ORIGINAL ARTICLE

ROLE OF BETA CAROTENE ON HISTOMORPHOLOGY OF LIVER IN ACETAMINOPHEN-INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: To study the protective role of beta carotene against acetaminophen-induced hepatotoxicity in rats.

Study design: Laboratory based randomized control trial

Place and duration of study: Department of Anatomy Army Medical College, Rawalpindi; in collaboration with National Institute of Health (NIH), Islamabad. The study duration was one year from Feb, 2009 to Jan, 2010.

Materials and methods: Sixty young adult (4-6 weeks old) Sprague-Dawley rats weighing 180-240 g were randomized into three groups. Control group C was given NIH laboratory diet, experimental group A was given toxic dose of acetaminophen 700 mg/kg body weight once daily and experimental group B was given beta carotene 30 mg/kg body weight once daily along with 700 mg/kg body weight acetaminophen once daily for one week. Liver specimens were collected 24 hours after the last dose. Five micron thick sections of liver were stained with H&E for histomorphological study.

Results: Microscopic examination demonstrated various grades of periportal and spotty necrosis in experimental group A as compared to control group C. In experimental group B, there was significant attenuation (P-value < 0.001) in periportal and spotty necrosis.

Conclusion: It was concluded that beta carotene has hepatoprotective role on histomorphology of liver in acetaminophen-induced hepatotoxicity in rats.

Key words: Acetaminophen, Beta carotene, Hepatotoxicity, Histomorphology, Necrosis

INTRODUCTION

Acetaminophen (APAP) is widely used analgesic and antipyretic with a few side effects when taken at curative doses. In massive doses, it is known to produce hepatic necrosis, extra hepatic lesions and even fatality both in experimental animals and in human beings[1]. The incidence of APAP poisoning varies throughout the world including Pakistan[2].

The APAP is mainly metabolized in the liver to excretable metabolites. APAP-induced hepatotoxicity has been attributed to the cytochrome P-450 generated reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI)[3]. Cell damage can be seen throughout the liver, predominantly in centrilobular and periportal areas, despite of the fact that individual lobules are variably affected[4]. Needless to say that APAP-induced toxicity and its management deserves serious attention. Recent advances in the understanding of the cellular events contributing to the development of toxicity have enabled us to devise novel strategies for APAP-induced hepatic damage. Such approaches included the removal of the causative agent and the use of anti-inflammatory or antioxidant drugs[5].

Carotene is the carotenoid containing no oxygen. It comes in two primary forms: alpha-carotene (α-carotene) and beta-carotene (β-carotene). These two primary isomers of carotene differ in the position of double bonds in the cyclic group at the end. Gamma, delta and epsilon isoforms (γ, δ and ε-carotene) also exist. Beta-carotene ($C_{40}H_{56}$) is an organic compound - a terpenoid, a red-orange pigment; and is the major carotenoid precursor of vitamin A. Beta carotene differs in the ability to quench singlet oxygen and free radicals. Vitamin A has little capacity to scavenge free

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radicals and oxygen species. Beta carotene ($C_{40}H_{56}$) has been thought of value to humans and other species for having excellent antioxidant properties[6]. Other carotenes are half as effective as beta carotene. Beta carotene is found in yellow, orange and green fruits and vegetables. Beta carotene has been shown to guard against arthritis, protoporphyria, long and short term morbidity in low birth weight infants, cancers and heart disease[7-8]. Since there is paucity of information regarding the role of beta carotene on histomorphology of liver in APAP-induced hepatotoxicity, therefore we proposed the possibility of protective role of beta carotene on histomorphology of liver in APAP-induced hepatotoxicity in rats.

MATERIAL AND METHODS

These lab 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 from Feb 2009 to Jan 2010. The study was carried out on young adult (4-6 weeks old) Sprague Dawley rats 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). Control group C rats were fed NIH laboratory diet for 7 consecutive days. Experimental group A rats were administered acetaminophen 700 mg/kg body weight orally once daily for 7 consecutive days. Experimental group B rats were given beta carotene 30 mg/kg body weight orally once daily along with acetaminophen administration 700 mg/kg body weight orally once daily for 7 consecutive days.

Twenty four hours after the last dose of APAP, the animals were euthanized under ether anesthesia. On dissection, the liver was identified in the right anterior portion of the abdominal cavity. The peritoneal connections of the liver were cut to remove the liver specimens which were fixed in 10% formalin. The tissue blocks of the right lobe of the liver were embedded in paraffin wax, cut in sections of 5 µm and stained with hematoxylin and eosin (H&E) for routine histomorphological study.

Grading of periportal necrosis was done at 10X by using subsequent criteria (Modified from Ishak *et al*., 1995)[9]: Absent = No necrosis; Mild = Focal, few portal areas; Mild/ moderate = Focal, most portal areas; Moderate = Continuous around < 50% of tracts or septa; and Severe = Continuous around > 50% of tracts or septa. Grading of focal (spotty) lytic necrosis was done at 10X by using following criteria: Absent = 0, One focus or less per field = 1, One to four foci per field = 2, Five to ten foci per field = 3 and More than ten foci per field = 4.

Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS) version 16. Frequency and percentages were calculated to describe the variables. The statistical significance of difference between the groups was evaluated using Chi-square test. Results were regarded significant if P-value was less than 0.05.

RESULTS

In control group C, H&E stained sections showed normal architecture of liver with no pathological changes. The histological examination of H&E stained preparations of the liver specimens in experimental group A illustrated areas of lesion around portal triad (Fig. 1). The cytoplasm of the hepatocytes in zone 1 and 2 (close to portal triad especially limiting plate) was intensely eosinophilic with increased density of nucleus in conjunction with extremely condensed and compact nuclear chromatin in either peripheral aggregates abutting the nuclear membrane or as extruded nuclear fragments free or within phagocytic cells. In some locations, boundaries between the cells were hardly discernible. Sporadically, areas of focal or spotty lesions involving single necrotic cells were observed in the region between central vein and portal triad with condensation of nuclei and strongly eosinophilic cytoplasm. The liver sections in experimental group B showed microscopic picture that closely approximated that of the control group with few exceptions (Fig. 2).

On semi quantitative scoring, no animal in control group C showed periportal necrosis. The liver specimens obtained in experimental

group A demonstrated mild/ moderate periportal necrosis in 5% of the animals. Thirty percent exhibited moderate periportal necrosis and 65% confirmed severe periportal necrosis. In experimental group B, only 15% of the animals revealed mild necrosis with highly significant difference (P-value < 0.001) (Table 1). Spotty necrosis was not observed in any animal of control group C. 55% of the animals in experimental group A demonstrated grade 2 spotty necrosis. Rest of the animals (45%) demonstrated grade 3 spotty necrosis. In experimental group B, only 20% of the animals showed grade 1 spotty necrosis (P-value < 0.001) (Table 2).

Table 1: Description of different grades of periportal necrosis of liver in groups

Groups	None	Mild	Mild/ moderate	Moderate	Severe
Control group C (n = 20)	20 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Experimental group A (n = 20)	0 (0%)	0 (0%)	1 (5%)	6 (30%)	13 (65%)
Experimental group B (n = 20)	17 (85%)	3 (15%)	0 (0%)	0 (0%)	0 (0%)

Table 2: Description of different grades of spotty necrosis of liver in groups

Groups	0	1	2	3
Control group C (n = 20)	20 (100%)	0 (0%)	0 (0%)	0 (0%)
Experimental group A (n = 20)	0 (0%)	0 (0%)	11 (55%)	9 (45%)
Experimental group B (n = 20)	16 (80%)	4 (20%)	0 (0%)	0 (0%)

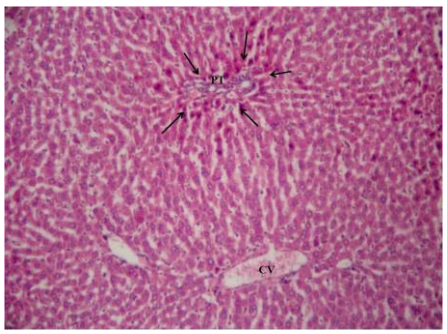

Fig. 1: Photomicrograph of liver of experimental group A showing areas of lesion (→) around portal triad (PT). Central vein (CV) H&E stain, (Approx X300)

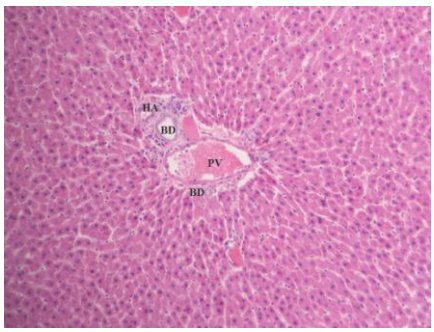

Fig. 2: Photomicrograph of liver of experimental group B showing portal triad having branches of hepatic artery (HA), portal vein (PV) and bile duct (BD). H&E stain, (Approx X300).

DISCUSSION

Acute adverse effect of APAP is dose dependent fatal hepatic necrosis[1]. To our knowledge the effect of beta carotene on histomorphology of liver in APAP-induced hepatic damage has not been demonstrated yet.

There was no necrosis in control group C; moderate to severe grades of periportal and spotty necrosis in experimental group A. These findings were consistent with the results of previous studies using multiple protective agents. Abdel-Zaher *et al*.[4] demonstrated necrosis in liver specimens of rats administered 750 mg/kg body weight APAP daily for 7 days. APAP could induce hepatocyte apoptosis in vitro as well as necrosis, while the quantitative resolution of cell death after introduction of hepatotoxic dose of APAP in vivo indicated that APAP caused broadly necrosis rather than apoptosis as observed by Gujral *et al*.[10].

In the present study, beta carotene administration along with APAP resulted in considerable protection by preserving parenchymal architecture of liver against APAP over dosage. These findings could be correlated with previous work, which reported treatment with multiple compounds such as alpha lipoic acid[4], beta glucan[11] and numerous substances derived naturally from plants[12] or synthetic[13], given alone[14] or in combinations[15], significantly improved histomorphology in APAP-induced hepatotoxicity[16].

Beta carotene has revealed hepatoprotective properties by improving liver function tests in APAP-induced hepatotoxicity[17,18]. Beta carotene has an edge over other compounds, as it scavenges a variety of free radical species in tissues at low partial pressures of oxygen while others are effective only at higher oxygen concentration[19]. Recently, it was considered that APAP damages the cells by producing oxidative stress[3]. There is significant experimental and clinical data regarding the protective role of beta carotene in case of oxidative stress via free radical scavenging and quenching of reactive oxygen species[6]. Manda *et al* demonstrated that beta carotene ameliorates radiation induced lipid peroxidation in mouse brain and testis[7]. Manda and Bhatia observed the antioxidant role of beta carotene against APAP-induced liver injury in mice in terms of lipid peroxidation[18].

In the light of above mentioned observations, the results of present study proved that beta carotene has potent hepatoprotective role in APAP-induced hepatotoxicity. These findings were correlated with previous research works; which reported that treatment with multiple compounds significantly reduced the histopathological findings in APAP-induced hepatotoxicity.

CONCLUSION

The results of our study indicate that toxic doses of APAP may prone rats more susceptible to periportal and focal necrosis. Beta carotene treatment play hepatoprotective role against APAP-induced hepatotoxicity. However, it remained to find the exact mechanism of protection. Additional studies are recommended to elucidate the precise mechanism of protection afforded by beta carotene during APAP-induced hepatotoxicity.

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