HISTOPATHOLOGICAL AND SERUM ENZYME ALTERATIONS IN RATS TREATED WITH CAMPTOTHECIN AND PROPHYLACTIC EFFECT OF α-TOCOPHEROL

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Abstract: The aim of the present study was to investigate the extent of change in level of serum marker enzymes and lipid peroxidation caused by camptothecin (CPT) administration and to determine whether vitamin E possess potentiality of reversing or suppressing CPT induced hepatotoxicity in Wistar rats. CPT is a cytotoxic quinoline alkaloid which inhibits the DNA enzyme topoisomerase I. This alkaloid reveals remarkable anticancer property in preliminary clinical trials but also low solubility and high adverse drug reaction. It induces cell damage by producing free radicals and reactive oxygen species. Liver damage was assessed by investigating serum marker enzymes like AST, ALT, LDH and ALP and was correlated to the histopathological observations in the liver. Treatment with CPT increased the levels of lipid peroxides, AST, ALT and LDH activity but decreased serum ALP. Pathological alterations were also observed in histology of the liver tissue with CPT treatment. Co-treatment of α-tocopherol significantly prevented the CPT induced pathological alterations in liver tissues with a decrease serum marker enzyme activity and a significant increase in antioxidant levels. The data obtained in the present study suggest that α-tocopherol afford significant protective effect over drug induced oxidative damage in rat liver.

Key words: Serum enzymes, Camptothecin, Vitamin E

INTRODUCTION

DNA topoisomerases are ubiquitous enzymes that catalyse the interconversion of topological isomers of DNA. They act by inducing breakage and reunion of either one strand (Topo I) or both the strands of DNA (Topo II) and are involved in many vital cellular processes for example, replication, transcription and recombination. These enzymes are essential for synthesis of nucleic acid but also they serve as molecular target for numerous clinically important antineoplastic agents like fluroquinolone, etoposide, teniposide and camptothecin [1].

Camptothecin (CPT), a class I topoisomerase inhibitor that stabilizes topo I DNA covalent specifically bind at the Top1- DNA interface and forms ternary cleavable complex, thus preventing DNA religation step. Stabilization of Top1- cleavage complex by Top 1 inhibitors arrests the replication fork which generates bulky DNA lesions [2].

Normal tissues are often affected by chemotherapeutic agents during cancer treatment which results in severe side effects [3]. CPT and its analog are broad spectrum antinoeplastic agents which had received FDA approval for use against ovarian, lung, breast, and colon cancers but occurrence of leucopenia, thrombocytopenia gastrointestinal malignancies, melanoma, fever, alopecia, vomiting, diarrhea and haemorrhagic cystitis were observed to be dose-limiting toxicities of CPT [4,5]. The present work was conducted in order to comprehend the ameliorative effect of vitamin E
against camptothecin induced changes in levels of serum enzyme and histopathological changes in liver. Vitamin E is previously reported to be beneficial in curbing the chemotherapy mediated side effects [6-8]. It is known to increase the cyto-activity of drugs like doxorubicin, cisplastin and 5-fluorouracil in vivo. Vitamin E also induces apoptosis in experimental tumor cell lines [9]. From the investigation, we uncover the partial protective effect rendered by vitamin E against CPT induced alterations in rat model.

MATERIALS AND METHODS

Drugs and chemicals: Camptothecin and α-tocopherol acetate, was purchased from Sigma Chemicals, St Louis, MO, USA. All other chemicals used were of high analytical grade and solvents were of Qualigen grade. Kits for enzyme assays were obtained from Span Diagnostics Ltd, India.

Animal model: Adult male albino rats of Wistar strain (120 ± 20 g) were obtained from Bharat Serum Pvt. Ltd, Thane, Navi Mumbai, India. The animals were maintained under standard conditions of temperature (25 ± 2°C), light (12 h light/12 h dark) and humidity. They were fed standard rat pelleted diet obtained from Lipolin, India and water ad libitum. Experimental animals were handled according to the Institutional legislation, regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Design: Following the acclimatization period, the rats were randomly divided into 4 groups consisting of six animals each.

Group I: Control rats were given saline solution (0.9% NaCl) for four consecutive days, intravenously.

Group II: Rats were injected CPT (6mg/kg body weight) dissolved in dimethyl sulphoxide for four consecutive days, intravenously.

Group III: Rats served as control group for vitamin E and received α- tocopherol (6 mg/kg body weight) orally daily for a period of 30 days.

Group IV: Rats received α- tocopherol prior to CPT injection as described for group 2 and 3 rats.

At the end of the experimental period the animals were killed by decapitation. Blood was collected and centrifuged at 3000 rpm for 10 minutes within one hour of collection and enzymes were assayed. Liver was excised immediately, washed with ice-cold saline and stored in formalin before processing for histopathological studies.

Biochemical analysis: Serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured spectrophotometrically by standard IFCC/UV kinetic method using commercial kits (Span Diagnostics Ltd.) and Robonik autobiochemistry analyser. Lipid peroxidation was determined by the procedure of Okhawa et al. [10]. Malondialdehyde (MDA), formed as an end product of lipid peroxidation, served as a measure of the intensity of oxidative stress. MDA reacts with thiobarbituric acid to generate a colored product that can be measured optically at 532 nm. Tetramethoxy propane was used as standard.

Statistical analysis: The Results were expressed as mean ± standard deviation (SD) for six animals in each group. Statistical significance of assay was analyzed by unpaired Students t-test and given respective symbols in the tables.

RESULTS AND DISCUSSION

Liver is a major target organ for toxicity of xenobiotics and drugs as most of the orally ingested drugs pass through the liver and some chemicals are metabolized into toxic intermediate in the liver. The target organs of CPT were determined to be liver, kidney and blood.

Table 1: Effect of Camptothecin and Vitamin E on serum enzymes and Lipid peroxides. Values are expressed as Mean ± SD for six rats in a group. Comparisons are made between: ‘a’ Groups I–II; ‘b’ Groups I and IV; ‘c’ Group II and IV. Statistical significance: * P < 0.05, ** P < 0.01, *** P < 0.001, NS- Non significant.
Fig. 1: Histology of normal control rat liver showing normal architecture after staining with hematoxylin and eosin stain, magnification X 400.

Fig. 2: Pronounced histopathological abnormalities seen in rats treated with Camptothecin (6 mg/kg body weight) showing presence of many spots of focal collected cellular granulomatous lesions, periportal inflammatory cells and loss of hepatic tissue structural pattern, magnification X 400.

Fig. 3: Histology of vitamin E treated rat showing normal architecture after staining with hematoxylin and eosin stain, magnification X 400.

Fig. 4: Histopathological abnormalities seen in rats treated with CPT (6 mg/kg body weight) and vitamin E (6mg/kg body weight) showing spots of focal collected cellular granulomatous lesions, periportal inflammatory cells and loss of hepatic tissue structural pattern, but the extent of alterations are lesser than that observed in Fig. 2 B, magnification X400
cells. In the present study we attempt to investigate the effect of CPT on liver cells by histopathological study and to co-relate the observations with serum marker enzymes.

Figure 1 shows the histology of control rats demonstrating normal architecture. In contrast, groups treated with CPT at doses of 6 mg/kg showed hepatotoxicity. The most pronounced histopathological abnormalities observed in rats treated with CPT involved dissolution of hepatic cords, which appeared as empty vacuoles aligned by strands of necrotic hepatocytes. The hepatic tissues showed the presence of dense focal inflammatory cells or necrotic tissues and tendency for liver fibrosis manifested by the presence of many spots of focal cellular granulomatous lesions (Fig. 2). Liver histology of the normal rats receiving vitamin E alone (Group III) did not show any significant change when compared with control rats (Group I), indicating that it does not have any adverse effects (Fig. 3). The liver histology of animals treated with vitamin E before CPT showed small amount of pathological alterations when compared to the CPT treated rats (Fig. 4).

Severe metabolic disturbances are known to occur as toxic manifestations including extensive alterations in enzyme levels in tissues and sera of hosts. The estimation of serum marker analysis namely AST, ALT, LDH and ALP are indicative of the damage caused to hepatic cells, the leakage causing increased levels of hepatospecific enzymes in serum [11].

Serum aminotransferase activities have long been considered to be sensitive indicators of hepatic injury [12]. Injury to the hepatocytes alters their transport function and membrane permeability, leading to leakage of enzymes from the cells [13]. Therefore, the marked release of AST and ALT into the circulation indicates severe damage to hepatic tissue membranes during intoxication. The significant increase of serum AST and ALT activities observed CPT treated group was considered to be treatment related (Table 1). The hepatotoxic effects induced by CPT administration were also confirmed by histopathological alterations. Animals that received vitamin E prior to CPT treatment (Group IV) showed significant decrease in the levels of transaminases when compared to enzyme levels of CPT treated rats (Group II). The reversal of increased enzymes in CPT-induced liver damage by vitamin E may be due to the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity. This is in agreement with the commonly accepted view that changed levels of transaminases return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes. These results are consistent with our previous reports of CPT induced hepatotoxicity [14,15]. Minami et al. [16] and Wang et al. [17] have also reported similar increases in AST and ALT after CPT administration.

LDH is an essential enzyme involved in anaerobic glycolysis and is responsible for the anaerobic transformation of pyruvate to lactate. Increased expression of LDH under hypoxic conditions has been demonstrated in various cell lines [18-20]. Concerning liver diseases, it is well known that dominant elevation of serum LDH is observed in hypoxic hepatitis caused by shock or heart failure [21-22]. Although the elevation of LDH activity in acute liver injury has been simply supposed to be enzyme leakage through damaged hepatocyte membranes, increased the LDH production could also be attributable to anaerobic conditions. The hepatocytes are expected to increase the production of LDH under anaerobic conditions, until they become necrotic.

The decrease in alkaline phosphatase levels may result from the increased need of energy through glycolytic and oxidative pathways of glucose 6 phosphate, rather than alkaline phosphatase activity [23].

Effect of camptothecin and vitamin E on the levels of serum lipid peroxides is shown in Table 1. Lipid peroxide status reveals significant (P<0.01) increase in group II, which highlights CPT induced oxidative damage. However vitamin E prevented CPT induced alterations in group IV. The accumulation of lipid peroxides can introduce hydrophilic moieties into the membrane hydrophobic phase and thus can result in alteration of membrane permeability and cell function. The present data reveal that CPT administration produced a marked oxidative impact as evidenced by the significant increase in lipid peroxides. The increase in lipid peroxides might result from increased production of free radicals. This finding is in agreement with other investigation that reported CPT as a strong inducer of oxidative stress and free radical detoxifying enzymes [24].
CONCLUSION

In summary, intoxication of rats with camptothecin impinged oxidative stress and liver damage, which is shown by elevation in serum enzyme levels. Indeed, the data presented here reveal the hepatoprotective role of vitamin E which is evidenced by the normalization of pathological parameters. The present study thus validates vitamin E as a potentially useful candidate in combinational chemotherapy against CPT mediated injury and oxidative stress. Further studies to elucidate the prophylactic role of vitamin E in CPT induced toxic manifestation are underway.

REFERENCE