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Cyclosporine A impairs wound healing of ureterocystoneostomy in rats

Scanning electron microscopic examination

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Summary. The effect of cyclosporine A (CsA), an immunosuppressive agent used in transplantation, on wound healing following microsurgical neoimplantation of a ureter in the bladder of 63 SIV ZUR rats was examined morphologically using the light and scanning electron microscopes and functionally by radiography. Following ureterocystoneostomy (UCN) on the right side, the animals in Group I (control group) received 1.0 ml CsA solvent (0.1 g ethanol and 0.3 g intralipid) per day. Group II received 12.5 mg/kg/day CsA and Group III 17.5 mg/kg/day CsA. All drugs were administered i.p. A third of the animals in each group were reoperated 7, 14 or 28 days after UCN. At these time intervals, there were no radiologically demonstrable differences in the operated side. Examination under the scanning electron microscope indicated delayed restitution of epithelium in the bladder for rats which had received CsA as compared to the control group. In the area of the UCN, CsA caused dose-independent retardation of the regenerative hyperplasia associated with wound healing (Group I: max. 7 days after UCN: Group II and Group III, max. 14 days after UCN). Hyperplastic areas had ropy microridges and uniform short microvilli. Where the hyperplasia exhibited nodular and papillary formation, also histologically more evident under CsA, occasional epithelial cells had pleomorphic microvilli on their luminal surface. Unlike other known premalignant changes of this kind, the frequent occurrence of pleomorphic microvilli under CsA was reversible. In general, CsA led to dose-unrelated protraction of UCN wound healing with no lasting functional disturbance in rats.

Key words: Wound healing – Ureterocystoneostomy – Cyclosporine A – Scanning electron microscopy

Introduction

Introduction of cyclosporine A (CsA), a fungal immunosuppressive metabolite with its sites of action in the cell-mediated immune system, especially in the T-helper cells, has permitted considerable reduction in allograft rejection in organ transplant patients [4, 5]. A possible influence of CsA on wound healing of skin, muscle and a model of the implanted polyvenyl alcohol sponge, ranging from retardation [10] to improvement [1, 20], has been reported. In kidney transplantation, wound areas with a potential for causing subsequent complications result from neoimplantation of the ureter in the bladder. Clinically, it is noteworthy that urine flow disturbances (stenosis or vesico-ureteral reflux) in the region of the ureterocystoneostomy are considerably more frequent (15–34%) in transplantation patients than in patients with autologous neoimplantation of the ureter [8, 9, 15]. The reasons would appear to lie in local rejection or disturbances in microvascular circulation due to problems of surgical technique.

A model of micro-surgical autoneoimplantation of a ureter in a rat is used to assess a possible influence of the immunosuppressive metabolite CsA on wound healing of a ureterocystoneostomy functionally, histologically, and by means of scanning electron microscopy.

Materials and methods

63 male SIV ZUR rats with body weights ranging from 200 to 250 g were used in the study. Four of five rats per cage were housed in stainless steel cages at room temperature of 23°C + 2°C. Commercial stock diet and tap water were available ad libitum. Control rats (Group I) received cyclosporine solvent (0.1 g ethanol and 0.3 g intralipid in 1 ml olive oil) in equivalent volume to CsA treated rats. CsA was administered to Group II at a dosage of 12.5 mg/kg/day and to Group III at 17.5 mg/kg/day. Drugs were administered as a

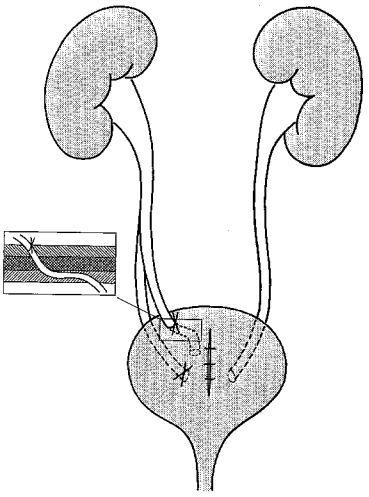

Fig. 1. Operative technique for the ureterocystoneostomy

Table 1. Excretory urography in rats 7, 14 or 28 days after ureterocystoneostomy. I control group, II CsA 12.5 mg/kg/day, III CsA 17.5 mg/kg/day; increase of normal i.v.p. (intra venous pyelography) in the temporal course of the experiment in all three groups ($n = 57$)

	I			II			III		
	7	14	28	7	14	28	7	14	28
i.v.p. normal	2	4	5	3	3	4	2	2	4
Hydron. I°	4	2	1	3	2	1	5	4	2
Hydron. II°	ø	1	ø	ø	1	1	ø	ø	1
Hydron. III°	ø	ø	ø	ø	ø	ø	ø	ø	ø

single daily intraperitoneal injection from UCN until relaparotomy and killing 7, 14 or 28 days after UCN. Weight checks were performed every 4 days. The CsA serum level concentrations were determined once weekly by high performance liquid chromatography (HPLC).

Microsurgical procedure for ureterocystoneostomy

Following anaesthesia (100 µg/g body weight Nembutal), a lower median abdominal incision was made. Using a Zeiss Diploscope with 2.5- to 15-fold magnification, the right ureter was mobilized, preserving the periureteral vessels and fat, and separated at the transition point to the bladder. A small stab wound was made in the fundus of the bladder (Fig. 1), allowing submucosal insertion of a small curved mosquito clamp which pierced the posterior wall. The tip of the ureter was grasped and drawn through a submucosal tunnel into the bladder, so that if fitted comfortably without tension (Fig. 1, detail) [12, 23]. Where the ureter passed into the bladder, a No. 9.0 silk stitch was placed in the serosa, securing it to the bladder wall. A short length of ureter (0.5–1.0 mm) was allowed to protrude into the vesicle lumen. The stab wound in the bladder was closed with a single suture Vicryl 7-0, containing serosa, muscle and mucosa. The initial intraperitoneal administration of the drug was performed and the abdomen closed. After a period of 7, 14 or 28 days, the rats were relaparotomised and killed.

Relaparotomy

After anaesthesia (100 µg/g body weight Nembutal), the left anterior carotis was exposed and a 0.5 mm polyethylene catheter introduced and pushed via the aorta thoracica to the level of the diaphragm. A prime of 0.3 to 0.4 ml Conray 70% was injected via the catheter under radiological control. The state of the renal vessels was assessed immediately, with subsequent radiological examination of the urinary tract after the elapse of 5, 15 and 30 min. The abdomen was re-opened via the existing median abdominal incision, and both the ureters and the bladder mobilised. 2% glutaraldehyde (in 0.1 M cacodylate buffer, pH 7.4, pressure 50 mmHg) was inflated into the bladder via a polyethylene catheter placed in the upper tract. The filled bladder and ureter were removed in toto. The urinary bladder was bisected and one half of the specimen used for scanning electron microscope examination as described by various authors [16, 17]. The remaining half was prepared for light microscopy by inflation with 10% phosphate buffer, embedding in paraffin and staining with haematoxilin and eosin.

Results

5/63 rats died either as a result of a peritoneal abscess (3/5) or of pneumonia (2/5). The cyclosporine serum level for Group I was 0, for Group II 2,200 to 3,000 ng/ml and for Group III 2,100 to 4,200 ng/ml. The blood count, kidney retention values and transaminases lay within the normal range for all groups; the gain in weight of the animals was synchronous.

Functional examination

Radiological presentation of renal drainage on the side with the neoimplanted ureter in the three groups revealed an excess of hydronephrosis grade I, i.e. dilation of the ureter with no tendency to dilation of the renal pelvis in the initial phase after UCN (7 days; Table 1). With increasing time after UCN, the number of non-pathological excretory urograms rose uniformly in all 3 groups (28 days after UCN: Group I:5; Group II:4; Group III:4 kidneys; Fig. 2). Simultaneously, slight dilation of the calyces in the region of the right kidney was observed in one animal in each group (second degree hydronephrosis). No worse distribution of the hydronephrosis was found for the CsA treated animals as compared either to one another or to the control animals. No third degree hydronephrosis and no non-functioning kidneys were observed.

Scanning electron microscope and histological findings

One week after UCN, epithelial lesions of the bladder were noted in all three groups (Table 2). In place of an exclusively superficial endothelial lining, the control

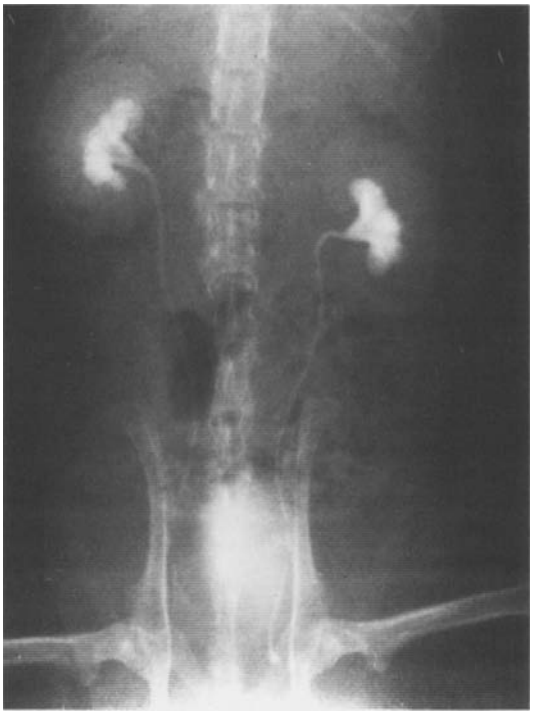

Fig. 2. Normal excretory urography 28 days after right-hand side ureterocystoneostomy and daily administration of 17.5 mg/kg CsA i.p.

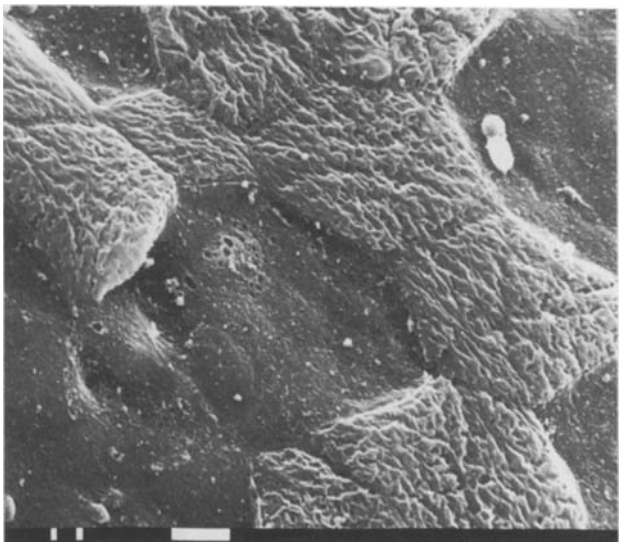

Fig. 3. Clump-like accumulation of the intermediate cell layer 14 days after UCN and daily administration of 12.5 mg/kg CsA, magnification ×840

animals had diffuse intermediate cells. Under cyclosporine there were even basal cell structures of the three-layer epithelium of the bladder, with presentation of the subendothelial capillaries. Exfoliative degeneration of damaged endothelial cells was equally frequent in all groups at this point. Whereas lining of the bladder with a normal superficial epithelium was fully complete in the control group 14 days after operation, cyclosporine treated animals still showed the intermediate cell layer, irrespective of the dose received (Fig. 3). After 28 days, however, the CsA treated groups also had an exclusively superficial intravesical layer. Restitution of the bladder epithelium was delayed under cyclosporine. Particularly in the region of the ureterocystoneostomy, the hyperplasia of the bladder mucosa defined by previous authors [13, 17] were noticeable. These formed a mixture of ropy and clump-like epithelial accumulations at the transition of the bladder and implanted ureter (Fig. 4). The cells varied from round to oval and were of differing size (Fig. 5). On their surfaces were numerous short uniform microvilli and a labyrinth of intertwined ropy microridges. Furthermore, clump-like accumula-

Table 2. SEM Findings of the urinary bladder epithelium following ureterocystoneostomy 7, 14, and 28 days. Under CsA appeared, irrespective of the dose received (II, III), a retardation of bladder restitution and of the regenerative hyperplasia associated with wound healing. The extent of pleomorphic microvilli accumulated in II and III compared to control rats (I)

	I			II			III		
	7	14	28	7	14	28	7	14	28
Superficial	+	+++	+++	+	+	+++	+	+	+++
Intermediate	++	ø	ø	+	++	ø	+	ø	ø
Basal	ø	ø	ø	+	ø	ø	+	ø	ø
Mucosa hyperplasia (UCN)	++	+	ø	+	++	+	+	++	+
Epithelial layers (histo)	3–5	3–5	3–5	4–5	5–7	5–7	4–5	5–7	5–7
Uniform microvilli	+	+	ø	+	+	+	+	+	+
Pleomorphic microvilli	+	ø	ø	+	++	+	+	++	+
Ropy microridges	++	+	ø	+	++	+	+	++	+

ø no change, +: slight, ++: moderate, +++: marked

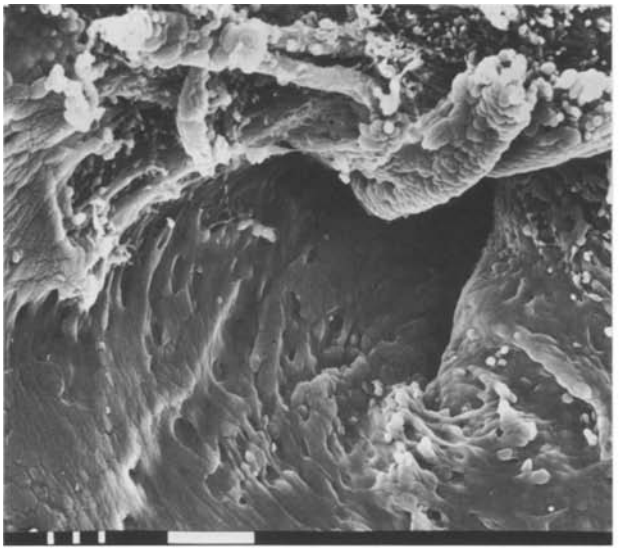

Fig. 4. Neoostium 14 days after UCN and daily administration of 12.5 mg CsA, showing epithelialisation disturbances and ropy hyperplasia, magnification ×126

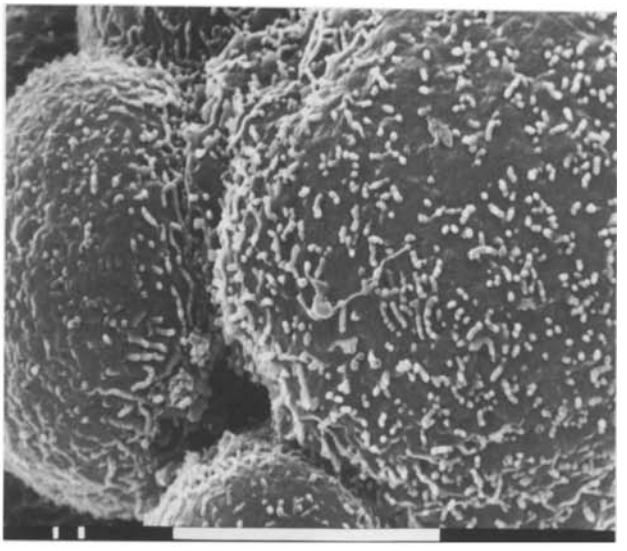

Fig. 6. Pleomorphic microvilli on the hyperplastic luminal surface (increased under CsA), magnification ×3800

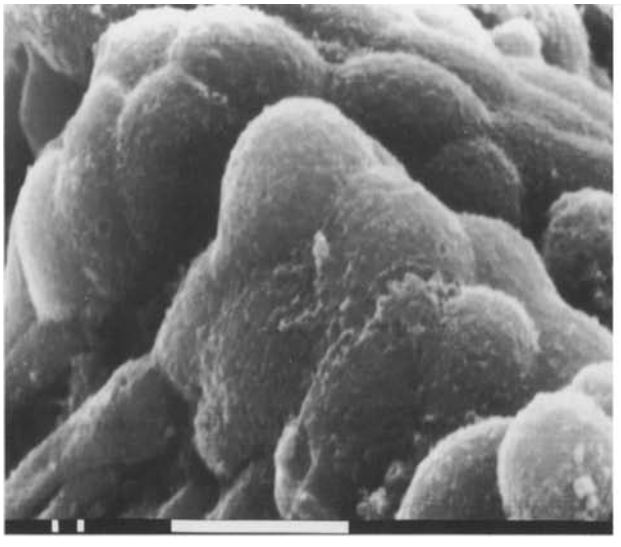

Fig. 5. Regenerative hyperplasia under 12.5 mg/kg CsA, magnification ×2530

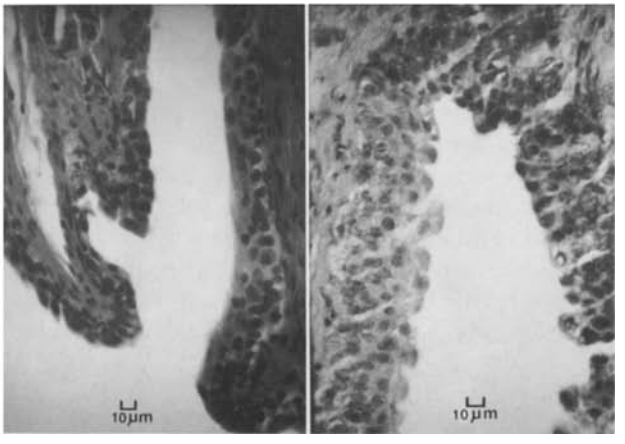

Fig. 7. Mucous hyperplasia in the region of the UCN

tions of hyperplasia had cell surfaces with irregularly arranged pleomorphic microvilli of varying length (Fig. 6). Numbers represented some 5–10% of those for the monomorphic forms.

In contrast to the control animals, which displayed the most pronounced hyperplasic forms after 7 days (Table 2), the most marked mucosal hyperplasia under cyclosporine appeared after 14 days, irrespective of the dose received, i.e. there was retardation of the regenerative hyperplasia associated with wound healing. Histologically, these hyperplasia had three to five layers in the control group, while there was multilayer epithelial cell activity (5–7) under CsA (Fig. 7). Whereas wound healing with normal epithelial lining of the UCN was completed after 28 days in the control animals (Fig. 8), residual hyperplasia were still detectable in the CsA groups. The monomorphic microvilli and ropy microridges located on the hyperplastic epithelia ran in parallel. They were equally evident in control and CsA treated animals. By contrast, the

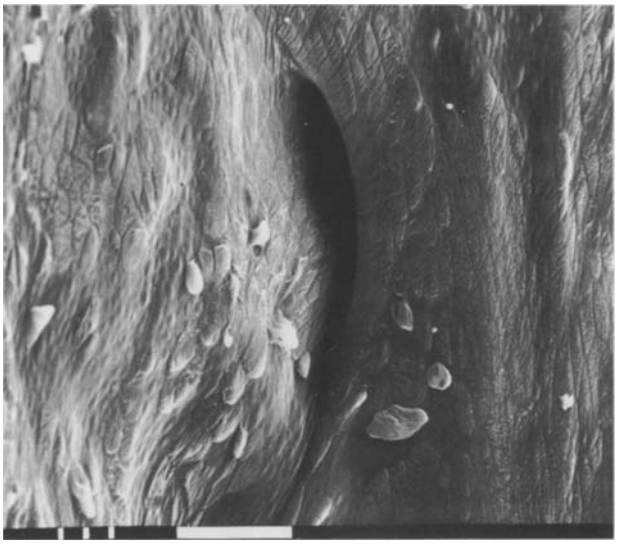

Fig. 8. Neoostium 14 days after UCN of a control animal, showing normal epithelium as opposed to Fig. 4, magnification ×126

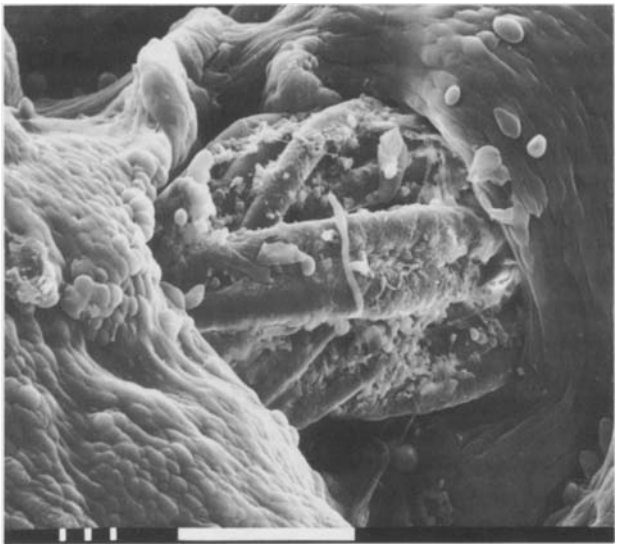

Fig. 9. Cystostomy suture 14 days after operation under daily administration of 17.5 mg/kg CsA with regenerative hyperplasia, magnification ×187

extent of pleomorphoc microvilli accumulations under CsA was considerably greater compared to control rats (Table 2), but receded slightly after 28 days.

Scanning electron microscope observation of the cystostomy suture revealed a similar picture of retarded wound healing and regenerative hyperplasia under Cyclosporine A. Whereas the epithelial layer was complete after 14 days in the control group, the CsA animals till had exposed intravesical suture material with hyperplasia. After 28 days, however, an epithelial lining with normal, polygonal, superficial cells of equal size had also been attained under CsA.

Discussion

Hyperplasia of the bladder mucosa may be induced either by mechanical manipulation or by chemical irritants [6, 16, 17]. Following operative incision of the bladder, hyperplasia associated with regenerative wound healing were observed over a period of approximately 14 days [13, 14], agreeing with our results for the control group (Table 2, Group I). By contrast, animals treated with Cyclosporine reached a maximum of regenerative hyperplasia after 14 days, irrespective of dose, with a tendency to recede after 28 days (Table 2, UCN Group II, III). Retardation of wound healing by CsA in the form protracted regenerative hyperplasia was also confirmed by a slowed restitution of the bladder epithelial lining (Table 2, Group II, III).

Various authors have described an interaction between the immune system and wound healing [2, 7, 11].

In association with a trauma, there is increased production of T-suppressor cells, which, unlike T-helper cells, induce protracted wound healing through reduction of lymphokine and monokine secretion [19, 24]. Where T-suppressor cells are eliminated by postnatal thymectomy, Barbul et al. [3] noted increased wound healing. The selective action site of the immunosuppressive agent Cyclosporine A in the region of the T-helper cells produces a relative predominance of T-suppressor cells, possibly causing the protracted healing process under CsA through the mechanism of reduced lymphokine and monokine secretion described above. Furthermore, CsA inhibits the secretion of macrophage activating mediators in T cells. This is followed by a decreased activity of C macrophages which delayed the first inflammation phase of wound healing. Fishel et al. [10] noted reduced collagen formation under CsA, though these findings were not confirmed by Nemlander's group [20]. Another possible cause of protracted wound healing may be found in negative effects of CsA on the microcirculation in the region of the ureterocystoneostomy, of the kind described for the kidney, with reduction of renal circulation [21].

The increased frequency of pleomorphic microvilli on cell lumina under CsA observed with the scanning electron microscope are not specific to neoplasia or irreversible damage [13, 16, 17]. Although pleomorphic microvilli are present in neoplastic and non-neoplastic bladder lesions in rats, recent studies of human bladder specimens indicate that there may be quantitative differences in surface features between the various grades of bladder lesion. Quantitative differences in pleomorphic microvilli may also exist between neoplastic and non-neoplastic lesions in rats. They also occurred more frequently in clumps in the CsA treated groups. The fact that they receded after 28 days tends

rather to indicate a benign hyperplasia, although our studies have demonstrated an increased incidence of chemically-induced bladder tumours under CsA [22].

Over an observation period of four weeks, a direct influence of CsA on the ureterocystoneostomy, causing protracted wound healing, was established. No functional urine flow disturbance was observed under CsA. The question of possible longterm CsA induced disturbances in this area must be resolved by further studies.

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