YENİ DOĞAN VE ERİŞKİN SIÇANLARDA ASETAMİNOFENE BAĞLI AKUT TÜBULER NEKROZ: IŞIK VE ELKTRON MİKROSKOPİK BİR ÇALIŞMA

ACETAMINOPHEN-INDUCED ACUTE TUBULAR NECROSIS IN NEW-BORN AND ADULT RATS: A LIGHT AND ELECTRON MICROSCOPIC STUDY

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Özet
Bu çalışmada 2 haftalık ve 6 aylık onar Wistar albino türü sıçan kullanıldı. Sıçanlara 300mg/kg acetaminophen intraperitoneal olarak uyguladı. Enjeksiyondan 8 saat sonra hayvanlar dekapite edilerek öldürüldüler ve böbrekleri ışık ve elektron mikroskopik inceleme için hazırlandı. Işık mikroskopik olarak hem yeni doğan hem de erişkin sıçan böbreklerinin korteksinde, bazen de medullanın dış bölgesinde tübüler nekroz izlendi. Elektron mikroskopik incelemede proksimal ve distal tübüllerde dejenerasyon vardı. Tübuller birbirinden geniş aralıklarla ayrılmıştı ve bu alanlarda bol interstisiyel materyal ve bağ dokusu hücresi bulunmaktaydı. Tübüllerin epitel hücrelerinin bazıları yanındaki hücrelerden ayrılmaya başlamıştı. Epitel hücrelerinin nükleuslarının çoğu piknotikti. Sitoplazmik matriksde yoğunlaşma gözlendi. Mitokondrilerde düzensizlik, şişme ve yoğunlaşma mevcuttu. Hücrelerin yüzeyinde mikrovilluslarda düzensizlik veya kısmi kayıp izlendi. Yer yer bazal membranda kalınlaşma ve düzensizlik dikkat çekiyordu.

Anahtar kelimeler: *Akut Tübüler Nekroz, Acetaminophen, Sıçan*

Summary
Ten 2-week-old and ten 6-month-old male Wistar albino rats were used in this study. Acetaminophen 300mg/kg was administered intraperitoneally. Eight hours following the injection, animals were killed by decapitation and their kidneys were prepared for light and electron microscopic examinations. By light microscope tubular necrosis in the cortex and sometimes in the outher zone of the medulla of both new-born and adult rat kidneys was observed. By electron microscope there was degeneration of epithelium of both proximal and distal convoluted tubules. The tubules were seperated from each others with wide spaces and these areas included increased amount of interstitial material and connentive tissue cells. Some cells of epithelium within the tubules were becoming detached from its neighbours. Most of the nuclei of the cells of the epithelium were pyknotic. There was condensation of cytoplasmic matrix. Disorganization, condensation and swelling of the mitocondria were detected. There was disorganization and partial loss of microvilli on the surfaces of the cells. Sometimes thickening and irregularity of basal lamina was observed.

Key words: *Acute Tubular Necrosis, Acetaminophen, Rat*

AÜTD 1995, 27:129-133 **MJAU 1995, 27:129-133**

Introduction
Acetaminophen (paracetamol) is a mild analgesic which has found increasing acceptance as an aspirin substitute over past decade. In addition to the characteristic, and sometimes fatal hepatic necrosis which occurs in man following massive acetaminophen ingestion, a significant proportion of individuals also develop acute renal failure (1,2,3). Renal complication due to the drug has been described with or without liver damage, but this is particularly common in chronic alcholics (2). Kidney is positioned in a primary site of expoure, since it receives a large blood supply and contains specialized transport processes for concentrating and secreting drugs (4). Acetaminophen bind covalently to cellular proteins and depletes renal glutathione, and thus may injure cells by covalent binding and damage to oxidative systems (5). Acute tubular necrosis after administiaration of toxic doses of acetaminophen has been reported in man and labratory animals (1,2,3,4).In the present study we investigated the histopathological alterations caused by acetaminophen in the kidney of new-born and adult Wistar albino rats using light and electron microscopes.

Methods
Ten 2-week-old and ten 6-month-old male Wistar albino rats were used in this study. Purified acetaminophen was obtained from Saba pharmaceuticals. Paracetamol 300mg was dissolved in a volume of 10ml/kg and was administered intraperitoneally. Two adult and two new-born rats were injected with an equal volume of saline. Eight

Fig.1. *Control Adult Kidney. G:Glomeruli. Hematoxylin and EosinX100.*

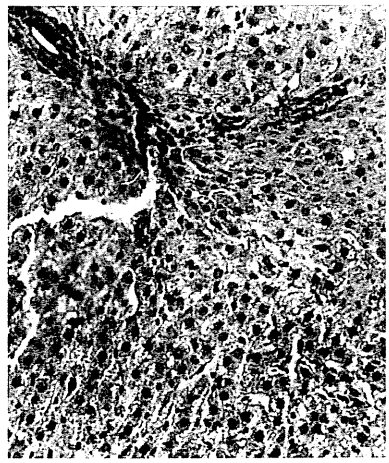

Fig.2. *Kidney of Acetaminophen Administered Adult Rat. G:Glomeruli. Tubular Necrosis is Seen (T). Hematoxylin and EosinX100*

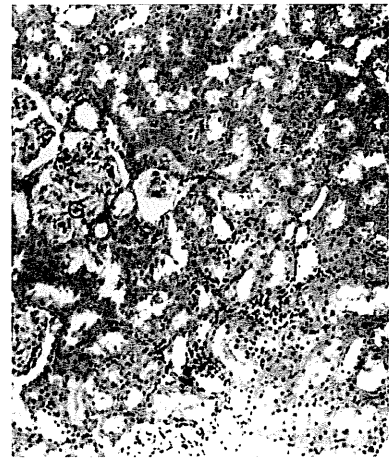

Fig.3. *Kidney of Acetaminophen Administered New-born Rat. G:Glomeruli. Tubular Necrosis is Seen (T). Hematoxylin and EosinX100.*

hours following the injection, animals were killed by decapitation. Kidneys were removed and placed in 10% buffered formal saline and 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 pictures were taken with Olympus BH-2 photomicroscope. On the other hand, kidneys of two animals from each group cut into smaller parts and fixed in 3% gluteraldehyde buffered with 0.2M NaH2PO4+NaHPO4(Ph=7.2-7.3) for electron microscopic examination. These samples postfixed in 2% OsO4, and dehydrated in acetone and embedded in Araldite CY212. Ultrathin sections were stained with uranyl acetate and lead citrate and examined in a Jeol 100SX electron microscope.

Results

The kidneys of Wistar albino rats were similar to human kidneys. They contained renal corpuscules, proximal tubules, distal tubules, thin segments, collecting tubules, renal interstitium and many blood vessels and cappillaries (Figure1). By light microscope tubular necrosis was observed in both new-born and adult rat kidneys within eight hours following the acetaminophen administiration. There was severe acute tubular necrosis principally in the cortex and sometimes in the outher zone of the medulla (Figure 2,3). In some of the glomeruli, necrosis occured. So, this toxicity was not age-dependent. By electron microscope, there was degeneration of epithelium of both proximal and distal convoluted tubules in the kidneys of acetaminophen administered rats. The tubules were seperated from each others with wide spaces and these areas included increased amount of interstitial material and connentive tissue cells (Figure 4). Some cells of epithelium within the tubules were becoming detached from its neighbours (Figure 5). It was difficult to see the Intercellular junctions because of the irregularity and rupture of cell membranes. (Figure 4,5,6).

Most of the nuclei of the cells of the epithelium were pyknotic. Heterochromatin condensation at the periphery of the nucleus occured, ırregularity of contour was dedicated (Figure 4). There was condensation of cytoplasmic matrix (Figure 4,5).

Sometimes swelling of the cells was observed, so cytoplasmic matrix contained electron lucent areas and cytoplasmic organelles were seperated from each others. Disorganization, condensation and swelling of the mitocondria were detected (Figure 5,6,7).

There was disorganization and partial loss of microvilli on the surface of the cells (Figure 4).Sometimes thickening and irregularity of basal lamina was observed (Figure 7).

Discussion

At normal doses, acetaminophen is readily detoxified largely by glucronidation and sulphation in the liver. A small fraction of the dose is oxidized in the liver by cytochrome P-450 dependent monooxygenase system to a highly reactive intermediate; acetyl-p-benzoquinoneimine(NABQI), this is very effectively detoxified by conjugation with glutathione. However, as the dose of acetaminophen increases, the amount and the proportion of the administered

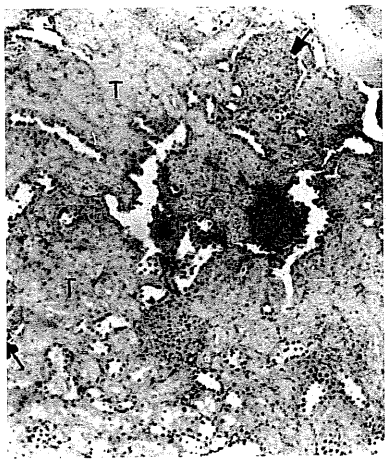

Fig.4. *Kidney of Acetaminophen Administered Adult Rat. the Tubules Are Seperated From Each Others By Wide Spaces, and There Fibroblast-Like Cells (F) Are Observed. it is Difficult to See The Intercellular Junctions. There Is Pyknosis (P) Or Irregularity Of The Nucleus (arrow). Disorganisation Or Partial Loss Of Microvilli Are seen (M). Lead Citrate and Uranyl AcetateX3000.*

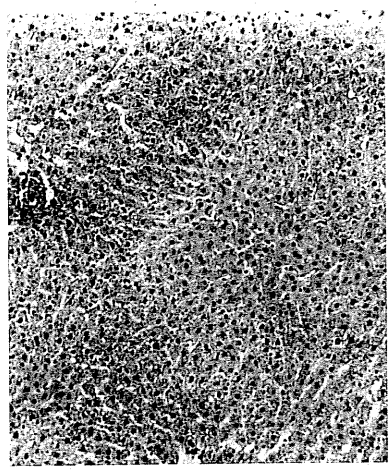

Fig.5. *Kidney of Acetaminophen Administered New-Born Rat. There is Heterochromatin Condensation At the Periphery of the Nucleus (arrow). Cytoplasmic Condensation is Seen (C). Cells Of Epithelium Within the Tubules Are Becoming Detached From Its Neighbours and Basal Lamina (double arrow). Lead Citrate and Uranyl AcetateX3000.*

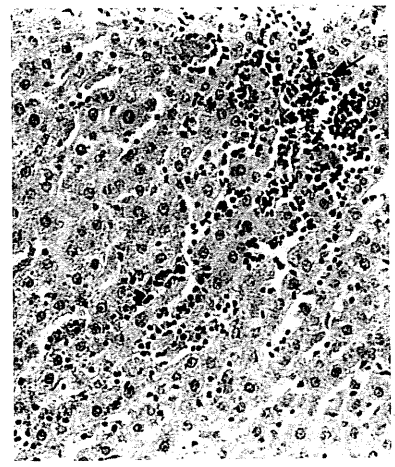

Fig.6. *Kidney of Acetaminophen Administered New-Born Rat. it is Difficult to See The Intercellular Junctions Because of Irregularity Or Loss of Plasma Memranes. Lead Citrate and Uranyl AcetateX2500*

dose that undergoes oxidation to NABQI increases due to the depletion of the sulphate pool. The levels of NABQI increase to an extent such that synthesis of glutathione is exceeded and the intermediate escapes detoxication.

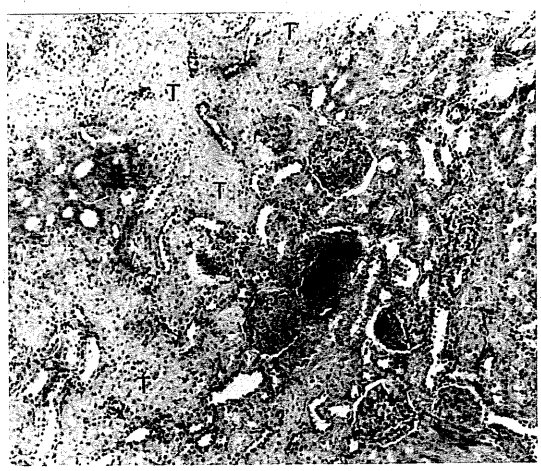

Fig.7. *Kidney of Acetaminophen Administered Adult Rat. the Thickening and Irregularity of Basal Lamina is Seen (arrow). There is Disorganisation of the Mitochondria. Lead Citrate and Uranyl AcetateX5000*

NABQI is also potential oxidizing agent so that when it interacts with thiol groups, two reactions can occur: the thiol groups can be oxidized to disulphide, one of the mechanisms of detoxification by glutathione; at least until the levels of hepatic glutathione are low; or they can undergo nucleophilic addition resulting in covalent binding by the reactive metabolite of cellular proteins. Binding correlates with the development of toxicity (5). In animals that have high concentration of microsomal cytochrom P-450 in their kidneys such as male Fischer rats and certain strains of mice, acute tubular necrosis develops after the administration of single, nonlethal doses of acetaminophen (1,2,4,6,7).Because cytochrom P-450 is the terminal oxygenase controlling most drug oxidations in the kidney, liver and other tissues and because this enzyme system is concentrated primarily in the renal cortex, which is selectively susceptible to acetaminophen-induced necrosis, it seems likely that metabolite might be responsible for the renal lesion just as it is for the hepatic injury (4). McMurtry et al (1) showed that the severity of the renal injury due to acetaminophen was prominent in the inner zone of the cortex and the outher zone of the medulla in Fischer rats. We observed acute tubular necrosis primarily in the cortex and sometimes in the outher zone of the medulla in Wistar albino rats. The metabolic pathway leading to nephrotoxicity differ significantly among the spaces. In Fischer rats, nephrotoxicity result from the enzymatically mediated N-deacetylation of acetaminophen to p-aminophenol. Both p-aminophenol and acetaminophen selectively damage the same nephron segment in the rat, providing early evidence that p-aminophenol mediated nephrotoxicity in the rat. In addition, both age and strain related differences in acetaminophen nephrotoxicity could be related to

differences in the relative quantity of p-aminophenol generated from acetaminophen by rat kidneys (2). de Marais et al (8) showed that Gunn rats were more susceptible to acetaminophen toxicity than normal Wistar controls from which the Gunn strains was derived. Tarloff et al (9) reported that middle-aged and senescent male Sprague-Dawley rats were more susceptible than young adult rats to kidney damage and they suggested that intrarenal detoxification capacity (via conjugation) was altered in aging male Sprague-Dawley rats. We observed severe acute tubular necrosis in new-born and adult male Wistar albino rats in our study. This nephrotoxicity was not age dependent. Probably the metabolic pathway of acetaminopen is not different significantly in new-born and adult Wistar rats. On the other hand, we think that single dose of acetaminophen causus severe nephrotoxicity in male Wistar albino rats. Trumper et al (10) also demonstrated the acetaminophen-induced nephrotoxicity after different single doses including 200, 500, and 1000mg/kg in male Wistar albino rats. Renal damage consisting of tubular edema and dilatation, degeneration of tubular epithelium and general congestion in rats surviving at least 24 hours after the administration of lethal doses of acetaminophen have been described(4). McMurtry et al(1) reported that early changes in proximal tubular cells consisting of nuclear pyknosis and karyorrhexis due to a toxic dose of acetaminophen occurs within six hours. Within eight hours we observed most of the nuclei of tubular eithelium pyknotic. Heterochromatin condensation at the periphery of the nucleus occured, ırregularity of contour was dedicated. Nuclei may swell prior to rupture in some forms of lethal injury, but more often the nuclei of degenerating cells become condensed and shrunken. Heterochromatin condensation producing the peripheral margination and clumping of chromatin often seen in dying and dead cells. Irregularity of contour may reflect rapid or unbalanced change in nuclear volume following an acute cytotoxic injury(5). It is reported that structural damage due to acetaminophen initially occurs in the mid-papillary region and spesifically involved the interstitial cells and interstitial matrix. Later cortical interstitial fibrosis and tubular atrophy occurs(11). Cortical changes include loss and atrophy of the convoluted tubules with interstitial fibrosis and variable numbers of chronic inflammatory cells (5). In our study the tubules were seperated from each others with wide spaces and these areas included increased amount of interstitial material and fibroblast like cells with irregular shape and cytoplasmic processes. The kidneys of control animals contained less interstitial matrix and less fibroblast-like cells. Some cells of epithelium within the tubules were becoming detached from its neighbours. It was difficult to see the Intercellular junctions because of the irregularity and loss of cell membranes. Cell death itself is only structurally recognizable after the event, by degree of disruption of cellular morphology so gross as to be clearly irreversible. This will usually include disintegration of the cell membrane, disorganization of the cytoplasmic organelles and shrinkage or fragmantation of the nucleus. The entire cell may become shrunken and condensed it s organelles barely recognizable. Dying cells often become dissconnected from their neighbours and detached or extruded from their position particularly in epithelial sheets which may in turn lead to disordered function and to the triggering of the defensive mechanisms of inflammation and reperative fibrosis(5). In our study generally there was condensation of cytoplasmic matrix. Rarely swelling of the cells was observed, so cytoplasmic matrix contained electron lucent areas and cytoplasmic organelles were seperated from each others. The range of cell response to given a challenge encompasses several possibilities. The adaptive potential of the cell may be exhausted, resulting in unequivocal functional and structural damage. Cell injury may therefore be defined as a failure of the cell, on challenge, to maintain itself within homeostatic tolerance limits. This may be an acute rapidly developing abnormality, such as the distension of intracytoplasmic membrane-limited spaces, sometimes accompanied by condensation of the interventing cytoplasmic matrix(5). Disorganization and the swelling of the mitocondria were observedin our study. Impairment of mitochondrial oxidative metabolism and energy generation is the common functional effect of many agents causing cell injury, including various chemicals. These functional events often result in an early stage of mitochondrial condensation and increased electron density, followed by progressive swelling which can ultimately result in mitochondrial rupture(5). Normal surface features of differentiated cells, such as microvilli and cilia may become swollen, irregular, attenuated, or entirely loss as a result of various forms of cell injury(5). There was disorganization and partial loss of microvilli on the surface of the cells of the tubular epithelium in our study.

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