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VITAMIN E PROTECTS INTESTINAL BASOLATERAL MEMBRANE FROM CMF-INDUCED DAMAGES IN RAT

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Abstract: CMF is a combination of anticancer chemotherapeutic agents Cyclophosphamide, Methotrexate and 5-Fluorouracil. Vitamin E protects the basolateral membrane (BSM) from CMF induced lipid peroxidative damages. Rats were treated intravenously with cyclophosphamide-10 mg, methotrexate-1.0 mg and 5-fluorouracil-10 mg per kg body weight for six cycles. Vitamin E (600 mg/kg body weight) was administered orally, daily. Intestinal basolateral membrane bound ATPases (3.6.1.3), Alkalinephosphatase (3.1.1) and 5'-Nucleotidase (3.1.3.5) were protected by co-administration of vitamin E with CMF. In CMF treated rats the lipid peroxidation levels were found to be elevated with a significant depletion in membrane sulfhydryl groups. In vitamin E co-administered animals, the enzyme activities were found to be restored with concomitant reduction in malondialdehyde levels and an increase in the sulfhydryl groups. The membrane cholesterol and phospholipid levels which were altered in CMF treated rats were bought back to the normal in co-administration of vitamin E.

Key words: vitamin E cyclophosphamide methotrexate 5-fluorouracil intestinal basolateral membranes

INTRODUCTION

Cyclophosphamide, Methotrexate and 5-fluorouracil (CMF) in a combination therapy is being extensively used for patients with breast cancer (1). The antimetabolic effect of methotrexate is known to give rise to the malabsorption syndrome (2). This syndrome is accompanied by histological changes in the intestine, such as shortened microvilli (3). This possibly results from the depressed generation of intestinal epithelial cells following damage to crypt cells by CMF. This syndrome causes biochemical changes such as a decrease in the content of membrane constituents. Methotrexate administration to rats were found to lessen interaction between protein and lipid. 5-Fluorouracil administration to rats were found to alter absorbtion, enzyme activity and mucosal lipid composition of intestine (4).

Generally, biological membranes have been reported to be susceptible to lipid peroxidation because of the presence of polyunsaturated fatty acid in their phospholipid (5). The epithelial cells of the mammalian small intestine are polarised in that the plasma membrane is divided into apical brush border and basolateral regions where digestion and absorption of nutrients takes place. Since the accumulation of lipid peroxides introduces hydrophilic moities into the membrane hydrophobic phase, in the present investigation, the effect of CMF on intestinal membrane bound enzymes were studied in rats. The sulphydryl groups are important for the functional integrity of membranes and hence the sulphydryl content was determined along with lipid peroxide levels. Since vitamin E has been proved to be very effective antioxidant, it was thought to use it in combination with CMF.

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METHODS

Chemicals: All chemicals used were of highest purity commercially available. Cyclophospha-mide, methotrexate and 5-fluorouracil were obtained from Astra Pharma, Frankfurt, Germany.

Animal study: Adult Wistar male rats weighing 180-200 g were maintained on standard rat pellet feed (Lipton India, Bombay) and water *ad libitum*. They were divided into 4 groups with 6 rats in each. Group 1 served as control. Group 2 rats were injected with CMF on day 1 and day 8 for 6 cycles intravenously with 14 days gap between each cycle. Rats were treated intravenously with cyclophosphamide 10 mg, methotrexate 1 mg and 5-fluorouracil 10 mg/kg. Group 3 rats were administered vitamin E 600 mg/kg orally, daily. Both CMF and vitamin E were given to Group 4 rats as described for Group 2 and 3.

Preparation of basolateral membrane: Rats were fasted overnight and were sacrificed by cervical decapitation. The intestine segment from the proximal end of the jejunum was isolated and the wet weight was noted. The basolateral membrane (BSM) of the small intestinal segment were prepared (6). Small intestine was homogenised using warren blender.

Biochemical assay: Protein content in the homogenate was determined (7). In BSM Na^+K^+-ATPase (8), Ca^{2+}-ATPase (9), Total-ATPase (10), 5'-Nucleotidase (11), Cholesterol (12), Phospholipid (13), Malondialdehyde (14), and Sulphydryl groups (15) were estimated.

RESULTS

Table I shows the decreased activity of Na^+K^+ATPase, Ca^{2+} ATPase, Total-ATPase and 5'-Nucleotidase in BSM of CMF treated rats. Vitamin E co-administered rats showed the normal value. Level of lipid peroxides, sulphydryl groups, cholesterol, phospholipid and cholesterol/phospholipid in BSM are depicted in Table II. In vitamin E co-administered rats lipid peroxidation was found to be decreased and TSH groups of the membrane were found to be increased significantly. Cholesterol levels increased significantly but the phospholipid levels were decreased in CMF treated rats. As a result of this the cholesterol/phospholipid ratio was increased. Cholesterol/phospholipid ratio is one of the main factors which maintains membrane fluidity. Cholesterol/phospholipid ratio in group 4 was found to be not significantly changed when compared to Group 1.

DISCUSSION

Methotrexate alters chemical constituents of the intestine leading to malabsorption syndrome (3). The lipid peroxidative nature of cyclophosphamide is responsible for the alterations observed in the small intestine BSM bound enzyme activities. Acrolin and phosphoramide mustard are the metabolites of cyclophosphamide, which are the causative agents for inducing lipid peroxidation (16). The

TABLE I : Activity of Na^+K^+ dependent ATPase, Ca^{2+} dependent ATPase, Total ATPase and 5' Nucleotidase activity in intestinal BSM of control and experimental rats. The values are expressed as mean ± S.D. for six animal in each group.

Parameters	*Control*	*CMF*	*Vitamin E*	*CMF + Vitamin E*
Na^+K^+ ATPase	9.1 ± 0.48	4.7 ± 0.23**	9.5 ± 0.57	8.3 ± 0.44*
$Ca^{2+}K^+$ ATPase	2.8 ± 0.16	1.5 ± 0.11**	2.9 ± 0.18	2.6 ± 0.16
Total ATPase	22.5 ± 1.8	13.1 ± 1.2**	23.1 ± 1.9	19.8 ± 1.7*
5' Nucleotidase	104.6 ± 0.04*	74.3 ± 6.9**	115.2 ± 10.3*	98.7 ± 9.2

The enzyme activities are expressed as μmoles of phosphorus liberated per min per mg protein. Values are compared to corresponding control groups. P values : ** < .001; * < .05

TABLE II : Levels of lipid peroxides, TSH group, cholesterol and phospholipid in intestinal BSM in control and experimental rats. Values are expressed as mean ± S.D.

Parameters	*Control*	*CMF*	*Vitamin E*	*CMF + Vitamin E*
Lipid peroxides (nmoles of MDA/mg protein)	7.8 ± 0.63	16.1 ± 1.24**	5.8 ± 0.48**	8.4 ± 0.81
TSH (μg/mg protein)	5.4 ± 0.42	2.6 ± 0.23**	5.8 ± 0.46	5.0 ± 0.39
Cholesterol (μmoles/mg protein)	0.610 ± 0.045	0.669± 0.053**	0.602 ± 0.048*	0.618 ± 0.050*
Phospholipid (μmoles/mg protein)	0.518 ± 0.047	0.375± 0.033**	0.503 ± 0.046	0.512 ± 0.044
Cholesterol/ Phospholipid ratio	1.178 ± 0.096	1.784± 0.091**	1.197 ± 0.104	1.207 ± 0.129

Values are compared to corresponding control groups. P values : ** <.001; * <.05

increase in lipid peroxidation on CMF treatment may also be due to the poor antioxidant system (17). The increased lipid peroxidation is responsible for the formation of the lipid hydroperoxides in membrane and would result in damage of the membrane structure and inactivation of membrane bound enzymes (18). The accumulation of lipid peroxides introduces hydrophilic moieties into the hydrophobic phase and thus alters membrane permeability and cell functions (19).

The metabolites of cyclophosphamide binds to the thiol groups and reduces the level of reduced glutathione, total thiol groups and activity of thiol containing enzymes. Nicotera et al (20) have associated the loss of critical protein sulphydryl groups with inactivation of Ca^{2+} dependent ATPase. Na^+K^+ dependent ATPase which is involved in the active transport was reported to contain essential sulphydryl groups.

Reduced glutathione and other sulphydryl groups protect the cell membrane against free radical attack. Free radical, produced in the membrane, rapidly react with α-tocopherol and cytosolic GSH (21). The total thiol content was well maintained in α-tocopherol co-administered rats.

Hence the observed decrease in the intestinal basolateral ATPase activities are due to the lipid peroxidative nature of the drug. This depletes the membrane sulphydryl groups (Table II). α-tocopherol has been reported to convert oxidised glutathione to reduced glutathione by glutathione reductase and thereby maintains the level of thiol groups in membrane.

CMF treatment resulted in a drastic change in membrane fluidity as a result of phospholipid degradation. Fluidity of the membrane is responsible for the functioning of membrane bound enzymes and various other transport processes. Molenaar et al (22) have demonstrated that Ca^{2+} ATPase requires phospholipid environment for its activity. Report shows that vitamin E stabilizes the lipid bilayers of artificial membrane through the interaction of it with arachidonic acid (23). The observed protection rendered by α-tocopherol on intestinal membrane is due to the effect of α-tocopherol on membrane lipid. This is further supported by the low level of lipid peroxides in Group 3 and Group 4 rats.

The observed defects in the basolateral membrane as a result of CMF administration is rectified by the administration of CMF supplemented with vitamin E.

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