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Ureterosigmoidostomy in Rats: A Model for the Study of Bladder Tumour Carcinogenesis and Cocarcinogenesis

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Summary. A modified Coffey I ureterosigmoidostomy has been developed in rats as a model of urinary diversion for studying bladder carcinogenesis and co-carcinogenesis. Diverted and sham-operated animals were killed at 1, 3 and 6 months. Excretory urograms revealed minimal hydroureteronephrosis in most diverted animals. Upper tract bacterial colonisation was 9 times more frequent in diverted animals. Approximately one-third of the diverted animals had focal cortical scarring; however, renal function was normal in all groups as assessed by serum creatinine and electrolytes. These studies indicate that ureterosigmoidostomy in rats is a satisfactory model of urinary diversion for studying carcinogenesis.

Key words: Ureterosigmoidostomy - Urinary diversion - Bladder carcinogenesis.

Urinary diversion as a means of treatment of bladder tumour has been used mostly as a palliative procedure (2, 8). Isolated reports are found in the literature in which urinary diversion was used as the primary means of treatment of bladder tumours. One such report was by Abeshouse who found regression of six cases of bladder tumour after urinary diversion (1). Davis noted the disappearance of superficial bladder tumours but the persistence of invasive tumours in two patients upon whom ureterosigmoidostomies were performed (5).

Experimental studies in dogs showed no beneficial effects of urinary diversion after gross tumours were induced with β-naphthylamine; however, if urine was diverted from the bladder before carcinogenic exposure, the bladder remained tumour free (11). Scott showed that a substituted isolated sigmoid loop did not form tumours in response to β-naphthylamine (12).

No reports can be found in the literature showing the effect of urinary diversion on the course of carcinoma in situ of the bladder. However, based on the report of regression of superficial tumour, one can make the hypothesis that urinary diversion would alter the natural history of intra-epithelial carcinoma and either eradicate the lesion or prevent its progression to invasive tumours.

An animal model of an induced multifocal intra-epithelial bladder carcinoma which is irreversible and progresses to invasive tumours has been described in detail (4, 6, 7, 13-15). Nitrofurylthiazolyl formamide (FANFT) induces intra-epithelial carcinoma of the bladder which progresses to invasive tumours despite cessation of exposure to carcinogen with a minimum exposure time in rats of 10 to 12 weeks (4). In order to test the hypothesis that urine has an effect on the course of intra-epithelial bladder tumours, a model of urinary diversion was needed which could be used with the FANFT-induced intra-epithelial carcinoma of the bladder. The technique and characterisation of this model are reported.

METHODS

Twenty-nine C. D. Fischer male rats (Charles River Breeding Laboratories, Wilmington, Mass.) weighing 140-250 gm, were used to characterise the model. All animals were given half-strength Vivonex (Eaton Laboratories, Norwich, N. Y.) for 2-3 days pre-operatively. A modified Coffey I ureterosigmoidostomy was performed on 15 animals through a lower midline incision under Ketaset (Bristol Laboratories, Syracuse, N. Y.) anaesthesia (3). The ureters were freed from the retroperitoneum by blunt dissection. The distal ureters were ligated with 8-0 chromic catgut and transected. A low colotomy was made and 8-10

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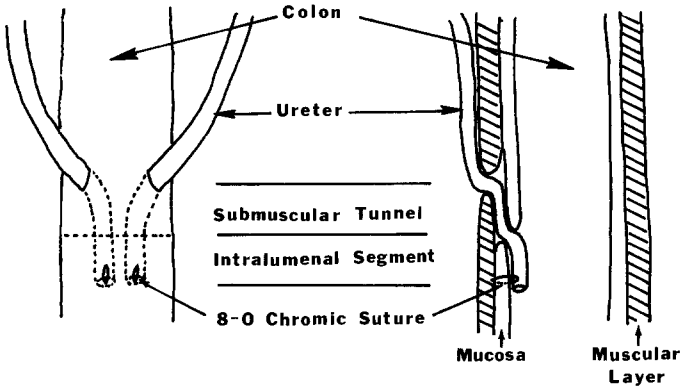

Fig. 1. Ureterosigmoidostomy by a modified Coffey I technique

Table 1. Animal weight (gm)

	Initial	Final	Change
1 Month			
Sham (n = 4)	163 ± 13	236 ± 10	+ 45%
Ureterosig. (n = 4)	148 ± 12	214 ± 23	+ 45%
3 Months			
Sham (n = 5)	170 ± 15	308 ± 19 [< .05]	+ 81%
Ureterosig. (n = 4)	184 ± 21	225 ± 70	+ 22%
6 Months			
Sham (n = 4)	194 ± 22	360 ± 15 [< .001]	+ 86%
Ureterosig. (n = 3)	200 ± 46	210 ± 28	+ 5%

Mean ± S. D. (Number of animals)

[p] = p value comparing sham to ureterosigmoidostomy animal values. If not shown, P > .05

mm submuscular tunnels were created for each ureter using a fine-tipped forceps. The ureters were then drawn through the submuscular tunnels. An 8-0 chromic catgut suture was then placed between the adventitia of the ureter and the distal colonic mucosa and muscular layers which, when tied, drew the distal ureter into the colonic lumen (Figure 1). The muscular layer was closed over the colotomy site with a running 8-0 chromic catgut suture and the skin with metal clips.

A sham precedure was performed on 14 animals through a similar incision. This procedure consisted of freeing the ureters from the retroperitoneum and making a colotomy which was closed with 8-0 chromic catgut as in the diverted animals.

All animals received 5 ml/100 gm body weight of 0.9% saline at 37° intraperitoneally at the end of the procedure. The animals were then given half-strength Vivonex for an additional 2-3 days before resuming water and rat chow. Urinary bladder urine was cultured following aspiration in all animals during surgery.

Four animals from each group were sacrificed at 1 month; 5 sham-operated and 4 diverted animals were sacrificed at 3 months; the remaining animals in each group were sacrificed at 6 months.

Two to 5 days before sacrifice, excretory urograms were performed by injecting 6 ml/kg of 60% contrast material by tail vein.

At the time of sacrifice, the animals were weighed and decapitated. The blood was collected, and serum Na, K, Cl, CO_2, BUN, and creatinine were performed. The bladder was aspirated and/or irrigated with 0.5 ml of 0.9% saline for culture. The kidneys and ureters were removed. The kidneys were bisected under sterile conditions, and 3-5 mg of each renal papilla was removed for culture. The bladder and sigmoid colon (with distal ureters in the case of the diverted animals) were removed. All tissue was fixed in formalin and representative pieces of tissue were processed for histological examination.

RESULTS

Of the 29 animals used, 1 sham-operated and 3 diverted animals died within 48 hours, and one additional diverted animal died at 4 months.

All diverted animals thrived in the first months after diversion; however, 2 of the 4 animals in the 3-month group developed perianal excoriation and lost weight. Three of the 4 animals in the 6-month group also developed excoriation and weight loss. One of these animals died in the fourth month after diversion. The cause of death could not be determined. The initial and final weights of the animals in each group are shown in Table 1.

Excretory urograms showed mild to moderate hydroureteronephrosis in all but 1 diverted animals and no abnormalities in sham-operated animals. The serum chemistry values as shown in Table 2 are normal except for elevation of the BUN in diverted animals at 1, 3, and 6 months and the elevation of the potassium in both groups at 6 months. Upper tract bacterial colonisation was 9 times more frequent in diverted animals as shown in Table 3.

Focal cortical scarring was found in one-third of the kidneys of diverted animals. Mild medullary collecting duct dilatation was noted in all diverted animals which showed hydronephrosis. The ureters of animals with hydronephrosis were di-

Table 2. Serum chemistries

	Bun	Creat	Na	K	Cl	CO_2
	mg%	mg%	mEq/l	mEq/l	mEq/l	mEq/l
1 Month						
Sham (n = 4)	16 ± 1 [< .01]	0.6 ± 0.1	147 ± 2	6.3 ± 0.1	100 ± 4	19.8 ± 1.8
Ureterosig. (n = 4)	20 ± 1	0.6 ± 0.0	147 ± 2	6.5 ± 0.2	103 ± 3	20.2 ± 3.1
3 Months						
Sham (n = 5)	19 ± 3	0.5 ± 0.1	147 ± 3	7.3 ± 1.0	106 ± 4	25.5 ± 1.3
Ureterosig. (n = 4)	30 ± 12	0.5 ± 0.0	149 ± 2	6.3 ± 0.3	108 ± 2	22.9 ± 3.0
6 Months						
Sham (n = 4)	18 ± 1 [< .02]	0.6 ± 0.1	142 ± 1	11.6 ± 3.0	104 ± 7	20.8 ± 5.2
Ureterosig. (n = 3)	45 ± 17	0.7 ± 0.2	144 ± 4	10.0 ± 1.5	106 ± 4	20.5 ± 3.4

Mean ± S. D. (Number of animals)

[P] = P value comparing sham to ureterosigmoidostomy animal values. If not shown P > .05

lated, but the histological features were normal. Renal pelvic stones were present in 2 and a ureteric stone in 1 of the 3 diverted animals studied for 6 months. No bladder or colonic abnormalities were noted in either group except for an apparent mild, diffuse hyperplasia of the diverted bladder mucosa.

DISCUSSION

Using this technique of ureterosigmoidostomy, the postoperative mortality rate was 20%. In pilot studies not reported here, intraperitoneal saline given at the end of the procedure seemed to be the most important factor in survival of the immediate postoperative period. The animals all have an adequate oral intake by the second postoperative day.

Initially the diverted animals thrived, but in the following months the animals developed excoriation of the perianal tissues. The extent of the excoriation varied with each animal. When the excoriation was extensive, the animals lost weight and one animal died. A dramatic improvement could be made by changing the cage bedding frequently and using coarse wood chips. This provided better mechanical cleansing of the scrotal and rectal areas. In future studies female rats may have advantages since the scrotum seemed to be the greatest hindrance to debridement of the area by the cage bedding.

Table 3. Bacterial colonisation rate*

	Pre-op	Post-op	
	Bladder	Bladder	Kidneys
1 Month			
Sham	2/4	3/4	1/8
Ureterosig.	1/4	3/4	2/8
3 Months			
Sham	2/5	0/5	0/10
Ureterosig.	1/4	0/4	1/8
6 Months			
Sham	1/4	0/4	0/8
Ureterosig.	0/3	0/3	5/6

*> 2000 colonies per total sample

Although ureterosigmoidostomy caused mild to moderate hydroureteronephrosis in all but 1 ani-

mal, the obstruction was not significant enough to cause a change in renal function as measured by serum creatinine and electrolytes. A clearance study would be needed to detect minor changes in renal function; however, a change of function of the magnitude that would effect survival of the animal would be reflected in the serum creatinine level. The elevation of BUN most likely reflects reabsorption of urea from the colon. The elevation of the potassium in all animals at six months was due to haemolysis.

A review of the literature on ureterosigmoidostomy in humans and dogs suggests that obstruction at the ureterocolonic function may be reduced by using a mucosa-to-mucosa anastomosis at the orifice and of the anti-refluxing tunnel, rather than tacking the ureter to the distal colonic mucosa (3, 9, 10). However, this seems to be technically impossible in the rat. As discussed above the degree of obstruction present is acceptable based on the persistence of grossly normal renal function.

The presence of stones, the colonised renal medulla, and the histological findings of cortical scarring and collecting duct dilatation can be explained by the stasis caused by the ureterocolonic obstruction. Reflux into the ureter is an alternative explanation which was not investigated in this study.

Despite the changes described above, this model of urinary diversion is satisfactory for the study of bladder carcinogenesis and the cocarcinogenic role of urine. This technique offers the advantages of being technically easy to perform, having an 80% survival rate, and preserving normal renal function . With careful management of the perianal region the animals will maintain a healthy appearance and gain weight.

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