

# Bladder Instillation of Liposomes for Bladder Coating and Drug Delivery Platform

Pradeep TYAGI,<sup>1\*</sup> Yao-Chi CHUANG,<sup>2</sup> Naoki YOSHIMURA,<sup>3</sup> Jonathan KAUFMANN,<sup>4</sup>  
and Michael B. CHANCELLOR<sup>1</sup>

<sup>1</sup>Department of Urology, William Beaumont Hospital, Royal Oak, MI, U.S.A., <sup>2</sup>Department of Urology, Chang Gung Memorial Hospital, Kaohsiung Medical Center, Chang Gung University College of Medicine, Taiwan, <sup>3</sup>Department of Urology, University of Pittsburgh School of Medicine, Pittsburgh, PA, U.S.A., and <sup>4</sup>Lipella Pharmaceuticals, Pittsburgh, PA, U.S.A.

The clinical use of exogenous polysaccharides for the treatment of interstitial cystitis (IC) has lent credence to the concept of a dysfunctional urothelium as the cause of lower urinary tract symptoms (LUTS). Studies have shown that lipids in the apical membrane of the urothelium form an integral component of the permeability barrier in the bladder. This premise is supported by the therapeutic effect of empty liposomes in the irritated bladder. Instillation of liposomes comprised of natural phospholipids can augment bladder barrier function and support repair following injury from protamine sulfate and irritation with high potassium concentration. The mechanism of action proposed for the therapeutic effect of empty liposomes is that liposomes form a coat on the injured urothelium and block irritation of submucosal afferent nerves. Reduced afferent excitation after liposome instillation is reflected in prolonged intercontractile interval in cystometry. Liposomes offer a powerful new treatment option for IC using an intravesical route and as a platform for intravesical drug delivery.

**Key words** cystitis, cystometrogram, frequency, liposomes

## 1. INTRODUCTION

Most of the path-breaking discoveries credited to Dr De Groat have been made possible through his collaboration and association with experts in the field of the lower urinary tract, except for one: the serendipitous discovery of placing empty liposomes into the bladder for the treatment interstitial cystitis. The discovery process also set the ball rolling for the application of nanotechnology for the study of pharmacology in the lower urinary tract and in the treatment of interstitial cystitis (IC).<sup>1</sup>

IC is a chronic disease of the bladder characterized by urinary frequency, urgency, nocturia, and suprapubic pain, affecting approximately 1 million Americans.<sup>2</sup> IC affects women much more frequently than men, with a ratio of 10:1. The onset of symptoms usually occurs from 30 to 40 years of age. Although the pathogenesis of IC is uncertain, it has been proposed that a dysfunctional epithelium allows the transepithelial migration of solutes, such as potassium, which can depolarize subepithelial afferent nerves and provoke sensory symptoms.<sup>3</sup> A compromised permeability barrier has been shown in a feline model of interstitial cystitis IC as well as in the majority of the IC patients.<sup>4,5</sup>

In recent years, treatments focused on techniques that coat the urothelium and maintain its integrity relative to permeability have gained tremendous interest. Parsons<sup>6</sup> successfully employed oral and intravesical

application of sulfated polysaccharides in the treatment of IC. The therapy of exogenous polysaccharides is based on the premise that bladder mucus and exogenous glycosaminoglycans (GAG) form a protective coating on the bladder surface.<sup>6</sup> Studies have shown that lipids in the apical membrane of the urothelium are also an integral component of the permeability barrier in the bladder.<sup>7</sup> The uppermost layer of the urothelium is comprised of umbrella cells, whose apical plasma membranes facing the urine have a unique lipid composition and transmembrane uroplakin proteins; both of which seem to play important roles in reducing the permeability of the apical membrane to water, ammonia, protons, and urea.<sup>8</sup>

The therapeutic effect of empty liposomes in the irritated bladder<sup>9</sup> argues that the dysfunctional urothelium not only involves depletion in the GAG layer, but is also likely to involve deficiency in the lipid structure of the urothelium. In animal models of bladder injury, exposure to protamine sulfate not only compromises the GAG layer, but also produces nonselective pores in the apical membrane, leading to irreparable cell damage.<sup>10</sup> Scanning and transmission electron microscopic examination of the

\*Correspondence: Pradeep Tyagi PhD, Department of Urology, William Beaumont Hospital, Royal Oak, MI, U.S.A. Tel: +1 248 5518 296; Fax: +1 248 551 0486. Email: pradeep.tyagi@beaumont.edu  
DOI: 10.1111/j.1757-5672.2009.00040.x

bladder have shown necrosis and sloughing of sheets of umbrella cells after exposure to protamine sulfate (PS).<sup>11</sup>

Studies on inflammatory disorders, such as pancreatitis and acute lung injury, have already demonstrated a protective role for exogenous supplementation of phospholipids.<sup>12,13</sup> Free phospholipids generated from cell membranes at the site of injury can attenuate inflammation and augment membrane barrier function. Based on those reports, we hypothesize that instillation of liposomes comprised of natural phospholipids can augment bladder barrier function and support repair of the injured lipid membrane.

Liposomes can be described as vesicles composed of concentric phospholipid bilayers separated by aqueous compartments; or in a jargon-free manner as bubbles of phospholipids filled with water instead of air. The walls of liposomes are made of layers of phospholipids identical to the phospholipids that comprise cell membranes, which explains their biocompatibility and lack of biological toxicity.<sup>14</sup> Liposomes were first discovered in 1961 by Alec D. Bangham, who was studying phospholipids and blood clotting. He noted that dried phospholipids self-assemble into a sphere after adding water because one end of each molecule is water-soluble, while the opposite end is water insoluble.

The ability of liposomes to adsorb on cell surfaces and fuse with cells has made them a favorite choice for topical drug carriers. Liposomes seem to have an innate ability for tissue repair due to their capacity to bind water and form a molecular film on cell surfaces.

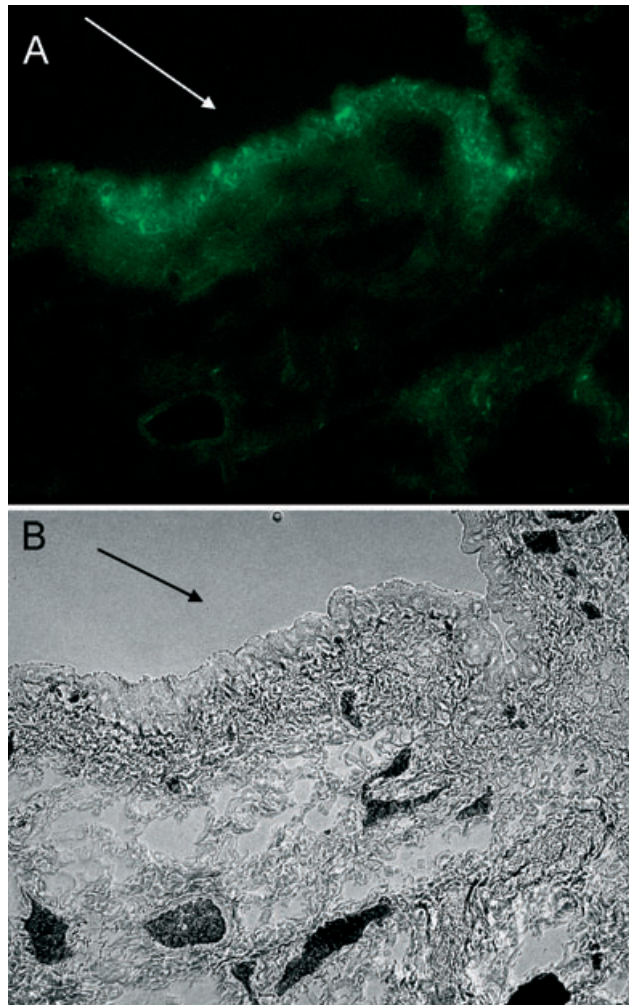
Liposomes provide a moisture film on the wound and promote wound healing without chronic inflammatory reaction in the neodermal layer.<sup>15</sup> Other investigators suggest that liposomes could interact with cells by stable absorption, endocytosis, lipid transfer and fusion.<sup>16</sup>

## 2. ABILITY OF LIPOSOMES TO FORM A FILM ON THE BLADDER SURFACE

Rats were instilled with liposomes containing trace amounts of fluorescent lipid (5% w/w). Eight hours after instillation bladder sections were taken of the whole bladder. A fluorescent signal detected by a microscope in bladder sections was localized in the top layers of the urothelium in the bladder (Fig. 1).

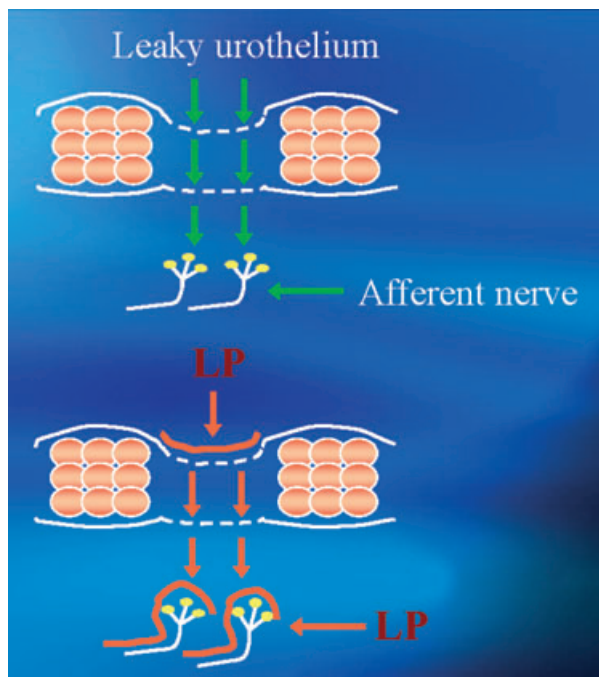
The pharmacology of empty liposomes in treating lower urinary tract symptoms has been evaluated in multiple rodent models of bladder irritation.<sup>9,17</sup> Animal models rely on producing tissue irritation to cause increased afferent excitability, which ultimately leads to increased urinary frequency and urgency in an effort to reduce the effect of urine on the bladder wall.<sup>18</sup> In our study, bladder instillation of liposomes protected against bladder injury and blunted the irritation induced by potassium.<sup>9,17</sup>

We measured an integrated response of the bladder to injury followed by irritation with potassium chloride.<sup>17</sup> Sequential administration of liposomes after PS allowed an unrestricted injurious effect of protamine sulfate on the bladder surface to occur before treatment with empty liposomes. Liposomes improve the barrier function of the



**Fig. 1** Liposomes instilled in a rat bladder formed a coating on the urothelium surface. Instilled liposomes contained 5% w/w of fluorescent phospholipids, along with natural phospholipids obtained from egg yolk. Whole bladder sections (20  $\mu$ ) were taken and photographed with a fluorescent microscope. (A) Fluorescent image with arrow indicating the urothelium and (B) bright field image of the same section. The fluorescent signal is mostly localized to the urothelium, indicating prolonged residence in the bladder.

injured bladder and the specific changes in cystometric parameters brought about by liposomes suggest that they modulate the afferent branch of the micturition reflex and do not act on the efferent branch (Fig. 2).<sup>19</sup> Afferent activity originating in the bladder primarily determines the intercontractile interval between bladder contractions and an increase in intercontractile interval (ICI) with liposomes suggests that liposomes attenuate the afferent activity induced by bladder irritation. Recent published studies performed at our lab have shown that certain molecular properties of phospholipids are preferred for an intravesical therapeutic effect, such as an absence of net charge in the headgroup and asymmetrical saturation in acyl chains.<sup>20</sup> Future studies in the clinic and lab will determine the duration of the therapeutic effect from liposome-based therapy.



**Fig. 2** Proposed mechanism of instilled liposome (LP) in bladder irritation. Liposomes form a coat on the injured uroepithelium and block irritation of the submucosal afferent nerves. Reduced afferent excitation after liposome instillation is reflected in the prolonged intercontractile interval in cystometry.

### 3. CONCLUSION

The diversity of IC therapies underscores the lack of understanding of the etiology and therapy of this syndrome.<sup>21</sup> None of the existing treatments uniformly eradicate the symptoms of urinary frequency, urgency, nocturia, and/or pain. The response to therapy is fraught with variation owing to the multifactorial nature of IC, and the clinical approach to treatment has been largely empirical and can involve a variety of oral and intravesical therapies.<sup>2</sup> Intravesical therapies for interstitial cystitis have been the mainstay of treatment for many years.<sup>22</sup> They provide high local drug concentrations in the bladder, low risk of systemic side-effects and eliminate the problem of low levels of urinary excretion with orally administered agents. Liposomes offer a powerful new treatment option for IC using an intravesical route.

### CONFLICT OF INTEREST

Michael Chancellor holds stocks in the company Lipella Pharmaceuticals developing liposomes as intravesical therapy.

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### My Journey in Pittsburgh, Pradeep Tyagi

Let me share with you how my involvement started in this exciting project. I came to Pittsburgh in July 2000, only a few months after my PhD advisor Dr Leaf Huang moved away from his location next door to Dr De Groat's office at the School of Pharmacy at the University of Pittsburgh, Pennsylvania, USA.



Dr Huang told me that although they were next door neighbors for over a decade, it was only in the latter part of that period that they started sharing research notes. I am glad that great minds came together to carve out a well thought-out project for PhD dissertation. This exciting project on liposomes introduced me to the field of neurourology. I thoroughly enjoyed working on this endeavor of taking an exciting concept from the benchtop to the bedside under the guidance of Dr Michael Chancellor and in collaboration with our longtime friend Dr Yao-chi Chuang. It is quite amazing how working in the company of Dr William Chet de Groat allows you to make lifelong friends on both sides of the Pacific Ocean.

**Biography** Pradeep Tyagi earned his PhD under Dr Leaf Huang at the University of Pittsburgh and worked with Drs William de Groat, Michael Chancellor and Naoki Yoshimura. After he successfully defended his thesis he was recruited to join the Department of Urology where his groundbreaking work on intravesical liposome research was awarded an NIH grant-funded and technology transfer. He was recruited to the William Beaumont Hospital Research Institute to collaborate with Dr Chancellor. Pradeep's wife, Shachi Tyagi, MD, is an internal medicine resident at the William Beaumont Hospital.