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LETTER TO THE EDITOR

EFFECT OF URSODEOXYCHOLIC ACID ON FREE RADICAL MEDIATED DNA DAMAGE – AN *IN VITRO* STUDY

Sir,

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There is a close correlation between hepatotoxicity and DNA damage in liver cells and the inhibition of oxidative DNA damage is the major mechanism of action a large number of chemoprotective drugs used for the treatment of various liver diseases. The effect of ursodeoxycholic acid (UDCA), a hepatoprotective agent on free radical medicated DNA damage was studied *in vitro* to find out its role against the free radicals generated during liver disorders.

The formation, behaviour and scavenging of oxygen free radicals and other oxygen derived species in biological system has received much attention, with increasing evidence that it is closely connected with a variety of pathological conditions including atherosclerosis, cancer, arthritis and liver disorders (1-4).

In these pathological conditions, DNA and lipid damage are of particular importance, and have been recognized in a large number of liver diseases (5,6). Ursodeoxycholic acid, a 7-beta hydroxy epimer of chenodexycholic acid is a recommended drug for the hepatic complications in obstructive jaundice (7). Besides affording protection against hepatic damage in obstructive jaundice it has also found to be effective for the treatment of primary biliary cirrhosis, gallstone and cholestatic diseases (8, 9).

The existence of a close correlation between hepatic disorders and oxidative stress in DNA (10) had led us to study the effect of UDCA on the free radicals mediated DNA damage and thereby to show a possible mechanism for the hepato protective action of the drug.

Ursodeoxycholic acid (UDCA), thiobarbituric acid (TBA), calf thymus DNA (E. Merck, India), ferric chloride, sodium chloride, ascorbic acid (BDH, India) were obtained in the highest purity commercially available. The drug was finely powdered and dissolved in physiological saline to a concentration of 1 mg/ml.

0.5 ml of the calf thymus DNA (1 mg/ml in 0.15 M Nacl), was incubated with 0-700µg of UDCA for one hr at 37°C. The method of Halliwell and Gutteridge (11) was adopted for the assay of DNA damage. The reaction mixture in a total volume of 2.0 ml, contained 0.5 ml of calf thymus DNA, 1.26 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of ascorbic acid (1 mM) and 0.04 ml of $FeCl_3$ (100 µM). The reaction mixture was incubated for 1 hr at 37°C in a water bath with intermittent shaking. After the incubation period, 1 ml of TBA (0.067%) was added and the reaction mixture was kept in a boiling water bath for 20 min. The TBA reactants so generated were extracted with

butanol and the colour intensity measured spectrophotometrically at 535 nm. The values were subjected to statistical analysis and student 't' test was conducted to asses the level of significance.

The *in vitro* antioxidant activity of UDCA was determined by means of its capacity to reduce DNA damage, assessed by the amount of TBARS formed. Table I shows a concentration dependent increase in the percentage inhibition of free radical mediated DNA sugar damage. A maximum inhibition of 70% could be recorded at the concentration of 700 µg suggesting that UDCA suppress the generation of reactive oxygen species.

TABLE I : Effect of various concentrations of UDCA on the level of TBARS formed in the Fenton reaction mixture.

Sl. No.	System+Drug (µg)	TBARS (n moles malondialdehyde)	% Inhibition
1.	0	1200±147	–
2.	100	1000±120	16.60
3.	200	910±100	24.16
4.	300	820±91	31.60
5.	400	659±73*	45.08
6.	500	521±69*	56.50
7.	600	410±50*	65.83
8.	700	350±42*	70.80

System, represents the reaction mixture containing 0.5 ml calf thymus DNA (1 mg DNA/ml), 1.26 ml phosphate buffer, 40 µl $FeCl_3$ (100 µm), 0.2 ml ascorbate (1 mM) and 1 ml TBA (0.067%); each value represents mean ± S.E. of 6 independent experiments. The value of TBARS are expressed as n mole malondialdehyde formed in the whole of the reaction mixture.

*P<0.001 when compared to system without drug.

Oxidative macromolecular damage has been reported in various degenerative liver diseases (12, 13). The observed inhibition of Fenton's reaction mediated degradation of DNA by the presence of UDCA suggest that the drug may counteract the free radical mediated degradation of the deoxyribose sugar moiety of DNA. Since the drug is known for its hepatoprotective action and has shown its efficiency in protecting against hepatic injury in cholestasis (14), it may be proposed that its efficiency may be attributed at least, in part, to its free radical scavenging ability.

The role of reactive oxygen metabolites in promoting cholesterol crystal formation in model bile has been reported (15). The free radical scavenging ability of UDCA observed could be one of the mechanisms for its gall stone desolating activity. This property may be due to its hydrophilic nature, because it has been reported that UDCA could antagonize macrophage activation by hydrphobic bile acids to blunt their capacity to generate reactive oxygen species (16).

The effect of UDCA on DNA damage suggest that the hepatoprotective and the gall stone desolating actions of UDCA may be due, at least in part to its ability to suppress the generation of reactive oxygen species. The hydrphilic nature of the drug may be accounted for this affect.

ARUMUGAM GEETHA*, SHANMUGAM KARTHIKA*, RAJAGOPAL SURENDRAN** AND GOPAL JAYALAKSHMI***

*,**Department of Biochemistry, Bharathi Women's College, Chennai-600 108 and

Departments of **Gastroenterology and ***Microbiology, Stanely Medical College & Hospital, Chennai- 600 108

*Corresponding Author

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