Overexpression of Erythrocyte Glutathione S-Transferase in Uremia and Dialysis

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Background: Overexpression of glutathione S-transferase (GST; EC 2.5.1.18) has been documented in the erythrocytes of patients with chronic renal failure, and this event may well be of relevance from a clinical standpoint. In fact, it could serve as a marker of uremic toxicity overall, which can contribute to impair the function and survival of the erythrocytes. However, the biochemical details of this phenomenon are poorly understood.

Methods: In this study, we characterized the expression of GST in erythrocytes of 118 uremic patients under different clinical conditions. The mechanisms responsible for the regulation of protein expression and enzyme activity were investigated in light of different dialysis approaches, oxidative stress, uremic toxins, erythrocyte age, and erythropoietin (EPO) supplementation.

Results: Mean GST activity in uremic patients was highly overexpressed with respect to controls, and this phenomenon was exclusively attributable to an increased expression of GST. Overexpression of GST did not appear to be dependent on oxidative stress and was not influenced by vitamin E supplementation. In the same manner, both erythrocyte age and EPO supplementation apparently did not interfere with the GST concentrations, which were the same in controls and patients. Preliminary experiments suggested that high-molecular weight or protein-bound toxins could play some role in the overexpression of GST.

Conclusions: GST expression may be a useful marker for the individual accumulation of uremic toxins as well as of the efficiency of new dialysis strategies in removing them. © 1999 American Association for Clinical Chemistry

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The ubiquitous enzyme glutathione S-transferase (GST; EC 2.5.1.18) is primarily involved in the neutralization of harmful exogenous or endogenous compounds by enzymatic conjugation with the scavenger peptide glutathione (GSH) and/or by direct binding of nonsubstrate ligands (1, 2). Other functions of GST include protection against oxidative damage to lipids and nucleic acids and participation in the metabolism of some steroids and leukotrienes (2). This enzyme is represented by a family of cytosolic proteins bearing a dimeric structure, the polymorphic expression of which has been widely studied in human tissues and in a wide series of cells (2–4).

In human erythrocytes, GST is present in large amounts (5) and in two forms: a highly cationic enzyme (designated with the Greek letter ρ), which accounts for <5% of the total GST activity; and the main anionic enzyme corresponding to the P form (π) (6, 7). Because of its abundance and overlapping substrate specificity with the ρ form, the GST P1-1 dimer is often considered the sole GST enzyme in the erythrocyte (2, 7).

In humans, increased concentrations of erythrocyte GST have been observed in uremia (8), where the role of GST expression may well be of relevance from a clinical standpoint. In fact, the exposure of cells to a uremic environment seems to determine the onset of various complications (9, 10), the intensity and characteristics of which differ from patient to patient as a function of the clinical conditions and of the efficiency/physiologic compatibility ratio of the dialysis procedures used, in which a critical prooxidant/antioxidant balance can be present (11).

The aim of the present study was to investigate GST expression in uremic patients under different clinical conditions. One of these was the use of a vitamin E-modified filter, which was expected to be both effective against oxidative stress-related toxicity and highly bio-

*Nonstandard abbreviations: GST, glutathione S-transferase; GSH, glutathione; EPO, erythropoietin; CAPD, continuous ambulatory peritoneal dialysis; HD, hemodialysis; D-HD, daily hemodialysis; S-HD, standard hemodialysis; Ht, hematocrit; Hb, hemoglobin; PDF, peritoneal dialysis fluid; RBC, red blood cell; CDNB, chloro- nitrobenzene; and MDA, malondialdehyde.
compatible (12). The role of a “protein-leaking” dialyzer able to eliminate high-molecular weight toxins, some of which have an inhibitory effect on erythropoiesis (13), was also investigated. Finally, we analyzed other aspects affecting GST activity, including erythropoietin (EPO) supplementation and dialysis fluid composition and biocompatibility in chronic ambulatory peritoneal dialysis (CAPD) patients.

These clinical and therapeutic approaches may influence the detoxification metabolism and, as a consequence, GST expression in erythrocytes and other cell types. In this context, the possibility of using GST expression either as an index of uremic toxicity or as a specific tool to evaluate the physiologic compatibility of some dialysis therapies in uremic patients is discussed.

Materials and Methods

Subjects

Four groups of uremic patients (total number of patients = 118) and an age- and sex-matched control group (n = 14) were studied. The patients were undergoing conservative therapy (predialysis; n = 10 and with a creatinine clearance <30 mg/L), daily hemodialysis (D-HD; n = 30), standard HD (S-HD; n = 29), or CAPD (n = 54). The clinical and anthropological conditions of the patients in the different subgroups were comparable. EPO supplementation was provided for the patients with the most severe cases of anemia [hematocrit (Ht) target, 30%], following the standard protocols. All other pharmacological treatments known to interfere with the parameters studied were avoided for a sufficient period of time before the study. Five patients (two in CAPD and three in S-HD) were excluded from the study because of chronic inflammatory diseases or other secondary conditions incompatible with the aim of the protocol.

Clinical Trials

Clinical trial 1: HD with vitamin E-modified filters. In a subgroup of patients in S-HD (n = 15), the effect of the treatment with two different types of dialysis membrane on GST expression was evaluated. The first was a cuprammonium rayon-based membrane, which was used for at least 1 month; after this period the same subjects were treated with a multilayer vitamin E-coated membrane (12), which was used for 3 months. Both membranes were provided by Terumo (Japan).

Clinical trial 2: HD with high-flux (protein-leaking) filters (BK-F). Four patients undergoing chronic HD with standard polymethylmethacrylate-based low-flux filters [two with normal GST activity and two with GST activity >35 U/g hemoglobin (Hb)] were treated for a period of 3 months with high-flux protein-leaking dialyzers (BK-F), which have a nominal cutoff of M, 70 000 (13). The different permeabilities of these two classes of membrane (pore diameter <30 Å for low-flux filters and 100 Å for BK-F, respectively) gave us preliminary information on the effect of toxins with molecular weights below and above 70 000 on GST expression.

Clinical trial 3: evaluation of GST expression in CAPD patients as a function of peritoneal dialysis fluid composition and EPO supplementation. In a subgroup of CAPD patients (n = 10), the effect of EPO therapy on GST expression was assayed as a function of red blood cell (RBC) age. At the same time, these patients (n = 21), all of whom had been stabilized with lactate-based peritoneal dialysis fluid (PDF), were shifted to treatment with bicarbonate-based PDF for a period of 3 months and then returned to treatment with lactate-based fluid for an additional 3 months. GST and GSH were measured at all the three steps.

Laboratory Techniques

Blood sampling and RBC preparation. Ten milliliters of venous blood (for predialysis or CAPD patients and controls) or arteriovenous blood (for HD patients) was drawn from either the antecubital vein or the arteriovenous fistula into heparin-containing Vacutainer Tubes. The cells and plasma were separated by centrifugation and treated as previously described to isolate the RBCs (14). An aliquot of plasma was stored at −20 °C until assayed in the kinetic experiments with purified GST; the remaining aliquot was used immediately for the other analyses described below, including assays of vitamin E and fatty acid content.

Human RBCs were fractionated by a slightly modified version of the density gradient procedures described by Rennie et al. (15). Cell counting and correction for the Ht values in each fraction were used to determine the amount of young and old cells. The different ages of the cell fractions were confirmed by evaluating the metabolic activity of the cells and the activity of the K⁺/Cl⁻ cotransport system, as well as the values of medium corpuscular volume and medium corpuscular hemoglobin content.

The reticulocyte count was performed on whole blood by optical microscopy after specific staining, and the concentrations of EPO in the serum were measured using a RIA method, as described previously (14, 16).

Analysis of the catalytic properties of GST. GST activity and kinetic parameters, namely maximal activity (Vₘₐₓ), apparent Michaelis constants (Kₘ) for GSH and chlorodinitrobenzene (CDNB), and optimum pH, were determined according to the method of Habig et al. (1) with some minor modifications as described by Carmagnol et al. (8).

GST was purified from a crude RBC lysate by the method described by Awasthi et al. (6) and affinity chromatography with a hexylglutathione gel (Sigma Chemicals). Alternatively, a purified preparation of human GST-π was purchased from Sigma. Kinetic, electrophoretic, and immunoblotting data confirmed the pres-
ence of GST-π in the purified enzyme preparations; specific activity was measured using CDNB as substrate.

The effect of the toxins contained in the uremic plasma, serum, and dialysis fluid samples on the kinetic parameters of the GST purified from human RBCs was also determined. These samples, obtained at different times of dialysis, were incubated for 30 min at 37 °C in the presence of partially purified GST-π. One volume of plasma or serum was mixed with 1 volume of enzyme suspension (0.1 g/L) in a final volume of 1 mL. Before GST activity was measured, the mixture was chromatographed on a Sephadex G-25 column (Pharmacia Biotech), with 10 mmol/L phosphate buffer (pH 7) as elution buffer. The fractions containing the enzyme were recovered and concentrated to a final protein concentration of 0.1 g/L. Enzyme activity was measured as above, with the exception that the concentration of CDNB was 0.5 mmol/L, the Km.

In some experiments, samples of plasma were subjected to ultrafiltration on PM10 membranes (Amicon) and/or deproteinization by boiling for 10 min or by extraction with 50 g/L trichloroacetic acid followed by decantation and filtration through 0.22 μm filters.

Analysis of GST protein expression. GST expression was measured as described previously (17) by immunoblotting on the RBC lysate, which was first subjected to a partial purification of the main protein (hemoglobin) by chloroform-ethanol extraction (18) to increase the sensitivity of the procedure. The clarified extract was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using 10% polyacrylamide gels, with 15 μg of protein loaded in each lane. Proteins were blotted onto a nitrocellulose sheet (Bio-Rad Laboratories), and a sheep anti-rabbit polyclonal antibody (Calbiochem-Novabiochem) was used to detect the amount of GST protein by the ECL enhanced chemiluminescence detection system (Amersham-Pharmacia Biotech). The signal was recorded with photographic films (Kodak; supplied by Sigma-Aldrich) and measured by laser scanning densitometry.

Analysis of free thiols, vitamin E, free fatty acids, and malondialdehyde. The presence of oxidative stress was tested by assay of the lipoperoxidative end-product malondialdehyde (MDA) as free fraction in the total RBC lysate and plasma. The thiobarbituric acid test was used, but its specificity was evaluated in some experiments by direct HPLC analysis of MDA as described previously (19), with only minor modifications. Briefly, RBC suspensions at a Ht of 30% were subjected to hypotonic lysis, and after perchloric acid extraction (20), the supernatant was filtered through 0.22 μm pore filters and subjected to HPLC. An LC-18T reversed-phase column (15 cm × 4.6 mm i.d.; Supelco) and isocratic conditions were used. The mobile phase was a mixture of 500 mL/L NaH2PO4, pH 8, and 500 mL/L methanol, and the flow rate was 1 mL/min. The elution time for MDA was 16.3 ± 0.6 min. An MDA calibrator was prepared by acidic hydrolysis of a solution of 1,1,3,3-tetrahydroxypropane.

In the S-HD subgroup treated with the vitamin E-modified filter, vitamin E and lipid composition in terms of the essential free fatty acids (arachidonic, linoleic, and linolenic acid), were measured in plasma and RBC lipid extracts, as described previously (12).

Free thiols in the RBC, of which GST made up >95%, and in the plasma, which were mainly protein thiols and to a lesser extent non-protein thiols such as GSH, were assayed with Elman’s reagent (14, 16).

Results

EXPRESSION OF GST ACTIVITY

The erythrocyte GST activity in healthy subjects and uremic patients is shown in Fig. 1 and in Table 1. Healthy subjects displayed a mean control value of 2.40 ± 0.89 U/g Hb (range, 1.22–4.00 U/g Hb). In the predialysis patients, a mean GST activity of 3.17 ± 1.17 U/g Hb was observed (range, 1.60–5.10 U/g Hb). This value was not statistically different compared with controls. On the other hand, in the patients undergoing different dialysis procedures, GST activity increased significantly with respect to the control values (P < 0.01). The rise in the mean

![Fig. 1. GST activity in healthy controls and in uremic patients in predialysis or undergoing different dialysis treatments.](image-url)

The enzymatic activity of GST was measured as described in Materials and Methods. Statistical analysis is reported in Table 1. CT, conservative therapy (predialysis); bars, SD.
GST activity in the dialysis patients followed this order: S-HD > CAPD > D-HD. These three subgroups showed mean GST activities (in U/g Hb) of: 5.13 ± 1.60 (range, 2.11–9.10), 5.07 ± 1.61 (range, 1.28–8.50), and 3.98 ± 1.41 (range, 1.56–6.89), respectively. The total population of dialysis patients (n = 103) displayed a GST activity of 4.77 ± 1.62 U/g Hb (range, 1.28–9.10 U/g Hb).

Regardless of the type of dialysis therapy, 65% of the patients studied had GST activity higher than the cutoff line value of 4.2 U/g Hb, which corresponded to the mean control value plus 2 SD. As for the mean activity, in the dialysis patients the relative incidence of GST overexpression, calculated as the fraction of patients in each subgroup with GST activity above the cutoff value, followed the order: S-HD (72%) > CAPD (57%) > D-HD (43%).

The mean GST activity in the D-HD patients was significantly lower than in the other two groups of dialysis patients (P < 0.05 in both cases).

GST activity was not correlated with GSH concentrations in the RBCs of the uremic patients or with hemoglobin concentrations and RBC number (not shown). In a similar way, no correlation was found between GST activity and the clinical and anthropological characteristics recorded, e.g., patient age, length of time in dialysis, or creatinine and blood urea nitrogen concentrations.

### Expression of GST Protein

The immunoblotting of the RBC lysate of controls and two groups of patients representative of the whole population studied is shown in Fig. 2. The analysis revealed the presence of two bands of M, ~31 000 and 245 000, respectively. The M, 31 000 protein, expressed in the same amount in both patients and controls, was demonstrated by further analysis to correspond to the cytosolic enzyme carbonic anhydrase (not shown).

A strict correlation between GST activity and protein expression in the RBC cytosol was observed in both the controls and patients (r = 0.889). Therefore, the increase in GST activity observed in the subgroups of uremic patients studied appears to be almost entirely a consequence of an increased GST protein expression.

### Kinetic Analysis of GST

Total erythrocyte GST activity and GSH concentrations in the S-HD patient group, as well as V_{max} and K_m for CDNB and GSH of the partially purified enzyme, were compared to those of the control group (Table 2). In the patients, in the presence of an increased total activity and of a slightly increased GSH concentration, the kinetic behavior of GST remained unmodified.

Erythrocyte GSH was also slightly increased in the S-HD patient group in comparison to controls.

### GST Inhibition by the Plasma of Patients Overexpressing GST

A dose-dependent inhibition of GST activity was observed in the plasma of patients overexpressing GST in their RBCs (activity, 5 U/g Hb), whereas the plasma obtained from healthy controls and from S-HD patients with a GST activity near the control interval (activity, 2.5 U/g Hb) did not exert this effect (not shown). After a HD session with conventional low-flux dialyzers (nominal cutoff ≤ 30,000), the inhibitory activity of the uremic plasma from subjects overexpressing GST decreased by 35% ± 23% (n = 14; P > 0.05). Ultrafiltration of the plasma drawn from the same subjects before the HD session with a membrane with a cutoff of 10,000 removed 27% ± 16% of the inhibitory activity (n = 3). Deproteinization by boiling or by acidic extraction permitted the recovery of 23% ± 9% and 31% ± 16%, respectively, of the inhibitory activity present in the whole plasma (n = 5).

### Lipoperoxidation and GSH Concentrations in Uremic Patients

The concentrations of the lipoperoxidative subproduct MDA showed a broad distribution in the RBCs of the four subgroups, and the mean concentration was not statistically different from that of the controls (Table 3). How-

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**Table 1. GST activity** in the erythrocytes of healthy controls and uremic patients.

<table>
<thead>
<tr>
<th></th>
<th>S-HD patients</th>
<th>D-HD patients</th>
<th>CAPD patients</th>
<th>Total dialysis patients</th>
<th>Predialysis patients</th>
<th>Healthy controls</th>
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<tbody>
<tr>
<td>n</td>
<td>28</td>
<td>30</td>
<td>45</td>
<td>103</td>
<td>10</td>
<td>14</td>
</tr>
<tr>
<td>Range</td>
<td>2.11–9.10</td>
<td>1.56–6.89</td>
<td>1.28–8.50</td>
<td>1.28–9.10</td>
<td>1.60–5.10</td>
<td>1.22–4.00</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>5.13 ± 1.60</td>
<td>3.98 ± 1.41</td>
<td>5.07 ± 1.61</td>
<td>4.77 ± 1.62</td>
<td>3.17 ± 1.17</td>
<td>2.40 ± 0.89</td>
</tr>
<tr>
<td>Median</td>
<td>4.96</td>
<td>3.64</td>
<td>4.50</td>
<td>4.48</td>
<td>2.9</td>
<td>2.31</td>
</tr>
</tbody>
</table>

*The activity was measured using the procedures described under Materials and Methods. GST activity was expressed as μmol of substrate consumed/min at 37 °C.

b–d Unpaired Student t-test: b P < 0.01 patients vs controls; c P < 0.01 dialysis patients vs predialysis patients; d P < 0.05 D-HD vs S-HD.*

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**Fig. 2. Erythrocyte GST expression in uremic patients and controls as assayed by Western blotting of cytosolic proteins.**

Left: lane 1, healthy control (Ctr); lanes 2 and 3, chronic HD patients (HD1 and HD2, respectively) overexpressing and normoexpressing GST. Right: lane 1, sample HD2 from the left panel; lanes 2–5, D-HD patients; lanes 6–9, S-HD patients with different degrees of expression of GST. 31 and 24.5 indicate M, 31 000 and 24 500 bands, respectively.
ever, in the S-HD group, a positive correlation between GST activity and MDA concentration was observed ($r = 0.619$; $P < 0.001$).

GSH concentrations in the RBC of the four subgroups examined did not differ or were slightly increased compared with those of the controls.

**Effects of Treatment with Vitamin E-Modified Dialyzers on Blood Vitamin E, Fatty Acid Composition, GSH, and GST Expression in S-HD Patients**

In the subgroup of S-HD patients treated with cuprammonium rayon-based filters, normal or slightly decreased concentrations of vitamin E and high concentrations of GST activity were observed. After the 3-month period of treatment with a vitamin E-modified filter, an increase in vitamin E from $0.30 \pm 0.11$ to $0.64 \pm 0.31 \text{mg/L RBCs}$ was observed ($113\%$; $P < 0.0001$). A comparable increase in the cell membrane content of polyunsaturated lipids, in particular arachidonic acid, was observed (not shown). These changes did not affect or induced only a slight decrease in the GST activity [from $3.8 \pm 1.4$ to $3.3 \pm 1.2 \text{ U/g Hb}$ (14%); $P > 0.05$] and the GSH concentration (from $8.6 \pm 1.1$ to $8.9 \pm 1.7 \text{ mmol/g Hb}$) in the RBCs.

**Effects of EPO Supplementation and RBC Age on GST Expression in CAPD Patients**

Younger cells had significantly higher GST activity and GSH concentrations but lower MDA concentrations with respect to older cells in both the controls and patients (Fig. 3). Regardless of the EPO supplementation, GST activity in the young and middle fractions obtained from the patients was higher than in healthy controls ($P < 0.01$ in both). However, the amount of EPO supplied to the patients did not correlate with the GST concentrations observed in the unfractonated cells even in the presence of a number of young cells ($12\% \pm 5\%$ higher than that of controls, not shown). The mean GST concentration displayed by the EPO-treated patients was not significantly higher than that of patients not treated with EPO (not shown).

In a subgroup of patients ($n = 7$) not responding to EPO therapy (Ht gain at the end of the protocol $<1\%$), a comparison with patients ($n = 21$) who responded to this therapy ($Ht$ gain of $6\% \pm 4\%$ after supplementation; range, 2–8%), demonstrated that these two subgroups of patients did not differ significantly in GST expression even in the presence of a significantly different reticulocyte count (respectively, $0.6\% \pm 0.5\%$ and $2.1\% \pm 1.6\%$; $P < 0.01$).

**Effect of PDF Composition on GST Expression**

Regardless of the type of PDF used, GST remained significantly higher in the patients vs controls over the entire prospective trial (not shown). GST remained in the control range in all three steps of the study.

**Effect of BK-F Filters on Erythrocyte GST Expression**

In three subjects, two with normal starting activity and one with high GST activity, the loss of high-molecular weight substances during dialysis caused a decrease in GST activity by $\sim 30\%$ at all experimental times considered (Table 4). A comparable decrease in the in vitro inhibitory activity of their plasma on the GST was observed (not shown). In the patients displaying the highest GST activity, the BK-F treatment did not modify, or only slightly increased, the activity observed during the treatment with the low-flux filter.

**Discussion**

GST overexpression has been documented in the RBCs of patients with chronic renal failure (8), and the possibility that this cell type could function as a circulatory detoxi-
A possible element responsible for the high GST expression in CAPD patients might be the bioincompatibility of the PDF used, which can impair RBC metabolism.
either directly or via a challenge of the peritoneal tissue (23). However, in this study, we demonstrated that bicarbonate-based PDF, even if considered the most biocompatible PDF, induced the same GST overexpression in vivo as lactate-based PDF, thus suggesting that other factors can affect expression of the enzyme.

RBC age (7) can influence GST expression in dialysis patients. In fact, GST concentrations were higher in young cells, which also had higher GSH and lower MDA concentrations than did older cells. However, the profile of the GST activity in the fractionated RBCs was modified in the S-HD patients overexpressing GST. Interestingly, the younger fractions in the RBCs of these patients displayed GST activities higher than those of the controls, whereas older fractions had the same activity as the control samples. This evidence seems to suggest that in the uremic environment, GST could be progressively inactivated during the RBC life span; thus, its overexpression could be a response to low efficiency of the GST-dependent detoxification system, which tends to become defective during the aging of uremic RBCs.

Supplementation with EPO, which can increase the number of young cells in circulation, did not interfere with the mean concentrations of GST in EPO-responsive patients, who had a GST expression profile (as a function of cell age) comparable to that of patients not treated with EPO and had mean GST concentrations comparable to those of EPO-unresponsive patients.

Oxidative stress could be included in the factors responsible for an overexpression of GSH-dependent enzymes in dialysis patients. In fact, lipophilic substances released during the oxidative damage to polyunsaturated lipids, such as short-chain aldehydes and alkenals, have been demonstrated to be substrates for GST (2) and to accumulate in the plasma and RBCs of dialysis patients (16,19). In this study, we measured the concentration of free MDA in the RBCs as an index of lipoperoxidation and found that MDA did not accumulate in the RBCs of the dialysis patients to a greater extent than that observed in the controls. Moreover, only a weak correlation between GST expression and MDA was found in S-HD, which is thought to be associated with a greater susceptibility to oxidative stress with respect to other types of dialysis, such as CAPD (16). Furthermore, in a subgroup of patients treated with vitamin E-modified dialysis filters and showing GST overexpression, although a significant increase in plasma and RBC vitamin E occurred, GST concentrations were only slightly lower. These data suggest that GST expression in uremic patients probably does not depend on a compensatory response of GST expression against oxidative stress and particularly lipoperoxidation. However, the data presented in this study do not exclude the presence of oxidative stress in dialysis patients, at least in some subjects undergoing HD procedures using poorly biocompatible materials (12).

Intriguingly, GST inhibitors are present in uremic plasma, and this could justify an overexpression of GST in erythroid cells. In in vitro experiments, we observed that whole plasma of patients with high GST concentrations exerted an inhibitory activity on GST purified from human RBCs. A major fraction of this inhibitory activity (>60%) was not removed by membranes with a cutoff of M$_{r}$ 10 000, and treatment with conventional (low-flux) HD filters eliminated the same amount of inhibitory activity.

The use of high-flux (protein-leaking) dialyzers, tested herein in a pilot study on four patients, slightly decreased (<30%) the GST activity of three patients regardless of the starting GST concentrations shown during treatment with low-flux dialyzers; a comparable decrease in the inhibitory activity of their plasma was observed.

Taken together, these findings are in agreement with previous observations that low-molecular weight substances present in the uremic blood and bound with high affinity to plasma proteins may be responsible for inhibition of hepatic GST (24). Among these endogenous ligands, one has been identified as 3-carboxy-4-methyl-5-propyl-furanopropanoic acid (24), which also inhibits mitochondrial respiration and acts as a hypoproliferative agent on the erythroid stem cells (25,26). Many other low-molecular weight toxins (27) are able to bind with high affinity to plasma proteins and could be responsible for an induction of the detoxification system. In fact, the possibility that during dialysis the steady concentration of the free form of these solutes undergoes rapid and significant changes able to affect GST expression in the erythroid cells cannot be ruled out. In this context, high-flux dialyzers might represent a solution for the removal of these protein-bound toxins and high-molecular weight toxins overall (28).

### Table 4. GST activity in four patients treated with protein-leaking filters (BK-F).$^a$

<table>
<thead>
<tr>
<th>Patient</th>
<th>GST activity range</th>
<th>Basal value</th>
<th>After 1 month of BK-F</th>
<th>After 3 months of BK-F</th>
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<tbody>
<tr>
<td>1</td>
<td>Normal</td>
<td>1.90</td>
<td>1.47 (−22.6)</td>
<td>1.85 (−2.6)</td>
</tr>
<tr>
<td>2</td>
<td>Normal</td>
<td>2.33</td>
<td>1.88 (−19.3)</td>
<td>1.86 (−20.2)</td>
</tr>
<tr>
<td>3</td>
<td>High</td>
<td>3.68</td>
<td>3.50 (−4.9)</td>
<td>2.64 (−28.3)</td>
</tr>
<tr>
<td>4</td>
<td>High</td>
<td>4.68</td>
<td>5.10 (9.0)</td>
<td>5.67 (21.1)</td>
</tr>
</tbody>
</table>

$^a$ Control activity determined in four healthy volunteers was 2.63 ± 0.75 U/g Hb. $^b$ Variations (%) from the basal values are given in parentheses.
In conclusion, this study provides new information on the mechanism of the overexpression of GST in uremic patients undergoing dialysis. The overexpression of GST and its related enzyme activity do not appear to be influenced by the presence of oxidative stress and do not depend on cell age in patients and controls or on supplementation and refractory responses to EPO. It seems probable that other factors linked to uremic toxicity are responsible for this phenomenon. In particular, some low-molecular weight toxins with electrophilic properties and bound to plasma proteins may play a key role in this context. Thus, the assay of GST expression can be useful to evaluate the individual accumulation of these molecules and to test the efficiency of new dialysis strategies in removing them.

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References


