α-Tocopherol and α-Lipoic Acid Enhance the Erythrocyte Antioxidant Defence in Cyclosporine A-Treated Rats

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Abstract: The aim of this study was to determine the effects of dietary antioxidant supplementation with α-tocopherol and α-lipoic acid on cyclosporine A (cyclosporine)-induced alterations to erythrocyte and plasma redox balance. Rats were randomly assigned to either control, antioxidant (α-tocopherol 1000 IU/kg diet and α-lipoic acid 1.6 g/kg diet), cyclosporine (25 mg/kg/day), or cyclosporine + antioxidant treatments. Cyclosporine was administered for 7 days after an 8 week feeding period. Plasma was analysed for α-tocopherol, total antioxidant capacity, malondialdehyde, and creatinine. Erythrocytes were analysed for glutathione, methaemoglobin, superoxide dismutase, catalase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, α-tocopherol and malondialdehye. Cyclosporine administration caused a significant decrease in superoxide dismutase activity ($P<0.05$ control versus cyclosporine) and this was improved by antioxidant supplementation ($P<0.05$ cyclosporine versus cyclosporine + antioxidant; $P<0.05$ control versus cyclosporine + antioxidant). Animals receiving cyclosporine and antioxidants showed significantly increased ($P<0.05$) catalase activity compared to both groups not receiving cyclosporine. Cyclosporine administration induced significant increases in plasma malondialdehyde and creatinine concentration ($P<0.05$ control versus cyclosporine). Antioxidant supplementation prevented the cyclosporine induced increase in plasma creatinine ($P<0.05$ cyclosporine versus cyclosporine + antioxidant; $P>0.05$ control versus cyclosporine + antioxidant), however, supplementation did not alter the cyclosporine induced increase in plasma malondialdehyde concentration ($P>0.05$ cyclosporine versus cyclosporine + antioxidant). Antioxidant supplementation resulted in significant increases ($P<0.05$) in plasma and erythrocyte α-tocopherol in both of the supplemented groups compared to non-supplemented groups. In conclusion, dietary supplementation with α-tocopherol and α-lipoic acid enhanced the erythrocyte antioxidant defence and reduced nephrotoxicity in cyclosporine treated animals.

A recent animal study from our group showed that 7 days administration of the commonly used immunosuppressant cyclosporine (25 mg/kg/day) induced changes to erythrocyte and plasma redox balance (Lexis *et al.* 2005). These results indicate that cyclosporine may contribute to the altered erythrocyte and plasma redox balance observed in transplant recipients (Cristol *et al.* 1996; Hussein *et al.* 1997). This is important since cyclosporine-induced oxidative stress is thought to play a role in the premature cardiovascular morbidity observed in transplant recipients (Morris *et al.* 2000; Heitzer *et al.* 2001). Furthermore, inverse associations have been found between cardiovascular disease and antioxidant levels in plasma and erythrocytes (Bonithon-Kopp *et al.* 1997; Nyyssonen *et al.* 1997). It is therefore of interest to determine if antioxidant supplementation can enhance antioxidant defences and reduce cyclosporine-induced oxidative stress in the plasma and erythrocytes of cyclosporine-treated animals.

It is widely believed that an optimum antioxidant supplement contains more than one nutrient (Upston *et al.* 2003), and the combination of α-tocopherol and α-lipoic acid has generated scientific interest (Haramaki *et al.* 1995; Coombes *et al.* 2000). In a study by Coombes *et al.* (2000) the dietary combination of α-tocopherol and α-lipoic acid reduced myocardial lipid peroxidation after ischaemia in aged rats. Haramaki *et al.* (1995) showed that α-tocopherol and dihydrolipoic acid exerted separate and synergistic protective effects against the hypoxic-reoxygenated rat heart.

In the circulation, α-tocopherol is the major lipid soluble antioxidant in plasma lipoproteins and the erythrocyte membrane (Constantinescu *et al.* 1993; Frei 1999). In animal studies, the cyclosporine-induced rise in renal malondialdehyde was prevented by α-tocopherol supplementation (Wang & Salahudeen 1995; Parra *et al.* 1998). It is therefore possible that α-tocopherol may also prevent the cyclosporine-induced rise in plasma malondialdehyde.

α-Lipoic acid is a naturally occurring cofactor within pyruvate dehydrogenase and α-keto-glutarate dehydrogenase and is soluble in both lipid and aqueous phases (Wollin & Jones 2003). Although very little free α-lipoic acid is thought to occur in unsupplemented conditions, free α-lipoic acid has the ability to scavenge superoxide, hydrogen peroxide, hydroxyl radicals, and peroxynitrite, and can also recycle glutathione (GSH), α-tocopherol, and ascorbic acid (Wollin & Jones 2003). *In vitro*, α-lipoic acid decreased plasma susceptibility to oxidation (Marangon *et al.* 1999), was protective against haemolysis of human erythrocytes

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induced by peroxyl radicals (Constantinescu *et al.* 1994), and increased GSH synthesis in isolated human erythrocytes (Han *et al.* 1997). In a study by Bhatti *et al.* (2005) α-lipoic acid attenuated superoxide generation and kidney expression of NADPH oxidase in diabetic rats, and it was concluded that α-lipoic acid improves pathology in diabetes by reducing oxidative stress.

Therefore, the present study was conducted to determine if a dietary combination of α-tocopherol and α-lipoic acid could attenuate the cyclosporine-induced alterations to erythrocyte and plasma redox balance that we have shown previously after 7 days of cyclosporine administration at a dose of 25 mg/kg/day (Lexis *et al.* 2005).

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. Forty-eight male Sprague Dawley rats (3 weeks old) weighing 91.5 ± 8.7 g (mean$\pm$S.D.) were randomly assigned ($n=12$ per group) to one of four experimental groups: 1) control, 2) antioxidant, 3) cyclosporine, or 4) cyclosporine and antioxidant. All rats were fed rat chow and water *ad libitum* and maintained on a 12 hr light/dark photo-period with a room temperature of $21 \pm 2°$. The rat chow given to groups 2) and 4) was supplemented with d-α-tocopheryl acid succinate (1000 IU/kg diet) and α-lipoic acid (1.6 g/kg diet). The supplemented diet was continued until sacrifice. After 8 weeks of antioxidant supplementation, cyclosporine-treated animals were administered with 25 mg/kg of the drug (30 mg/ml dissolved in extra light olive oil and 4% ethanol) via intraperitoneal injection for 7 days. Control and antioxidant groups were injected daily for 7 days with the same volume of the vehicle. Animal weights were recorded daily and cyclosporine doses adjusted to changes in body mass. All injections were performed at approximately the same time of the day. The concentration of α-tocopherol (1000 IU/kg diet) in the supplemented rat chow and the supplementation period (8 weeks) was based on a study reporting significant increases in α-tocopherol concentration in the plasma and erythrocytes of rats after 8 weeks of α-tocopherol supplementation (Machlin & Gabriel 1982). The concentration of α-lipoic acid (1.6 g/kg diet) in the supplemented rat chow was the same as a study by Coombes *et al.* (2000) in aged rats. Food intake was not monitored in the present study because we have shown previously that food intake is similar in rats consuming standard rat chow and those consuming antioxidant-supplemented chow (Coombes *et al.* 2000). It has also been shown that weight loss occurred in cyclosporine-treated rats despite similar food intake to that of rats not receiving cyclosporine (Wang & Salahudeen 1995).

Blood collection. On the day of sacrifice, blood was collected 3 hr after drug administration as it has previously been shown that cyclosporine reaches peak levels approximately 3 hr after intraperitoneal injection in the rat (Ibarra *et al.* 1996). Just prior to exanguination 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 exanguinated via intracardiac puncture. Approximately 5 ml of blood was collected in glass EDTA vacutainer tubes and aliquots of whole blood removed for analysis of cyclosporine concentrations and methaemoglobin. The remaining whole blood was centrifuged at 4° for 10 min. at 800$\times$g. The plasma was then removed, divided into aliquots, and stored at $-80°$ until biochemical analysis of total antioxidant capacity, 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 $-80°$ until biochemical analysis of α-tocopherol and malondialdehyde, and the activities of superoxide dismutase, catalase, glucose-6-phosphate dehydrogenase (G6PD) and GSH peroxidase. Because plasma contains comparatively little superoxide dismutase, catalase and GSH peroxidase compared to the erythrocyte (Halliwell & Gutteridge 1986), these measurements were made only in the erythrocyte.

Cyclosporine A. Peak blood cyclosporine concentrations were determined at the Hunter Area Pathology Service (Hunter Hospital, NSW, Australia) using the EMIT 2000 Specific Assay (Dade Behring – Syva, Deerfield, ILL, 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 glutathione 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) 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 modification of the Sigma Diagnostics kit method (procedure #345-UV) which is based on the spectrophotometric method of Lohr & Waller (1974). G6PD catalyses the first step in the pentose phosphate pathway, oxidising glucose-6-phosphate to 6-phosphogluconate and reducing 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 1μM 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 H_2O_2 produced by the action of uricase on uric acid. Peroxidase uses available H_2O_2 to catalyse the formation of a coloured quinoneimine, however catalase inhibits this reaction by eliminating H_2O_2. 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 H_2O_2 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 min.

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 of enzyme required to inhibit MTT reduction by 50%. All erythrocyte enzyme activities were normalised to haemoglobin concentration.

Malondialdehyde. High performance liquid chromatography (HPLC) was used to determine plasma and erythrocyte malondial-

dehyde 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 concentrations using a modification of the method of Hatam & 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 concentrations were determined using a modification of the method of Taibi & 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). α-Lipoic acid was not assayed in the present study as it has been shown to have a short elimination half-life and there is a wide intersubject variation in time to peak concentration in plasma after oral administration (Teichert *et al.* 1998). This is supported by unpublished observations in rats from our laboratory.

Plasma total antioxidant capacity. Total antioxidant capacity 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 hydrogen peroxide. Antioxidant compounds suppress the absorbance of the ABTS radical cation 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).

Statistical analysis. Comparison between groups for cyclosporine concentrations after the dosing period was made using an independent t-test. Comparison between groups for animal body weight data, and for all markers of antioxidant status and oxidative stress was made by one way ANOVA. If significance was determined, a Tamhane's (antioxidant status/oxidative stress data) or Tukey HSD (body weight data) post hoc test was used. All biochemical data is shown as the mean±S.D. Significance was established at the 95% confidence level ($P<0.05$).

Results

Changes in body mass throughout the 7 day cyclosporine administration period were significantly different ($P<0.05$) between cyclosporine-treated and placebo groups. Placebo-treated animals continued to gain weight (2.9% increase in control group, 4.7% increase in antioxidant group) at the same rate ($P>0.05$ control versus antioxidant) during the cyclosporine administration period. Cyclosporine-treated animals failed to gain weight (0.7% decrease cyclosporine, 0.4% decrease cyclosporine + antioxidant) during the drug treatment period. Mean peak blood cyclosporine concentrations of the cyclosporine treated, and cyclosporine + antioxidant treated animals at sacrifice were not significantly different ($P>0.05$) between groups, and were 3899±1465 μg/l and 3127±1292 μg/l respectively.

Markers of plasma oxidative stress and antioxidant status are presented in table 1. Antioxidant supplementation resulted in a significant increase in α-tocopherol concentration in both of the antioxidant supplemented groups ($P<0.05$ control versus antioxidant; control versus cyclosporine + antioxidant). Cyclosporine administration caused a significant increase in malondialdehyde concentration in both of the cyclosporine-treated groups ($P<0.05$ control versus cyclosporine; control versus cyclosporine + antioxidant). Antioxidant supplementation did not significantly alter the cyclosporine-induced increase in plasma malondialdehyde concentration ($P>0.05$ cyclosporine versus cyclosporine + antioxidant). Plasma creatinine, measured as an assessment of renal function at sacrifice, was significantly increased in the cyclosporine-treated animals ($P<0.05$ control versus cyclosporine). The mean creatinine concentrations of cyclosporine + antioxidant-treated animals were not significantly increased compared to controls ($P>0.05$), and were significantly lower than cyclosporine treated animals ($P<0.05$ cyclosporine versus cyclosporine + antioxidant).

Markers of erythrocyte antioxidant status and oxidative stress are presented in table 2. Cyclosporine administration resulted in a significant decrease in superoxide dismutase activity ($P<0.05$ control versus cyclosporine). Antioxidant supplementation attenuated the cyclosporine-induced decrease in superoxide dismutase activity ($P<0.05$ cylosporine versus cyclosporine+antioxidant), however, activity remained significantly less than controls ($P<0.05$ control versus cyclosporine+antioxidant). The combination of antioxidant supplementation and cyclosporine administration resulted in a significant increase in erythrocyte catalase activity when compared to both groups not receiving cyclosporine ($P<0.05$ control versus cyclosporine + antioxidant;

Table 1.

Measures of antioxidant status, oxidative stress, and renal function in the plasma after 8 weeks antioxidant supplementation and 7 days cyclosporine/placebo administration. Values are means±S.D. Statistical difference at the level of $P<0.05$ using a Tamhane's post hoc test: [c]=different from control, [ao]=different from antioxidant, [csa]=different from cyclosporine.

	Control	Antioxidant	Cyclosporine	Cyclosporine + antioxidant
α-Tocopherol (μM)	22.1±7.3	39.6±7.8[c, csa]	25.3±3.7	37.4±6.7[c, csa]
Total antioxidant capacity (mM)	1.59±0.04	1.64±0.14	1.59±0.06	1.7±0.14
Malondialdehyde (μM)	23.9±2	22.8±2	32.5±5[c, ao]	29.5±4.9[c, ao]
Creatinine (μM)	53.5±1.4	48.6±3.2	68.3±5.2[c, ao]	60.6±6.6[ao, csa]

Table 2.

Measures of erythrocyte antioxidant status and oxidative stress after 8 weeks antioxidant supplementation and 7 days cyclosporine/placebo administration. Values are means±S.D. Statistical difference at the level of P<0.05 using a Tamhane's post hoc test: [c]=different from control, [ao]=different from antioxidant, [csa]=different from cyclosporine.

	Control	Antioxidant	Cyclosporine	Cyclosporine + antioxidant
Superoxide dismutase (U/gHb)	6035±443	3858±528[c]	3053±906[c]	4450±383[c, ao, csa]
Catalase (U/gHb)	4855±931	4981±403	5232±860	6045±598[c, ao]
Glutathione peroxidase (U/gHb)	167.2±28.9	182.9±18.5	167.3±18.1	177.7±19.7
Glucose-6-phosphate dehydrogenase (U/gHb)	22.9±2	24.7±2.1	23±2	22.5±4.4
Glutathione (mg/100 ml RBC)	107.6±11.1	123.9±15.9	103±14.8	119.6±40
Met Hb (% total Hb)	0.12±0.14	0.19±0.13	0.11±0.15	0.07±0.12
α-Tocopherol (µg/ml packed cells)	2.82±0.47	4.86±0.35[c]	3.17±0.28[ao]	5.09±1.22[c, csa]
Malondialdehyde (µM)	16.9±4	17.3±1.5	20.4±2.1	17.8±1.7

antioxidant versus cyclosporine + antioxidant). Antioxidant supplementation resulted in a significant increase in α-tocopherol in both of the supplemented groups (P<0.05 control versus antioxidant; control versus cyclosporine + antioxidant). Antioxidant supplementation alone caused a significant decrease in superoxide dismutase activity (P<0.05 control versus antioxidant).

Discussion

These experiments showed that dietary supplementation with α-tocopherol and α-lipoic acid enhanced the erythrocyte antioxidant defence and reduced nephrotoxicity in cyclosporine-treated rats. Antioxidant supplementation attenuated the cyclosporine-induced decrease in superoxide dismutase activity, and the combination of antioxidant treatment and cyclosporine administration resulted in an increased erythrocyte catalase activity. Antioxidant supplementation prevented the cyclosporine-induced increase in plasma creatinine, however, it did not alter the cyclosporine-induced increase in plasma malondialdehyde concentration.

In the present study, antioxidant supplementation attenuated the cyclosporine-induced decrease in erythrocyte superoxide dismutase activity, indicating an improved ability of the erythrocyte to remove superoxide enzymatically. It is hypothesised that α-lipoic acid provided an additional mechanism for the direct removal of superoxide, thus sparing superoxide dismutase under conditions of elevated reactive oxygen species. A limitation of the present study is the failure to measure reactive oxygen species and future studies designed to test this hypothesis via the direct measurement of superoxide are warranted.

Animals receiving both cyclosporine and antioxidants had higher erythrocyte catalase activity compared to animals not receiving cyclosporine, indicating an enhanced ability to remove hydrogen peroxide from the circulation. We hypothesise that the increased activity is due to increased oxidative stress in conjunction with antioxidant supplementation. A potential source of plasma hydrogen peroxide is the endothelium (Griendling *et al.* 2000) and Longoni *et al.* (2001) reported an increased production of cellular oxidants after incubation of human endothelial cells with cyclosporine. It has been shown that human erythrocyte catalase contains tightly bound NADPH which is effective in preventing and reversing inactivation of the enzyme (Kirkman *et al.* 1987). It is possible in the present study that antioxidant supplementation indirectly caused an increased availability of erythrocyte NADPH leading to increased catalase activity. A limitation of the present study is the failure to measure hydrogen peroxide levels. Future studies designed to test this hypothesis via the direct measurement of hydrogen peroxide and NADPH concentrations are warranted.

Supplementation with α-tocopherol and α-lipoic acid prevented the cyclosporine-induced rise in plasma creatinine. This supports the findings of previous studies showing that α-tocopherol treatment prevented the cyclosporine-induced reduction in creatinine clearance in rats (Wang & Salahudeen 1995; Parra *et al.* 1998). These results are not surprising given that kidney dysfunction is a major complication of cyclosporine treatment, and available evidence suggests a therapeutic role for antioxidants in the prevention of cyclosporine-induced nephrotoxicity (Parra Cid *et al.* 2003). It has been shown that α-tocopherol inhibited the synthesis of renal reactive oxygen species and improved renal function and histological alteration in cyclosporine-treated animals (Parra Cid *et al.* 2003).

Supplementation with α-tocopherol and α-lipoic acid failed to attenuate the cyclosporine-induced increase in plasma malondialdehyde, indicating that either the dose or antioxidant combination was insufficient to prevent lipid peroxidation. In contrast, Naidu *et al.* (1999) showed that co-administration of lacidipine (a calcium channel blocker with an antioxidant effect) and cyclosporine to rats prevented the drug induced rise in plasma malondialdehyde. Similarly, Kumar *et al.* (1999) showed that co-administration of melatonin and cyclosporine had the same effect. Potential sources of enhanced reactive oxygen species formation after cyclosporine administration include the vasculature (Griendling *et al.* 2000) and the superoxide producing P450 enzyme systems in the kidney and liver (Yoshimura *et al.* 1993). Future studies should be designed to determine the relationship between reactive oxygen species in the circulation and lipid peroxidation products.

In the present study, antioxidant supplementation resulted in significant increases in plasma and erythrocyte α-tocopherol concentration. These results support a previous study showing increases in α-tocopherol concentration in the plasma and erythrocytes of rats after a similar supplementation protocol (Machlin & Gabriel 1982). This finding may prove to be important for transplant recipients, as epidemiologic studies have shown inverse associations between both plasma and erythrocyte α-tocopherol concentration, and the incidence of cardiovascular disease (Gey & Puska 1989; Bonithon-Kopp *et al.* 1997). Indeed, it has been reported that transplant recipients show decreased concentrations of erythrocyte α-tocopherol (Cristol *et al.* 1996). Furthermore, a primary prevention study of 40 cardiac transplant recipients 0–2 years after transplantation showed that a combination of α-tocopherol and vitamin C supplementation for 1 year retarded the progression of coronary artery arteriosclerosis (Fang *et al.* 2002).

The cyclosporine-induced alterations to erythrocyte redox balance shown in the present study differ from a previous study from our group despite the use of the same cyclosporine administration protocol (Lexis *et al.* 2005). In our previous study, cyclosporine administration for 7 days resulted in increased methaemoglobin, increased superoxide dismutase and catalase activities, and decreased GSH concentration and G6PD activity (Lexis *et al.* 2005). The mean peak cyclosporine levels (5726 μg/l) were significantly higher ($P<0.05$) than the levels of the present study (3899 μg/l and 3127 μg/l for the cyclosporine, and cyclosporine+antioxidant-treated groups, respectively). The source of cyclosporine A in our previous study (Lexis *et al.* 2005) (Research Biology Institute (RBI) product #C224) differed from that of the present study (Fluka product #30024), and although unexpected, may explain the significantly different mean blood concentrations. It is possible that the lower concentrations of cyclosporine in the present study resulted in decreased concentrations of reactive oxygen species in the circulation and more moderate alterations to erythrocyte redox balance. A potential source of vascular superoxide is the endothelium (Griendling *et al.* 2000) and Lopez-Ongil *et al.* (1998) reported a cyclosporine-induced dose-dependent increase of reactive oxygen species synthesis in bovine cultured aortic endothelial cells. To our knowledge, the mechanism to explain alterations to erythrocyte antioxidant enzyme activity in the absence of cellular protein synthesis is not known, however, it is hypothesised that it is related to changing levels of oxidative stress within the erythrocyte. This may explain the controversial results between studies and is an exciting area for future research.

In summary, these experiments show that dietary supplementation with α-tocopherol and α-lipoic acid enhanced the erythrocyte antioxidant defence and reduced nephrotoxicity in cyclosporine-treated rats. Antioxidant supplementation attenuated the cyclosporine-induced decrease in superoxide dismutase activity, and the combination of antioxidant treatment and cyclosporine administration resulted in an increased erythrocyte catalase activity. Antioxidant supplementation prevented the cyclosporine-induced increase in plasma creatinine, however, it did not alter the cyclosporine-induced increase in plasma malondialdehyde. Future studies should measure reactive oxygen species concentrations as a result of cyclosporine administration to test the hypothesis that a cyclosporine-induced increase in vascular reactive oxygen species alters erythrocyte redox balance. Additional studies should also determine the individual effects of dietary supplementation with α-lipoic acid and α-tocopherol on cyclosporine-induced oxidative stress in the erythrocyte because the mechanism by which α-lipoic acid exerts its antioxidant effects are not completely understood. Although the relevance of these findings to the clinical setting is yet to be established, the results suggest that antioxidant supplementation with α-tocopherol and α-lipoic acid may be beneficial in the management of cyclosporine-dependent patients.

Acknowledgements

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