SUPEROXIDE DISMUTASE ACTIVITIES OF BLOOD PLATELETS IN TRISOMY 21

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SUMMARY. - Mitochondrial and cytoplasmic superoxide dismutase activities have been determined in blood platelets from normal subjects and from trisomy 21 patients. The cytoplasmic enzyme (erythrocuprein) shows the same increase of 50 % in trisomy 21 compared with controls, previously noted in erythrocytes, whereas the mitochondrial manganese containing enzyme is significantly decreased by one third in platelets from cases of trisomy 21. The biological significance of these results is discussed.

We have previously reported an increase of human erythrocyte superoxide dismutase activity (SOD) (EC. 1.15.1.1.) in trisomy 21 (1). This result has since been confirmed in several other laboratories (2, 3). This cytoplasmic enzyme (SOD - 1) containing copper and zinc, is present in all human cells (4, 5) and catalyses the dismutation of superoxide radicals according to the reaction : $O_2^{\cdot-} + O_2^{\cdot-} + 2\,H^+ \longrightarrow H_2O_2 + O_2$ (6). Because the SOD - 1 gene has been located on chromosome 21 (7, 8), this finding supports the hypothesis that the increase of the erythrocyte SOD in trisomy 21 is due to a direct gene-dosage effect. However, in cells other than erythrocytes, there exists another type of SOD, a mitochondrial enzyme (SOD - 2) containing manganese (4, 5, 9) for which the gene has been sited on chromosome 6 (10). The cuprozinc enzyme is inhibited by cyanide whereas the manganoenzyme is not (5, 9). These properties allow measurement of the two types of SOD in cell extracts.

In quest of a relationship between gene dosage and gene expression, we have measured the SOD - 1 and SOD - 2 activities in platelets from nine normal subjects and eleven trisomic 21 patients of similar age.

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MATERIAL AND METHODS

The platelets from 5 ml of venous blood were prepared as described by Pinot et al. (11). The platelet pellet was suspended in 1 ml of 10 mM phosphate buffer, pH 7, 2, containing 0,14 M NaCl, and was frozen at - 70° C. After thawing, lysis of the platelets and the mitochondria was completed as follows : one volume of 1 % (v/v) "Triton X-100" was added to ten volumes of platelet suspension and gently shaken at 4° C for 2 hours. After centrifugation (15, 000 g) the supernatant was used for the different assays. Protein concentration was determined by the Lowry method (12). The SOD activities were measured by an indirect method, previously described (13, 14) where the following principle is used : inhibition of the chemiluminescent reaction produced in the system oxygen-hypoxanthine-xanthine oxidase-luminol, in which SOD in competition with luminol for superoxide anions generated by the xanthine oxidase decreases light emission. The assay of the SOD activity, slightly modified, is carried out in 0. 1 M glycine-NaOH buffer pH 9, containing 100 μM EDTA, 10 μM luminol, 10 μM hypoxanthine and suitable aliquots of cell extracts (final volume 3 ml). The reaction is initiated by injection of 15 μl of xanthine oxidase (Boehringer) (1 mg/ml). Under these conditions 50 % inhibition of maximal light intensity is considered as corresponding to one unit of SOD activity. The SOD activity was measured in absence and in presence of 2 mM cyanide added to the reaction mixture in order to inhibit any cuprozinc enzyme activity. SOD - 1 activity was obtained by the difference between the two assays.

For gel electrophoresis, organic treatment of crude extracts was also carried out. The lysates were mixed with cold ethanol-chloroform (0. 25 and 0. 15 volumes) and the heavy precipitate which occurs was removed by centrifugation (15, 000 g) and the supernatant then used for electrophoresis.

Staining for SOD activity after polyacrylamide gel electrophoresis was done according to the technique of Beauchamp and Fridovich (15) modified by Salin and McCord (5).

RESULTS AND DISCUSSION

The results of activity stained gel electrophoresis or crude extracts of human platelets are presented in figure 1. An identical profile

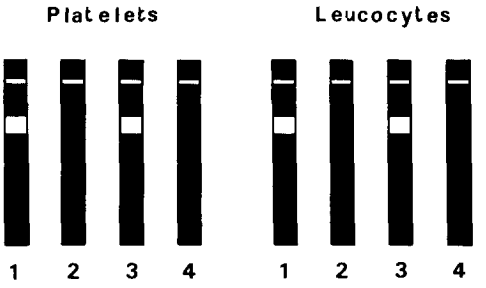

Fig. 1: Results of polyacrylamide gel electrophoresis stained for superoxide dismutase activity from human platelets and leucocytes. Electrophoresis was performed in 7.5 % acrylamide gels with Tris-glycine buffer, pH 8.5 and a constant current of 4 mA per gel. Human leucocytes were isolated by the procedure of Pinot et al. (11) ; lysis and organic solvent treatment was carried out as described for the platelets.

Crude extracts 1) without cyanide 2) with 10^{-3} M cyanide : extracts after organic solvent treatment 3) without cyanide 4) with 10^{-3} M cyanide.

Table 1

	SOD activities in units/mg protein (mean ± standard deviation)		P	Ratio : $\frac{\text{Trisomy 21}}{\text{Controls}}$
	Controls n = 9	Trisomic 21 patients n = 11		
Total SOD	46.73 ± 11.5	67.10 ± 13.52	< 0.01	1.44
SOD - 2 (mitochondrial)	6.44 ± 1.65	4.31 ± 1.70	< 0.02	0.67
SOD - 1 (cytoplasmic) = Total SOD minus SOD - 2	40.30 ± 10.90	62.79 ± 12.93	< 0.001	1.56

SOD activities in platelets from normal controls and trisomic 21 patients.

is obtained with platelets from normal or trisomic 21 subjects. Two distinct bands are present and have Rf values corresponding to those of cytoplasmic and mitochondrial SOD from human leukocytes (5). In addition, we found that all the SOD activity is fully retained after the organic solvent treatment, both enzymes being resistant to chloroform-ethanol. Human mitochondrial SOD thus appears to be different from other mitochondrial SODs, such as the chicken liver enzyme, which are definitely destroyed by such treatment (9). As shown in figure 1, cyanide inhibition is observed for the cytoplasmic enzyme.

The table shows the results of estimations of SOD activity in platelets from controls and from trisomy 21 patients. The cytoplasmic SOD activities are significantly increased in platelets of trisomic 21 patients. This finding might be related to modifications of platelet properties in trisomy 21 such as their density (16) and perhaps their life span. Thus, in leucocytes which have a modified life span in trisomy 21, enzymes such as alkaline phosphatase and even glucose-6-phosphate dehydrogenase which is sited on the X-chromosome, are increased (17). However, the levels of these enzymes are unaltered in the platelets (17). On the other hand, the ratio of the average values of trisomic 21 SOD - 1/control SOD - 1 is 1.56, which is quite comparable with the ratio observed in erythrocytes, cells in which no differences in maturity characteristics have been discerned between normal subjects and trisomy 21 patients (18, 19). Thus, in platelets as in the erythrocytes, the increase of the SOD - 1 activity in trisomy 21 is most likely due to a direct gene-dosage effect.

The mitochondrial SOD - 2 activity is significantly decreased in platelets from trisomic 21 subjects. The analysis of this finding is made difficult by the lack of reports relating to the properties of mitochondria in trisomy 21. Only the platelet mono-amine oxidase has been studied (20, 21, 22) but the results are conflicting, and depend on the type of substrates used for assays. Yet these data, which may appear surprising because the SOD - 2 gene is located on chromosome 6, raise the hypothesis of a possible regulation of this enzyme by the intra-cytoplasmic levels of SOD - 1 and/or superoxide anions. Moreover, an increase in the superoxide radical concentration in mitochondria could well occur as a consequence of the reduced SOD - 2

level. In view of the highly reactive and toxic properties of these radicals (23, 24), this might be the origin of damage in the mitochondria and consequently in cell function in trisomy 21.

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