A feasibility study of a thermally sensitive elastin-like polypeptide for submucosal injection application in endoscopic resection in 3 animal models

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Background and Aims: Endoscopic submucosal dissection (ESD) can successfully resect large lesions en bloc by using a submucosal injection solution, but the cost of currently available submucosal injection solutions is not satisfactory. The authors' aim was to evaluate the feasibility and effectiveness of a thermally sensitive elastin-like polypeptide (ELP) used as submucosal injection solution in ESD.

Methods: We conducted an ex vivo study to determine the optimal concentration of ELPs in rabbits, an in vivo study to evaluate the effectiveness of mucosal elevation in rats, and a large animal study to confirm the feasibility of preclinical application by using conventional clinical procedure in pigs.

Results: ELP (500 μM) was proved to be the optimal injectable submucosal injection solution and elevated mucosa more efficiently than any control. The same concentration of ELP exhibited an equivalent effectiveness of mucosal elevation, the retention of the elevation, and minimal bleeding with sodium hyaluronate. The ESD procedure time with 500 μM ELP in a preclinical study with pigs was significantly shorter than with any other concentration of ELP and normal saline solution.

Conclusions: Use of ELP as submucosal injection solution was feasible, with higher and longer-lasting elevation and fewer adverse events.

Endoscopic submucosal dissection (ESD) was developed to provide minimally invasive treatment for early GI tumors and even some large lesions.[1,2] However, perforation and bleeding occurred, being the most common adverse events during an ESD procedure.[3] In an attempt to prevent these adverse events, many efforts have been made to maintain a sufficiently thick cushion in the submucosal layer by injecting fluid into the submucosa endoscopically.[4,5] Use of a solution to elevate the mucosal lesion and maintain this elevation during ESD procedure facilitates the separation of the mucosal and muscular layers for resection of a neoplasm and allows reliable and complete en bloc resection to reduce surgical adverse events, such as perforation and bleeding, and allow an accurate histopathological diagnosis.[5] Much preclinical and clinical research concluded that the ideal solution should elevate the lesion sufficiently, maintain the elevation, and allow less bleeding during the ESD procedure.[6,7] In addition, the cost of the submucosal injection solution should be acceptable. Normal saline solution (NS) was the first solution used for this purpose, but was not an ideal one because it was difficult to maintain the desired elevation, and the elevation lasted only a few minutes due to its isotonic property leading to rapid absorption by the surrounding tissue.[8,9] To overcome this disadvantage and meet these criteria, a solution with higher viscosity to inject submucosa is used such as sodium hyaluronate (SH), a promising submucosal injection solution during endoscopic mucosal resection (EMR), but its high cost prevents its more widespread use.[10,11] Therefore, it is necessary to develop an effective, safe, and inexpensive agent for submucosal injection.

Abbreviations: ESD, endoscopic submucosal dissection; ELP, elastin-like polypeptide; NS, normal saline solution; SH, sodium hyaluronate.

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Recombinant peptide polymers, such as elastin-like polypeptides (ELPs), provide an attractive route for the design of such materials because they are injectable, biodegradable, biocompatible, and nonimmunogenic.[12,13] ELPs are a class of artificial peptide polymers inspired by the amino acid sequence of tropoelastin, which is composed of oligomeric repeats of the pentapeptide sequence Val-Pro-Gly-Xaa-Gly, where Xaa is any amino acid except Pro that is found in tropoelastin. ELPs are attractive as injectable biomaterials undergoing a soluble to insoluble phase transition when heated above a tunable transition temperature (T_t, also called lower critical solution temperature).[14,15] ELP is the product of genetic engineering and required a lot of preparation without liquid chromatography, another complex extraction, and purification processes so that it is easy to obtain at low cost.[16]

We hypothesized that ELPs are injectable (soluble in a syringe) at room temperature and undergo a phase transition to become a solution with high viscosity at the injection site (gastric submucosa) immediately after submucosal injection if the T_t of the ELP is $25°C < T_t < 37°C$, which will have longer retention at a higher and longer-lasting elevation of lesions. ELP was designed and synthesized to spontaneously undergo a soluble–insoluble phase transition (forming viscous coacervates) between room temperature and body temperature on submucosal injection. ELP as a submucosal injection solution was prepared by a series dilutions of the maximum injectable concentration of 1000 μM ELP into 500, 250, and 125 μM with phosphate-buffered saline solution. The in vivo trial was performed to investigate the feasibility of injecting ELP as a submucosal injection solution for ESD compared with glycerin fructose, SH, and NS in the rat, rabbit, and pig, respectively. The feasibility and effectiveness were evaluated by analyzing the height and duration of the mucosal elevation, bleeding during resection, and the amount of time to perform a resection.

MATERIALS AND METHODS

Preparation of ELP for submucosal injection

The ELP was designed and synthesized at the Department of Biomedical Engineering at Duke University (Durham, NC). A 50-kDa ELP comprising the pentapeptide sequence $(VPGVG)_{120}(GY)_7$ ($ELP_{120}Tyr_7$) was encoded and expressed in *Escherichia coli*. This formulation in a concentration ranging from 62.5 to 1000 μM targets a transition temperature range of $20°C < T_t < 30°C$, enabling depot formation at physiological body temperatures of 37°C.[17] ELP as a submucosal injection solution was prepared by a series of dilutions of the maximum injectable concentration of 1000 μM ELP into 500, 250, and 125 μM with phosphate-buffered saline solution and kept at 4°C to ensure its injectable. The 0.5% SH (Pharmaceutical Co, Ltd., Shandong Bausch & Lomb Freda Pharmaceutical Co, Jinan, China) was used as a positive control and NS and glycerin fructose (Baxter Healthcare Ltd, Shanghai, China) as a negative control. The ELP solution was loaded into a plastic syringe and kept on ice before injection.

Ex vivo study on the effect of injection pressure and ELP concentration on the performance of mucosal elevation

Twenty healthy New Zealand White rabbits obtained from the People's Liberation Army, Military Academy of Medical Sciences (Beijing, China) were randomly divided into 5 groups (n = 4) for submucosal injection of different concentrations of ELP and glycerin fructose. The rabbits were euthanized by an intraperitoneal injection of pentobarbital, after which the stomach was removed. The resected rabbit stomach was cut into approximately 5 × 5-cm sections. Two milliliters of each of different concentrations of ELP (1000, 500, 250, or 125 μM or glycerin fructose as a control with a small amount of indigo carmine for easy visibility) was injected into the submucosa through the resected margin by using a 5-mL syringe connected to a pressure meter. During injection, the input pressure was recorded. After recording the pressure of injecting the different concentrations of ELP, we also observed and recorded the height of mucosal elevation and changes at 0, 5, 10, and 30 minutes after the submucosal injection. The mean height of mucosal elevation achieved with each solution was compared with each other.

In vivo comparison study of mucosal elevation of maximal injectable concentration of ELP with SH in rats

Male Sprague-Dawley rats aged 9 to 10 weeks were obtained from the People's Liberation Army, Military Academy of Medical Sciences. The rats were randomly divided into 3 groups (n = 6) based on various injection solutions, ie, ELP, SH, and NS. After 24 hours of fasting, rats were anesthetized by intraperitoneal injection with 5% chloral hydrate (0.7 mL/100 g). The mucosa of the glandular stomach was exposed by sterile anterior gastrotomy after laparotomy after a submucosal injection of each of the aforementioned solutions into the submucosa of the posterior wall via a 25-gauge stainless steel needle. In the ELP group, 0.2 mL of the 500-μM ELP solution was injected. The rats in the other 2 groups were similarly injected with either 0.5% SH or NS. The submucosal thickness was defined as an increase in thickness. With the rats under anesthesia, the rat gastric wall thickness from the inner layer to the outer layer at the maximum height within 5 mm in diameter surrounding the injection site was measured by using Vernier calipers and recorded at 0, 5, 10, 30, and 60 minutes after injection of the various solutions. The mucosal elevation after injection of submucosal injection solution into the submucosa was determined by subtracting the initial thickness (before injection) from