Glutathione Transferases in the Urine: Sensitive Methods for Detection of Kidney Damage Induced by Nephrotoxic Agents in Humans

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With the aid of immunohistochemical methods the localization of the various isoenzymes of glutathione S-transferase was investigated. The α isoenzyme was present solely in the proximal tubular cells of the human kidney, while the π form was restricted to the distal convoluted tubules, the thin loop of Henle, and the collecting ducts. Damage to the epithelial cell membranes results in the increased excretion of these enzymes with the urine. The α and π isoenzymes have been isolated in a highly purified form and used for the production of polyclonal antisera. Subsequently, radioimmunological and ELISA techniques were developed for quantitation of these proteins in the urine; the methods exhibited a high specificity and were sufficiently sensitive to determine nanogram quantities or less. Disease affecting tubular function, cyclosporine A treatment, administration of nephrotoxic antibiotics, and exposure to cadmium all resulted in characteristic changes in the pattern of the glutathione transferase isoenzymes present in urine. Such effects were seen also in patients who had previously been exposed to nephrotoxic agents, but in whom conventional tests for kidney function were apparently normal. Thus, it appears that radioimmunologic or immunohistochemical quantitation of α and π forms of the enzyme can be used as sensitive and relatively simple markers for the early detection of toxic effects with respect to the renal tubuli. — Environ Health Perspect 102(Suppl 3):293–296 (1994).

Key words: nephrotoxicity, clinical diagnosis, tubular damage, glutathione S-transferase, cadmium

Introduction

In several organs—mainly heart and liver—cell damage is followed by release of a number of cytoplasmic enzymes to the blood, a phenomenon that provides the basis for clinical diagnosis of heart and liver disease. Although attempts in this direction have been made previously using, for example, the Tamm-Horsfall glycoprotein, corresponding assays for kidney damage have, for various reasons, had little success (1).

Three major classes of human isoenzymes of glutathione S-transferases (ligandin, GST) have been identified; α, π, and μ. Human kidney contains the α and π forms in relatively high amounts. Although there have been some previous attempts to use GST as a marker of kidney damage (2), earlier methods have not been sufficiently specific and sensitive to be clinically func-

This paper was presented at the Second International Meeting on Molecular Mechanisms of Metal Toxicity and Carcinogenicity held 10–17 January 1993 in Madonna de Campiglio, Italy.

This project has been supported by the National Swedish Chemicals Inspectorate and the Swedish Research Council for Engineering Sciences.

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Figure 1. Immunohistochemical staining of a human kidney biopsy for α- and π-glutathione S-transferases. Formalin-fixed slices were incubated with the antiserum, and the antigen-antibody complex visualized by the peroxidase-antiperoxidase technique (5). (A) Antiserum against human liver α-GST was used. Staining was only found in the proximal convoluted tubular epithelium. (B) Antiserum against human placental π-GST was employed. Positive reaction was obtained in epithelial cells of the thin segments of the loop of Henle, the distal convoluted tubules and the collecting ducts. Magnification × 550.
tional. The aim of our investigations has been to study the localization of the α and π isoenzymes of GST in the kidney, isolate the proteins in pure form, and develop radioimmunologic and immunochemical methods to be employed as sensitive and relatively simple markers for the assessment of toxic effects on the human kidney (3–5).

**Experimental**

Isolation of the α and π isoenzymes of GST from human liver and placenta, the production and purification of polyclonal antibodies in rabbit, development of immunochemical procedures for quantitation, and clinical diagnosis and selection of patients have been described elsewhere in detail (3–5).

**Purification of GST**

Highly purified α and π isoenzymes were obtained by using chromatographic procedures involving affinity chromatography with hexylglutathione coupled to epoxy-activated Sepharose as the ligand as a final step. Upon chromatography the purified proteins behaved as single components of 27,000 and 25,000 Da for the α and π isoenzymes, respectively.

**Localization in the Kidney**

Localization, as visualized by the peroxidase-antiperoxidase method (6), was studied in histological sections using the appropriate antibody (4). In fresh kidney biopsies, α GST is found exclusively in the proximal tubuli (Figure 1A), whereas the π enzyme (Figure 1B), absent from this tubular section, is localized to the distal convoluted tubulus and the thin loops of Henle and the collecting ducts.

**RIA and ELISA**

Using established principles, a competitive RIA was developed for both GST proteins. Routine measurements could be performed regularly in the range 2 to 1000 ng/ml in urine. An even more sensitive solid-phase ELISA for both isoenzymes was also developed. The titration curves for the α (Figure 2A) and π-GST (Figure 2B) demonstrate that quantitation down to around 0.5 ng/ml is feasible. The high quality of the antibodies is indicated by the steep slope of the titration curves.

**Specificity**

The specificity of the antibodies was tested with respect to the three isoenzymes, α, π, and μ, and with regard to other proteins that may be present in human urine, such as albumin, fibrinogen, prothrombin, hemoglobin and β-2-microglobulin. As can be seen from Table 1, the antisera of α and π proteins, reacted exclusively with the corresponding enzyme with no detectable cross-reactivity.

The same was true for several other tested proteins and enzymes. The specificity could also be confirmed by employing Western blot analysis of the cytosol prepared from various human organs. Figures 3A and 3B demonstrate that each antibody only reacts with one single protein band, also in preparations from tissues in which both isoenzymes are present. The band width is proportional to the relative levels of the isoenzymes present in liver, kidney, lung, and adrenals, respectively.

**Stability**

Samples kept in the refrigerator at 4°C for 5 days showed no significant decrease in enzyme content (Figure 4A). Storage at room temperature resulted in a gradual decrease, but after 5 days 35% of the original amount could still be detected. As seen in Figure 4B, stability at room temperature is not appre-

![Image](https://via.placeholder.com/150)

*Figure 2. ELISA titration of purified α- and π-GST. (A) α-GST; (B) π-GST*

*Figure 3. Western blot analysis of cytosolic proteins from human tissues with antibodies for α- and β-GST. (A) Reaction with antibodies towards α-GST. Lane 1, liver; lane 2, kidney; lane 3, lung; lane 4, adrenals; and lane 5, standard (mixture of purified α- and π-GST). (B) Reaction with antibodies against π-GST. Lanes 1to 5 as described for (A).*

*Figure 4. Stability of π-GST in human urine. A) Urine samples stored at 4°C or 20°C prior to measurement. B) Urine samples stored at pH 5.3, 7.0, and 8.5 (20°C).*

| Protein          | % of π-GST | % of α-GST |
|------------------|------------|------------|
| GST, π           | 100        | 0.3        |
| GST, α           | 0.2        | 100        |
| GST, μ           | 0.3        | 0.4        |
| Albumin          | 0.2        | 0.2        |
| Fibrinogen       | 0.4        | 0.4        |
| Prothrombin      | 0.1        | 0.2        |
| Hemoglobin       | 0.3        | 0.4        |
| β-2-microglobulin| 0.1        | 0.2        |

*The values for α and π-GST were taken as 100%. The values presented are the means of three experiments.*
induce tubular damage (7). Upon treatment with CsA, considerable amounts of α-GST are found in the urine, while π-GST remains unaffected. As expected, renal infarction, which destroys various types of kidney tissue, results in a more than 100-fold increase of both enzymes in the urine. Figure 5 shows the levels of α-GST in a patient treated with the aminoglycoside Netilmicin. After treatment for 5 days, the enzyme concentration in the urine was increased 15-fold. However, no changes in the serum creatinine level could be observed.

**Heavy Metal Nephrotoxicity**

Preliminary studies of cohorts of workers exposed to nephrotoxic heavy metals have indicated that quantitation of GST proteins in the urine constitute a valuable diagnostic tool. In Figure 6 the π-GST levels have been recorded in 15 accumulator workers who had been exposed to cadmium by inhalation 5 to 10 years previously, and who at the time of our investigation were diagnosed as clinically healthy using conventional criteria. However, in comparison with the control group, a continued increased excretion of the tubular marker, α-GST, was still evident.

Currently, workers in Sweden and Poland with a history of exposure to high levels of lead, cadmium, and mercury are being investigated to characterize the dose-response relationship for exposure to nephrotoxic heavy metals as well as the resulting changes in the GST isoenzyme pattern in urine.

**Discussion**

In the case of tubular dysfunction, the measurement of plasma proteins like β-2-microglobulin and the retinol-binding protein (α-2-microglobulin) have so far provided the most sensitive indices of tubular damage. The instability, especially in acid urine, constitutes the main problem with assays based on β-2-microglobulin. Although the retinol-binding protein is more stable, it is not a constitutive protein of the kidney, but is synthesized in liver; its presence in urine only provides indirect evidence of tubular damage (1). The persistence of signs of tubular damage in humans in whom cadmium exposure had been stopped many years before is consistent with previous observations based on determination of β-2-microglobulin in cadmium exposed workers (8). Our investigations have demonstrated that the determination of the isoenzymes of GST using RIA and ELISA techniques provides a reliable and sensitive alternative to conventional methods. The most important advantages of this technique are: α and π GST isoenzymes are constitutive proteins of the kidney. The markers are stable in urine. The method is simple, sensitive and reproducible. Due to selective localization of the two isoenzymes, differential diagnosis is possible.

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**Table 2. Glutathione S-transferase levels in urine of normal individuals and in patients with kidney disease.**

| Patients         | α-GST, ng/ml urine | π-GST, ng/ml urine |
|------------------|--------------------|--------------------|
| Controls         | 0.6                | 5.9                |
| Rejection after  | 0.9                | 20.5               |
| transplantation  |                    |                    |
| Cyclosporin A    | 6.2                | 6.3                |
| Tubular necrosis | 21.3               | 246                |
| (acute form)     |                    |                    |
| Renal infarct    | 148                | 808                |

*Mean values (n=11–49).
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