patient’s bodily condition. Therefore, no patient underwent endoscopic resection at the same time as colonoscopy. We added this explanation to the methods section.

2. The primary outcome measure is unclear. On page 8 the authors write “The primary outcome measure was assessed by comprehensively evaluating en bloc complete resection…and the lifting and maintaining of a mucosal lesion during endoscopic resection…and (Table 1)”. Table 1 indicates that the primary endpoint is a kind of score along two dimensions: completeness of en bloc resection AND need for additional injections (if that is what count stands for in the table). Then the authors define “usefulness rate” as the percentage of en bloc complete resections that needed a maximum of 1 additional injection. The usefulness rate corresponds to the sum of percentages with total evaluations being Excellent or Good. Which one of these measures was considered as the primary outcome measure?

(Response)
   The primary outcome measure was defined such that it evaluated the proportion of the patients completing en bloc resection with zero or one additional injection of 0.4% SH during operation. In other words, the primary outcome measure (usefulness rate) indicated the ratio of patients who were evaluated “Excellent” or “Good”.

3. The report on the size of the study is difficult to understand. The authors state that the a "sample size of 35 patients was required in order to bound the hypothesized rate of resection with 95% confidence interval". What do you
mean? In fact any sample with more than 2 observations can yield a 95% confidence interval. Since the measure is a proportion, the size of the study will mainly affect the width of the 95% confidence interval. Please explain why 35 patients were needed! What was it that you wished to achieve by including 35 patients rather than 70 or 90 or any other number?

(Response)

The intention of calculating a sample size of 35 patients was to keep the length (precision) of the confidence interval to ±10% from the success rate when the anticipated en-bloc resection rate was assumed to be 90%. In this case, the CI length of ±10% was deemed appropriate because it allowed for a reasonable degree of precision on the result and it bounded the hypothesized rate of resection without exceeding the parameter boundary of [0, 1] (ie. The upper limit of the confidence interval of a rate should not exceed 1.).

Then:

$$\text{Half-Length of CI} = 1.96 \times \text{SE(success rate in a binomial sample of size n)} = 0.1$$

Thus, $$1.96 \times \sqrt{(0.9) \times (1 - 0.9)/n} = 0.1$$

Therefore, $$n = 1.96^2 \times 0.9 \times (1 - 0.9)/0.1^2 \approx 35.$$  

We have revised the sentence in question in order to add clarity as below:

“sample size of 35 patients was required in order to bound the hypothesized rate of the 90% resection rate with 95% confidence interval of the length ±10%.”

4. The discussion contains no comparative data. The only place I could find some kind of comparative data was in the description of statistical methods where the authors state that injections with normal saline enables en bloc resection in 72.9%-85% of cases. This is an interesting figure with which the results of this study should be compared. I think this information should be included somewhere in the discussion. Is the "usefulness rate" of the present study any better?

(Response)

This issue is addressed in the Discussion section.

Added reference


5. The discussion mainly repeats some of the results but I find very little discussion of findings in relation to results from other studies. I also find very little reasoning of what the findings from the present study should lead up to. Should we use sodium hyaluronate rather than hypertonic saline or any other injection fluid in the future?
6. Results/Table 2. Age is usually not a normally distributed variable. It would be better to give the median and the range.

(Response)
The table was modified as suggested.

7. Discussion. The reasoning in the beginning of the discussion includes a statement "Endoscopists often could not adequately lift and maintain lesions..." that lacks verification or a reference. If the authors have data on other types of injections this would have been interesting for the reader. Please, rephrase or add reference.

(Response)
We added references in the sentence.
1. Why were drugs contraindicated in the treatment of peptic ulcer prohibited?

(Response)

It would be difficult for us to judge the cure rate of ulcer after endoscopic resection if peptic ulcer drugs were used. Therefore, such drugs were contraindicated to help with clinical evaluation.

2. Most of the polyps were small and one could have removed without injection.

(Response)

This clinical trial was conducted on the patients with colorectal mucosal neoplasm to evaluate using 0.4% sodium hyaluronate solution in EMR or ESD. The other purpose of this study was ensuring the same results as those from the Gastric study* so that its results could become part of the application to Ministry of Health, Labor, and Welfare of Japan for the manufacture of the said medical device. Therefore, this study was conducted with the same study design, sites, and study periods as those for the Gastric study*. As mentioned in Methods section in the manuscript, the guideline of the Japanese Society of Colorectal Cancer Research stated that the size of colorectal neoplasm that can be resected en bloc with reasonable effort is 20 mm or less. Moreover, neoplasms of more than 20 mm in size are frequently resected in multiple pieces in typical operations. Therefore, we defined the size of limit to be less than 20 mm.

While small tumors are certainly easier for resection than large tumors, successful en-bloc resection or complete resection are not guaranteed for the tumors with size less than 20mm when the technique of standard polypectomy is used. The article “Effectiveness of Glycerol as a Submucosal injection for EMR” (Gastrointestinal Endoscopy 2005, Vol 61, No. 6) reports the normal saline en-bloc resection rate of 66.7% (46/69) and the complete resection rate of 34.8% (24/69) for laterally spreading tumors smaller than 20mm. Our data shows that HA can achieve a rate of complete resection (62.5%, 5/8 for laterally spreading tumor smaller than 20mm), which is much higher than the rate of 34.8% for the historical placebo data reported in Gastrointestinal Endoscopy 2005 article.


3. The usefulness rate is arbitrary, subjective, and has not been validated.

(Response)

As there were no published clinical outcome measures that could indicate the performance of injection solutions, we defined an outcome measure using complete en bloc resection that was an indicator of the degree of completion endoscopic resection and the counting of additional injections. However, the counting of additional injections was a measurement that an operating surgeon could have a subjective influence on. Therefore, we addressed it in the Discussion section as a limitation.
Yamamoto et al had already published the result of the use of 0.4% SH using the same outcome measures.

4. Steepness is also subjective.

(Response)
  It's true that evaluation of steepness could be subjective. The Discussion section was modified to include it as one of potential limitations.

5. This is a single-arm, non-blinded study.

(Response)
  The Discussion section was modified to include it as one of potential limitations.

6. I don't see how the sample size calculation was of any benefit. The numbers are not large enough to show a difference – at best would be an equivalency to data published on saline.

(Response)
  The research study was a single-arm study. Thus, our intention in calculating the sample size was not for the purpose of proving superiority of HA against a placebo. Rather, it was for the purpose of securing a sample size that is capable of yielding results with a reasonable amount of precision with which we can make an inference on the effectiveness of HA as a surgical aid.

To be more specific, we wanted to keep the length (precision) of the confidence interval to ±10% from the success rate when the anticipated en-bloc resection rate was assumed to be 90%. In this case, the CI length of ±10% was deemed appropriate because it allowed for a reasonable degree of precision on the result and it bounded the hypothesized rate of resection without exceeding the parameter boundary of [0, 1] (ie. The upper limit of the confidence interval of a rate should not exceed 1.).

7. The types of EMR differed amongst endoscopists making it difficult to tell what the true benefit of the HS is.

(Response)
  By establishing and limiting the size of neoplasms to be resected en bloc in this study, we aimed to control one potential source of bias that varying neoplasm sizes may make resections considerably different in difficulty levels of the operation. In addition, all techniques of mucosal resection and dissection involved submucosal fluid cushion during endoscopic resection. Therefore, we think that there is negligible difference among those techniques used as far as the evaluation of the effectiveness of the HA injection in en bloc resections is concerned.
8. Adverse events occurred in almost half the patients.

(Response)
Adverse events were assessed in all 41 patients who underwent endoscopic resection. In this study, adverse events were seen in 19 of the 41 patients (46.3%). This appears to be a high rate of adverse events, because we collected all minor adverse events that were recorded in the medical records in strict compliance with GCP (Good Clinical Practice).

9. Healing at 8 weeks would be expected regardless of whether HS was used or not.

(Response)
Some report showed that SH stimulated wound healing after endoscopic resection. However, there was no statistically significant difference between using saline and 0.4% SH in the gastric study that Yamamoto et al reported. However, because this study was not a controlled study, we could not describe this issue in this manuscript.
【Referee 5】
Thank you very much for your review.

We thank the reviewers for their detailed comments and hope that the revised manuscript will be accepted for publication in BMC Gastroenterology.

Sincerely,

Shoji Hirasaki, M.D.