

INTRAVESICAL LIPOSOME ADMINISTRATION—A NOVEL TREATMENT FOR HYPERACTIVE BLADDER IN THE RAT

MATTHEW O. FRASER, YAO-CHI CHUANG, PRADEEP TYAGI, TERUHIKO YOKOYAMA, NAOKI YOSHIMURA, LEAF HUANG, WILLIAM C. DE GROAT, AND MICHAEL B. CHANCELLOR

ABSTRACT

Objectives. To examine the effect of intravesical administration of liposomes (LPs) on chemically induced bladder hyperactivity in the rat. It has been suggested that interstitial cystitis (IC) is associated with a dysfunctional or leaky epithelium. Thus, enhancement of epithelial barrier function might be useful in the treatment of IC. LPs are vesicles that are concentric phospholipid bilayers separated by an aqueous compartment and can fuse with cells to provide a molecular film that can promote wound healing.

Methods. The intravesical pressure was recorded using a transurethral catheter in adult female Sprague-Dawley rats anesthetized with urethane (1.2 g/kg subcutaneously). Some animals were pretreated with capsaicin (125 mg/kg subcutaneously) 4 days before the experiments. Continuous cystometrograms were performed by slowly filling the bladder (0.04 mL/min) with solutions of varying compositions, including saline, acetic acid (AA, 0.1%), potassium chloride (KCl, 500 mM), protamine sulfate (PS, 10 mg/mL), LPs, PS/KCl, or LPs/KCl. The parameters measured included the intercontraction interval (ICI), amplitude of bladder contractions, compliance, and micturition pressure threshold.

Results. The ICI was decreased after exposure to AA (79.8% decrease) or PS/KCl (81% decrease); however, the ICI was not changed after LPs, PS, or KCl alone. The decreased ICI was partially reversed after infusion of LPs (172.8% increase) or LPs/KCl (63% increase), but was not significantly changed after switching to saline or KCl administration. Pretreatment with capsaicin delayed the onset of the irritative effects of AA by approximately 30 to 60 minutes, but had not changed the magnitude after 2 hours of infusion.

Conclusions. Intravesical administration of PS/KCl or AA activates capsaicin-sensitive and capsaicin-resistant afferents in a time-dependent sequence that is partially reversed by LP infusion. We hypothesize that LPs might enhance the barrier properties of a dysfunctional uroepithelium and increase resistance to irritant penetration. Thus, intravesical LP administration could be a novel treatment of patients with IC. *UROLOGY* 61: 656–663, 2003. © 2003, Elsevier Science Inc.

Interstitial cystitis (IC) is a clinical syndrome characterized by bladder pain, irritative voiding symptoms, and sterile urine. Although the pathogenesis of IC is uncertain, it has been proposed that a dysfunctional epithelium allows the transepithe-

lial migration of solutes, such as potassium, that can depolarize subepithelial afferent nerves and provoke sensory symptoms.^{1–4}

Liposomes (LPs) are vesicles composed of concentric phospholipid bilayers separated by aqueous compartments.^{5,6} Because LPs adsorb to cell surfaces and fuse with cells, they are being used as vehicles for drug delivery and gene therapy.^{5,7,8} In addition, they create a molecular film on cell surfaces and therefore are being tested as possible therapeutic agents to promote wound healing.^{5,6}

Previous reports have shown that patients with IC have a defect in the glycosaminoglycan (GAG) layer of urothelium.^{1–3} Those therapies, such as intravesical administration of heparin, hyaluronic acid, or pentosan polysulfate,^{2,9,10} which appear to restore the GAG layer on the surface of the epithe-

This study was supported by the Interstitial Cystitis Association and Fishbein Family Foundation and Chang Gung Memorial Hospital Grant CMRP 1120.

From the Departments of Urology and Pharmacology, University of Pittsburgh School of Medicine; University of Pittsburgh School of Pharmacy, Pittsburgh, Pennsylvania; Division of Urology, Chang Gung Memorial Hospital, Kaohsiung; and Department of Urology, National Yang Ming University School of Medicine, Taipei, Taiwan

Reprint requests: Michael B. Chancellor, M.D., Department of Urology, University of Pittsburgh School of Medicine, 3471 Fifth Avenue, Kaufman Building, Suite 700, Pittsburgh, PA 15213

Submitted: April 2, 2002, accepted (with revisions): September 30, 2002

lial barrier, may reduce the leakage of irritants and result in palliation of IC symptoms. Similarly, intravesical administration of LPs, which adhere to the cell, may improve the dysfunctional uroepithelium and provide an alternative treatment of IC. In this study, we evaluated the effects of intravesical LP administration on two types of chemically induced bladder hyperactivity (potassium chloride [KCl]/protamine sulfate [PS] combined or acetic acid [AA]) in the rat that are attributable in part to activation of afferent nerves. Intravesical instillation of KCl solutions has been used clinically as a provocative diagnostic test for IC,⁴ and AA-induced irritation has been used extensively to study bladder nociceptive pathways in the rat.^{11,12} The results have indicated that LP treatment can reduce the irritative bladder symptoms in both models.

MATERIAL AND METHODS

ANIMAL PREPARATION

Thirty-four female Sprague-Dawley rats (250 to 300 g) anesthetized with urethane (1.2 g/kg subcutaneously) were used in this study. Their body temperature was maintained in the physiologic range using a heating lamp.

CYSTOMETROGRAPHY

A transurethral bladder catheter (PE-50) connected by a three-way stopcock to a pressure transducer and to a syringe pump was used to record intravesical pressure and to infuse solutions into the bladder. A control cystometrogram (CMG) was performed by slowly filling the bladder with saline (0.04 mL/min) to elicit repetitive voiding. The parameters recorded were amplitude, pressure threshold (PT), compliance, and intercontraction interval (ICI) of the reflex bladder contractions. The measurements in each animal represent the average of 3 to 5 bladder contractions.

INDUCTION OF A HYPERACTIVE BLADDER

After performing control CMGs with saline infusion, five types of experiments were performed by intravesical infusion of various solutions: (a) infusion of PS (Sigma Chemical; 10 mg/mL) for 1 hour (n = 6) to increase epithelial permeability; (b) infusion of KCl (500 mM) for 1 hour and then infusion with PS/KCl for another hour, followed by infusion of either KCl for 2 hours (n = 6) or LPs/KCl for 2 hours (n = 6); (c) infusion of AA (0.1%) for 1 hour, followed by infusion of saline (n = 6) or LPs (n = 6) for 2 hours; (d) infusion of LPs for 1 hour, followed by infusion of AA for 1 hour (n = 6); and (e) infusion of AA (n = 4) for 2 hours in rats subcutaneously injected with capsaicin (125 mg/kg in 10% ethanol, 10% Tween-80, and 80% saline) 4 days before the experiment.¹³ The experimental design is illustrated in Figure 1. The KCl concentration was within the range of concentrations present in normal rat urine.¹⁴

PREPARATION OF LPs

LPs were prepared as described by Kirby and Gregoriadis.¹⁵ LPs were constructed as a 2:1 molar ratio of L- α -phosphatidylcholine and cholesterol (Sigma Chemical, St. Louis, Mo) to a final lipid concentration of 2 mg/mL in saline. Lipids in chloroform were dried together in the proper ratio under nitrogen. The residues were reconstituted as LPs in saline or 500 mM

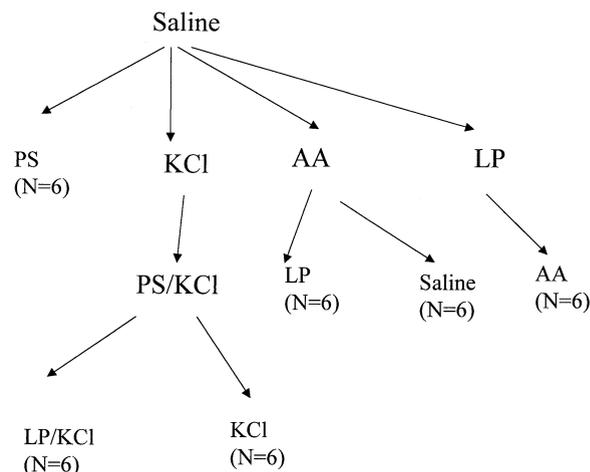


FIGURE 1. *Experimental design.*

KCl by intense sonication. This lipid composition produces LPs with no net charge.

STATISTICAL ANALYSIS

All data are expressed as the mean \pm SE. Statistical analyses were performed using Student's *t* test for paired or unpaired data, as applicable. A *P* value less than 0.05 was considered statistically significant.

RESULTS

CMGs IN PS/KCl INFUSION GROUP

As shown in Tables I through III, infusion of PS (10 mg/mL) or KCl (500 mM) alone did not significantly change the CMGs. However, infusion of PS/KCl resulted in an irritative effect after a delay of 30 to 40 minutes (Fig. 2). The ICI and compliance were significantly reduced by 79% to 83% (from 15.8 ± 1.4 to 2.7 ± 1.0 minutes or from 16.3 ± 1.5 to 3.4 ± 0.7 minutes) and 58% to 75% (from 0.284 ± 0.028 to 0.070 ± 0.019 mL/cm H₂O or from 0.226 ± 0.050 to 0.096 ± 0.037 mL/cm H₂O) in two series of experiments. The bladder contraction amplitude increased significantly (23%) in one series (Table III), but not in the other series (Table II). However, in the average of the two series, the bladder contraction amplitude was significantly increased by 16%. The PT was not significantly changed. When the infusion fluid was switched to LPs/KCl, the ICI increased significantly (63%, from 2.7 ± 1.0 to 4.4 ± 1.2 minutes) after a delay of 10 to 20 minutes. Switching to KCl alone did not alter the ICI for periods as long as 120 minutes (Fig. 2 and Tables II and III). The PT was significantly increased after shifting to LPs/KCl or KCl infusion. Compliance was not significantly changed after shifting to LPs/KCl infusion, but was reduced further (from 0.096 ± 0.037 to 0.043 ± 0.014 mL/cm H₂O) after shifting to KCl infusion.

TABLE I. Effects of saline and protamine sulfate on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	13.8 ± 3.1	0.197 ± 0.030	7.6 ± 0.7	23.5 ± 1.7
PS (10 mg/mL)	14.9 ± 1.7	0.210 ± 0.031	5.9 ± 0.9	27.5 ± 0.7

KEY: CMG = cystometrography; ICI = intercontraction interval; PT = pressure threshold; PS = protamine sulfate. Data presented as the mean ± SE; n = 6. No statistically significant differences were observed between saline and 1 hour of PS treatment.

CMGs IN AA INFUSION GROUP

The irritative effect of AA was evident about 20 to 30 minutes after the start of infusion. ICI and compliance were significantly reduced by 75% to 84% (from 15.3 ± 2.2 minutes to 2.4 ± 0.5 minutes and 12.6 ± 2.0 minutes to 3.2 ± 1.3 minutes) and 71% to 76% (from 0.296 ± 0.040 mL/cm H₂O to 0.071 ± 0.016 mL/cm H₂O and 0.275 ± 0.048 mL/cm H₂O to 0.079 ± 0.026 mL/cm H₂O) in two series of experiments (Fig. 3 and Tables IV and V). The amplitude was less affected, showing a slight but not significant increase, and the PT was not significantly changed. The ICI and compliance increased significantly (179%, from 2.4 ± 0.5 to 6.7 ± 1.5 minutes and 38%, from 0.071 ± 0.016 mL/cm H₂O to 0.114 ± 0.020 mL/cm H₂O, respectively) approximately 10 to 20 minutes after switching to LP infusion, but persisted for as long as 120 minutes after switching to a saline infusion (Fig. 3, Tables IV and V). LP infusion alone for 1 hour did not change the micturition reflex in untreated animals (Table VI); and the effect of a subsequent infusion of AA was not reduced by prior intravesical administration of LPs.

CMGs IN ANIMALS PRETREATED WITH CAPSAICIN

In capsaicin-pretreated animals, the bladder hyperactivity evoked by AA was delayed for 0.5 to 1 hour. ICI and compliance were reduced in magnitude by 51% and 33%, respectively, at 1 hour (from 21.0 ± 2.4 to 10.2 ± 3.0 minutes and 0.226 ± 0.033 to 0.152 ± 0.028 mL/cm H₂O, respectively) but, at 2 hours, was similar to the effect in untreated animals (78% decrease to 4.6 ± 1.2 minutes and 64% decrease to 0.082 ± 0.024 mL/cm H₂O; Table VII). In addition, the reduction in ICI (10.2 ± 3.0 minutes) after a 1 hour application of AA in capsaicin-pretreated rats (Table VI) was significantly (*P* < 0.05) longer than the ICI (2.9 ± 0.9 minutes) reduced by AA, which was measured within 1 hour in untreated rats (Table V). This indicates that C fiber desensitization by capsaicin pretreatment suppressed AA-induced bladder hyperactivity in addition to the delaying effect on the onset of AA-induced hyperactivity.

COMMENT

The major findings of the present study are that intravesical administration of LP suppresses chemically induced bladder hyperactivity and low-dose PS treatment in the presence of physiologic KCl can lead to sustained bladder hyperactivity. The former raises the possibility of new treatments for a damaged or leaky urothelium, and the latter provides a pharmacologic model for the examination of drugs that might restore the leaky urothelium. In addition, it is of interest that AA-induced hyperactivity was also reduced by LP administration, indicating that irritation due to chemically induced irritation/inflammation of the bladder mucosa, as well as direct breakdown of the urothelial barrier by PS, can be partially reversed by LPs. It is tempting to speculate that LPs exert their effect by producing a film on the urothelium and thereby reducing the influx of irritants. However, LPs could also possibly stabilize neuronal membranes and reduce the hyperexcitability of afferent receptors. Therefore, in future experiments, it seems reasonable to examine the effect of intravesical LPs in patients with IC, in whom a breakdown in urothelial barrier function may be a significant contributor to their symptoms.

Although the site and mechanism of action of AA to induce bladder hyperactivity is still uncertain, it is clear that bladder afferents must play a key role. Previous experiments have showed that infusion of AA into the bladder stimulates nociceptive afferent fibers, induces an inflammatory reaction, and evokes a hyperactive bladder.^{11,12,16} The stimulation of silent C fibers has been implicated to play a central role in the pathogenesis of some hyperactive bladders; A δ afferents are usually thought to be primarily responsible for triggering normal voiding function.^{11,13,16} However, capsaicin pretreatment at a dose known to desensitize C fiber bladder afferents delayed and reduced, but not completely eliminated, the effect of intravesical AA. These results raise the possibility that sensitization of myelinated A δ afferents may also play a role in the bladder hyperactivity induced by AA. The concen-

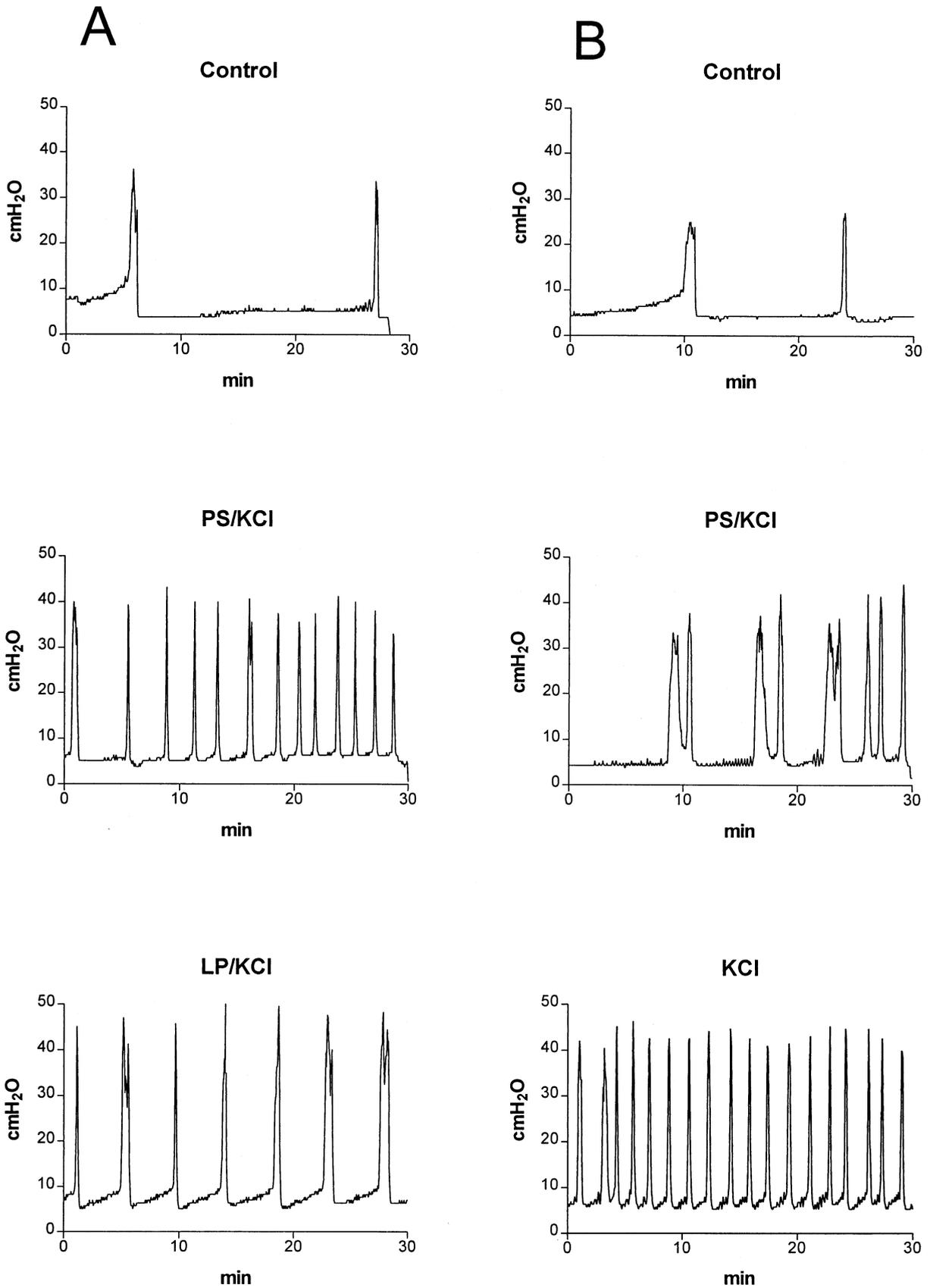


FIGURE 2. CMG tracing during control, PS/KCl, and LPs/KCl or KCl treatment. PS/KCl elicited bladder hyperactivity. LPs/KCl partially reversed the irritative effect of LPs/KCl, which was maintained after switching to KCl.

TABLE II. Effects of saline, KCl, PS/KCl, and LP/KCl on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	15.6 ± 2.1	0.270 ± 0.020	7.3 ± 0.8	26.5 ± 1.1
KCl	15.8 ± 1.4	0.284 ± 0.028	8.2 ± 0.9	28.8 ± 1.2
PS/KCl	2.7 ± 1.0*	0.070 ± 0.019*	6.7 ± 0.7	31.2 ± 1.0
LP/KCl	4.4 ± 1.2*	0.065 ± 0.029	10.3 ± 0.6*	31.3 ± 2.8

KEY: KCl = potassium chloride; LP = liposome; other abbreviations as in Table I.
Data presented as the mean ± SE; n = 6.
*P < 0.05 compared with before treatment.

TABLE III. Effects of saline, KCl, PS/KCl, and KCl on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	18.0 ± 2.0	0.270 ± 0.022	6.3 ± 0.7	25.7 ± 1.6
KCl	16.3 ± 1.5	0.226 ± 0.050	6.8 ± 0.6	28.8 ± 2.2
PS/KCl	3.4 ± 0.7*	0.096 ± 0.037*	7.3 ± 0.4	35.3 ± 2.6*
KCl	2.9 ± 0.5	0.043 ± 0.014*	11.8 ± 1.6*	33.0 ± 2.3

Abbreviations as in Tables I and II.
Data presented as the mean ± SE; n = 6.
*P < 0.05 compared with before treatment.

tration of AA used in this experiment was 0.1%, and the pH value measured by litmus paper was about 3 to 4. The acid solution could dissolve the GAG layer, damage the urothelial barrier, and facilitate deeper penetration of irritant.¹⁷ Furthermore, AA could also produce bladder hyperactivity through the VRI receptors or proton-sensitive channels.¹⁸

The most prevalent theories to explain the pathogenesis of IC appear to be a leaky and dysfunctional urothelium, which allows transepithelial migration of irritants, such as potassium, into the deep layers of bladder wall, where they depolarize afferent nerves and induce abnormal sensations, as well as frequent voiding.^{1,2,3,9} In the present study, PS, which increases epithelial permeability,¹⁶ was used to increase the penetration of KCl through the urothelial barrier and induce a similar activation of afferent neurons. Before PS treatment, the same concentration of KCl did not alter voiding function. In addition, PS alone did not elicit bladder hyperactivity. Thus, it seems reasonable to assume that PS is not a direct bladder irritant and that under normal conditions KCl in the bladder lumen does not change the excitability of afferent nerves in the bladder wall. However, combined treatment with the two agents may mimic the condition occurring in patients with IC.

The use of PS/KCl in our study showed a decrease in ICI and compliance, an increase in bladder contraction amplitude, and no change in PT. A

high concentration of potassium has been used as a provocative test for patients with IC.⁴ A previous study has shown that a high concentration of potassium triggers C afferent fibers and causes additional release of neurotransmitters or neuromodulators.¹⁹ Subsequently, potassium induces the depolarization of detrusor muscle and provokes muscle contraction or tissue damage.^{3,4} The acute exposure of a high concentration of potassium to the detrusor would cause a decrease in bladder compliance and capacity.²⁰ Thus, the ICI and compliance decreased, but PT did not change in our experiment. However, a high concentration of potassium can still irritate the urethra and cause high outlet resistance.³ Therefore, the bladder contraction amplitude was elevated in the combination of our two series of study.

The surface GAG layer has been proposed as a protective barrier that coats the transitional cell surface.^{9,21,22} A GAG layer defect has been suggested in a subset of patients with IC.^{1,2} LPs are phospholipids in a system of concentric closed membranes and are used as a carrier for drugs or genes.^{5,8,23} LP-based drug products provide a moisture film onto the wound and result in outstanding wound healing without a chronic inflammatory reaction in the neodermal layer.^{5,6} Other investigators have suggested that LPs could interact with cells by stable absorption, endocytosis, lipid transfer, and fusion.²⁴ The application of LPs into the

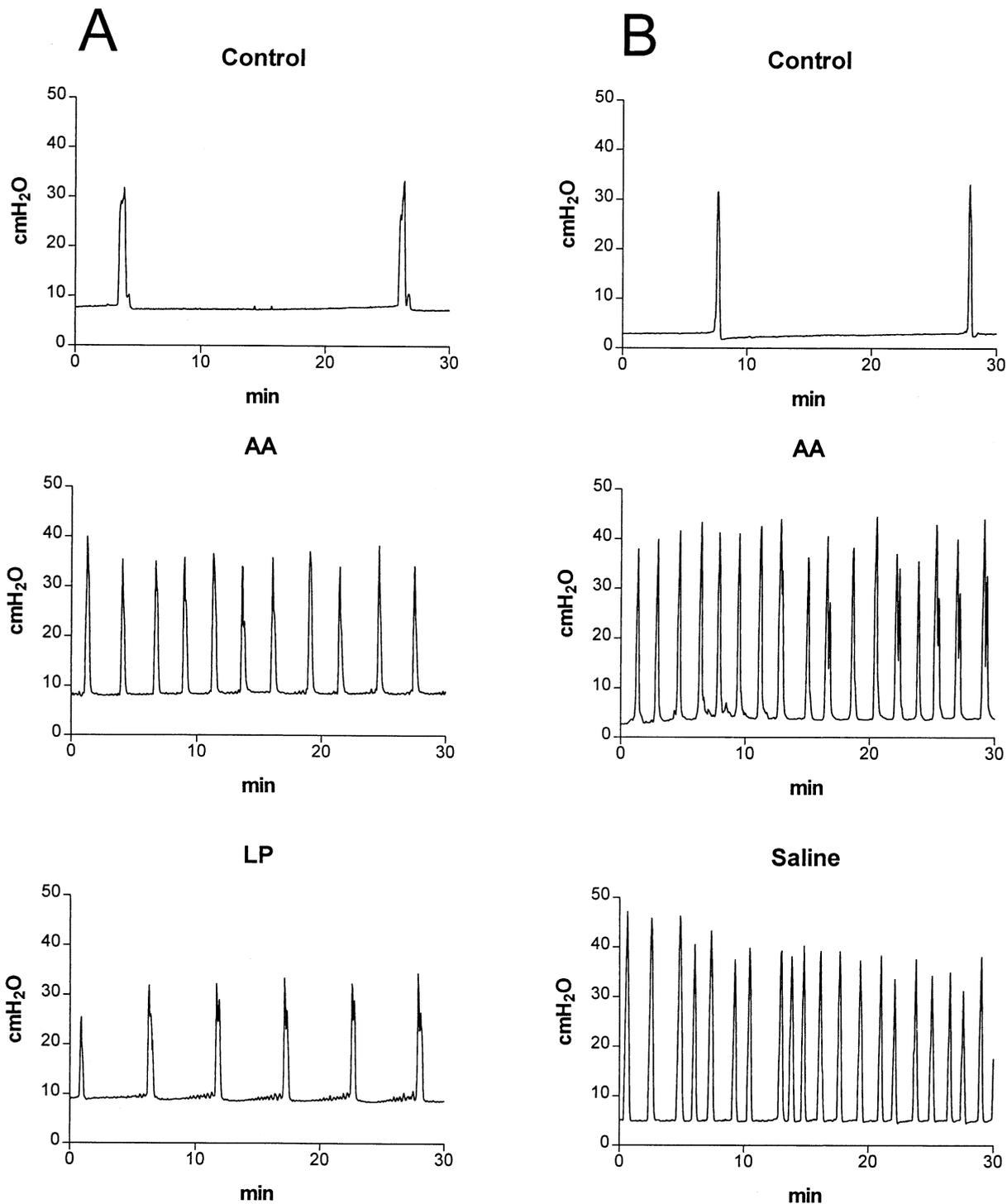


FIGURE 3. CMG tracing during control, AA, and LP or saline treatment. AA elicited bladder hyperactivity. LPs partially reversed the irritative effect of AA, which was maintained after switching to saline.

wounded urothelium might be reasonable for a subset of patients with hyperactive bladder.

In our studies, LP pretreatment did not prevent the irritative effect on the bladder by AA infusion. We assume that a weakened urothelium enhances the attachment and deeper penetration of LPs, which might be only loosely adherent to intact urothelium and be easily degraded by AA.

One weakness of this study was the lack of histologic visualization of the instilled LPs. Rose-necker *et al.*²⁵ have used encapsulated colloidal gold-containing LPs to investigate the anatomic localization of LPs. Additional experiments for future reports are underway to visualize the site, depth, and duration of liposomal attachment to the bladder.

TABLE IV. Effects of saline, acetic acid, and liposomes on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	15.3 ± 2.2	0.296 ± 0.040	6.6 ± 0.9	28.7 ± 1.7
AA	2.4 ± 0.5*	0.071 ± 0.016*	8.3 ± 0.9	36.8 ± 6.5
LP	6.7 ± 1.5*	0.114 ± 0.020*	10.2 ± 1.8	30.8 ± 6.5

KEY: AA = acetic acid; LP = liposomes; other abbreviations as in Table I.
Data presented as the mean ± SE; n = 6.
*P < 0.05 compared with before treatment.

TABLE V. Effects of saline, AA, and saline after AA on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	12.6 ± 2.0	0.275 ± 0.048	7.2 ± 1.0	28.2 ± 3.2
AA	2.9 ± 0.9*	0.079 ± 0.026*	9.1 ± 1.4	37.0 ± 7.0
Saline after AA	3.2 ± 1.3	0.063 ± 0.011	8.6 ± 1.2	28.2 ± 7.0*

Abbreviations as in Tables I and IV.
Data presented as the mean ± SE; n = 6.
*P < 0.05 compared with before treatment.

TABLE VI. Effects of saline, LP, and AA on CMG parameters

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	14.7 ± 3.0	0.254 ± 0.028	7.0 ± 0.8	29.8 ± 2.4
LP	16.0 ± 1.9	0.255 ± 0.023	6.7 ± 0.6	30.0 ± 2.3
AA	3.1 ± 0.8*	0.071 ± 0.022*	6.7 ± 0.7	38.0 ± 1.3*

Abbreviations as in Tables I and IV.
Data presented as the mean ± SE; n = 6.
*P < 0.05 compared with before treatment.

TABLE VII. Effects of saline and AA on CMG parameters in capsaicin-pretreated rats

	ICI (min)	Compliance (mL/cm H ₂ O)	PT (cm H ₂ O)	Amplitude (cm H ₂ O)
Saline	21.0 ± 2.4	0.226 ± 0.033	7.9 ± 0.8	21.1 ± 1.3
AA (1 hr)	10.2 ± 3.0*	0.152 ± 0.028*	6.7 ± 0.2	20.9 ± 2.5
AA (2 hr)	4.6 ± 1.2	0.082 ± 0.024*	6.7 ± 0.7	23.6 ± 5.7

Abbreviations as in Tables I and IV.
Data presented as the mean ± SE; n = 4.
*P < 0.05 compared with before treatment.

Although LPs did not completely reverse the irritation by infusion of AA or PS/KCl, they did significantly reduce the effects. Our study revealed that 1 hour of continuous exposure to AA or PS/KCl causes high sensitization of bladder afferents, which can produce additional urothelial barrier breakdown by the release of inflammatory neuropeptides. A vicious circle is thus established, such as the late stage of patients with IC. LPs could probably ease the injured urothelium and reduce additional irritation, but could not acutely abolish

the whole cascade that has occurred in the hyperactive bladders.

CONCLUSIONS

We used LPs as a therapeutic agent for hyperactive bladder in a rat model and demonstrated an effect in reducing bladder hyperactivity. We hypothesize that intravesical administration of LPs reduces the defect of urothelial barrier breakdown and decreases penetration of irritants into the

deeper layers of the bladder wall. Intravesical administration of LPs may be a novel way to treat patients with IC. Additional study is required to determine the mechanisms by which LPs exert their effect.

REFERENCES

1. Parsons CL, Lilly JD, and Stein P: Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). *J Urol* **145**: 732–735, 1991.
2. Parsons CL, Housley T, Schmidt JD, *et al*: Treatment of interstitial cystitis with intravesical heparin. *Br J Urol* **73**: 504–507, 1994.
3. Hohlbrugger G: Urinary potassium and the overactive bladder. *Br J Urol* **83**(suppl 2): 22–28, 1999.
4. Parsons CL, Greenberger M, Gabal L, *et al*: The role of urinary potassium in the pathogenesis and diagnosis of interstitial cystitis. *J Urol* **159**: 1862–1867, 1998.
5. Reimer K, Fleischer W, Brogmann B, *et al*: Povidone-iodine liposomes—an overview. *Dermatology* **195**(suppl 2): 93–99, 1997.
6. Schafer-Korting M, Korting HC, and Braun-Falco O: Liposome preparations: a step forward in topical drug therapy for skin disease? *J Am Acad Dermatol* **21**: 1271–1275, 1989.
7. Tsuruta T, Muraishi O, Katsuyama Y, *et al*: Liposome encapsulated doxorubicin transfer to the pelvic lymph nodes by endoscopic administration into the bladder wall: a preliminary report. *J Urol* **157**: 1652–1654, 1997.
8. Nishikawa M, and Huang L: Nonviral vectors in the new millennium: delivery barriers in gene transfer. *Hum Gene Ther* **12**: 861–870, 2001.
9. Bade JI, Laseur M, Nieuwenburg A, *et al*: A placebo-controlled study of intravesical pentosanpolysulphate for the treatment of interstitial cystitis. *Br J Urol* **79**: 168–171, 1997.
10. Nickel JC, Downey J, Morales A, *et al*: Relative efficacy of various exogenous glycosaminoglycans in providing a bladder surface permeability barrier. *J Urol* **160**: 612–614, 1998.
11. Yu Y, and de Groat WC: Effects of ZD6169, a K_{ATP} channel opener, on bladder hyperactivity and spinal c-fos expression evoked by bladder irritation in rats. *Brain Res* **807**: 11–18, 1998.
12. Birdler LA, and de Groat WC: Increased c-fos expression in spinal neurons after irritation of the lower urinary tract in the rat. *J Neurosci* **12**: 4878–4889, 1992.
13. Cheng CL, Ma CP, and de Groat WC: Effects of capsaicin on micturition and associated reflexes in rats. *Am J Physiol* **265**: R132–R138, 1993.
14. Ohnishi M, Yajima H, Takeuchi T, *et al*: Mechanism of urinary tract crystal formation following biphenyl treatment. *Toxicol Appl Pharmacol* **174**: 122–129, 2001.
15. Kirby CJ, and Gregoriadis G: A simple procedure for preparing liposomes capable of high encapsulation efficiency under mild conditions, in Gregoriadis G (Ed): *Liposome Technology*. Boca Raton, Florida, CRC Press, 1984, vol 1, pp 20–36.
16. Thor KB, and Muhlhauser MA: Vesicoanal, urethroanal, and urethrovesical reflexes initiated by lower urinary tract irritation in the rat. *Am J Physiol* **277**: R1002–R1012, 1999.
17. Leppilahti M, Teuvo MK, and Tammela LJ: Duration of increased mucosal permeability of the urinary bladder after acute overdistension: an experimental study in rats. *Urol Res* **27**: 272–276, 1999.
18. Welch JM, Simon SA, and Reinhart PH: The activation mechanism of rat vanilloid receptor 1 by capsaicin involves the pore domain and differs from the activation by either acid or heat. *Proc Natl Acad Sci USA* **97**: 13889–13894, 2000.
19. Morrison J, Wen J, and Kibble A: Activation of pelvic afferent nerves from the rat bladder during filling. *Scand J Urol Nephrol Suppl* **201**: 73–75, 1999.
20. Stein PC, Pham H, Ito T, *et al*: Bladder injury model induced in rats by exposure to protamine sulfate followed by bacterial endotoxin. *J Urol* **155**: 1133–1138, 1996.
21. Hohlbrugger G: The vesical blood-urine barrier: a relevant and dynamic interface between renal function and nervous bladder control. *J Urol* **154**: 6–15, 1995.
22. Parsons CL, Stauffer C, and Schmidt JD: Bladder surface glycosaminoglycans: an efficient mechanism of environmental adaptation. *Science* **208**: 605–607, 1980.
23. Gregoriadis G: The carrier potential of liposomes in biology and medicine. *N Engl J Med* **295**: 704–710, 1976.
24. Egerdie RB, Reid G, and Trachtenberg J: The effect of liposome encapsulated antineoplastic agents on transitional cell carcinoma in tissue culture. *J Urol* **142**: 390–398, 1989.
25. Rosenecker J, Zhang W, Hong K, *et al*: Increased liposome extravasation in selected tissues: effect of substance P. *Proc Natl Acad Sci USA* **93**: 7236–7241, 1996.