ACUTE ACETAMINOPHEN NEPHROTOXICITY AND URINARY GAMMA-GLUTAMYL TRANSFERASE ACTIVITY IN RATS

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SUMMARY

In order to determine the relationship between the nephrotoxicity of acetaminophen and urinary gamma-glutamyl transferase (GGT) excretion, a single dose of 900 mg/kg acetaminophen (APAP) was administered to rats intraperitoneally. Following drug administration, 24 hour urine was collected and the kidneys were removed under ether anesthesia for histological examination. GGT activity measurements and quantitative analysis for creatinine was carried out on urine samples. Urinary GGT activity in the APAP administered group (n=12) (1.88 ± 0.21 U/mg creatinine) was significantly higher than in the control group (n=16) (0.77 ± 0.05 U/mg creatinine) ($p<0.0002$). Histological examination of the kidneys under light microscopy showed only very slight tissue damage. Further use of urinary GGT activity measurements in experimental nephrotoxicity studies has been suggested.

KEY WORDS

nephrotoxicity, gamma-glutamyl transferase, acetaminophen

INTRODUCTION

N-Acetyl-p-aminophenol (APAP), also called acetaminophen or paracetamol, is an antipyretic analgesic which is widely used. Although APAP is a safe drug when consumed at therapeutic doses, its consumption at large overdoses may cause massive hepatic necrosis and acute renal failure /1,2/.

Berg /3/ suggested that the available clinical and epidemiological data, including several population based case-control studies, provide inadequate evidence of carcinogenicity of acetaminophen in the kidney or urinary tract in humans. In the same review it was also stated that the chronic use of high doses of this drug should be avoided.

Due to increase in the urinary excretion of renal enzymes in case of kidney damage, these enzymes are included in the list of "nephrotoxicity markers" /4/. It has been shown in many studies in humans /5,6/ and especially in rats /7-10/ that the nephrotoxic effects of some drugs can be determined by the changes in urinary enzyme activities. Gamma-glutamyl transferase (GGT, EC 2.3.2.2), which has maximal activity in the brush border membranes of proximal tubular epithelium, is an enzyme investigated as a pre-indicator of acute nephrotoxicity /6-9/.

Studies conducted on the nephrotoxicity of acetaminophen have generally investigated its metabolites and the role of these metabolites in its nephrotoxic effect /2,11/ or their nephrotoxic mechanisms /12/. Although there have been some studies on acetaminophen's effects of parameters such as glomerular filtration rate (GFR), clearance on p-aminohippuric acid (CIPAH) /13/, blood urea nitrogen (BUN), urinary glucose and osmolality /14-16/, studies measuring the activity of specific urinary enzymes, such as urinary N-acetyl-β-glucosaminidase (NAG), alanine aminopeptidase (AAP) /17/, alkaline phosphatase (ALP) /18/ or GGT /12/, have been limited.

This study was undertaken to determine the changes in activity of urinary GGT, which is accepted as a nephrotoxicity marker, and the tubular damage induced by the application of a single high dose of APAP (900 mg/kg, i.p.) in rats, and to confirm this damage by histological examination.

MATERIALS AND METHODS

Male Swiss Albino rats (E.Ü. Faculty of Pharmacy Experimental Animal Laboratories, Bornova, Izmir), weighing 125-250 g were used in this study.

Acetaminophen, glycylglycine, and gamma-L-glutamyl-p-nitroanilide (Sigma Chemical Co., St. Louis, MO, USA) were of analytical grade.

The rats were housed in metabolic cages and were supplied with food and water *ad libitum* before experiment. The experimental group of 12 rats (180-230 g) was treated with a 900 mg/kg single dose of APAP (35 mg/ml suspension in distilled water, 40°C) intraperitoneally /2/. The control group was injected with the same volume of 0.9% NaCl (16 rats, 125-250 g). After injection, no food was given to both groups and 24-h urine was collected. Following the urine collection, the kidneys of the rats were removed under light ether anesthesia, into 0.2 M sodium cacodylate containing 8% paraformaldehyde (pH 7.3) /8/.

The 24-h urine samples were centrifuged for 10 min at 3000 *g* (4°C) /7/, the supernatants were diluted with distilled water (1:10) /8/, and measured for GGT content. GGT activity was determined by a modification of the method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology /19/. The final reaction mixture contained in 2.4 ml total volume: 0.2 ml diluted urine; 75 mM glycylglycine and 10 mM $MgCl_2$ in 2 ml 100 mM Tris-HCl buffer, pH 8.2; 4 mM final concentration of gamma-L-glutamyl-p-nitroanilide in 0.2 ml HCl (0.15 mol/l). After incubation for 10 min at 37°C, the reaction was stopped by addition of 1 ml diluted glacial acetic acid (1.4 mol/l) /20/. Measurements were made at 405 nm. One unit of GGT activity was defined as the amount of enzyme that catalyzes the formation of 1 μmol of p-nitroanilide per minute under the assay conditions. Urinary creatinine concentrations were also measured using the Jaffé reaction /21/. Urinary GGT activities found in the study were evaluated as ratios against urinary creatinine.

The results are expressed as the mean ± standard error of the mean. They were statistically analyzed by the non-parametric Mann-Whitney U test.

Histological examination of renal tissue was made on 3-4 μm thick cross sections stained with hematoxylene-eosin, PAS, Masson trichrome and methenamine silver, using a light microscope.

RESULTS AND DISCUSSION

The mean values for urinary GGT activities of the groups treated with APAP and controls are given in Table 1. The mean urinary GGT activity (1.88 ± 0.21 U/mg creatinine) in the treated group was found to be significantly higher ($p<0.0002$) than in the control group (0.77 ± 0.05 U/mg creatinine).

TABLE 1

Urinary GGT activities of the control group and the acetaminophen (900 mg/kg x single dose, i.p.) treated rats

	No. of rats	GGT/creatinine (U/mg)	Proximal tubular necrosis#
Control	16	0.77 ± 0.05	–
Acetaminophen	12	1.88 ± 0.21*	±

Results are expressed as mean ± SEM
Control vs acetaminophen group: *$p<0.0002$
#Arbitrary grade: – no change, ± slight change

Histological examination by light microscope showed that there was only a minimal level of damage in the kidney tissues of the group treated with APAP (Figure 1).

Although urinary GGT activity measurements have been used in many studies to determine nephrotoxicity induced by drugs such as gentamicin /9,10/, neomycin /7/ and *cis*-platin /7-9,22/ in experimental animals, there is only one investigation of acetaminophen nephrotoxicity using urinary GGT activity measurements /12/. In this study the hepatotoxic and nephrotoxic effects of APAP were suggested to involve different mechanisms; it was hypothesized that the

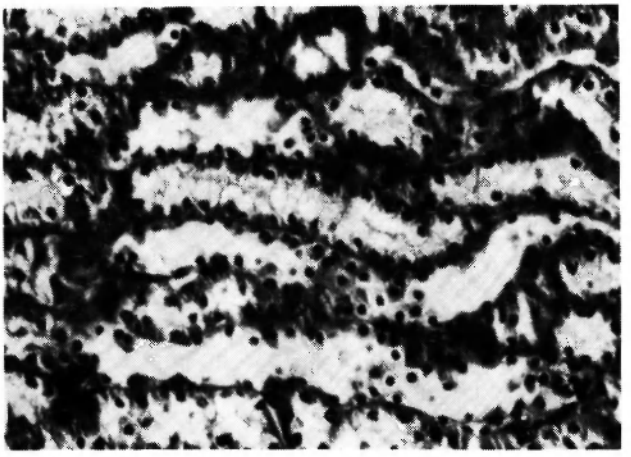

Fig. 1: Microphotograph of kidney histology of rat exposed to acetaminophen (900 mg/kg x single dose, i.p.). Note hydropic degeneration of tubular epithelium and some other tubules displaying focal desquamations of the epithelium (H.E. x400).

metabolism of acetaminophen-S-conjugates by a C-S-lyase may be responsible for the nephrotoxicity.

This study showed that a single dose APAP injection significantly increased urinary GGT excretion ($p<0.0002$) (Table 1). An average of 144% increase in urinary GGT activity was observed in the group treated with APAP in comparison with the control group.

The method of application of the high dose of APAP to rats has been found to be important. Colin *et al.* /11/ found no adverse hepatic or renal effects when APAP was applied orally. Due to the toxic effects observed in that study /11/ after i.p. application, this method of application was used in the present study.

For determination of urinary GGT activity, the removal of inhibitors in urine by pretreatment such as dialysis /6/ or gel filtration /10/ has been suggested. However, since it was also found that 1/10 dilution of urine samples led to 20% increase in enzyme activity, without any pretreatment /8/, the GGT activity was measured in 1/10 diluted urine samples in the present study. In order to minimize errors arising from the collection of urine samples and the effects of changes in urine flow /6/, GGT activity values are given as ratios against urinary creatinine (Table 1).

In spite of the significant increase find in urinary GGT, histological examination showed only little degenerative change in the kidney tissue (Figure 1). It has been stated that nephrotoxicity induced by APAP is related to the strain of rats and that renal damage is observed in Fischer 344 rats but not in Sprague-Dawley rats /2/. In other studies, it has been suggested that there is a relationship between proximal renal damage and the rat's age; the effects of differences in strain decrease as the rat's age increases; it is possible that these effects are related to changes in the pharmacokinetics of the drug on aging /14,15,23/. The lack of histological evidence of kidney damage in this study may be related to the strain of rats used; however, it may also be due to the sensitivity of GGT as a marker, as effects might be seen to occur in this parameter prior to histological evidence of tissue damage.

It is recommended that further studies be performed to evaluate acute acetaminophen nephrotoxicity using different strains of rats and measuring also other urinary enzymes which are "nephrotoxicity markers" as well as urinary GGT, with electron microscopic examination for kidney damage. In order to confirm the clinical relevance of this non-invasive method, urinary GGT excretion should be measured in different patient groups after APAP treatment.

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