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INCREASED RATE OF SUPEROXIDE ION GENERATION IN FANCONI ANEMIA ERYTHROCYTES

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The rate of generation of superoxide ion, the concentration of Cu, Zn superoxide dismutase and the hematalogical parameters were measured in red blood cells obtained from Fanconi anemia patients and from healthy individuals. No significative difference in the superoxide dismutase concentration was found, while the rate of generation of the superoxide ion doubled in Fanconi anemia patients. The steady-state concentration of the superoxide ion was calculated from these data and was found to be 2.3 times higher in Fanconi anemia erythrocytes than in controls. The possible consequences with respect to the alterations in FA are discussed.

FA is an autosomal recessive disease characterized by chromosomal instability, pancytopenia, elevated incidence of cancer and a variety of clinical symptoms (1). Several arguments suggest that the primary defect in FA could be in the repair system for DNA interstrand crosslinks (2). However, the presence of an abnormality in DNA repair remains controversal (3, 4). Studies on red blood and nucleated cells point to additional causes for FA such as defects of the nucleotide metabolism (5-8), an abnormal intracellular distribution of topoisomerase I (9, 10), and alteration of oxygen metabolism (11-14). In particular some of these studies indicate that the defect of FA cells could be explained by a diminished defence against some DNA damaging agents which are spontaneously produced. However this idea has been supported only by indirect evidence and the possibility

Abbreviations: FA, Fanconi Anemia; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume, SOD, superoxide dismutase.

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of an abnormality in the free radicals scavenging system and/or an increased production of oxygen reactive species in FA cells remain to be proved. Since we obtained experimental evidence that superoxide ion, O_2^-, is continuously generated in red blood cells of healthy individuals (15) we studied oxygen metabolism in FA erythrocytes, measuring both the O_2^- production and the activity of Cu, Zn superoxide dismutase, which is assumed to be one of the cellular defences against oxygen toxicity since it efficiently scavenges O_2^- (16).

MATERIALS AND METHODS

FA patients. The diagnosis of FA was confirmed cytogenetically for all the 5 patients (2 females and 3 males) included in this study. Case reports and cytogenic details were described previously (17-18). The patient ages ranged from 16 to 22 years. Hematological data showed normal MCHC values, while MCV ranged from 94 to 112 fl (normal 87 5 fl) and MCH from 20.2 to 40.5 pg (normal 29 2 pg). The percentage of reticulocytes was ranging from 0.8% to 1.4%.

Biochemical studies. Blood was obtained by a venipuncture from the 5 FA patients and 7 healthy subjects (controls). Heparin 15 units ml^{-1} was used as anticoagulant. All specimens were mantained in ice and processed within 24 hours from collection. The white blood cells and platelets were removed by filtration according to Beutler et al. (19).

Bovine Cu Zn superoxide dismutase was kindly supplied by Dr. Loschen (Grunenthal, GmbH Achen). The concentration of superoxide dismutase was calculated from activity measurements carried out, according to the catalytic current method, by an Amel 461 polarographic unit (20), while superoxide production was measured, as described by Scarpa et al. (15), utilizing a Brucker ER 200 D EPR spectrometer.

RESULTS AND DISCUSSION

FA patients were examined for hematological data, superoxide dismutase concentration and O_2^- flux (Ro), over an 8 month period. In particular superoxide dismutase concentration and O_2^- fluxes were measured in lysates of packed erythrocytes separated from white blood cells. The lysates were obtained by addition of Triton X 100, to a final concentration of 0.5%, to packed erythrocytes.

The hematological parameters for the FA patients and for the controls are reported in Table 1, while the concentration of superoxide dismutase and the Ro values expressed per g of Hb are reported in Table 2.

TABLE 1

Hematological parameters of Fanconi's anemia patients and of healthy individuals: A statistical analysis

Variable		Classes		Mean differences		Variance analysis	
		FA	Control	$\frac{\bar{X}_{FA}-\bar{X}_C}{\bar{X}_C}$ %	P.L.[b]	$\frac{(MS)f.cl.}{(MS)w.cl.}$[a]	P.L.[b]
hematocrit	N[c]	14	5	---	---	---	---
%	R	18.3-47.3	37.5-47.4	---	---	110/76.9	n.s.[d]
	$\bar{X}$	31.7	41.2	-30.0	0.90	---	---
RBC	N	14	6	---	---	---	---
cells μl^{-1}	R	1.94-5.25	3.92-5.35	---	---	14.5/0.46	0.995
x 10^6	$\bar{X}$	3.37	4.50	-25.1	0.995	---	---
$[Hb]_{blood}$	N	14	6	---	---	---	---
	R	64-176	126-158	---	---	3384/863	0.95
g l^{-1}	$\bar{X}$	109.8	137.8	-20.3	0.90	---	---
[Hb]	N	14	6	---	---	---	---
g l^{-1}	R	327-377	331-338	---	---	591/202	n.s.
	$\bar{X}$	348	335	+3.8	0.90	---	---

7 healthy individuals (control) and 5 Fanconi anemia patients (FA) were examined. Three different sampling were obtained from FA, over a period of 8 months. Where, it is not specified, the data are referred to the red blood cells volume.
a) (MS)f.cl. = mean square for classes.
(MS)w.cl. = mean square within classes.
b) P.L. = probability level.
c) N = size of the samples, R = values range, $\bar{X}$ = mean of the values.
d) n.s. = not significative.

Since from an inspection of these data it appeared that the Hb concentration in the blood is on the average lower in FA patients than in controls, the superoxide dismutase concentration and the O_2^- production were also expressed per liter of packed red cells. The latter data are listed in the last rows of Table 2, together with the Ro/[superoxide dismutase] ratio and the superoxide dismutase concentration in the whole blood. The comparison of the means, their probability level together with the analysis of variance are reported in the last columns of Table 1 and Table 2.

TABLE 2

Superoxide dismutase (SOD) concentration and rate of O_2^- production (Ro) in the blood of Fanconi's anemia patients and of healthy individuals: A statistical analysis

Variable		Classes		Mean differences		Variance analysis	
		FA	Control	$\frac{\bar{X}_{FA}-\bar{X}_C}{\bar{X}_C}$ %	P.L.[b]	$\frac{(MS)f.cl.}{(MS)w.cl.}$[a]	P.L.[b]
[SOD]/[Hb]	N[c]	15	7	---	---	---	---
mole g^{-1}	R	4.25-8.50	6.03-9.59	---	---	9.50/1.48	0.990
x 10^9	$\bar{X}$	6.30	7.65	-21.4	0.98	---	---
Ro/[Hb]	N	15	7	---	---	---	---
M $sec^{-1}g^{-1}$	R	4.27-11.7	2.48-5.34	---	---	57.7/6.99	0.995
x 10^{11}	$\bar{X}$	7.34	3.87	+90.0	0.99	---	---
[SOD]	N	13	6	---	---	---	---
M	R	1.46-3.01	2.03-3.14	---	---	0.04/0.19	n.s.[d]
x 10^6	$\bar{X}$	2.19	2.35	-3.4	n.s.	---	---
Ro	N	13	5	---	---	---	---
M sec^{-1}	R	1.56-5.80	0.82-1.80	---	---	6.12/1.04	0.975
x 10^8	$\bar{X}$	2.60	1.21	+108	0.95	---	---
[SOD]	N	14	6	---	---	---	---
M ^{blood}	R	0.27-1.16	0.82-1.49	---	---	0.30/0.05	0.975
x 10^6	$\bar{X}$	0.70	0.98	-27.5	0.975	---	---
Ro/[SOD]	N	15	7	---	---	---	---
sec^{-1}	R	0.60-2.36	0.28-0.89	---	---	2.01/0.23	0.995
x 10^{-2}	$\bar{X}$	1.20	0.50	+129	0.99	---	---

Samplings and statistical analysis as in Table 1.

The analysis of the experimental data suggests the following conclusions:

i) the hemoglobin content of the blood of FA patients is in average lower (about 20%) than that of healthy individuals, while the Hb concentration per liter of packed red blood cells is slightly higher.

ii) The production of O_2^- in the red blood cells, expressed as mole of O_2^- per liter of packed red blood cells per sec, appears twice as high in FA patients than in controls.

iii) The superoxide dismutase content expressed per g of Hb appears, in average, lower in FA patients (about 20%). This is in accordance with already reported results (12, 21). However, if the superoxide dismutase is effective only in scavenging the O_2^- produced inside the erythrocytes, the concentration of this enzyme inside the red blood cells must be taken into account. In this case, according to the steady-state hypothesis, the O_2^- concentration is given by:

$$[O_2^-] = \frac{Ro}{k\ [SOD]} \qquad (1)$$

where SOD is the superoxide dismutase concentration in the erythrocytes and $k = 2.3 \times 10^9\ M^{-1} sec^{-1}$ (22, 23), is the kinetic rate constant of dismutation of O_2^- by the enzyme. Since the superoxide dismutase concentration in the erythrocytes of FA and of controls is practically the same, as shown in Table 2, according to the equation 1, no superoxide dismutase effect should be present in FA. This result appears in contrast with the conclusions of the authors claiming that superoxide dismutase scavenging effect is defective in FA cells (12, 21). However it must be noted that superoxide dismutase is defective in FA if this enzyme, which is located inside the red blood cells, reacts also with the extracellular O_2^-. This possibility has been considered by some authors but it has not been clearly demonstrated yet (24). In this case we must take into account the superoxide dismutase concentration in the whole blood, which, according to Table 2, is significantly lower (about 20%) in FA patients.

iiii) The Ro/[SOD] ratio, is in average higher, by a factor of 2.3, in FA than in controls. This result appears very interesting, since on the basis of eqn. 1, the steady-state O_2^- concentration doubles in Fanconi erythrocytes.

This study has shown a hiterto unrecognized aspect of FA cell metabolism, that is an increased production of superoxide ion in red blood cells. If this result is confirmed in the nucleated cells, the hypothesis that the chromosomal aberrations are generated by activated oxygen species will receive direct support. Finally, since no evidence is available for obvious hemolysis in FA anemia (25) it appears that an increased endogenous O_2^- production and, as a consequence, a higher O_2^- steady-state concentration, does not result in a damage of the membrane. This observation should

be taken in consideration in the debate on O_2^- toxicity in different cell components.

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