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The Sustained effects on tear volume of pilocarpine hydrochloride in gelatin by hydrogel administered by an implant-mediated drug delivery system

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Abstract

Pilocarpine hydrochloride is commonly prescribed to patients with dry mouth and eye using a frequent dosing schedule. The aim of this study was to evaluate the sustained effects of this highly soluble drug carried by a gelatin hydrogel, which was administered by an implant mediated drug delivery system (IMDDS). The IMDDS was installed in a total of 24 rabbits. After complete healing, pilocarpine hydrochloride was administered as 30 mg as raw powder (Group 1; n = 8), 30 mg in gelatin hydrogel (Group 2; n = 8), and 60 mg in gelatin hydrogel (Group 3; n = 8). The effects were evaluated by tear volume measured using the Schirmer tear test for 2 weeks after administration. All 3 groups showed an increase in tear volume from the initial measurement at 1 hour. Group 1 exhibited this increase for 24 hours, while Groups 2 and 3 sustained this increase for 5 days and 7.5 days, respectively. When provided in gelatin hydrogel, highly water-soluble pilocarpine hydrochloride administered through IMDDS resulted in sustained effects with increased tear volume in normal rabbits.

Keywords

implant-mediated drug delivery system; pilocarpine hydrochloride; dry eye; sustained release; osseointegration

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

1. INTRODUCTION

Titanium implants have evolved as one of the most preferred choices for replacement of missing teeth, due to their proven biocompatibility and stability [1]. However, their application is still limited to restorative purposes in the oral and maxillofacial area, despite several attempts to expand their utility [2]. Recently, a new application for this implant as a drug delivery system (DDS) was introduced, in which pharmacologic substances diffuse into the intrabony space [3, 4]. A DDS is a formulation or a device that conveys drug molecules into the body and maximizes the efficacy to safety ratio by controlling every aspect of pharmacokinetics and pharmacodynamics [5]. This implant-mediated drug delivery system (IMDDS) was devised for use with chronic systemic diseases with notable sustained release feature, not just for the purpose of local delivery of drugs. Several candidate drugs are being evaluated the efficacy of the delivery through the IMDDS with promising results [6].

Secretion of natural fluids, like tears and saliva, is essential for tissue health. These fluids serve to protect, lubricate, moisturize, prevent dehydration and desiccation of mucosal surfaces, induce growth of epithelial cells, heal wounds, and maintain mucosal homeostasis [7].

Disturbance or impairment of secretory function causes discomfort, reduces quality of life and increases comorbidity especially in patients with severe degree of xerostomia and xerophthalmia, which are usually radiation-induced or result from Sjögren's syndrome [8].

Sjögren's syndrome (SjS) is a chronic, autoimmune, rheumatic disorder characterized by progressive destruction of exocrine glands, such as salivary and lacrimal glands, accompanied by leukocytic infiltration [9]. Due to the progressive damage to the exocrine glands, patients with SjS typically present clinically xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eyes) [10]. These symptoms of dryness can also be caused by another etiology, radiation injury to the exocrine glands. In addition to general discomfort, morbidity from the gland hypofunction results from alteration of mucosal and ocular surfaces and failure of the host defense to infection. Numerous complications include oral ulcers, weight loss, candidiasis, bacterial sialadenitis, sleep disruption, rampant dental caries, corneal ulcer or perforation, and bacterial conjunctivitis [11].

Replacement therapies using artificial tears or saliva have, in general, achieved only limited success and patient satisfaction and compliance have been low [12]. Patients often find these over-the-counter remedies costly, ineffective, inconvenient, or irritating. Pilocarpine is a naturally occurring compound extracted from the South American shrub *Pilocarpus jaborandi*. This plant alkaloid is a nonselective muscarinic agonist with a parasympathomimetic effect. It can induce pharmacological smooth muscle contraction, as well as stimulation of the exocrine glands [13]. Use of pilocarpine has also been reported to alleviate xerostomia in patients with chronic graft-versus-host disease after bone marrow transplant [14].

The pilocarpine hydrochloride, along with cevimeline, is the drug of choice for symptomatic treatment of patients with Sjögren's syndrome and radiation-induced dry mouth/eye [15–17]. Several multicenter studies have demonstrated sufficient safety and great efficacy of

oral pilocarpine administered up to 4 times a day and 20–30 mg daily total [11]. Sometimes, delays in symptomatic relief occur. Despite the significant symptomatic relief of dry mouth, a too-frequent dosing schedule, along with cholinergic side effects, can be considered disadvantages. Patient compliance is known to dramatically decrease as the prescribed dose frequency increases [18]. Poor compliance may be related to the delay in symptom improvement.

In this regard, the use of IMDDS can be suggested a new delivery route of pilocarpine hydrochloride administration for xerophthalmia/xerostomia. The IMDDS is advantageous due to the sustained release of drug and the painless needle-free delivery of drugs to the systemic circulation without a first pass effect [3]. However, the physicochemical properties of specific drugs affect their release profiles, and highly water-soluble drugs are known to dissolve fast into the blood stream of intrabony space. Pilocarpine hydrochloride is one of these highly soluble drugs. Therefore, a special strategy to slow the dissolution of the drug is required when using the IMDDS [19].

In the present study, we use gelatin hydrogels as carriers of pilocarpine hydrochloride to decrease the release speed. The aim of this study was to evaluate the sustained effects of pilocarpine hydrochloride in gelatin hydrogel when administered by an IMDDS.

2. MATERIALS AND METHOD

2.1. Animals

A total of 24, 10-week-old male New Zealand White rabbits, each weighing 2.5 to 3.0 kg, were used in this study. Animal selection, management, and surgical protocols were approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, Korea. Before the start of the experiment, the rabbits were quarantined and acclimated for seven days. They were housed in separate cages and were provided a standard rabbit diet and water ad libitum. Temperature and relative humidity were kept at $22 \pm 4^{\circ}\text{C}$ and 50 – 60%, respectively, with a 12-hour light/dark cycle.

2.2. Implant-mediated drug delivery system (IMDDS)

IMDDS devices were custom made on a milling machine using grade IV titanium to facilitate osseointegration. The fixture part of the IMDDS (diameter, 4.1; length, 7.0 mm) has external threads for initial stability in bone and is designed as a hollow cylinder with multiple diffusion holes with a diameter of 0.2mm. The drug cartridge, pilocarpine hydrochloride or pilocarpine hydrochloride in gelatin hydrogel in the present study, is accommodated inside the hollow cylinder. Multiple holes are located in both the circumferential axial wall and the apex of the fixture and allow for drug diffusion into the intrabony space. The top of the fixture is equipped with a cover to separate the inside of the IMDDS from the external environment (Fig. 1).

2.3. Surgical procedures

Before the rabbits were brought to the operating room, their hind legs were shaved. The animals were anesthetized with an intramuscular injection of 10 mg/kg zolazepam and 0.15

ml/kg xylazine hydrochloride. Local anesthesia, consisting of 2% lidocaine with 1:100,000 epinephrine (0.5 to 1 ml/site), was applied around the surgical site. The site was accessed with 3-cm-long periosteal incisions through the skin. The flat lateral surface of the proximal tibia metaphysis was selected for IMDDS installation. The IMDDS was placed after sequential site preparation using a 2.0-, 3.0- diameter twist drill and a screw tap. Each rabbit received one IMDDS placed randomly in the left or right tibia. The surgical site was closed in layers with appropriate absorbable sutures. The surgery procedure was performed using aseptic routines to prevent infection. After surgery, each rabbit received a non-narcotic, non-steroidal analgesic agent for pain control and a broad spectrum antibiotic for infection control.

2.4. Loading of pilocarpine hydrochloride into the IMDDS

Pilocarpine hydrochloride (P6503-5G) and gelatin (G6650-Type B) were purchased from Sigma-Aldrich (St Louis, MO). Pilocarpine hydrochloride is highly water soluble. Thus, to reduce the release rate from the IMDDS, the cartridges were prepared with the drug dissolved into the sol state of gelatin at 50°C. The gelatin powder was mixed with distilled water at a 1:1 weight ratio. Pilocarpine hydrochloride was mixed into the gelatin sol at two different concentrations: 30 mg and 60 mg per single cartridge of administration. The test rabbits were divided into 3 groups. In Group 1, 30 mg pure pilocarpine hydrochloride powder was loaded in the IMDDS. For Groups 2 and 3, gelatin hydrogels containing 30 mg and 60 mg of pilocarpine hydrochloride were used, respectively. The respective individual cartridges of drug were inserted within the IMDDS and sealed with a cover screw (Fig. 1).

2.5. Measurement of the efficacy of pilocarpine hydrochloride (Schirmer test)

Schirmer strips were purchased from Merck Animal Health (Madison, NJ, USA). Each animal was manually restrained in a standard polycarbonate restrainer. This test was carried out by inserting a notched end of a standardized sterile Schirmer strip over the lower eyelid margin at the junction of the temporal and middle third of eyelid without topical anesthesia. The lids were held shut during the test period. After one minute, the paper strip was removed and the length of the wet portion of the strip, as designated by the advancement of dye, was measured in millimeters as an index of tear volume. Control tear volumes for all 3 groups were measured 3 times and averaged before initiation of the experiment. The tests were performed for each rabbit at 1, 2, 4, 6, 8, 12, and 24 hours on the first day of the experiment and twice daily thereafter for 2 weeks. The left and right eyes were tested sequentially. All the test strips were from the same lot and manufacturer. The Schirmer test is noninvasive and causes minimal distress to the animals. The tests were done indoors in a controlled environment.

2.6. Statistical analysis

Serial measurements were correlated for each rabbit because of the repeated observations at each time point. To control for this correlated data structure, a mixed-effects analysis was applied. Statistical analyses were performed using the R statistical language, and p-values < 0.05 were considered statistically significant, but significance was also assessed at the 0.01 level of confidence.

3. RESULTS and DISCUSSIONS

There were no abnormalities, mobility, or inflammation of the surgical sites during the observation period after the placement of the implants.

Both the control and test tear volumes showed great individual variability. The pooled average baseline tear measurement of 3 groups was 10.89 mm with a standard deviation of 2.10 mm. The effect of pilocarpine hydrochloride was observed even at the initial measurement in all 3 groups of rabbits. In particular, the rabbits in Group 1 showed substantial salivation during the loading of the drug, in addition to lacrimation. However, the 3 groups of rabbits showed significant different patterns of tear volume change over time ($p < 0.01$). The average tear volume of Group 1 was high until 8 hours of measurement; however, after that a decreasing tendency was evident and after 36 hours the value had nearly returned to the control measurement. In contrast, the average tear volumes of Groups 2 and 3 exhibited more sustained effects of the drug after a smaller initial peak, compared with that observed in Group 1. The average tear volume of Group 2 approached the control value around day 5 and that of Group 3 decreased to the level of control after 7.5 days (Fig. 2). Considering the weight of experimental animals and the way of administration into blood stream, the amount of loaded drug was considered sufficient to have effects on them. However, additional studies might be necessary to confirm the effects with dry eye/mouth induced animal model since the present study used a normal rabbit model.

In the pilot experiments prior to the present study, using only 3 rabbits, the sustained plasma concentration of pilocarpine derivatives was found after pilocarpine hydrochloride administration through IMDDS in the high performance liquid chromatography analysis (data unrepresented). The confirming results of the present study provide a clue to the solution to this dosing frequency problem. If it were possible to extend the effects of pilocarpine hydrochloride sufficiently without severe side effects, it would be very beneficial for both patients and healthcare providers. Less frequent dosing schedules are known to increase patient compliance and therefore would decrease problems related to skipped dosing. If the prolonged effects could be made last long enough up to recall interval, doctors could change drug cartridges at regular recall visits. At the same time, he/she could perform routine maintenance procedures to the IMDDS, as are done with conventional dental implants.

Because the prototype IMDDS used in the present study were not equipped with any active delivery control components, the release profiles of drugs administered using the IMDDS were largely dependent on the physicochemical characteristics of the drugs. In the present experiment, the Group 1 animals showed instant lacrimation and salivation upon the loading of pilocarpine. It means that the loaded drug diffuse into the intrabony blood stream when it contact with the interface between IMDDS and bone marrow of tibiae since the pilocarpine itself has very soluble characteristics. Practically speaking, this prototype IMDDS acts only as a permanent gate between the outside and the inside of the body, particularly with respect to the bone marrow tissue. Consequently this route of delivery has been termed “Osseogate”. Thus, if we want more elaborate drug delivery, additional technology would be necessary. Control of drug delivery in this system can also be realized at the level of the drug

cartridges, not only at the level of the implant fixture. The present study used gelatin hydrogel as a carrier for pilocarpine hydrochloride.

Gelatin is a natural polymer derived from collagen by acid or alkaline hydrolysis [20], and is commonly used for pharmaceutical and medical applications because of its biodegradability, biocompatibility and great capacity for modification at the level of amino acids [21–23]. Gelatin hydrogels can be formed by physical crosslinking in water; during the process, the molecules aggregate and undergo conformational change from a random coil to a triple helix [24]. The noncovalent bonds formed from this process are not strong and are easily broken at a physiological temperature of 37°C. Therefore, the gels are usually crosslinked by aldehyde or carbodiimides to increase their stability for other biomedical uses [25, 26]. However, we put pilocarpine hydrochloride powder into the sol state of gelatin with water, without the addition of any crosslinking agents, because it was our intention to determine the appropriate degree of delay with respect to clinical efficacy. According to the results, gelatin encapsulation of pilocarpine made the drug effect a little bit weak at initial stage and sustained after that. Interestingly, doubling the dose of pilocarpine in Group 3 demonstrated the increase in lacrimation and more prolonged duration. However, the effects does not reach two times probably because several factors could affect the drug delivery. The pilocarpine in gelatin of Group 3 may have more concentrated than that of Group 2, release rate would be fast. On the other hand, the hydrogel containing pilocarpine use in this study was not exposed to the entire surface of the tissue, which is different from subcutaneous hydrogel implants. The results of the study might suggest the need for some additional modifications to the carrier such as cross-linking of gelatin in order to decrease the drug infusion speed, but we still achieved substantially prolonged drug action.

In this type of drug delivery, excessive drug release is also undesirable. Although pilocarpine is a very safe and well-tolerated drug, it can be used as an epilepsy-inducing pharmaceutical in animal models. However, intraperitoneal injection of as high as 320 mg/kg, is known to be required for this purpose unless there is pretreatment of lithium chloride; this equals at least 13 times the amount administered in Group 3 (60 mg/cartridge per minimum 2.5 kg rabbit). The pharmacokinetics of pilocarpine have not been well investigated and the metabolism of the drug is thought to occur in plasma and neuronal synapses with pilocarpic acid being the primary metabolite [27, 28]. Further studies are recommended to evaluate the plasma concentration profiles of this drug.

Dry eye symptoms are usually evaluated with either the Schirmer test or the Rose Bengal test. The Schirmer test involves placing a sterile filter paper strip beneath the lower eyelid [29]. To acquire acceptable reproducibility, a test of 1 minute without the use of topical anesthesia has been recommended for animal experiments [30]. According to Abrams et al. [31], the average ($\pm$ SD) value of the Schirmer tear test without use of topical anesthesia performed on 142 normal rabbit eyes was 5.30 ($\pm$ 2.96) mm/min; (range, 0 to 15 mm/min). They suggested that the Schirmer tear test is more useful in increased lacrimation rather than the decreased one. The control values before the experiment in the present study were high compared with the previous study. Although the higher volume measured in the present study may have been caused by irritation due the test itself [32], the measurement was done using special caution. Nevertheless, the sustained effects after administration of pilocarpine

by IMDDS were generally demonstrated with even higher amount of tear compared with the high control value by the present study.

The IMDDS as a permanent drug delivery gateway have a lot of utility. It could be installed for the purpose of increase moisture in the mouth like in the present study but its usage can be expanded into nearly any kinds of drug. Especially, the drugs for chronic systemic disease such as insulin for diabetes will be the best candidates because the IMDDS can get rid of the needle pains of everyday injection. In addition, it can be reserved as a route similar to intraosseous injection when there is no need of drug delivery. They can even be used for dental restorative purpose. Recently, the installation of IMDDS into the canine mandible was reported and demonstrated sustained release into systemic circulations [33]. In fact, the oral cavity is the most studied anatomic site for titanium implants. For more than 50 years, the titanium implants have served as stable anchors of dental prostheses for edentulous area. Extensive researches and accumulated experiences made the installation of titanium implants routine procedures of every practice with minimum risk except in compromised cases. Most of the complications reported are mainly in conjunction with restoration/prosthesis [34]. Therefore, the risk of IMDDS would not be any greater than that of conventional dental implants since it does not accommodate load-bearing prosthetic units. Oral cavity is one of strong candidate for IMDDS installation because there is an advantages regarding self-cleansing and maintenance. Furthermore, many patients suffering dry eye and mouths are seniors who usually end up receiving implants due to the loss of teeth. Currently, various kinds of drugs are under investigation for the purpose of IMDDS delivery, including insulin.

The surgery procedure of IMDDS might be a burden to patients of need since there are many alternatives such as eye drops or artificial saliva. However, the installation surgery is relatively simple and flapless if it is done at uncompromised oral cavity. In addition, it takes less than 30 minutes in most cases and the pain is considered below moderate. Moreover, once installed, the IMDDS requires no pain every time the drug is administered. Therefore, the advantage of this device becomes more evident to the patients of chronic disease and to the drug of parenteral administration. Patients of dry eye/mouth could also benefit from this type of administration especially when the symptom is not mild enough to be taken care of with easily accessible over-the-counter remedies.

4. CONCLUSION

Highly water-soluble pilocarpine hydrochloride which was administered by IMDDS demonstrated prolonged effect on lacrimation in normal rabbit model when it is provided in gelatin hydrogel carrier. Compared with pilocarpine (30mg) administered as raw powder showing sharp drop in lacrimation around 8 hours, the pilocarpine in gelatin hydrogel showed much prolonged effects on lacrimation until 5 days (30mg) and 7.5days (60mg) respectively.

The sustained effect could reduce the dosing frequency of this drug, which may be beneficial to both patient and doctor of xerostomia/xerophthalmia, although more elaborate control of delivery is required for clinical purpose in the future.

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Biography

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Fig. 1. Implant-mediated drug delivery system (IMDDS) (top left), schematic diagram of its drug release into bone marrow space (top right) and installed IMDDS at rabbit tibia (bottom)

Fig. 2.
Schirmer test results of 3 groups. The dotted horizontal lines denote the 95% confidence interval of pooled control measurements of 3 groups. The bottom graph show the superimposition of results from 3 groups.