Elevation of Whole-Blood Glutathione in Peritoneal Dialysis Patients by L-2-Oxothiazolidine-4-Carboxylate, a Cysteine Prodrug (Procysteine®)

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Abstract. Glutathione is a major cellular antioxidant that protects protein thiols and inhibits cellular damage due to oxygen free radicals. It has been reported previously that patients undergoing dialysis have low levels of blood glutathione, which may lead to increased susceptibility to oxidant stress. L-2-oxothiazolidine-4-carboxylic acid (OTZ) is a cysteine prodrug that raises cellular glutathione levels by increasing delivery of cysteine, the rate-limiting substrate for glutathione synthesis. This study investigates the effect of OTZ on blood glutathione in a blinded, placebo-controlled study of patients with chronic renal failure treated by peritoneal dialysis. Twenty patients were randomly selected to receive OTZ (0.5 g three times a day orally with meals) or placebo for 14 d. Patients visited the clinic for predose blood collection and safety evaluation at baseline (days 3, 7, and 14 and again at 14 d from the last dose [follow-up]). Glutathione concentrations were determined in whole blood by HPLC. OTZ resulted in a significant rise in whole-blood glutathione at days 7 (594 ± 129 µmol/L) and 14 (620 ± 108 µmol/L) compared with baseline (544 ± 139 µmol/L) (P < 0.01 and P < 0.05, respectively). Glutathione was also significantly increased at days 7 and 14 when normalized by hematocrit (Hct) or hemoglobin to correct for anemic status (e.g., 20.7 ± 5.7 µmol/L per % Hct [day 7] and 20.9 ± 4.0 µmol/L per % Hct [day 14] versus 18.0 ± 4.2 µmol/L per % Hct [baseline]; P < 0.05). Glutathione levels did not change in the placebo group at any patient visit, and levels in the OTZ-treated group returned to baseline at follow-up. There were no serious adverse events attributable to OTZ, and the drug appeared to be well tolerated by patients with renal failure treated by continuous ambulatory peritoneal dialysis. Our results show that OTZ increases blood glutathione levels, which may improve antioxidant status in dialysis patients. (J Am Soc Nephrol 9: 1093-1099, 1998)

Glutathione is the most widespread cellular thiol and an important intracellular antioxidant. Reduced glutathione (GSH) inhibits free radical-mediated injury by eliminating toxic peroxides and protects protein sulfhydryl groups from oxidation by serving as a biological redox reagent (1,2). GSH also preserves cellular levels of other antioxidants (3) and participates in the detoxification of xenobiotics that cause cellular injury by generating free radicals (4). Evidence for GSH deficiency has been found in a variety of diseases, including diabetes (5), HIV infection (6), cystic fibrosis (7), acute respiratory distress syndrome (8), and chronic renal failure (9,10).

We and others have previously reported low levels of whole-blood glutathione in patients undergoing both hemodialysis and peritoneal dialysis (9,10). Although the clinical significance of this depletion in blood glutathione is unclear, decreased antioxidant capacity in dialysis patients may contribute to red blood cell (RBC) fragility, anemia, or other pathologic conditions associated with chronic renal failure (11). Decreased blood antioxidant capacity could also be a marker of depleted antioxidants in other tissues, such as the liver and kidney (12), or the peritoneal membrane in the case of peritoneal dialysis (13). Supplementation of blood glutathione levels by parenteral glutathione administration may be useful in the treatment of anemia in patients affected by chronic renal failure (14). For example, infusion of GSH into hemodialysis patients for 90 d at the end of each dialysis session increased RBC glutathione levels, prolonged RBC survival, and decreased the need for erythropoietin (15). Direct administration of glutathione is limited, however, because it must be delivered parenterally to prevent hydrolysis and because it is inefficiently transported into cells (16).

L-2-oxothiazolidine-4-carboxylate acid (OTZ) is a cysteine prodrug that raises cellular glutathione levels by providing a source of cellular cysteine, the rate-limiting substrate for glutathione biosynthesis (17,18). OTZ is stable in plasma and is
readily transported into cells, where it is converted into cysteine by the enzyme 5-oxo-L-prolinase (17). In vitro, OTZ raises cellular glutathione and reduces cellular damage due to free radicals generated by ionizing radiation or other sources (19). In mice, OTZ has been shown to raise hepatic glutathione levels during protein deficiency (20), to protect against acetaminophen toxicity, and to restore normal hepatic glutathione levels in animals that had been depleted of hepatic glutathione by buthionine sulfoximine, an inhibitor of glutathione biosynthesis (21). OTZ has also been shown to be effective as a nontoxic cysteine delivery system for glutathione synthesis in neonatal pigs, chicks, and rats on a cysteine-free diet (22,23). Oral OTZ has been shown to raise blood glutathione in HIV-infected subjects who have diminished levels of blood cell glutathione, which is hypothesized to contribute to their impaired immune response (24).

We investigated the effect of OTZ on blood glutathione in a group of patients with chronic renal failure treated by peritoneal dialysis. A preliminary study of safety and pharmacokinetics after administration of a single oral dose of OTZ in continuous ambulatory peritoneal dialysis (CAPD) patients indicated that the drug was rapidly eliminated and well tolerated (25). The present study was undertaken to extend these preliminary results and to investigate whether oral OTZ administration could raise blood glutathione in patients with chronic renal failure and thus improve their blood antioxidant status.

Materials and Methods

Patients and Study Design

Subjects were enrolled from a population of stable, chronic renal failure patients managed by peritoneal dialysis and attending the outpatient clinic at the Western Division of The Toronto Hospital, Toronto, Canada. Patients were offered the opportunity to participate in the study if they were between the ages of legal consent and 75 yr, if they were adequately dialyzed and in stable condition, if they were considered to have a history of good compliance to patient management instructions, if they had been treated with CAPD for at least 4 mo, and if they had no episode of peritonitis for at least 1 mo. Patients were excluded from the study if they had an illness requiring hospitalization within 30 d, if they were likely to receive a kidney transplant within the time of the study, if they were participating in another study that could interfere with the study, if they were pregnant or nursing an infant, or if they were known to be positive for hepatitis B, hepatitis C, or HIV. The study was conducted in compliance with federal regulations and in accordance with the ethical principles described by the Declaration of Helsinki. The study was approved by The Toronto Hospital Independent Ethics Committee, and all patients gave written informed consent before enrollment.

The study was a prospective, randomized, controlled, double-blind trial comparing OTZ with placebo. Twenty patients were enrolled and randomly selected to receive either 500 mg of OTZ or placebo capsules with an identical appearance. Patients were instructed to take one capsule three times each day (with meals) for 14 d. Patients visited the hospital clinic at day 0 (baseline) and days 3, 7, and 14 while on the medication. Patients were reevaluated at approximately 14 d after the last dose of study drug (follow-up) to ensure that there were no persistent effects of the drug. Safety was monitored by measurement of vital signs, venous blood gases (pH, PCO₂, total CO₂, and bicarbonate) blood chemistries (sodium, potassium, chloride, calcium, phosphorus, aspartate aminotransferase, alanine aminotransferase, urate, creatinine, urea, glucose, alkaline phosphatase, and albumin), hematology (RBC, white blood cells, platelets, hemoglobin, and hematocrit), and a complete review of adverse events. Blood samples were obtained the morning of each visit, before the morning dose, and at least 8 h after the evening dose on the previous day. Blood and plasma samples for measurement of whole-blood glutathione and cysteine and plasma OTZ were collected and stored frozen until shipment to the appropriate analytical laboratory.

Materials

OTZ (Procysteine®, 500-mg capsules) and placebo capsules were obtained from Transcend Therapeutics (Cambridge, MA). Each hard gelatin capsule contained either 500 mg of OTZ or 500 mg of sodium and potassium phosphate (placebo) in combination with 167 mg of corn starch, 64 mg of lactose, and 3.6 mg of ascorbic acid. Treatment compliance with study medication was monitored by patient diaries, return capsule count, and patient interviews.

Analytic Methods

Serum chemistries, venous blood gases, and hematologic parameters were measured in the clinical laboratories of The Toronto Hospital. Plasma OTZ was determined by HPLC, using a method established and validated in the Applied Sciences Laboratory of Baxter Healthcare Corp. (26). Briefly, samples were mixed with 5% metaphosphoric acid, and supernatants were chromatographed using a reversed-phase analytic column (AsteC18, Advanced Separation Technologies, Whippany, NJ). OTZ was determined spectrophotometrically (230 nm) after isocratic elution, using a mobile phase of 0.1 M sodium phosphate buffer, pH 3.0, and a flow rate of 1 ml/min. Concentrations of whole-blood glutathione (oxidized plus reduced) and cysteine were determined by HPLC after derivatization with the fluorescent tag, monobromobimane, as described previously (27). The method was performed and validated in the Metabolic Assessment Laboratory, University of Florida (Gainesville, FL). Venous blood samples were acidified within 5 min from the time of blood draw by mixing with an equal volume of 10% sulfosalicylic acid. Samples were vortexed vigorously and stored at −20°C until analysis. Whole-blood thiols were reduced by incubation with diithiothreitol and then derivatized in the dark by incubation with 2 mM monobromobimane (15 min, room temperature). Samples were transferred in the dark to centrifuge tubes containing thiol-Sepharose 4B (Sigma) and centrifuged for 10 min at 3000 rpm, and supernatants were analyzed in duplicate on a Beckman Altex Ultrasphere ODS column using a System Gold (Beckman Instruments, Fullerton, CA). Thiols were eluted from the column using methanol as the mobile phase and a flow rate of 1.5 ml/min. Calibration standards, included in each batch of samples that were derivatized, were diluted from a solution containing 0.5 mM GSH and 0.5 mM Cys to final concentrations of 5 to 500 nmol/ml.

Statistical Analyses

Comparisons of groups at baseline were performed using t test for continuous variables and Fisher’s exact test for dichotomous variables. ANOVA with repeated measures was used to determine possible interactions between treatment and patient visit for glutathione, glutathione normalized to hematocrit and hemoglobin, cysteine, and other laboratory values. For significant interactions, the Wilcoxon rank sum test was used to assess differences between treatment groups at each patient visit. Comparisons with baseline values within the
treatment group were performed using the \( t \) test. Analyses of safety parameters were performed with the intent-to-treat population, and comparison of adverse events was performed by Fisher’s exact test. Statistical significance was defined as \( P < 0.05 \).

**Results**

**Patients**

Baseline patient characteristics are shown in Table 1. Patients in the placebo and treatment groups were similar in terms of age, gender, and race. A higher proportion of patients in the placebo group were male and had diabetes as their primary cause of renal failure, whereas the most frequent primary diagnoses in the treatment group were glomerulonephritis and hypertension. Patient compliance, based on the number of capsules reported taken, was more than 99% in both the OTZ and placebo groups. One patient (randomized to the OTZ group) chose to withdraw from the study after 2 d of treatment and was not included in the efficacy analysis. This patient was included in all safety analyses.

**Drug Concentrations**

OTZ was not detected in plasma from any patient during any visit. The absence of OTZ in plasma from samples obtained more than 8 h after the last dose is consistent with results from a preliminary study of the pharmacokinetics of OTZ in CAPD patients, which demonstrated the absence of plasma OTZ by 6 h after a single 1500-mg dose (25).

**Effect of OTZ on Blood Glutathione and Cysteine**

ANOVA with repeated measures identified a significant interaction between treatment group and patient visit for whole-blood glutathione, whole-blood cysteine, and glutathione normalized by either hematocrit or hemoglobin. Values of whole-blood glutathione for the placebo and OTZ-treated groups are shown for each patient visit in Table 2. Baseline values of blood glutathione varied by more than twofold among individual patients (range: 342 to 794 \( \mu \text{mol/L} \) for patients randomized to receive OTZ and 412 to 877 \( \mu \text{mol/L} \) for patients randomized to receive placebo). Mean baseline glutathione levels were lower in the OTZ group (544.2 \( \pm \) 138.8 \( \mu \text{mol/L} \)) compared with placebo (627.7 \( \pm \) 163.1 \( \mu \text{mol/L} \)); however, this difference was not statistically significant and was eliminated when glutathione values were normalized by hematocrit or hemoglobin (e.g., 18.0 \( \pm \) 4.2 versus 18.4 \( \pm \) 2.5 \( \mu \text{mol/L} \) per % hematocrit [Hct]).

OTZ treatment resulted in a significant increase in whole-blood glutathione when compared with baseline at day 7 (+57.8 \( \pm \) 49.1 \( \mu \text{mol/L} \)) and day 14 (+84.0 \( \pm \) 104.4 \( \mu \text{mol/L} \)) (Table 2). There was no significant change in blood glutathione at any visit in the placebo group. The greatest increase in blood glutathione in the OTZ-treated group occurred at day 14, and the data suggest a tendency toward greater increases in glutathione with longer treatment times with OTZ. Evidence for an increase in blood glutathione had disappeared by the follow-up visit, approximately 14 d after removal of study drug. When mean change-from-baseline values for blood glutathione were compared between the OTZ and placebo groups using the Wilcoxon rank sum test, the values were significantly greater in the OTZ-treated group at days 7 and 14 (\( P < 0.001 \) and \( P < 0.01 \), respectively) (Figure 1).

Because approximately 99% of blood glutathione is contained within erythrocytes (28), levels of blood glutathione may be expected to vary with anemic status. Consistent with this expectation, baseline glutathione levels were significantly correlated with hematocrit (\( r^2 = 0.45, P < 0.01 \)). To correct for the variability in glutathione levels due to anemic status, we examined glutathione values after normalization by hematocrit (\( \mu \text{mol/L} \) per % Hct) and by hemoglobin (\( \mu \text{mol/g} \) hemoglobin [Hb]). Mean glutathione values were significantly increased by OTZ treatment at day 7 and 14 whether normalized by hematocrit (Table 3) or hemoglobin (Table 4). Change-from-baseline values for these parameters were also significantly different between placebo- and OTZ-treated groups when compared by

**Table 1.** Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n = 10)</th>
<th>OTZ (n = 10)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (yr)</td>
<td>48.9</td>
<td>60.0</td>
<td>0.13</td>
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<td>Gender (M/F)</td>
<td>8/2</td>
<td>5/5</td>
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<td>Race</td>
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<td></td>
<td>0.78</td>
</tr>
<tr>
<td>Caucasian</td>
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<td>8</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>black</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>1</td>
<td>0</td>
<td></td>
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<tr>
<td>Primary diagnosis</td>
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<td>0</td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>glomerulonephritis</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>cystic kidney disease</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>other</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \) OTZ, 1,2-oxothiazolidine-4-carboxylic acid.

\( ^b \) \( P \) value calculated by two-sample \( t \) test.

\( ^c \) \( P \) value calculated by Fisher’s exact test.

**Table 2.** Whole-blood glutathione (\( \mu \text{mol/L} \)) in placebo- and OTZ-treated patients

<table>
<thead>
<tr>
<th>Group</th>
<th>Visit</th>
<th>( n )</th>
<th>Mean ( \mu \text{mol/L} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Baseline</td>
<td>10</td>
<td>627.7 ± 163.1</td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>579.8</td>
<td>150.2</td>
</tr>
<tr>
<td>Day 7</td>
<td>10</td>
<td>563.9</td>
<td>104.2</td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>635.3</td>
<td>150.9</td>
</tr>
<tr>
<td>Follow-up</td>
<td>10</td>
<td>632.2</td>
<td>123.4</td>
</tr>
<tr>
<td>OTZ</td>
<td>Baseline</td>
<td>10</td>
<td>544.2 ± 138.8</td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>585.0</td>
<td>120.4</td>
</tr>
<tr>
<td>Day 7</td>
<td>9</td>
<td>594.0</td>
<td>128.7</td>
</tr>
<tr>
<td>Day 14</td>
<td>9</td>
<td>620.2</td>
<td>107.5</td>
</tr>
<tr>
<td>Follow-up</td>
<td>9</td>
<td>559.8</td>
<td>133.0</td>
</tr>
</tbody>
</table>

\( ^a \) \( P \) values based on paired \( t \) tests using change-from-baseline values (see Results).
Figure 1. Change from baseline for whole-blood glutathione in l-2-oxothiazolidine-4-carboxylic acid (OTZ) and placebo-treated continuous ambulatory peritoneal dialysis (CAPD) patients. Groups were compared by Wilcoxon rank sum test, and values were significantly different for visits at day 7 (P < 0.001) and day 14 (P < 0.01).

Table 3. Glutathione in whole blood normalized by hematocrit (μmol/L per % Hct)

<table>
<thead>
<tr>
<th>Group</th>
<th>Visit</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Baseline</td>
<td>10</td>
<td>18.4</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>17.4</td>
<td>3.2</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>10</td>
<td>17.2</td>
<td>2.4</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>10</td>
<td>18.7</td>
<td>2.1</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>10</td>
<td>18.9</td>
<td>2.3</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>OTZ</td>
<td>Baseline</td>
<td>10</td>
<td>18.0</td>
<td>4.2</td>
<td></td>
</tr>
<tr>
<td>Day 3</td>
<td>10</td>
<td>19.6</td>
<td>3.9</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Day 7</td>
<td>9</td>
<td>20.7</td>
<td>5.7</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Day 14</td>
<td>9</td>
<td>20.9</td>
<td>4.0</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>Follow-up</td>
<td>9</td>
<td>17.9</td>
<td>4.0</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

* P values based on paired t tests using change-from-baseline values.

Wilcoxon rank sum test (P < 0.05, P < 0.01, and P < 0.05 at days 3, 7, and 14 for glutathione/Hct and P < 0.001 and P < 0.01 at days 7 and 14 for glutathione/g Hb, respectively). ANOVA with repeated measures failed to detect any significant interaction between patient visit and treatment with respect to blood hemoglobin or hematocrit. Hematocrit levels were somewhat (but not significantly) lower in the treatment group at baseline (30.6 ± 4.7 versus 34.1 ± 7.5 for OTZ and placebo groups, respectively) and remained relatively constant at each visit.

ANOVA for repeated measures also revealed a significant interaction (P = 0.0167) for cysteine, the primary metabolite of OTZ. Mean concentrations of whole-blood cysteine were significantly increased at day 3 (+52.6 ± 50.0 μmol/L) and day 7 (+65.1 ± 69.2 μmol/L) in the OTZ-treated group compared with baseline. Change-from-baseline values were also significantly different between the treated and placebo groups at days 3 and 7 (P < 0.05 for both by Wilcoxon rank sum test). There was a tendency toward elevated blood cysteine at day 14 (+68.2 ± 105.1), but this value was not significant due to higher variability on this day. Mean blood cysteine levels returned to baseline values by follow-up (−4.7 ± 45.2 μmol/L). There were no significant changes in blood cysteine in the placebo group.

One patient exhibited a greater rise in whole-blood cysteine in response to OTZ (Figure 2); cysteine levels for this patient increased from 268 μmol/L at baseline to 388 at day 3, 500 at day 7, and 610 at day 14. This rise in cysteine appeared to be drug-dependent, because levels returned to near baseline (350 μmol/L) after withdrawal of the drug. Exclusion of this patient from the cysteine analysis resulted in a loss of statistical significance for difference between groups on day 3 but not on

Figure 2. Whole-blood cysteine levels in CAPD patients treated with OTZ. Each symbol represents an individual patient. Cysteine levels in patients treated by placebo did not change over time and are not shown.
day 7; the increase in blood glutathione remained significant on days 7 and 14 even when this patient was excluded.

**Safety**

OTZ and placebo treatment groups showed similar adverse event profiles, and there was no significant difference in the number of patients with adverse experiences (90% in the treatment group and 60% in the control group, \( P = 0.30 \) using Fisher’s exact test). One patient in the OTZ group had a serious adverse event (peritonitis), which occurred during the follow-up period (after drug administration had ceased) and was not considered related to the study treatment. Other adverse events that were reported in both groups were consistent with adverse experiences commonly associated with chronic renal failure. These adverse events included peritonitis (one incident in each group), hypertension (two incidents in the placebo group), diarrhea (one incident in each group), nausea (one incident in the treatment group), anemia (one incident in the placebo group and two in the treatment group), hypercalcemia (one incident in the placebo group and two in the treatment group), hyperphosphatemia (two incidents in the placebo group), hyperglycemia (one incident in the placebo group), and pruritus (one incident in each group). There were no drug-related, clinically relevant changes in BP, vital signs, hematology parameters, or venous blood gases at any patient visit. There was no evidence for an interaction between treatment and patient visit for serum chemistries except serum sodium and serum phosphorus, using ANOVA with repeated measures. When groups were compared at each patient visit by the Wilcoxon rank sum test, only serum phosphorus (moderately higher in the placebo group at days 3, 7, and 14, \( P < 0.05 \)) and uric acid (moderately higher in the placebo group at day 14, \( P < 0.05 \)) were significantly different. The elevation in serum phosphorus in placebo-treated patients likely was caused by the presence of potassium- and sodium-phosphate as an excipient in the placebo capsules. There was no evidence for an effect of OTZ on urinary output or urinary creatinine, protein, and urea levels.

**Discussion**

Blood glutathione concentrations are reported to be low in patients with chronic renal failure (9,10), and other reports suggest depleted blood antioxidant capacity in these patients (29,30). Blood glutathione levels in healthy subjects are reported to vary between 670 and 1900 \( \mu \)mol/L (mean = 1020 \( \mu \)mol/L, from a study of more than 700 healthy adult subjects) (31). Another recent study, using HPLC methods similar to those used in our study, found mean blood glutathione levels of 941 ± 155 \( \mu \)mol/L in more than 200 healthy adults (28). Baseline concentrations of blood glutathione for CAPD patients in our study varied from 342 to 877 \( \mu \)mol/L (mean, 586 \( \mu \)mol/L), clearly lower than levels reported for healthy adults. Although anemia contributes to the low levels of blood glutathione in dialysis patients, we reported previously that blood glutathione levels are low even when normalized by hemoglobin or hematocrit (10), suggesting a specific decrease in the intra-erythrocyte concentration of glutathione. This impairment of erythrocyte antioxidant defenses may increase membrane fragility and decrease RBC life span, which in turn can contribute to anemia in patients with chronic renal failure (9). In support of this hypothesis, a recent study showed that intravenous glutathione administration to hemodialysis patients at the end of each dialysis session increased RBC glutathione content, improved RBC survival, and caused a partial reduction in the need for erythropoietin (15).

Low levels of blood glutathione may also result in impaired glutathione transport to other tissues. Both the liver and kidney normally release substantial amounts of glutathione in the form of precursor amino acids, which are then extracted by erythrocytes, resynthesized into glutathione, and transported to tissues with a high rate of GSH utilization, including the lung, heart, gut, and brain (12). Other tissues that require glutathione may also depend on erythrocytes for delivery of GSH or GSH precursors (e.g., lymphocytes to ensure proper immune function [32] and artery wall macrophages to reduce toxicity of oxidized LDL [33]). Low levels of erythrocyte glutathione in patients with chronic renal failure could therefore indicate impaired GSH transport to tissues and directly contribute to pathologies associated with poor antioxidant status, including infection and atherosclerosis.

In this study, oral OTZ administration resulted in a significant increase in whole-blood glutathione in CAPD patients, consistent with its proposed mechanism of action (17). This increase in whole-blood glutathione was associated with an increase in erythrocyte glutathione concentration. There was no evidence that OTZ increased hematocrit or reduced the need for erythropoietin; a longer-duration study may be required to observe such changes in anemic status. Nevertheless, the increase in blood glutathione suggests an improvement in erythrocyte antioxidant status and may indicate evidence for an increase in glutathione in other, inaccessible tissues.

Another potential benefit of an increase in glutathione in CAPD patients is the protection of the peritoneal membrane from oxidative damage. During peritoneal dialysis, oxygen free radicals contribute to local damage to mesothelial cells lining the peritoneal membrane, ultimately resulting in long-term consequences such as peritoneal fibrosis and ultrafiltration failure (34,35). Damage to the peritoneal membrane may be especially severe during peritonitis, when oxygen radicals are produced by leukocytes that infiltrate the peritoneal cavity in response to a bacterial infection (36). In vitro, OTZ has been shown to protect human peritoneal mesothelial cells against free radicals and to reduce cytotoxicity due to exposure to peritoneal dialysis solutions (37,38). Treatment with OTZ during CAPD could limit oxidative damage to the peritoneal membrane during chronic peritoneal dialysis or bacterial infection of the peritoneum.

Oral OTZ was well tolerated in CAPD patients and did not result in any serious adverse events. Both treatment and placebo groups were similar with respect to the relationship to the seriousness and severity of reported adverse events. OTZ administration did cause a transient rise in blood cysteine, particularly in one patient; however, this increase was not associated with any adverse reaction. An increase in blood cysteine
is not surprising because cysteine is the primary metabolite of OTZ; however, the magnitude of the increase in cysteine may reflect the impairment in cysteine metabolism in patients with chronic renal failure. Cysteine levels are known to be elevated in patients with chronic renal failure (39), and, consistent with this observation, baseline cysteine levels for patients in this study were 2 to 4 times normal levels. The baseline cysteine level was highest in the patient with the greatest rise in blood cysteine, suggesting that this patient had a greater impairment of cysteine metabolism or perhaps a higher dietary cysteine intake. There was no statistical relation between cysteine concentrations (or changes in cysteine concentrations) and blood glutathione levels in this study, consistent with our previous findings that dialysis patients have low blood glutathione levels despite adequate or even elevated levels of blood cysteine. Our data suggest that extracellular cysteine may not be available for glutathione synthesis in dialysis patients. Homocysteine, which is also known to be elevated in patients with end-stage renal disease (40), was not affected by OTZ treatment in this study (J. Moberly, unpublished observations).

In conclusion, OTZ administration to CAPD patients resulted in a significant increase in whole-blood glutathione while demonstrating a satisfactory safety profile. The elevation of blood glutathione is consistent with the mechanism of action of this drug, which is to raise cellular glutathione by increasing the supply of intracellular cysteine. Further investigation will be required to determine whether this increase in blood glutathione will have therapeutic benefits related to: (1) the protection of erythrocytes from oxidative damage and improvement of anemia; (2) the protection of the peritoneal membrane during dialysis; and (3) the protection of other tissues by improving cellular antioxidant status.

References
26. Webb LE, Gechman M, Story K: Determination of L-2-oxothiazolidine-4-carboxylic acid (Procytaste®) in human plasma by