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LETTER TO THE EDITOR

EFFECT OF INHIBITORS ON GLUTATHIONE METABOLIZING ENZYMES

Sir,

(Received on October 28, 2001)

Jaundice is observed during first week of life in nearly 60 percent of term infants and 80 per cent of preterm infants (1). Jaundice is observed in children with age group between 3 to 4 years though the infants have been not suffering for jaundice previously (2). Human erythrocyte are rich in glutathione and glutathione peroxidase (3). Studies have been done on the effect of $MgCl_2$ and $SnCl_2$ on the glutathione metabolizing enzymes.

Eight normal patients (mean age 3–6 years) and eight jaundiced patients were selected for our study attending out at the Department of Institute of Child Health, Calcutta. Whole blood was drawn by vein puncture, one part of which was collected in EDTA vial, the other part was allowed to clot separately. From EDTA vial plasma was separated by centrifugation at 2500 g for 10 min at 0°C. The packed red cells were washed thrice with isotonic saline, and the buffy coat was removed, washed erythrocytes were lysed by adding distilled water to it and allowing it to stand for 15 min at (0–4°C). This hemolysate was used as a source of enzyme in RBC. The activity of glutathione peroxidase was assayed according to the method of Pagila and Valentine (4). The assay system contained the enzyme, glutathione (reduced) glutathione reductase and NADPH in 50 mM phosphate buffer (pH–7.0) and sodium azide. The activity of glutathione reductase was assayed according to the method of Pinto and Bartley (5). The assay system contained the enzyme, oxidised glutathione, NADPH in 50 mM phosphate buffer (pH–7.4) containing 5 mM EDTA. The reaction was followed upto 3 min at 340 nm. The activity of glutathione-S-transferase was assayed according to the method of Warholm et. al. (6). *In vitro* study was carried out with magnessium chloride and stanus chloride. The inhibitor was directly added to the erythrocyte and then kept under incubation for different time intervals like 5, 10, 15 min and finally enzyme activity was estimated. Three different concentrations of each salt (1–10) nmol/dl was utilised. It was already found that $MgCl_2$ shows no inhibitory effect in *in vitro* conditions.

The results presented in Table I show control and patient values and inhibition of GSH-peroxidase, GSH-reductase and GSH-S-transferase by $SnCl_2$ accordingly. It is evident from Table I that at 5 nM concentration of inhibitor 20% inhibition of glu-peroxidase activity, 50% inhibition of glu-reductase activity and 25% inhibition of glu-s-transferase activity take place accordingly. At 10 nM concentration, 30% inhibition of glu-reductase, 25% inhibition of glu-peroxidase and 40% inhibition of glu-

TABLE I: Inhibitory activities of glutathione metabolizing enzymes values are means ± SD.

Source	*Inhibitor concentration (n mole)*	*GSH reductase (a)*	*GSH peroxidase (a)*	*Glu-s-transferase (b)*
Control (8)	–	5.3±0.35	13.3±0.07	4.1±0.3
	1	–	–	–
Patient (8)	5	2.8±0.25*	2.6±0.5	2.4±0.2
	10	1.59±0.17	3.9±0.31*	1.62±0.4*

(a) n mole of NADPH oxidised/min/mg of protein (b) Unit/mg protein

*P<0.001

s-transferase take place accordingly. The results of the present investigation clearly indicates the inhibition of antioxidant enzymes at a certain range of inhibitor concentration respectively in comparison to their adjacent control set. We had demonstrated earlier that the activity of glutathione peroxidase was reduced in erythrocyte of jaundiced children (2). It was explained that glutathione reductase activity had greatly increased in erythrocytes of jaundiced children to maintain steady state concentration of glutathione within the cell (7).

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REFERENCES

1. Behrman RE, Vanghen VC III, WE, Nelson WB, Saunders WB. Text Book of Paediatrices 14th edn., Philadelphia, USA, 1992: 476.
2. Pandit M, Mazumdar S, Sengupta D. Glutathione status in Paediatric Jaundice, *69th Annual Meeting SBC (1), Calcultta,* November 2000.
3. Rao Maddipati K, Gasparski C, Marnett LJ. Characterization of the Hydroperoxides-Reducing Activity of Human Plasma. *Arch Biochem Biophys* 254; 1987: 9–17.
4. Pagila DE, Valentine WN. Glutathione reductase assay. *J Lab Clin Med* 70; 1967: 158.
5. Pinto PE, Bartley W. *Biochem J* 112; 1969: 109.
6. Warholm M, Guthenberg C, Bahr CV, Mannervik B. Methods in Enzymol Glutathione Transferase from human liver. 1985; 113, p.499.
7. Majumder S, Sarkar U, Sengupta D. Jaundice in new born and erythrocyte and plasma antioxidant defence system. *Indian Journal of Experimental Biology* 1995; 33: 303–305.