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Acetaminophen intoxication is associated with decreased serum paraoxonase and arylesterase activities and increased lipid hydroperoxide levels

S Karadas¹, M Aslan², H Gonullu¹, C Kati³, L Duran³, S Olmez⁴, ME Kucukoglu² and H Demir⁵

Abstract

Background: Acetaminophen is at present one of the most commonly used analgesics and antipyretics. Recent evidence has suggested that oxidative stress is involved in the mechanism of acetaminophen intoxication. Paraoxonase-I (PONI) plays an important role as an endogenous free-radical scavenging molecule. The aim of this study was to evaluate the influence of serum PONI activity and oxidative stress in patients with acetaminophen intoxication.

Methods: A total of 20 patients with acetaminophen intoxication and 25 healthy controls were enrolled. Serum total antioxidant capacity (TAC), lipid hydroperoxide (LOOH) levels, and paraoxonase and arylesterase activities were measured spectrophotometrically.

Results: The serum TAC levels and the paraoxonase and arylesterase activities were significantly lower in patients with acetaminophen intoxication compared with controls (all, $p < 0.001$), while the serum LOOH levels were significantly higher ($p < 0.001$).

Conclusions: Our results suggest that decreased PONI activity seems to be associated with increased oxidative stress in patients with acetaminophen intoxication. Measuring serum PONI activity may be useful in assessing the development of toxicity risk in acetaminophen toxicity. It would be useful to recommend vitamins with antioxidant effects such as vitamins C and E along with medical treatments.

Keywords

Acetaminophen intoxication, PONI activity, oxidative stress, total antioxidant capacity

Introduction

Acetaminophen (*N*-acetyl-*p*-aminophenol), also known as paracetamol, is at present one of the most commonly used analgesics and antipyretics. It is considered to be a safe drug when taken at therapeutic doses. However, acetaminophen overdose can cause hepatotoxicity.¹

Acetaminophen is primarily metabolized by sulfation and glucuronidation, but these pathways become saturated with increased doses, and a greater proportion of the drug is available for oxidation by the microsomal cytochrome P-450 system.² Hepatic damage in acetaminophen toxicity is induced by *N*-acetyl-*p*-benzoquinoneimine (NAPQI), a reactive metabolite that is the primary metabolite responsible for hepatotoxicity.³

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Glutathione (GSH) depletion in paracetamol-induced hepatotoxicity is a consequence of high reactive oxygen species (ROS) formation.⁴ GSH, the most abundant low-molecular-weight thiol in animal cells, plays a central role in the antioxidant defense against ROS. ROS generation and GSH depletion in the early stage of the hepatic metabolism of acetaminophen have been proposed as the possible mechanisms mediating the hepatocellular injury process.⁴ Therefore, identifying the mechanism of acetaminophen toxicity is an important matter for medical and pharmaceutical research. The precise mechanism involved is not entirely unknown, though oxidative damage and redox imbalance have been suggested as the mechanisms behind acetaminophen toxicity.⁵

Paraoxonase-1 (PON1), a calcium-dependent esterase, is synthesized in liver and released into the blood where it circulates with high-density lipoprotein (HDL). The enzyme has both arylesterase and paraoxonase activities.⁶ Serum PON1 catalyzes the hydrolysis of many organophosphates and aromatic carboxylic acid esters.⁷ PON1 prevents the accumulation of lipid peroxides in low-density lipoprotein cholesterol (LDL-C).⁸ PON1 is believed to be involved in protecting against oxidative stress. It has been suggested that decreased serum PON1 activity might be associated with increased oxidative stress.⁹ Furthermore, oxidative stress has been shown to decrease PON1 activity and downregulate the serum expression of PON1.^{10,11} The antioxidant activity of paraoxonases is well established, and lower PON1 activity has been reported in the etiology of various disorders such as cardiovascular diseases.^{12,13}

To our knowledge, there is limited information in the literature about serum PON1 enzyme activity and oxidative stress in acetaminophen intoxication.¹⁴ Therefore, the aim of this study was to investigate the influence of serum PON1 activity and oxidative stress in patients with acetaminophen intoxication.

Materials and methods

Subjects

In this prospective study, 20 (15 females and 5 males) patients with acetaminophen intoxication and 25 (14 females and 11 males) healthy controls were enrolled. Eight patients were married, while other patients were single.

All intentional drug overdose patients were included in the study. Patients were asked for both generic and specific brand names with emphasis on

the commonest products. In addition, they were thoroughly questioned on the number of medications taken, amount, time of ingestion, past medical history, current drug therapy, and availability of medications at home. Family members or attendants were questioned and pill packages were sought, whenever possible.

Vital signs of all patients were normal. None of the patients had been taking antioxidant vitamin supplements, including vitamins E or C. All the patients were nonsmokers.

Serum acetaminophen levels could not be measured because of technical difficulties. Dosage of ingested acetaminophen was 10.20 ± 4.95 g. At the beginning of the treatment, all the patients were given a first high dose of oral *N*-acetylcysteine (NAC; 140 mg/kg). Then, 17 additional oral doses of 70 mg/kg NAC were given every 4 h, comprising a total dose of 1330 mg/kg over 72 h.

The control group was selected from 25 healthy volunteers. All the control subjects were asymptomatic with unremarkable medical histories and normal physical examinations. None of the control subjects had been taking antioxidant vitamin supplements, including vitamins E or C. All the control subjects were nonsmokers. Of the total control subjects, 12 were married and the remaining were single.

The study protocol was conducted in accordance with the Helsinki Declaration as revised in 2000 and was approved by the local ethics committee. All the subjects were informed about the study, and a written consent was obtained from each one.

Exclusion criteria

The exclusion criteria included a history of alcohol abuse, habitual smoking, intravenous drug abuse, pregnancy, antioxidant supplements, hypertension, diabetes mellitus, liver or renal disease, rheumatoid arthritis, pulmonary disease, and coronary artery disease.

Blood samples

Blood samples were collected at least 4 h after drugs ingestion in all patients prior to NAC treatment and immediately stored at 4°C. The serum samples were then separated from the cells by centrifugation at 3000 r/min for 10 min. The serum samples were stored in plastic tubes at -80°C and were used to analyze the total antioxidant capacity (TAC) levels, PON1 activity, and lipid hydroperoxide (LOOH) levels.

Table 1. Demographic characteristics of the patients with acetaminophen intoxication and the control subjects.^a

Parameters	Controls (n = 25)	Patients (n = 20)	p Value
Age (years)	33.20 ± 11.16	28.15 ± 11.97	0.151
Sex (female/male)	14/11	15/5	0.186
Marital status (married/single)	12/13	8/12	
Cause of poisoning (intentional)		20	

^aValues are expressed as mean ± SD.

Measuring paraoxonase and arylesterase activities

The paraoxonase and arylesterase activities were measured by reading the absorbances with a spectrophotometer (Genesys 10 UV Scanning ultraviolet–visible (UV/Vis) spectrophotometer, Shimadzu, Tokyo, Japan) using assay kits (Rel Assay Diagnostics kit, Mega Tip, Gaziantep, Turkey). The PON1 activity was assayed using two different substrates.¹⁵ The paraoxonase activity was expressed in units per liter. Phenylacetate was used as a substrate to measure the arylesterase activity. The arylesterase activity was expressed in kilounits per liter and defined as one micromole of phenol generated per minute under the reaction conditions.¹⁶

Measuring serum TAC

The serum TAC levels were determined spectrophotometrically (Genesys 10 UV Scanning UV/Vis Spectrophotometer) at 660 nm using assay kits (Rel Assay Diagnostics kit, Mega Tip) that were developed by Erel.¹⁷ Hydroxyl radicals (the most potent biological radicals) are produced using this method. In the assay, ferrous ion solution, which is present in reagent 1, is mixed with hydrogen peroxide, which is present in reagent 2. The radicals, such as the brown-colored dianisidiny radical cation that is sequentially produced by the hydroxyl radical, are potent radicals. Using this method, the antioxidative effect of the sample against the potent free radical reactions, which is initiated by the generated hydroxyl radical, is measured. The results are expressed in milli moles of Trolox equivalent per liter.

Measuring LOOH levels

Serum LOOH levels were measured spectrophotometrically with the ferrous ion oxidation–xylene orange assay.¹⁸ The principle of the assay depends on the oxidation of ferrous ion to ferric ion through various oxidants, and the produced ferric ion is measured with

xylene orange. LOOHs are reduced by triphenyl phosphine (TPP), which is a specific reductant for lipids. LOOH levels are affected by using or not using the TPP pretreatment.

Other parameters

The serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by commercially available assay kits (Roche[®], Mannheim, Germany) with an autoanalyzer (Roche[®]/Hitachi modular P-800, Mannheim, Germany). The HDL cholesterol (HDL-C) and LDL-C levels were determined using commercially available assay kits (Roche) with an autoanalyzer (Roche[®]/Hitachi modular P-800). The serum LDL-C levels were calculated with the Friedewald formula.

Statistical analysis

The results were expressed as mean ± SD. Parametric variables were compared using Student's *t* test. Qualitative variables were assessed by χ^2 test. The correlation analyses were performed using Pearson's correlation analysis. The results were considered to be statistically significant when $p < 0.05$. The data were analyzed using Statistical Package for Social Sciences for Windows computing program (Version 11.0; SPSS[®], Chicago, Illinois, USA).

Results

The demographic characteristics of the patients with acetaminophen intoxication and the controls are shown in Table 1. There were no significant differences between the groups with respect to age and gender ($p > 0.05$; Table 1).

The timing of ingested acetaminophen was 2.95 ± 1.43 h. Dosage of ingested acetaminophen was 10.20 ± 4.95 g.

The levels of serum total cholesterol and LDL-C were significantly higher in patients with acetaminophen intoxication compared with the controls, but there

Table 2. Serum lipid parameters, AST, and ALT levels in the patients with acetaminophen intoxication and the control subjects.^a

Parameters	Controls (n = 25)	Patients (n = 20)	p Value
TG (mg/dl)	157.6 ± 23.2	187.9 ± 26.6	0.001
TC (mg/dl)	173.5 ± 33.9	178.8 ± 30.6	0.584
HDL-C (mg/dl)	47.3 ± 9.8	35.3 ± 8.6	0.001
LDL-C (mg/dl)	94.7 ± 35.6	105.9 ± 30.6	0.270
AST (U/l)	22.76 ± 6.15	29.25 ± 8.97	0.036
ALT (U/l)	23.12 ± 5.75	27.05 ± 6.85	0.006

TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; ALT: alanine aminotransferase; AST: aspartate aminotransferase.

^aValues are expressed as mean ± SD.

Table 3. Serum oxidant and antioxidant levels in patients with acetaminophen intoxication and the control subjects.^a

Parameters	Controls (n = 25)	Patients (n = 20)	p Value
LOOH (nmol/ml)	13.41 ± 1.41	25.65 ± 5.83	0.001
TAC (mmol Trolox equivalent/l)	3.31 ± 0.21	2.38 ± 0.12	0.001
Paraoxonase (U/l)	74.13 ± 4.03	42.99 ± 2.96	0.001
Arylesterase (kU/l)	39.38 ± 8.61	19.12 ± 3.35	0.001

LOOH: lipid hydroperoxide; TAC: total antioxidant capacity.

^aValues are expressed as mean ± SD.

was no statistically significant difference (all, $p > 0.05$). Moreover, serum triglyceride levels were significantly higher in the patients with acetaminophen intoxication compared with the controls ($p < 0.001$). Serum HDL-C levels were significantly lower in the patients with acetaminophen intoxication compared with the controls ($p < 0.001$; Table 2).

The serum AST and ALT levels were significantly higher in patients with acetaminophen intoxication compared with the controls (all $p < 0.01$; Table 2).

The serum TAC levels and the paraoxonase and arylesterase activities were significantly lower in the patients with acetaminophen intoxication compared with the controls (for all, $p < 0.001$), while the LOOH levels were significantly higher ($p < 0.001$; Table 3).

No significant correlation was found between the amount of acetaminophen ingested and the TAC, LOOH, paraoxonase, and arylesterase activities ($p > 0.05$) in the patient group. Moreover, no significant correlation was found between the serum LOOH and the TAC levels and paraoxonase and arylesterase

activities ($p > 0.05$) in the patient group. No significant correlation was found between the serum lipid parameters and the oxidant and antioxidant levels ($p > 0.05$) in patients with acetaminophen intoxication.

Discussion

In this study, we measured serum TAC levels and paraoxonase and arylesterase activities. In addition, we measured serum LOOH levels as one of the end products of lipid peroxidation. We also aimed to determine the relationship between these parameters and the amount of acetaminophen ingested.

In the present study, we observed decreased serum TAC levels and paraoxonase and arylesterase activities in patients with acetaminophen intoxication than in healthy controls. We also found that patients with acetaminophen intoxication had increased LOOH levels compared with those of healthy subjects. Decreased serum PON1 enzyme activity may have a role in the etiopathogenesis of acetaminophen intoxication, and the increased susceptibility to oxidative stresses was observed in acetaminophen intoxication.

Information regarding serum PON1 activity in patients with acetaminophen intoxication is very limited.¹⁴ Alagoz et al.,¹⁴ in their study, investigated serum PON1 activity and oxidative stress levels after acetaminophen intoxication. They suggested that acetaminophen intoxication does not appear to induce serum PON1 activity and oxidative stress levels and also investigated the relationship among serum PON1 activity and oxidative stress levels and serum liver function tests for AST and ALT levels in acetaminophen intoxication and finally reported that no significant differences were found in serum PON1 activity and oxidative stress levels and AST and ALT levels in acetaminophen intoxication.

Acetaminophen is widely used as an analgesic and antipyretic agent. Acetaminophen overdose is a very common poisoning; moreover, the drug is widely used in suicide attempts.¹⁹ However, the accidental or intentional intake of high doses often causes acute hepatocellular necrosis with high morbidity and mortality.²⁰

Acetaminophen is rapidly absorbed from the gastrointestinal tract,²¹ and peak levels of 10–20 mg/l are achieved within 1–2 h after orally administering a 1000-mg dose. Acetaminophen is widely distributed throughout most body fluids. Although protein binding appears to be insignificant at therapeutic concentrations, it does increase to approximately 20% when

the drug is present in toxic concentrations. The half-life of acetaminophen elimination ranges from 1 to 4 h.²¹

At therapeutic doses, the major pathway for acetaminophen removal appears to be through glucuronidation and sulfation, which makes acetaminophen more water soluble and allows its removal from the liver and blood via the urine.²² The remaining portion of the acetaminophen dose is oxidized by the cytochrome P450 system to NAPQI (a toxic metabolite agent). Under normal conditions, NAPQI is reduced via conjugation with GSH to a nontoxic substance and excreted. In toxic acetaminophen levels, the glucuronidation and sulfation pathways are saturated; acetaminophen is oxidized to NAPQI to a much greater extent, and hepatic GSH stores become depleted. NAPQI causes oxidative damage to the liver. This damage can result in fulminant hepatic failure if prompt measures are not taken to regenerate GSH.²³ Consequently, conjugation at higher doses causes the critical depletion of GSH, which is essential for maintaining the cellular redox metabolisms that cause acute oxidative stress and cellular toxicity. Increased mitochondrial stress, the production of ROS, and reactive nitrogen species (RNS) have been implicated in a number of acetaminophen-induced toxicity studies.^{23,24} Acetaminophen toxicity is one of many human disease processes that are widely believed to involve ROS.²⁵ Lipid peroxidation via free radical formation and mitochondrial dysfunction via increased permeability of the mitochondrial permeability transition have been postulated as causes of acetaminophen-associated hepatotoxicity.²⁶

Several antioxidants, such as β -carotene²⁷ and α -tocopherol, have been shown to protect against acetaminophen toxicity.²⁸ In addition to the studies of these chemicals, the exogenous administration of antioxidant enzymes, such as catalase and superoxide dismutase,²⁹ has been shown to dramatically protect against acetaminophen toxicity.

The effects of various antioxidants in the plasma and other biological samples are additive, and the cooperation of antioxidants in human serum protects against attacks by free radicals. TAC measurements may accurately reflect the antioxidant activity of the organism.¹⁷ In the present study, we observed decreased serum TAC levels in patients with acetaminophen intoxication compared with the controls.

PON1 is first synthesized in the liver and subsequently released into the blood as a HDL-associated protein,³⁰ and chronic liver diseases are associated with increased oxidative stress and inflammation.³¹ It has also been reported that serum paraoxonase and

arylesterase activities may be significantly reduced in patients with chronic liver disease.^{32,33} Furthermore, serum paraoxonase is thought to protect lipoproteins against oxidative modification.⁸ PON1 is one of the antioxidants that act as an enzymatic defense against LOOH and lipid peroxides in LDL under in vitro oxidizing conditions.⁸ In some diseases, the serum PON1 activity was found to be inversely correlated under oxidative stress.^{34,35} In addition, oxidative stress has been reported to affect the expression of PON1 and its activities.^{10,11} This decrease could be related to enhanced lipid peroxidation because oxidized lipids reportedly inhibit PON1 activity. Furthermore, PON1 is an HDL-associated enzyme, and it has been suggested that reduced serum PON1 activity might be associated with decreased HDL-C levels.^{36,37} In the current study, we did not find any correlation between PON1 activity and HDL-C levels.

Acetaminophen caused liver damage as evident by significant increase in the activities of aspartate and alanine transferases.¹ Acetaminophen is often used in animal experiments to establish acute liver injury models. The serum levels of ALT and AST are the main indices that reflect liver injury.³⁸ In the current study, we found increased serum AST and ALT levels in patients with acetaminophen intoxication compared with the controls.

There were several limitations in the present study. First, this study is a cross-sectional study. Second, the number of patients with acetaminophen toxicity who were enrolled in the study was relatively small. However, a large sample would have increased the power to detect serum PON1 activity in patients with acetaminophen toxicity. Third, serum acetaminophen levels were not determined in the study population.

Our results suggest that decreased PON1 activity seems to be associated with increased oxidative stress in patients with acetaminophen intoxication. Measuring serum PON1 activity may be useful in assessing the development of toxicity risk in acetaminophen toxicity. It would be useful to recommend vitamins with antioxidant effects such as vitamins C and E along with medical treatments. Further studies are necessary to confirm this observed relationship.

Conflict of interest

The authors declared no conflicts of interest.

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