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# Nephrotoxicity of Acetaminophen and Gentamicin in Combination in Rats

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***ABSTRACT :*** *Acetaminophen (APAP) and gentamicin are widely used for many patients, but little information is available regarding the combined effects of APAP and gentamicin. This study was aimed to investigate the potent nephrotoxicity following combined-treatment with APAP and gentamicin. Serum biochemical parameters and histopathological changes in the kidney were observed in female SD rats after continuous daily treatment with either 600 mg/kg/day APAP, and/or 300 mg/kg/day gentamicin for 3 days, and compared with saline sham-treated control animals. APAP and gentamicin combination-treated rats exhibited inconsistent increasing tendency in blood urea nitrogen (BUN) by 96 hours after the last treatment, compared to control or the animals treated with each drug. The relative kidney weights were also increased. Histopathological findings of kidneys revealed that necrosis of proximal convoluted tubules were higher in rats treated with APAP and gentamicin combination than the rats treated with each drug alone. These results suggest that combination use of both drugs have more severe nephrotoxicity than treating each drug alone.*

***Key Words :*** *APAP, Combination, Gentamicin, Nephrotoxicity, Rat*

## I. INTRODUCTION

Acetaminophen (APAP; paracetamol; N-acetyl-p-aminophenol) is widely used as antipyretic-analgesic drug, which is considered safe at therapeutic doses. But following an overdose it causes hepatic and renal toxicity both in man (Boyer *et al.*, 1971) and laboratory animals (McMurtry *et al.*, 1978) and in some cases acute renal proximal tubular necrosis has been described with or without liver damage (Cobden *et al.*, 1982). Also it has been described that a single large dose of APAP administered to male Fischer 344 rats induced necrosis of the proximal tubules and other evaluations of APAP-induced renal functional changes were elevation in blood urea nitrogen (Tarloff *et al.*, 1989) or plasma creatinine(Siegers *et al.*, 1989). Although these data suggest that APAP is a nephrotoxicant, limited mechanism was found about sequential aspects of the development of the APAP-induced nephrotoxicity in vivo. McMurtry *et al.*, (1978) postulated that APAP toxicity could be due to a chemically reactive metabolite which could bind to cellular macro-molecules after a substantial loss of glutathione (GSH) in rats and mice. APAP administration results in depletion of renal GSH and covalent binding of an APAP metabolite to proteins as precursors to necrosis (Mitchell *et al.*, 1973).

Aminoglycoside antibiotics such as gentamicin have proven to be of considerable value for the treatment of severe infections caused by Gram-negative bacteria (Neu, 1982). These antimicrobials are also known for their two major untoward effects, ototoxicity and nephrotoxicity, which has been repeatedly reported in both clinical and animal studies (Appel *et al.*, 1978; Kahlmeter *et al.*, 1984). In experimental animals, aminoglycoside nephrotoxicity is associated with acute tubular necrosis (Houghton *et al.*, 1976) predorminantly affecting proximal convoluted tubules of renal cortex, where these antibiotics are actively reabsorbed and concentrated (Fabre *et al.*, 1979). In ultrastructural and biochemical studies the animals exposed to low doses of aminoglycosides have revealed that these drugs accumulate within lyso-

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somes of renal proximal tubular cells and induce therein a phospholipid accumulation leading to typical lysosomal alteration (Tulkens *et al.*, 1986). Although several mechanisms have been proposed to explain the pathogenesis of aminoglycoside-induced tubular necrosis, there is circumstantial evidence that the lysosomal phospholipidosis associated with drug accumulation in renal proximal tubules may be a major factor contributing to tubular injury (Laurent *et al.*, 1990).

The nephrotoxicity of APAP is as well known as gentamicin, but little information is available regarding the combined effects of gentamicin plus APAP. Thus, this study was conducted in order to clarify the severity of nephrotoxicity in the combined use of the drugs.

## II. MATERIALS AND METHODS

### 1. Animals and treatment

Fifty female Sprague-Dawley rats weighing 130~180 g were purchased from Bio-Safety Research Institute of Chonbuk National University, and housed in a regular 12-hr light/dark cycle and allowed 1 week for acclimation prior to the beginning of the treatment. The rats had free access to laboratory rodent chow pellet (Sam Yang) and tap water. The rats were distributed at random in cages of six rats and divided four experimental groups: APAP, Gentamicin, Combination of APAP and gentamicin, and Control. Each of them has twelve rats.

APAP (minimum 99.0%; Sigma) was dissolved in sterile 0.15 M NaCl and 5 M NaOH to give a final pH 10, and the volume of injected solution (approx. APAP group: 4 m*l* of APAP, Combination group: 3 m*l* of APAP) was adjusted according to the body weight of each animal to reach a daily dosage of exact 600 mg/kg (Laura *et al.*, 1992). Gentamicin sulfate (potency: approx. 655 μg/mg Chong Kun Dang) was dissolved in sterile 0.15 M NaCl and the volume of injected solution (approx. Gentamicin group: 4 m*l* of gentamicin, Combination group: 1 m*l* of gentamicin) was adjusted according to the body weight of each animal to reach a daily dosage of exact 300 mg/kg. Sham-treated control animals received an equivalent volume of sterile 0.15 M NaCl, pH 10. All drugs were injected i.p. for 3 days.

Animals were observed daily for the check of clinical signs such as vitality, and three treated animals of each experimental group were terminated at different time intervals after last treatment: 24, 48, 72, 96 hrs.

Blood sample was collected from the abdominal vena cava during anesthesia and the kidneys and livers were promptly removed. The kidney GSH contents were assayed using whole tissue homogenates.

### 2. Serum analysis

Serum samples were analyzed using a blood autoanalyzer (Spotchem SP-4410, Daiichi Kagaku Co., Kyoto, Japan) for blood urea nitrogen (BUN), creatinine, total protein, alanine transferase (ALT), and aspartate transferase (AST).

### 3. Glutathione assay

24 hours prior to euthanized each rat was fasted. Removed kidney tissues were washed with ice-cold isotonic KCl and frozen in liquid nitrogen. Tissues were homogenized in 20% KCl (w/v) and glutathione concentration was determined as described by Asaoka (Asaoka *et al.*, 1981).

### 4. Histopathologic observation

After euthanasia animals were immediately necropsied and left kidneys were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 4 μm, stained with hematoxylin and eosin, and then examined under light microscope.

### 5. Statistical analysis

Quantitative data were analyzed using the paired student's *t*-test and were considered significant at $p<0.05$. All data were expressed as mean SE.

## III. RESULTS

APAP (600 mg/kg) and/or gentamicin (300 mg/kg) were administered daily for 3 days and the ef-

**Table 1.** The effects of APAP and/or gentamicin-treatment on serum biochemical parameters†

| Group[a] | Control | APAP | Gentamicin | Combination |
|---|---|---|---|---|
| 24 hours | | | | |
| BUN | 18.7±2.26 | 22.3±0.88 | 32.3±1.20* | 28.7±3.18 |
| Creatinine | 1.3±0.03 | 1.4±0.09 | 1.7±0.03** | 1.4±0.13 |
| 48 hours | | | | |
| BUN | 17.3±2.32 | 29.3±3.22 | 24.7±2.71* | 39.7±3.24* |
| Creatinine | 1.3±0.07 | 2.9±1.12 | 1.8±0.25 | 1.4±0.14 |
| 72 hours | | | | |
| BUN | 17.7±2.03 | 21.7±0.88 | 33.3±6.06 | 28.0±5.69 |
| Creatinine | 1.5±0.07 | 1.8±0.22 | 1.8±0.12 | 1.9±0.12 |
| 96 hours | | | | |
| BUN | 17.3±2.03 | 17.3±2.03 | 26.0±4.04 | 32.7±4.67* |
| Creatinine | 1.6±0.03 | 1.7±0.07 | 1.5±0.03* | 1.6±0.06 |

†Each value is the mean ±SE.
*Significantly different from control; *p<0.05, **p<0.01.
[a]Hours after the last treatment.
BUN (mg/dl), Creatinine (mg/dl).
APAP (Acetaminophen), Combination (APAP plus gentamicin).

fects on various biochemical parameters are presented in Table 1. In 24 hours after the last treatment, significant increases in BUN and creatinine were observed in gentamicin-treated group. Also the APAP-treated group showed increased BUN level in 48 hours after the last treatment. Also great increases in BUN were observed in APAP and gentamicin combination-treated group during the experiment. In 96 hours, the APAP-treated group was similar to that observed in control group, furthermore BUN was significantly increased in the combination-treated group. Besides, ALT level showed slightly increasing tendency in the combination-treated group. Meanwhile the total protein of the group was decreased comparing with control group. The relative kidney weights were presented in Fig. 1. The relative kidney weights of combination-treated group were higher than control or single drug-treated group throughout the experiment.

The histopathological changes following treatment of APAP and gentamicin combination are presented in Fig. 2~5. In APAP-treated group acute tubular necrosis was mild and the tubules were filled with an heterogenous materials. Also pyknotic cells were observed in 24 hours after the last treatment. A number of eosinophilic cells found sporadically and widespread among the proximal tubules in 48 hours. Thereafter the tubules returned to

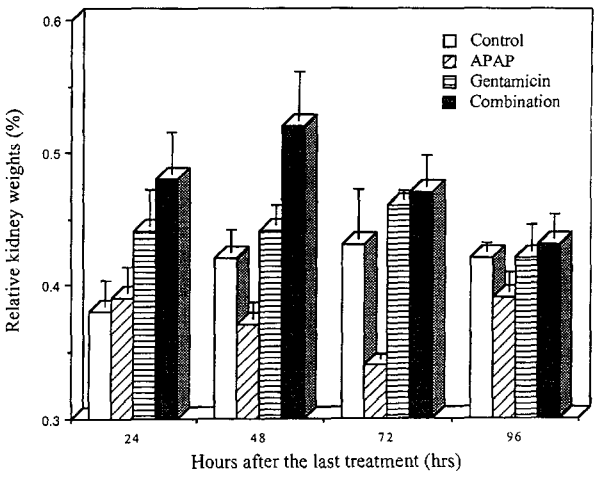


**Fig. 1.** Relative kidney weights to body weights. Data are expressed as mean±SE.

normal structure in 72 hours. In gentamicin-treated group morphological evidence of drug-induced toxicity was mostly confined to the proximal tubules, whereas glomeruli and distal portions of the nephrons were remained unremarkable during the experiment. In 48 hours after the last treatment eosinophilic cells began to appear typically. Although cytological changes associated with the drug nephrotoxicity were already presented by 48 hours, but signs of tubular necrosis could not be found

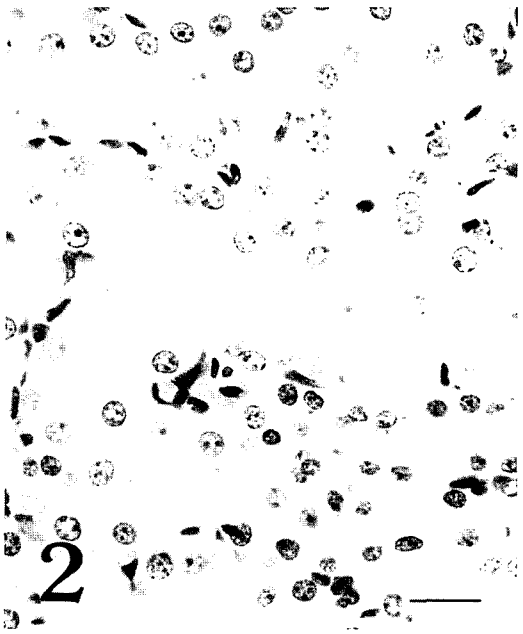


**Fig. 2.** Proximal convoluted tubules of kidney; APAP and gentamicin combination- treated rat. 48 hours after the last treatment. H&E, ×400(Bar=220).

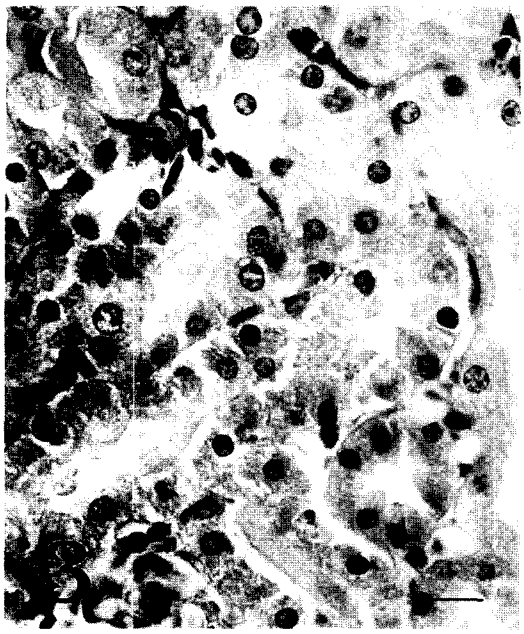

**Fig. 3.** Proximal convoluted tubules of kidney; combination-treated rat. 72 hours after the last treatment. eosinophilic cell appears and some swelling cells are sloughed in lumen. H&E, ×400(Bar=220).

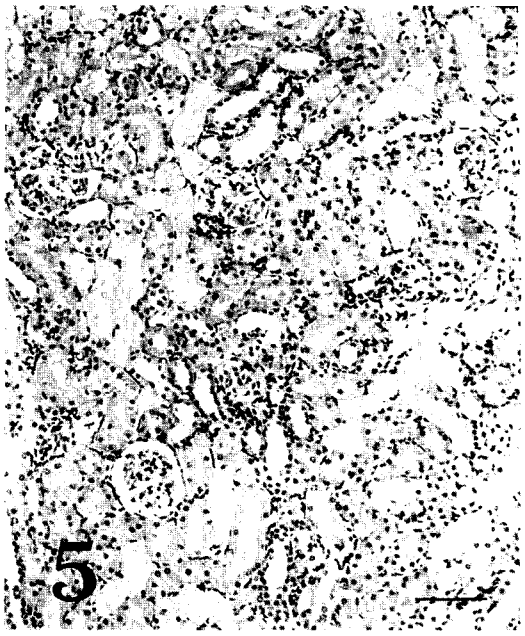

**Fig. 5.** Proximal convoluted tubules of kidney; combination-treated rat. 96 hours after the last treatment. low power of Fig. 4. proximal tubular epithelia show massive necrosis. H&E, ×100(Bar=880).

directly after the end of gentamicin dosing. In contrast, numerous tubular sections exhibited evidence of necrotic lesions with dying cells sloughed and disappearance of tubular outline in 72 hours, and finally was followed by a progressive repair to normal structure. In APAP and gentamicin combination-treated group, the pyknotic and eosinophilic cells appeared and the proximal tubules were devoid of epithelia in 72 hours after the last treatment. Some tubules were filled with amorphous cell debris and a few epithelial cells were sloughed. In 96 hours after the last treatment the tubular arrangement of epithelial cells were demolished and the epithelial cells were disappeared together. Although many degenerated and necrotic cells were still remained, and most lumina had proteinous granular debris. These tubular necrosis of combination-treated group culminated at this time compared with each single drug-treated or sham-treated control.

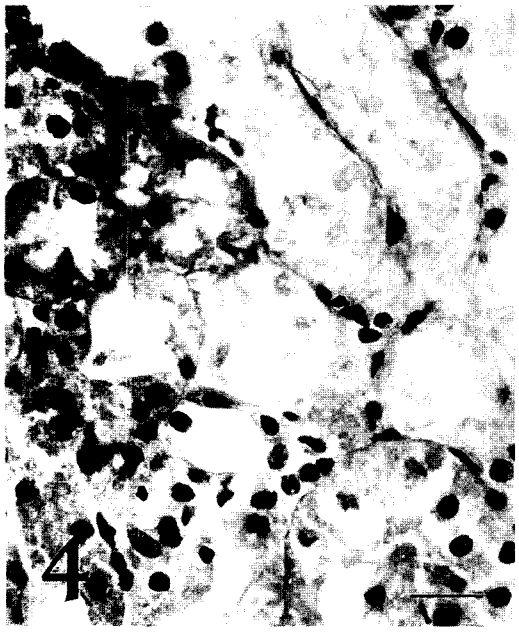

**Fig. 4.** Proximal convoluted tubules of kidney; combination-treated rat. 96 hours after the last treatment. the arrangement of tubular cells are perfectly destroyed and tubules contain cell debris. H&E, ×400(Bar=220).

## IV. DISCUSSION

Early morphological and biochemical observation have shown that APAP is not only hepatotoxicity but also nephrotoxicity (Prescott *et al.*, 1971). In experimental animals, hepatotoxicity has been associated with cytochrom P450-mediated

biotransformation of APAP to the toxic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (Hoffman *et al.*, 1985). However the nature and formation of the toxic metabolite in APAP nephrotoxicity is differ from hepatotoxicity. Formation of reactive intermediates from APAP within the renal cortex is dependent upon deacetylation prior to renal metabolism of the deacetylated product, p-aminophenol (PAP), to an electrophilic intermediates. PAP was approximately 5 to 10 times more potent as a nephrotoxicant than APAP (Newton *et al.*, 1982b). As for gentamicin, it was also nephrotoxicity and ototoxicity (Sullivan *et al.*, 1987). Many previous reports have shown that tubular necrosis caused by aminoglycoside antibiotics is related to lysosomal phospholipidosis predominantly affecting proximal tubular cells (Houghton *et al.*, 1978; Tulkens *et al.*, 1986). There is circumstantial evidence that the inhibitory effect of aminoglycosides on phospholipid catabolism eventually leads to cell necrosis.

In this study it was demonstrated that the APAP and gentamicin combination-treatment effect was synergism of the two drugs and the target site of kidney was proximal convoluted tubules in the rats.

In APAP and gentamicin combination-treated group, the serum BUN and creatinine could be a sensitive marker of concerned renal injury. The values of BUN were significantly increased in 96 hours after last treatment compared to single drug-treated group or sham-treated group, which suggested that the damage might be peak in 96 hours after last treatment. Because morphological changes were remarkable at this time. This data was similar to that of the previous studies (Tarloff *et al.*, 1989; Newton *et al.*, 1982b). Renal tubules revealed various changes throughout the experiment, and especially the proximal convoluted tubules underwent severe necrosis. Thus the kidney function might be disordered, that the filtered water was not resorbed and retention. This resulted in swelling of the kidney tissue. It revealed that the relative kidney weights of the combined group are higher than single drug-treated group or sham-treated group throughout the experiment. Grossly, a few ischemic coagulation necrosis of tissue called anemic infarction was visible at this time.

The gentamicin-treated group was also severely damaged in the proximal tubules in 72 hours after last treatment but was less severe than combination-treated group. The APAP-treated group showed more mild nephrotoxicity throughout the experiment. The peak time was at 48 hours after the last treatment. Some previous studies have demonstrated that the animal strain differences in susceptibility to APAP-induced nephrotoxicity (Tarloff *et al.*, 1989). Young adult male F-344, but not SD, rats were more susceptible to APAP-induced nephrotoxicity induced by PAP, a proposed nephrotoxicity metabolite of APAP, than weight-matched SD rats (Newton *et al.*, 1983). Corresponding to previous studies the APAP effects were not severed so much in this study and it might be related to strain differances in susceptibility. The mechanism mediating strain differences in APAP nephrotoxicity are not entirely clear, but may be related to differences in APAP metabolism via deacetylase-dependent pathways.

We can conclude that the acute nephrotoxicity in APAP and gentamicin combination-treatment to rats is very higher than in each single durg-treated alone and the target site is proximal convoluted tubules.

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