Evaluation of renal enzymuria and cellular excretion as a marker of acute nephrotoxicity due to an overdose of paracetamol in Wistar rats

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Abstract

Introduction: The present study was conducted to determine whether the urinary levels of excreted enzymes, γ-glutamyltransferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate (AST) and alanine aminotransferases (ALT), can efficiently indicate, within 24 h, an acute nephrotoxicity due to an overdose of paracetamol (PAR).

Methods: A baseline urine was collected from the experimental group. Thereafter, blood collected from the orbital sinus (1.0 ml) and paracetamol (650 mg/kg of body weight) was administered by gavage. After the drug administration, animals were returned to the metabolic cages and then urine was collected in the next 22 h. Blood and urine collection was performed at time 0+24 h (T24), as well as at times 48 and 72 h (T48 and T72). After the last urine and blood collection, the rats were killed and the kidneys removed and prepared for histological examination. Plasma creatinine and urinary levels of creatinine (to determine glomerular filtration rate—GFR), GGT, ALP, LDH, ALT and AST were measured. Kidney tissues were stained with hematoxylin and eosin stain for histological assessment.

Results: Urinary levels of GGT, ALP and LDH enzymes were significantly higher (P<0.05) at T24 when compared to the levels at T0 and returned to basal levels at T48 and T72. The number of urinary epithelial cells at T24 was significantly higher when compared to the control time (T0) (P<0.001). The GFR was significantly reduced 24, 48 and 72 h after the drug administration.

Conclusion: The number of urinary epithelial cells and urinary enzymes levels are a simple and low cost procedure that is available and can help in the detection of renal acute lesions.

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1. Introduction

Paracetamol (also known as acetaminophen) is a widely used over-the-counter analgesic, an antipyretic drug that is safe at therapeutic dosages (10–15 mg/kg). Even though paracetamol is considered a safe drug, in overdose situation, it produces hepatic necrosis and renal failure in both humans [1,2] and experimental animals [3]. In a recent study, Bessems and Vermuelen [4] described the molecular aspects of paracetamol-induced toxicity showing that the main clinical finding produced by high doses of paracetamol is the hepatotoxicity related to the P-450 cytochrome route. Renal tubular damage and acute renal failure can also occur, even in the absence of liver injury [5]. This implies that the hepatic and renal metabolic capabilities are independent, even though both require NADPH, oxygen and the P-450 cytochrome [6,7]. The main characteristic features of paracetamol-induced renal damage are acute tubular necrosis, increase in plasma creatinine levels and decrease in GFR. Tubular cell injury is the main feature observed during paracetamol-induced renal failure and the main functional evidence of proximal tubular injury is phosphaturia and low-molecular-weight proteinuria [8–10].

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The renal tissue is the main source of the excreted urinary enzymes and the evaluation of these enzymes level is known to be a good and sensitive noninvasive method to measure the tubular cells integrity [11,12]. These renal enzymes are characteristic and located at different specific sites. GGT is mainly located at proximal tubule and Henle loop. ALP is found on the epithelial cells of proximal tubule and the LDH enzyme mainly at distal tubule cells.

Another manner by which acute renal injury is evaluated is by monitoring the number of epithelial cells excreted in the urine. Stetinova et al. [13] demonstrated that monitoring the urinary excreted cells, during a fixed time interval, could be a good and sensible tool to evaluate the acute injury produced by a nephrotoxic agent.

2. Materials and methods

2.1. Ethics

All experiments were performed in accordance with the University Research Ethic Committee guidelines for experiments with animals.

2.2. Chemicals

Paracetamol and polyethylene glycol-400 was obtained from Sigma Chem. Co.. (St. Louis, MO). All other chemicals were of analytical grade and of current use in our laboratory.

2.3. Animals

Adult male Wistar rats weighing 200–250 g were used for the study. The rats were housed in individual metabolic iron cages, with free access to water, in a thermostatically controlled room under 12-h dark/light cycle. By design, we used just one group (n=6) for the entire experiment. As a control, we used the data collected at time zero (T0), just before the administration, by gavage, of the drug (650 mg/kg of body weight of paracetamol, diluted in polyethylene glycol-400 at 50%).

2.4. Animal treatment

We collected urine from the experimental group during 22 h, with the animals having free access to water but no food. After that, blood was collected (1.0 ml) and diluted paracetamol was administered by gavage. After this administration, the animals were feed for 2 h (protein chow; Nuvilab-CR1, Nuvital Nutrientes S/A, PR, Brazil) and then the urine was collected during the following 22 h with the rats having no access to food. We collected blood at time = 0+24 h (T24) and repeated this procedure at 48 and 72 h (T48 and T72). After the last urine and blood collection, the rats were killed and the kidneys removed and used for histological examination.

2.5. Biochemical studies

The blood and urine samples (T0, T24, T48 and T72) were centrifuged at 3000×g for 5 min and the supernatant frozen at −70 °C for late analysis. We used blood for plasma creatinine measurement. In the urine, we measured creatinine, GGT, ALP, LDH, ALT and AST. All enzymes quantification was made by using commercial kits (Labtest Diagnostica S.A. Minas Gerais, Brazil) according to the manufacturer’s instructions and expressed by gram creatinine. The measurements of GFR were calculated through the measurement of creatinine clearance using standard formulae.
2.6. Histologic studies

Kidney tissues were fixed in buffered formalin, processed and stained with hematoxylin and eosin stain for histological assessment (25 μm slices).

2.7. Statistical analysis

Results are expressed as mean±S.E.M. Significance at 95% level was established using paired MANOVA with Newman–Keuls post hoc test, taking \( P<0.05 \) as significant. For correlation studies, Pearson rank correlation was used. The presence of significant differences between test points (\( T_0, T_{24}, T_{48} \) and \( T_{72} \)) was examined by the mean of the SPSS 11.5 statistical software package (SPSS Inc., Chicago, IL).

3. Results

3.1. Enzymes excretion

The urinary excreted enzymes measured at each time-point are shown in Fig. 1. GGT, ALP and LDH were significantly higher (\( P<0.05 \)) at \( T_{24} \) when compared to the values obtained at \( T_0 \), and returned to the basal levels at \( T_{48} \) and \( T_{72} \). ALT and AST did not show statistical significant difference in any time-point, when compared to the values obtained at the control time-point (\( T_0 \)).

3.2. Excreted cells

We counted the excreted epithelial cells, as per Stetinova et al. [13], at each time-point and noticed that the value obtained at \( T_{24} \) was significantly higher than the value measured at control time (\( T_0, P<0.001 \)). The count was returned to the basal value after 48 and 72 h (Fig. 2).

3.3. Glomerular filtration rate

GFR was significantly reduced 48 h after the drug administration and did not return to the basal level up to 72 h (\( T_0 = 0.67±0.06, T_{24} = 0.35±0.05, T_{48} = 0.21±0.02, T_{72} = 0.24±0.03 \) ml/min) (Fig. 3).

![Fig. 3. GFR at time 0 h (basal rate) and 24, 48 and 72 h after intragastric administration of paracetamol 650 mg/kg in Wistar rats. Data are expressed as mean S.E. of the mean (n=6). *\( P<0.001 \).

3.4. Correlation between enzymuria – \( T_{24} \) – and GFR at \( T_{48} \)

A highly significant inverse correlation was obtained between the excreted renal enzymes GGT, ALP and LDH, measured at \( T_{24} \), and the GFR (GFR), measured 48 h after the drug administration (\( r=0.76, 0.73 \) and 0.79, respectively, \( p<0.001 \)).

3.5. Histology

Histological analysis of the kidney samples showed only minor evidence of renal damage, not enough to confirm explicit injury.

4. Discussion

Paracetamol-induced renal injury may be due to the metabolic activation of paracetamol to the reactive metabolite \( N \)-acetyl-\( p \)-benzoquinone imine [14]. This metabolite can directly react with glutathione and, at overdose of paracetamol, depletion of cellular glutathione may occur [15]. Depletion of glutathione may have two adverse consequences. First, it reduces the inactivation of the reactive metabolite and tends to increase its covalent binding to macromolecules. Second, it may have several deleterious effects on cell homeostasis and may therefore aggravate the toxic effects of reactive metabolite [16].

The detection of enzymes liberated during the lesion of tubular cells has been studied to evaluate acute and chronic lesion in many clinical and experimental studies. Most of the urinary enzymes, such as GGT and ALP, are located in the brush border of the proximal tubule epithelium. The increase of the concentration of these enzymes in the urine denotes lesion in the brush border membrane and structural alterations due to loss of the microvule, which reduces the reabsorption of proteins filtered as for \( β2 \)-microglobulina [17].

Westhuyzen et al. demonstrated that enzymuria monitoring can detect acute renal failure up to 4 days before significant alterations in GFR can be detected. Among the studied enzymes, GGT and ALP stand out as being particularly important ones to identify patients with high risk of acute renal impairment [18].

The number of urinary epithelial cells showed similar changes between GGT, ALP and LDH enzymes. Stetinova et al. demonstrated that the excretion of urinary tubular cells is more sensitive for nephrotoxicity than urinary excretion of \( N \)-acetyl-\( β \)-d-glicosaminidase (NAG) [13].

In the present study, using Wistar rats as a model, we monitored GFR and urinary excreted enzymes and cells to determine if enzymuria measurement could be a good tool for estimation, within 24 h, of the acute renal injury produced by an overdose of paracetamol. We found that paracetamol-induced renal damage was accompanied by a significant increase of enzymuria, especially of GGT and ALP enzymes, well in advance to the major manifestation of an acute reduction on the glomerular filtration rate. Therefore, although the greatest enzymuria increase might have preceded the greatest GFR reduction (\( T_{24} \) vs. \( T_{48} \), a clinically significant GFR reduction was already evident at the time of maximum enzymuria. These findings are in agreement with those reported earlier by others [19,20].
Urinary GGT, ALP, LDH and cells count, determined 24 h after the drug administration, correlated very well with the measured GFR. ALT and AST did not present significant correlation (data not shown). These findings suggest that GGT, ALP, LDH and cells count determination could be a sensible and low cost alternative to evaluate, within 24 h, the risk to have an acute renal failure due to an overdose of paracetamol.

The cells count and enzyme concentration determination are a very simple and low cost procedure that are available at every hospital; we are encouraged to proceed our studies aiming to determine if we can extrapolate our findings to humans.

In conclusion, in the acute paracetamol nephrotoxicity, the urinary enzymes analysis can be a faster and relevant method in the detection of renal damage.

References


