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EFFECT OF METHYLENE BLUE ON OXIDATIVE STRESS AND ANTIOXIDANT DEFENCE PARAMETERS OF RAT HEPATIC AND RENAL TISSUES

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Abstract : The objective of the present study was to investigate the effect of administration of 1 mM methylene blue (MB) in drinking water for 30 days on hepatic and renal antioxidant status in female adult Wistar strain rats (n=5). MB failed to induce significant change in any of the measured antioxidant defence parameters namely, superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH). However, a marginally significant ($P<0.05$) increase in the level of lipid peroxidation (LPx) was recorded in liver, while a reduction ($P<0.05$) in its level in the kidney was noticed. Serum alanine amino transferase (AlaAT) and creatinine levels significantly ($P<0.001$) decreased in MB treated rats without any change in blood urea nitrogen (BUN) level. Our findings suggest that the effect of MB as administered in the present study was tissue specific with regard to the level of LPx, however, in general, it does not impair liver and kidney functions as evidenced by serum parameters.

Key words : methylene blue (MB), oxidative stress (OS), superoxide dismutasea (SOD), catalase (CAT), reduced glutathione (GSH), lipid peroxidation (LPx), alanine amino transferase (AlaAT), blood urea nitrogen (BUN), creatinine

INTRODUCTION

Methylene blue (MB), a redox dye, is clinically used in the treatment of various ailments such as septic shock (1), ifosfamide encephalopathy (2) and methaemoglobinaemia (3). MB is also known as an effective inhibitor of guanylate cyclase (4). Exposure to MB has been demonstrated to prevent ethanol-induced redox changes and fat accumulation in isolated hepatocytes (5) and HeLa cells (6). Effects of MB on human erythrocytes have been studied in detail under *in vitro* condition (7–9). Effect of MB with regard to oxidative stress however is controversial. *In vivo* effect of MB has been shown to reduce oxidative stress in kidney of ciclosporine A treated rats (10). On the contrary, MB at a concentration range of 5 µM has been shown to increase intracellular oxidant stress in cultured endothelial cells (11). Recently, the dye has been shown to delay cellular senescence and enhances key mitochondrial

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pathways in cell culture studies by decreasing oxidant production (12). Above controversies regarding the effect of MB on oxidative stress parameters emerged mainly due to the various systems used to study its effect, the dose and mode of application of the drug used in different studies. However, it is not clear how MB influences oxidative stress in tissues of rats. In the present study, effect of 1 mM MB in drinking water for 30 days was assessed on antioxidants like superoxide dismutase, catalase, and reduced glutathione and oxidant parameter like lipid peroxidation in liver and kidney of adult female rats.

METHODS

Chemicals

Methylene blue was obtained from E. Merck, Germany. Sodium dodecyl sulphate (SDS), Butylated hydroxyl toluene (BHT), Triton-X 100, Hydrogen peroxide (H_2O_2), L-methionine, Hydroxylamine Hydrochloride, N-(1-Napthylethylene diamine), and Sulfanilamide, 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB) were procured from SRL, India. Thiobarbuturic acid (TBA) and Bovine serum albumin (BSA) were purchased from Sigma Chemical Company, USA. Bradford reagent was obtained from Biogene Reagents Inc. USA. All other chemicals used were of the analytical grade.

Animals and treatments

Ten female adult rats of Wistar strain weighing about 200 g were used in the present experiments. The rats were divided into two groups each having five individuals. Group I served as control, and group II received MB at concentration of 1 mM in drinking water for 30 days. Animal care, maintenance and experiments were done under the supervision of the Institutional Animal Ethics Committee (IAEC) regulated by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Sample preparation

After recording the body weights rats were sacrificed by decapitation. Trunk blood was collected to obtain serum and the serum was stored at –20°C until measurement of alanine aminotrasferase (AlaAT), creatinine and blood urea nitrogen (BUN). Liver and kidney were dissected out immediately, cleaned in cold normal saline and stored at –80°C till further use.

A 10% homogenate of tissues were prepared in ice-cold phosphate buffer (50 mM, pH 7.4). Homogenate was centrifuged at 900 × g for 5 minutes at 4°C to separate nuclei and cellular debris and further centrifuged at 10,000 × g for 20 minutes at 4°C (13). Lipid peroxidation (14) and reduced glutathione (15) levels were measured in 900 × g supernatant fraction, while the activities of superoxide dismutase (16) and catalase (17) were measured in 10,000 × g supernatant fraction of samples. The activity of AlaAT in serum was determined by standard procedures using commercially available diagnostic laboratory test kit (Merck, India). Concentrations of BUN and creatinine were assessed using diagnostic laboratory test kit (Crest Biosystems, India).

Statistical analysis

Data are expressed as means ± SD. Students' unpaired *t* test was performed to find level of significance between groups ($P<0.05$ and $P<0.001$).

RESULTS

Administration of MB to rats in drinking water for 30 days did not affect activities of two principal antioxidant enzymes SOD and CAT of liver and kidney. Also the treatment failed to influence level of GSH in these organs. Interestingly, lipid peroxidation level of liver was augmented while that of the kidney decreased significantly ($P<0.05$) in response to MB indicating an organ specific effect of the drug. There were no change in the level of serum BUN, but serum concentration of creatinine and AlaAT decreased significantly ($P<0.001$) in MB treated group as compared to the control group (Table I).

TABLE I: Effect of methylene blue (1 mM in drinking water for 30 days) on liver and kidney of rat.

Parameters	*Groups*	
Body weight (g)	**Control**	**MB**
Initial	182±9.79	186±6.61
Final	186.2±9.68	181±6.63
Liver		
SOD (U/mg protein)	9.43±0.92	10.22±0.94
CAT (µkat/mg protein)	6.85±0.31	7.46±0.77
LPx (TBARS/mg protein)	0.66±0.09	0.82±0.08*
GSH (µmol/g protein)	4.39±0.33	4.64±0.15
Kidney		
SOD (U/mg protein)	10.15±0.82	10.95±0.89
CAT (µkat/mg protein	2.95±0.35	2.76±0.113
LPx (TBARS/mg protein)	2.29±0.009	2.07±0.1*
GSH (µmol/g protein)	2.21±0.51	2.78±0.5
Serum		
AlaAT (U/L)	26.97±3.94	15.66±2.29**
Creatinine(mg/dL)	0.414±0.02	0.313±0.026**
BUN (mg/dL)	19.44±1.51	18.00±0.12

Data were expressed as means±SD. *$P<0.05$; **$P<0.001$ as compared to controls.

DISCUSSION

MB potentially represents a new class of antioxidant drugs that competitively inhibit the reduction of molecular oxygen to superoxide by acting as an alternative electron acceptor for tissue oxidases (18). It can accept electrons from pyrimidine nucleotides and transfers them to oxygen non-enzymatically (19) and reduced MB inside the cell can be oxidized by molecular oxygen. It is also oxidized by reduction of Fe^{3+} in prosthetic groups of numerous cellular proteins and enzymes (20) and in due course can generate ROS. MB has been shown to prevent ethanol induced redox changes in isolated hepatocytes and HeLa cells (5, 6) and in rats fed with ethanol for long time (8).

Peter *et al.* (21) have reported that distribution of MB is much higher in liver than other organs after the oral administration of the drug in rats. MB at a concentration more than 5 µM has been shown to increase intracellular oxidant stress in endothelial cells (11). In liver of rats, MB has been shown to stimulate mitochondrial respiration (22). Therefore, high content of MB in liver and high metabolic activities of liver may be the reason for observed enhanced lipid peroxidation content in the present study. However, at this point, it is difficult to access the mechanism by which MB enhanced liver malondialdehyde content. The dose used in the present study is not hepatotoxic as evident by low serum alaline amino transaminase (AlaAT) activity.

MB is used to treat urolithiasis (23). Oxidative stress is implemented in several kidney pathophysiology including urolithiasis (24) and MB has been reported to protect kidney tissue from oxidative damage induced by Ciclosporin A treatment (10). The decrease in creatinine level in the serum and lipid peroxidation level in the kidney of MB treated rats also supports the above view. However, the activity of antioxidant enzyme SOD and CAT and level of GSH did not show any change after administration of MB for 30 days.

In conclusion, the present investigation indicates that MB has organ specific effect and is better in protecting kidney than liver.

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