FANCONI'S ANAEMIA: CASE HISTORY OF SIX SPANISH FAMILIES

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FANCONIHO ANÉMIA: KAZUISTIKA ŠIESTICH ŠPANIELSKYCH RODÍN

We report on the results obtained in 6 Fanconi's anaemia families (FA) (parents, brothers and sisters) affected by at least one of the symptoms usually observed in FA. The 6 FA families were studied from 1974 to 1990, all having located in Madrid (Spain) but with different ethnic origin: 3 families are of Spanish descent and the other 3 are gipsy families. All showed characteristics of the disease, including malformations, stunted growth, microcephaly, skin hyperpigmentation, high incidence of chromosomal breaks in lymphocyte cultures, and hematological and biochemical abnormalities: pancytopeny, increased fetal hemoglobin levels and significantly decreased superoxide dismutase (SOD) activity. *(Ref. 17.)*

Key words: ethnic origin, Fanconi's anaemia, fetal hemoglobin, superoxide dismutase.

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Uvádzame výsledky získané od šiestich FA pacientov a ich rodín (rodičia, bratia a sestry) psotihnutých aspoň jedným zo symptómov obyčajne pozorovaných v prípadoch Fanconiho anémie (FA). Štúdia bola vykonaná v období rokov 1974—1990 a zahrnula šesť rodín, všetky z Madridu (Španielsko), ale s rôznym etnickým pôvodom: tri rodiny sú španielskeho a tri rómskeho pôvodu. Všetci pacienti preukazovali charakteristiky ochorenia, vrátane malformácií, zakrpateného vzrastu, mikrocefálie, kožnej hyperpigmentácie, vysokým výskytom chromozomálnych zlomov v kultúrach lymfocytov, hematologických a biochemických abnormalít: pancytopénia, zvýšené hodnoty fetálneho hemoglobínu a významne znížená aktivita superoxiddismutázy. *(Lit. 17.)*

Kľúčové slová: etnický pôvod, Fanconiho anémia, fetálny hemoglobín, superoxiddismutáza.

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The autosomal recessive disorders Fanconi's Anaemia (FA) is characterized by progressive insufficiency of the bone marrow (Fanconi, 1927) in combination with congenital malformations, as skeletal and kidney deformities, skin hyperpigmentation, mental retardation, microcephaly, impaired growth, short stature, dwarfism, proneness to leukemia and the other malignancies (Lambert, 1982). Chromosome breakage is characteristic: gaps, breaks, and fragments (Schroeder et al., 1974; Schroeder et. al., 1974) and DNA is sepair is senously impaired (Poon et al., 1974; Hirsch-Kauffmann et al., 1978), although the enzymatic anomalies remain unclear. However, an alternative possibility should be considered: a deficiency of superoxide dismutase (SOD) activity be an alternative explanation for the increased risk of chromosomal anomalies borne by the patient with FA. Norderson's observation (Norderson, 1977) that FA cells show less chromosomal aberrations when cultured in presence of added SOD and/or catalase points in this direction.

Superoxide radical and other activated oxygen species (singlet excited oxygen, hydroxyl radicals and hydrogen peroxide) are generated in probably all aerobically living cells (Fridovich, 1975; Halliwell, 1974). These forms of oxygen are highly reactive and capable of damaging cellular components, e.g. they may initiate lipid peroxidation or cause inactivation of enzymes. Cells are protected against superoxide radicals by superoxide dismutase (E.C.1.15.1.1.) which specifically scavenge these radicals. In the erythrocyte a continous background of superoxide radicals is supposedly produced by the oxyhemoglobin auto-oxidation to methemoglobin (Carrel et al., 1975). The erythrocyte components at risk, i.e. hemoglobin and the cytoplasmic membrane, are protected by superoxide dismutase.

We report on the results obtained in 6 FA patients and their families (parents, brothers and sisters) affected by at least one of the symptoms usually observed in FA.

Material and methods

The 6 FA patients and their families (parents, brothers and sisters) were studied from 1974 to 1990. The patients who was diagnosed as FA by hematological (pancytopeny) and cytogenetic studies which showed high chromosomal instability (25 %) with

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structural aberrations (gaps, breaks, acentrics, etc) and triradials forms. The brothers and the sisters also showed high chromosomal instability (11 %) with structural aberrations, while the parents had a normal karyotype.

Blood samples were obtained by venipuncture. Fresh blood (5 ml) was collected in tubes containing lithium heparin and stored cold (0—4 °C). All assays were carried out within 24 h of sampling.

The superoxide dismutase (SOD) activity assay was performed with the method of Minami and Yoshikawa (1979). This assay is based on the inhibition by superoxide dismutase of nitroblue tetrazolium (NBT) reduction produced by the superoxide radical generated in the pyrogallol autoxidation. The rate of the superoxide reaction by SOD was calculated according to the definition of McCord and Fridovich (1969).

Alkali-resistant (F) hemoglobin was determined by the method of Singer et al. (1951).

Results and discussion

The patients show a significant decrease in the SOD activity, ranged between 2.45 and 2.82 units/ml of blood, mean 2.68 standard deviation 0.20, his brothers and his sisters had reduced SOD activity ranged netween 2.87 and 3.18 units/ml of blood, mean 2,97 standard deviation 0.15, while the parents had normal SOD activity ranged between 3.76 and 3.92 units/ml of blood, mean 3.86 standard deviation 0.09. The normal spanish population SOD activity levels is 4.16±0.89 (De la Torre et al., 1990). These reduction of SOD activity support the hypothesis that the enhanced spontaneous chromosomal damage in FA could be due to decreased levels of cellular SOD.

SOD, a free radical-metabolising enzyme, protects the cell membrane from damage by the highly reactive superoxide free radical (Halliwell et al., 1992; Aruoma et al., 1991). According to Joenje et al. (1981), and Joenje and Oostra (1983) the frequency of chromosome aberrations in FA is dependent on the concentration of free oxygen radicals and SOD reduces the cellular level of these radicals. As there is a deficiency in SOD activity in FA patients, the free radical level is higher than normal. Although this free radical level may not be the primary defect in FA, SOD may protect the cells against the genotixicity of free oxygen radicals. It is more likely that the primary defect in FA in an especific repair mechanism (Izakovic et al., 1985).

Fetal hemoglobin was elevated in the patients ranged between 8.3 and 9.1 %, mean 8.7 standard deviation 0.08, and to a lesser extent in his brothers and his sisters ranged between 4.1 and 4.5 standard deviation 0.04, while it was normal in the parents.

All 6 FA families having been located in Madrid (Spain) but with different ethnic origin: 3 families are normal spanish population and the other 3 families are gipsy. Those cases are first described in Spain although FA is the most widely recognized congenital aplastic anaemia worldwide. The most significant subjects in our cases are: a) Parents are consaguineous couples in all 6 families (first cousins). b) Not all FA patients exhibit the same clinical features. In all 6 families the youngest son is most severly affected. c) Clinical manifestations are more pronounced in the gipsy families than in the other families. It was probably caused by increased exposure the heavy metals (gipsy are usually scrap-metal merchants).*

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