WISTAR ALBINO TÜRÜ SIÇANLARDA ASETAMİNOFENİN HEPATOTOKSİSİTESİ VE NEFROTOKSİSİTESİ ÜZERİNE N-ASETİLSİSTEİNİN ETKİSİ

EFFECT OF N-ACETYLCYSTEINE ON ACETAMINOPHEN-INDUCED EPATOTOXICITY AND NEPHROTOXICITY IN WISTAR ALBINO RATS

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Özet
Bu çalışmada Wistar albino sıçanlarda asetaminofenin hepatotoksik ve nefrotoksik etkilerinden N-acetilsisteinin koruyuculuğunu inceledik. Hayvanlara 300mg/kg asetaminofen intraperitoneal olarak uygulandı. Hayvanların bir kısmına asetaminofen enjeksiyonundan dört saat sonra 300mg/kg veya 800mg/kg N-acetilsistein intraperitoneal yolla verildi. Histopatolojik olarak asetaminofen verilmiş sıçanların karaciğerinde orta şiddette sentrilobüler hepatik nekroz belirgindi. Böbreklerinin korteksinde ve bazen de medullanın dış bölümünde ciddi akut tübüler nekroz izlendi. Asetaminofene ilaveten N-acetilsistein verilmiş şıçanların karaciğerlerinde nekroz izlenmedi. Öte yandan böbrek kesitlerinde yalnız asetaminofen verilmiş sıçanlarda olduğu kadar belirgin tübüler nekroz izlendi. Çalışmamızın sonucunda N-asetilsisteinin acetaminofene bağlı renal hasarı engellemediğini düşünüyoruz. Fakat bir antidot olarak kullanılabilir, çünkü asetaminofene bağlı hepatik nekroz ölüme sebep olabilir ve akut renal lezyonlar genellikle fulminant karaciğer yetmezliğine sekonder olarak gelişir.

Anahtar kelimeler: *Acetaminofen, N-acetilsistein, Hepatotoksisite, Nefrotoksisite, Sıçan.*

Summary
In the present study we investigated preventing effects of N-acetylcysteine on the hepatotoxic and nephrotoxic effects of acetaminophen in male Wistar albino rats. Acetaminophen 300 mg/kg was administered intraperitoneally. N-acetylcysteine (300 or 800mg/kg) was administered intraperitoneally four hours after the acetaminophen injections to one group of the rats. Moderate centrilobular necrosis was the common histopathological feature of the livers of the acetaminophen administered rats. In the kidneys of these rats there were severe acute tubular necrosis in the cortex and sometimes in the outher zone of the medulla. In the livers of acetaminophen plus N-acetylcystein adminstered rats centrilobular necrosis was not observed. On the other hand, in the sections of the kidneys of these rats acute tubular necrosis was as prominent as that of the only acetaminophen injected rats. As a result of the present study, we think that N-acetylcysteine do not prevent renal damage due to acetaminophen. But, it can be used as an antidote because hepatic necrosis due to acetaminophen may prove fatal and acute renal lesions is usually secondary to fulminant hepatic failure.

Key words: *Acetaminophen, N-acetylcysteine, Hepatotoxicity, Nephrotoxicity, Rat*

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Introduction

Adverse effects of acetaminophen (paracetamol), an analgesic and antipyretic, are extremely rare when it is taken recommended therapeutic doses. However, it is an overdose that acute lethal injury of hepatocytes can result from acetaminophen intoxication both in humans and in experimental animals. This result in hepatic necrosis which may prove fatal. Following a large overdose of acetaminophen acute renal necrosis also occurs occasionally, but this is usually secondary to fulminant hepatic failure (1). The species differences in susceptibility to acetaminophen are due largely to differences in the rate of formation of the toxic metabolite,N-acetyl-p-benzoquinoneimine (NABQI). There are also intra-species differences in acetaminophen toxicity, in most rat strains acetaminophen is primarily hepatotoxic, but in Fischer 344 strain it is also nephrotoxic (1,2). NABQI normally rapidly inactivate by conjugation with glutathione. It is also a potent oxidizing agent so that when it interacts with thiol groups two reactions occur: the thiol groups can be oxidized to disulphide, one of the mechanisms of detoxication by glutathione, at least until the levels of hepatic glutathione are low; or they can undergo nucleophilic addition, resulting in covalent binding by reactive metabolite of cellular proteins. Procursors of glutathione, such as N-acetylcysteine can prevent the toxicity of acetaminophen without affecting its covalent binding to protein, even when added after covalent binding has reached a

Figure 1. *The Liver of Healthy Rat. C:Vena Centralis, P:Portal Space. Hematoxylin and EosinX100*

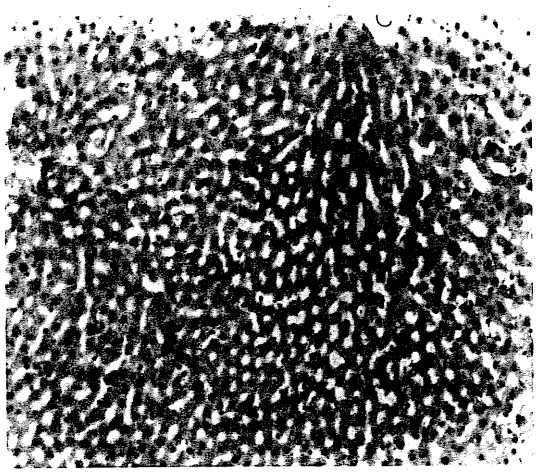
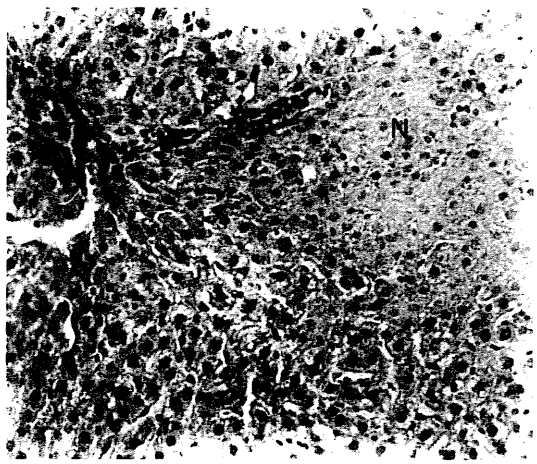


Figure 2. *The Liver of Acetaminophen Administered rat. Hepatic Necrosis is Observed (N). Hematoxylin and EosinX250.*



maximum.(1,3) In the present study we investigeted preventing effects of N-acetylcystein on the hepatotoxic and nephrotoxic effects of acetaminophen in Wistar albino rats.

Material and Methods

Wistar albino rats weighting 220-260gr were used in this study. Five to six rats were housed together at 23-25 °C and 60% humidity with a 12/12 hour light and dark(light on 06.00, off at 18:00) cycle. Rats were given rat chow and tap water ad libitum. Acetaminophen was obtained from Saba pharmaceuticals and N-acetylcystein from Zambon phermaceuticals-West Germany. Acetaminophen 300mg/kg was dissolved in 10mls of 0,9% NaCl freshly for every day and heated until 32°C to obtain clear solution. For all groups, 300mg/kg acetaminophen was administered intraperitoneally at 9.00 o'clock every day. This dose was chosen on the basis of previous experience to be a threeshold toxic dose in young adults (4). Either vehicle or N-acetylcystein(300 or 800mg/kg) was administered in 0.5 ml volumes intraperitoneally four hours after the acetaminophen injections. For control group, 0.9% NaCl was administered in the same volumes intraperitoneally as well. Twenty-four hours folowing the acetaminophen injections, animals were sacrified by decapitation and their livers and kidneys were removed and fixed in 20% of formaldehyte solution, then embedded in parrafin wax. Sections cut from parrafin blocks and mounted on glass slides were stained with hematoxylin and eosin for light microscopic examination. Preparations were examined for the histopathological changes and the

Figure 3. *The Kidney of Healthy Rat. G:Glomeruli. Hematoxylin and EosinX250.*

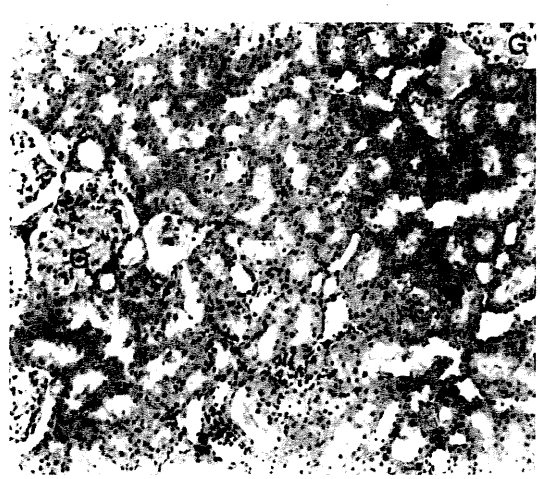


Figure 4. *The Kidney of Acetaminophen Administered rat. G:Glomeruli. Tubular Necrosis is Observed (T). in Some of the Glomeruli Necrosis is Seen (arrow). Hematoxylin and EosinX250.*

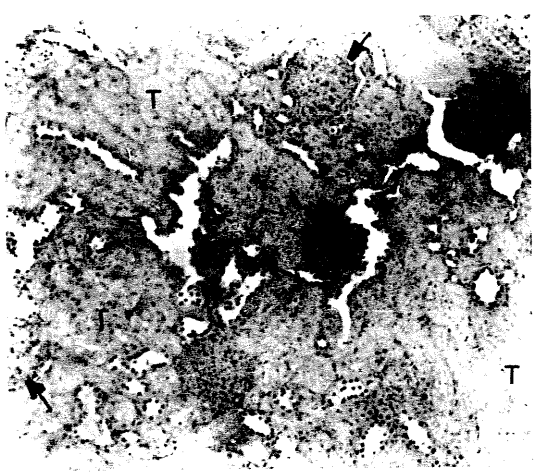


Figure 5. *The Liver of Acetaminophen and N-Acetylcystein Administered Rat. There is No necrosis. Hematoxylin and EosinX100*

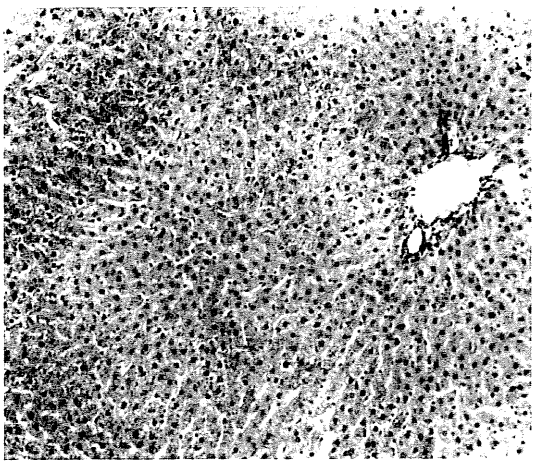
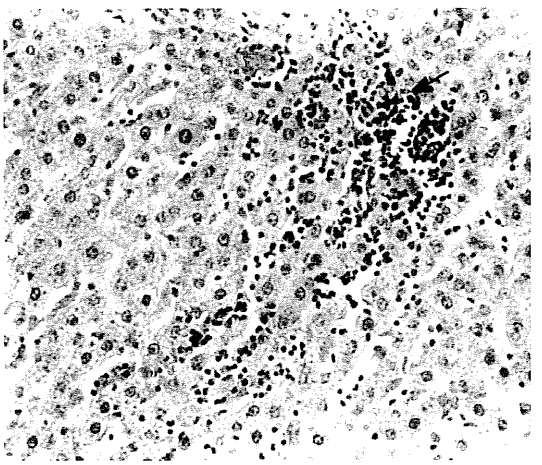


Figure 6. *The Liver of Acetaminophen and 300mg/kg N-Acetylcystein Administered Rat. There is No necrosis. Leukocyte Infiltration is Observed (arrow). Hematoxylin and EosinX250*



pictures were taken with Olympus BH-2 photomicroscope.

Results

We observed the histopathological alterations caused by acetaminophen and acetaminophen plus N-acetylcystein in the livers and the kidneys of Wistar albino rats as follows:

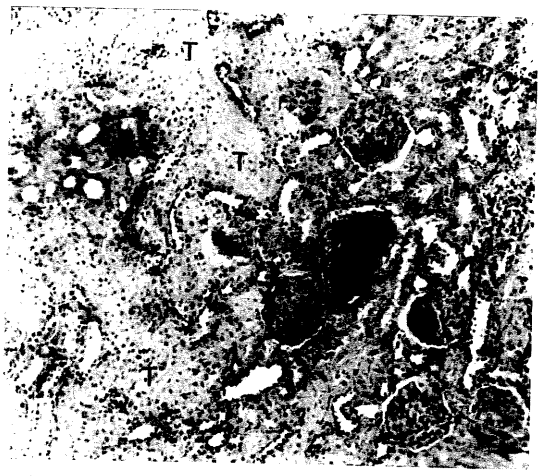
Acetaminophen: The histological structure of the livers and kidneys of the acetaminophen administered rats was highly different from that of the controls (Figure1,2,3,4). Moderate centrilobular necrosis was the common feature of the livers of the acetaminophen administered rats. In the liver sections some of the lobules were morphologically deranged. The other histopathological alterations were sinusoidal congestion and dilatation and leukocyte infiltration. In the kidneys of these animals there was severe acute tubular necrosis in the cortex and sometimes in the outher zone of the medulla. In some of the glomeruli necrosis occured (Figure 4).

Acetaminophen plus N-acetylcystein: In the livers of acetaminophen and N-acetyl-cystein adminstered rats centrilobular necrosis was not observed(Figure 5). But in the 300mg/kg N-acetyl-cystein dose group, sometimes there were sinusoidal dilatation and leukocyte infiltration (Figure 6). On the other hand, in the sections of the kidneys acute tubular necrosis was as prominent as that of the only acetaminophen injected rats. There was tubular necrosis in the cortex and sometimes in the outher zone of the medulla (Figure 7). In some of the glomeruli necrosis occured too.

Discussion

The major use of N-actylcysteine in clinical toxicology is in the treatment of acetaminophen overdose. The hepatorenal toxicity of acetaminophen is mediated by reactive metabolite normally detoxified by reduced glutathione. If glutathione is depleted, covelent binding to macromolecules and/or oxidation of thiol enzymes can lead to cell death. Oral or intravenous N-acetylcysteine mitigates acetaminophen-induced hepatorenal damage if given within 10 hours; but becomes less effective thereafter. In vivo N-acetylcysteine forms L-cysteine, L-methionine, glutathione and mixed disulfides; L-methionine also forms cysteine, thus giving rise to glutathione and other products (5). Boberg et al (6) reported that N-acetylcysteine also seems to improve survival when given 36-80 hours following acetaminophen ingestion. Presscott et al (7) demonstrated that with the administiration of 150mg/kg dose of N-acetylcysteine given in 15 minutes followed by 50mg/kg in 4 hours and 100mg/kg over the next 16 hours , acetaminophen-induced liver damage was absent or mild, on the cases of severe poisoning with acetaminophen. We administered 300mg/kg or 800mg/kg doses of N-acetylcysteine, four hours after the acetaminophen injections. We observed moderate hepatic necrosis in the livers of acetaminophen administered rats, whereas with N-acetylcysteine injection there was no necrosis. But sometimes we observed sinusoidal dilatation and mild leukocyte infiltration in the livers of acetaminophen plus 300mg/kg N-acetylcysteine injected rats. So we think that the N-acetylcysteine mitigates the hepatotoxic effects of acetaminophen Acetaminophen-induced renal lesion is acute tubular necrosis (8,9). In animals such as male Fischer rats and certain strains of mice necrosis develops after administiration of single nonlethal doses of acetaminophen (8). Trumper et al (10) observed acute acetaminophen nephrotoxicity in male Wistar

Figure 7. *The Kidney of Acetaminophen and N-Acetylcystein Administered Rat. There is Tubular Necrosis (T). Hematoxylin and EosinX250.*



albino rats one hour after the administration of different single doses of acetaminophen intraperironeally (200mg/kg,500mg/kg and 1000mg/kg body weight) both in the presence or absence of hepatic damage. We observed both hepatic and renal damage due to acetaminophen in Wistar albino rats within 24 hours. Tubular necrosis occured in the cortex and sometimes outher zone of the medulla. In the study of McMurtry et al (11) tubular necrosis was reported prominent in the combined areas of renal cortex and the outher stripe of the outher zone of the medulla in the kidneys of Fischer rats. In our study, N-acetylcysteine administration did not prevent the renal damage produced by acetaminophen. Because we observed severe tubular necrosis both in the absence and presence of N-acetylcysteine. Moller-Hatman et al (12) demonstrated that N-acetylcysteine, an antidote known to protect againist hepatotoxicity of acrtaminophen, is not effective in preventing acetaminophen-induced kidney damage in rats. Jones et al (13) suggested that antidotal therapy with agents such as N-actylcysteine may not prevent renal toxicity and indeed on the basis of animal work may actually potentiate tubular damage. As a result we think that N-acetylcysteine do not prevent renal damage due to acetaminophen. But, it can be used as an antidote because hepatic necrosis due to acetaminophen may prove fatal and acute renal lesions is usually secondary to fulminant hepatic failure.

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