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Duration of increased mucosal permeability of the urinary bladder after acute overdistension: an experimental study in rats

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Abstract The duration of damage to the mucosal barrier of the urinary bladder after overdistension was investigated in a rat model. Overdistension was induced for 3 h in 48 female Sprague-Dawley rats by forced diuresis and balloon obstruction of the bladder neck. In 24 rats 0.5 ml of 2% solution of Trypan blue in 0.9% NaCl solution was instilled into the bladder for 1 h at 0 h, 24 h, 48 h, 7 days and 21 days after overdistension. After dyeing, full-thickness samples were taken from the wall of the bladder dome and body immediately above the ureteral orifices for histological studies. Inflammatory reaction was investigated histologically without Trypan blue dyeing in 24 rats at 0 h, 24 h, 48 h, 7 days and 21 days after overdistension. At 0, 24 and 48 h after overdistension the bladder wall was deep blue throughout. The dome and body were similar. At 7 days there was only slight staining of the bladder surface urothelium and subjacent connective tissue, while at 21 days there was no longer any dye in the bladder wall or urothelium. Oedema reached its maximum at 48 h, and large numbers of inflammatory cells were seen in the submucosa at 48 h. These changes had normalized by 7 days. After overdistension urothelial integrity is destroyed for several days, making it possible for different substances in the urine to penetrate into the bladder wall. This renders questionable the use of bladder distension in the treatment of interstitial cystitis, as it may only increase leakage of the urothelium and accelerate inflammatory reaction in the bladder wall. However, in the present study of healthy rat bladders the integrity of the urothelium had recovered to a large extent after 1 week and completely after 3 weeks.

Key words Trypan blue · Urothelial permeability · Glycosaminoglycan · Interstitial cystitis

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Introduction

The bladder has both anatomical and biochemical mechanisms of defence against urine and bacterial adherence. The umbrella cell layer, three to seven cells thick, has unique tight junctions that in part serve to reduce the permeability of the urothelium [81]. In addition, the mucosal surface of the urinary bladder is lined with a highly anionic glycosaminoglycan (GAG) layer that has been reported to prevent bacterial adherence and penetration of substances from the urine across the mucosal epithelium into the bladder wall [14, 15, 17, 22, 23] . The bladder surface proteoglycans may actually be the principal mechanism in maintaining the barrier. Sulphated polysaccharides are negatively charged and have a high affinity to bond ionically with water. When GAG is bound to the bladder surface the water molecules become interposed between bladder surface and urine [6]. Any mechanism by which the mucosal lining or surface GAG layer is disturbed may result in both increased microbial attachment to the bladder wall and the penetration of urinary solutes into the bladder wall [13, 20].

A considerable body of evidence has accumulated to suggest that a defect in the permeability control of the bladder is an aetiological factor in interstitial cystitis, the symptom complex resulting from penetration of the urothelium by urinary solutes [7, 15, 16, 18].

Overdistension of the bladder has been used as a method for treating urgency [1, 21], bladder instability [9, 19] and interstitial cystitis [2]. Some patients experience symptomatic relief following distension [5], presumably due to damage to sensory [24] or cholinergic innervation [10]. In any case, a rapid reappearance of the

symptoms is often observed after initial success with distension therapy [2, 9].

The colloidal dye Trypan blue can be used as a marker for defects in the integrity of the mucosal epithelium [12, 13]. The aim of the present work was to investigate the effect of bladder distension on urothelial permeability of normal rat bladder, focusing especially on the recovery time of urothelial integrity after damage.

Materials and methods

Animals

Three-month-old female Sprague-Dawley rats (250–315 g) were used throughout. All animals received water and food ad libitum.

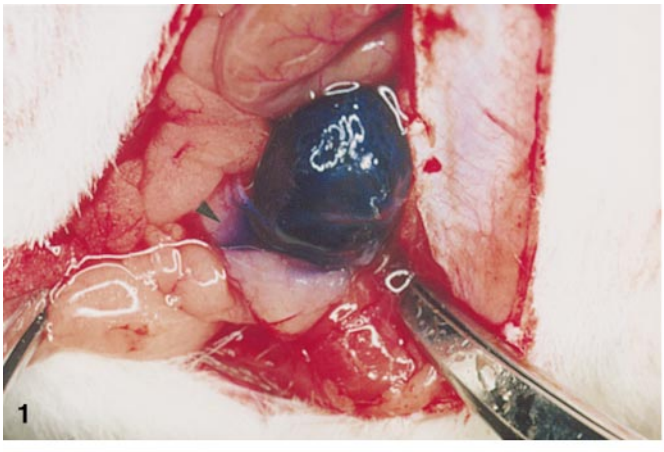
Overdistension

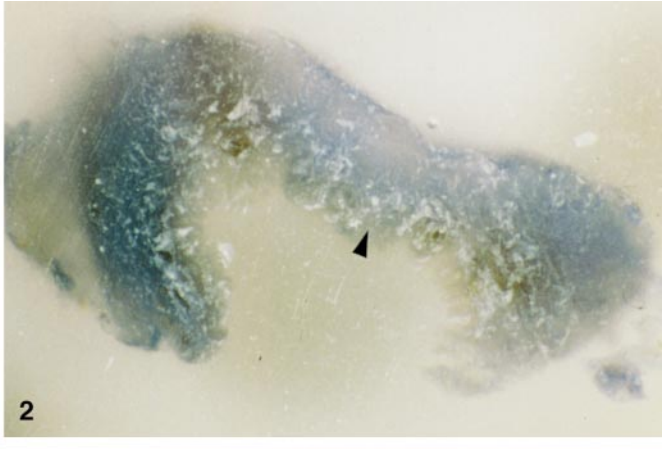
The rats were anaesthetized with pentobarbithal sodium (35 mg/kg, i.p.) and the bladder was catheterized with a Fogarty (3 Fr) arterial embolectomy catheter (Baxter, Santa Ana, Calif., USA). The balloon was filled with 0.05 ml water and pulled into the bladder neck. The rats were then given furosemide, 12 mg/kg i.m., and Ringer solution, 12 ml/kg i.p., to induce diuresis and maximal bladder distension, which was palpated as a hard resistance in the lower abdomen. After distension for 3 h, the bladders were emptied (2.5–3 ml; normal cystometric capacity in female rats of similar body weight is known to be around 0.4 ml [26]) and the rats allowed to recover. Each rat was then given i.m. cefuroxime, 30 mg/kg, to prevent infection. Buprenorphine, 0.1–0.3 mg/kg s.c., was given if the animals seemed to be in pain. They were then watched carefully to check bladder emptying. Control animals were anaesthetized similarly but their bladders were not distended.

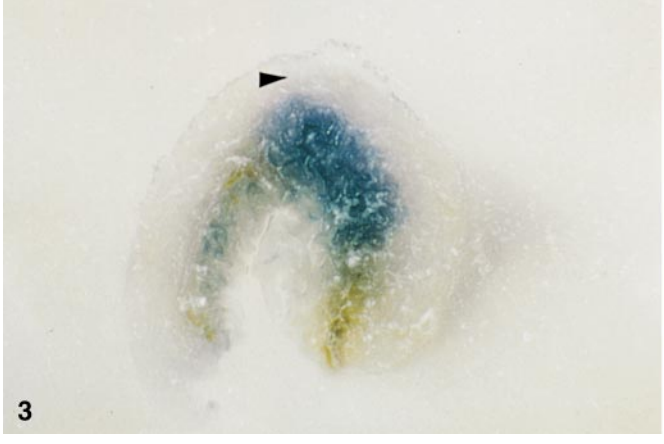
Samples

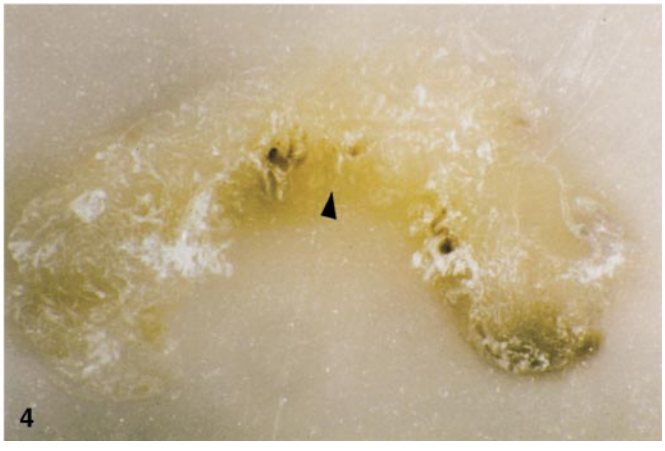
Using the same anaesthesia as previously described, 0.5 ml of 2% solution of Trypan blue in 0.9% NaCl solution was instilled and left in the bladder for up to 1 h. After 1 h the dye was removed and the bladders washed with four 0.5 ml saline washes to remove all unabsorbed dye. Full-thickness samples were taken from the wall of the bladder dome and posterior corpus immediately above the ureteral orifices of 24 rats at 0 h, 24 h, 48 h, 7 days and 21 days after distension. The rats were then killed with an overdose of the same drug. Specimens were fixed in buffered 10% formalin (pH 7), embedded in paraffin and blocks photographed at ×10 magnification. A semiquantitative scoring system was used for Trypan blue staining of the bladder wall: 0 = negative, 1 = weak, 2 = intermediate, 3 = strong. Similar samples were taken without intravesical Trypan blue instillation from 24 rats for histological studies with haematoxylin and eosin.

Fig. 1 At 0 h after overdistension. The whole bladder is deep blue. The peritoneal cavity is also slightly stained in the neighbourhood of the bladder (*arrow*)
Fig. 2 At 48 h after overdistension. The bladder wall is deep blue throughout. The mucosa is marked with an *arrow*. Trypan blue, ×10
Fig. 3 At 7 days after overdistension. There is still marked staining of the urothelium, but dyeing of the muscle (*arrow*) is very mild. Trypan blue, ×10
Fig. 4 At 21 days after overdistension. There is no discernible staining in the bladder wall or urothelium (*arrow*). Trypan blue, ×10









Principles of Laboratory Animal Care (NIH publication no. 86–23, revised 1985) was followed, as well as specific national laws were applicable.

Results

Mortality during the study was 0. No bladder ruptures occurred. There was no evidence of the staining of tissues by Trypan blue in any of the control bladders.

There was no difference in appearance between samples from the bladder dome and posterior corpus. Immediately after overdistension there was even slight staining of the peritoneal cavity in the neighbourhood of the bladder (Fig. 1). At 0, 24 and 48 h after overdistension the bladder wall was deep blue throughout (Fig. 2). At 48 h there was a multifold increase in the number of inflammatory cells in the submucosa (Fig. 5b).

At 7 days after overdistension there was still considerable staining of the bladder surface urothelium and subjacent connective tissue, but dyeing of the muscle was very mild (Fig. 3). The number of inflammatory cells had normalized (Fig. 5c). At 21 days after overdistension there was no longer any evidence of staining of the bladder wall or urothelium (Fig. 4).

The results of semiquantitative scoring are shown in Table 1.

Fig. 5 **a** Control rat. Normal urothelium and submucosa. **b** At 48 h after overdistension submucosal oedema, haemorrhages (*small arrowhead*) and an abundance of polymorphonuclear (inflammatory) cells (*large arrowheads*) are seen. **c** At 7 days inflammatory cells have disappeared. Urothelial nuclei are slightly granulated (*arrowhead*), probably due to increased metabolic activity. Haematoxylin and eosin, ×400

Discussion

Previous studies have shown that dye penetration into and through the bladder wall does not occur until the mucosal surface is damaged, either by overdistension, anoxia or acid treatment [13, 25]. We demonstrated in the present study that overdistension destroyed urothelial integrity in the urinary bladder for several days. On the other hand, after acid instillation the mucin layer has been found to be fully regenerated in 24 h [14]. The difference in the recovery times is probably due to a difference in the damage caused by these two manipulations. Acid instillation merely dissolves the mucin layer but epithelial cells stay undamaged, so reformation of the GAG layer is very rapid, whereas overdistension not only destroys the mucin layer but also damages the urothelial cells and repair time is, therefore, much longer. In overdistension the urothelial damage is probably caused partially by mechanical overstretching of the urothelium, while interruption of capillary perfusion for 3 h is presumably another reason for the damage [11].

In a recent study overdistension was found to cause a rapid proliferative reaction within the bladder wall [26]. Its initial effects occurred within the urothelium and were already maximal within 16 h, and the later involvement of the subendothelial smooth muscle and connective tissue was directly proportional to the degree of bladder overdistension [26]. However, in spite of the rapid proliferative reaction in the urothelium its integrity was not completely recovered in 1 week, which suggests either that the damaged cells in the urothelium were not able to produce sufficient GAG or that other structures in the bladder wall are necessary for integrity. These other structures include tight junctions (unique to

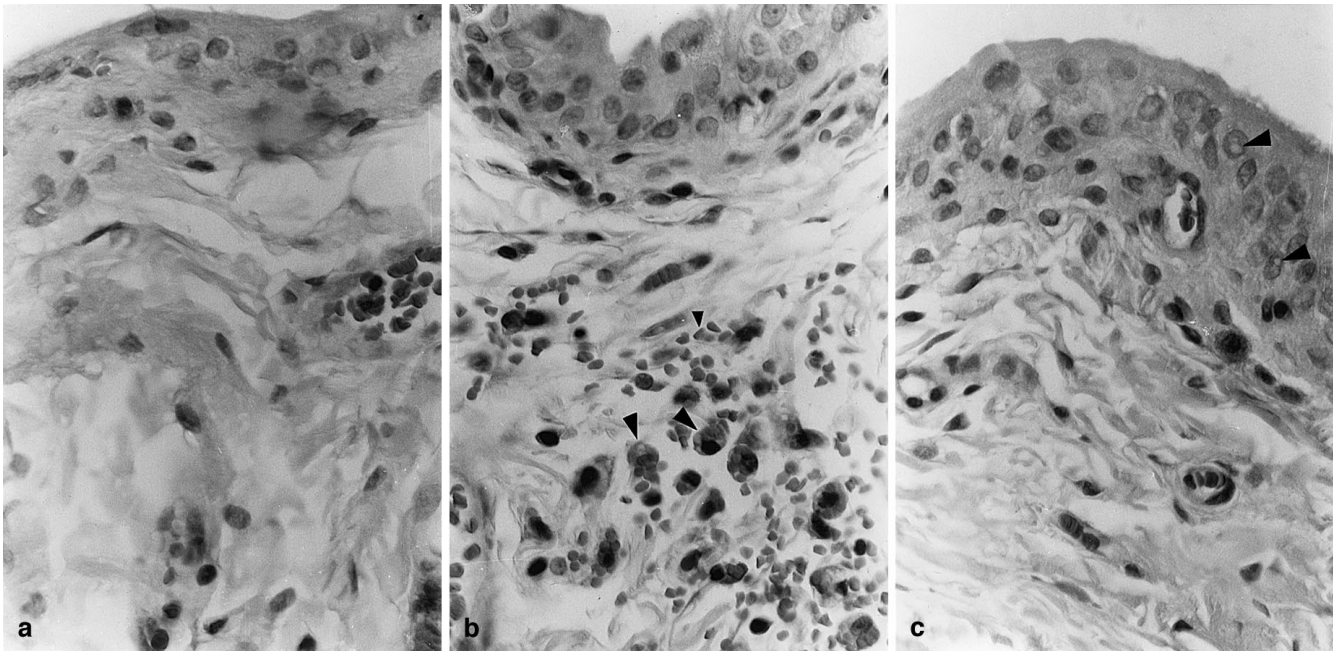


Table 1 Bladder wall staining with intravesical Trypan blue instillation after overdistension. The intensity of stainig was scored semiquantitatively: 0 = negative, 1 = weak, 2 = intermediate, 3 = strong

Rat no.	Controls	Time after Trypan blue installation				
		0 h	24 h	48 h	7 days	21 days
1	0	3[a]	3	3[b]	1[c]	0[d]
2	0	3	3	2	1	0
3	0	3	3	3	2	0
4	0	3	3	3	1	0

[a] Fig. 1; [b] Fig. 2; [c] Fig. 3; [d] Fig. 4

the bladder epithelium) and ion pumps, which have previously been perceived as the primary mechanism by which the transitional cells remain impermeable [3]. Obviously the mucin layer is highly susceptible to injuries because even catheterization induced a proliferative reaction [26].

The long-lasting destruction of urothelial integrity following overdistension makes it possible for different toxic waste products of metabolism in the urine to penetrate the mucosa into the wall. When the barrier between the bladder wall and urine is damaged it no longer prevents the adherence of bacteria, crystals, proteins and ions to the bladder wall. Evidences accumulating that a leaky urothelium is an aetiological factor underlying inflammatory conditions in the bladder in patients suffering from interstitial cystitis [7, 15, 16, 18]. Therefore, although bladder distension has been used as a method for treating an unstable bladder and interstitial cystitis [1, 2] and may produce some relief of symptoms via a mechanical increase in bladder capacity and temporary damage to the innervation of the bladder wall, there is a potential to increase the penetration of irritative substances into the bladder that might adversely affect the bladder function and inflammatory reaction at a later time. Also in the present study an inflammatory reaction was found, reaching a maximum 48 h after overdistension. However, in these healthy bladders it had completely disappeared after 1 week.

The induction of mucosal damage could perhaps be avoided, and fluid infiltration into the wall of the urinary bladder prevented, if the bladder is filled neither too rapidly nor to a high pressure during distension therapy [25]. Also anionic polyelectrolytes such as heparin and pentosanpolysulphate have been found to bind to the areas of focal damage and thus restore resistance of the mucosa to bacterial adherence when instilled intravesically [4, 22, 23], and further to prevent dye penetration following acute overdistension. However, heparin is not able to eliminate dye penetration completely but only reduces it after acetone treatment [13, 25].

In conclusion, overdistension of the urinary bladder destroys urothelial integrity for up to 1 week, which makes it possible for different substances in the urine to penetrate into the wall. It is thus possible that overdistension in patients with interstitial cystitis may further damage the leaking urothelium and even accelerate the inflammatory reaction, rendering this therapy even harmful. However, healthy bladders have an excellent ability to recover, so that the mucosal barrier was mostly recovered within 1 week, and 3 weeks after distension it was intact. Further studies are needed to investigate in more detail the volume-damage relationship of the wall of the urinary bladder.

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References

1. Dunn M, Smith JC, Ardran GM (1974) Prolonged bladder distension as a treatment of urgency and urge incontinence of urine. Br J Urol 46:645
2. Dunn M, Ramsden PD, Roberts JBM, Smith JC, Smith PJB (1977) Interstitial cystitis, treated by prolonged bladder distension. Br J Urol 49:641
3. Fellows GJ, Marshall DH (1972) The permeability of human bladder epithelium to water and sodium. Invest Urol 9:339
4. Hanno PM, Parson CL, Shrom SH, Fritz R, Mulholland SG (1978) The protective effect of heparin in experimental bladder infection. J Surg Res 25:324
5. Hanno PM, Wein AJ (1887) Medical treatment of interstitial cystitis. Urology Suppl 29:22
6. Hurst RE, Rhodes SW, Adamson PB, Parsons CL, Roy JB (1987) Functional and structural characteristics of the glycosaminoglycans of the bladder luminal surface. J Urol 138:433
7. Hurst RE, Parson CL, Roy JB, Young JL (1993) Urinary glycosaminoglycan excretion as a laboratory marker in the diagnosis of interstitial cystitis. J Urol 149:31
8. Jost SP, Gosling JA, Dixon JS (1989) The morphology of normal human bladder urothelium. J Anat 1867:103
9. Jörgensen L, Mortensen SO, Colstrup H, Andersen JT (1985) Bladder distension in management of detrusor instability. Scand J Urol Nephrol 19:101
10. Lasanen LT, Tammela TLJ, Kallioinen M, Waris T (1992) Effect of acute distension on cholinergic innervation of the rat urinary bladder. Urol Res 20:59
11. Leppilahti M, Tammela TLJ, Hirvonen J (1997) Influence of transient overdistension on bladder wall morphology and enzyme histochemistry. Scand J Urol Nephrol 31:517
12. Lillie RD (1977) HJ Conn's biological stains, 9th edn. Williams and Wilkins, Baltimore
13. Monson FC, Wein AJ, McKenna BAW, Whitmore K, Levin RM (1991) Indigocarmine as a quantitative indicator of urothelial integrity. J Urol 145:842
14. Parson CL, Greenspan C, Moore SW, Mulholland SG (1977) Role of surface mucin in primary antibacterial defense of the bladder. Urology 9:48
15. Parson CL, Boychuk D, Jones S, Hurst R, Callahan H (1990) Bladder surface glycosaminoglycans: an epithelial permeability barrier. J Urol 143:139
16. Parson CL, Hurst RE (1990) Decreased urinary uronic acid levels in individuals with interstitial cystitis. J Urol 143:690
17. Parsons CL, Mulholland G (1978) Bladder surface mucin. Am J Pathol 93:423
18. Parsons CL, Lilly JD, Stein P (1991) Epithelial dysfunction in nonbacterial cystitis (interstitial cystitis). J Urol 145:732
19. Pengelly AW, Stephenson TP, Milroy EJG, Whiteside CG, TurnerWarwick R (1978) Results of prolonged bladder distension as treatment for detrusor instability. Br J Urol 50:243

20. Perlow DL, Gikas PW, Horowitz PM (1981) Effect of vesical overdistension on bladder mucin. Urology 18:380
21. Ramsden PD, Smith JC, Dunn M, Ardan GM (1976) Distension therapy for the unstable bladder: later results including an assessment of repeat distension. Br J Urol 48:623
22. Ruggieri MR, Hanno PM, Levin RM (1985) Further characterization of bacterial adherence to urinary bladder mucosa: comparison with adherence to anion exchange resin. J Urol 134: 1019
23. Ruggieri MR, Hanno PM, Samadzadeh S, Johanson E, Levin RM (1986) Heparin inhibition of increased bacterial adherence following overdistension, ischemia and partial outlet obstruction of the rabbit urinary bladder. J Urol 136:132
24. Tammela T, Lasanen L, Waris T (1990) Effect of distension on adrenergic innervation of the rat urinary bladder. Urol Res 18:345
25. Tammela T, Wein AJ, Monson FC, Levin RM (1993) Urothelial permeability of the isolated whole bladder. Neurourol Urodyn 12:39
26. Tammela TLJ, Levin RM, Monson FC, Wein AJ, Longhurst PA (1993) The influence of acute overdistension on rat bladder function and DNA synthesis. J Urol 150:1533

ANNOUNCEMENTS

1999

17th World Congress on Endourology & SWL
2–5 September 1999, Rhodes, Greece

Congress Secretariat: Erasmus Conference Center, 227 Kifisias Avenue, 14561 Kifisia, Athens, Greece. Tel.: +30 1 612502213, Fax: + 30 1 612 5021, e-mail: Erasmus@netit.gr, Website: www.netit.gr/WCEgg.gr.org

Dresdner Urologie – Tage
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27th Munich Endourological Symposium with Nursing Staff Seminary
14–15 October 1999, Munich, Germany

Information: Prof. Dr. H. Leyh, Dr. R. Paul, Mrs. U. Wagner, Department of Urology, Technische Universität Mnchen, Klinikum rechts der Isar, Ismaninger Str. 22, D-81675 Mnchen, Germany Tel.: 089/41402587, Fax: 089/41402585, e-mail: tb81taa@mail.lry-muenchen.de, Internet: http://www. med.tu-muenchen.de/uro/endo.html

3. International Symposium on Uro–Oncology "Advances in diagnosis and therapy of bladder cancer"
5–6 November 1999, Marburg, Germany

Topics: Pathology and molecular genetics of pediatric and adult bladder tumors; Diagnosis and staging of bladder cancer; Management of superficial bladder cancer; Therapy of muscle invasive bladder cancer (surgical demonstrations); Management of advanced bladder tumors

Information: Priv. Doz. Dr. med. A. Heidenreich, Dr. med. V. Knobloch, Department of Urology, Philipps Universität Marburg, Baldingerstrasse, D-35033 Marburg, Tel.: +49 6421 286239, Fax: +49 6421 285590

2000

Urolithiasis 2000
The IXth International Symposium on Urolithiasis
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