ROLE OF BETA CAROTENE ON HISTOMORPHOLOGY OF RAT KIDNEYS IN SUBACUTE APAP INDUCED RENAL DAMAGE

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ABSTRACT

Objective: This study was conducted to evaluate the role of beta carotene on histomorphology of rat kidneys in subacute Acetaminophen (APAP)-induced renal damage.

Study Design: Lab based randomized control trial

Place and Duration of Study: The study was carried out in the department of Anatomy Army Medical College, Rawalpindi; in collaboration with National Institute of Health (NIH), Islamabad for one week in June 2009.

Material and Methods: Sixty young adult (4-6 weeks old) Sprague-Dawley rats of both sexes weighing 180-240 g were randomized into three groups. Experimental group A was treated with 700 mg/kg body weight subacute APAP orally once daily for 7 consecutive days. Experimental group B was administered beta carotene 30 mg/kg body weight once daily one hour before 700 mg/kg body weight subacute APAP once daily for 7 consecutive days. Control group C animals were fed NIH laboratory diet. Kidney specimens were collected 24 hours after the last dose. Five micron thick sections of kidney were stained with H&E for histomorphological study. Frequencies and percentages were calculated to describe the variables *p* values less than 0.05 was considered statistically significant

Results: Microscopic examination in experimental group A demonstrated tubular necrosis of level 2 (35% animals) and level 3 (65% animals). Mild vacuolar degeneration was also observed in 90% of the experimental group A animals. In experimental group B, there was statistically significant difference (*p*-value < 0.001 in levels of renal tubular necrosis (15% animals) and grades of vacuolar degeneration (5% animals) as compared to experimental group A. Findings in experimental group B were not significantly different from that of control group C.

Conclusion: Beta carotene has protective role on histomorphology of kidneys in subacute APAP-induced renal damage in rats.

Keywords: Acetaminophen; Beta carotene; Nephroprotective; Necrosis; Renal damage; Vacuolar degeneration

INTRODUCTION

Acetaminophen (APAP), commonly known as paracetamol (N-acetyl-para-aminophenol), is an over-the-counter analgesic and antipyretic with not many side effects when taken at remedial doses[1]. In acute, subacute and chronic massive doses, it is known to produce hepatic and renal tubular damage; and even death both in experimental animals and in human beings[2]. Accurately predicting the risk of renal damage following APAP overdose is essential for several reasons. A careful perusal of literature has revealed that APAP-induced liver necrosis has been studied extensively. Nevertheless, the extra hepatic manifestations of APAP toxicity are currently not described well in the literature. Acute renal failure can occur even in the absence of hepatic injury[3]. Owing to unique metabolism in kidneys, even therapeutic doses of APAP have been reported to produce renal lesions[4]. APAP-induced renal failure becomes evident after hepatic damage in most cases, but can be differentiated from the hepatorenal syndrome, which may complicate fulminant hepatic failure[5].

Research has demonstrated that a range of organic compounds with antioxidant properties contribute to the protection of cells and tissues against deleterious effects of APAP and its reactive metabolites[6]. In the past, the kidneys were paid no attention in studies aimed at preventing APAP-induced damage. At present,

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studies are being carried out universally to identify compounds that can protect the kidneys with few or no side effects[7, 8]. Beta-carotene is an organic compound - a terpenoid, red-orange pigment; and is the major carotenoid precursor of vitamin A. Beta carotene has been thought of value to humans and other species because of excellent antioxidant properties[9] and has been shown to guard against APAP-induced hepatic damage, cancers, and heart diseases[10,12]. Beta carotene affords significant protection against gamma radiation-induced oxidative stress which was measured in the terms of lipid peroxidation[13]. Beta carotene has demonstrated antioxidant, anticancer, anti-radiation properties and hepatoprotective role against APAP-induced hepatic damage, however there is paucity of information regarding the protective role of beta carotene in APAP-induced renal damage. Extensive research has demonstrated that acute renal failure due to APAP-induced renal damage has been prevented by the same means which provide protection against hepatic damage[3,7,8]. In the light of above mentioned observations, it was proposed to study the potential protective role of beta carotene on histomorphology of kidneys in subacute APAP-induced renal damage which is one of the common causes of morbidity and mortality in drug poisoning cases in our emergencies.

MATERIAL AND METHODS

These laboratory based randomized controlled trials were carried out in the Department of Anatomy Army Medical College, Rawalpindi in collaboration with National Institute of Health (NIH), Islamabad for one week in june 2009. The study was carried out on young adult (4-6 weeks old) Sprague Dawley rats of both sexes weighing 180–240 g. Rats were housed in controlled environment of Animal house of NIH. They were randomly divided into three groups (n = 20 animals in each group, 10 males and 10 females). Control group C rats received vehicle for 7 consecutive days. Experimental group A rats were administered subacute acetaminophen 700 mg/kg body weight (dissolved in distilled water) orally via gavage tube once daily for 7 consecutive days. Experimental group B rats were given beta carotene 30 mg/kg body weight orally once daily one hour prior to subacute acetaminophen 700 mg/kg body weight orally once daily for 7 consecutive days. Beta carotene was mixed in diet pellets and compliance was ensured.

Twenty four hours after the last dose of APAP, the animals were euthanized under ether anesthesia. Kidney specimens were dissected, washed and fixed in 10% formalin. The tissue blocks of the kidneys were embedded in paraffin wax. Five micron thick sections were cut and stained with hematoxylin and eosin (H & E) for routine histomorphological study of renal cortex and medulla both.

Slides were studied for both qualitative and semiquantitative histomorphological parameters. Qualitative parameters included general architecture (undisrupted or disrupted), inflammatory infiltrate (present or absent), necrosis (present or absent), vascular changes (e.g., congestion); and individual cell morphology regarding cellular swelling, cytoplasmic staining (normal, pale or dark) and nuclear changes (shape, position and staining).

Levels of tubular necrosis were scored at 10x by using semi quantitative criteria (Modified from Hadjipour et al.[14]: Level 0 (none) = no necrosis in 3 views of any slide by light microscope; Level I (light necrosis) = average of 1-2 tubules necrosis in 3 views of any slide by light microscope; Level II (medium necrosis) = average of 3-5 tubules necrosis in 3 views of any slide by light microscope; Level III (severe necrosis) = average of 6-10 tubules necrosis in 3 views of any slide by light microscope; & Level IV (extra severe necrosis) = average of more than 10 tubules necrosis in 3 views of any slide by light microscope.

Vacuolar degeneration was scored at 40x on a Semi Quantitative Scale (modified from Abdel-Zaher et al[8]: 0 = No pathological change; + = Mild (less than 25 percent of the tissues affected); ++ = Moderate (25–50 percent of the tissues affected);

+++ = Severe (More than 50 percent of the tissues affected).

Statistical analysis

Data had been analyzed by using Statistical Package for Social Sciences (SPSS) version 16. Frequencies and percentages were calculated to describe the variables. The statistical significance of difference between the groups was evaluated using Chi-square test. Results were considered to be statistically significant at *p*-value < 0.05.

RESULTS

Qualitative findings

In experimental group A, H & E sections demonstrated lesions of whole tubules in the parenchyma as well as individual cell damage at X4, X10 and X40 respectively (Fig. 1). At X4, renal parenchyma revealed necrosis of tubules but individual tubules were identified at high magnification X10. At X40, individual cell damage in the renal tubules was studied.

At X40, the proximal convoluted tubules showed swelling of cells, apical blebbing and blunting along with loss of brush border in early cases of cell injury. In extreme stages, shrinkage and shedding of the entire cells were seen along with the narrowing of the lumen in majority of the tubules. A few renal tubules illustrated only foci of single epithelial cell desquamation. Severely necrosed tubules appeared dilated in conjunction with epithelial desquamation. Intraluminal cast formation together with apoptotic bodies was seen in severely damaged tubules. Exfoliated tubular epithelial cells in tubular lumen appeared as eosinophilic deposits of granular debris lacking recognizable cellular details. Proximal convoluted tubules were the main site of tissue damage. Inflammatory cells infiltration was also observed at X40 around the degenerating tubules and areas of cell injury at places of the medullary part of the kidney and at these sites the inflowing cells blurred the tubular structure. In some cases, tip of renal papilla also showed necrosis and even sloughing of the tip of the papillae was observed in rare cases. Mild vacuolar degeneration of the distal tubules was seen in most of the animals. Micro vascular changes (i.e., hyperemia and congestion of blood vessels) were also observed. Capillaries appeared dilated and overfilled with red blood cells in the cortical and medullary parts of the kidney. Basement membrane was found to be of normal thickness. The renal glomeruli showed no remarkable changes (Fig-1).

In experimental group B, the cytoarchitecture was almost analogous to control group C in H & E stained sections (Fig. 2 and 3 respectively). The histomorphological changes in renal cortex and medulla of experimental group B were less marked as compared to experimental group A. Beta carotene co administration along with subacute overdose of APAP resulted in marked attenuation of the tubular damage induced by subacute APAP overdose. In experimental group B, few animals showed foci of cell injury and evidence of mild vacuolar degeneration. But cellular injury did not involved the entire renal tubules; rather foci of damaged cells were observed in only a small number of tubules.

Quantitative findings

On histomorphological examination, there was no tubular necrosis seen in kidneys in control group C. In experimental group A, 35% of the animals revealed tubular necrosis of level 2. Remaining 65% of the animals demonstrated level 3 necrosis. In experimental group B, there was statistically significant difference in tubular necrosis as compared to group A Figure 4. Only 15% of the animals in experimental group B exhibited level 1 necrosis (*p*-value < 0.001.

There was no vacuolar degeneration in control group C. Mild vacuolar degeneration was observed in experimental group A in 90% of the animals. Whereas remaining 10% of the animals did not show vacuolar degeneration. In experimental group B, only 5% of the animals in experimental group B displayed mild vacuolar degeneration (*p*-value < 0.001). Rest of the animals exhibited findings which were not different from control group C.

DISCUSSION

Overdose of acetaminophen causes hepatic and renal damage both in experimental animals and humans[1,15]. Beta carotene has demonstrated relatively fair hepatoprotective properties in APAP-induced hepatic damage (12). The role of beta carotene on APAP-induced renal damage was not observed. The present study was planned to observe the potential protective role of beta carotene in APAP-induced renal damage in

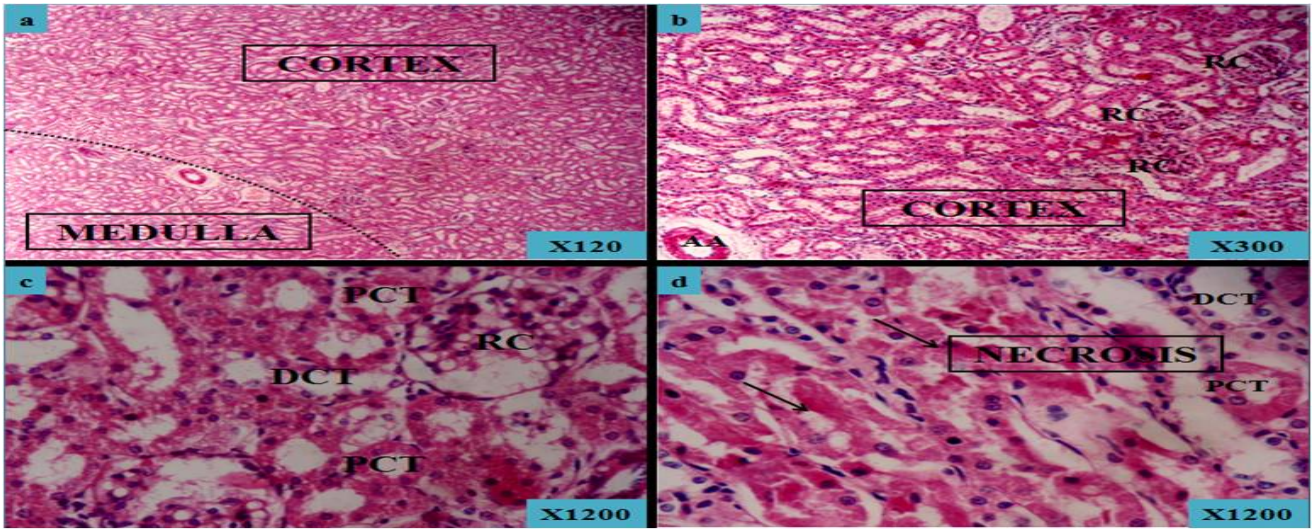

Figure-1: Sections of specimen of experimental group A showing renal architecture. a) Damaged tubules in both renal cortex and medulla; b) Cortex containing damaged renal tubules and unaffected renal corpuscles. Renal corpuscles (RC) arcuate arteries (AA); c) Cortex containing damaged renal tubules and unaffected renal corpuscles at higher magnification, Proximal convoluted tubules (PCT) exhibiting loss of brush border and extensive cell damage, Distal convoluted tubules (DCT) also exhibited severe tubular damage; d) Tubular necrosis (→).
H&E stain, Photomicrographs, Approx X120, X300 and X1200

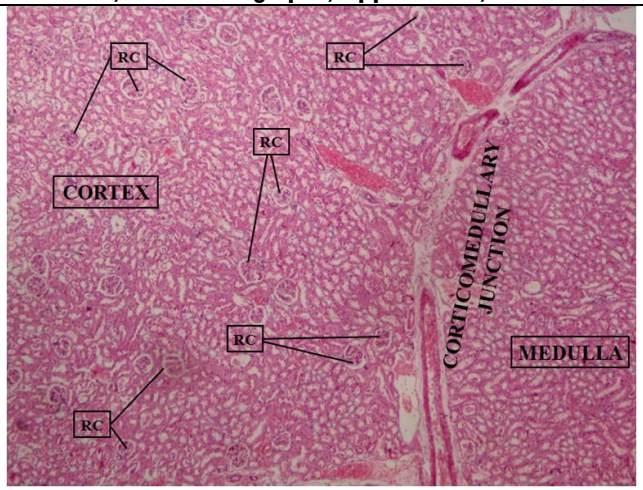

Figure-2: Section of specimen of experimental group B showing renal cortex and medulla having renal corpuscles (RC) and normal renal tubules. H&E stain, Photomicrograph, Approx X300

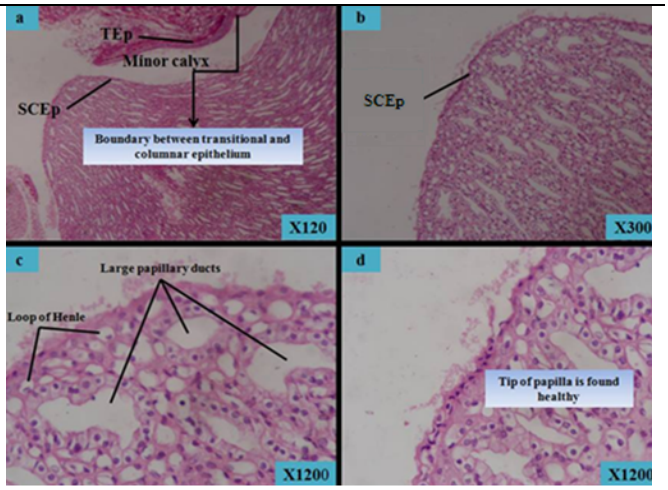

Figure-3: Sections of specimen of control group showing renal medulla containing loop of henle, collecting ducts, collecting tubules and large papillary ducts. Simple columnar epithelium (SCEp) and transitional epithelium (TEp). H&E stain, Photomicrographs, Approx X120, X300 and X1200.

rats as evidenced by histomorphological observations with subacute dosage of 700 mg/kg body weight daily for one week.

Results of the present study demonstrated two main histomorphological findings (qualitative and quantitative) in kidneys of Experimental group A rats i.e., necrosis and vacuolar degeneration after administration of subacute toxic doses of APAP. Experimental group A showed tubular necrosis, shedding of the entire cells into the lumen, vacuolar degeneration and inflammatory cells infiltration on histomorphological examination. More or less similar findings were observed by Abdel Zaher et al.,[8] who also noted tubular necrosis and vacuolar degeneration with acute single overdose of APAP (i.e., 2.5 g/ kg body weight). Abdel-Zaher et al.[8] also demonstrated cloudy swelling of proximal convoluted tubules (PCT) and congestion of distal tubules in rats when administrated APAP at dose of 750 mg/kg body weight per day for 7 days.

Histomorphological findings of Experimental group A in our study also demonstrated initial plasma membrane alterations which included: apical blebbing and distortion of brush border. Microvascular changes observed included hyperemia of renal vasculature in both cortex and medulla. Price et al.[16] also demonstrated coagulative necrosis of the proximal tubule cells, vacuolar degeneration, epithelial desquamation, collections of cellular debris within damaged tubules, intraluminal cast formation mainly in distal convoluted tubules, rupture of tubular membranes, interstitial edema and infiltration with lymphocytes and plasma cells together with apoptotic bodies. Similar changes have been noted by Ghosh and Sil[3] in kidney histomorphology under the influence of toxic doses of APAP. Similar findings were noticed by Fouad et al.[17] in a histological study of the kidneys after administration of single dose of 2.5 g/kg body weight of APAP and described severe tubular necrosis along with tubular dilatation in proximal convoluted tubules, vacuolar degeneration, epithelial desquamation and intraluminal cast formation mainly in the distal convoluted tubules, together with apoptotic bodies.

Cytoarchitecture of Experimental group B in our study demonstrated features not different from control group C. Only few animals showed foci of mild cell injury and evidence of mild vacuolar degeneration. These observations were similar to the findings by many other researchers in the past with several other compounds (natural and synthetic)[8,17,20]. Five distinct properties of beta carotene have been elucidated in previous studies i.e., guard against cancers[9], free radical scavenging and singlet oxygen quenching role of beta carotene in the prevention and treatment of APAP-induced depletion of glutathione in APAP-induced hepatotoxicity in mice[11], protective role of beta carotene on liver enzymes in APAP treated rats[12] shielding role of beta carotene in the pathological conditions mediated via oxidative stress e.g. radiation hazards[13] and heart diseases[10]. In view of these considerations, we investigated the potential protective role of beta carotene in subacute APAP-induced renal damage. Our results demonstrated that treatment of rats in Experimental group B with beta carotene along with administration of subacute toxic dose of APAP markedly protected against renal damage as assessed by qualitative and quantitative histomorphological features. In experimental group B, there was insignificant difference in tubular necrosis and vacuolar degeneration as compared to control group C but significant difference from experimental group A. Similar protective results were observed in a study for effect of beta carotene on serum transaminase levels after concurrent administration of beta carotene with dose of APAP daily for 7 days to rats[12]. Manda and Bhatia also[11] demonstrated the ameliorating capacity of beta carotene in the APAP-induced depletion of glutathione, glutathione peroxidase Manda et al[13] reported that beta carotene affords significant protection against γ-radiation-induced oxidative stress

which was measured in the terms of lipid peroxidation.

Our results demonstrate that beta carotene has ability to protect against renal damage induced by subacute APAP overdose by improving histomorphological features.

CONCLUSION

Results of our study helped to conclude that beta carotene has significant protective role on histomorphology of kidneys in subacute APAP-induced renal damage. However, precise mechanism of protection afforded by beta carotene during APAP-induced renal damage still remains to discover.

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