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Epidermal growth factor in urine after kidney transplantation in humans

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Summary. The urinary excretion of epidermal growth factor (EGF) was studied after kidney transplantation in 15 patients. At the final follow-up (between 38 and 173 days after transplantation) the 9 patients with a successful transplantation excreted EGF in the urine, but the concentration was low (0.13–1.1 nmol/l) compared to individuals with two kidneys (1.0–9.8 nmol/l). In all of the patients the urine creatinine increased prior to the increase in urine EGF. Patients who received cyclosporine A as immunosuppressive treatment excreted little or no EGF during the first month, while patients who received prednisone and azathioprine excreted EGF as early as four days after the transplantation. Our results together with earlier published data suggests that urinary EGF originates from the kidney.

Key words: Epidermal growth factor – Renal transplantation

Introduction

Epidermal growth factor (EGF) is a growth promoting peptide originally isolated from the male mouse submandibular gland [4]. Later the peptide was identified in urine from several species [12, 13], and human EGF has been purified from the urine [5]. In humans EGF was identified first by immunohistochemistry both in the submandibular gland and in Brünner glands [6], and urinary EGF was thought to originate from these glands. Recent studies in rats and mice questioned this concept. The amount of EGF in the urine does not decrease after removal of the submandibular gland, but is halved after removal of one kidney [12, 13]. By immunohistochemistry EGF can be visualized in the tubular cells [14, 16], and cDNA encoding for EGF has been identified in the human kidney [2]. Together these observations suggest that the EGF recovered in the urine is synthesized in the kidneys.

In this paper we investigate the excretion of urinary EGF following kidney transplantation. Our results support the view that EGF is produced in the kidney, and they suggest that quantitation of the excretion of urinary EGF after kidney transplantation may be of clinical relevance.

Materials and methods

Patients

Fifteen patients, 11 men and 4 women aged 20–64 years underwent renal transplantation. All suffered from end stage renal failure. Patient data are summarized in Table 1.

Fourteen patients received cadaveric grafts. One received a graft from a member of the family (patient no. 6).

The transplantation technique employed was described by Ladefoged et al. [7].

The immunosuppressive treatment was either azathioprine and prednisone or cyclosporine A.

In case of graft rejection the prednisone dosage was either increased or the treatment was changed from azathioprine and prednisone to cyclosporine A, eventually supplemented with prednisone.

Rejection episodes were diagnosed by conventional clinical and laboratory observations: malaise, fever, swelling and tenderness of the graft, decreasing creatinine clearence, increasing serum creatinine, decreasing sodium excretion, increasing proteinuria, weight gain, hypertension and change in the pattern of the renogram, the excretory urogram and the renal angiogram [3, 7].

Collection of samples

In all patients the collection of urine started postoperatively. Twentyfour hour urine samples were collected and kept at 5°C for 24 h. The volume was measured and an aliquot of the well mixed 24 hour urine collection was kept at −20°C until analyzed for EGF.

Table 1. Data on 15 patients submitted for renal transplantation

Patient no.	Age (years)	Sex	Diagnosis	Kidneys in situ	Serum creatinine prior to transplantation (mmol/l)
1	60	M	Chronic interstitial nephritis	+	0.58
2	30	F	Congenital malformations	+	1.07
3	64	M	Chronic interstitial nephritis	+	0.51
4	30	M	Diabetes	+	1.17
5	40	F	Polycystic kidneys	+	1.44
6	20	F	Chronic interstitial nephritis	−	1.52
7	33	M	Glomerulonephritis	+	0.87
8	60	M	Renes contracti	+	1.11
9	31	M	Glomerulonephritis	−	1.79
10	48	M	Polycystic kidneys	+	0.94
11	57	M	Glomerulonephritis	+	0.94
12	48	M	Hypertension	+	0.68
13	29	M	Diabetes	+	1.03
14	46	M	Polycystic kidneys	+	1.18
15	41	F	Chronic interstitial nephritis	+	0.57

Table 2. Urinary output of EGF in 9 patients following a successful kidney transplantation

Patient no[a]	Day after transpl.	U-EGF nmol/l	U-EGF nmol/24 h	U-EGF/Creat. nmol/mmol	S.-Creat. mmol/l	Immunosuppr. therapy	Rejection therapy (day after transpl.)
1	38	0.28	0.73	0.12	0.29	CyA	P(7)
2	173	0.20	–	0.02	0.30	CyA	P(30)
3	82	0.13	0.29	0.14	0.25	CyA	P(13)
4	74	0.25	–	0.05	0.17	CyA	–
5	43	0.13	0.14	0.03	0.39	P+A	CyA(6)P(9)
6	84	0.48	0.96	0.20	0.16	P+A	CyA(11)P(56)
7	71	0.60	1.38	0.20	0.15	P+A	P(8)
8	76	0.63	1.35	0.24	0.16	P+A	–
9	61	1.06	1.48	0.17	0.13	P+A	P(8)
Controls $n=25$	Range (median)	1.0–9.8 (3.5)	1.7–12.3 (6.2)	0.13–1.07 (0.53)	0.040–0.140		

[a] The same numbers as employed in Table 1

Abbreviations employed: Creat = creatinine; CyA = cyclosporine A; P+A = prednisone + azathioprine; Transpl. = transplantation; Immunsuppr. = immunosuppressive

In addition 24 h urine samples were collected from 25 healthy volunteers aged 20–65 years.

Laboratory analyses

EGF was determined by radioimmunoassay as previously described [11]. Rabbit-antihuman-EGF (serum 4554-0186) in a final dilution of 1 + 30,000 was employed. Labeled ligand and calibrators were prepared from EGF purified by immunoaffinity chromatography [10]. The peptide was pure as judged by SDS polyacrylamidgel electrophoresis and by aminoterminal analyses. The sensitivity of the assay was 0.04 nmol/l. The interassay precision was 8.5% as calculated from a urinary control ($\bar{x} = 1.6$ nmol/l) run at 15 different occasions during a 3 month period. The intraassay precision was 5.5% ($n = 36$, $\bar{x} = 1.7$ nmol/l).

Results

Fifteen patients aged 20–64 years underwent kidney transplantation. The transplantation was successful for 9 patients (nos. 1–9, Tables 1 and 2), and in all of these, EGF could be quantitated in the urine at the final examination. The concentration (nmol/l) and the output (nmol/24 h) of EGF varied, but was in each case low compared to the group of controls (Table 2).

The patients were divided into 2 groups according to the immunosuppressive treatment given. In the first group cyclosporine A was given as immunosuppressive drug (patient nos. 1–4, 10 and 11). In this group serum-creatinine remained high, the urinary excretion of

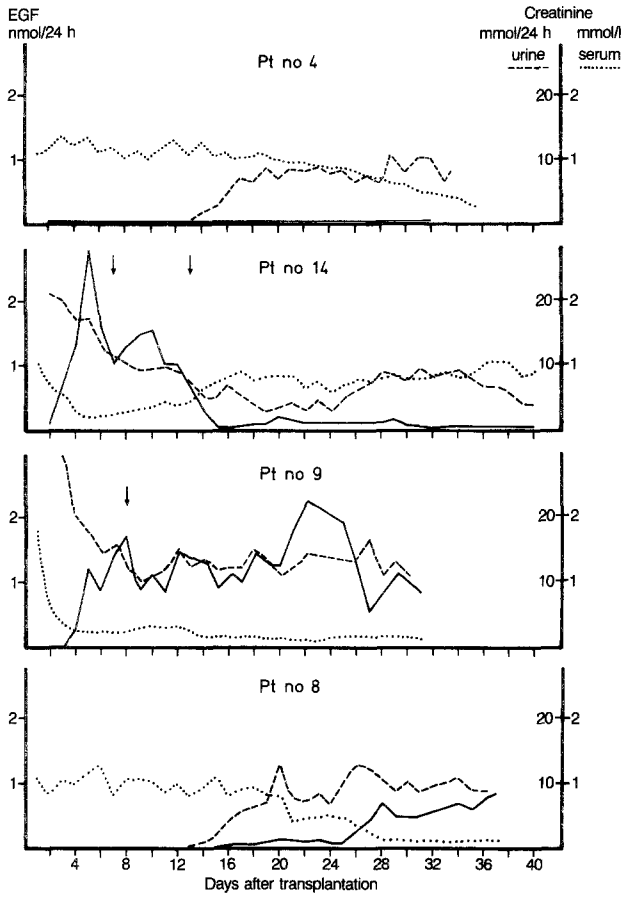

Fig. 1. Urinary output of epidermal growth factor (EGF) following kidney transplantation. The urinary output of EGF (——) is compared to the output of creatinine (– – –) and to serum creatinine (·····), in a patient receibing cyclosporine A is immunosuppressive drug (no. 4), a patient in whom the transplanted kidney was removed after 45 days (no. 14), and two patients receiving prednisone and azathioprene as immunosuppressive drug (nos. 8 and 9). ↓ Indicates the start of rejection therapy

creatinine remained low, and the urinary output of EGF was undetectable during the first month of treatment as exemplified in Fig. 1 (patient no. 4). The same pattern occured in 2 patients (patient nos. 5 and 13), who both received cyclosporine A as additional therapy within the first 10 days after transplantation.

The second group received prednisone and azathioprine as immunosuppression (patient nos. 6–9, 12, 14 and 15). All the patients in this group excreted EGF in the urine during the first month after transplantation, and in all 7 patients the initial excretion of creatinine in urine preceded the excretion of EGF.

In three of the patients (nos. 12, 14, 15) the final outcome was unsuccessful. In these patients the excretion of EGF started within the first week, and the 24 h output remained above 1 nmol during the initial phase. In patient no. 15 the urinary excretion of EGF ceased at the same time as the excretion of creatinine decreased and the serum-creatinine increased. The patient had the graft removed on day 12. In patient no. 14 (Fig. 1) an initial high level of EGF in urine decreased at the time of rejection, but as the patient responded positively to rejection therapy, the level of EGF in the urine increased. After a new rejection episode which was treated with cyclosporine A, little or no EGF was found in the urine. The patient had the graft removed on day 45. In patient no. 12 the initial course was comparable to patient nos. 14 and 15, but the output of urinary-EGF ceased several days prior to the increase in S-creatinine. At the end of the study the patient was on hemodialysis with the kidney left in situ.

Patient nos. 9 (Fig. 1) and 7 showed an almost identical pattern. The output of urinary EGF started at day 5, and the excretion remained around or above 1 nmol/24 h.

The remaining 2 patients both suffered from complicating diseases. Patient no. 8 (Fig. 1) had cardiac failure. In this patient urinary excretion of creatinine preceded the excretion of EGF by more than a week. The other patient (no. 6) had a necrotic ureter. After ureteric reimplantation the excretion of EGF slowly reached values of around 1 nmol/24 h.

Discussion

The present paper suggests that urinary EGF is derived from the kidney rather than from the circulation, and it shows that the transplanted kidney restores the ability to synthesize EGF.

The circulatory origin of urinary EGF has been debated for several years. If urinary EGF is derived from the circulation one would expect the pattern of excretion for creatinine and EGF to be the same following kidney transplantation. We found this pattern to differ. The initial excretion of creatinine always precedes the output of EGF. If urinary EGFs is derived from the circulation, one would expect plasma-EGF to be increased prior to kidney transplantation. A recent paper shows no increase in plasma-EGF in patients with chronic renal failure [8].

The concept that urinary EGF is synthesized by the kidney is directly supported by our finding that humans with one functioning kidney excreted no more than half the amount of EGF excretes by individuals with two kidneys. The same has been observed in rats [3]. Two additional lines of study support that EGF is synthesized in the kidney. By immunohistochemistry EGF has been localized in the distal tubules, and recently cDNA encoding for the EGF precursor has been isolated from the human kidney [2, 14, 15].

The above data taken together make us believe that urinary-EGF is derived from the kidney and not from the circulation. Accepting urinary EGF to be derived from the tubular cells of the kidney poses the next question: Will quantitation of urinary EGF reflect the function of the tubular cells. The present study does not answer this question, but the differences observed in the excretion of EGF during different types of immunosuppressive treatment are of interest. Patients treated with cyclosporine A excretes little if any EGF during the first month following kidney transplantation, and the output at the final follow-up is low compared to the output in patients receiving prednisone and azathioprine. The reason for this phonomenon may well reflect the nephrotoxity of cyclosporine A [1]. Patients treated with cyclosporine A after kidney transplantation have a decreased renal blood flow and an increased renal vascular resistance, even though the graft function judged by serum-creatinine appears excellent [9]. The histological lesions induced by cyclosporine A involve a variable degree of tubulu-interstitial injury accompanied by focal glumerular sclerosis [1, 9]. It is thus possible that cyclosporine A directly effects the cells that produce EGF.

In conclusion the present paper supports a renal origin of human urinary EGF. Further studies are needed to evaluate whether quantitation of this peptide will be useful for predicting rejection periods and/or the final outcome of kidney transplantation.

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