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Urinary endothelin (ET1) in complete ureteric obstruction in the miniature pig

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Summary. Segmental renal scarring occurs in experimental obstructive uropathy in the multipapillary porcine kidney, and segmental abnormalities in renal perfusion are likely to be responsible. This preliminary study examines the urinary excretion of the potent locally active vasoconstrictor endothelin 1 (ET1) in a pig model of renal obstruction and subsequent relief. Significant urinary excretion of ET1 from the postobstructive kidney was found to occur after longstanding obstruction. Preglomerular arteriolar stenosis may be the cause of the renal ischaemia in ostruction that is at first reversible but later becomes irreversible if the stimulus persists. ET1 may be implicated in the pathogenesis of this injury.

The endothelins (ET1, ET2 and ET3) are a family of recently discovered polypeptides that have remarkable physiological characteristics [10, 11]. ET1 was originally cultured from pig aortic vascular endothelial cells and is the most potent in vitro vasoconstrictor that is known to man. It is thought to be released by vascular endothelial cells in response to injury and is known to lead to a fall in both glomerular filtration rate and renal blood flow through locally active preglomerular vasoconstriction [2, 4]. It acts predominantly on the efferent arteriole and to a lesser extent the afferent arteriole [4]. It is excreted in the urine at approximately five times its plasma concentration and when its antibody is infused into the renal artery of postischaemic rat kidney, the expected subsequent vasoconstriction is ameliorated [1, 4]. ET1 has recently been implicated in the progression of renal disease towards end stage renal failure [7].

Both the renin angiotensin system and the prostaglandin derivatives, the thromboxanes, have been implicated in the vascular injury that is associated with renal obstruction [5, 6, 12]. There is little doubt that both are involved, but variable results have been reported when specific inhibitors of these pathways have been used to influence the outcome of experimental obstructive nephropathy [5, 9, 12].

It is a currently held belief that the parenchymal damage in renal obstruction is diffuse and uniform in nature. We have observed, however, in hydronephrosis secondary to pelvi-ureteric junction obstruction in children and in experimental hydronephrosis following ureteric ligation in the miniature pig, that parenchymal damage may be segmental in nature. This damage appears as photon-deficient areas in the kidney, particularly the upper pole on ^{99m}Tc-DMSA (technetium-labelled dimercaptosuccinic acid) imaging, and on histophatological examination these areas correspond to parenchymal scars that are indistinguishable from those seen in sterile reflux nephropathy, and are thus likely to be related to the underlying papillary morphology [3, 8].

An earlier preliminary study from this unit strongly implicated segmental abnormalities in renal perfusion as the likely cause of this parenchymal damage [3]. As it is known that ET acts at a local level and is produced in response to trauma and shearing stress, it could be proposed that compound papillae allowing free pressure interchange between the renal pelvis and the tubules exposes the related nephrons to an early obstructive insult. One could postulate that the release of ET1 could produce segmental hypoperfusion resulting in the observed segmental defects demonstrated on isotope scanning both clinically and experimentally. The mechanism through which damage may be mediated would be a preglomerular stenosis which may be reversible in the early stages but becomes irreversible with time. Progression of obstructive nephropathy could then occur as the segmental response becomes generalized.

We have therefore investigated the excretion of this substance in a porcine model of renal obstruction. Urinary ET1 content was estimated before and at the end of varying periods of complete ureteric ligation in the miniature pig, and also following relief of obstruction by ureteric reimplantation into the urinary bladder.

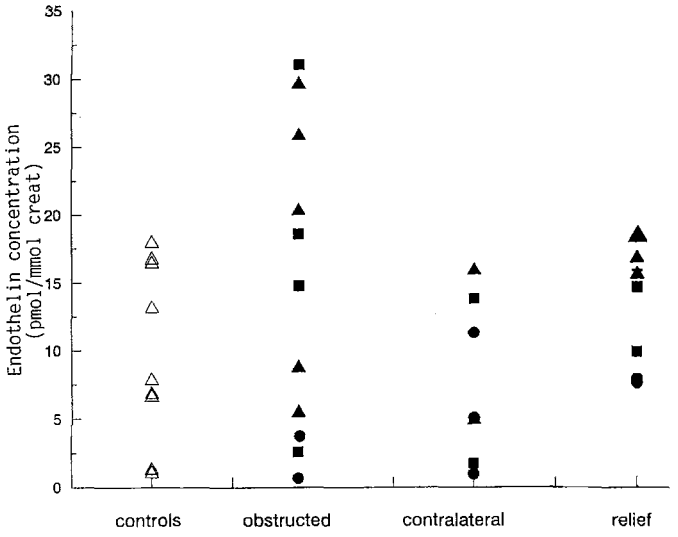

Fig. 1. Urinary endothelin levels in renal obstruction in the pig. △ = controls; ● = 5th day; ■ = 10th day; ▲ = 20th day

Materials and methods

The study material is comprised of 38 ET1 estimations in 23 Gottingen miniature pigs. As these are preliminary data accrued from a larger study and collected retrospectively, not all pigs had urine saved and therefore urinary ET1 results are missing at some points in some animals.

Pigs were obstructed by ureteric ligation for 5, 10 or 20 days, following which the obstruction was relieved by ureteric reimplantation into the urinary bladder. Two months later a contralateral nephrectomy was performed and after a further month the animal was killed. Urine was obtained from the animals at the following intervals: (i) prior to intervention – control urine, (ii) aspirated from the hydronephrosis after 5, 10 and 20 days of complete ureteric obstruction – obstructed urine; (iii) aspirated from the urinary bladder at the same time as reimplantation – contralateral urine; (iv) aspirated from the urinary bladder 3 months following reimplantation – relief urine.

Urinary endothelin was estimated using an Amprep (Amersham International) kit. The procedure involves acidification, extraction of the ET in equilibrated columns and then a radioimmune assay.

Urinary ET is expressed in relation to the urinary creatinine concentration in all cases. Results are given in pmol/ml/mmol creatinine. Statements of significance were based on a paired *t*-test.

Results

Figure 1 demonstrates that there is a broad spread of ET levels in all groups. There was no significant difference between urinary ET excretion in the hydronephrotic urine or the contralateral urine or the relief urine when compared to controls. However, if the hydronephrotic urine from the samples taken from the 10- and 20-day obstructed animals are considered as a subgroup, mean urinary ET excretion is significant when compared to controls. Also examination of contralateral urinary ET excretion is low in those pigs with very high ipsilateral values, indicating that ET may have a local action in the ipsilateral obstructed kidney (Table 1).

Table 1. Longitudinal study of urinary endothelin (ET1) levels in complete urinary obstruction in the miniature pig: selected results

Pig No.	Control	Obstructed	Contra-lateral	Relief
12 (20)	6.8	20.3	13.8	16.8
20 (20)	1.24	29.6	0.96	
19 (10)		> 30	1.74	
6 (10)		25.9		14.7
9 (5)		0.67		7.7

Results expressed in pmol/mmol creatinine; figures in brackets represent duration of obstruction in days

Discussion

Segmental renal scarring has been detected in the miniature pig under the differing circumstances of high pressure sterile urinary reflux and sterile upper urinary tract obstruction [3, 8]. Regarding the distribution of renal scarring, the importance of papillary morphology has been emphasized in reflux but not in obstruction. In this retrospective study we have examined creatinine corrected urinary ET levels in four groups of pigs in consideration of the hypothesis that the pathogenesis of segmental scarring may be primarily vascular in origin.

This work demonstrates that it is possible to isolate ET1 in the urine of the hydronephrosis of completely obstructed pig kidneys. The broad spread of values in the control group limits any firm conclusion, but significant elevation of the urinary ET1 excretion in more prolonged obstruction was clearly identified. Since we believe that the vascular injury sustained by the kidney in complete urinary obstruction is early (as early as 24 h following ligation), we anticipated that urinary ET levels may be elevated in the 5-day obstructed pigs. This was not so.

The low contralateral urinary ET1 levels do indicate that the substance acts locally. Although ET1 was not found in high levels in the 5-day obstructed group, this does not necessarily exclude its production at this stage. It may be that it is only when the obstructive injury is established that significant urinary excretion of ET1 occurs. Certainly it is known that in prolonged renal obstruction a generalised cortical hypoperfusion exists, which is associated with a preglomerular arteriolar stenosis which can persist despite relief of obstruction.

This work has stimulated further investigation into ET as a mediator of vascular damage in obstructive uropathy. Although little is understood regarding the actions of this substance in vivo, its involvement in progressive renal pathology regardless of aetiology cannot be ignored.

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