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Decreased Oxidized Glutathione with Aerosolized Cyclosporine Delivery

AVIVA KATZ, M.D., ARNOLD G. CORAN, M.D., KEITH T. OLDHAM, M.D.,* AND KAREN S. GUICE, M.D.*

*Section of Pediatric Surgery, Department of Surgery, University of Michigan, Ann Arbor, Michigan 48109-0245; and *Department of Surgery, Duke University, Durham, North Carolina 27706*

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Cyclosporine immunosuppression remains vital for successful lung transplantation. Cyclosporine also functions as a membrane active biological response modifier and has been noted to have a variable effect on ischemia–reperfusion (I/R) injury in various tissues. Glutathione plays an important role in the endogenous antioxidant defense system; plasma oxidized glutathione (GSSG) levels are useful as a sensitive indicator of *in vivo* oxidant stress and I/R injury. Lung transplantation results in ischemia, followed by a period of reperfusion, potentially producing functional injury. This study was designed to evaluate the effect of cyclosporine on oxygen radical generation in a model of single-lung transplantation. Single-lung transplantation was performed in 12 mongrel puppies, with animals assigned to receive either intravenous or aerosolized cyclosporine. Arterial blood and bronchoalveolar lavage fluid (BALF) samples were obtained to determine GSSG levels via a spectrophotometric technique. Samples were obtained both prior to and following the revascularization of the transplanted lung. Whole blood and tissue cyclosporine levels were determined via an high-performance liquid chromatography technique 3 hr following the completion of the transplant. Aerosolized cyclosporine administration resulted in greatly decreased arterial plasma and BALF GSSG levels, whole blood cyclosporine levels, and equivalent tissue cyclosporine levels when compared to intravenous cyclosporine delivery. These findings support the hypothesis that the transplanted lung is a source of GSSG production and release into plasma. Additionally, these findings suggest that cyclosporine may have a direct antioxidant effect on pulmonary tissue, with this activity occuring at the epithelial surface, an area susceptible to oxidant injury.

INTRODUCTION

Significant progress has been made in the field of lung transplantation since the initial work resulting in the first human lung transplant in 1963 by Hardy [1]. Indications for lung transplant in the pediatric population now include cystic fibrosis, lymphocytic interstitial pneumonitis, obliterative bronchiolitis, bronchopulmonary dysplasia, and pulmonary hypoplasia [2, 3]. The introduction of cyclosporine has allowed improved control of rejection and prolonged graft survival, although cyclosporine nephrotoxicity, hepatotoxicity, and central nervous system side effects remain significant complications. Lung tissue has a high avidity for cyclosporine and local aerosolized therapy with cyclosporine provides high tissue levels of cyclosporine with low systemic delivery [4, 5]. Previous work with aerosolized delivery of cyclosporine has demonstrated that the drug may also act along other, nonimmunologic, pathways [4]. Cyclosporine functions as a membrane active biological response modifier, with an ability to disturb phospholipid membranes [6]. Additionally, cyclosporine may also act as an inhibitor of mitochondrial respiration [7]. The toxicity of cyclosporine to the non-T cell and other nonimmunosuppressive functions remain unclear.

Lung tissue becomes ischemic during clinical transplantation with hypoxia of alveolar and pulmonary endothelial cells. Reperfusion injury follows reoxygenation [8–10]. The tissue damage with reperfusion is rapid; free oxygen radicals produce lipid peroxidation of cell membranes with cytolysis and edema formation. When compared with other solid organs, the lung appears particularly sensitive to ischemia–reperfusion injury [11, 12]. The problem of whole organ preservation for transplantation may be improved by modification of free radical metabolism [13]. Glutathione, L-γ-glutamyl-L-cysteinylglycine, the most abundant low-molecular-mass intracellular thiol, and the glutathione redox cycle play an important role in the endogenous antioxidant defense system [14, 15]. The turnover rate of glutathione in the lung is high, and glutathione is required to maintain lung mitochondrial integrity [16]. Oxidized glutathione, GSSG, is toxic to cells and is extruded into plasma by active transport via a membrane bound Ca/ATP mechanism. The rate of this is dependent on intracellular GSSG concentrations [17, 18]. With this system, small

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changes in intracellular GSSG and glutathione redox state are amplified in the plasma [19]. Plasma GSSG may therefore be utilized as a reliable, repeatable, and sensitive indicator of *in vivo* oxidant stress and ischemic–reperfusion injury [20, 21, 22, 23].

This study was designed to evaluate the effect of cyclosporine on oxygen radical generation, as measured by oxidized glutathione production, in a canine model of single-lung transplantation. The use of both aerosolized and intravenous drug delivery systems allows differentiation of the local effects from the systemic effects of cyclosporine. The data presented suggest a direct, local antioxidant effect of cyclosporine.

METHODS

Animal Model

Twelve 3- to 5-kg mongrel puppies were studied. Experimental protocols were approved by the University of Michigan Committee on the Use and Care of Animals in accordance with guidelines from the Federal Animal Welfare Act and The Guide for the Care and Use of Laboratory Animals. All animal experiments were conducted using barbiturate anesthesia, with final organ harvesting conducted following hyperkalemic euthanasia under level III pentobarbital anesthesia in compliance with the Panel on Euthanasia of the American Veterinary Medical Association.

The experimental animals were ventilated with a Harvard volume-cycled ventilator at volumes of 15 to 20 cc/kg at rates of 20 to 25 breaths/min titrated to an arterial carbon dioxide tension of 30 to 40 mm Hg. Additionally, supplemental oxygen was blended into the circuit resulting in final inspired oxygen fractions of 0.30 to 0.70 to maintain arterial oxygen tensions between 60 and 120 mm Hg. All animals were maintained with a central intravenous infusion of lactated Ringers, requiring approximately 100 to 150 cc/kg of crystalloid throughout the periods of anesthetic induction, surgery, and postoperative resuscitation and study. Animals were monitored with arterial pressure, central venous pressure, pulmonary artery wedge pressure, and cardiac output determinations. Arterial and venous blood gases were monitored throughout the surgery and the study period.

Surgical Methods

All experimental animals underwent a standard left posterolateral thoracotomy with left pneumonectomy. This was followed by implantation of the donor lung with sequential atrial cuff, pulmonary artery, and bronchial anastomosis. The vascular anastomoses were completed with running 7-O prolene suture using microvascular technique. The bronchial anastomosis was completed using interrupted 6-O vicryl suture; the left mainstem bronchus was used for implantation, and as these were acute models, no further attempt was made to protect this anastomosis with a pedicle of omentum or pleura. The chest wall was loosely approximated by skin closure to decrease evaporative losses during the remainder of the study period.

The donor lung was prepared by immediate irrigation with cold EuroCollins solution via the pulmonary artery, each lung receiving approximately 150 cc of flush to clear it of all remaining blood. The lung was maintained in a deflated state in 4°C preservative solution for a 2-hr ischemic period prior to implantation.

Cyclosporine Administration

Animals were assigned to receive either intravenous or aerosolized cyclosporine. Intravenous cyclosporine, (Sandimmune; Sandoz, East Hanover, NJ) was obtained from the hospital pharmacy. Animals assigned to receive intravenous cyclosporine received a single dose of 7 mg/kg following anesthetic induction and the placement of monitoring lines. Cyclosporine for intravenous use was diluted in 20 cc of normal saline and administered via a central venous/fluid resuscitation line over 20 to 30 min. All animals were monitored during this period of drug administration and there were no episodes of hemodynamic instability or respiratory distress.

For aerosol treatment, 5 mg/kg of pure cyclosporine (Sandimmune) in powdered form was dissolved in 5 cc of normal saline by continuous mixing for 24 hr at room temperature. This solution was administered with the use of a standard respiratory nebulizer connected in series with the ventilator circuit, with a continuous 2- to 3-liter flow of oxygen providing adequate pressure for drug delivery. Twenty to 30 min were required to complete drug administration via this route, with aerosolized cyclosporine delivery started immediately upon completion of the bronchial anastomosis. Again, all animals were monitored during the period of nebulizer therapy, and no respiratory or hemodynamic complications were observed; specifically, there was no disturbance of ventilation or oxygenation during this time period. The nebulizer was removed from the ventilator circuit following completion of the period of drug delivery.

Tissue Sampling

Cyclosporine levels. Whole blood obtained from an indwelling central venous monitoring line, separate from the cyclosporine infusion line, was collected in heparinized syringes 3 hr following completion of transplantation in all animals. Blood was immediately transferred to EDTA-prepared tubes and stored at room temperature prior to analysis.

The native and transplanted lungs from the recipient animal were harvested immediately following hyperkalemic euthanasia under deep barbiturate anesthesia, 3 hr following completion of the lung transplant. Approximately 30 g of tissue was sharply excised from the infe-

rior portion of the upper lobe from both the right (native) and left (transplanted) lungs. This tissue was placed immediately on ice and stored frozen at −70°C until tissue cyclosporine level analysis.

In preparation for analysis of tissue cyclosporine levels, the frozen tissue was finely ground under liquid nitrogen. One gram of this prepared tissue was stored in 0.1 *N* HCl for 48 to 72 hr prior to sample analysis, to aid in tissue homogenization.

Glutathione analysis. The donor lung underwent bronchoalveolar lavage (BAL) at the following times: harvest, after irrigation with EuroCollins solution, prior to implantation, and after the completion of the vascular anastomoses. Bronchoalveolar lavage was carried out using a soft rubber catheter with instillation of 10 cc of normal saline into a dependent bronchial segment, with a consistent return of approximately 7 cc of lavage fluid. This return was immediately centrifuged with the supernatant kept iced in preparation for glutathione assay.

Central venous and aortic arterial blood samples were obtained from the experimental animals following the induction of anesthesia (T0), after pulmonary artery ligation (T1), at the completion of recipient pneumonectomy (T2), following reestablishment of blood flow to the donor lung (T3), at the completion of the transplant (T4), and 1 (T5), 2 (T6), and 3 (T7) hr postoperatively. All samples were prepared for glutathione assay.

Cyclosporine level analysis, blood samples. Whole blood cyclosporine levels were determined by high-performance liquid chromatographic analysis utilizing a microbore column. This allowed determination of cyclosporine levels with minimal sample volume (0.5 ml) and excellent limits of cyclosporine detection (to 20 ng/ml).

Specimens were acidified with HCl and then extracted using methyl-*t*-butyl ether. Following centrifugation, the ether portion was transferred to another extraction tube and further purified by the addition of and mixing with NaOH. The ether portion was transferred again and evaporated to dryness under nitrogen. The dried extract was reconstituted with mobile phase and hexane, vortexed, and centrifuged to separate the two phases, and the lower mobile phase was injected onto the microbore column. The amount of cyclosporine injected onto the column was quantitated from the standard curve created with a known working standard of cyclosporine created with heparinized whole blood [24].

Cyclosporine level analysis, tissue samples. Tissue cyclosporine levels were prepared by homogenization in 0.1 *N* HCl to a fine suspension. An aliquot of this homogenate was then assayed in a manner similar to the blood chromatographic procedure following tissue extraction with methyl-*t*-butyl ether. The amount of cyclosporine was quantitated with the standard curve of the blood cyclosporine assay and the tissue concentration was calculated from the tissue weight and initial dilution of the sample.

Glutathione Assay

The method of Adams *et al.* [19] was modified as outlined. Whole blood was collected in heparin-coated syringes. For total glutathione (GSH), 200 μl of fresh whole blood was added to 200 μl of a buffer containing 10 m*M* 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 17.5 m*M* disodium EDTA, and 100 m*M* potassium phosphate, pH 7.5. Samples were mixed by tilting and then centrifuged 6 min at 2000*g*. The supernatant was frozen at −70°C until analysis.

To assay for GSSG, 200 μl of fresh whole blood was added to a buffer containing 10 m*M* *N*-ethylmaleimide (NEM), 17.5 m*M* disodium EDTA, and 100 m*M* potassium phosphate, pH 6.5. Mixing and centrifugation were as previously detailed. NEM derivatizes all reduced GSH, leaving only GSSG for measurement by the fluorometric assay. The sample was then passed through a C18 Sep Pak cartridge (Waters Associates, Framingham, MA) which was prewashed with 1 ml methanol followed by 2 ml distilled water. Each sample was rinsed from the cartridge with 1 ml phosphate–imidazole buffer (0.01 *M* Na_2HPO_4 and 0.017 *M* imidazole, pH 7.2). The elute was frozen for analysis.

Plasma GSH undergoes rapid autooxidation to GSSG *in vitro* and this can be prevented by the use of NEM in sample preparation, with NEM removed on the Sep Pak cartridge prior to final fluorometric assay [17, 19, 21].

A standard curve was generated using known amounts of GSH (Sigma, St. Louis, MO). Determination of GSH equivalents was made in plastic cuvettes containing 700 μl 0.3 m*M* DTNB, 700 μl 0.4 m*M* NADPH (Sigma), and 1.2 units/ml glutathione reductase (Sigma, Type III) to which 700 μl of the experimental sample was added for NEM-treated samples for GSSG determination. For total GSH determination, 50 μl of the supernatant was used with the volume difference corrected with phosphate–imidazole buffer. The difference in optical density was determined at 412 nm on an LKB Ultraspec II spectrophotometer (Model 4050; Biochrom, Cambridge, England). Concentrations of GSSG and total GSH were then determined by reverse linear regression against the standard curve.

BAL fluid was collected in prepared cuvettes containing the appropriate buffers with equivalent sample analysis.

RESULTS

Statistical evaluation of all specimen collections was by an analysis of variance (ANOVA) with the assistance of Stat View II computer software. Significance was defined as $P < 0.05$ and individual comparisons are indicated in the text.

This is a reproducible model of single-lung transplantation in an immature animal model with a size approximating that of a human infant. Arterial plasma and

bronchoalveolar lavage fluid (BALF) GSSG levels were chosen as end points because previous work with this canine model demonstrated that changes in plasma or tissue GSSG levels were more sensitive indicators of an ischemia–reperfusion injury than changes in GSH. Additionally, changes in arterial plasma GSSG levels preceeded changes in the venous compartment. We were able to demonstrate significant differences in whole blood cyclosporine levels between animals receiving intravenous vs aerosolized cyclosporine, and this is correlated with a significant difference in GSSG production.

Arterial Plasma Oxidized Glutathione Levels

GSSG was barely detectable in baseline, anesthetized canine plasma. Despite the significant surgical stresses of thoracotomy and pneumonectomy, there was no significant increase in arterial GSSG level until completion of the vascular anastomoses (T3) and reestablishment of blood flow to the ischemic transplanted lung. The arterial GSSG level continued to rise throughout the study period, despite completion of the bronchial anastomosis and return of ventilation and oxygenation to the pulmonary parenchyma. Following completion of the vascular anastomoses there was a significant difference in GSSG levels between the animals receiving aerosolized versus intravenous cyclosporine. Animals which received aerosolized cyclosporine had greatly decreased GSSG levels, with a minimal, insignificant rise during the surgical and postoperative observation period. These animals did not demonstrate the significant increase in GSSG levels seen in the animals which received intravenous cyclosporine and had almost complete inhibition of the rise in GSSG seen with pulmonary reperfusion (Fig. 1).

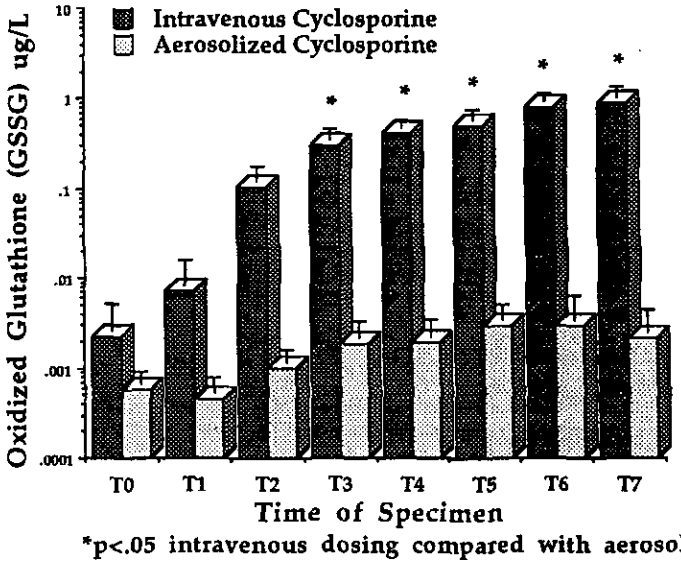

FIG. 1. Arterial plasma GSSG levels: aerosolized vs intravenous cyclosporine administration.

Bronchoalveolar Lavage Fluid Oxidized Glutathione Levels

There was minimal detectable GSSG in the BALF collected at the time of lung harvest and immediately following EuroCollins perfusion. Again, when evaluating BALF GSSG, significant differences were noted between the animals which received aerosolized versus intravenous cyclosporine. Animals which received intravenous cyclosporine demonstrated an increase in BALF GSSG following the period of cold ischemia, with a significant increase occurring with completion of the vascular anastomoses. Animals which received aerosolized cyclosporine demonstrated no increase in BALF GSSG during the periods of cold ischemia and transplantation. This response was significantly different from that of the animals receiving intravenous cyclosporine, again demonstrating inhibition of the rise in GSSG seen with pulmonary reperfusion (Fig. 2).

Cyclosporine Levels, Tissue and Whole Blood

Animals receiving intravenous cyclosporine attained a mean whole blood cyclosporine level of 410 ng/ml approximately 5 to 6 hr following drug administration, with a therapeutic whole blood cyclosporine level throughout the peritransplant period. The animals which received aerosolized cyclosporine had a mean whole blood cyclosporine level of only 52 ng/ml, with several animals having whole blood levels too low to detect with the chromatographic assay. In the absence of data concerning tissue levels of cyclosporine, this would be considered a subtherapeutic, nonimmunosuppressive whole blood level. These differences are significant with $P < 0.001$.

Lung tissue cyclosporine levels were not significantly different in animals receiving intravenous vs aerosolized therapy. The drug level in the transplanted lung in animals which received aerosolized cyclosporine was 2133 ng/g tissue (mean), while the corresponding level in the intravenously treated group was 1890 ng/g tissue (mean). These differences were not statistically significant, with $P > 0.05$ (Fig. 3).

DISCUSSION

Transplantation involves the reperfusion of an organ rendered ischemic, usually in conjunction with an attempt to prolong the acceptable period of ischemia by modifying the extent of cellular deterioration [9]. Studies in liver, kidney, and heart tissue have demonstrated a loss of tissue glutathione in warm ischemia and tissue glutathione catabolism during cold storage. As depletion of glutathione sensitizes cells to oxidative injury, this loss of tissue glutathione, or the inability to regenerate glutathione, may be a cause of the loss of organ viability [25]. *De novo* glutathione biosynthesis and reduction from its oxidized state may be inadequate in these conditions and this loss of intracellular glutathione levels may be critical determinants of the early cellular response to injury [26]. The adequacy of antioxidant protection at the epithelial surface is of primary importance

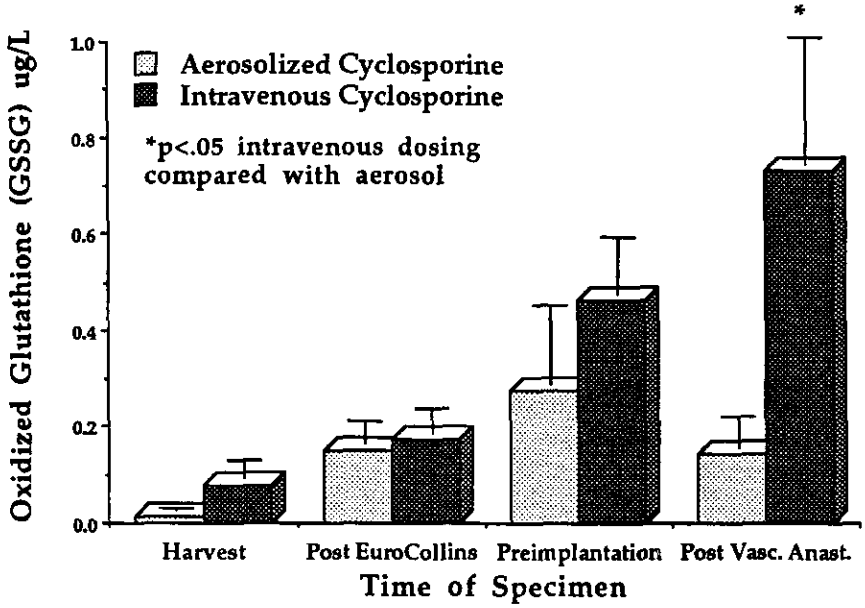

FIG. 2. Bronchoalveolar lavage fluid GSSG levels: aerosolized vs intravenous cyclosporine administration.

in the lung. Intracellular glutathione stores may be inadequate to protect this epithelial surface, which faces an oxygen tension three times higher than that of other tissues [27, 28]. Inadequate reduced glutathione stores may lead to pulmonary parenchymal injury, with type 2 alveolar cell lamellar body damage and disintegration, and a resultant decrease in surfactant production with associated pulmonary dysfunction [28, 29]. Glutathione is also active in the process of reductive and conjugative detoxification of cyclosporine and its metabolites, with active oxygen formed during drug metabolism [30, 31].

The data provided demonstrate significant reduction in oxidized glutathione levels in both arterial plasma and bronchoalveolar lavage fluid in a canine single-lung transplant model treated with aerosolized cyclosporine. The use of aerosolized cyclosporine, which resulted in direct lung exposure to cyclosporine but minimal whole blood levels of cyclosporine, abolished any increase in BALF GSSG in response to cold ischemia or transplantation. This was associated with an insignificant rise in arterial plasma GSSG during the surgical and postoperative study period. Intravenous cyclosporine administration resulted in therapeutic whole blood cyclosporine levels, with equivalent tissue levels of cyclosporine as compared to the aerosolized group. Animals which received intravenous cyclosporine developed a significant rise in BALF and arterial plasma GSSG despite tissue cyclosporine levels equivalent to animals receiving aerosolized drug. These findings support the hypothesis that the transplanted lung is the source of oxidized glutathione production and extrusion in this single-lung transplant model. Additionally, these findings suggest that cyclosporine may have a direct antioxidant effect on pulmonary tissue, with this activity occuring at the epithelial surface, an area susceptible to oxidant injury.

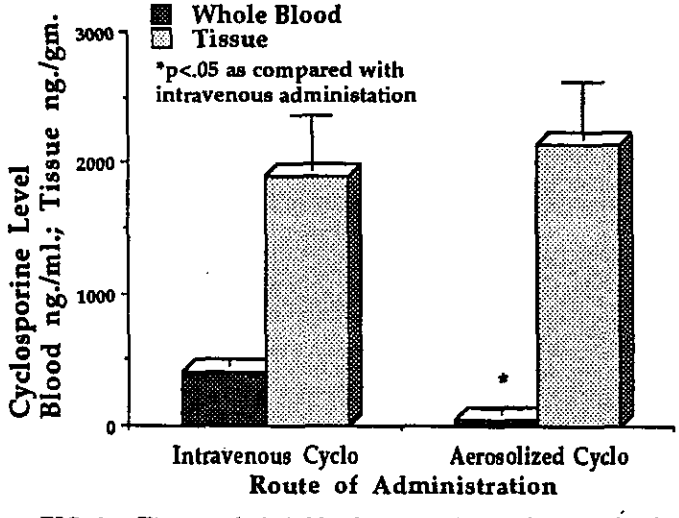

FIG. 3. Tissue and whole blood cyclosporine levels: aerosolized vs intravenous administration.

Cyclosporine has been noted to have a variable effect on intracellular glutathione levels and ischemia–reperfusion injury in various tissues [32]. In a model of hepatic ischemia, cyclosporine was noted to ameliorate reperfusion, although not isolated ischemic, liver injury [33]. Others using models in which cyclosporine is administered have correlated a decrease in intracellular glutathione stores with increased systemic cyclosporine levels [33, 34]. The inconsistencies among these studies which suggest that cyclosporine may be either an antioxidant or a prooxidant may involve a variety of other factors. Cyclosporine is a very lipophilic agent and demonstrates a differential avidity for various tissues, which

will alter its effect on each organ system obviously [30, 33]. Route and timing of drug administration also vary the effect of cyclosporine on intracellular glutathione stores [31]. The data presented here suggest that aerosolized cyclosporine may function as a direct antioxidant at the epithelial surface in a single-lung transplant model. Furthermore, these data suggest that the aerosolized route may have important advantages, not only in terms of diminished systemic toxicity, but in providing enhanced antioxidant activity at the alveolar epithelial surface during a time of reperfusion. As cyclosporine administration remains a vital component of the lung transplantation protocol, and ischemia–reperfusion injury continues to be a significant factor in early graft dysfunction, we feel that this potential nonimmunosuppressive function of cyclosporine deserves further investigation.

REFERENCES

1. Hardy, J. D., Webb, W. K., Dalton, M. L., Jr., *et al.* Lung homotransplantation in man: Report of the initial case. *J. Am. Med. Assoc.* **186:** 1065, 1963.
2. Bolman, R. M., Shumway, S. J., Estrin, J. A., *et al.* Lung and heart–lung transplantation. *Ann. Surg.* **214:** 456, 1991.
3. Backer, C. L., Ohtake, S., Zales, V. R., *et al.* Living-related lobar lung transplantation in beagle puppies. *J. Pediatr. Surg.* **26:** 429, 1991.
4. Keenan, R. J., Duncan, A. J., Yousem, S. A., *et al.* Improved immunosuppression with aerosolized cyclosporine in experimental pulmonary transplantation. *Transplantation* **53:** 20, 1992.
5. Dowling, R. D., Zenati, M., Burckart, G. J., *et al.* Aerosolized cyclosporine as single-agent immunotherapy in canine lung allografts. *Surgery* **108:** 198, 1990.
6. Cairo, M. S., Toy, C., Sender, L., *et al.* Effect of idarubicin and epirubicin on in vitro polymorphonuclear function: Diminished superoxide radical formation compared to their parent compounds daunorubicin and doxorubicin. *J. Leukocyte Biol.* **47:** 224, 1990.
7. Humes, H. D., Jackson, N. M., O'Conner, R. P., *et al.* Pathogenetic mechanism of nephrotoxicity: Insights into cyclosporine nephrotoxicity. *Transplant. Proc.* **17:** 51, 1985.
8. Jenkinson, S. G., Marcum, R. F., Pickard, J. S., *et al.* Glutathione disulfide formation occurring during hypoxia and reoxygenation of rat lung. *J. Lab. Clin. Med.* **112:** 471, 1988.
9. Paull, D. E., Keagy, B. A., Kron, E. J., *et al.* Reperfusion injury in the lung preserved for 24 hours. *Ann. Thorac. Surg.* **47:** 187, 1989.
10. Bryan, C. L., Cohen, D. J., Dew, J. A., *et al.* Glutathione decreases the pulmonary reimplantation response in canine lung autotransplants. *Chest* **100:** 1694, 1991.
11. Heberer, M., Jorgensen, J., Mihatsch, M. J., *et al.* Protective effect of allopurinol and superoxide dismutase in renal isografts in Cyclosporin A treated rats. *Renal Failure* **13:** 233, 1991.
12. Keenan, R. J., Griffith, B. P., Kormos, R. L., *et al.* Increased perioperative lung preservation injury with lung procurement by Euro-Collins solution flush. *J. Heart Lung Transplant.* **10:** 650, 1991.
13. Parks, D. A., Bulkley, G. B., and Granger, D. N. Role of oxygen free radicals in shock, ischemia, and organ preservation. *Surgery* **94:** 428, 1983.
14. Mannervick, B. The enzymes of glutathione metabolism: An overview. *Biochem. Soc. Trans.* **15:** 717, 1987.
15. Harlan, J. M., Levine, J. D., Callahan, K. S., *et al.* Glutathione redox cycle protects cultured endothelial cells against lysis by extracellularly generated hydrogen peroxidase. *J. Clin. Invest.* **73:** 706, 1984.
16. Martensson, J., Jain, A., Frayer, W., *et al.* Glutathione metabolism in the lung: Inhibition of its synthesis leads to lamellar body and mitochondrial defects. *Proc. Natl. Acad. Sci. USA* **86:** 5296, 1989.
17. Chang, S. W., Lauterburg, B. H., and Voelkel, N. F. Endotoxin causes neutrophil-independent oxidative stress in rats. *J. Appl. Physiol.* **65:** 356, 1988.
18. Kondo, T., Kawakami, Y., Taniguchi, N., *et al.* Glutathione disulfide stimulated Mg2+-ATPase of human erythrocyte membranes. *Proc. Natl. Acad. Sci. USA* **84:** 7373, 1987.
19. Adams, J. D., Jr., Lauterburg, B. H., and Mitchell, J. R. Plasma glutathione and glutathione disulfide in the rat: Regulation and response to oxidative stress. *J. Pharmacol. Exp. Ther.* **227:** 749, 1983.
20. Jackson, R. M., and Veal, C. F. Effects of hypoxia and reoxygenation on lung glutathione system. *Am. J. Physiol.* **259:** H518, 1990.
21. Hughes, H., Jaeschke, H., and Mitchell, J. R. Measurement of oxidant stress in vivo. *Methods Enzymol.* **186:** 681, 1990.
22. Nemith, J., and Boda, D. The ratio of oxidized/reduced glutathione as an index of oxidative stress in various experimental models of shock syndrome. *Biomed. Biochem. Acta* **48:** 553, 1989.
23. Abdalla, E. K., Caty, M. G., Guice, K. S., *et al.* Arterial levels of oxidized glutathione (GSSG) reflect oxidant stress in vivo. *J. Surg. Res.* **48:** 291, 1990.
24. Annesley, T. M., Matz, K., Balogh, L., *et al.* Liquid chromatographic analysis for cyclosporine with use of a microbore column and small sample volume. *Clin. Chem.* **32:** 1407, 1986.
25. Vreugdenhil, P. K., Belzer, F. O., and Southard, J. H. Effect of cold storage on tissue and cellular glutathione. *Cryobiology* **28:** 143, 1991.
26. Linas, S. L., Shanley, P. F., White, C. W., *et al.* Oxygen metabolite mediated injury in perfused kidneys is reflected by consumption of DMTU and glutathione. *Am. J. Physiol.* **253:** F692, 1987.
27. Cantin, A. M., North, S. L., Hubbard, R. C., *et al.* Normal alveolar epithelial lining fluid contains high levels of glutathione. *J. Appl. Physiol.* **63:** 152, 1987.
28. Canitn, A. M., and Begin, R. Glutathione and inflammatory disorders of the lung. *Lung* **169:** 123, 1991.
29. Jain, A., Martensson, J., Mehta, T., *et al.* Ascorbic acid prevents oxidative stress in glutathione deficient mice: Effects on lung type 2 cell lamellar bodies, lung surfactant, and skeletal muscle. *Proc. Natl. Acad. Sci. USA* **89:** 5093, 1992.
30. Duruibe, V. A., Okonmah, A., and Blyden, G. T. Effect of ciclosporin on rat liver and kidney glutathione content. *Pharmacology* **39:** 205, 1989.
31. Edkins, R. D., Rybak, L. P., and Hoffman, D. W. Comparison of cyclosporine and FK506 effects on glutathione levels in rat cochlea, brain, liver and kidney. *Biochem. Pharmacol.* **43:** 911, 1992.
32. Yamanoi, A., Nagasue, N., and Kohno, H. Failure of pretreatment with cyclosporine to reduce ischemic liver injury after hepatic artery ligation in pigs. *Transplantation* **52:** 569, 1991.
33. Duruibe, V. A., Glyden, G. T., and Okonmah, A. Effect of cyclosporin A on the content of glutathione in the brain of the rat. *Neuropharmacology* **30:** 353, 1991.
34. Walker, R. J., Lazzaro, V. A., Cuggin, G. G., *et al.* Evidence that alterations in renal metabolism and lipid peroxidation may contribute to cyclosporin nephrotoxicity. *Transplantation* **50:** 487, 1990.