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José A. Amado
Angel L. M. De-Francisco
Manuel A. Botana
Carlos Pesquera
José A. Vázquez-de-Prada
Manuel Arias

Influence of kidney or heart transplantation on the urinary excretion of epidermal growth factor

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J. A. Amado (✉) · M. A. Botana
C. Pesquera
Endocrinology Section, Hospital Universitario Marqués de Valdecilla, University of Cantabria, E-39010 Santander, Spain

A. L. M. De-Francisco · M. Arias
Nephrology Service, Hospital Universitario Marqués de Valdecilla, University of Cantabria, E-39010 Santander, Spain

J. A. Vázquez-de-Prada
Cardiology Service, Hospital Universitario Marqués de Valdecilla, University of Cantabria, E-39010 Santander, Spain

Abstract We studied urinary epidermal growth factor (uEGF) in kidney transplant patients with normal and elevated serum creatinine, in cardiac transplant patients with normal serum creatinine, and in patients with chronic renal failure. Patients with chronic renal failure had the lowest uEGF levels. uEGF was reduced in normally functioning kidney transplant patients. If the kidney graft was failing, this reduction was more marked. Cardiac transplant patients had normal uEGF. The type of immunosuppressive therapy did not influence the uEGF excretion. Kidney function and kidney tissue mass appeared to be the most important factors in uEGF excretion.

Key words Epidermal growth factor, in urine, transplant patients
Urinary excretion, epidermal growth factor, in transplant patients

Introduction

Epidermal growth factor (EGF), or β-urogastrone, is a mitogenic 53 amino acid polypeptide found in higher concentrations in urine than in any other body fluid [9]. Though initially it was thought that urinary EGF was derived from plasma via glomerular filtration, recent data suggest that almost all urinary EGF is derived from kidney synthesis [1]. EGF synthesis has been immunolocalized to the apical portion of the cells lining the thick, ascending limb of Henle and the distal, convoluted tubule [2]. Its physiological role is obscure, but EGF may be important in maintaining the integrity of tubules and urothelium and in modulating distal nephron transport processes [2]. Furthermore, i.v. exogenous EGF may accelerate the recovery phase of experimental acute renal failure [3].

Urinary immunoreactive EGF (uEGF) levels are low in patients with renal failure, and there is a significant inverse correlation between uEGF and serum creatinine concentrations [6]. We could find only one report about uEGF levels after kidney transplantation, but eight out of nine patients studied had increased serum creatinine levels, so that the low uEGF levels found could be related to renal insufficiency [4]. Furthermore, it is not known if uEGF levels are normalized in patients with long-term normally functioning kidney grafts or if the different types of immunosuppressive therapy influence urinary EGF excretion.

The aim of this study was to assess the clinical relevance of measuring uEGF levels in a large group of kidney transplant patients with different degrees of renal function and the factor(s) responsible for their alterations. We also measured uEGF levels in a group of heart transplant patients with normal kidney function to evaluate the effect of immunosuppressive therapy on normal kidneys.

Table 1 Serum creatinine (SCR), urinary EGF, and EGF/CR in control and chronic renal failure patients of both sexes

Group	*N*	Age (years)	SCR[a] (μmol/l)	uEGF[b] (nmol/l)	EGF/CR[c] (nmol/mmol)	*P* (significance)
Female controls	53	46.3 ± 12.9 (25–73)	79.5 ± 17.7	3.57 ± 2.74	0.49 ± 0.25	not significant
Male controls	44	45.5 ± 14.6 (25–80)	88.4 ± 8.8	5.46 ± 4.44	0.41 ± 0.22	
Female controls	44	49.9 ± 10.9 (34–73)	79.5 ± 8.8	3.29 ± 2.72	0.49 ± 0.28	< 0.0001
Female controls with chronic renal failure	10	53.2 ± 12.8 (34–69)	671.8 ± 362.4	0.1 ± 0.22	0.03 ± 0.05	
Male controls	17	61.5 ± 8.1 (50–80)	88.4 ± 8.8	2.8 ± 1.79	0.32 ± 0.2	< 0.0001
Male controls with chronic renal failure	10	60.9 ± 6 (49–69)	618.8 ± 371.3	0.05 ± 0.05	0.01 ± 0.01	

[a] To convert to mg/dl, multiply by 0.01131
[b] To convert to ng/ml, multiply by 5.988
[c] To convert to ng EGF/mg creatinine, multiply by 52.694

Materials and methods

We collected fasting urinary samples from the following groups of patients:

1. 44 healthy male controls with serum creatinine levels less than 106.1 μmol/l (1.2 mg/dl)

2. 69 healthy female controls with serum creatinine levels less than 97.2 μmol/l (1.1 mg(dl)

3. 42 male renal transplant patients with stable normal serum creatinine levels

4. 21 female renal transplant patients with stable normal serum creatinine levels

5. 25 male renal transplant patients with varying degrees of renal failure

6. 12 female renal transplant patients with varying degrees of renal failure

7. 11 male controls with chronic renal failure

8. 10 female controls with chronic renal failure

9. 21 male cardiac transplant patients under immunosuppression with normal kidney function

Before statistical comparisons were made, controls and patients were classified according to their sex and matched for age, since it has been reported that uEGF is higher in females than in males and that uEGF decreases with age [5, 12]. Immunosuppression in the renal and cardiac transplant patients was achieved with cyclosporin A (CyA) plus prednisone, azathioprine plus prednisone, or CyA plus azathioprine and prednisone.

Urinary EGF was measured by direct radioimmunoassay, using a kit furnished by Biomedical Technologies (Stoughton, Mass., USA). In this assay ^{125}I-labelled human EGF competes with unlabelled human EGF for rabbit anti-human EGF antibody-binding sites. The antigen-antibody complex is separated by the addition of goat anti-rabbit IgG plus polyethylene glycol. In our hands, inter- and intra-assay coefficients of variation were 8.6% and 5.2%, respectively. Crossreactivity with α-transforming growth factor was negligible. Serum and urinary creatinine were measured by routine automatic methods (Beckman Creatinine Analyzer 2). Kidney volume (KV) in renal transplant patients was studied by echography within a period of no longer than 3 months in relation to the uEFG determination. Kidney volume was determined using the equation: KV = anteroposterior diameter × longitudinal diameter × transverse diameter × 0.58 (correction factor for ellipsoid volume). This method was validated at our institution via echography to measure renal graft volume before nephrectomy, comparing it with the real anatomical volume. Mean difference observed were ± 9% in twenty cases.

We used Stata software (Computing Resource Center, Los Angeles, Calif., USA) for the statistical analyses. The results are given as mean ± SD. Spearman's correlation coefficient (r_s) was calculated with linear regression analysis. Statistical comparisons were made with the Kruskal-Wallis and Mann-Whitney tests when appropriate.

Results

Table 1 shows the age, serum creatinine (SCR), urinary EGF expressed as a concentration (uEGF) and urinary EGF expressed as a function of urinary creatinine (EGF/CR) in the male and female control groups with and without chronic renal failure; it also shows the *P* values for EGF/CR comparisons between groups. There was no statistical difference between the male and female control groups, although the EGF/CR of men tended to be lower than that of women ($P < 0.13$). For the entire group of normal subjects, a significant inverse correlation ($r_s = 0.32$) was found between EGF/CR and SCR, but not for male or female groups alone. Control women ($P < 0.0001$) and men ($P < 0.0001$) with chronic renal failure had a very low urinary excretion of EGF.

Table 2 shows the age, SCR, uEGF and EGF/CR in female controls, transplanted females with and without renal failure, male controls, transplanted males with and without renal failure, and male cardiac transplant pa-

Table 2 Serum creatinine (SCR), urinary EGF, and EGF/CR in control and transplant groups

Group	*N*	Age (years)	SCR[a] (μmol/l)	uEGF[b] (nmol/l)	EGF/CR[c] (nmol/mmol)	*P* (significance)
Female controls	69	40.6 ± 15.3 (20–73)	70.7 ± 17.7	4.51 ± 3.5	0.57 ± 0.3	
Transplanted females with stable normal SCR	21	39.8 ± 13.6 (24–69)	79.6 ± 17.7	1.67 ± 1.5	0.29 ± 0.1	< 0.0001
Transplanted females with renal failure	12	36.1 ± 11.8 (21–61)	274 ± 229.8	0.58 ± 0.5	0.14 ± 0.1	< 0.0005
Male controls	44	45.5 ± 14.6 (25–80)	88.4 ± 8.8	5.46 ± 4.4	0.41 ± 0.2	
Transplanted males with stable normal SCR	42	45 ± 13.8 (21–70)	88.4 ± 8.8	2.42 ± 1.6	0.32 ± 0.2	< 0.05
Transplanted males with renal failure	25	36.1 ± 10.1 (22–58)	397.8 ± 318.2	0.82 ± 1.9	0.1 ± 0.1	
Male cardiac transplant patients	21	46.5 ± 10.5 (27–62)	88.4 ± 8.8	4.24 ± 2.1	0.4 ± 0.2	< 0.0001

[a] To convert to mg/dl, multiply by 0.01131
[b] To convert to ng/ml, multiply by 5.988
[c] To convert to ng EGF/mg creatinine, multiply by 52.694
[d] There is no difference C. M. and C. T. M.

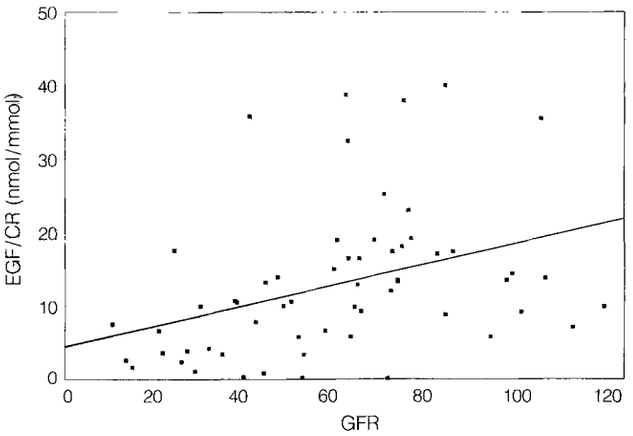

Fig. 1 Correlation between EGF/Cr and GFR (creatinine clearance) in kidney transplant patients. $P < 0.01$, $r_s = 0.37$

tients; it also shows the *P* values for comparisons of the EGF/CR in different groups. EGF/CR in transplanted women with stable normal SCR was decreased to about half, that of healthy control women ($P < 0.0001$) and it was even lower in transplanted women with renal failure ($P < 0.0005$; transplanted females with vs without renal failure). Transplanted males with stable normal SCR had a lower EGF/CR than healthy male controls ($P < 0.05$), and transplanted males with renal failure also had a lower EGF/CR than those without renal failure ($P < 0.0001$).

In all renal transplant patients, the EGF/CR was negatively correlated with plasma creatinine ($r_s = -0.348$, $P < 0.01$) and positively with GFR measured by creatinine clearance ($r_s = 0.368$, $P < 0.01$; Fig. 1).

The post-transplant period in men was 32.8 ± 40.9 months and in women it was 20 ± 27.1 months ($P < 0.06$). There was no correlation between EGF/CR and the period after kidney transplantation either in men ($r_s = 0.2$, P = NS) or in women ($r_s = 0.07$, P = NS), nor between donor age and EGF levels.

There was no correlation between kidney mass volume as assessed by echography and uEGF concentrations ($r_s = 0.16$; P = NS). Furthermore, no significant differences were found in kidney volume between male and female renal transplant patients with stable, normal SCR 199 ± 48) and those with renal failure (222 ± 74; $P = 0.147$).

There was no statistical difference between healthy controls and male cardiac transplant patients with regard to EGF/CR.

The type of immunosuppressive therapy did not induce any differences in the EGF/CR in the renal transplant and cardiac transplant groups. (There were no differences in EGF/CR levels when comparing the three different immunosuppressive regimens).

Discussion

Other authors have found that normal males have lower levels of uEGF than females [5, 10]. We also found this trend, although it did not reach statistical significance ($P < 0.13$). For the whole control group, a significant inverse correlation was found between EGF/CR and SCR, indicating that even within the normal SCR range there is a significant influence of kidney function on uEGF excretion. In patients with chronic renal failure,

there was a marked reduction in uEGF, as previously reported [6].

As far as we are aware, there has been only one previous report about urinary excretion of EGF after kidney transplantation [6], in which most patients (8/9) had renal failure. We studied uEGF excretion in kidney transplant patients with normal and elevated SCR levels. We found that uEGF was reduced in patients with normal SCR levels, who had only one transplanted kidney. This reduction was comparatively more marked in female than in male patients. If the graft was failing, the reduction in uEGF was considerably greater. It has been reported that there is a functional correlation between glomerular filtration and tubular excretion of EGF [1]; we think that kidney tissue mass is another important factor in uEGF excretion, as these data and previous reports in rats [8] show. Moreover, Mattila et al. reported that after unilateral nephrectomy the urinary EGF concentration fell by approximately 50% [7].

In our group of female kidney transplant patients, uEGF was halved; it was not reduced as much in the male group. This could be due to the fact that the post-transplant period in males was longer than that in women. This raises the possibility that, with time, graft hypertrophy can induce an increment in the tubular synthesis of EGF, although we found no correlation between EGF/CR and time after transplantation.

Cyclosporin A nephrotoxicity is observed in clinical practice. The pathogenesis remains speculative: a tubular toxic effect has been suggested [11]. The mechanism of the tubular injury is uncertain; it may be due to a direct effect of increased serum CyA levels with deposition in tubular cells. Since it is difficult to study CyA-induced nephrotoxicity in human renal transplantation where rejection and the existence of only one kidney can complicate the findings, we selected cardiac transplant recipients to investigate the possible effect of CyA on uEGF. Yet, our cardiac transplant patients, under CyA therapy, had normal levels of uEGF, evidence against a possible effect of immunosuppressive therapy on uEGF levels. We did not find any differences in uEGF when comparing several types of immunosuppressant agents either in cardiac or in renal transplant patients.

We conclude that kidney function and kidney tissue mass are the most important factors in uEGF excretion. When administered within therapeutic doses, a CyA tubular effect on urinary excretion of epidermal growth factor was not observed.

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