COMPARATIVE RESULTS OF GASTRIC SUBMUCOSAL INJECTION WITH HYDROXYPROPYL METHYLCELLULOSE, CARBOXYMETHYLCELLULOSE AND NORMAL SALINE SOLUTION IN A PORCINE MODEL

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ABSTRACT – ***Context*** - Endoscopic mucosal resection is an established modality for excision of sessile lesions in the gastrointestinal tract. Submucosal fluid injection creates a cushion and may prevent thermal injury and perforation. ***Objectives*** - This blind study investigated the performance of three different solutions to create submucosal fluid cushions in porcine stomach. ***Methods*** - Three solutions were injected in the stomach of nine pigs BR1: normal saline solution, carboxymethylcellulose 0.5% and hydroxypropyl methylcellulose 0.25%. In each pig, submucosal injections with 6 mL per test-solution were performed. One drop of methylene blue was added to all injections for better visualization. The time for the bleb to disappear was recorded. ***Results*** - The overall median time of visible submucosal cushion was 37 minutes (range 12–60 min) for hydroxypropyl methylcellulose, 31 minutes for carboxymethylcellulose (range 10–43 min) and 19 minutes for normal saline solution (range 8–37 min). There was no statistically significant difference neither between normal saline solution and carboxymethylcellulose ($P = 0.146$) nor carboxymethylcellulose and hydroxypropyl methylcellulose ($P = 0.119$) but the median duration of hydroxypropyl methylcellulose was significantly longer than normal saline solution ($P = 0.039$). ***Conclusions*** - The length of hydroxypropyl methylcellulose submucosal fluid cushion is longer in comparison with normal saline solution. The median time for carboxymethylcellulose was not longer than normal saline solution. Hydroxypropyl methylcellulose, in the concentration of 0.25%, may be a durable alternative for submucosal injection.

HEADINGS – Endoscopy, digestive system. Gastrointestinal neoplasms. Saline solution, hypertonic. Carboxymethylcellulose. Methylcellulose. Swine.

INTRODUCTION

Since 1984, endoscopic mucosal resection (EMR) has been used in the treatment of early cancers and precancerous lesions of the gastrointestinal tract[1, 11, 12, 14, 21, 28]. The objectives of performing EMR are to remove superficial neoplastic lesions and to obtain specimens for accurate pathologic staging[20].

One of the major complications of EMR is perforation[15, 16]. The most effective method of preventing perforation is to create an adequate submucosal fluid cushion (SFC) between the lesion and the muscle layer by submucosal injection[4]. A SFC lifts the mucosa around the target lesion facilitating resection techniques and protecting from deeper tissue injury.

Normal saline solution (NSS) is the most popular solution for use in EMR, it is considered safe and has low cost[9, 22]. Even though, it is difficult to maintain a suitable level of tissue elevation after injection of NSS[18]. A long-lasting SFC is necessary in lengthy procedures or in piecemeal resection of large sessile polyps. Many solutions have been tested for submucosal injection during EMR[2, 3, 7, 13, 19, 21, 23, 24, 25, 26]. Recently, solutions with high viscosity, such as sodium hyaluronate (SH), hydroxypropyl methylcellulose (HPMC) and fibrinogen have been used. Although the duration of mucosal elevation has improved, the ideal solution has not been founded yet[8]. SH is reported to create longer lasting SFCs[4, 5, 6, 25]. However, it is expensive, needs specific storage requirements and has to be manipulated before

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use. Hence, it is necessary to find a less expensive and long-lasting solution as an alternative to SH.

This blind study compared the durability of three cheap solutions with SH, regarding the length of SFCs in porcine stomach. There are no previous reports on the use of HPMC and carboxy methylcellulose (CMC) in this concentration to evaluate its duration. Both solutions, which have high viscosity, have been used in ophthalmology.

METHODS

The gastric wall of nine female "BR1" pigs, about 35 kg, was injected with each of the three tested solutions. Endoscopy was performed with diagnostic endoscopes (GIF-v2- Olympus®, Melville, NY, United States). The animals were kept in a liquid diet for 12 hours and submitted to tracheal intubation and general anesthesia with acepromazin, ketamine, thiopental and halothane.

In each pig, three injections (6 mL per test-solution) were administered into submucosa at separate sites (minimal distance of 2 cm) in the animal stomach.

The stomach was chosen as the test site because it is technically easy and it is possible to study more than one SFC simultaneously. A standard 23-gauge, working length of 200 cm with outer diameter of 2.3 mm (Boston Scientific®, Natick, MA, United States) catheter injection needle was used.

Three solutions were studied: NSS, CMC and HPMC, in concentration 0.9%, 0.5% and 0.25%, respectively. All solutions were at room temperature. One drop of methylene blue was added to each solution for better visualization of submucosal diffusion. If the mucosa did not elevate after injection of 2 mL, the needle was reinserted at a different place until a successful SFC was created.

A SFC was defined as the prompt appearance of a spreading and enlarging bleb of solution with a semitransparent appearance (Figure 1). Timing began after the specific test solution (6 mL) was injected into the submucosa. Timing was stopped when the bleb had completely flattened. SFC time was reported rounded off to the nearest minute.

The same endoscopist evaluated all bleb duration and he did not have knowledge which solution was being injected.

This study was approved by Ethic Committee of our institution. All animals were painless killed at the end of study in accordance with the Internationals Norms of Protection to the Animals.

Statistical analysis

Continuous variables were expressed as mean (95% confidence interval) and median. Submucosal cushions time were plotted using the Kaplan-Meier method and compared with log-rank test[10]. The statistical analysis was made with Minitab 12.2 (Minitab, State College, PA). *P* values less than 0.05 were considered statistically significant.

RESULTS

Normal saline cushions had median duration of 19 min (range 8-37 min). The median length for CMC injection was 31 min (range 10-43 min). The time of HPMC injection was 37 min (median) with 12 to 60 minutes. There was no difference between SFC length between NSS and CMC ($P = 0.146$) or between CMC and HPMC ($P = 0.119$). However, the median length of HPMC was significantly longer than NSS ($P = 0.039$). Table 1 shows individual results and Figure 2 shows the comparative analysis using survival curve according to the Kaplan-Meier method.

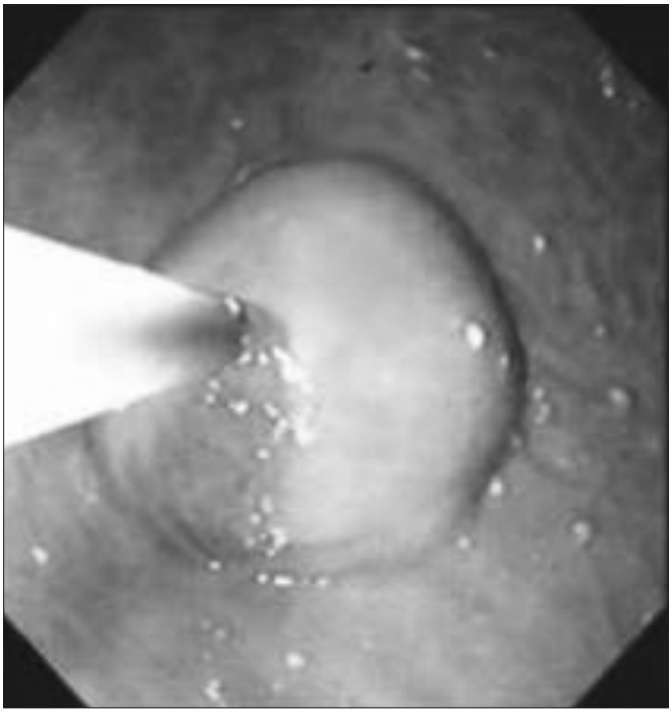

FIGURE 1. Gastric submucosal injection

TABLE 1. Durability of submucosal fluid cushions (SFC) in minutes

Animal	HPMC	CMC	NSS
1	*	28	18
2	12	15	13
3	>30	19	19
4	28	26	16
5	>30	10	11
6	35	29	38
7	37	33	25
8	25	31	42
9	>60	59	13
9	>60	44	8

HPMC = hydroxypropyl methylcellulose; CMC = carboxymethylcellulose; NSS = normal saline solution

DISCUSSION

Medical technology development and improvement in medical knowledge enable us to choose a minimally invasive treatment for early cancer patients[5]. EMR was developed as a less invasive method to treat superficial gastrointestinal tumors, and led to a considerable improvement in patient's quality of life[17]. Recently, various EMR devices have been

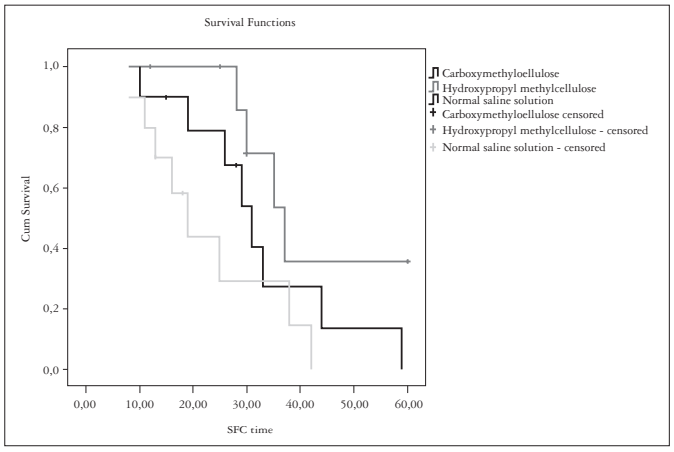

FIGURE 2. SFC Survival curve according to the Kaplan-Meier method

introduced. Improvements in endoscopic resection techniques determined good results regarding local cure and long-term outcome after resection[25].

A predictable SFC is essential for safe EMR. Repeated injection is frequently necessary during EMR to isolate the mucosal tissues and to prevent deep tissue injury. Then, a more durable SFC may result in safer procedures. Normal saline is the usual solution used for EMR. However, it diffuses quickly with consequent disappearance of the bleb[5]. To overcome this problem, several other solutions were tested, but the ideal injection solution has yet to be identified. According to Gostout[6], an ideal solution should be: cheap, nontoxic, readily obtainable in bulk, storable at room temperature, not requiring any mixing other than dilution, highly fluid outside of the body, easily injected through any standard needle catheter and almost impenetrable when in place.

Several studies have compared various solutions regarding their ability to maintain mucosal elevation during EMR, but most of them were not blinded or was not in vivo. These two aspects can be a potential source of bias.

Although SH should be used as a first-line solution, its high cost limits its use in clinical practice. Besides that, SH is a synthetic product that could potentially cause antigenic reactions[4]. HPMC has been identified as an economical alternative to SH, being similarly effective at a dramatically lower cost, with no storage requirement, no need for reconstitution before use and minimal tissue reaction[3]. HPMC is cellulose derivate with viscoelastic characteristics. We used HPMC in lower concentration (0.25%) than were used in previous studies[3, 8].

CMC has high viscosity and is used as eye drops and in ophthalmologic practice. Recently, Yamasaki et al.[27] showed that CMC was technically efficient method for dissection of gastric lesions and did not cause damage to muscular layer and surrounding. However, this study had used high concentration (2.5%), which was difficult to inject through a standard needle. Thus, we preferred a lower concentration. In spite of the SFC median time for CMC was not significantly longer than NSS, CMC is inexpensive, nontoxic, storable at room temperature, not requiring any mixing other than dilution, easily injected through any standard needle catheter and easy to buy in Brazil.

In conclusion, in this present study, the length of HPMC submucosal fluid cushion is longer in comparison with NSS. HPMC in the concentration of 0.25% may be a durable and available alternative for submucosal injection. This is the first blinded and further comparative studies will be necessary to find the best solution for submucosal injection.

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RESUMO – ***Contexto*** - A ressecção endoscópica mucosa é uma modalidade estabelecida para a excisão de lesões sésseis no trato gastrointestinal. A injeção de fluídos na submucosa cria uma coxim que pode prevenir lesão térmica e perfuração. ***Objetivo*** - Este estudo cego investiga o desempenho de três diferentes soluções para criar um coxim fluído submucoso no estômago suíno. ***Métodos*** - Três soluções foram injetadas no estômago de nove porcos BR1: soro fisiológico, carboximetilcelulose 0.5% e hidroxipropil metilcelulose 0.25%. Em cada porco, injeções submucosas com 6 mL por solução-teste foram realizadas. Uma gota de azul de metileno foi adicionada a cada injeção para melhor visualização. O tempo de desaparecimento de cada coxim foi registrado. ***Resultados*** - O tempo mediano total do coxim submucoso visível foi de 37 minutos (faixa 12–60 min) para hidroxipropil metilcelulose, 31 minutos para carboximetilcelulose (faixa 10–43 min) e 19 minutos para soro fisiológico (faixa 8–37 min). Não houve significância estatística entre soro fisiológico e carboximetilcelulose ($P = 0.146$), assim como entre carboximetilcelulose e hidroxipropil metilcelulose ($P = 0.119$), mas a duração mediana de hidroxipropil metilcelulose foi significativamente maior que a do soro fisiológico ($P = 0.039$). ***Conclusão*** - A duração do coxim submucoso com hidroxipropil metilcelulose é maior em comparação com o do soro fisiológico. O tempo mediano da carboximetilcelulose não foi maior que do soro fisiológico. A hidroxipropil metilcelulose, na concentração de 0.25%, pode ser uma alternativa durável para injeção submucosa.

DESCRITORES – Endoscopia do sistema digestório. Neoplasias gastrointestinais. Solução salina hipertônica. Carboximetilcelulose. Metilcelulose. Suinos.

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