

# Instillation of liposomes vs dimethyl sulphoxide or pentosan polysulphate for reducing bladder hyperactivity

Pradeep Tyagi, Vincent C. Hsieh\*, Naoki Yoshimura<sup>†</sup>, Jonathan Kaufman<sup>‡</sup> and Michael B. Chancellor

Department of Urology, William Beaumont Hospital, Royal Oak, MI, \*Department of Anaesthesiology, Stanford University School of Medicine, Stanford, CA, <sup>†</sup>Department of Urology, School of Medicine, University of Pittsburgh, and <sup>‡</sup>Lipella Pharmaceuticals Inc., Pittsburgh, PA, USA

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## OBJECTIVE

To investigate the efficacy of intravesical liposomes against dimethyl sulphoxide (DMSO), and pentosan polysulphate (PPS) in reducing chemically induced bladder hyperactivity in rats.

## MATERIALS AND METHODS

Bladder reflex activity of female Sprague-Dawley rats was evaluated by continuous cystometry under urethane anaesthesia (1.0 g/kg). After obtaining a control cystometrogram (CMG) with normal saline (0.04 mL/min) for 2 h, bladder hyperactivity was then induced by 1 h infusion of

protamine sulphate (10 mg/mL) followed by a 1-h infusion of KCl (500 mM). Six rats each were then infused with KCl-based preparations containing either 50% DMSO, PPS (6 mg/mL), or liposomes (2 mg/mL) for 2 h. The variables measured included the intercontraction interval (ICI), pressure threshold (PT) and baseline pressure (BP).

## RESULTS

Sequential infusion of protamine sulphate/KCl induced hyperactive bladder with no significant difference in ICI, PT or BP among groups before initiating treatment. ICI was significantly increased after infusion of PPS (58.1% increase) and liposomes (156.8% increase) but there was no increase with DMSO. PT was not significantly affected by liposome infusion but slightly increased with PPS (12.4% increase). There was a large and

significant increase in PT and BP with DMSO (116.5% increase) and BP largely remained unchanged after instillation with liposomes or PPS.

## CONCLUSIONS

Intravesical liposomes and PPS have a beneficial effect in a bladder hyperactivity rat model, while acute instillation of DMSO does not. Intravesical liposomes were effective in doubling the ICI compared with PPS, and might be a new treatment option for bladder hyperactivity.

## KEYWORDS

interstitial cystitis, women, health, liposome, bladder, overactive

## INTRODUCTION

The overactive bladder (OAB) syndrome and interstitial cystitis/painful bladder syndrome (IC/PBIS) are clinical syndromes characterized by urinary urgency, frequency, with and without painful bladder, respectively, in the context of sterile urine [1]. Pathological changes in urothelial permeability are thought to allow the passage of solutes, such as potassium, from urine to deeper layers of the bladder wall, where subepithelial afferent nerve fibres are depolarized to produce urinary symptoms [2–4]. Mast cell abnormalities and neurogenic inflammation are also attributed as possible factors leading to the pathogenesis of IC/PBIS. The unknown

aetiology of OAB and IC/PBIS has resulted in various treatment regimens for its management.

The most common first line of treatment for OAB are antimuscarinic agents and most common for IC/PBIS is replenishment of the protective glycosaminoglycan (GAG) layer by exogenous GAGs, e.g. heparin, chondroitin sulphate, hyaluronate or the semisynthetic pentosan polysulphate (PPS) [5]. This line of treatment is only effective in a third of patients and severe cases frequently require instillation of drugs into bladder. Instillation of dimethyl sulphoxide (DMSO) is one such treatment, approved by the USA Food and Drug Administration, that provides

symptomatic relief, probably via anti-inflammatory properties and mast cell-stabilizing effects [6]. However, none of these treatments uniformly eradicates the symptoms of urinary frequency, urgency, nocturia and/or pain [7].

In a previous study we reported the effectiveness of liposomes to reverse bladder hyperactivity after intravesical administration in an acute rat model of bladder hyperactivity [8]. Liposomes are microscopic vesicles composed of concentric phospholipid bilayers separated by aqueous compartments. The nontoxic biocompatible nature of liposomes had made them a potential topical coating therapy, and possible carriers for drugs [9].

The extensive safety record in widespread clinical use of liposomes as vehicles via the systemic route, and preclinical effectiveness after intravesical administration, makes them a promising tool in the clinical treatment of urinary urgency and frequency symptoms. Thus we investigated the efficacy of intravesical liposomes against DMSO and PPS in reducing chemically induced bladder hyperactivity in rats.

## MATERIALS AND METHODS

Twenty-four female Sprague-Dawley rats (250–300 g) were anaesthetized with urethane (1.0 g/kg s.c.) for use in this study. Heating lamps were used to maintain physiological body temperature throughout the duration of the experiment.

For the cystometrogram (CMG), a transurethral bladder catheter (PE-50) was connected by a three-way stopcock to a pressure transducer and syringe infusion pump to facilitate simultaneous intravesical infusion and pressure monitoring. The pressure transducer was linked to a computer running software for continuous data recording. A control CMG was obtained by slowly filling the bladder with saline (0.04 mL/min) to elicit repetitive voiding. The variables measured included the intercontraction interval (ICI), pressure threshold (PT) and baseline pressure (BP), which is representative of the pressure nadir after contraction. The measurements in each rat represent the mean of 10 sequential bladder contractions.

The acute model of overactive bladder was induced as previously described [10]. After control CMGs were recorded for each rat, a hyperactive bladder was induced by a sequential infusion of protamine sulphate (PS; Sigma Chemical Co., St Louis, MO, USA; 10 mg/mL) for 1 h, followed by KCl (500 mM) for an additional 1 h. Six rats each were then randomized into one of three treatment options: (i) DMSO 50% v/v (Edwards Life Science Research Medical, Inc., Irvine, CA); (ii) PPS (6 mg/mL) (Ortho-McNeil Pharmaceutical, Inc., Titusville, NJ); and (iii) liposomes, with a lipid concentration of 2 mg/mL (2.84 mM; Lipella Pharmaceuticals Inc., Pittsburgh PA). All three infusion solutions were prepared in 500 mM KCl. Continuous infusion at 0.04 mL/min was maintained in each of the three groups while CMG tracings were recorded for 2 h.

FIG. 1. Representative CMG tracings from the treatment groups after the infusion. The start of treatment for >1 h is marked by a black arrow; note that compared to untreated controls (bottom tracing) the rats treated with liposome and PPS had a significant increase in the interval between peaks after the start of treatment.

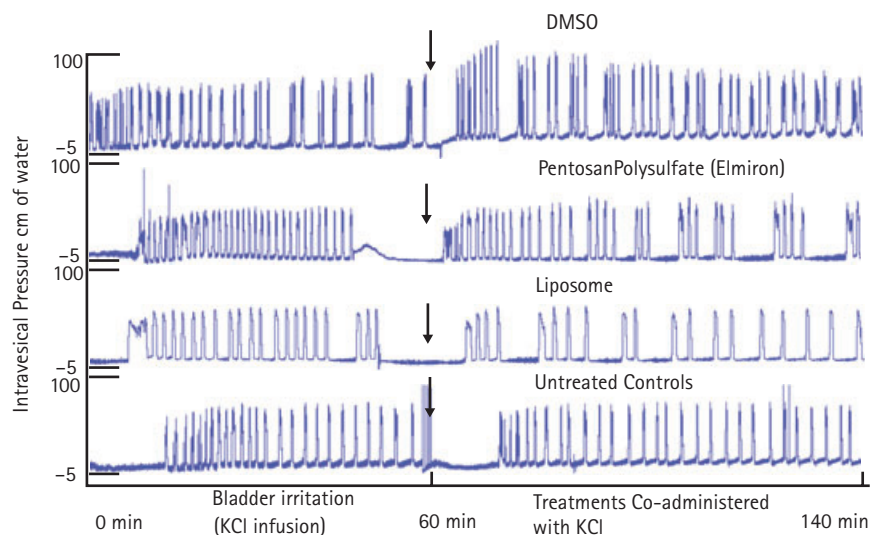


TABLE 1 CMG variables before and after the start of treatment

Mean (SEM) variable	DMSO + KCl	PPS	Liposome
ICI, s			
Before	138.33 (19.23)	139.38 (9.06)	136.45 (11.21)
After	141.35 (16.61)	217.53 (24.34)*	338.97 (38.89)*
% change	5.0 (6.6)	58.1 (9.8)	156.8 (17.8)
PT, cmH <sub>2</sub> O			
Before	16.00 (1.90)	17.38 (1.34)	12.80 (1.08)
After	31.17 (2.39)*	20.32 (1.74)	14.17 (0.79)
% change	106.0 (26.7)	17.7 (7.8)	13.6 (8.9)
BP offset, cmH <sub>2</sub> O			
Before	13.36 (2.16)	14.19 (0.88)	11.05 (0.98)
After	25.32 (2.26)*	14.37 (1.01)	11.36 (0.89)
% change	110.1 (34.4)	1.1 (3.3)	5.4 (9.0)

\* $P < 0.05$ .

All data are expressed as the mean (SEM) and analysed by pair-wise comparison between groups with Student's *t*-test;  $P < 0.05$  was considered to indicate statistical significance.

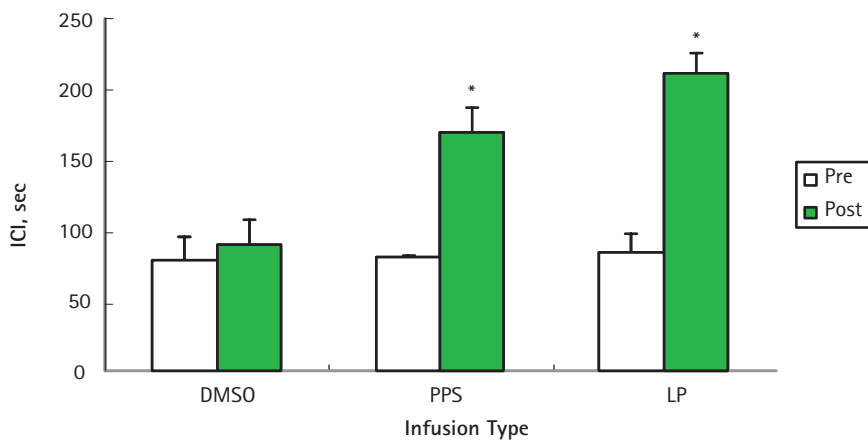
## RESULTS

Sequential infusion of PS/KCl into bladder of rats from all the four groups produced a decrease in ICI compared to that measured during the baseline CMG with saline infusion (Fig. 1). Rats in the group that only received 500 mM KCl in saline showed no change in the

ICI during the treatment period of the other groups (bottom tracing of Fig. 1). Similarly, infusion of 50% DMSO using 500 mM KCl solution in saline also failed to elicit any changes in the ICI of treated rats (top tracing of Fig. 1; Table 1). These results were not unexpected, as it is known from clinical experience and studies on rats that acute administration of DMSO causes irritation [11].

By contrast, we expected the ICI to increase after infusion of PPS and indeed there was a 58.1% increase relative to the ICI before treatment (Table 1). Comparatively, the

FIG. 2. Bar graph of mean ICI before (pre) and after (post) treatment in different groups. The treatments of PPS\* and LP\* significantly reversed the ICI decrease induced by KCl following PS injury. There was no change in the DMSO treatment group. \* denotes  $P$  value  $< 0.05$ .



infusion of liposome in hyperactive bladder more than doubled the pretreatment ICI (Table 1, Fig. 2, 156.8% increase,  $P < 0.05$ ). The degree of increase in the liposome group above the PPS group was unexpected. These observations with liposomes corroborates our previous results [7]. PT was not significantly affected by liposome or PPS infusion, but was increased significantly 106.0% increase,  $P < 0.05$ ; Table 1) with instillation of DMSO. Similarly, BP was increased with DMSO (110.1%,  $P < 0.05$ ) but not with liposome or PPS. There was no significant difference in the values of ICI, PT, or BP measured among groups before the start of treatments (Table 1).

## DISCUSSION

The transitional cell epithelium of the bladder lined by a thin GAG layer is believed to shield the bladder surface from toxic constituents in urine [12]. The pathogenesis of IC/PBIS, and perhaps all urgency and frequency sensory symptoms, might originate from epithelial leakage of toxic substances into the bladder wall that would subsequently induce the resulting characteristic symptoms of pain, frequency, urgency and nocturia. A marked increase in urea absorption from the bladder of patients with IC and normal subjects (after mucosal injury induced by PS) was reported [1,13]. In addition, the injured bladders of normal subjects showed a significant increase in potassium absorption [14]. Exogenous supplementation of PPS and hyaluronic acid is presumed to restore the GAG layer on bladder surface, lost as a result of the disease.

The present rat model of bladder hyperactivity is particularly suited to assessing the effectiveness of liposomes, as it allows co-administration of liposomes with the irritant KCl into the bladder to mimic the real-life situation. The rate and concentration of irritant infusion (KCl) can be controlled during the experiments with this model, unlike the model of cyclophosphamide-induced cystitis, where the rate and urinary concentration of irritant (acrolein) varies with rat's rate of metabolism.

The concentration of intravesical PPS used in our study (6 mg/mL) was chosen based on a previous report [15]. Moreover, dose escalation of PPS by infusion in patients did not lead to the expected results and the concentration used here is well above the expected urinary concentration of unmetabolized PPS, given a standard dose of 300 mg PPS per day. Acute administration of DMSO with KCl failed to show any therapeutic benefit in the present study. The ICI after DMSO treatment remained unchanged, but the PT and BP were significantly increased. These observations made in the DMSO-treated group are consistent with the ability of DMSO to desensitize nociceptive bladder afferents [11]. Isolated rat bladder strips exposed to 40% DMSO lost the ability to contract on electrical-field stimulation and bladder compliance was also decreased [16].

The results obtained with liposomes in hyperactive bladder might be related to their ability to adsorb and create a molecular film on cellular surfaces [17]. Bladder injury

induced by PS leads to increase in lipid peroxidation in the bladder, and the anti-inflammatory and antioxidant potential of liposomes against lipid peroxidation might be a unique effect of intravesical instillation. Free phospholipids generated from cells at the site of injury might attenuate inflammation and augment membrane barrier function. It is probable that intravesical exposure of natural phospholipids via liposomes improves the barrier function in hyperactive bladder.

In conclusion, the present study in a rat model showed that liposomes are superior to DMSO and PPS in producing immediate beneficial effects after intravesical instillation in rats with chemically induced bladder hyperactivity. Acute intravesical instillation of DMSO lacks the therapeutic benefit shown by PPS and liposomes, as the latter doubled the ICI over that of the former. Intravesical liposomes might be considered a potential therapy for patients with sensory urgency and frequency. Further research and controlled human trials will be needed.

## CONFLICT OF INTEREST

Michael B. Chancellor is a patent holder for the mentioned product, a paid consultant to the sponsor and a board member with the sponsor; Jonathan Kaufman is an employee of the sponsor.

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**Correspondence:** Michael B. Chancellor, 3535 West 13 Mile Road, Suite 438, Royal Oak, MI 48073, USA.  
e-mail: michael.chancellor@beaumont.edu

**Abbreviations:** OAB, overactive bladder; IC, interstitial cystitis; PBIS, painful bladder syndrome; GAG, glycosaminoglycan; PPS, pentosan polysulphate; DMSO, dimethyl sulphoxide; CMG, cystometrogram; ICI, intercontraction interval; PT, pressure threshold; BP, baseline pressure; PS, protamine sulphate.