PAPERS AND ORIGINALS

Possible effect of time on renal allograft rejection

M S KNAPP, J R COVE-SMITH, R DUGDALE, N MACKENZIE, R POWNALL

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Summary and conclusions

The change in plasma creatinine concentrations from decreasing values after successful renal transplantation to increasing values after the onset of rejection occurs as a sudden event. Twenty-two such episodes in 16 renal allograft recipients were studied by extrapolating sequential measurements of plasma creatinine concentrations to see when the change occurred. Seventeen of the episodes occurred between 2300 and 1100 and the rest at other times. This difference was significant.

The results suggest that rejection is more common at night and apparently has a circadian rhythm, being likely to first influence creatinine clearance at around 0600.

Introduction

Ratte *et al*[1] performed renal transplantation on rats at various times of the day and night. Survival was longest in animals transplanted at 2000 and there was a significant correlation between the duration of transplant survival and the time of operation. Our studies on rats[2] and man[3] provided new information suggesting that the severity of an immune response may be more intense if the challenge that elicits the response is given at certain times of the day. When intradermal purified protein derivative (PPD) in tuberculin-sensitive subjects was studied the maximum response was observed at 0700.[3] In rats the maximum response to a cutaneous challenge with oxazolone was at 1000 in previously sensitised animals.[2]

These observations raise questions about whether the timing of transplantation should be more carefully considered, and also whether the timing of immunosuppression should be adjusted to take account of the time when the immune response in an untreated patient is strongest. The possible effect of the time of operation on transplant survival in patients will be considered elsewhere.[4] The case for reconsidering the timing of immunosuppressive treatment would be stronger if it could be shown that a rejection episode was more likely to occur at a particular time of day.

We find that renal allograft rejection occurs as a sudden event, the change from progressive improvement in renal function to progressive deterioration occurring over less than 24 hours and probably over a period of hours or possibly minutes. This was not fully appreciated until we analysed graphically the sequential plasma creatinine concentrations in a retrospective study of transplant recipients.[5] The sudden transition from improving function to deteriorating function is usually most obvious when reciprocals of the serum creatinine concentrations are plotted against time.[5] We have analysed the data mathematically to calculate a probable "time of rejection" in individual patients.

Methods

Venous samples were taken daily between 0900 and 1000 from 35 patients after transplantation. Plasma creatinine concentrations, measured with an AutoAnalyzer or an EEL creatinine analyser (coefficient of variation $<3\%$ on batch-to-batch assessments), were recorded daily together with the patient's weight. Creatinine values were "corrected" for the estimated "ideal" body weight in each case to adjust for the effect of change in body water.[5] Analysis and graphical display of plasma creatinine, its logarithm, and reciprocal were plotted against time.

Rejection episodes observed in the first 60 days after transplantation (see figure) were selected for study when the linear regression of the reciprocal of weight-corrected decreasing plasma creatinine values, plotted against time, provided a correlation coefficient exceeding 0·8 in a period of improving function before rejection, with a similar line of increasing values developing immediately in the days after onset of rejection. The line of best fit was calculated and plotted on large-scale graph paper. Lines of improvement and deterioration displayed

Renal Unit, City Hospital, Nottingham NG5 1PB

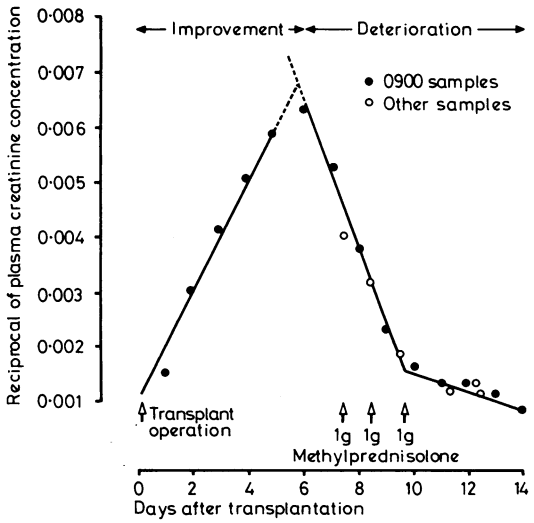
M S KNAPP, MD, FRCP, consultant physician

J R COVE-SMITH, MB, MRCP, senior registrar in medicine (now consultant physician, North Ormesby Hospital, Middlesbrough)

R DUGDALE, BSC, PHD, medical physicist (now at Bradford Royal Infirmary, Yorkshire)

N MACKENZIE, BSC, research assistant (now at department of zoology, University of Nottingham)

R POWNALL, BSC, PHD, senior scientific officer



Example of rejection episode showing lines of improvement and deterioration when reciprocals of serum creatinine concentrations are plotted against time. Lines extrapolated to intercept at estimated time of rejection. (Serum creatinine concentrations measured in μmol/l; 1 μmol/l ≈ 0·01 mg/100 ml.)

were extrapolated and the point of intersection taken as the time of sudden transition from improvement to deterioration (figure).

Results

Twenty-two rejection episodes in 16 patients fulfilled the selection criteria. The lowest correlation coefficient of any line used to calculate the time of rejection was 0·83, most exceeding 0·96.

Seventeen of the rejection episodes (77%) occurred during the night and early morning (2300-1100) compared with 5 (23%) during the remainder of the day ($P < 0·025$—see table). When the data were analysed by the method of least squares to test for similarity to a sine wave with a frequency of 24 hours[6] a significant rhythm was found ($P < 0·01$), the maximum of the best fitting sine wave having its maximum (acrophase) at 0558.

Distribution of 22 rejection episodes in a 24-hour span

Time (hours):	2300 -0200	0200 -0500	0500 -0800	0800 -1100	1100 -1400	1400 -1700	1700 -2000	2000 -2300
No of rejections/3h	4	4	6	3	2	1	1	1

Number of rejections between 2300 and 1100 versus number at other times: $P < 0·025$.

Discussion

The circadian rhythms of many body functions have been extensively studied, but there are few examples of such studies influencing clinical practice. A loss of diurnal variation in adrenocortical activity was noted in Cushing's syndrome, and investigation for this is now routine in suspected cases. Di Raimondo and Forsham reported that the timing of steroid administration influences adrenocortical activity the next day.[7] This was confirmed and has slowly influenced clinical practice. Morning or alternate-day doses are now often prescribed for patients receiving long-term corticosteroid treatment unless suppression of adrenocortical activity is the objective, as in the adrenogenital syndrome, when an evening dose is appropriate. In some conditions—for example, asthma and rheumatoid arthritis—a nocturnal increase in symptoms may lead to evening administration, and adrenopituitary suppression is then accepted as a likely side effect. The timing of drug administration may also be influenced, as in renal transplant units, by the organisation of clinical practice. If dosage depends on laboratory results, which may not be available until the evening, treatment will often include an evening dose.

When deciding the timing of drug administration it is also logical to consider whether there is a time of day when the disease is especially active. The few reported studies on this include examples related to the chemotherapy of cancer and treatment of allergy.[8,9] There have been even fewer therapeutic trials and most of these were on laboratory animals, but some provide results suggesting that at certain times drugs may either be more effective or less toxic.[8-10]

Our discovery of a circadian rhythm in cell-mediated immune responses[2,3] made it important to try to define the time when renal graft rejection was most common. The decrease in renal clearance of creatinine, reflected by the increase in plasma creatinine observed in association with graft rejection, must have occurred abruptly to provide the "rejection" peaks seen when appropriate plots of plasma creatinine concentrations were drawn against time (figure). Analysis of the changes that we observed permits an estimate of the time when the rejection process may first have affected this aspect of renal function.

This method of calculating "time of rejection" is, we believe, novel and entails extrapolating collected information. The absence of more-detailed study close to the time of rejection and the extrapolation needed to permit an estimate of when the change from improvement to deterioration took place must create some uncertainty about the timing of the change, and future studies should, if possible, provide more frequent plasma sampling to reduce the need to extrapolate. The data on plasma creatinine, collected in our patients after operation, were also analysed by a method developed by Professor Adrian Smith (department of mathematics, University of Nottingham). This analysis calculates the most likely time of sudden change and the probability of that change being at that time. The analysis and the results obtained will be presented elsewhere, but provide support for rejection being a sudden event which may be most frequent at night.

A time when renal allograft rejection occurs can be determined only if there is a sudden event rather than slow changes from a non-rejecting to a rejecting state. The few detailed studies of renal function that have been performed during rejection suggest that there is a sudden change in renal perfusion[11] which could cause an abrupt alteration in the clearance of creatinine as suggested by our analyses. A report on the blood flow in skin allografts described sudden changes in blood flow at the time of rejection,[12] and skin grafting may be an experimental model in which circadian rhythmicity in the onset of sudden vascular change could be studied.

We have no information from which to estimate when the immunological responses that cause a rejection episode first take place or the time of day when these are most active. The onset of physiological responses to rejection may be simultaneous with the maximum intensity of the immune response, although certain changes in lymphocyte activity precede it.[13] Interestingly the most usual time for the development of a change in renal function due to rejection was similar to the time when the maximum skin response was observed in tuberculin-sensitive subjects challenged with PPD.[3]

Plasma concentrations of administered glucocorticosteroids and of other immunosuppressive agents are usually low just before waking. The last dose may have been taken in the early evening or the previous morning, and the plasma half life is short. The time of maximum immune response will often coincide with the time of minimum plasma concentrations of immunosuppressive agents. This helps to explain why renal allograft rejection may occur most often at around this time. Plasma concentrations of immunosuppressive drugs may be important for preventing rejection, and Wassner *et al*[14] attributed the failure of renal transplants in children receiving anticonvulsants to the shorter half life of immunosuppressive drugs resulting from enzyme induction.[15] These children would have had lower plasma drug concentrations by the time of waking

than those receiving not anticonvulsants, perhaps accounting for the greatly increased incidence of rejection observed.

It seems logical to provide maximum immunosuppression at the time when the immune response is most active and when transplant rejection may be most likely. Some of the drugs could be taken at night to avoid the low morning levels of immunosuppression which probably occur with most regimens. Suitable studies are needed, both in the experimental laboratory and in the transplant unit, to test whether rejection would then be less likely or less severe.

Our investigations were initiated to determine whether rhythms of physiological or pathological activity need to be considered when treating patients with renal transplantation. Analysis of the results provides some support for the hypothesis that there is a circadian rhythm in allograft rejection. Data collected prospectively are needed to confirm this and provide information relevant to planning treatment. Immune responses are important in many other conditions, and our observations suggest that circadian variations should be considered when treating them. Seven-day rhythms of renal allograft rejection may occur,[16] and preliminary analysis of our results supports this suggestion. Thought should be given to the most appropriate times to administer treatment to achieve the maximum effect and minimum toxicity.[17] More research is needed into the relevance of circadian and other rhythms to the diagnosis, management, and treatment of disease.

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Metabolic consequences of atenolol and propranolol in treatment of essential hypertension

J L DAY, N SIMPSON, J METCALFE, R L PAGE

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Summary and conclusions

A six-month study of triglyceride, cholesterol, free fatty acid (FFA), glucose, insulin, growth hormone, and glucagon concentrations was carried out in asymptomatic hypertensive normal-weight men randomly allocated to treatment with atenolol or propranolol. A highly significant increase in the basal plasma triglyceride concentration was observed in propranolol-treated patients after three and six months' treatment, with a smaller but significant increase in atenolol-treated subjects after six months' treatment. The changes in triglyceride concentration could not be ascribed to variations in plasma insulin, growth hormone, or glucagon concentrations. Basal FFA concentrations were reduced during the first three months of treatment in both groups but returned to pretreatment levels after six months. Plasma cholesterol concentrations were unchanged by either agent.

Propranolol had a greater effect on triglyceride concentrations than atenolol, but probably all beta-blocking agents have similar effects of different magnitudes. These effects should be investigated further in view of the postulated association between plasma triglyceride concentrations and cardiovascular disease.

Department of Medicine, Ipswich Hospital, Suffolk IP4 5PD
J L DAY, MD, MRCP, consultant physician
N SIMPSON, MB, MRCP, clinical assistant
J METCALFE, BSC, biochemist
R L PAGE, DM, MRCP, senior registrar (present appointment: senior registrar, Addenbrooke's Hospital, Cambridge)

Introduction

Intravenously administered β-adrenergic antagonists (beta-blockers) may significantly alter plasma insulin, free fatty acid (FFA), and possibly glucose concentrations in man.[1 2] The consequences of long-term treatment with such agents have not been adequately studied,[3] however, and reports have given conflicting results. Failure to show major metabolic changes may in some instances be due to the study of heterogeneous groups of subjects[4 5] or of patients with angina or recent myocardial infarction,[6-8] in whom glucose tolerance and plasma lipid concentrations may be influenced by changes in ambulation, irrespective of any medication that they might receive. More recently Waal-Manning and Simpson[9] reported an increase in the plasma triglyceride concentration during treatment with metoprolol (a selective beta-blocker), while Newman[10] reported a significant fall in basal FFA concentrations after short-term treatment with acebutolol (a selective beta-blocker) and propranolol (an unselective beta-blocker) but not with metoprolol.

We undertook this study to determine whether atenolol (a relatively selective beta-blocker) or propranolol given by mouth over