doses of methotrexate combined with other cytotoxic drugs. Seven out of 10 patients with localised adult sarcomas were free of disease for a mean interval of 7.3 months. This is comparable with the results of Townsend *et al*,¹⁷ who used adjuvant chemotherapy with adriamycin and high-dose methotrexate given intravenously and found that 68% of patients with adult sarcoma were disease free after a mean follow-up period of 9.3 months. Of four patients with small-cell carcinoma of the lung treated with the present schedule, two showed objective response, a rate similar to that reported by Gilby *et al*,¹⁸ who used a similar combination of drugs including high-dose intravenous methotrexate (200 mg over 24 hours), vincristine, adriamycin, cyclophosphamide, and prednisolone combined with radiotherapy.

We have shown that our regimen may be safely administered on an outpatient basis with the co-operation of a home-visiting nursing service. The plasma concentrations achieved and the bioavailability of methotrexate given by mouth with this method are comparable to those after intravenous administration, and extrapolation from in-vitro studies on malignant cells indicates that the plasma concentrations achieved are adequate for an antitumour effect. Patient tolerance was good and the cost of treatment considerably reduced. These data provide a sound pharmacokinetic basis for further clinical evaluation of modified high-dose oral methotrexate regimens.

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Urinary excretion of factor VIII after renal transplantation

Z M RUGGERI, Y B GORDON, NICOLE ARDAILLOU, MARIE-JOSÉ LARRIEU, A TARANTINO, RAFFAELLA COPPOLA, C PONTICELLI, P M MANNUCCI

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Summary and conclusions

The urinary excretion of factor-VIII-related antigen (VIII_{IR}Ag) was measured in 72 patients with kidney transplants and compared with that of two end-products of fibrin-fibrinogen lysis (fragments D and E) to assess their usefulness in monitoring the onset of rejection episodes. Specific and sensitive radioimmunoassays were used to measure the three proteins. Unconcentrated urine samples of 24-hour collections were obtained from 20 healthy subjects, 48 patients with stable transplants,

and 24 patients with recent transplants serially followed up from the day of transplantation. Factor VIII_{IR}Ag and fragments E and D were not detectable in the urine from healthy subjects but were present in 39%, 60%, and 100% respectively of samples from patients with stable transplants. During 33 acute rejection episodes in 19 patients with recent transplants factor VIII_{IR}Ag and fragments E and D were significantly increased above the values observed in patients with stable transplants in 82%, 73%, and 64% of samples respectively; in patients with recent transplants showing no clinical sign of rejection increased excretion of these proteins was observed in 11%, 26%, and 22% of samples respectively. The presence of factor VIII_{IR}Ag in urine from patients with kidney allografts suggests that endothelial cell-factor VIII-platelet interactions might play a key part in the pathogenesis of acute rejection.

The results suggest that the assay of factor VIII_{IR}Ag in urine is more useful than assays of fragments D and E as a corroborative index of transplant rejection.

Introduction

Studies on man^{1,2} and animals^{3,4} have shown the existence of local activation of the coagulation pathway during acute and hyperacute rejection of renal allografts. In addition, urinary excretion of fibrin-fibrinogen degradation products (FDP), which are considered to be an index of secondary activation of the fibrinolytic system after fibrin deposition, was shown to be increased during acute rejection and its measurement thought to be of predictive diagnostic value.⁵⁻¹¹

In the sequence of pathological reactions leading to transplant

Haemophilia and Thrombosis Centre "Angelo Bianchi Bonomi" and Third Department of Internal Medicine, University of Milan and Policlinico Hospital, Milan

Z M RUGGERI, MD, senior registrar
RAFFAELLA COPPOLA, BSc, research assistant
P M MANNUCCI, MD, professor

Department of Reproductive Physiology, St Bartholomew's Hospital, London

Y B GORDON, MD, MRCOG, consultant

Institut de Pathologie Cellulaire (Inserm U143), Hôpital de Bicêtre, Paris

NICOLE ARDAILLOU, MD, senior research assistant
MARIE-JOSÉ LARRIEU, MD, professor

Division of Nephrology and Dialysis, Policlinico Hospital, Milan
A TARANTINO, MD, senior registrar
C PONTICELLI, MD, consultant

rejection, however, the earlier events involve immunologically mediated lesions of the vascular endothelium, which may then be followed by platelet adhesion and aggregation, fibrin deposition, and fibrinolysis in the kidney vessels.¹² Thus a marker of endothelial damage might be a more selective index than FDP of the reactions leading to rejection episodes. In the past few years factor-VIII-related antigen (VIIRAg) has been shown to be synthesised and released from endothelial cells,^{13 14} and its measurement has been proposed as a useful tool to monitor the occurrence of endothelial injury in renal and other diseases.^{15 16} We therefore decided to use a sensitive radioimmunological technique to see whether factor VIIRAg was excreted in the urine of patients with renal allografts and if its excretion might be related to the appearance of rejection episodes. Two fibrin-fibrinogen fragments (D and E), which are end-products of fibrin-fibrinogen lysis, were also measured by radioimmunoassay and the validity of these different variables in monitoring the appearance of transplant rejection evaluated.

Present study

SUBJECTS

Seventy-two patients with renal allografts were studied. They were divided into two groups.

The *stable transplant group* comprised 48 patients (four with transplants from live donors, and 44 with cadaver kidneys) who had had their operations three months to six years before the investigation and had good renal function. Fifteen were studied on one occasion, and 33 on two to 10 random occasions over three months.

The *recent transplant group* comprised 24 patients (one with a live-donor transplant) who were studied prospectively from the day of transplantation. They were studied daily throughout the postoperative inpatient period and then at each outpatient visit for up to three months. Rejection episodes were diagnosed, and immunosuppressive treatment instituted when the serum creatinine concentration rose by at least 30% with no other possible explanation. Increased body temperature, tenderness over the allograft, and decreased urinary output were regarded as supportive but not essential evidence of the diagnosis. Rejection was diagnosed at the renal unit without knowledge of the results of the haemostasis studies carried out at the haemophilia and thrombosis centre. Immunosuppressive treatment was given according to a protocol described.¹⁷

Twenty healthy volunteers served as controls. Six were studied for six consecutive days, and 14 only once.

METHODS

Urine collection—Twenty-four-hour urine samples, collected at room temperature without enzyme inhibitors or pH correction, were centrifuged at 5000 g and the supernatants stored at -20°C in plastic tubes until assayed. Samples were allocated random numbers at the renal unit and assayed at the haemophilia and thrombosis centre without knowledge of the clinical and laboratory findings in each case.

Immunoradiometric assay of factor VIIRAg—Factor VIIRAg was measured by a two-site immunoradiometric assay, as described.¹⁸ Polystyrene tubes were coated with a specific anti-factor-VIII rabbit antiserum and the samples to be tested incubated in the coated tubes (first stage); factor VIIRAg, if present, was bound by the antibody in solid phase and could then be quantified by adding specific ¹²⁵I-labelled anti-factor-VIIRAg IgG (second stage). The amount of radioactivity bound to the tubes was proportional to the concentration of factor VIIRAg in the sample. The minimum detectable concentration corresponded to 0.1 U/l (one unit being the amount present in one ml of average normal plasma). Results in urine samples were expressed as units excreted in 24 hours.

Radioimmunoassay of fragments D and E—Fragments D and E were measured as described¹⁹ by classical radioimmunoassays with purified fragments D and E labelled with ¹²⁵I and specific antisera raised in rabbits. Results in urine samples were expressed as mg excreted in 24 hours.

Total urinary protein and other assays—Total urinary protein was measured by the biuret method. Other laboratory assays were performed according to routine techniques.

Statistical—Since factor VIIRAg and fragment E were unmeasurable in some urine samples non-parametric distribution-free

tests were adopted for statistical evaluation of the results.²⁰ Increased excretion was defined as any value exceeding the 95% confidence limit of the 90th percentile calculated from the ranked results in patients with stable transplants. Correlation was evaluated with Kendall's rank correlation coefficient (τ) test. The corrected χ^2 test was used to compare frequencies.

Results

NORMAL SUBJECTS

Concentrations of factor VIIRAg and fragments D and E were below the minimum detectable (0.1 U/24 h for factor VIIRAg, and 0.01 mg/24 h for fragments D and E) in all 50 urine samples from the 20 controls.

STABLE TRANSPLANT GROUP

In contrast to the findings in normal subjects, fragment D was detectable in all 92 urine samples from the 48 patients with stable transplants. Fragment E was present in 55 samples (60%), and factor VIIRAg in only 36 (39%) (fig 1). No correlation was found between the excretion of factor VIIRAg and that of fragment D or between the excretion of fragment E and that of fragment D ($P > 0.1$). A significant correlation was observed, however, between the excretion of factor VIIRAg and that of fragment E ($P < 0.01$). The arbitrary limits for the definition of increased excretion of factor VIIRAg, fragment D, and fragment E, calculated from the ranked results in these patients, were 1.28 U/24 h, 0.38 mg/24 h, and 0.24 mg/24 h respectively (fig 1). Increased excretion of the three proteins was thus defined as daily excretion exceeding these limits.

FIG 1—Urinary excretion of factor VIIRAg and fragments D and E in patients with stable transplants. Horizontal lines represent 95% confidence limits of 90th percentile of each distribution of values (see text).

Statistical analysis (Kendall's rank correlation test)—Factor VIIRAg v fragment D: $\tau = 0.098$; $P > 0.1$. Factor VIIRAg v fragment E: $\tau = 0.227$; $P < 0.01$. Fragment D v fragment E: $\tau = 0.043$; $P > 0.1$.

RECENT TRANSPLANT GROUP

The 24 patients in the recent transplant group were studied for 90 days from the day of operation. Thirteen grafts were still functioning at the end of the observation period, but 11 had to be removed because of irreversible rejection (5 cases), accelerated rejection (2), septic arteritis (2), tubular necrosis (1), and acute pyelonephritis (1). Fig 2 shows the patterns of excretion of factor VIIRAg and of fragments D and E in the early postoperative period in 14 patients whose transplants functioned without early evidence of rejection. Increased excretion of

all three proteins was present until the onset of the diuretic phase, but factor VIIIIRAg showed a tendency to return to values characteristic of the stable transplant group before fragments D and E. Complications such as macroscopic haematuria (one case) and urinary fistula (three cases) were associated with persistently increased excretion of the three proteins.

A total of 361 urine samples were obtained from the 24 patients with recent transplants at a time when renal function was satisfactory and there was no clinical sign of rejection. Excretion of factor VIIIIRAg was found to be increased in 40 of the samples (11%), fragment D in 78 (22%), and fragment E in 95 (26%; false-positive result) (see table).

Acute rejection episodes—Thirty-three acute rejection episodes were diagnosed and immunosuppressive treatment instituted in 19 patients (one patient had four episodes, two patients had three, six patients had two, and 11 patients had one). Only six of the 33 episodes were characterised by an isolated, short-lasting increase in serum creatinine concentrations of dubious significance. In the remaining 27 episodes the clinical and laboratory evidence of rejection (see above) was clear-cut and unequivocal. On the day of clinical diagnosis of acute rejection increased urinary excretion of factor VIIIIRAg was observed in 27 out of 33 samples (82%), fragment E in 24 (73%), and fragment D in 21 (64%) (see fig 3 and table). Excretion of factor VIIIIRAg was significantly correlated with that of fragments D and E ($P < 0.01$); the correlation between urinary factor VIIIIRAg and total protein excretion

Incidences of increased excretion of factor VIIIIRAg and fragments D and E in urine samples (A) from all 48 patients in stable transplant group, (B) from all 24 patients in recent transplant group at time of satisfactory function with no evidence of rejection, and (C) in 19 patients in recent transplant group during 33 episodes of rejection. (Corrected χ^2 test used for analysis)

	No (%) of samples showing increased excretion of:		
	Factor VIIIIRAg	Fragment D	Fragment E
(A) Stable transplant group (No of samples = 92)	3 (3)	3 (3)	3 (3)
(B) Recent transplant group— no evidence of rejection (No of samples = 361)	40 (11)	78 (22)	95 (26)
(C) Recent transplant group— during rejection (No of samples = 33)	27 (82)	21 (64)	24 (73)

Factor VIIIIRAg—A v B: $\chi^2 = 4.00$; $0.025 < P < 0.05$. B v C: $\chi^2 = 102.25$; $P < 0.001$.
Fragment D—A v B: $\chi^2 = 26.63$; $P < 0.001$. B v C: $\chi^2 = 26.20$; $P < 0.001$. Fragment E—A v B: $\chi^2 = 42.71$; $P < 0.001$. B v C: $\chi^2 = 28.73$; $P < 0.001$.

FIG 2—Urinary excretion of factor VIIIIRAg and fragments D and E in early postoperative period in 14 patients with uncomplicated early postoperative course. Points are means $\pm$ SE. Horizontal lines represent arbitrary upper limits of excretion in patients with stable transplants (see fig 1).

FIG 4—Urinary excretion of factor VIIIIRAg and fragments D and E in representative patient from day of operation. Horizontal lines represent arbitrary upper limits of excretion in patients with stable transplants (see fig 1). R=Rejection episode. T=Transplant removal.

FIG 3—Urinary excretion of factor VIIIIRAg and fragments D and E during acute rejection of transplanted kidneys. Horizontal lines represent arbitrary upper limits of excretion in patients with stable transplants (see fig 1).

Statistical analysis (Kendall's rank correlation test)—
Factor VIIIIRAg v fragment D: $\tau = 0.419$; $P < 0.01$.
Factor VIIIIRAg v fragment E: $\tau = 0.532$; $P < 0.01$.
Fragment D v fragment E: $\tau = 0.406$; $P < 0.01$.

was of borderline significance ($\tau = 0.354$; $0.01 < P < 0.05$). Nine rejection episodes occurred in the early postoperative period when excretion of the three proteins was already at high levels owing to the operation; a further increase in factor VIIIIRAg occurred in all nine patients, whereas the excretion of fragments D and E increased in only seven. When anti-rejection treatment was successful the urinary excretion of the three proteins decreased in 1-16 days, whereas irreversible rejection was always associated with persistently raised excretion (fig 4).

Discussion

This is the first documented evidence that factor VIIIIRAg can be detected in the urine of patients with kidney allografts, particularly during acute rejection episodes. The appearance of factor VIIIIRAg in urine might be explained by passive leakage of the molecule through the abnormal basal membrane, since increased glomerular permeability with changes in the selectivity of proteinuria are constant features of rejection episodes.²¹ In our patients, however, a poor correlation was found between urinary factor VIIIIRAg and total protein output; moreover, patients

with totally unselective proteinuria but no clinical evidence of rejection had absent or low excretion of factor VIIIIRAg. These observations, although not conclusive, suggest that the presence of factor VIIIIRAg in urine is not dependent solely on passive filtration through diseased glomeruli. Immunologically mediated lesions of vascular endothelium might be responsible for local release of factor VIIIIRAg, followed by glomerular deposition and filtration through the altered basal membrane. This view agrees with the observation that endothelial factor VIIIIRAg is increased on immunofluorescence in biopsy specimens obtained during rejection of transplanted kidneys^{22 23} and with the finding of high plasma concentrations of factor VIIIIRAg in patients with severe glomerulonephritis and extensive endothelial damage in affected kidneys.¹⁵ Moreover, during experimental hyperacute rejection in primates a pronounced increase in factor VIII has been observed in the venous effluent from the graft.⁴ Thus urinary excretion of factor VIIIIRAg might be considered to be a marker of endothelial injury in the transplanted kidney and its measurement an index of the early events responsible for rejection.

In only six out of 33 cases was there a discrepancy between the clinical diagnosis of acute rejection and the urinary excretion of factor VIIIIRAg, which was not increased (false-negative result). All these cases were clinically mild and characterised by a modest, isolated increase in serum creatinine concentrations, with no other corroborative sign of rejection; kidney function returned to normal within one day after steroid treatment. Whether in these patients the clinical diagnosis of acute rejection was incorrect or functional impairment of the transplanted kidney occurred through mechanisms not including the release of factor VIII from endothelial cells is unknown. Several patients with kidney allografts, particularly patients in the recent transplant group, showed urinary excretion of minute amounts of factor VIIIIRAg without any clinical evidence of rejection. This might be either a non-specific phenomenon or the expression of low-grade endothelial damage not resulting in the sequence of reactions leading to graft rejection.

The measurement of factor VIIIIRAg in urine appears to be more discriminating than that of other variables of haemostasis such as fragment D or E for monitoring the occurrence of rejection episodes. Other studies have shown that the excretion of fibrin-fibrinogen-related material is not uncommon in patients with transplants even in the absence of clinically evident rejection.^{6 9-11} It was also shown that fibrin-fibrinogen derivatives of high molecular weight constitute the predominant portion of urinary FDP detected in these cases²⁴ and that clottable fibrinogen is occasionally excreted in almost all patients with kidney transplants showing no clinical evidence of rejection.²⁵ Our results in patients with stable transplants—namely, the detection of fragment D in all urine samples and fragment E in 60% of them—agree with these observations, since the radioimmunoassay of fragment D is more affected than that of fragment E by cross-reactivity with intact fibrinogen

and its early breakdown products.¹⁹ During acute rejection the excretion of both fragments was increased in most patients, but less consistently than that of factor VIIIIRAg. Increased excretion of FDP was also less specific, since in patients with recent transplants episodes of increased excretion, not associated with clinical evidence of rejection, were half as frequent for factor VIIIIRAg than for fragments D and E.

In conclusion our results show that during acute rejection of renal allograft there is concomitant urinary excretion of factor VIIIIRAg. This may be the expression of endothelial damage in the transplanted kidney and supports the view that endothelial cell-factor VIII-platelet interactions play a key part in initiating the events leading to activation of the haemostatic mechanisms and graft rejection. The diagnostic usefulness of this new test is limited by its technical complexity and lack of complete specificity; however, the fewer false-positive and false-negative results makes it preferable to assays of urinary FDP as an index of haemostasis corroborating the diagnosis of rejection made on the basis of the serum creatinine concentration and other clinical signs.

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ONE HUNDRED YEARS AGO At the meeting of the Académie de Médecine on January 18th, M Broca presented the upper portion of the skull of a boy, who, notwithstanding the terrible injury was still alive and well. The patient, a shepherd boy, had always enjoyed good health, but from his birth had presented the peculiarity that, if he once went to sleep in the evening, it was impossible to awaken him. He would spontaneously awake in the morning, and go about his work. One evening, having been left sitting on a chair by the fire, he fell asleep, and was found almost in the fire. His large felt hat was smouldering on his head, which had also been burnt; but he had gone on sleeping as usual. He was put to bed, and the next morning awoke and went out to tend his flock without complaining of any pain. This went on for some weeks, until a large eschar fell off and exposed the bones of the skull, which appeared black and mortified, while a line of demarcation separated the dead bone from the living. One day, a sequestrum fell off which comprised the whole of the external surface

and a portion of the diploë of both parietal bones, the upper portion of the frontal, and part of the occipital bone. The temporal bones, which were still covered by their muscles, had not suffered. About a year later, the wound began to be covered with granulations. In one particular spot, which corresponded to the middle region of the right parietal bone, pulsations could be felt, which were isochronous with those of the heart. The next year, these pulsations had disappeared, showing that ossification had taken place. The wound now is almost as large as it was a year ago, as the cicatrization progresses very slowly; but the boy, although he had lost the greater part of his skull, has continued to tend his flock every day. From time to time, his wound is dressed by covering it with a cloth dipped in oil, over which he wears a felt hat. He often carries heavy burdens on his head, or heavy branches of trees, and is said to rejoice very much at not feeling the thorns, which used to prick his scalp rather painfully when he was in full possession of his skull. (*British Medical Journal*, 1879.)