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ORIGINAL ARTICLE

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Early cellular and ultrastructural response of the mouse urinary bladder urothelium to ischemia

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Abstract An experimental ischemic model of mouse urinary bladder was developed to study urothelium permeability and changes in cell ultrastructure. The bladder permeability barrier response to experimental ischemia (30–120 min) was investigated by means of indigo carmine dye, trypan blue and lanthanum nitrate tracer, which were used as quantitative and qualitative indicators of urothelial integrity. Changes to the urothelium were studied by light microscopy, and by scanning and transmission electron microscopy. It was established that ischemia primarily induces breakdown of the blood–urine permeability barrier by disruption of the tight junctions. It causes focal interruption of the contacts between the cells, which is followed by detachment and desquamation of viable urothelial cells. Urothelial damage occurs as funnel-shaped wounds, which can extend into the lamina propria. They are proportional to the duration of ischemia and to the extent of reperfusion induced. Desquamated cells in the bladder lumen, when exposed to hypertonic and toxic urine, gradually become irreversibly changed.

Key words Urinary bladder · Urothelium · Ischemia · Permeability barrier · Desquamation

Introduction

To function properly, the urinary bladder requires adequate amounts of oxygen and nutrients, which are supplied through the arterial system. Ischemia compromises supplies of both and can result in increased susceptibility to infection [15] or even in gangrenous necrosis [32]. Thus, very recently, Buttyan et al. [3] and Levin et al. [18] proposed that the development of various stages of bladder dysfunction subsequent to partial outlet obstruction (secondary to benign prostatic hypertrophy) [37] is essentially driven by ischemia. In experimental rabbit models, ischemia and partial outlet obstruction have similar effects on bladder morphology, biochemistry, urodynamics and contractile function [13, 19, 20, 22, 36]. At the molecular level both ischemia and partial outlet obstruction result in a very similar pattern of gene activation [2, 4] and in elevation of intracellular Ca^{2+} [39]. Although bladder responses to reduced blood flow have been studied extensively, much less has been written about the ischemic damage to the bladder urothelium. It is known that 1 h of bladder ischemia causes a functional defect of the urothelium, which leads to increased bacterial adherence in a very focal fashion [30], and that after a few days or 1 week of unilateral or bilateral ischemia atrophy of the urothelium occurs [8, 20].

The urothelium serves as an effective blood–urine permeability barrier to the penetration of urine into the bladder wall. The barrier function is maintained by the tight junctions that seal the space between adjacent superficial cells and by the asymmetrical luminal plasma membrane covered with glycosaminoglycans [10, 28]. Recently it was demonstrated that the permeability of the bladder blood–urine barrier increases as a result of 1 h of in vitro anoxia [33]. Although this study suggested a failure of the permeability barrier, it did not explore the mechanism involved. During renal ischemia in vivo or in ATP depletion in vitro, alterations in cell junctions lead to detachment of viable tubular epithelial cells [9, 14, 24, 29]. However, in the urinary bladder interruption of junctional attachments, which result in desquamation of urothelial cells, may occasionally occur following various specific inductions [1, 6, 12]. It is doubtful whether short-duration bladder ischemia involves similar changes and induces desquamation. To investigate these problems an in vivo ischemic model of mouse urinary bladder was developed. The purpose of the current study was to determine the effects of experimental bladder isch-

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emia on the blood–urine permeability barrier and to analyze the early ischemic cellular and ultrastructural changes to the bladder urothelium. Permeability studies were performed with indigo carmine dye [26, 33] and trypan blue [16], which were used as quantitative or qualitative indicators of urothelium integrity, while paracellular permeability properties of urothelium were analyzed by using lanthanum nitrate tracer [5].

Materials and methods

Induction of ischemia

Adult male Albany strain mice (30–38 g) were anesthetized with a ketamine/xylazine/atropine (i.p. 150 mg ketamine–10 mg xylazine–0.1 mg atropine/kg) mixture. The urinary bladder was exposed with a midline incision and a 4-0 silk ligature was secured tightly around the ureters and urethra in the trigonal region to occlude the vesicle vasculature.

Indigo carmine dye

After 30, 40, 60, 80 or 100 min of ischemia (followed by reperfusion) the loop was removed and a 0.45-mm (outer diameter) needle was placed intravesically through a dome of the bladder. The bladder was then emptied and 1% (w/v) indigo carmine (Sigma) in normal saline (0.9% NaCl) was instilled for 1 h. Sham operation performed in the control animals involved anesthesia, bladder exposure and 1 h of dye instillation, but no ligature was placed. At the end of the 1-h period, the dye solution was drained and the bladder was rinsed five times with instillations of saline to remove unabsorbed dye. Volumes of the dye solution instilled and the washing instillations were the same as the urine volume at the outset to ensure that overdistension did not influence this study. Following the final rinse, the bladder was removed (the mouse was then sacrificed), washed in saline for 5 min, weighed and stored in 6% formalin. During 1 week of storage, all the dye was extracted into formalin. The indigo carmine concentration in the formalin (and thus in the bladder) was determined spectrophotometrically (610 nm, Beckman DU 65) using a standard curve of indigo carmine in formalin. The concentration of the dye in the bladder was calculated and the results for various groups were compared by means of Student's *t*-test.

Trypan blue

After 30, 60, 90 or 120 min of ischemia (followed by reperfusion via removal of the loop) or in control animals, the urine was removed and 1% (w/v) trypan blue in saline was instilled to the same volume and left for 1 h. At the end of the 1 h period the bladder was washed five times with the same volume of saline, removed and then fixed with 4% paraformaldehyde and 2% glutaraldehyde, dehydrated in graded ethanol and embedded in paraffin. Sections were taken at 6 µm and examined unstained (except for entrained trypan blue).

Scanning electron microscopy

The excised ischemic (after 30, 60, 90 or 120 min of ischemia) or control bladders were bisected longitudinally and flooded with 2% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 3–4 h at 4°C. In an additional two experimental groups, after 90 min of ischemia or in control animals, urinary bladders were emptied and filled five times with the same volume of urine as was present at the outset, removed, and fixed in the same fixative. The tissue samples were rinsed with 0.1 M cacodylate buffer (pH 7.4) and postfixed in buffered 1% osmium tetroxide for 1 h at 4°C. Specimens were immersed in Freon 13, critical-point dried, sputter coated with gold, and examined at 15 kV under a scanning electron microscope (Jeol JSM 84A).

Ultrathin sections and lanthanum nitrate penetration

Ligated bladders at the beginning of the period of ischemia or at 30 or 60 min during ischemia were injected via a 0.40-mm (outer diameter) needle with 15 µl of 6% indigo carmine in saline for 1 h. After 1 h, at 60, 90 or 120 min of ischemia, the bladder was washed with 1 ml saline at a constant volume and removed. Control sham-operated mice underwent the same procedure except for ligation (anesthesia, bladder exposure, injection of dye and washing). Blue spots of indigo carmine, which appeared on the ischemic bladders, indicate focal areas of damage to the bladder urothelium. These areas were dissected and the further procedure with lanthanum nitrate followed as detailed below.

Lanthanum nitrate (1%) was added to 2.5% glutaraldehyde and 4% paraformaldehyde in cacodylate-buffered (0.1 M, pH 7.2) fixative. Tissue samples were incubated in this solution for 2.5 h, then washed with 1% lanthanum nitrate in 0.1 M cacodylate buffer, followed by 1 h postfixation in 2% osmium tetraoxide dissolved in 0.5% lanthanum nitrate buffered in 0.1 M cacodylate. Samples were then rinsed with 1% lanthanum nitrate in 0.1 M cacodylate buffer for 1 h, stained with uranyl acetate, dehydrated and embedded in Epon. Ultrathin sections were examined in a Jeol 100 CX electron microscope.

Results

Effect of ischemia/reperfusion on the permeability barrier

After 1 h of exposure to indigo carmine dye, there was visually no staining on control bladders. After 40 min of ischemia or more followed by 60 min of reperfusion (exposure to indigo carmine dye), blue areas appeared on the bladder, indicating local penetration of indigo carmine dye into the bladder wall (Fig. 1).

The effect of ischemia/reperfusion on the concentration of the indigo carmine dye in the bladder wall

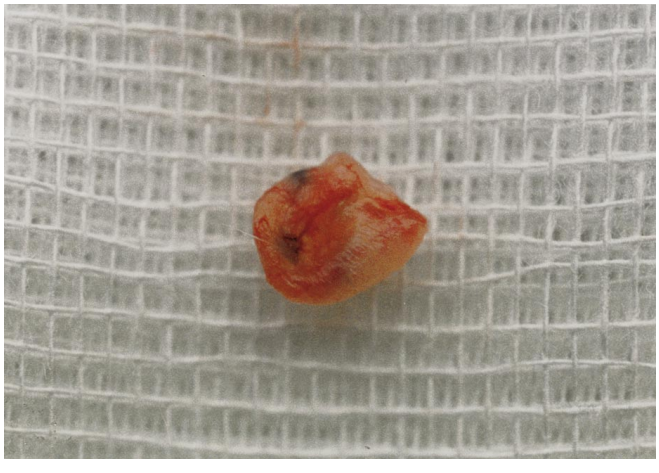

Fig. 1 Photograph of a urinary bladder after 40 min of ischemia followed by 60 min of reperfusion (exposure to indigo carmine dye)

showed significantly increased dye penetration after 40 or 60 min of ischemia (followed by 60 min of reperfusion) and further increased penetration after 80 or 100 min of ischemia (Fig. 2).

After 1 h exposure to trypan blue, control bladders visually showed no staining and the dye had not penetrated the urothelium. In animals subjected to 60 min of ischemia or more followed by 60 min of reperfusion (exposure to trypan blue), areas of blue appeared on the bladders (the extent of these areas was largest after 120 min of ischemia). At those sites, desquamation of all three urothelial strata was evident and trypan blue penetrated the lamina propria (Fig. 3). It colored certain remaining intermediate and/or basal urothelial cells and various connective tissue elements, but not the underlying smooth muscle.

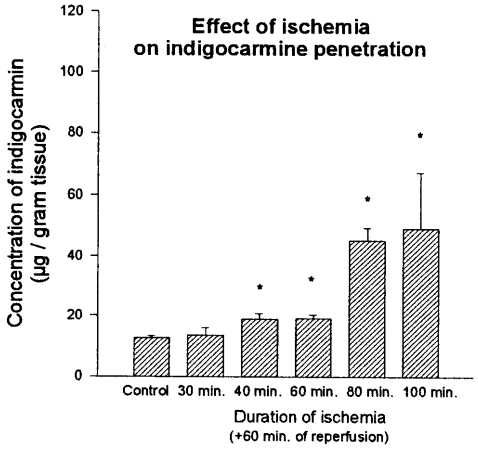

Fig. 2 Effect of ischemia on indigo carmine dye penetration into the bladder wall. *Bars* represent mean±SEM for control and different periods of ischemia followed by 60 min of reperfusion (exposure to dye). Control checks and those at 40, 60 and 100 min were done in four individual preparations, and the 30- and 80-min tests in 2. *Asterisk* indicates a significant difference from the control ($P<0.05$)

Luminal bladder surface

In control experiments the luminal bladder surface was covered with characteristically flattened polygonal superficial cells. Identical results were observed in animals subjected to 30 min of ischemia, while after 60 min of ischemia detachment of single superficial cells was detected (Fig. 4a).

After 90 min of ischemia, areas of focal damage to the urothelium caused by detachment (Fig. 4b) and desquamation (Fig. 4c) of superficial and intermediate cells were observed. The damage was seen as funnel-shaped wounds extending to the basal cells (Fig. 4c). In addition, detachment of single superficial cells became more frequent. In experiments where after 90 min of ischemia the urinary bladder was emptied and filled, desquamation of all strata was evident in focal areas and the lamina propria was exposed to the lumen. Furthermore, a marked number of leukocytes was noted on the exposed lamina propria of the focal areas (Fig. 4d).

In animals subjected to 120 min of ischemia the extent of bladder surface alternations was somewhat larger, but still in agreement with the extent of those in the group of animals subjected to 90 min of ischemia.

Paracellular permeability barrier and cell ultrastructure

The ischemic areas of focal damage to urothelium were localized by the procedure described in Materials and

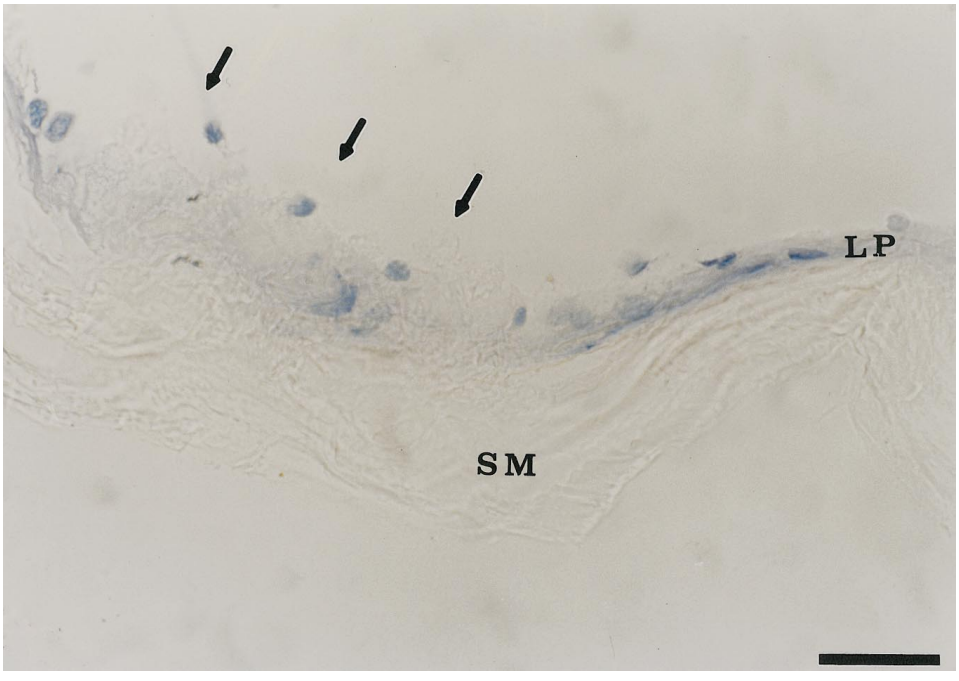

Fig. 3 Photomicrograph of a damaged area after 90 min of ischemia followed by 60 min of reperfusion (exposure to trypan blue). Note intermediate and/or basal cells (*arrows*), lamina propria (*LP*) and smooth muscle (*SM*). ×320 *Bar* 50 µm

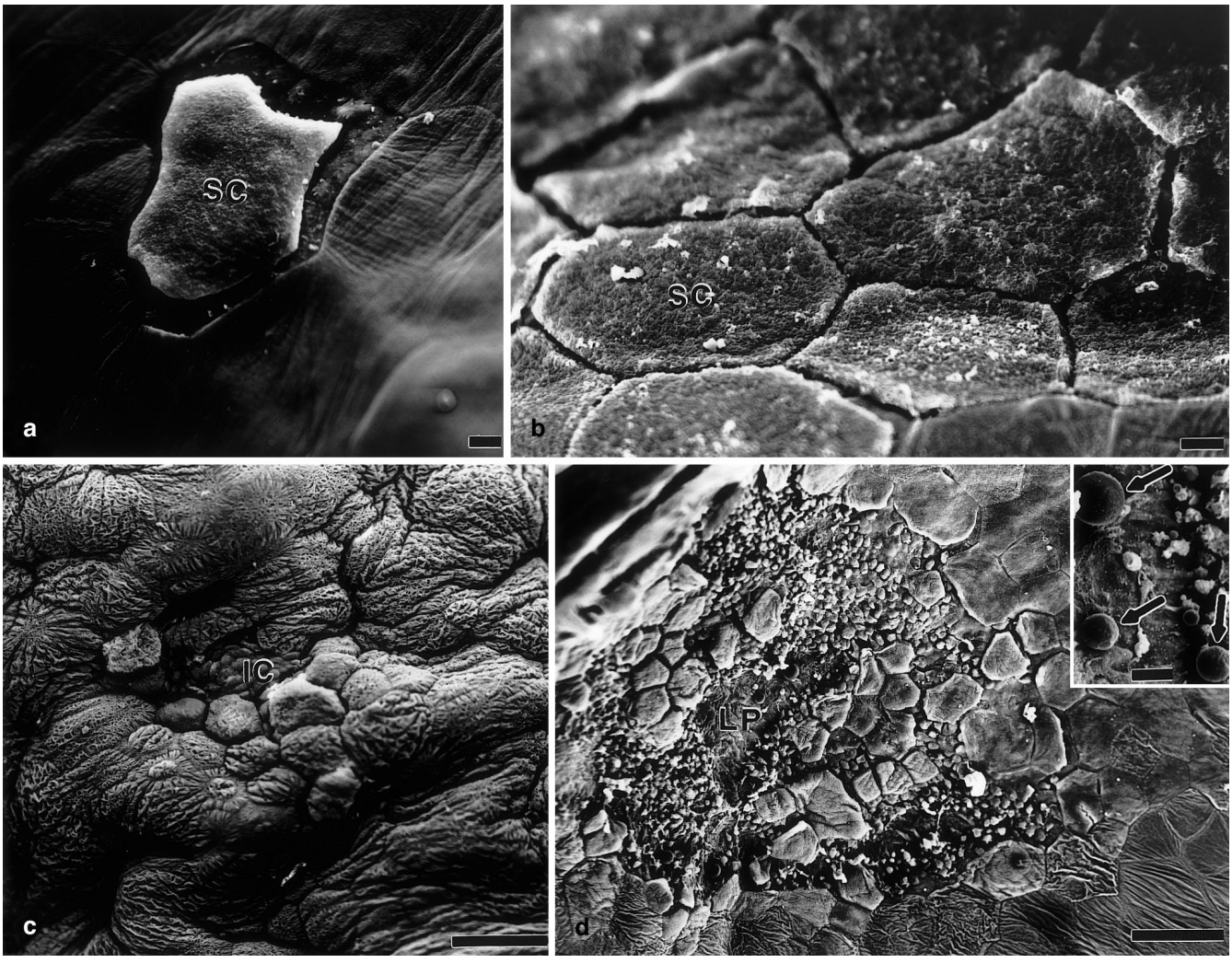

Fig. 4a–d Luminal surface of urothelium after **a** 60 min or **b–d** 90 min of ischemia. **d** experimental group in which ischemia was followed by emptying and filling of the bladder. Note detaching superficial cells (*SC*), intermediate cells (*IC*), lamina propria (*LP*) and leukocytes (*arrows*). **a** ×440, *bar* 10 µm **b** ×550, *bar* 10 µm **c** ×260, *bar* 50 µm **d** ×130, *bar* 100 µm; *insert* ×520, *bar* 10 µm

methods. With this procedure areas of color were easily found, and they appeared only on the bladders of animals subjected to more than 60 min of ischemia.

Following 1 h of exposure to indigo carmine dye, the urothelium of the control animals clearly showed strata of superficial, intermediate and basal cells. Lanthanum nitrate was found only on the apical surface of the urothelium and did not penetrate into the bladder wall. This supports the idea of an intact blood–urine barrier. In animals subjected to 60 min of ischemia no alterations were visible and the urothelium looked the same as in the control group.

After 90 min of ischemia lanthanum nitrate penetrated the tight junctions between superficial cells localized at the boundary of the focal areas (Fig. 5a). In these superficial cells, no ultrastructural changes were identified. At the central part of the focal areas, detachment (Fig. 5b) and desquamation (Fig. 5c) of adjacent superficial cells and some intermediate cells were clearly visible. In detached and desquamated superficial cells dilatation of vacuoles, chromatin condensation and cytoplasmic blebbing were observed (Figs. 5b, c). Some of the exposed intermediate cells gradually became lytic, while the others showed no ultrastructural changes (Fig. 5c, d). Between intermediate and basal cells, enlarged intercellular space often appeared (Fig. 5c, d). In the focal areas penetration of lanthanum nitrate both between intermediate and basal cells and into the lamina propria was evident (Fig. 5d).

In focal areas in animals subjected to 120 min of ischemia irreversible ultrastructural changes gradually appeared in desquamated superficial cells. Interruptions of plasma membrane continuity, pronounced condensation of chromatin followed by dissolution, and flocculent densities of already swollen mitochondria were observed in these cells (Fig. 5e).

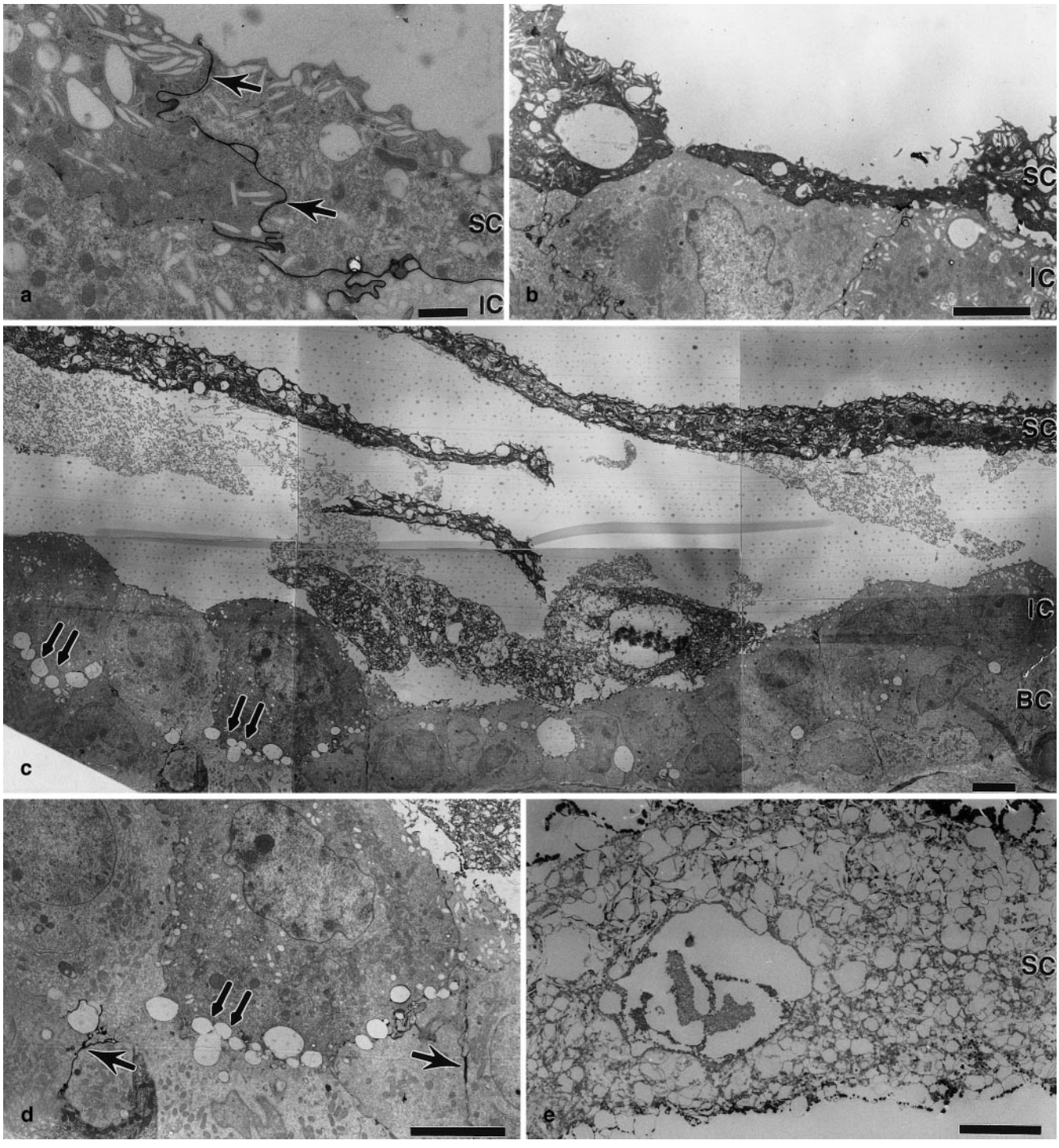

Fig. 5a–e Ultrathin sections of the areas of focal damage after **a–d** 90 min or **e** 120 min of ischemia. Note superficial cells (*SC*), intermediate cells (*IC*), basal cells (*BC*), lanthanum nitrate penetration (*arrows*) and enlarged intercellular spaces (*double arrows*). **a** ×7700, bar 1 µm **b** ×2600, bar 5 µm **c** ×1490, bar 5 µm **d** ×3050, bar 5 µm **e** ×2640, 5 µm

Discussion

The current study indicates that alterations of cell junctions are responsible for early ischemia-induced urothelium damages. By scanning electron microscopy, which gives an overview of the urothelium surface, focally distributed areas where detachment and desquamation of urothelium cells took place become evident. However, this technique did not make it possible to assess the viability of these cells or their functional integrity. Transmission electron micros-

copy, on the other hand, did give at least some of this information, but unfortunately there was almost no chance to find small focal areas of ischemically damaged urothelium. Indigo carmine dye can be used intravesically to study urothelial permeability and does not penetrate the bladder wall unless the urothelium is damaged [26, 33]. Therefore, by using indigo carmine we developed an in vivo model that provides an opportunity to locate damaged areas of the urothelium and thus the parts with the damaged blood–urine barrier. For this reason, it was feasible to examine the earlier ischemic changes applying techniques by which it is only possible to view a thin cross section of a very small bladder area each time. In none of the experiments performed with indigo carmine was there any evidence that this dye caused any untoward effects, and this is consistent with the studies of Monson et al. [26] and Tammela et al. [33].

The effects of short-term ischemia of the bladder urothelium were certainly distributed in a very focal fashion, and this is consistent with previous report of Ruggieri et al. [30]. We think the reason for this is the considerable regional variation in bladder wall blood flow and oxygenation as a result of bladder filling [31]. In our study, almost all the damage appeared in the urothelium of the bladder dome, which is consistent with the findings of Siroky et al. [31]: their study suggests that bladder perfusion and oxygen tension are greater at the bladder base than at the bladder dome.

After 1 h of ischemia, the first changes in the urothelium were noted in our in vivo model, which is consistent with the time of early ischemic molecular changes in the rabbit bladder tissue [4, 36, 39]. Hypolite et al. [11] showed significantly higher basal glycolytic and mitochondrial metabolic rates for bladder mucosa than for bladder smooth muscle and significantly higher specific activity of citrate synthase in mucosa than in smooth muscle. Furthermore, in the mucosa (and therefore in the urothelium) the creatine phosphate concentration is only a quarter of that in smooth muscle [11], and this also emphasizes the importance of oxidative metabolism for adequate urothelial function. It is known that 1 h of ischemia causes an 80% decrease in the bladder intracellular ATP content [36], and it has recently been shown that in vitro anoxia results in a significantly and substantially greater degradation of high-energy phosphates in the mucosa than in the smooth muscle [17]. All these studies point to a very high sensitivity of the urothelium to ischemic alterations, which is in agreement with our results.

It is known that reperfusion (by generation of reactive oxygen species) causes further damage to the tissue. Recently, reperfusion/reoxygenation injury in both bladder muscle and mucosa was made evident by the presence of malon dialdehyde (MDA), a marker for lipid peroxidation [21, 27]. In our study, urothelial damage was also proportional to in vivo-induced reperfusion. That means that the permeability of the blood–urine barrier increased even after only 40 min of ischemia followed by 60 min of reperfusion and that the extent of damage (in area) after 60 min of ischemia followed by 60 min of reperfusion was rather similar to that seen after 90 min of ischemia.

In the present study we demonstrate that ischemia induced significant indigo carmine penetration into the bladder wall. It has been reported that indigo carmine penetration increases as a result of 1 h of in vitro anoxia [33]. Although these experiments suggest a failure of the permeability barrier proved by movement of the vital dye, they provide only indirect evidence and did not explore the mechanisms involved or what components of the barrier were damaged. The tight junctions do not allow the passing of lanthanum tracer [5], and our results obtained on permeability to lanthanum clearly reveal ischemic disruption of tight junctions. The leaky tight junctions between superficial cells manifested focal loss of the paracellular blood–urine barrier of the urothelium, and it was evident that disruption occurred without obvious ultrastructural changes. The loss of tight junction integrity during renal ischemia in vivo or cellular ATP depletion in cell culture models has been described by many investigators [7, 23, 25, 35]. Our observations consistent with these studies suggest that disruption of tight junctions is probably a key event in the ischemic urothelial alternations.

Desquamation appears as a result of the disruption of cell junctions, and our results undoubtedly prove that ischemia induces urothelium damages due to desquamation. It has been shown that the desquamation in the bladder could be induced by stress [6, 12] or treatment with LPS [1, 12]. Desquamated cells in the lumen of the bladder after this experimental procedure are mainly viable [12]. In desquamated superficial cells after 90 min of ischemia, only ultrastructural changes characteristic for an early, reversible phase (stage) of cell injury [34] were found. However, after 120 min of ischemia irreversible ultrastructural changes [34] gradually appeared. For this reason, we conclude that desquamation in response to ischemia is not connected with irreversible changes, and that these changes appear later as a result of the exposure of desquamated superficial cells to the hypertonic and toxic urine. Hence, it becomes obvious that bladder ischemia results in alterations at the level of specialized junctions and leads to detachment and desquamation of viable nonlethally damaged urothelial cells. Emptying and filling of the ischemic bladder with intravesical physiological volumes results in denuded patches of mucosa in the focal areas. These suggest that ischemic changes probably involve alteration of junctional attachment to adjacent cells of all three urothelium strata as well as to extracellular matrix.

Our observations are supported by the earlier findings of Gill et al. [8] and Lin et al. [20]. In the works mentioned, it was established that as a result of ischemia the urothelium was not dying or degenerating, but either present or absent, so Gill and Lin suggested that the urothelium dissociated.

Urine contains many potentially noxious substances, such as urea, ammonia and excreted toxins. Disruption of the bladder permeability barrier may irritate the bladder wall layers underlying the urothelium causing or promoting inflammation, as well as increasing urinary frequen-

cy, urgency and bladder pain [38]. In our study, leukocyte influx observed in the areas of focal damage probably demonstrates ischemic inducement of an inflammatory process. Siroky et al. [31] suggest that smaller degrees of ischemia may be more common, while any condition producing urinary retention, increased intravesical pressure or detrusor instability may result in decreased bladder wall blood flow and oxygenation. Therefore, our findings may be applicable in the pathophysiology of several clinical problems. Further studies will define the mechanism of cell detachment in this model and examine how the damaged urothelium can recover.

References

1. Aronson M, Medalia O, Amychay D, Nativ O (1988) Endotoxin-induced shedding of viable uroepithelial cells is an antimicrobial defense mechanism. Infect Immun 56:1615–1617
2. Buttyan R, Jacobs BZ, Blaivas JG, Levin RM (1992) Early molecular response to rabbit outlet obstruction. Neurol Urodyn 11:225–238
3. Buttyan R, Chen LV, Levin RM (1997) Animal models of bladder outlet obstruction and molecular insights into the basis for the development of bladder dysfunction. Eur Urol 32:32–39
4. Chen MW, Buttyan R, Levin RM (1996) Genetic and cellular response to unilateral ischemia of the rabbit urinary bladder. J Urol 155:732–737
5. Claude P, Goodenough DA (1973) Fracture faces of zonula occludens from "tight and leaky" epithelia. J Cell Biol 58: 390–400
6. Dalal E, Medallia O, Harari O, Aronson M (1994) Moderate stress protects female mice against bacterial infection of the bladder by eliciting uroepithelial shedding. Infect Immun 62:5505–5510
7. Fish EM, Molitoris BA (1994) Alterations in epithelial polarity and the pathogenesis of disease states. N Engl J Med 330:1580–1587
8. Gill HS, Monson FC, Wein AJ, Ruggieri MR, Levin RM (1988) The effects of short-term in vivo ischemia on the contractile function of the rabbit urinary bladder. Neurourol Urodyn 139:1350–1354
9. Goligorsky MS, Lieberthal W, Racunsen L, Simon EE (1993) Integrin receptors in renal tubular epithelium: new insight into pathophysiology of acute renal failure. Am J Physiol 264 [Renal Fluid Electrolyte Physiol 33]:F1–F8
10. Hicks RM (1975) The mammalian urinary bladder: an accommodating organ. Biol Rev 50:215–246
11. Hypolite JA, Longhurst PA, Gong C, Briscoe J, Wein AJ, Levin RM (1993) Metabolic studies on rabbit bladder smooth muscle and mucosal epithelium. Mol Cell Biochem 125:35–42
12. Jezernik K, Medalia O, Araonson M (1995) A comparative study of the desquamation of urothelial cells during gestation and in adults mice following moderate stress or endotoxin treatment. Cell Biol Int 19:887–893
13. Kato L, Lin AT, Haugaard N, Longhurst P, Wein AJ, Levin RM (1990) Effects of outlet obstruction on glucose metabolism of the rabbit urinary bladder. J Urol 143:844–847
14. Kellerman PS, Bogusky RT (1992) Microfilament disruption occurs very early in ischemic proximal tubule cell injury. Kidney Int 42:896–902
15. Lapides J (1979) Mechanisms of urinary tract infection. Urology 14:217–225
16. Levin RM, Wein AJ, Whitmore K, Monson FC, McKenna BAW, Ruggier R (1990) Trypan Blue as an indicator of urothelial integrity. Neurourol Urodyn 9:269–279
17. Levin RM, Levin SS, Wein AJ (1996) Etiology of incontinence: a review and hypothesis. Scand J Urol Nephrol 179 [Suppl]:15–25
18. Levin RM, Levin SS, Zhao Y, Buttyan R (1997) Cellular and molecular aspects of bladder hypertrophy. Eur Urol 32:32–39
19. Lin AT, Wein AJ, Gill HS, Levin RM (1988) Functional effect of chronic ischemia on rabbit urinary bladder. Neurourol Urodyn 7:1–12
20. Lin AT, Monson FC, Kato K, Haugaard N, Wein AJ, Levin RM (1989) Effect of chronic ischemia on glucose metabolism of rabbit urinary bladder. J Urol 142:1127–1133
21. Lin AT, Yang CH, Chen KK, Chang LS (1995) Oxygen free radical-induced lipid peroxidation in overdistension of the rabbit urinary bladders. Neurourol Urodyn 14:553–554
22. Malkowicz SB, Wein AJ, Elbadawi A, Arsdalen K van, Levin RM, Ruggieri MR (1986) Acute biochemical and functional alterations in the partially obstructed rabbit urinary bladder. J Urol 136:1324–1329
23. Mandel LJ, Bacallao R, Zampighi G (1993) Uncoupling of the molecular "fence" and paracellular "gate" function in epithelial tight junctions. Nature 361:552–555
24. Mandel LJ, Doctor RB, Bacallao R (1994) ATP depletion: a novel method to study junctional properties in epithelial tissues. II. Internalization of Na, K, ATPase and E-cadherin. J Cell Sci 107:3315–3324
25. Molitoris BA, Nelson WJ (1990) Alternations in the establishment and maintenance of epithelial cell polarity as a basis for disease processes. J Clin Invest 85:3–9
26. Monson FC, Wein AJ, McKenna BAW, Whitmore K, Levin RM (1991) Indigocarmin as a quantitative indicator of urothelial Integrity. Neurourol Urodyn 145:842–845
27. Ohnishi N, Liu SP, Horan P, Levin RM (1998) Effect of repetitive stimulation on the contractile response of rabbit urinary bladder subjected to in vitro hypoxia or in vitro ischemia followed by reoxygenation. Pharmacology 57:139–147
28. Parsons CL, Boychuk D, Jones D, Hurst R, Callaha H (1990) Bladder surface glycosaminglycans: an epithelial permeability barrier. J Urol 66:139–142
29. Racusen LC, Fivush YL, Li YL, Slatnik I, Solez K (1991) Dissociation of tubular cell detachment and tubular cell death in clinical and experimental "acute tubular necrosis". Lab Invest 64:546–556
30. Ruggieri MR, Hanno PM, Samadzdeh S, Johnson EW, Levin RM (1986) Heparin inhibition of increased bacterial adherence following overdistension, ischemia and partial outlet obstruction of the rabbit urinary bladder. J Urol 136:132–135
31. Siroky MB, Azadzoi KM, Pontari M, Vlachiotis J (1996) Canine bladder blood flow and oxygenation: changes induced by filling, contraction and outlet obstruction. J Urol 155:1459–1465
32. Stirling WC, Hopkins J (1934) Gangrene of the bladder. J Urol 31:517
33. Tammella T, Wein AJ, Monson FC, Levin RM (1993) Urothelial permeability of the isolated bladder. Neurourol Urodyn 12:39–47
34. Trump BF, Berezesky IK (1995) Calcium -mediated cell injury and cell death. FASEB J 9:219–228
35. Tsukomoto T, Nigam SK (1997) Tight junctions proteins form large complexes and associate with the cytoskeleton in an ATP depletion model for reversible junction assembly. J Biol Chem 272:16133–16139
36. Van Arsdalen KN, Wein AJ, Levin RM (1983) The contractile and metabolic effects of acute ischemia upon the rabbit bladder. J Urol 130:180–182
37. Wein AJ, Levin RM, Barrett DM (1991) Voiding function and dysfunction. Relevant anatomy, physiology and pharmacology. In: Gillenwater JY, Grayhack JT, Howards SS, Duckett JW (eds) Adult and pediatric urology, pp 933–999
38. Zeidel ML (1997) Functional anatomy of the mammalian urinary bladder. In: Sips IG, McQueen CA, Gandolf AJ (eds) Comprehensive toxicology. Elsevier, Oxford
39. Zhao Y, Levin SS, Wein AJ, Levin RM (1997) Correlation of ischemia/reperfusion or partial outlet obstruction-induced spectrin proteolysis by calpain with contractile dysfunction in rabbit bladder. Urology 49:293–300