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SUPEROXIDE DISMUTASE, GLUTATHIONE PEROXIDASE AND CATALASE IN OXIDATIVE HEMOLYSIS. A STUDY OF FANCONI'S ANEMIA ERYTHROCYTES.

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Summary. Superoxide dismutase, glutathione peroxidase and catalase were assayed in the erythrocytes of three patients of Fanconi's anemia. Superoxide dismutase was found to be significantly decreased, as previously reported. The enzymes metabolizing H_2O_2 are normal (glutathione peroxidase in the higher limits of the normal value). The abnormal erythrocytes were found to be as resistant (perhaps more resistant) as normal red blood cells to oxidative hemolysis induced by drugs. Malonyl dialdehyde production was found to be comparable to that of normal erythrocytes. It is concluded that a significant (30-40%) deficiency of superoxide dismutase, when associated to normal values of H_2O_2-removing enzymes, does not affect the antioxidative defense capability of erythrocytes, even in conditions of augmented oxidative injury.

Fanconi's anemia (FA) is an autosomal recessive disease characterized by a high frequency of chromosomal aberrations in cultured lymphocytes (1), apparently associated with defect in the DNA repair process. It has recently been reported that the frequency of chromosomal aberrations in FA lymphocyte cultures is positively related to oxygen tension (2), thus suggesting that the site primarily affected by the FA mutation is some component of the cellular system of defense against oxygen cytotoxicity. On the other hand, one enzyme of this system, namely superoxide dismutase, has been reported to be deficient in the FA erythrocytes (3). Therefore FA red blood cells can provide information on the role of superoxide dismutase in the protection of erythrocytes against oxidative injury. In this context it would be important to know whether other enzymes involved in the "antioxygenic" defense, such as glutathione peroxidase and catalase, are altered in FA erythrocytes. A preliminary investigation (4) has shown that a number of glycolytic

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TABLE I

Specific activity of superoxide dismutase, glutathione peroxidase and catalase in FA erythrocytes

	Superoxide dismutase		Glutathione peroxidase		Catalase	
	μg/mg Hb	mg/U LDH	(U/mg Hb)x10^5	U/U LDH	(U/mg Hb)x10^5	U/U LDH
P.(1) (12-year-old male)	0.28	11.8	1.47	0.62	15.7	6.6
R. (12-year-old male)	0.22	10.0	1.57	0.70	12.5	5.6
S.(1) (9-year-old female)	0.38	14.2	2.77	1.04	15.7	5.9
P.(2) (12-year-old male)	0.31	12.0	1.56	0.61	11.8	4.6
S.(2) (9-year-old female)	0.35	10.9	2.85	0.94	12.6	3.9
rmal (n = 20)	0.37±0.04	19.0±4.5	1.40±0.39	0.71±0.20	13.4±4.3	6.8±2.3

e subscripts (1 and 2) refer to separate assays of the same patient (5 months interval).

enzyme activities are high in FA erythrocytes. We report here that FA erythrocytes show a normal, if not higher resistance, to oxidative damage as tested by a number of parameters. This behavior may be related to the finding that the other "antioxygenic" enzymes are not deficient in FA erythrocytes, and glutathione peroxidase, in particular, is in the higher limit of normal values.

MATERIALS AND METHODS

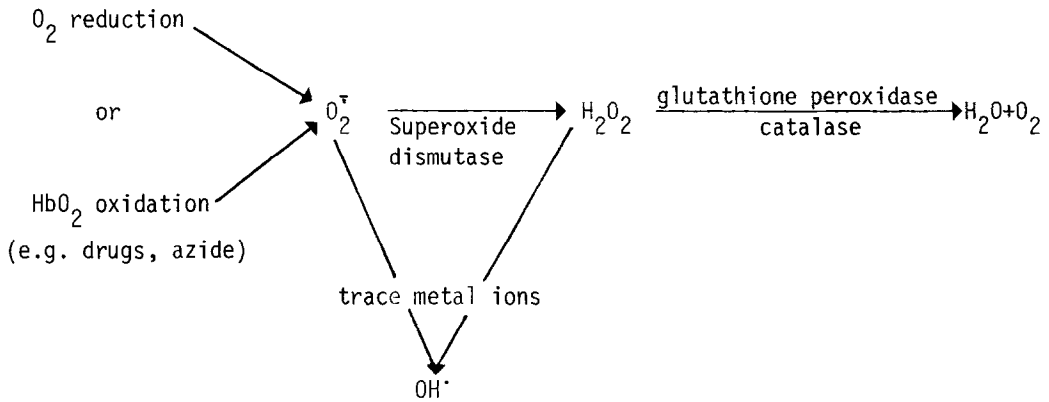
Erythrocytes were separated from freshly drawn blood, washed three times in isotonic NaCl and lysed by fourfold dilution with H_2O, followed by repeated freezing-thawing cycles. Superoxide dismutase was determined by polarography (5), glutathione peroxidase, catalase and lactate dehydrogenase spectrophotometrically (6-8). Hemoglobin was determined as the cyano-met derivative (9). Malonyldiadehyde (MDA) was measured by the colorimetric procedure involving reaction with thiobarbituric acid after oxidative challenge of erythrocytes with azide and H_2O_2 as previously described (10). Drug-induced hemolysis was followed in the presence of acetylphenylhydrazine and 1,4-naphthoquinone-2-sulfonate as previously described (11,12). Percent hemolysis was expressed as $\frac{100 \times Hb}{Hb_{100\%}}$, where Hb is the hemoglobin content of the supernatant of the red cell suspensions after centrifugation; $Hb_{100\%}$ is the total hemoglobin content of the cells as evaluated after complete hemolysis in H_2O. FA erythrocytes were assayed at least one month after last transfusion, when transfused cells do not exceed 10% of the total cell population.

RESULTS AND DISCUSSION

Assays of superoxide dismutase, glutathione peroxidase and catalase in FA erythrocytes (Table I) show that in the three patients examined superoxide dismutase is actually less than in normal individuals and that the decrease amounts to 20-30%, as previously reported (2). The deficiency is even more significant ($\simeq$ 40%) when referred to lactate dehydrogenase (LDH), an enzyme reported to be unaltered

in FA patients (4). Glutathione peroxidase and catalase were found to be within the normal values although the former enzyme activity was always in the higher limit

The relevance of these results to possible damaging effects by partially reduced forms of oxygen, that is $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, is better understood by considering the relation of these molecules in the process of biological oxygen activation, which is reported in the following scheme:



According to this scheme redox drugs and nucleophiles that oxidize hemoglobin can generate $O_2^{\cdot-}$ and H_2O_2 in erythrocytes (13). An elevated superoxide dismutase coupled with deficient glutathione peroxidase and catalase activities may build up H_2O_2 in cells, with consequent oxidative damage to membranes (14) and other cell components. On the contrary deficient superoxide dismutase coupled with normal H_2O_2-removing enzyme activities should not affect the defense capabilities of FA erythrocytes against oxidative stress, also in view of the fact that a proper supply of GSH is maintained by the normal activities of glutathione reductase and glucose-6-phosphate dehydrogenase in FA red blood cells (4). To test this hypothesis, erythrocytes from the FA patients and from normal individuals were exposed to two drugs known to interact with oxyhaemoglobin giving $O_2^{\cdot-}$ and H_2O_2 with consequent hemolysis (12, 15) although the detailed mechanism of action may differ for each drug. The FA patients resulted to be as resistant as the normal cells, and in the case of acetylphenylhydrazine more resistant, to the hemolytic action of the drugs (Fig. 1, A,B). Oxidative hemolysis is known to be associated to increased concentrations of a secondary product of peroxides of membrane lipids, MDA. This is, for example, the case of thalassemia major (10). In this disease, red blood cells contain unstable monomeric hemoglobin chains, which produce $O_2^{\cdot-}$ much more easily than the tetrameric molecule (16). This situation is considered to be a primary factor for the increased membrane peroxidation leading to hemolysis. MDA

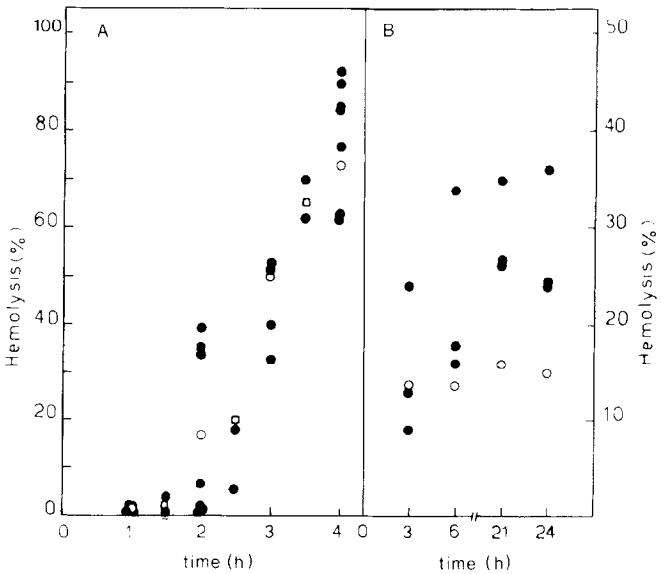


Fig. 1. Effect of hemolytic drugs on FA and normal erythrocytes. Panel A: 1,4-naphtoquinone-2-sulfonate; ●: normal controls; ○: FA patient D.S.(2); □: FA patient C.P.(2). Panel B: acetylphenylhydrazine; ●: normal controls; ○: FA patient D.S.(1).

production by FA red blood cells (Table II) was the same as by normal cells, in contrast with the approximate tenfold increase in thalassemia (10).

CONCLUSIONS

Activities of "antioxygenic" enzymes in FA red blood cells provide further elements to current theories on the cellular systems of protection against oxygen toxicity. In particular our results indicate that even a 40% decrease of supero-

TABLE II

MDA production by FA erythrocytes upon treatment with azide and hydrogen peroxide (10).

	2 hr MDA (nmol/g Hb)	Ref.
FA patients		
C.P.(1)	87	This work
D.S.(2)	137	" "
Normal variations	138±71	10
Untransfused thalassemia major patients (n=14)	967±229	10

xide dismutase is not deleterious to the "antioxygenic" defense of erythrocytes. It is reasonable that the relatively normal, and even higher than normal, activity of enzymes removing H_2O_2 plays a role in the increased resistance to oxidative stresses displayed in some circumstances by FA erythrocytes. This would confirm the prominent role in the "antioxygenic" cell response of the ratio between superoxide dismutase and the enzymes removing H_2O_2 rather than of any enzyme specifically acting on a single "oxygen radical" (17). Therefore the oxygen-mediated damage observed in other cells of FA patients (2) should be associated with other conditions than those present in erythrocytes. Work is in progress to elucidate this point.

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