Use of hollow microneedles for targeted delivery of phenylephrine to treat fecal incontinence

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ABSTRACT

A hollow microneedle (HM) was prepared to deliver a phenylephrine (PE) solution into the anal sphincter muscle as a method for treating fecal incontinence. The goal of this study was the local targeted delivery of PE into the sphincter muscle through the perianal skin with minimal pain using hollow microneedles, resulting in the increase of resting anal sphincter pressure. PE was administered on the left and the right sides of the anus of a rat through the perianal skin using 1.5 mm long HM. An in vivo imaging study was conducted after injection of Rhodamine B, and a histological study was performed after injection of gentian violet. The resting anal sphincter pressure in response to various drug doses was measured by using an air-charged catheter. Anal pressure change produced by HM administration was compared with change produced by intravenous injection (IV), subcutaneous (SC) injection and intramuscular (IM) injection. The change in mean blood pressure produced by HM administration as a function of PE dose was compared with change produced by PBS injection. A pharmacokinetic study of the new HM administration method was performed. A model drug solution was localized in the muscle layer under the perianal skin at the injection site and then diffused out over time. HM administration of PE induced significant contraction of internal anal sphincter pressure over 12 h after injection, and the maximum anal pressure was obtained between 5 and 6 h. Compared to IV, SC and IM treatments, HM treatment produced greater anal pressure. There was no increase in blood pressure after HM administration of PE within the range of predetermined concentration. Administration of 800 μg/kg of PE using HM produced 0.81 ± 0.38 h of tmax. Our study suggests that HM administration enables local delivery of a therapeutic dose of PE to the anal sphincter muscle layer with less pain. This new treatment has great potential as a clinical application because of the ease of the procedure, minimal pain, and dose-dependent response.

1. Introduction

Fecal incontinence is a disease involving the repetitive, involuntary excretion of solid or liquid feces or gas. It is reported that 15% of the United State population aged 50 years or older suffer from fecal incontinence [1–5]. However, people with fecal incontinence are often afraid or embarrassed to talk about this condition with their family, friends, and medical doctor. Fecal incontinence affects quality of life through physical, psychological, and social isolation [6,7]. It is primarily caused by dysfunction or deformity of the anal sphincter muscle. A variety of treatments have been attempted to reduce symptoms and restore functions, but currently there is no effective way to treat fecal incontinence.

Surgery improves anal sphincter muscle function [8]; however, surgery is not suitable for patients older than 70 years because of infection and pain. Non-surgical methods such as oral drug administration and biofeedback treatment alleviate diarrhea by reducing the amount of water in feces [9]. But these non-surgical methods have limited efficacy due to side effects and lack of control of the sphincter muscle [10,11].

In an effort to overcome previous limitations in treatment, several studies have applied a gel formulation of phenylephrine (PE), an alpha 1 adrenergic agonist, to the anal canal. In one study, 5 of 18 patients (28%) reported improvement in symptoms after the application of PE gel twice a day for 4 weeks [12]. Another study reported a remarkable increase in mean resting anal pressure after a high concentration of PE gel was applied [13,14]. However, other studies have found that topical PE gel application is ineffective for increasing resting pressure in patients with fecal continence [15]. These contradictory experimental results may be due to an insufficient dose of PE. Thus, a new system was needed that can deliver PE effectively to the anal sphincter muscle.
a previous study, we introduced an enhanced delivery of PE by applying PE gel after a microneedle array treatment on the perianal skin. PE was effectively delivered through the micro-holes created by the microneedle array, resulting in a significant increase in resting anal sphincter pressure for 1 h after treatment. However, PE penetration decreases gradually as the micro-holes close. This closure prevents delivery of the proper drug dose. Moreover, dozens of micro-holes in perianal skin can lead to bacterial infection [16].

In the present study, we introduced a new treatment method using a minimally invasive hollow microneedle (HM) for local delivery of a specific dose of PE into the anal sphincter muscle. This new treatment method maximizes drug efficacy and clinical utilization. We measured the anal sphincter pressure profiles in terms of PE dose. We also developed a new in vivo animal model by using a disposable air-charged catheter to accurately measure the anal sphincter pressure. In addition, through a pharmacokinetic study, we demonstrated the efficacy of this new delivery system. Finally, to confirm the clinical safety of this new system, we investigated blood pressure levels as one of the potentially adverse effects of PE administration.

2. Materials and methods

2.1. Animals

Female Sprague–Dawley rats (Charles River Technology, Yokahama, Japan) 8–10 weeks old and weighing 250–300 g were used for in vivo studies. The animals were housed in standard facilities with two rats per cage, and maintained with free food and water. All animal protocols were performed in accordance with Gachon Animal Experiment Guidelines and approved by the Animal Care Committee.

2.2. Materials

Phenylephrine was provided by Il-Dong Pharmaceutical Co. Ltd. (Seoul, Korea) and the different concentrations of PE solution were prepared by dissolving the appropriate amounts of PE in phosphate-buffered saline (PBS). Rhodamine B was purchased from Sigma-Aldrich (St. Louis, MO).

2.3. Administration with a hollow microneedle

A hollow microneedle was prepared by modifying a 34-gauge beveled NanoFil needle (WPI, Sarasota, FL, USA) 5 mm in length, with an outer diameter of 185 μm and an inner diameter of 85 μm. The modified syringe was attached by tubing to a Hamilton syringe with a volume of 100 μl. The microneedle tip was wrapped with a parafilm to make a protrusion of 1.5 mm from the 5-mm length of the microneedle (Fig. 1(a)). A PE solution was administered at the rate of 1 μl/s for a predetermined time with a Micro Flow Rate syringe pump (Longer Pump, Baoding, China). As seen in Fig. 1(b), the rats were injected with PE by inserting the hollow microneedle into the perianal skin at two sites 3 mm on either side of the anus at a depth of 1.5 mm. The injections were made without anesthesia.

2.4. In vivo bio-distribution of a model drug

To study the distribution of a drug in tissue near an injection site using a hollow microneedle, we injected 200 μg/kg of the model drug, Rhodamine B into the left and right perianal regions of rats in identical injection conditions. At 1, 2, 3, 4 and 6 h after drug administration, the non-treatment group (negative control) and the treatment group of rats were placed on the stage under isoflurane anesthesia and imaged using a Xenogen In-Vivo Imaging System IVIS-200 (Xenogen Corporation, Alameda, CA) with DsRed filter. Images were acquired using 60-s exposure. To minimize undesired fluorescence caused by surroundings, the hair in the perianal region was removed before treatment and rats were fed non-fluorescent food.

2.5. Histological study

Using the same protocol, another model drug, gentian violet (Sigma Aldrich), was injected into the perianal skin with a hollow microneedle after the rats were euthanized with carbon dioxide. The perianal tissues were carefully excised, embedded in an optimal cutting temperature compound (Tissue-Tek, Sakura Finetek, Torrance, CA), and sectioned into 50-μm-thick slices using a cryostat microtome (Microm HM 550P, Thermo Scientific, Rockford, IL, USA). The samples were examined by optical microscope (Nikon, Japan) without staining, and identical samples were investigated repeatedly after staining with hematoxylin and eosin (H&E).

2.6. In vivo resting anal sphincter pressure measurement

Resting anal sphincter pressures of rats were measured with a disposable T-DOC air-charged catheter (7FA, Abdominal Catheter, T-DOC, Wilmington, DE, USA) connected to a Delphis IP Urodynamic System (Laborie Medical Technologies, Toronto, Canada). Six groups (n = 5) were examined for dose-dependent response: 1) the nontreated, negative control (NC) group; 2) group administered PBS (HM PBS); 3) group administered 200 μg/kg of PE with a hollow microneedle (HM 200); 4) group administered 400 μg/kg of PE with a hollow microneedle (HM 400); 5) group administered 800 μg/kg with a hollow microneedle (HM 800); and 6) group administered 1600 μg/kg with a hollow microneedle (HM 1600). Subcutaneous injection (SC 200), intramuscular injection (IM 200), and intravenous injection (IV 200) of 200 μg/kg of PE were performed on three groups of rats (n = 5 each group) to compare the efficacy of these methods with that of HM administration. Experiments were conducted on separate days. At 0, 1, 2, 3, 4, 5, 6, 9, and

Fig. 1. (a) Comparative optical image of a 1.5 mm length of hollow microneedle (bottom) with a 26-gauge needle (top). (b) Image showing two injection sites on the left and right sides of a rat’s anus.
12 h after drug administration, rats were placed in the prone position in a restrainer (universal holder, Jeungdo Bio & Plant Co., Seoul, Korea) and resting anal sphincter pressure was monitored. Analysis of variance was performed to determine the percentage of subjective improvement (p < 0.05 was considered statistically significant). Student’s t-test was used to compare the mean values between the two groups (p < 0.05 was considered statistically significant).

The air-charged catheter has a 2.33-mm diameter and once it inflates, the maximum diameter of the balloon is 6.73 mm. The uninflated air-charged catheter was gently inserted into the anus and positioned at the anal entrance (Fig. 2). Upon insertion and calibration, the balloon was filled with air and the pressure exerted on the balloon catheter was transmitted to a monitoring system. The mean pressure value over about a 1-min period was recorded at determined intervals up to 12 h. Statistical comparisons were performed using Student’s t-test or analysis of variance. p < 0.05 was considered statistically significant.

2.7. In vivo blood pressure measurement

Assessment of blood pressure was carried out in the HM PBS, HM 200, HM 400, HM 800 and HM 1600 at 0 min, 15 min and 30 min and at 1, 2, 4, 6, 9, and 12 h after administration using the CODA™ non-invasive blood pressure acquisition system (Kent Scientific Corporation, Torrington, CT, USA). Rats were gently restrained with a nose cone in an animal holder. An occlusion tail cuff connected with a Volume Pressure Recording (VPR) sensor was put in the tail as the given protocol [17]. The average pressure was obtained from 10 measurements per animal at 25 °C and in a quiet environment.

2.8. Pharmacokinetic study

A pharmacokinetic study was conducted with three groups of rats (n = 5 in each group). In this study, one group of rats received a hollow microneedle injection of 200 μg PE/kg body weight, and another group received an injection of 800 μg PE/kg body weight. A negative control group did not receive an injection. After the injections, blood samples were harvested from the jugular vein at 5, 20, and 40 min, then at 1, 2, 4, 8, and 12 h. Collected blood samples were centrifuged for 10 min at 4000 rpm and the clear supernatants were transferred to 1.5 mL Eppendorf tubes. They were immediately frozen in liquid nitrogen and stored at −80 °C until assayed. The plasma drug concentrations were analyzed with a Triple Quadrupole LC–MS/MS system (Thermo Fisher Scientific, CA, USA) and determined from a standard curve.

3. Results

3.1. Drug delivery to anal sphincter muscle by a hollow microneedle

To facilitate delivery of the correct drug dose, the length of the microneedle needs to be adjusted to be minimally invasive and to prevent leakage of the drug. A length of 1.5 mm was finally selected based on trials with microneedles of various lengths (0.5 mm to 2 mm). The hollow microneedle used in this study has much smaller inner and outer diameters and a shorter length than a 26-gauge needle (as shown in Fig. 1(a)). The small diameter and short length of microneedles minimize the pain at insertion, which improves the patient’s acceptance of treatment [18,19]. However, we found that PE leaks out of the skin when the needle was shorter than 1.5 mm.

3.2. Drug distribution

Fig. 3 shows the perianal tissue anatomy composed of epidermis, dermis, hypodermis, and muscle, which is similar to other skin tissues. After administration of PE, gentian violet was spread out along the intramuscular layer (Fig. 3) below the anal skin. A portion of the drug was found in the adipose tissue layer and in the interface of the adipose tissue layer and the muscle layer because of backflow to the dermis layer from the muscle layer.

In order to evaluate in vivo bio-distribution of a model drug, Rhodamine B, the fluorescence intensity of Rhodamine B was measured in real time after the drug was administered with a hollow microneedle. A strong localized signal was detected for the first 2 h, and a gradual decrease was observed over time, as shown in Fig. 4. This result indicates that the highly concentrated drug was located around the injection site and that the high concentration decreased rapidly.

Fig. 2. (a) Air charged catheter for circumferential recording of urethral pressure 1. before balloon expansion and 2. after balloon expansion (b) Diagram of measurement of resting anal sphincter pressure using air charged pressure catheter for rat anus.

Fig. 3. Histological images by optical microscope after administration of gentian violet solution using hollow microneedle; a. epidermis b. dermis and hypodermis c. gentian violet at interface and d. muscle.
3. Resting anal sphincter pressure measurement

Fig. 5 shows the time course of change in the resting anal sphincter pressure according to dose of phenylephrine (HM 200, HM 400, HM 800, and HM 1600). The hollow microneedle injection of PE showed a dose-dependent increase in the resting anal sphincter pressure, with the highest pressure occurring between 4 and 6 h.

Administration of a 1600 μg/kg dose of PE (HM 1600) showed an approximately fivefold increase over the PBS administration level (9.38 ± 1.73 cm H2O vs. 50.14 ± 4.64 cm H2O) at 5 h. High doses (HM 800 and HM 1600) of PE kept anal pressure significantly higher over 12 h, even though the high doses showed a parabolic profile (18.81 ± 3.16 cm H2O and 24.38 ± 4.39 cm H2O) at 12 h.

A dose lower than 200 μg/kg was allowed for comparison between administration methods because pulmonary edema was observed in 100% of the rats when 200 μg/kg of PE was administered by IV injection (IV 200). At 5 h after administration, HM 200, IM 200, SC 200, and IV 200 showed 15 ± 1.6 cm H2O, 10 ± 1.5 cm H2O, 10 ± 2.5 cm H2O and 11 ± 1.9 cm H2O, respectively (Fig. 6), and that of HM 200 was significantly different from that of IM 200, SC 200, and IV 200 individually (t-test, p < 0.05). However, there was no significant difference between HM PBS, IV 200, IM 200, and SC 200 (by ANOVA, p > 0.1).

The anal pressure measured by a disposable-air-charged catheter was 10.8 ± 1.0 cm H2O, indicating a lower standard deviation than that measured by a four-channel water-perfused catheter, 9.5 ± 2.8 cm H2O, which was used in previous work. As shown in Fig. 7, at 5 h using the disposable-air-charged catheter produced only small variations in pressure measurement between individuals in the HM PBS group and those in the 1600 HM group.

3.4. In vivo blood pressure measurement

A high dose of PE caused side effects, including an increase in mean blood pressure. The change in mean blood pressure in the HM PBS, HM 200, HM 400, HM 800, and HM 1600 groups and there was no significant differences at 1 h between groups (ANOVA, p = 0.32).

Pulmonary edema was observed in 100% of the rats when 200 μg/kg of PE was administered by IV injection, and IV administration of 400 μg/kg caused the death of some rats. Acute pulmonary hemorrhage was found in the autopsies of dead rats. The lungs showed a dark-red color caused by pulmonary hemorrhage.

3.5. Pharmacokinetics

Fig. 9 shows the time course curve of plasma PE concentration after HM administration of different doses (200 and 800 μg/kg). The plasma PE concentration increased immediately after administration of HM 200 and HM 800 of PE. The peak time (tmax) of HM 200 and HM 800 appeared about 0.354 ± 0.38 h and 0.81 ± 0.38 h and showed 15.12 ± 4.78 ng/ml and 104.7 ± 38.33 ng/ml of mean Cmax, respectively, after administration of HM 200 and HM 800.
4. Discussion

Previous trials of the topical application of PE on anal skin showed the possibility of increasing resting anal sphincter pressure. However, there has been controversy over the efficacy of local delivery of PE by topical application. In our previous study, local delivery of PE into the sphincter muscle was performed by generating holes by a solid microneedle array [16]. This method produced a significant increase in resting anal sphincter pressure. However, there were limitations in making it clinically feasible. For example, the number of micro-holes created by the solid microneedle array and the slow healing of the micro-holes could lead to infection. In addition, variable closure of the micro-holes altered skin permeability, leading to the delivery of inaccurate PE doses. Furthermore, treatment using micro-holes followed by drug application for long period was inconvenient and painful for patients. Thus, we developed a new delivery system using a hollow microneedle to overcome the limitations of solid microneedles.

Hollow microneedles are designed for efficient and fast delivery of liquid medication and to enhance patient compliance. In this study, the length of a hollow microneedle was adjusted for drug delivery to a specifically targeted site near the sphincter muscle without leakage and to minimize pain in order to reduce patients’ anxiety. Administering the drug with a short hollow microneedle is required to prevent leakage and to penetrate the dense dermal tissue. Therefore, to eliminate drug leakage, the length of the microneedle and the flow rate were optimized. A microneedle shorter than 1 mm caused leakage onto the skin surface at a rate lower than 1 μl/s. In addition, the level of insertion pain depends on the length of the needle. A pain score of a 1.5 mm long microneedle was 12 mm, and that of a 26-gauge syringe was a mean value of 39 mm [19]. Thus, the shortest possible HM length of 1.5 mm was selected. This length allowed an acceptable, fairly slow flow rate of 1 μl/s. The infiltration depth of the drug solution can be affected if the flow rate is too fast. Thus, injection speed should be determined in order to avoid a negative effect on the therapeutic region. In addition, an administration rate below 8 μl/s was reported to be less painful [18]. In order to distribute PE into the sphincter muscle around the anus, 100 μl of PE solution was administered at two spots on either side of the anus of rats for 100 s. Two PE solutions of 50 μl injected for 50 s per spot were found to be a feasible clinical treatment. This new treatment can be utilized more efficiently by local administration of the drug into the targeted site when the damaged muscle region is detected by magnetic resonance imaging (MRI).

The distribution of PE in the perianal region after HM administration can be predicted by investigating the distribution of gentian violet and fluorescence. In an ex vivo study, gentian violet was distributed in the muscle layer and in the interface between the adipose tissue and the muscle layer. The fluorescent intensity of IVIS images does not mean the therapeutic dose, but the decay of the model drug by diffusion can be estimated from the images. The one-compartment model can be applied to the decay of fluorescence, showing rapid decay of drug concentration from the center of the injection site [20]. Local delivery of higher concentration of PE solution might maintain therapeutic concentration in the local region for a longer time. Also, a small volume of higher concentration of PE solution might be suitable for maintaining therapeutic concentration in the specific region and producing less pain. A locally higher amount of PE in the same volume of tissue (HM 200) showed higher resting anal pressure as shown in Fig. 6 compared to other administration methods. This result confirmed that the increased anal pressure was caused by local delivery of PE with a hollow microneedle. The profile of anal resting pressure in Fig. 5 was different from the dilution profile of Rhodamine B in Fig. 4. The profile of anal pressure with the highest value between 5 and 6 h presented the peripheral compartment of a two-compartment model and rapidly perfused tissues around the injection site belongs in the central compartment of two compartment as shown in IVIS images in Fig. 4 [20]. As shown in Fig. 5, HM administration of 100-μl volume of PBS did not change anal pressure. This showed that the change in anal pressure was caused not by the liquid volume but by the delivery of PE.

A high dose of PE can effectively increase anal sphincter pressure, as shown in the dose–response relationship graph in Fig. 5, but a high dose of PE can also cause cardiovascular problems. Elderly people (aged over 60) are more likely to develop fecal incontinence; thus, the side effects of PE administration should be considered. There was a significant correlation between the dose administered by a hollow microneedle and an increase in anal pressure; however, there was no correlation between the change in blood pressure and the same dosage. Also, the change in blood pressure produced by HM 1600 corresponded to the profile of changes produced by HM PBS as shown in Fig. 8. These results demonstrate that local delivery of concentrated PE can be a safe administration method because the therapeutic dosage of PE can be reduced by local targeting, resulting in a decreased PE amount absorbed into the bloodstream. A higher concentration of PE was recommended for efficiency when side effects did not exist. Thus, HM 1600 was recommended in this study for greater efficiency and longer lasting effect.

Our study found that the PK profile of hollow microneedle injection showed a pattern similar to conventional IM or SC injection, and a mean t_{max} of 60 min in the HM profile was observed. In case of epinephrine with similar molecular weight and comparable pharmacokinetics with phenylephrine by oral administration, the mean time at which maximum epinephrine concentration was reached by IM was 8 min in adults and children and 34 min by SC [21]. Thus, PE administration into perianal skin by HM showed delayed t_{max} compared to administration by IM and SC. A delayed t_{max} may be caused by the slower adsorption
of locally administered PE into the bloodstream. Intradermal injection of liquid insulin into children with a 1 mm long hollow microneedle showed fast adsorption into the bloodstream, peaking at 30 min. Compared to SC and IM injections, HM injection showed higher glucose efficacy because of the microneedle’s greater effectiveness in reaching dermal capillaries [22]. The conflicting PK results in these studies may be due to the differences in sample volume (100 μl vs. 1 ml) with different concentrations, feeding rate (1 μl/s vs. 17 μl/s), length of microneedle (1.5 mm vs. 1 mm), and injection site (perianal skin vs. arm).

There was no well-established animal model for measuring anal sphincter pressure. Our previous study provided an animal model for measuring resting anal sphincter pressure by using a water-perfused four-channel catheter. This catheter was positioned in the anus and the average pressure from four micro-holes was measured. The critical limitations of the water-perfused system were caused by escaping water. Long exposure to water caused the inner tissue to swell, which made it difficult to accurately measure anal pressure over a few hours. A drug gel applied around the anus could be removed by water, also leading to inaccurate measurement over hours. Moreover, the water could enter the large intestine, which made measurements unstable. To overcome these limitations, we developed a new system based on an air-charged balloon catheter. This system enabled the balloon to be manipulated and positioned in the anus quickly and a single measurement could be performed rapidly. Measurement using the disposable-air-charged catheter allowed the fast recovery of anal pressure to steady-state within 1 min after insertion of the catheter, whereas the four-channel water-perfused catheter required relatively longer stabilization because of water flow in the anus. Keeping the rats in a stable state was important in case the interval between measurements was not long enough. As shown in Fig. 7, extended observation over more than 12 h was possible by using the disposable-air-charged catheter. This new in vivo animal model can also be utilized for studying other muscle responses to drug delivery.

5. Conclusion

Our study suggests that hollow microneedle administration enables local delivery of the right dose of PE to the anal sphincter muscle layer with minimal pain. Compared to other treatments, this new treatment showed significantly increased contraction of the anal sphincter muscle for a few hours and did not make blood pressure increase and pain. This treatment has great potential as an effective clinical application because of the convenient procedure, reduced chance of contamination, minimal pain, and dose-dependent response to anal pressure. A 12 hour increase in the resting sphincter muscle pressure shows the possibility of treatment of fecal incontinence. However, this finding is not enough to support clinical treatment, and the extension of lasting drug effects needs to be studied to understand how to achieve steadier and longer reductions in fecal incontinence. The sol–gel transition system has been developed to improve sustained drug release because this system uses an injectable formulation and intelligent behavior with respect to phase transition and drug release profile. We believe that an HM-optimized sol–gel transition system will provide the solution to achieve longer intervals between HM administrations. A new animal model provides reliable measurement of anal pressure using a balloon catheter to verify the efficacy of the treatments. This study can be widely applied to evaluate other treatments for improving symptoms by delivering a drug into a certain muscle or nerve.

Phenylinephrine HCl has been approved and used for various clinical applications. An HM can be classified as class II such as syringine. Thus, administration of PE using an HM does not have serious limitations for clinical application. However, a clinical trial is needed to define HM-PE in order to ensure the safety and effectiveness of this treatment method. Clinical tests with patients should be well designed. In particular, the right injection site should be defined using rectal MRI, sonography, and manometry in order to determine a viable muscle portion adjacent to the damaged region.

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