PLASMA PREDNISOLONE MEASUREMENTS IN RENAL TRANSPLANT PATIENTS

by

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In recent years the monitoring of plasma drug levels carried out with a view to relating these with the clinical response produced by drugs has emerged as one of the expanding areas of medicine. There are now a number of examples of drugs where this approach has provided a basis for the appropriate adjustment of the dosage given and contributed to the understanding of drug toxicity.

Prednisolone (\(\Delta^1\)-cortisol), a synthetic glucocorticoid is widely used as an immunosuppressive in renal transplant patients. The amount given is between 50-300 mg/daily for the first few days after transplantation. This is gradually reduced over the first three months to a maintenance dose of 10-20 mg/day. The details of the schedule may vary depending on the local preference. Little is known about the circulating drug concentration achieved in these patients after steroid therapy and whether any derangement of the plasma drug kinetics may be implicated in rejection episodes or in the exaggerated action of corticoids observed in some patients.

MEASUREMENT OF PREDNISOLONE

The analytical techniques that have been applied to the measurement of prednisolone include fluorimetry/colorimetry, gas chromatography, competitive protein-binding assay, radioimmunoassay, high performance liquid chromatography. Among the procedures that have been reported for such determination in body fluids, those based on competitive protein building and radioimmunoassay (RIA) have been most widely used. One limitation of the former is that prednisolone has to be isolated by a chromatographic step in order to eliminate the interference from cortisol and other steroids. The RIA on the other hand allows high sample throughput and, from various other practical considerations, is the method of choice. The more recent use of high performance liquid chromatography (HPLC) with UV-detection system (254, 239 nm) for the determination of prednisolone although less sensitive that RIA, looks promising. The important advantage of this over other procedures is that cortisol and prednisolone (and possibly its metabolites) may be measured simultaneously.

Radioimmunoassay of prednisolone

The method developed at the University of Surrey is as follows. The antiserum was raised in sheep against prednisolone-21-hemisuccinate conjugated to bovine serum albumin and used at an initial dilution of 1:2,250. The cross-reactivity of the antiserum with various steroids is given in Table 1. The sample (0.2-1 ml) is extracted
TABLE 1
Cross-reactivity of various steroids with prednisolone antiserum in absence of prednisolone

| Cross-reactivity (%) | Prednisolone | Prednisone | 20-Dihydroprednisolone | Cortisol | Cortisone | Progesterone | Testosterone | Cholesterol |
|----------------------|--------------|------------|------------------------|----------|-----------|-------------|--------------|-------------|
| Prednisolone         | 100          |            |                        |          |           |             |              |             |
| Prednisone           | 11.1         |            |                        |          |           |             |              |             |
| 20-Dihydroprednisolone| 20.6        |            |                        |          |           |             |              |             |
| Cortisol             | 3.9          |            |                        |          |           |             |              |             |
| Cortisone            | 2.8          |            |                        |          |           |             |              |             |
| Progesterone         | 0.6          |            |                        |          |           |             |              |             |
| Testosterone         | 0.1          |            |                        |          |           |             |              |             |
| Cholesterol          | 0.1          |            |                        |          |           |             |              |             |

with ethyl acetate (3 ml × 2), the extracts pooled and dried by evaporation at 40° under a stream of nitrogen. The residue is reconstituted in 0.5 ml and 0.1M phosphate buffer, pH 7.4 and aliquots (0.1 ml, either neat or appropriately diluted) analysed for prednisolone by RIA. Detailed information on the RIA has been lodged with the Editor. The sensitivity of the assay is 1 ng/ml. Replicate assays carried out on pooled normal human plasma to which known amounts of prednisolone was added gave coefficient of variation 3.6-5.9% within batch and 5.1-7.3 between batch.

HUMAN STUDIES

Prednisolone absorption

After ingestion of plain prednisolone tablets orally, peak plasma drug levels are achieved, according to most reports, within the first hour and the plasma half-life of prednisolone is 2.5-3.5h.6, 7, 8, 9, 10, 11 Most published studies on plasma prednisolone levels were carried out with 10-30 mg doses. The values quoted for the same doses, however, vary between reports and this, at least in part, may be attributed to differences in methodology used for prednisolone and in the experimental protocol. Another feature of prednisolone bioavailability which appears to be prominent is inter-individual variation even in healthy subjects, as illustrated in Fig. 1. After an oral dose of 10 mg (plain tablets) plasma peak prednisolone levels in these six subjects ranged between 165 and 260 ng/ml with a mean value of 202 ± S.D. 34.6. The amount of unchanged prednisolone isolated from 24h urine by thin layer chromatography (polystyrene-backed silica gel plates; dichloromethane/ethanol/water, 150:10:1, v/v) followed by RIA varied between 11.1 and 19.4% of the administered dose (mean value 13.6 ± S.D. 3.2).

From the available data it is hardly possible to construct a useful dose-plasma prednisolone level curve for reasons mentioned before. Some examples of the values collected from literature2, 4, 7-15 are shown in Table 2.

Enteric-coated tablets

There have been some conflicting reports on the bioavailability of enteric-coated prednisolone compared with that of the plain tablets. According to Lee et al.,16 the
Plasmaprednisolone levels in healthy subjects after an oral dose (10 mg, plain tablets)

![Graph showing prednisolone levels over time]

TABLE 2
Some reported\(^2,4,7-15\) values of plasma prednisolone level after plain tablets given by mouth

| Dose (mg) | Plasma prednisolone (ng/ml) |
|-----------|----------------------------|
| 10        | 248                        |
| 10        | 227                        |
| 10*       | 250                        |
| 10        | 246                        |
| 15        | 139                        |
| 20        | 375                        |
| 20        | 185                        |
| 50        | 500                        |
| 50*       | 807                        |
| 60        | 684                        |
| 90        | 1,343                      |

* Prednisone given.
pattern of absorption and plasma prednisolone levels depend on the formulation of the enteric coating. They concluded that the bioavailability of the more recent CAP-based preparation, unlike the previously used shellac-based tablets, is consistent and similar to that of plain prednisolone. They also found that the presence of food in the stomach at the time of the drug ingestion (20 mg) did not alter the absorption of CAP-enteric-coated tablets (Fig. 2).

**Mean plasma prednisolone concentrations after enteric-coated tablets to fasted () and non-fasted subjects ( ). Dose range 15-22.2 mg. (Lee et al)**

*Renal transplant recipients—Study 1*

Plasma prednisolone levels were monitored in 8 renal transplant patients, 5 men and 3 women within three weeks after transplantation. No restriction was imposed on their food and drink intake and they received by mouth their respective doses of CAP-based enteric-coated prednisolone tablets. Some of the findings are given in Tables 3, 4 and 5. These show a gross variation in the rate of appearance of prednisolone in the blood and peak values achieved irrespective of the dosage used. After the same dose given to different patients or to the same patients on two separate occasions, there was a difference in the time required to reach peak plasma prednisolone concentrations and, more strikingly, in the magnitude of the values obtained.

Breakfast taken before dosing resulted in a delay of 7-10 hours before peak values were reached. Furthermore, fasting before taking prednisolone produced up to eightfold higher peak concentrations after 175 and 150 mg doses, but not after 20 or 50 mg doses.
TABLE 3

Plasma prednisolone levels (ng/ml) after oral doses in renal transplant patients

| Time (h) | Dose (mg) and plasma prednisolone (ng/ml) | 200 mg | 200 mg | 175 mg | 175 mg |
|----------|------------------------------------------|--------|--------|--------|--------|
| 0        |                                          | 67     | 0      | 630    | 400    |
| 0.5      |                                          | 900    | 90     | 500    | 325    |
| 1        |                                          | 1,067  | 100    | 490    | 250    |
| 2        |                                          | 900    | 3,000  | 1,470  | 188    |
| 4        |                                          | 1,017  | 3,350  | 4,700  | 163    |
| 8        |                                          | 1,267  | 2,550  | 3,350  | 423    |

TABLE 4

Plasma prednisolone levels (ng/ml) after oral doses in renal transplant patients

| Time (h) | Dose (mg) and plasma prednisolone (ng/ml) | 175 mg | 150 mg | 125 mg | 100 mg |
|----------|------------------------------------------|--------|--------|--------|--------|
| 0        |                                          | 1 I    | 375    | 933    | —      | 70     |
| 0.5      |                                          | 295    | 1,067  | 450    | 1,070  |
| 1        |                                          | 898    | 227    | 1,333  | 300    | 570    |
| 2        |                                          | 852    | 841    | 1,367  | 250    | 660    |
| 4        |                                          | 943    | 1,875  | 1,400  | 613    | 1,300  |
| 8        |                                          | 1,057  | 716    | 1,017  | 688    | 670    |

TABLE 5

Plasma prednisolone (ng/ml) concentrations in renal transplant patients after an oral dose

| Time (h) | Dose 125 mg | Dose 100 mg |
|----------|-------------|-------------|
|          | I I*         | I*           | II           |
| 0        | 267 133     | 160          | 70           |
| 0.5      | 183 67       | 170          | 1,070        |
| 1        | 117 550      | 240          | 570          |
| 2        | 183 717      | 201          | 660          |
| 4        | 900 1,533    | 350          | 1,300        |
| 8        | 917 833      | 1,350        | 670          |

Drug was measured on two consecutive days.  
*: Haemodialysis was carried out.
Renal transplant recipients—Study 2

Ten patients with stable renal function two years after transplantation who were receiving prednisolone orally (2 × 5 mg enteric-coated tablets) as the only immunosuppressive treatment were included in this investigation. The prednisolone dose was reduced by 1 mg at monthly intervals by replacing one of the 5 mg tablets with the appropriate number of 1 mg tablets. After the patient had been on a dose for one week, plasma drug levels were determined at 1, 3 and 6h following the daily dose. Some of these results are given in Table 6.

**Table 6**

Patients with renal transplant—Stepwise reduction of prednisolone dose

| Patient | Case No. | Creatinine clearance at start (ml/min) | Lowest prednisolone dose (mg/day) | Plasma prednisolone (ng/ml) at 3 h. |
|---------|----------|----------------------------------------|---------------------------------|-----------------------------------|
|         |          |                                        | 10 mg                           | 8 mg                              |
| Non-rejection | 1       | 72.7                                   | 1                               | 88                                |
|           | 2       | 61.5                                   | 1                               | 11                                |
|           | 3       | 64.0                                   | 5                               | 86                                |
| Rejection | 4       | 61.0                                   | 3                               | 140                               |
|           | 5       | 72.8                                   | 6                               | 0                                 |
|           | 6       | 79.6                                   | 5                               | 116                               |
|           | 7       | 80.0                                   | 4                               | 91                                |
|           | 8       | 80.0                                   | 2                               | 0                                 |
| Others   | 9       | 53.0                                   | 6                               | 192                               |
|           | 10      | 86.6                                   | 5                               | 93                                |

The patients (1, 2 & 3) in the non-rejection group remained well at daily doses of 1, 1 & 5 mg respectively. The reduction of prednisolone dose in patients 9 and 10 had to be stopped because of acute pyelonephritis and symptoms of cortisol deficiency respectively. The plasma prednisolone values obtained after 7-10 mg oral doses were not proportional to the size of the dose; in this respect, no clear and consistent pattern of differences emerged between the groups or between individual patients. On some occasions (Case Nos. 2, 5 & 8, 10 mg; Case No. 4, 8 mg) prednisolone was hardly detectable in plasma.

**CONCLUSION**

With the availability of RIA methods for prednisolone and other synthetic corticoids it should now be possible to examine critically the various aspects of the use of immunosuppressive steroids in renal transplant patients. It is clear from the studies presented here that the continued use of enteric-coated prednisolone tablets, especially when a large number of tablets make up the required dose, needs careful appraisal. Considerations have also got to be given to the effect of food on intestinal absorption and the patient’s gastrointestinal activity which may be contributory factors in the gross variation in plasma drug levels observed in the transplant.
patients. Low concentrations of prednisolone in plasma noted in some instances (Study 1) might be potentially hazardous, possibly favouring graft rejection.

Whether the concept of a standard 'minimum threshold dose' compatible with graft survival can be generally applicable remains to be established. The preliminary data presented here provide a useful basis for future more elaborate studies of patients who sustain stable renal function with low maintenance doses of prednisolone. Any progress made in understanding the 'therapeutically effective' plasma prednisolone concentration, determined preferably by using plain tablets throughout, should be an important step forward towards adjusting dosage according to individual capacity to handle prednisolone. Such information may also prove to be a useful adjunct to the assessment of the undesirable side effects of corticoids and chrono-biological evaluation of the standard protocol used for steroid therapy.

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