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Superoxide dismutase activity in the Spanish population

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Summary. Superoxide dismutase is an enzyme that catalyzes the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen. This superoxide radical is produced by all aerobic cells as a normal metabolic intermediate of molecular oxygen, and is dangerous for the cell because it induces the inactivation of various enzymes, lipid peroxidation and mutations. Superoxide dismutase can therefore be considered as a protective enzyme. The purpose of this work was to determine the level of superoxide dismutase activity in the Spanish population, and to study the factors that influence this activity. The superoxide dismutase activity of 2397 individuals was determined using the method described by Minami and Yoshikawa. The superoxide dismutase activity level in the adult Spanish population was found to be 4.16 ± 0.89 Units/ml of blood. No significant variations with respect to sex were detected. But it was observed that the superoxide dismutase activity level was 9% higher in the young urban Spanish population.
Key words. Superoxide; superoxide dismutase; sex; age; aging processes.

There is an enzymatic activity universally present in all aerobic cells. The substrate is an unstable free radical that can be present only in minuscule amounts at any instant, and the reaction catalyzed proceeds at a rapid rate even in the absence of the enzyme. Yet the enzyme, superoxide dismutase, is essential for the survival of aerobic cells. It catalytically scavenges the superoxide radical, which is an important agent of the toxicity of oxygen, and thus provides a defense against this aspect of oxygen toxicity[1].

Superoxide radical can act either as a reducing agent, giving up its extra electron, or as an oxidizing agent, becoming reduced to hydrogen peroxide. For example, it reduces cytochrome c, but it oxidizes molecules such as ascorbic acid and adrenalin. It can also decarboxylate ketoacids and react with certain phenols. In comparison with other oxygen radicals, superoxide is rather unreactive, with a lifetime of milliseconds at physiological pH values. But superoxide can react with hydrogen peroxide to produce hydroxyl radicals and excited state oxygen (singlet oxygen $^1\Delta$ g), which are among the most reactive species known to organic chemistry; they will attack and damage almost every molecule found in living cells. For example, they can attack olefinic bonds and so initiate lipid peroxidation. Once initiated, lipid peroxidation is autocatalytic. Lipid peroxides are powerful inhibitors of many enzymes, causing severe damage to membranes and eventual loss of membrane integrity. Hydroxyl radicals and singlet oxygen can also hydroxylate the purine and pyrimidine bases present in DNA, resulting in mutations[2].

Superoxide radical is a common intermediate of oxygen reduction. A number of reactions of biochemical interest have been shown to generate superoxide radical. Among these are the autoxidation of hydroquinones, leucoflavins, catechol amines, reduced ferredoxins, hemo-

proteins, etc. Furthermore, the catalytic actions of several enzymes have been shown to evolve superoxide radical[3].

Thus, it can be concluded that superoxide radical is an important agent of oxygen toxicity and that superoxide dismutase provides an essential defense. Levels of superoxide dismutase must therefore be very strictly controlled in order to avoid lesions due to excessive quantities of superoxide radical, without seriously inhibiting essential processes involving this radical as an intermediate.

But there exist some health and disease processes where superoxide dismutase activity is decreased. This happens in aging processes and in Fanconi's anemia, characterized by high frequencies of chromosome aberrations[4,5]. It has been observed that superoxide dismutase has a protective effect on Fanconi's anemia[6,7], on Werner's syndrome, which is characterized by precocious aging[8], and against radiation damage[9,10], decreasing the frequency of chromosome aberrations. Also, superoxide dismutase has an anticancer effect, as the superoxide anion is carcinogenic, and can have a very important role in cancer incidence[11]. Superoxide dismutase activity seems also to be decreased in thalassemia[12].

Since the gene for superoxide dismutase is located in chromosome 21 in humans, it was of considerable interest to study trisomy 21 mongoloids, which led to the finding of a direct gene dosage effect[13]. Also, as chromosome 21 seems to be related to Alzheimer syndrome and superoxide dismutase has a protective effect in aging processes it would be interesting to look for a relationship between superoxide dismutase activity and Alzheimer syndrome.

The intention of this work was to determine the superoxide dismutase activity level in the adult Spanish population, and to find out whether there are differences between sexes and among different ages.

Materials and methods

The sample was made up of 2397 hematologically normal individuals of both sexes and different ages. The blood samples were supplied by the National Institute of Hematology and Hemotherapy, Madrid.

Cu/Zn Superoxide dismutase (E.C.L. 15.1.1) activity was measured in red blood cells. Whole blood was obtained by venipuncture and prevented from coagulating by heparin. 0.1 ml of blood was hemolyzed by 0.9 ml of cold (4 °C) water. Hemoglobin was removed by adding 0.25 ml of chloroform and 0.5 ml of ethanol followed by vigorous mixing. The mixture was centrifuged at 18.000 × g for 60 min. The clear supernatant was used for SOD assay. This assay was performed using the method described by Minami and Yoshikawa in 1979[14], which is based on the inhibition by superoxide dismutase of the nitro blue tetrazolium reduction produced by the superoxide radical generated by the autoxidation of pyrogallol. The rate of inhibition of the superoxide reaction by SOD was calculated according to the definition of McCord and Fridovich[15].

Results and discussion

The superoxide dismutase activity level in the adult Spanish population was found to be 4.16 ± 0.89 Units/ml of blood. As shown in table 1, no significant variations with respect to sex were detected.

Superoxide dismutase activity seems to be different at different ages. As can be seen in table 2, the age-group between 18 and 27 years shows a significantly higher level of superoxide dismutase activity. These data are different from those of Michelson et al.[16], who did not detect any significant variations with respect to age. This could be explained by the fact that the population studied by Michelson et al.[16] was only rural, while the population considered in this work was both rural and urban, so the two populations considered were different.

It has already been shown that superoxide dismutase activity in the urban Spanish population is 10 % higher than in the rural Spanish population[17]. When the superoxide dismutase activity levels in rural and urban populations were compared, taking account of age, it was found (table 3) that the 9 % increase detected in the superoxide dismutase activity of the Spanish population between 18 and 27 years took place only in the urban population.

Table 1. Superoxide dismutase activity in the Spanish population, considering both sexes. Student's t-test was carried out, and no significant variations were found.

	Total	Male	Female
Sample size	2397	1539	858
Superoxide dismutase activity mean (Units/ml of blood)	4.16	4.2	4.09
Standard deviation	0.89	0.97	1.01

Table 2. Superoxide dismutase activity in the Spanish population with respect to age. A Student's t-test was carried out. **Increase significant ($p \leq 0.001$).

	Total	18–27	28–37	38–47	48–57	58–65
Sample size	2397	836	537	541	399	94
Superoxide dismutase activity mean (Units/ml of blood)	4.16	4.40**	4.01	4.03	4.06	4.04
Standard deviation	0.89	0.91	1.02	0.94	0.91	0.83

Table 3. Superoxide dismutase activity in the urban Spanish population with respect to age. Student's t-test was carried out. **Increase significant ($p \leq 0.001$).

	Total	18–27	28–37	38–47	48–57	58–65
Sample size	2047	756	457	446	321	70
Superoxide dismutase activity (Units/ml of blood)	4.22	4.49**	4.07	4.07	4.05	4.20
Standard deviation	0.97	1	1	0	0	0

Table 4. Superoxide dismutase activity in the rural Spanish population, considering the age. Student's t-test was carried out. No significant variations were found.

	Total	18–27	28–37	38–47	48–57	58–65
Sample size	348	81	79	94	67	25
Superoxide dismutase activity mean (Units/ml of blood)	3.81	3.79	3.61	3.89	4.05	3.59
Standard deviation	0.9	0	0	0	0	0

The reason for this increase is unknown. Furthermore, the level in those younger than 18 has not yet been studied; it would be interesting to do this.

Considering the rural population, no significant differences in the superoxide dismutase activity level among different age groups were found (table 4). However, as found by Michelson et al.[16], a small decrease in the superoxide dismutase activity was detected in the rural Spanish population between 58 and 65 years. This little difference could be due to the fact that the number of individuals is smaller in this group. But the hypothesis proposed by Michelson et al.[16] that aging processes occur more rapidly under low superoxide dismutase activity cannot be rejected. This could be due to the modification of repair enzymes, cross-linking and covalent linkage of lipids or nucleic acids to protein caused by an excess of superoxide radical, as well as direct modification of DNA.

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Increased angiogenesis in diabetes

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Summary. Rats with streptozotocin-induced diabetes mellitus showed a 3.4–4.5 times increased angiogenic response following mast-cell activation in situ as compared with age-matched normal controls. The test tissue used was the mesenteric window, which we have previously exploited as a quantitative angiogenesis assay. In the present study two independent techniques for quantifying the angiogenic response showed essentially the same result. The finding of a pathologically increased angiogenic reaction in the diabetic animals is noteworthy since some of the most harmful complications of diabetes in man relate to proliferative vascular lesions.
Key words. Angiogenesis; diabetes; mast cells; mesentery; quantification; rat.

The most important clinical complications in human diabetes include late developing proliferative lesions in the kidney and retina, as well as advanced atherosclerosis[1]. Examples of such lesions include growth of capillary vessels in proliferative retinopathy[2], one of the most common causes of blindness, the pathogenesis of which is unknown[3,4]; proliferation of smooth muscle cells; and the formation of new vessels in atherosclerotic plaques in advanced atherosclerosis[5].

As we have demonstrated earlier, rats suffering from 4 weeks of experimental diabetes show an augmented mast-cell-mediated mitogenic reaction in various cell types in the test tissue used in the present study, the mesenteric window, in vivo[6,7], as well as in organ culture[8]. This augmented cell proliferation is unaffected by insulin[6,9] and is not due to hyperglycemia per se[10]. Increased mitogenesis appears to be causally related to some cellular and/or extracellular factor that slowly manifests its effect during the course of the disease[8]. Mast cells are not only able to stimulate surrounding cells to synthesize DNA and divide, by a paracrine mode of action in normal and diabetic tissues, but also, when