Transpl Int (2002) 15: 546–549
DOI 10.1007/s00147-002-0462-5

ORIGINAL ARTICLE

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Functional relationship of α-glutathione *S*-transferase and glutathione *S*-transferase activity in machine-preserved non-heart-beating donor kidneys

Received: 20 March 2001
Revised: 24 June 2002
Accepted: 2 July 2002
Published online: 3 October 2002

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Abstract α-Glutathione *S*-transferase (α-GST) is a biochemical parameter used to estimate the amount of proximal tubule damage to a kidney. In normal clinical practice, the concentration of α-GST in urine is determined by a rather time-consuming immunochemical test, the enzyme-linked immunosorbent assay (ELISA). Kidneys from non-heart-beating (NHB) donors are perfused prior to transplantation. The determination of α-GST concentration in the perfusate to monitor damage is also done by means of an ELISA test. However, because this is a time-consuming method, it would be helpful to find a parameter proportional to GST concentrations that would be available within minutes. We therefore compared the method of determining α-GST concentration via ELISA with that of determining the enzymatic activity of GST, which is much faster (results available within 10 min). The comparison was made using 150 preserved kidneys that had been perfused for 6 h. The correlation was found to be very good, as indicated by the linear regression data: $r = 0.954$, $P < 0.001$. π-Glutathione *S*-transferase (π-GST) was also determined by means of an ELISA test, and the concentration of π-GST was compared with the enzymatic activity of the "total" GST. The precision of the enzymatic method, given by intra- and interassay variation, was 1.5% and 10.5%, respectively.

Keywords GST activity · α-GST · Acute tubule necrosis · Proximal tubule damage

Glutathione transferases (GSH transferases) play an important role in conjugation reactions and are present at high levels in the liver and kidney. Three major forms of glutathione S-transferase (GST) – basic (α-GST), neutral (μ-GST), and acidic (π-GST) – have been identified and isolated from human tissues, and it appears likely that each major form consists of subgroups of isoenzymes [2, 9, 13, 21, 28]. The basic GSH transferases (molecular weight 51,000), formerly known as ligandin, have been reported to be localized in the proximal tubule epithelium in the kidney [7, 21]. Earlier investigations detected the enzyme in urine, which is normally free from GSH transferases, as a result of leakage from damaged tubule cells and not as a result of increased glomerular filtration of blood enzymes [1, 22, 25]. Neutral GST (human μ-class) [14] was first identified in the human liver in 1980. Acidic GSTs (π-class isoenzymes) are widely distributed, represent the most thoroughly characterized extra-hepatic form, and have been purified from a number of sources including placenta [2, 15, 23] and erythrocytes [21].

Kidneys are procured from donors who have suffered irreversible cardiac arrest, i.e., whose deaths were of cardiac origin. These are called non-heart-beating

(NHB) donor kidneys. Warm ischemia time (WIT), defined as the period between cardiac arrest and the start of organ cooling, is often of unknown duration. Therefore, it is important to be able to estimate ischemic damage for the safe use of kidneys from NHB donors.

The appearance of α-GST in the perfusate of continuously perfused kidneys was found to predict the development of post-transplant acute tubule necrosis [8]. The aim of this study was to develop a rapid method of determining α-GST concentration. We therefore compared α-GST, a typical proximal tubule damage marker, with the enzymatic GST activity test.

The substrate most commonly employed in activity measurements is 1-chloro-2,4-dinitrobenzene (CDNB) [10, 11]. GST catalyzes the transfer of the SH-group of GSH to a wide variety of hydrophobic acceptor molecules, rendering the product more water-soluble. Although the different isoenzymes may be distinguished from each other by the spectrum of molecules they can use as acceptors, all isoenzymes use CDNB as the substrate for transfer reactions. The method described by Habig et al. [10] was modified and optimized for automation and reproducibility.

Materials and methods

Kidneys

All kidneys were from NHB donors and were preserved by machine perfusion at the University Hospital of Maastricht [8]. Samples for the determination of GST activity and α-GST concentration were taken from the perfusate at time points 0, 1, 2, 4, 6, and 8 h after preservation and immediately stored at –80 °C.

GST chemicals

All chemicals for GST activity were of analytical grade: dimethylsulfoxide 99% (DMSO, Merck), CDNB (gradel, approximately 99%), reduced-form glutathione, and GST from rabbit liver (G8261) (Sigma-Aldrich).

Buffer used

We prepared the buffer just before it was required, by mixing 4.5 ml potassium phosphate buffer (mix 500 ml 100 mmol/l KH_2PO_4 with 60 ml 100 mmol/l $K_2HPO_4 \times 3H_2O$, adjusted to pH 5.9), 300 µl glycerin 43.5% (diluted glycerin 87% 1:1 with 100 mmol/l potassium phosphate buffer), and 1.2 mL CDNB (27.5 mmol/l in DMSO 100%, frozen in portions of 1.2 mL).

Determination of α-GST concentration

The glutathione solution contained 13 mmol/l GSH and 43.5% glycerol (w/v) in 100 mmol/l KH_2PO_4 pH 5.9. α-GST was quantitatively determined by NEPHKIT-Alpha immunoassay (Biotrin International, Dublin, Ireland) designed for urine analysis, which has a range of detection from 0 to 10 µg/l and utilizes anti-α-GST IgG antibodies. Perfusate samples were assayed by the standard assay procedure after being diluted from 25 to 1,000 times in the buffer.

Determination of enzymatic GST activity

Reactions were carried out in a Cobas Bio or Cobas Fara centrifugal analyzer at 37 °C (Hoffmann-La Roche, Basle, Switzerland). In the Cobas system, the volumes were: 5 µl sample preservation bath solution, 20 µl diluent (bidest), and 160 µl reagent mix. After 60 s of incubation, which is sufficient for temperature and substrate equilibration, the reaction was started with 18 µl of reduced glutathione solution and 20 µl diluent (bidest). The final pH of the test was 6.5. After the start of the reaction, the optical density at 340 nm was recorded after a lag time of 60 s at intervals of 10 s, for 60–160 s [molar extinction coefficient CDNB = 9.6 mmol^{-1} ×cm^{-1}×l]. We corrected the rate for spontaneous hydrolysis by omitting the preservation fluid in a sample blank. This rate was then subtracted from the rate obtained in the samples containing perfusion fluid.

We modified the conditions given by Habig et al. [10] to give better substrate solubility. We also automated the method to give better reproducible results. DMSO was used instead of ethanol, which gives better solubility; however, the adsorption of CDNB itself limits the concentration to 4 mmol/l. The *K*m measured was 1.0 mmol/l. This is in agreement with the findings of Habig et al. [10]. The optimal CDNB concentration should be approximately ten times *K*m, but solubility allows a concentration of only 4.0 mmol/l.

π-GST

π-GST was quantitatively determined by NEPHKIT-Pi Human GST-Pi immunoassay (Biotrin), which has a range of detection from 0 to 50 µg/l. Antibody anti-π-GST IgG was utilized in the assay. The detection limit of Biotrin's NEPHKIT-Pi is 3 µg/l.

Statistical analyses of the results

Using linear regression, we calculated a regression line for the prediction of α-GST from GST activity. The cut-off point for GST activity corresponding to the cut-off point 2,500 µg/100 g kidney for α-GST concentration (ELISA method) follows from the prediction formula. The accuracy of this prediction is represented by 95% prediction limits [26]. This cut-off point is the concentration of α-GST obtained from 100 g of tissue after 6 h of perfusion.

Results

Storage conditions

Concentration of samples, immediately frozen at –80 °C, did not change during the course of 1 year or more.

Correlation between enzymatic GST and α-GST levels

Figure 1 shows the correlation between α-GST levels (range 50–15,000 µg/l) and GST activity (range 25–600 U/l) from 150 kidneys at T = 6 h with 95% prediction limits. Data are expressed for α-GST and GST activity in µg/100 g kidney and in U/100 g kidney,

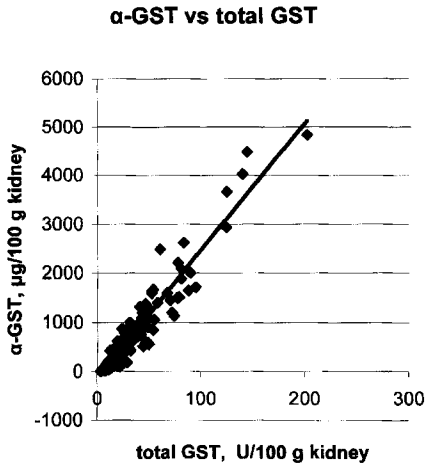

Fig. 1 Correlation between GST activity and α-GST from 150 kidney preservation samples at T = 6 h. Using linear regression, we calculated a calibration line for the prediction of α-GST in µg/100 g kidney from GST activity in U/100 g kidney

Table 1 Correlation between GST activity and 95% prediction limits

Parameter	Coefficient	Standard error	*P*	Lower 95%	Upper 95%
Intercept	−145.13	32.70	< 0.001	−209.77	−80.489
GST	26.037	0.700	< 0.001	24.65	27.42

respectively. The correlation coefficient was 0.952 ($P < 0.001$). A regression line was calculated for the prediction of α-GST from GST activity (Table 1). The cut-off point for GST activity corresponding to the cut-off point for α-GST was 101.6 U/100 g kidney, with prediction limits ranging from 82.5 to 121.0.

Kidney preservation time curves

Figure 2 shows the typical GST release curves during kidney preservation at T = 1, 2, 4, 6, and 8 h. These curves are independent of flow.

Correlation between GST activity and π-GST levels

Twenty-eight kidneys were used to correlate GST enzymatic activity and π-GST concentration in the perfusion fluids after 6 h. A correlation coefficient of $r = 0.477$ was found.

Discussion

Several viability parameters have been proposed in the past. However, currently, none of these parameters for

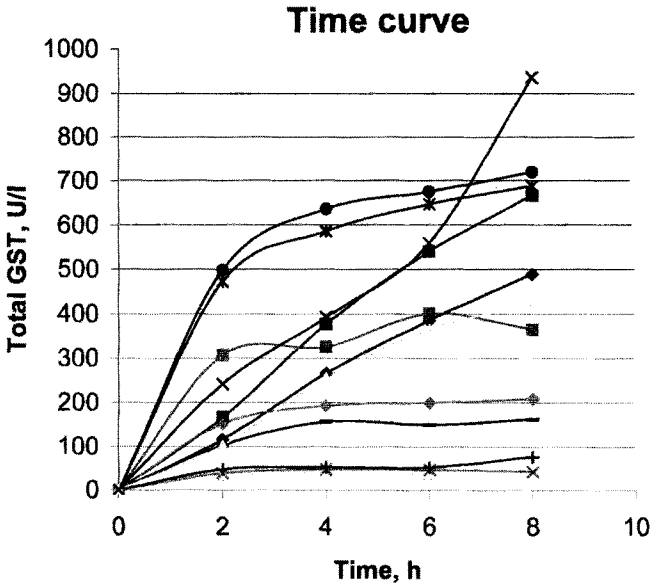

Fig. 2 Typical GST activity curves for different kidneys. The samples were taken at T = 0, 1, 2, 4, 6, and 8 h. GST activity is expressed in U/l. This indicates the variation between kidneys

estimating warm ischemic damage and predicting transplant outcome is routinely used in clinical practice [5, 6, 17]. One parameter, α-GST, had already been used in an evaluation study [8]. α-GST is found in the proximal tubulus [24], while π-GST is confined mainly to the distal tubulus [5, 6, 17]. The third isoform (µ-GST) is expressed only in the liver in 60% of the population [3, 4, 12, 16, 18, 19, 20, 27].

It has been shown that a strong correlation exists between WIT and the release of α-GST, and that α-GST can be used to define margins for the safe use of NHB donor kidneys. Post-transplant function was found to worsen with increasing α-GST concentration [8]. However, the determination of α-GST concentration was done using an enzyme-linked immunosorbent assay (ELISA). The long incubation times required by this technique prohibit its use as a viability parameter for donor kidneys. We have now shown that instead of determining the concentration of α-GST (this is very low and can only be determined with immunochemical methods), we can also use its enzymatic activity for this purpose. This method is fast (10 min), inexpensive, and requires no special instruments. Thus, it can easily be implemented in centers that perform kidney transplantations.

Acknowledgements We would like to thank Arnold Kester (Department of Methodology and Statistics, Maastricht University) for his help with the statistical analysis.

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