INCREASE IN GLUTATHIONE PEROXIDASE ACTIVITY IN ERYTHROCYTES FROM TRISOMY 21 SUBJECTS

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SUMMARY. - Glutathione peroxidase activity has been measured in erythrocytes from normal subjects and from trisomy 21 patients. The latter cases show about 50 % increase of this enzyme similar to the increase observed for superoxide dismutase (erythrocuprein) suggesting either localisation of the gene for glutathione peroxidase on chromosome 21 (as is the case for erythrocuprein) or regulation of this enzyme by intracellular levels of $O_2^{\cdot}$, H_2O_2 or superoxide dismutase.

We have earlier described the increase of erythrocuprein (superoxide dismutase, E. C. 1. 15. 1. 1.) for which the gene is located on chromosome 21 (1, 2) in erythrocytes (3 -6) and platelets (7) from trisomy 21 patients compared with controls. As demonstrated in the pioneer work of McCord and Fridovich (8) this enzyme catalyses the dismutation of superoxide radicals to oxygen and H_2O_2.

As part of a programme of study of oxygen metabolism in trisomy 21 cases we have also examined the activity of erythrocyte glutathione peroxidase.

In the red blood cell, H_2O_2 produced biochemically or otherwise, can be eliminated by the action of various enzymes such as catalase and glutathione peroxidase (GPX). It is generally considered (9) that in erythrocytes,

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at low concentrations of H_2O_2 the second enzyme catalyses reduction of H_2O_2 preferentially by the reaction

$$2\ GSH + H_2O_2 \xrightarrow[GPX]{} GSSG + 2\ H_2O$$

Previous studies have shown that catalase activity in erythrocytes from cases of trisomy 21 is completely normal (5, 10). It was thus of interest to examine levels of glutathione peroxidase in such patients.

MATERIAL AND METHODS

Twelve trisomy 21 subjects free of congenital cardiopathy were compared with eighteen normal individuals of similar age, all older than three years.

Dosage of glutathione peroxidase (11) was by the modified method described by Gunzler (12) using t-butyl hydroperoxide as substrate. Oxidised glutathione (GSSG) produced by action of glutathione peroxidase and peroxide was reconverted to reduced glutathione (GSH) by glutathione reductase (GR) and nicotinamide adenine dinucleotide phosphate (NADPH). Decrease in concentration of NADPH was recorded at 340 nm in a Gilford 2400 spectrophotometer.

Blood was drawn by veinous puncture using heparin as anti-coagulant. The red cells were separated by low speed centrifugation and washed three times in 0.154 M NaCl, then lysed by freezing (at -70° C) and thawing. The lysates were adjusted by dilution with distilled water to a uniform concentration of 5 g hemoglobin per 100 ml (using the method of Drabkin (13) for estimation of hemoglobin) then mixed with an equal volume of a solution of 4×10^{-3} M potassium ferricyanide and 2×10^{-2} M KCN in 0.1 M phosphate buffer pH 7.0.

The incubation mixture (3 ml) maintained at 37° C contained 5×10^{-2} M phosphate pH 7.0, 2×10^{-4} M NADPH, 10^{-3} M reduced glutathione, 2 units of yeast glutathione reductase (Sigma type III) and 0.1 ml of the above erythrocyte lysate mixture. After 10 min pre-incubation, the

reaction was initiated by addition of t-butyl hydroperoxide (to a final concentration of 10^{-3} M). Kinetics of oxidation of NADPH were calculated using a molar extinction coefficient for NADPH of 6.22×10^{3} at 340 nm. The slow oxidation of NADPH observed before addition of t-butyl hydroperoxide was substracted from the values obtained in presence of the peroxide.

As control, the same technique was used throughout except that the erythrocyte lysate mixture was replaced by an equivalent amount of hemoglobin free of glutathione peroxidase, obtained by treatment of a red blood cell lysate in 3×10^{-3} M phosphate buffer pH 7.0 with DEAE-Sephadex A-50 to absorb the peroxidase. This blank was then substracted from the rates observed in presence of the various samples.

RESULTS AND DISCUSSION

The results are shown in figure 1, expressed as μMoles of NADPH consumed per min per mg of hemoglobin. Athough activities are relative, the technique described above, with a molar ratio of K_3FeCN_6 : KCN : heme of

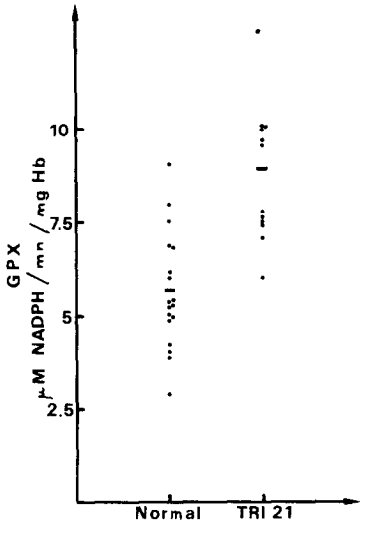

FIGURE 1

Glutathione peroxidase activity in normal and trisomy 21 subjects.

1.2 : 6 : 1 and a corresponding heme control (see methods) gives a relatively reliable estimate of glutathione peroxidase activity in erythrocytes (12).

The difference between average levels of glutathione peroxidase in the two populations is highly significant (table I) and the ratio of the averages for trisomy 21 compared with normal subjects is 1.55, closely similar to that observed (3, 5) in the case of determinations of erythrocuprein (SOD - 1).

In contrast with the clear cut difference in distribution for erythrocuprein, the levels of glutathione peroxidase activity for trisomy 21 cases and normal subjects partially overlap. It is not excluded that the distribution of glutathione peroxidase values observed with trisomy 21 is bimodal (see fig. 1).

Two hypotheses may be presented at the moment to explain the concurrent increase of activity to the same extent for glutathione peroxidase and erythrocuprein. The first implies that the genes for expression of both proteins are located on chromosome 21 (as is the case for erythrocuprein) while the second invokes participation of intracellular $O_2^{\cdot-}$ or H_2O_2, or of superoxide dismutase in the regulation of erythrocyte glutathione peroxidase

TABLE I

Comparison of glutathione peroxidase activity in erythrocytes from normal subjects and trisomy 21 patients.

	Number of subjects	μM NADPH/min/mg Hb			P
		Average	Standard deviation	Standard deviation of the mean	
Normal	18	5.730	1.544	0.364	< 0.001
Trisomy 21	12	8.893	1.907	0.551	

activity. With respect to the effect of superoxide dismutase, it is to be noted that this enzyme can in fact increase intracellular levels of H_2O_2 by catalysed dismutation of $O_2^{\cdot-}$ as opposed, not to spontaneous dismutation (which gives the same quantity of H_2O_2), but to elimination of $O_2^{\cdot-}$ by oxidative processes or by diffusion (given the relatively long life time of $O_2^{\cdot-}$ and the kinetics of dismutation by superoxide dismutase).

Apart from direct roles in the metabolism of activated oxygen derivatives played by these two enzymes, glutathione peroxidase forms a link between destruction of H_2O_2 and the hexose monophosphate metabolic pathway via glutathione reductase and NADPH. Thus the often contradictory observations of glucose metabolism (14, 15) in cases of trisomy 21 could well be reconsidered in the light of the results presented in this communication.

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