Cyclosporine A-Induced Changes to Erythrocyte Redox Balance is Time Course-Dependent

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Abstract: Cyclosporine A-treated transplant recipients develop pronounced cardiovascular disease and have increased oxidative stress and altered antioxidant capacity in erythrocytes and plasma. These experiments investigated the time-course of cyclosporine A-induced changes to redox balance in plasma and erythrocytes. Rats were randomly assigned to either a control or cyclosporine A-treated group. Treatment animals received 23 mg/kg of cyclosporine A via intraperitoneal injection for either 7 days or a single dose. Control rats were injected with the same volume of the vehicle. Three hours after the final injections, plasma was analysed for total antioxidant status, α-tocopherol, malondialdehyde, and creatinine. Erythrocytes were analysed for reduced glutathione (GSH), α-tocopherol, methaemoglobin, malondialdehyde, and the activities of superoxide dismutase, catalase, GSH peroxidase, and glucose-6-phosphate dehydrogenase (G6PD).

Cyclosporine A administration for 7 days resulted in a significant increase (P<0.05) in plasma malondialdehyde, methaemoglobin, and superoxide dismutase and catalase activities. There was a significant decrease (P<0.05) in erythrocyte GSH concentration and G6PD activity in cyclosporine A animals. There were no significant differences (P>0.05) between groups following a single dose of cyclosporine A in any of the measures. In summary, cyclosporine A alters erythrocyte redox balance after 7 days administration, but not after a single dose.

Cardiovascular disease is significantly increased in organ transplant recipients and remains the largest single factor limiting long-term survival in these patients (Kendrick 2001). In renal transplant recipients, the risk of cardiovascular disease mortality is as high as 10 times that of age-matched controls in the 25 to 34 year old age group (Kendrick 2001). After heart transplantation, cardiovascular disease occurs in approximately 50% of patients at 5 years from the date of transplantation (O’Neill et al. 2004). Epidemiologic studies have shown inverse associations between cardiovascular disease and both plasma antioxidants and erythrocyte α-tocopherol (Bonithon-Kopp et al. 1997; Nyysönen et al. 1997). Available evidence suggests that the potent antioxidant defence system of the erythrocyte is pivotal to the maintenance of the overall antioxidant status of the blood, as the erythrocyte performs a vital role in maintaining plasma antioxidants in the reduced state (May et al. 2000), and eliminates superoxide and hydrogen peroxide derived from the plasma (Hatherill et al. 1991).

In transplant recipients, decreased concentrations of α-tocopherol (Cristol et al. 1996) and membrane polysaturated fatty acids (Phair et al. 1994) have been reported, as well as increased erythrocyte malondialdehyde (Simic-Ogrizovic et al. 1998). Decreased concentrations of erythrocyte glutathione (GSH) (Hussein et al. 1997) and altered activities of superoxide dismutase (Cristol et al. 1998) and GSH peroxidase (Simic-Ogrizovic et al. 1998) have also been reported. In the plasma, transplant recipients show decreased thiol concentrations (Simic-Ogrizovic et al. 1998), increased malondialdehyde (Cristol et al. 1998; Simic-Ogrizovic et al. 1998), and an increased susceptibility of low density lipoprotein (LDL) to oxidation (Hussein et al. 1997). Indeed, it has been shown that oxidised plasma LDL is a prognostic marker of transplant coronary artery disease (Holvoet et al. 2000).

Cyclosporine A is a commonly administered immunosuppressive agent administered to organ transplant recipients to prevent rejection (Santori et al. 1997) and evidence is accumulating to implicate this agent in the altered redox balance observed in the plasma and erythrocytes of transplant recipients. In vitro, it has been shown that cyclosporine A increases concentrations of reactive oxygen species and malondialdehyde, and the addition of antioxidants decreases concentrations (Serino et al. 1994; Wolf et al. 1997). In support of in vitro studies, it has been shown in animal models that cyclosporine A administration increases renal and plasma malondialdehyde (Wang & Salahudeen 1995), and impairs antioxidant capacity in the kidney (Durak et al. 1998) and liver (Duruibe et al. 1989). It has also been shown in animal studies that antioxidant supplementation reduces cyclosporine A induced renal toxicity (Wang & Salahudeen 1995). Given this information, it is not surprising that results of a recent study from our laboratory showed that cyclosporine A administration (25 mg/kg/day) for 18 days decreased plasma antioxidant capacity and altered the antioxidant defence in the erythrocytes of female rats.

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(Fletcher et al. 2005). The present experiments were designed to extend these findings by investigating the effect of cyclosporine A administration for more acute time periods than 18 days. In addition, a male model was chosen based on reports from previous studies showing the ability of estrogen to improve the antioxidant status of plasma and erythrocytes (Massafra et al. 2002). Therefore, the primary purpose of the first series of experiments was to determine the effects of cyclosporine A administration for 7 days on plasma antioxidant capacity and erythrocyte redox balance. On the basis of previous studies, we hypothesised that cyclosporine A administration would alter erythrocyte and plasma redox balance. Our results showed a cyclosporine A induced change to erythrocyte redox balance. These findings prompted a second series of experiments designed to elucidate whether a single dose of cyclosporine A would also alter erythrocyte redox balance.

**Materials and Methods**

**Animals and experimental design.** After approval was given from Charles Sturt University’s Animal Care and Ethics Committee, the following experiments were conducted. Male Sprague Dawley rats (2 months old) weighing 213±19 g (mean±standard deviation (S.D.)) were randomly assigned to either a control (prolonged) or a cyclosporine A treated (acute) group (n=12 per group). Rats were fed rat chow and water ad libitum and maintained on a 12 hr light/dark photo-period with a room temperature of 21±2°C. For one week prior to the beginning of the experiments, animals were handled daily by laboratory technicians to reduce contact stress. Treatment group animals were administered with 25 mg/kg body mass cyclosporine A (dissolved in extra light olive oil and 4% ethanol) via intraperitoneal injection for 7 days. Control rats were injected with the same volume of the vehicle for 7 days. Animal mass was recorded daily and cyclosporine A doses were adjusted to changes in body mass. All injections were performed at approximately the same time of the day. The findings from the prolonged study prompted a second series of experiments designed to determine the effect of a single dose (acute) of cyclosporine A. With the exception of the drug administration time period, the experimental procedures described above were repeated. Male Sprague Dawley rats (2 months old) weighing 335±30 g (mean±S.D.) were randomly assigned to either a control (acute) or a cyclosporine A treated (acute) group (n=12 per group). Treatment group animals were administered with a single dose of 25 mg/kg body mass cyclosporine A (dissolved in extra light olive oil and 4% ethanol) via intraperitoneal injection. Control animals were administered with a single dose containing the same volume of vehicle. The differences in animal body weight between studies is due to inter-study variation.

**Blood collection and sample preparation.** On the day of sacrifice, blood was collected 3 hr after drug administration as it has previously been shown that cyclosporine A reaches peak levels approximately 3 hr after intraperitoneal injection in the rat (Ibarra et al. 1996). Just prior to exsanguination rats were administered with 90 mg/kg sodium pentobarbital via intraperitoneal injection. After reaching a surgical plane of anaesthesia, the chest cavity was opened and rats were exsanguinated via intracardiac puncture. Approximately 5 ml of blood was collected in glass EDTA vacutainer tubes and aliquots of whole blood removed for analysis of peak cyclosporine A concentration, methaemoglobin (metHb), and glucose-6-phosphate dehydrogenase (G6PD) activity. The remaining whole blood was centrifuged at 4°C for 10 min. at 800 × g. The plasma was then removed, divided into aliquots, and stored at −80°C until biochemical analysis of total antioxidant status, α-tocopherol, malondialdehyde, and creatinine. The buffy coat was then removed and discarded. Erythrocytes were subsequently washed three times with phosphate buffered saline. An aliquot of washed cell suspension was used to determine the concentration of erythrocyte GSH. Erythrocyte aliquots were stored at −80°C until biochemical analysis of α-tocopherol, malondialdehyde, and the activities of superoxide dismutase, catalase, and GSH peroxidase. Because plasma contains comparatively little superoxide dismutase, catalase, and GSH peroxidase compared to the erythrocyte (Halliwell & Gutteridge 1986), these measures were made only in the red cell.

**Cyclosporine A.** Peak blood cyclosporine A levels were determined at the Hunter Area Pathology Service (John Hunter Hospital, Newcastle, NSW, Australia) using the EMT 2000 Specific Assay (Dade Behring – Syva, Deerfield, IL, USA). The method was based on a homogenous enzyme immunoassay and analysis was carried out on a Roche Cobas Mira chemistry system (Roche, Basel, Switzerland).

**Erythrocyte glutathione.** Erythrocyte GSH was determined by the method of Beutler et al. (1963). This method is based on the principle that GSH reduction of 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) forms a yellow coloured anion which can be measured spectrophotometrically at 412 nm.

**Methaemoglobin.** Methaemoglobin was determined by the method of Evelyn & Malloy (1938). Methaemoglobin has an absorption peak at 630 nm, which disappears upon addition of potassium cyanide. The difference in absorbance is therefore proportional to the concentration of methaemoglobin. Total haemoglobin was measured by conversion to cyanmethaemoglobin by the addition of potassium cyanide and potassium ferricyanide.

**Erythrocyte antioxidant enzymes.** G6PD activity was determined using a Sigma Diagnostics kit (procedure #345-UV) which is based on a modification of the spectrophotometric method of Loehr & Waller (1974). G6PD catalyses the first step in the pentose phosphate pathway, oxidising glucose-6-phosphate to 6-phosphogluconate and reducing nicotinamide adenine dinucleotide phosphate (NADP) to NADPH. The rate of formation of NADPH is proportional to G6PD activity and was measured spectrophotometrically as an increase in absorbance at 340 nm. One unit of G6PD activity is defined as 1 μM NADPH produced per minute.

Catalase activity was measured using a modification of the method of Slaughter & O’Brien (2000). This method is based on the competition of catalase with peroxidase for H₂O₂ produced by the action of uricase on uric acid. Peroxidase uses available H₂O₂ to catalyse the formation of a coloured quinoneimine, however catalase inhibits this reaction by eliminating H2O2. The procedure was carried out on an automated spectrophotometer (Cobas, Mira, Roche Diagnostics, Switzerland) and one unit of catalase activity defined as the concentration of catalase that inhibits quinoneimine colour formation from H₂O₂ by 50%.

GSH peroxidase activity was determined using a modification of the method of Andersen et al. (1997) which measures the oxidation of NADPH to NADP. The procedure was carried out on an automated spectrophotometer (Cobas, Mira, Roche Diagnostics, Switzerland) and one unit of GSH peroxidase activity defined as 1 μmol NADPH oxidised per minute.

Superoxide dismutase activity was determined based on the method of Madesh & Balasubramanian (1998) which measures the ability of the enzyme to inhibit reduction of the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-dimethyltetrazolium bromide (MTT) by superoxide. The procedure was carried out on a microplate reader (Titertek Multiskan MCC340, Flow Laboratories, North Ryde, Australia) and one unit of superoxide dismutase defined as the amount required to inhibit MTT reduction by 50%. All erythrocyte enzyme activities were normalised to haemoglobin (HB) concentration.
Malondialdehyde. High performance liquid chromatography (HPLC) was used to determine plasma and erythrocyte malondialdehyde using the method of Sim et al (2003). The principle of this method is that malondialdehyde contained in plasma or erythrocytes is derivatised with 2,4-di-nitrophenylhydrazine which forms stable hydrazones that can be easily separated by HPLC using diode array detection (Shimadzu, Kyoto, Japan).

α-tocopherol. HPLC was used to determine erythrocyte α-tocopherol using a modification of the methods of Hatam and Kayden (1979). Proteins were precipitated from haemolysate with HPLC grade methanol and α-tocopherol extracted from the supernatant using HPLC grade hexane. Plasma α-tocopherol was determined using a modification of the method of Taibi and Nicotra (2002). Proteins were precipitated from solution with HPLC grade ethanol and α-tocopherol subsequently extracted using HPLC grade hexane. α-Tocopherol extracted from erythrocytes and plasma was measured using fluorometric detection (Shimadzu, Kyoto, Japan).

Total antioxidant status. Total plasma antioxidant status was determined by the method of Miller et al. (1993) which is based on the inhibition by antioxidants of the absorbance of the radical cation 2,2'-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS-•⁻). The ABTS radical cation is formed by the interaction of ABTS with the ferrylmyoglobin radical species, generated by the activation of metmyoglobin with H₂O₂. Antioxidant compounds suppress the absorbance of ABTS•⁻ to an extent and on a time scale dependent on the antioxidant capacity of the plasma. The assay was carried out on an automated spectrophotometer (Cobas, Mira, Roche Diagnostics, Switzerland).

Plasma creatinine. Plasma creatinine was determined using the Jaffe Reaction method. Absorbance was measured at 520 nm using an automated spectrophotometer (Cobas, Mira, Roche Diagnostics, Switzerland).

Data analyses. Comparisons between groups for the dependent variables were made by independent t-tests. Significance was established at the 95% confidence level (P<0.05). All data is shown as mean±S.D.

Results

Seven day study.
Changes in body weight over the 7 day drug administration period (measured as % of initial body weight), were significantly different between groups (P<0.05). Control animals were 110.0±2.9% after 7 days of vehicle administration, whereas treatment animals were 92.7±5.2% after 7 days of cyclosporine A administration. Mean peak blood cyclosporine A concentrations in the treated animals at sacrifice were 5726±1513 μg/l. Plasma creatinine, measured as an assessment of renal function at sacrifice, was 30% greater (P<0.05) in treatment animals (83.7±5.3 μM) compared to controls (64.3±6.2 μM).

Table 1 indicates that cyclosporine A administration resulted in a significant 15% increase (P<0.05) in plasma malondialdehyde. Plasma total antioxidant status and α-tocopherol were not significantly different (P>0.05) between groups.

Markers of erythrocyte antioxidant status and oxidative stress are presented in table 2. Erythrocyte superoxide dismutase and catalase activity were significantly increased (P<0.05) by 38% and 44% respectively in the treatment group compared to controls. Cyclosporine A administration resulted in significant reductions (P<0.05) in GSH concentration and G6PD activity by 21% and 8% respectively. α-Tocopherol concentrations and GSH peroxidase activity were not significantly different (P>0.05) between groups. Methaemoglobin, measured as a marker of haemoglobin oxidation, was significantly increased (P<0.05) by 63% in cyclosporine A treated animals. Erythrocyte malondialdehyde, measured as a marker of red cell membrane lipid peroxidation, was not significantly different (P>0.05) between groups.

Single dose study.
Mean peak blood cyclosporine A concentrations in the treated animals at sacrifice were 1085±599 μg/l. Plasma creatinine was not significantly different (P>0.05) in cyclosporine A treated animals (56±2.8 μM) compared to controls (54.3±5.9 μM). There were no significant differences (P>0.05) between groups in any of the antioxidants or markers of oxidative stress measured in the plasma and erythrocytes. Results are presented in table 3 and 4. In our hands, the coefficient of variation for these assays are all less than 5%. The two studies described in this manuscript were conducted independently, therefore, inter-assay variation may exist between studies.

Table 2.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Cyclosporine treated</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase (U/gHb)</td>
<td>4336±1324</td>
<td>5998±1298</td>
<td>0.008*</td>
</tr>
<tr>
<td>Catalase (U/gHb)</td>
<td>2633±506</td>
<td>3782±941</td>
<td>0.004*</td>
</tr>
<tr>
<td>Glutathione peroxidase (U/gHb)</td>
<td>48.8±16.3</td>
<td>53.7±4.2</td>
<td>0.33</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (U/gHb)</td>
<td>19.3±1.9</td>
<td>17.7±1.2</td>
<td>0.03*</td>
</tr>
<tr>
<td>Glutathione (mg/100 ml red blood cells)</td>
<td>92.5±10.2</td>
<td>72.8±15.3</td>
<td>0.002*</td>
</tr>
<tr>
<td>α-Tocopherol (μg/ml red blood cells)</td>
<td>3.45±0.83</td>
<td>2.67±1.36</td>
<td>0.12</td>
</tr>
<tr>
<td>Methaemoglobin (% total Hb)</td>
<td>0.59±0.32</td>
<td>0.96±0.44</td>
<td>0.03*</td>
</tr>
<tr>
<td>Malondialdehyde (μM)</td>
<td>16.8±6.2</td>
<td>19.8±7.3</td>
<td>0.35</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05).
Discussion

The results of these experiments provide further evidence to implicate cyclosporine A as a cause of the altered erythrocyte redox balance observed in transplant recipients. Our results show that 7 days of cyclosporine A administration (25 mg/kg/day) increases oxidative stress and alters antioxidant defences in erythrocytes of male rats. Animals administered for 7 days showed significant increases in erythrocyte methaemoglobin, and the activities of superoxide dismutase and catalase. Cyclosporine A also caused significant decreases in erythrocyte GSH concentration and G6PD activity. In contrast, the administration of a single dose of cyclosporine A (25 mg/kg) did not significantly alter markers of oxidative stress and antioxidant protection.

To our knowledge, this is the first study to examine the effect of cyclosporine A administration on methaemoglobin concentration. Methaemoglobin, a marker of haemoglobin oxidation, is formed when the haem iron loses an electron. This free electron can be transferred to an oxygen molecule, resulting in the formation of the superoxide radical (Winterbourn 1990). Our results show that methaemoglobin was increased after 7 days of treatment, providing evidence of cyclosporine A-induced oxidant stress in the erythrocyte. While the exact cause of increased methaemoglobin in the present study is not known, it has been shown that superoxide and hydrogen peroxide can oxidise haemoglobin to methaemoglobin (Hatherill et al. 1991). Given the increased superoxide dismutase and catalase activity observed in the present study, it is possible that cyclosporine A administration resulted in increased levels of erythrocyte superoxide and hydrogen peroxide, with the resultant oxidation of haemoglobin. Future studies designed to test this hypothesis via the direct measurement of reactive oxygen species are warranted.

Seven days of cyclosporine A administration increased the activities of erythrocyte superoxide dismutase and catalase by 38% and 44% respectively. In our previous study, 18 days of cyclosporine A induced a 30% increase in catalase activity, but showed no change in superoxide dismutase activity (Fletcher et al. 2005). Increased erythrocyte superoxide dismutase activity has also been reported in human transplant recipients (Taylor et al. 1993). The mechanism to explain the increased activity of superoxide dismutase and catalase is not known, although because erythrocytes lack a nucleus, the increased activity indicates that changes in inhibition or activation of the enzymes occurred. It is hypothesised that increased activities of these two enzymes are an adaptive response to increased levels of superoxide and hydrogen peroxide. Future studies designed to test this hypothesis via the direct measurement of reactive oxygen species are warranted. Superoxide and hydrogen peroxide are derived either directly from the plasma (Hatherill et al. 1991), or from oxidative processes within the erythrocyte (Winterbourn 1990). Within the erythrocyte, formation of superoxide can occur when haemoglobin is oxidised, with the transfer of an electron from haem iron to oxygen (Winterbourn 1990). Hydrogen peroxide is also formed within the red cell when superoxide dismutase enzymatically converts superoxide to hydrogen peroxide (Taniguchi 1992). This adaptive response in times of high oxidant stress may protect the integrity of the red cell, as studies have shown that excess superoxide and hydrogen peroxide can lead to lipid peroxidation of the cell membrane and oxidative damage to haemoglobin and membrane proteins (Winterbourn 1990). In contrast to the results of superoxide dismutase and catalase, erythrocyte G6PD activity was reduced by 8% in cyclosporine A treated animals. This finding contrasts that of our most recent study showing no change in G6PD activity after cyclosporine A administration (Fletcher et al. 2005). Differences in the drug administration period and the sex of the animals may explain the discrepancy. While the mechanism to explain the decreased activity in the present study is not known, a decrease in G6PD activity may represent an overwhelmed enzyme system in a cell unable to synthesise the protein. Indeed, a reduction in G6PD activity is likely to decrease the ability of the pentose phosphate pathway to produce NADPH, an important reducing equivalent in the erythrocyte (Chance et al. 1979).

In addition to the antioxidant enzymes, we investigated the effects of cyclosporine A on the important erythrocyte antioxidant GSH. After 7 days of cyclosporine A treatment

| Table 3. Measures of antioxidant status and oxidative stress in the plasma of control and cyclosporine A-treated rats after a single dose of placebo/cyclosporine A. Values are means±S.D. |
|----------------|----------------|----------------|
| Control | Cyclosporine treated | P value |
| Total antioxidant status (mM) | 1.57±0.16 | 1.58±0.13 | 0.89 |
| α-Tocopherol (µM) | 31.1±3.5 | 29.8±4.2 | 0.43 |
| Malondialdehyde (µM) | 21.4±2.4 | 21.7±2.5 | 0.81 |

| Table 4. Measures of antioxidant status and oxidative stress in erythrocytes of control and cyclosporine A-treated rats after a single dose of placebo/cyclosporine A. Values are means±S.D. |
|----------------|----------------|----------------|
| Superoxide dismutase (U/gHb) | 4308±977 | 4318±533 | 0.98 |
| Catalase (U/gHb) | 2563±253 | 2544±188 | 0.84 |
| Glutathione peroxidase (U/gHb) | 54.6±3.9 | 52.6±2.5 | 0.14 |
| Glucose-6-phosphate dehydrogenase (U/gHb) | 15.2±3 | 15.2±2.5 | 0.97 |
| Glutathione (mg/100 ml red blood cells) | 72.8±8.5 | 73.2±10.2 | 0.91 |
| Alpha tocopherol (µg/ml red blood cells) | 4.17±0.41 | 3.91±0.7 | 0.33 |
| Methaemoglobin (% total Hb) | 0.33±0.16 | 0.39±0.22 | 0.45 |
| Malondialdehyde (µM) | 15.5±1.7 | 15.9±1.7 | 0.57 |
erythrocyte GSH concentration was decreased by 21%. Hussein et al. (1997) also reported a decrease in total red cell GSH of 23% as well as an increase in oxidised GSH in cyclosporine A-treated human transplant recipients. Given the importance of GSH for GSH peroxidase activity (Yu 1994) and recycling of erythrocyte ascorbic acid and α-tocopherol (Nutall et al. 1999), it is logical that decreased GSH concentrations may lead to decreased non enzymatic antioxidant protection and a reduced capacity of the red cell to remove hydrogen peroxide.

The increase in plasma creatinine concentration observed in the cyclosporine A treated rats after 7 days has also been reported in previous animal studies (Parra Cid et al. 2003) and is not surprising given that kidney dysfunction is the main complication of cyclosporine A treatment (Parra Cid et al. 2003). The cyclosporine A-treated animals failed to maintain body weight during the 7 day drug treatment phase of the study, a finding previously reported in this model (Wang & Salahudeen 1995). While the mechanism to explain the cyclosporine A-induced weight loss is not known, evidence suggests that oxidative stress may play a role. Nath and Salahudeen (1990) reported somatic weight loss in antioxidant deficient rats. In addition, a study by Wang and Salahudeen (1995) showed that co-administration of α-tocopherol with cyclosporine A administration limited the cyclosporine A-induced weight loss.

The administration of a single dose of cyclosporine A did not alter erythrocyte and plasma redox balance. This findings contradicts that of a previous animal study investigating cyclosporine induced nephrotoxicity (Wang & Salahudeen 1995). An increase in arterial malondialdehyde peaked 2 hr after a single intraperitoneal injection of cyclosporine A (25 mg/kg) and receded to baseline at 6 hr (Wang & Salahudeen 1995). The cyclosporine A dose, vehicle, route of administration, and species were the same as the present single dose study. The method used for the determination of malondialdehyde was the only difference in methodology and may explain the discrepant results. The present study used an HPLC method (Sim et al. 2003) whereas the study by Wang and Salahudeen (1995) used thiobarbituric acid reactive substances (TBARS). It is also possible that absorption of cyclosporine A from the peritoneal cavity was faster in the study by Wang and Salahudeen (1995), explaining the increased concentration of arterial malondialdehyde 2 hr after drug administration. Indeed, it is well established that cyclosporine A has a highly variable absorption in man (Lindholm 1991).

The difference in mean peak drug levels between the 7 day study (5726 μg/l) and the single dose study (1085 μg/l) is due to the cumulative pharmacokinetics of cyclosporine A (Wagner et al. 1987). Therefore, it cannot be ruled out that a single dose of cyclosporine A resulting in a peak level of approximately 6000 μg/l may induce alterations to erythrocyte redox balance, while a single dose resulting in peak levels of approximately 1000 μg/l does not. This point remains to be clarified. Peak cyclosporine A concentrations in human transplant recipients are approximately 1500 μg/l just after transplantation, and are gradually reduced to 600–800 μg/l (Oellerich & Armstrong 2002). Although the peak cyclosporine A levels achieved in the present 7 day study are higher than those used in human transplantation, it is difficult to extrapolate these findings to humans as it has previously been reported that rats are more resistant than humans to the toxic effects of cyclosporine A (Pell et al. 1998).

In summary, the purpose of these experiments was to compare an acute dose (single dose) with prolonged (7 days) cyclosporine A administration (25 mg/kg/day) on erythrocyte redox balance and plasma antioxidant capacity in male rats. Our experiments demonstrate that cyclosporine A administration (25 mg/kg/day) for 7 days alters erythrocyte redox balance but does not alter plasma antioxidant capacity. A single dose of cyclosporine A (25 mg/kg) had no effect on any of the variables measured. These results provide further evidence to suggest that cyclosporine A may be responsible for the altered redox balance observed in the erythrocytes of transplant recipients. Further animal studies are warranted to determine if antioxidant supplementation concurrent with cyclosporine A administration can attenuate the drug induced changes to erythrocyte redox balance.

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References


