Review Article

Intravesical liposome drug delivery and IC/BPS

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Abstract: Intravesical therapy has previously shown to be effective in delaying or preventing recurrence of superficial bladder cancer. This local route of drug administration is now demonstrating promise in the treatment of interstitial cystitis/bladder pain syndrome (IC/BPS) with the benefit of minimal systemic side effects. Liposomes (LPs) are lipid vesicles composed of phospholipid bilayers surrounding an aqueous core. They can incorporate drug molecules, both hydrophobic and hydrophilic, and vastly improve cellular uptake of these drug molecules via endocytosis. Intravesical LPs have therapeutic effects on IC/BPS patients, mainly due to their ability to form a protective lipid film on the urothelial surface and repair the damaged urothelium. This review considers the current status of intravesical LPs and LP mediated drug delivery for the treatment of IC/BPS.

Keywords: Liposome (LP); bladder; interstitial cystitis (IC)

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Introduction

Intravesical therapies provide a high concentration of drugs to the diseased bladder with minimal or undetectable systemic levels. Evidence to date shows that there is a low risk of systemic side effects (1,2). Intravesical therapy is commonly used to treat superficial bladder cancer; therefore, it seems reasonable to apply these methods to improve the treatment of functional bladder conditions such as interstitial cystitis/bladder pain syndrome (IC/BPS). The urothelium is a highly impermeable surface and many drugs are not stable in the hostile urine environment (2,3). Liposomes (LPs) are lipid vesicles composed of phospholipid bilayers surrounding an aqueous core (4). Empty LPs can protect damaged urothelium and have shown therapeutic benefits for IC/BPS patients (5). In addition, LPs can carry various drugs to penetrate urothelium and modulate afferent neurotransmission (2,6).

Interstitial cystitis/bladder pain syndrome (IC/BPS)

IC/BPS is a chronic disease characterized by suprapubic/bladder discomfort accompanied by urinary frequency, urgency, or nocturia in the absence of infection or other pathological conditions (7,8). The debilitating condition of IC/BPS results in diminished quality of life (9). IC/BPS is not a rare condition, and it occurs more frequently in women than men in a 5:1 ratio. Recent studies have revealed that perhaps over 3 million men and women in the U.S. have symptoms of IC/BPS (10).

One hypothesis of IC/BPS pathology is that dysfunctional epithelium allows the transepithelial migration of toxic solutes, such as potassium, which can depolarize subepithelial afferent nerves and provoke sensory symptoms (8). Dysfunction of the superficial layer of the glycosaminoglycan (GAG) layer, activation of mast cells in the bladder wall, and down-regulation of tight junctional proteins have also been shown to contribute to the pathophysiology of IC/BPS (6,7,11). Pain-sensing C-fibers located within the uroepithelium and submucosa of the bladder can be activated by either a GAG layer deficiency, release of histamine via mast cells, or release of sensory neurotransmitters from urothelium cells. Neurogenic

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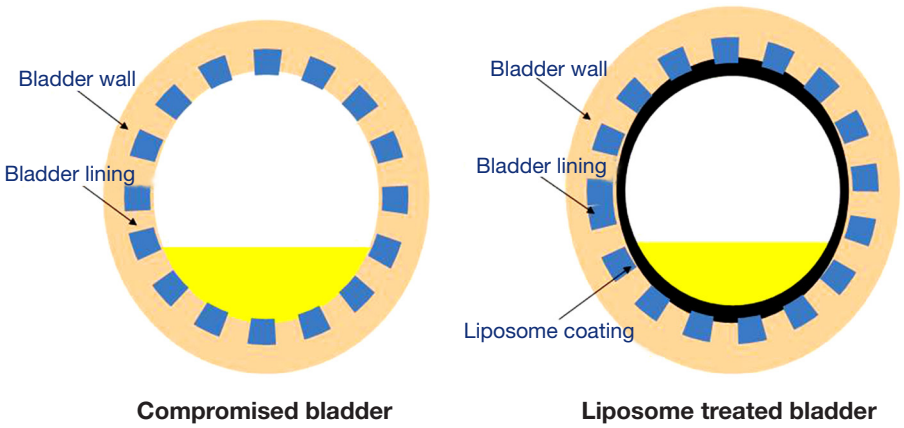

Figure 1 Mechanism of action with intravesical liposome instillation.

inflammation, primary afferent nerve activation, and central nervous system sensitization may all occur and lead to increasing pain, urinary urgency, and frequency.

Rationale for intravesical treatment of IC/BPS

Most of the therapeutic agents for functional bladder disorders are administered orally. These medications may be poorly absorbed and/or metabolized by the liver, and they often fail to have a therapeutic effect at the diseased bladder wall without also producing significant unwanted systemic side effects. Their primary elimination route may not be through the urinary system, which further reduces the amount of drug delivered to the urothelium. The rationale for intravesical treatment for IC/BPS is to apply an effective dose of a therapeutic drug to the diseased organ and only to that organ (*Figure 1*). The anatomy of the urinary bladder and urethra allows easy access and manipulation with a catheter and allows for increased agent exposure via intravesical therapy (12). The advantages of intravesical treatment include:

(I) Coating and repair of bladder urothelium;
(II) High drug concentrations in the bladder;
(III) Minimal incidence of systemic side effects;
(IV) Modulation of urothelial sensory nerve function and neurotransmission.

Limitations to intravesical therapy

IC/BPS patients may be unable to hold a volume of drug in the bladder long enough for the drug to be efficacious. A reduced drug residence time will most likely attenuate therapeutic effects. Another potential shortcoming of intravesical therapy is the dilution of the instilled drug solution due to the continual flow of urine into the bladder. Patients receiving intravesical therapy are advised to decrease fluid intake and empty their bladder before drug administration.

Despite these limitations, it is likely that intravesical therapy can have a positive effect on many IC/BPS patients, especially those with less severe symptoms. Drug delivery to the urothelium via LPs overcomes traditional disadvantages of intravesical bladder therapy (i.e., lack of drug penetration through the urothelium) by bypassing the protective GAG layer.

The urothelium

The structure of the bladder wall, from the luminal to outer surface, consists of the urothelium, detrusor muscle, and adventitia. The urothelium serves as a permeability barrier and prevents urine and waste solute from penetrating into the submucosal layer (13). The urothelium is composed of three different cells: umbrella cells, intermediate cells, and basal cells. Barrier function is established by the arrangement of uroplakins (tight junctional proteins) and is further enhanced by a mucin layer composed of GAG on the luminal surface. The GAG layer is hydrophilic, and forms an aqueous layer on umbrella cells. The GAG layer has been suggested to prevent urine substances from adhering to the bladder lumen. The barrier structure of urothelium restricts the movement of drugs after intravesical administration and restricts the action of the active drug fraction in the urine. Hence, many drugs fail to reach the bladder at desired therapeutic levels and

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ultimately lack pharmacological effects (14).

Liposomal drug delivery

To overcome the limited permeability of the bladder wall, the intravesical approach is able to modulate the release and absorption characteristics of instilled drugs through coupling them to novel carriers such as LPs. LPs are lipid vesicles composed of synthetic or natural phospholipid bilayers that self-assemble to enclose an aqueous interior. They can incorporate hydrophilic and hydrophobic drug molecules of various sizes and promote cellular drug uptake via endocytosis (4). The nontoxic nature of the lipids improves the delivery of various drugs by altering pharmacokinetics, and they have been widely used as drug carriers for a variety of chemotherapeutic agents (15). There is a long history of pharmaceutical agents with improved safety, and sometimes efficacy, when delivered by LPs (16).

Non-clinical studies of liposome (LP) for IC/BPS

Intravesical delivery of hyaluronic acid (HA), heparin, and chondroitin sulfate (CS) restores the barrier function lost due to epithelial dysfunction in IC/BPS. The same concept can be applied to LPs. LPs may aid in the formation of a lipid film on the luminal surface of the urothelium that protects it from penetration by irritants, stabilize neuromembranes of damaged nerves, and reduces hyperexcitability.

Fraser *et al.* (17) reported the effect of intravesically administered LPs of L-α-phosphatidylcholine: cholesterol at 2:1 in a rat model of hyperactive bladder induced by protamine sulfate (PS) followed by KCl or acetic acid infusion to mimic the IC state. The cystometrographic results showed that the bladder hyperactivity was partially reversed by treatment with the LP formulation.

Tyagi *et al.* (18) evaluated the comparative efficacy of LPs against intravesical instillation of dimethyl sulfoxide (DMSO) and pentosan polysulfate (PPS) in chemically induced bladder hyperactivity in rats by sequential infusion of PS and KCl. Intravesical LPs were effective in doubling the intercontractile interval (ICI) compared with PPS, while acute instillation of DMSO failed to produce any protective effect in this animal model.

A recent study showed that LPs carrying a trace amount of near-infrared (NIR) lipophilic fluorescent dye could be tracked microscopically (19). The LPs coating the bladder surface was indicated by blue-colored coating on the bladder luminal surface in NIR light. The study provides evidence to support that LPs form a protective film coating on the injured bladder lumen surface and assist in the repair of leaky and inflamed uroepithelium.

Clinical studies of liposomes (LPs) for IC/BPS

Chuang *et al.* (5) published the first information on the clinical safety and efficacy of LPs in the urinary bladder in an open-label prospective study of 24 IC/BPS patients. The effect of intravesical LPs (80 mg/40 CC distilled water) once weekly was compared to oral PPS sodium (100 mg) 3 times daily for 4 weeks each. No short- or long-term treatment-related adverse events were reported. Comparable efficacy of significant decreases in urinary frequency and nocturia were observed in each treatment group. Statistically significant decreases in pain, urgency, and the O'Leary-Sant symptom index were observed in the LP group with the effect being most profound on urgency (*Figure 2*). None of the patients reported urinary incontinence, retention, or infection due to LP instillation.

Peters *et al.* reported the results of 14 symptomatic IC/BPS subjects treated with intravesical LPs once a week for 4 weeks in an open-label study (20). No treatment-related adverse events were found over the course of the study. The most frequently reported pain score reduced by 80% at 8 (P=0.01) and 12 weeks (P=0.29). Urgency scores showed significant improvement (57% reduction) at 8 (P=0.076) and 12 weeks (P=0.084). The multilamellar sphingomyelin LPs used in this study (LP-08) were well-tolerated and their effects were associated with improvement in pain, urinary urgency and overall symptom scores (*Figure 3*).

LP delivery of botulinum toxin

The use of botulinum neurotoxin (BoNT) for the treatment of neurogenic detrusor over-activity (NDO) and idiopathic detrusor over-activity (IDO) has recently been approved by the U.S. FDA. BoNT-A acts by cleaving the soluble *N*-ethylmaleimide-sensitive fusion attachment protein receptor (SNARE) protein, SNAP-25 (21) and inhibiting release of various neurotransmitters at the presynaptic vesicle by binding to the synaptic vesicle protein, SV2, during neurotransmitter exocytosis. BoNT has been shown to modulate pain and inhibit afferent neurotransmission including substance P, glutamate, nerve growth factor, calcitonin gene related peptide and adenosine triphosphate (22). Given the function of chemical denervation, BoNT has

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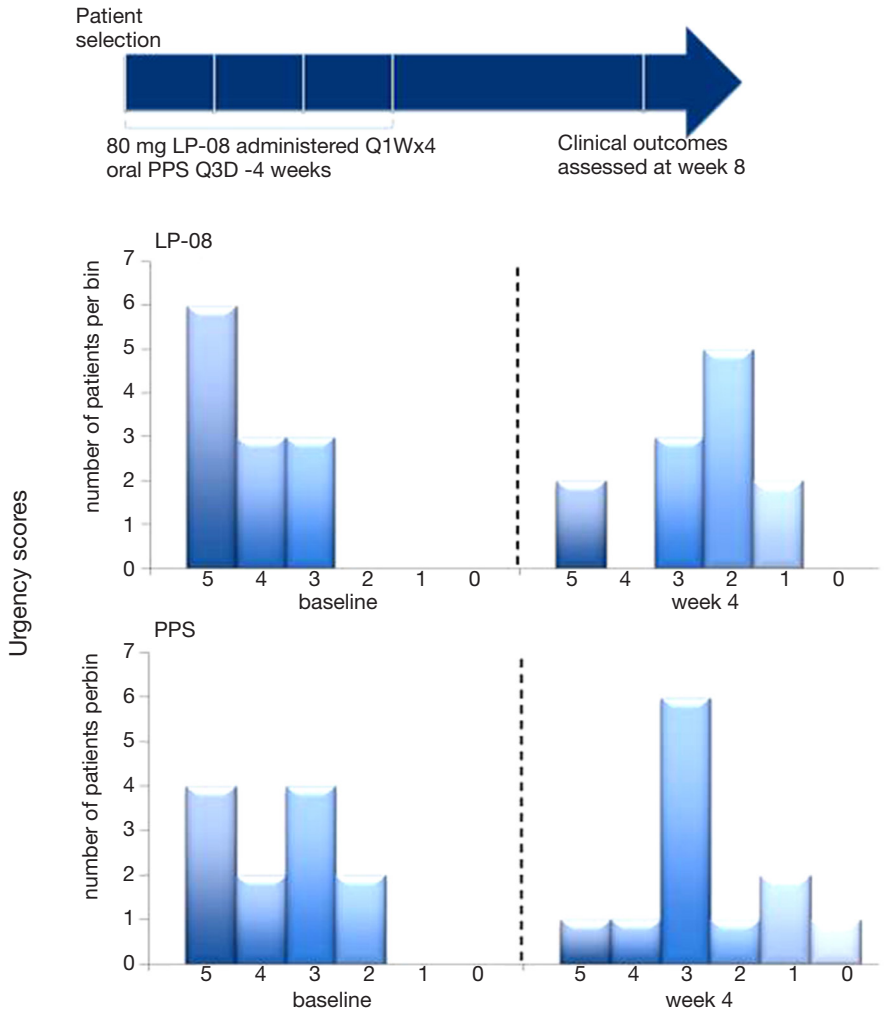

Figure 2 Intravesical liposome (LP-08) *vs.* standard of care oral pentosan polysulfate (PPS) (5).

been successfully used to treat overactive bladder (OAB) as well as IC/BPS through a cystoscopically guided injection. However, the method of intravesical injection has the potential for adverse events, such as urinary tract infection, urinary retention, pain, and hematuria.

BoNT serotype A is a neurotoxin with high molecular weight of 150 kDa. BoNT is generally provided in a saline solution. In this form, it cannot gain access to the submucosal nerve plexus without direct injection through the urothelium. Pretreatment of the urothelium with PS was attempted in rats with the goal of improving the permeability to BoNT (23-25). The cationic PS interacts with the anionic GAG layer, leading to a slight increase in permeability of the urothelium (26). Based on LP's carrier potential and characteristics of adsorption and fusion with cells, the transport of BoNT into urothelium via LPs was studied and confirmed by detection of its unique effect on neurotransmitters and proteolysis of SNAP-25 through western blotting and immunohistochemistry. BoNT encapsulated within LPs is protected from degradation by proteases and proteinases in the urine without compromising efficacy (23). Therefore, instillation of liposomal mediated BoNT (lipo-BoNT) into the bladder is an exciting approach to achieve sustained duration of chemical neuromodulation of afferent neurotransmission underlying IC/BPS and OAB.

Kuo *et al.* (6) reported a double-blind randomized parallel controlled pilot trial in 24 OAB patients at a single tertiary center. Patients were randomly assigned to intravesical instillation of lipo-BoNT containing 80 mg LPs and 200 U

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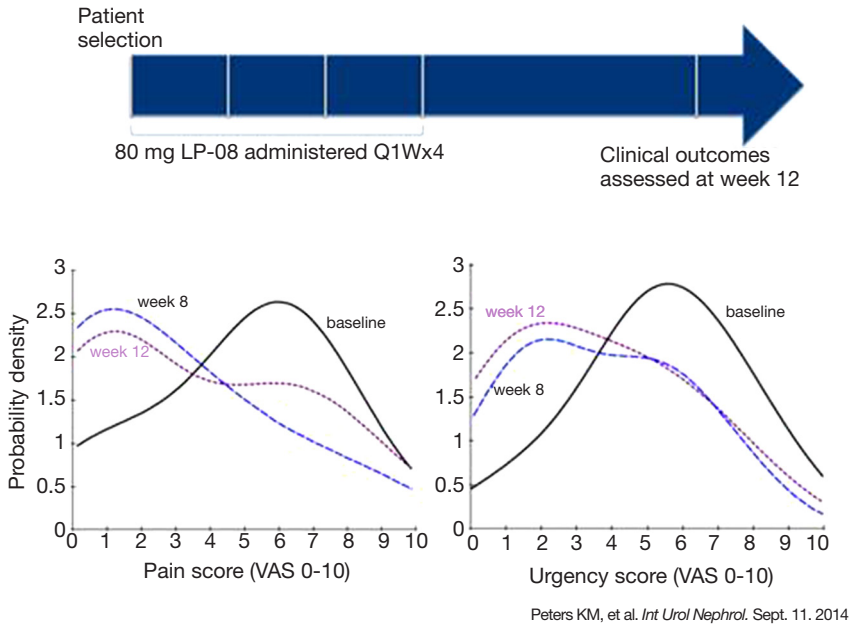

Figure 3 Intravesical liposome (LP-08) reduces pain and urgency scores in symptomatic IC/BPS patients. Probability density functions for pain and urgency scores of patients at baseline, 8 and 12 weeks. The leftward shift of the curves following LP-08 treatment indicates reduced pain and urgency symptoms (20).

BoNT serotype A or normal saline (N/S). Patients were retreated with lipo-BoNT 1 month later if they failed the first treatment. At 1 month post-treatment, the change of urinary frequency as reported on bladder diaries, which was the primary end point, significantly improved in the lipo-BoNT group (n=12; P=0.008) but not in the N/S group. (n=12; P=0.79). Urgency episodes also showed a significant decrease in the lipo-BoNT group (P=0.01) but not in the N/S group (P=0.2). SV2A and SNAP-25 were expressed in urothelial cells and suburothelial tissues. However, the protein expression did not significantly differ between responders and non-responders at 3 months after treatment. It is possible that the SNAP-25 proteins will have recovered by 3 months after treatment (6).

Chuang *et al.* (27) reported a two-center, double-blind, randomized, placebo controlled study enrolled patients with OAB inadequately managed with anti-muscarinics. Patients were assigned to intravesical instillation of lipo-BoNT or N/S. At 4 weeks after treatment, the lipo-BoNT instillation was associated with a statistically significant decrease in micturition events per 3 days (–4.64 for lipo-BoNT *vs.* –0.19 for placebo, P=0.025). The lipo-BoNT instillation was also associated with a statistically significant decrease in urinary urgency events with respect to baseline but not placebo. However, lipo-BoNT instillation was associated with a statistically significant decrease in urgency severity scores compared to placebo (P=0.0181). This study demonstrated that the lipo-BoNT instillation was not accompanied by an increased risk of urinary retention, and none of the patients at either site required intermittent catheterization. Currently, there is an international multicenter prospective double-blind placebo controlled study of lipo-BoNT in IC/BPS that is listed on ClinicalTrials.gov.

LP delivery of tacrolimus

Tacrolimus is a potent hydrophobic immunosuppressive agent that is involved in the inhibition of IL-2-dependent T-cell activation and has a direct inhibitory effect on cell-mediated immunity. Local treatment with tacrolimus has been shown to be beneficial in an ointment or lotion formulation against inflammatory skin conditions without systemic side effects (28). Tacrolimus has poor aqueous solubility; however, a liposomal formulation of tacrolimus greatly increases its solubility within the bladder, and it increases endocytosis and delivery of the drug. A previous study demonstrated that liposomal tacrolimus significantly inhibited cyclophosphamide-induced inflammatory cystitis through modulating interleukin (IL)-2, prostaglandin (PG) E_2, and prostaglandin E receptor 4 (EP) function (29).

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Nirmal *et al.* (30) evaluated the pharmacokinetics of tacrolimus encapsulated in LPs (lipo-tacrolimus). They found the area under the curve of lipo-tacrolimus in serum at 0-24 h was significantly lower than that of tacrolimus instillation or injection, and maximum concentration of lipo-tacrolimus in serum and urine was at 1 and 2 h, respectively. Urine area under the curve after intravesical administration was significantly higher than in the intraperitoneal injection group ($P<0.05$). Single dose pharmacokinetics revealed that bladder instillation of liposomal tacrolimus significantly decreased systemic exposure to instilled tacrolimus. Taken together, these findings support investigation of local tacrolimus in cases of inflammatory bladder disorders refractory to conventional therapy.

A recent study by Rajaganapathy *et al.* (31) examined creating a radiative cystitis rat model and observed the effects of lipo-tacrolimus treatment *vs.* placebo. To generate a radiative cystitis rat model, the animals were attached to a small animal radiation research platform (SARRP). A CT contrast agent was injected to target the SARRP radiation to the rodent bladder. A 40 Gy radiation most reliably produced cystitis symptoms within the bladder, and this level was used for the efficacy study. The radiation significantly lowered inter-micturition interval (IMI) values ($P<0.05$). Four weeks after the radiation, the rats were treated with a lipo-tacrolimus instillation (saline in placebo). The average IMI 4 weeks post-treatment for the lipo-tacrolimus treatment group returned to baseline levels ($P>0.5$; baseline *vs*. treatment) while the saline still showed decreased IMI levels ($P<0.5$; baseline *vs.* placebo). Histology showed that the lipo-tacrolimus treated bladder was identical to a healthy bladder with no features of note, whereas the placebo bladder showed degenerative type epithelial changes, urothelial swelling, and evidence of pseudo-carcinomatous epithelial hyperplasia.

Conclusions

Intravesical LPs have shown safety and efficacy in non-clinical and clinical IC/BPS studies. A prospective, double-blind, and placebo-controlled phase two study of using LPs for IC/BPS is currently ongoing. LPs may also improve vesicular trafficking in the urothelium and aid in improving the delivery of agents across the bladder permeability barrier. Encapsulation of botulinum toxin and tacrolimus inside LPs protected them from urinary degradation without compromising the efficacy of the active drug. The safety of intravesical LP therapy in the studies mentioned has been excellent, with no serious adverse events reported. Intravesical LP and liposomal drug delivery may be an exciting new treatment option for IC/BPS and other urology and women's health disorders.

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None.

Footnote

Conflicts of Interest: The authors are Employees of Lipella Pharmaceuticals, Inc.

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