

Stability of cyclosporin A in human serum

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SUMMARY Cyclosporin A (CyA), a new immunosuppressive drug, is used for the prevention of organ graft rejection in man. The main side effect of CyA is nephrotoxicity which is usually reversible when the drug is stopped. Nephrotoxicity may be avoided in patients by careful monitoring of serum CyA concentration by radioimmunoassay, in addition to the routine biochemical tests of renal function. There is a good correlation between trough (12 hour) serum concentrations of CyA and plasma creatinine.

Cyclosporin A was stable in serum at room temperature for up to seven days and on storage at -20°C for up to five months. Delayed separation of blood stored at room temperature led to increased serum CyA concentrations while blood stored at 4°C before separation was much more stable.

Blood samples should be separated as quickly as possible to avoid the release of CyA from the cellular fraction into the serum. Haemolysed samples should be avoided. CyA is sufficiently stable at room temperature for serum samples to be sent in the first class post to a control laboratory for CyA assay, after patients are discharged from the transplant centre. Control and patient serum samples should be stored for a maximum of five months at -20°C prior to assay for CyA.

Cyclosporin A (CyA) is a fungal metabolite with a cyclic structure consisting of 11 amino acids and has a molecular weight of 1202.6 daltons.¹ The powerful immunosuppressive effect of CyA has been used clinically to prevent rejection of organ grafts in man² and to prevent graft versus host disease in allogeneic bone marrow transplantation.³

Mild nephrotoxicity occurs in most patients treated with CyA and cases of established renal failure have been reported.³

We have found careful monitoring of serum CyA concentration using radioimmunoassay (RIA) useful in the management of bone marrow transplant patients receiving the drug. There is a good correlation between predose (trough) CyA serum concentration and nephrotoxicity which is most apparent when other nephrotoxic drugs are given concurrently with CyA.⁴

Bone marrow transplantation is carried out in specialised centres but after discharge from hospital, patients may be followed up at other hospitals some distance from the transplant centre. It is important that pharmacological monitoring should continue at this stage. The most effective way to continue monitoring patients is for blood samples to be sent by post to the transplant centre where the RIA for CyA is routinely performed.

The purpose of this study was to investigate the stability of CyA in serum on storage under different conditions and to define the best method of handling

blood samples which are sent to transplant centres for assay. In addition we present some data on the long-term stability of CyA in serum samples stored at -20°C .

Material and methods

Patients were given CyA orally every 12 h. Venous blood was collected from patients just prior to the next dose to measure 12 h (trough) concentrations of CyA in the serum. Blood samples were separated at 1100 g for 10 min at 4°C .

MEASUREMENT OF CYA

Cyclosporin A was measured by RIA using tritiated CyA (H^{-3}CyA) as a tracer and an antibody supplied in kit form by Sandoz Ltd, Basle. Determinations were performed in triplicate using 20 μl serum samples diluted 1/50 with 0.05 M Tris buffer, pH 8.5.⁵

The interassay variation was $\pm 5\%$ and the assay was sensitive in the range 60–4000 ng/ml. Concentration of CyA in the unknown samples was calculated from a standard curve. The standard error and 95% confidence limits ($\pm 2\text{SEM}$) were calculated for each result.

STABILITY OF CYA IN SERUM AFTER DELAYED SEPARATION OF WHOLE BLOOD

Aliquots of clotted blood were kept at room temperature or 4°C for 24 h before separation. Control samples were separated immediately and stored at -20°C .

STABILITY OF CyA IN SERUM AFTER IMMEDIATE SEPARATION OF WHOLE BLOOD

Storage of serum at room temperature: Clotted blood samples were separated within 1 h of collection. Fresh serum was aliquoted and kept at room temperature for 24 and 48 h. A control sample was immediately frozen after separation for comparison.

The stability of a previously frozen (-20°C) serum sample was assessed at room temperature by measuring aliquots of serum left at room temperature for 1–7 days.

Effect of freeze-thawing: Aliquots of serum were frozen at -20°C and thawed in a uniform fashion from 1–5 times before assaying for CyA.

LONG-TERM STORAGE OF SERUM AT -20°C

The long-term stability of CyA in serum was assessed by storing two sera containing CyA in 200 μl aliquots at -20°C for up to eight months. Aliquots were thawed and assayed at regular intervals over this period.

Results

STABILITY OF CyA IN SERUM AFTER DELAYED SEPARATION OF WHOLE BLOOD

Table 1 shows an increase of serum CyA measured in blood samples stored at room temperature for 24 h before separation. This effect was greatest in a haemolysed sample (PR₁) with an increase of 42% over the control. However, a repeat sample from the same patient (PR₂) was not haemolysed and only a small increase in CyA (2%) was seen. CyA is more stable in serum when whole blood samples are stored at 4°C before separation (Table 2).

STABILITY OF CyA IN SERUM AFTER IMMEDIATE SEPARATION

Serum at room temperature for 24 and 48 h was stable whether the samples were freshly separated or frozen and

Table 1 Stability of CyA in serum after delayed separation of whole blood (clotted blood at room temperature for 24 h before separation)

Patient	Sample	Serum CyA (ng/ml) $\pm 2\text{SEM}$	% change treated sample	Visible haemolysis
PR (1)	a	170 \pm 27		No
	b	242 \pm 13	42	Yes
PR (2)	a	130 \pm 1		No
	b	133 \pm 5	2	No
WK	a	162 \pm 20		No
	b	189 \pm 28	17	No
JC	a	74 \pm 20		No
	b	84 \pm 16	14	No
PH	a	112 \pm 33		No
	b	152 \pm 26	36	No
MH	a	117 \pm 1		No
	b	162 \pm 11	38	No

a = serum immediately separated and stored at -20°C (control serum).
b = whole blood at room temperature for 24 h before separation of serum (treated serum).

Table 2 Stability of CyA in serum after delayed separation of whole blood (clotted blood at 4°C for 24 h before separation)

Patient	Sample	Serum CyA (ng/ml) $\pm 2\text{SEM}$	% change treated sample	Visible haemolysis
AZ	a	76 \pm 29		No
	b	81 \pm 37	7	No
PR	a	130 \pm 1		No
	b	135 \pm 3	4	No
MS	a	204 \pm 36		No
	b	174 \pm 62	-15	No
GZ	a	340 \pm 1		No
	b	325 \pm 5	-4	No
PR	a	98 \pm 10		No
	b	100 \pm 3	2	No
MH	a	117 \pm 1		No
	b	119 \pm 13	2	No

a = serum immediately separated and stored at -20°C (control serum).
b = whole blood at 4°C for 24 h before separation of serum (treated serum).

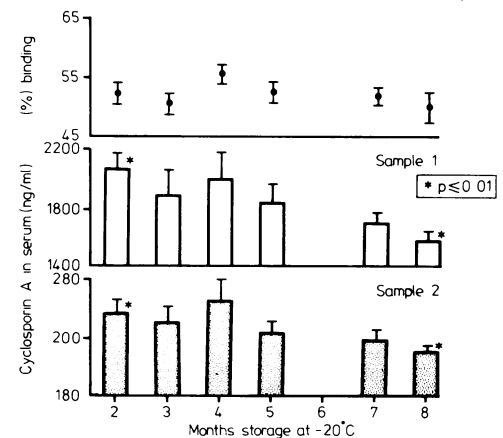
thawed before standing at room temperature (Tables 3 and 4). Repeated freezing and thawing (Table 4) of serum sample did not affect the stability of CyA as measured by RIA. CyA was stable in a previously frozen serum sample for up to seven days at room temperature (Table 5).

LONG-TERM STABILITY OF SERUM SAMPLES STORED AT -20°C

Aliquots of two serum samples stored at -20°C for up to eight months were assayed. Aliquots stored for more than five months were found to have a significant decrease in measurable CyA ($p < 0.01$). The Figure shows the mean CyA concentration ($\pm 2\text{SEM}$) for aliquots measured during several months of the storage period. The mean percentage binding of tritiated CyA by antiserum for each month is also shown as a measure of the reproducibility of the experimental conditions over the observation period.

Discussion

Delayed separation of blood causes a rise in the serum CyA concentration (Tables 1 and 2). This change is more



Long-term storage of CyA in serum at -20°C .

Table 3 Stability of CyA in serum after immediate separation (effect of freeze/thawing)

Patient	Serum CyA (ng/ml) ± 2SEM				
	No of times thawed				
	1	2	3	4	5
WK	242 ± 31	259 ± 14	259 ± 14	289 ± 44	286 ± 16
SS	557 ± 50	581 ± 49	532 ± 54	542 ± 92	548 ± 77

marked when the unseparated blood is left at room temperature than when it is stored at 4°C. The greatest increase in CyA concentration was noticed in a haemolysed sample (Table 1). It has been shown that 58% of whole blood CyA is bound to the red cell membrane with 33% in the plasma fraction (T Beveridge, personal communication 1981). This suggests that the rise in serum CyA seen after delayed separation of blood samples is due to release of CyA from the cellular fraction. Ideally blood samples to monitor serum CyA should be carefully taken to avoid haemolysis just before the drug is given (12 hours post dose). Samples should be separated within one hour of collection. If separation is delayed the clotted blood should be kept at 4°C.

Cyclosporin A appears to be stable on short term storage of serum samples which have been separated immediately (Tables 3–5). Repeated freeze-thawing and storage of separated serum samples for up to one week at room temperature does not affect the measurement of CyA by RIA. Separated serum samples can therefore be sent through the post without freezing, provided reason-

able precautions are taken to avoid bacterial contamination.

Long-term storage of serum samples appears satisfactory at -20°C for up to three months (Figure). However, some deterioration of the samples occurs after this period. These data should be considered when samples are assayed retrospectively and care should be taken to replace quality control samples stored at -20°C every three months.

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Table 4 Stability of CyA in serum after immediate separation (serum at room temperature for 24 and 48 h after separation)

Patient	Serum CyA (ng/ml) ± 2SEM		
	*Control sample	†24h at room temp	‡48h at room temp
WK	242 ± 31	232 ± 37	261 ± 4
SS	557 ± 50	565 ± 60	580 ± 4
AZ	123 ± 17	103 ± 20	113 ± 25
GZ	215 ± 17	205 ± 20	211 ± 7

*Frozen immediately after separation.
 †Room temperature: min 21°C and max 23°C for first 24h.
 ‡Room temperature: min 19°C and max 23°C for second 24h.

Table 5 Stability of CyA in serum at room temperature for 7 days

	Days at room temperature							
	0	1	2	3	4	5	6	7
Serum CyA (ng/ml ± 2SEM)	399 ± 175	312 ± 87	394 ± 31	401 ± 32	396 ± 54	374 ± 21	385 ± 44	331 ± 52
Room temperature (°C)	Min 18	18	18	20	19	19	19	19
	Max 20	22	23	22	20	21	23	22