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Copper/zinc superoxide dismutase activity in newborns & young people in Spain

Ángela Casado, Rosario de la Torre & Mª Encarnación López-Fernández

Departamento de Fisiopatología y Genética Molecular Humana, Centro de Investigaciones Biológicas (CIB), Consejo Superior de Investigaciones Científicas (CSIC), Madrid (España)

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***Background & objectives*: There is evidence that reactive oxygen species (ROS) plays an important role in the pathophysiology of many paediatric disorders. We carried out this study to see whether superoxide dismutase (SOD) activity was associated with age, sex and rural or urban status in three groups of Spanish people (newborns, children and young).**

***Methods*: SOD activity was measured in red blood cells in newborns, children and young Spanish people (n=1212, divided in six groups) using the Minami and Yoshikawa method.**

***Results*: The newborns had high levels of SOD activity, but among all age groups studied, SOD showed the highest activity in groups 1 and 2. We also observed that this activity decreased gradually with age until achieving adult levels. No significant variations with respect to sex were detected, except for the ≥14 to 18 yr age group, in which SOD activity decreased significantly in females.**

***Interpretation & conclusion*: Our findings show that SOD activity in newborns, children and young Spanish people is affected by age but not by gender (except from ≥14-18 yr) or rural or urban status.**

Key words Children - copper/zinc superoxide dismutase - newborns - reactive oxygen species - sex - young

Reactive oxygen species (ROS) are substances that are released during oxidative metabolism. ROS include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^·$)[1]. The reactions of ROS with macromolecules can lead to DNA mutations, changes in the structure and function of proteins, and peroxidative damage of cell-membrane lipids[2]. Abundant evidence exists that ROS play an important role in the pathogenesis of many paediatric neurologic diseases, including retinopathy of prematurity[3], Down syndrome, epilepsy, and mitochondrial encephalopathies[4,5].

The biological effects of these highly reactive compounds are controlled *in vivo* by a wide spectrum of antioxidative defense mechanisms such as vitamins E and C, carotenoids, metabolites such as uric acid or glutathione and antioxidants enzymes. Cells have an enzymatic antioxidant pathway against ROS which are generated during oxidative metabolism: firstly, superoxide dismutase (SOD) catalyzes the formation of hydrogen peroxide from superoxide radicals. Hydrogen peroxide can generate toxic hydroxyl radicals, but it is removed by a reaction catalyzed by catalase (CAT) and glutathione peroxidase (GPx)[6]. Any increase in SOD catalytic activity produces an excess of hydrogen peroxide that must be efficiently neutralized by CAT or GPx. The activity of first and second step antioxidant enzymes must, therefore, be balanced to prevent oxidative damage in cells, which may contribute to various pathological processes[7]. The trace elements Selenium (Se), Copper (Cu) and Zinc (Zn) are essential for the proper functioning of these enzymes.

SOD previously known as erythrocuprein (human) or hemocuprein (bovine), exists as a family of metalloproteins and is widely distributed in mammalian tissues[8,9]. Erythrocytes only contain the copper/zinc SOD isoenzyme, which is coded by a gene located on chromosome 21[10]. By virtue of their physiological role, erythrocytes are exposed to continuous oxidative stress, because oxygen radicals are continuously generated by autoxidation of haemoglobin[11]. There are previous reports regarding the SOD activity in human erythrocytes from normal individuals[12,13]; however, most of the studies involved a restricted population.

The aim of the present study was to see whether SOD activity was associated with age, sex and rural or urban status in the three groups of Spanish people (newborns, children and young).

Material & Methods

Subjects: A sample of 1212 healthy subjects (657 males and 555 females) aged from newborns to 18 yr were included in this study with a significance level of $P<0.05$ and the minimum confidence level the required sample size was calculated to be 955. Six groups were included according to age. In group 1 healthy term newborns of both sexes with a normal Apgar score 5 min after birth were included. In group 2 healthy children of both sexes aged from 0 to 1 yr were included. Group 3 comprised healthy children of both sexes aged from ≥2 to 5 yr. In group 4 healthy children of both sexes aged from ≥6 to 9 yr were included, group 5 had young healthy people of both sexes aged from ≥10 to 14 yr, and group 6 consisted of young healthy people of both sexes aged from ≥15 to 18 yr.

Recruitment of consecutive newborns took place in San Francisco de Asis Hospital (Madrid, Spain) in the Neonatology service. All babies (61 males, 64 females) born after a normal gestation at full term with normal birthweight from normal single pregnancies were included. The children and young people were selected from students of a kindergarten and a primary and secondary school in Madrid (Spain), and considered as healthy, normally developing children by their physicians. Subjects were excluded if they had any history of chronic disease (such as asthma or diabetes), neurological disease, developmental delay or chronic medication use.

Written informed consent from the parents were obtained for all participants. The study was approved by the Ethical Committee of Superior Council of Scientific Investigations.

Blood sampling: Blood samples (4 ml) in newborns were taken from their umbilical cordon and

collected into 5 ml vacutainers containing lithium heparin. Blood samples of children and young people were also collected into 5 ml vacutainers containing lithium heparin from the cubital vein (right arm).

Superoxide dismutase activity determination: The SOD activity was measured in red blood cell, 0.1 ml of blood was haemolyzed by 0.9 ml of ice cold water (0-4ºC). The haemoglobin was removed by adding 0.25 ml of chloroform and 0.5 ml of ethanol followed by vigorous mixing. The mixture was centrifugated at 18,000 *g* for 60 min. The clear supernatant was used for superoxide dismutase assay performed using the method of Minami and Yoshikawa[14]. The rate of inhibition of the superoxide reaction by SOD was calculated according to the definition of McCord and Fridovich[8].

Statistical analysis: Data were analyzed by using SPSS 10.0 (SPSS Inc. Chicago, IL USA) computing program. The results are expressed as mean ± standard deviation. The hypothesis of normality of the quantitative variables was tested using a Kolmogorov-Smirnov test. As the group showed normal distribution, parametric statistical methods. Student's t test and analysis of variance (ANOVA) were used. The statistical significance was defined at $P<0.05$.

Results & Discussion

Our results indicated that newborns had a high SOD activity, but among all groups of age, ±SOD showed the highest activity in groups 1 (≥0-1yr) and 2 (≥2-5 yr). SOD activity decreased gradually in the rest of groups until achieving adult levels (Table I).

Aliakbar *et al*[15] and Huston *et al*[16] observed that SOD activity was lower in neonates than in adults. This difference in results can be partly explained by a large difference in the size of the population studied (10 newborns by Aliakbar *et al* and 6 by Huston *et al*). On the other hand, the process of childbirth is accompanied by an increase in oxidative aggression. The foetus exchanges an intrauterine environment that is hypoxic for another with a greater oxygen content. This change results in greater oxidative stress simply due to the existence of normoxic levels in the new intrauterine environment[17], and the oxygen tension in prenatal life is about five times lower than that in postnatal life[18]. The high challenge that occurs at birth might lead to increased formation of reactive oxygen species (ROS)[19].

The oxidative aggression suffered by the neonate is counteracted by maturation of effective antioxidant mechanisms such as the enzymatic systems (superoxide dismutase, catalase, glutathione peroxidase, *etc.*), it might be expected that the SOD activity would be lower in foetal blood than in neonates and that the adults levels would be achieved within a few months after birth. Frank *et al*[20] determined the increase in antioxidant mechanisms at the pulmonary level that are produced at the end of the gestation, a process that is concurrent with the

Table I. SOD activity in children and young Spanish people according to age

	Total	Newborns	0-1 yr	≥5 yr	≥9 yr	≥14 yr	≥18 yr
Sample size	1212	125	131	255	206	126	369
SOD activity (units/ml blood)	4.65 ± 0.9	4.67 ± 0.85	5.46 ± 0.87*	5.14 ± 0.81*	4.78 ± 0.71	4.66 ± 0.83	4.14 ± 0.79*

Values are mean ± SD
*$P<0.001$

increase in the substrates derived from the increase in free radicals.

Further, similar situation has been observed in other antioxidant enzymes, we previously found the highest CAT activity in Spanish newborns[21], and we also observed that this activity decreased in children aged 1-3 and 4-9 yr. McElroy *et al*[22] have shown that CAT is the only antioxidant to increase in activity with progressing gestational age.

Our findings in children and young Spanish people in the present study agree well with those of Inal *et al*[23] who found a negative correlation between SOD activities and age. Anderson *et al*[24] reported an age related decrease in SOD activity.

No significant variations with respect to sex were detected except for the >14 to 18 yr age group, in which group SOD activity decreased significantly in females (Table II). Our findings are in agreement with those reported by Guemouri *et al*[25] in females aged 10-16 yr, after menarche. In our previous study[13] we reported significant variations in SOD activity with respect to sex in Spanish population from 68-93 yr. SOD activity in males remained constant or slightly decreased with age, whereas in females SOD activity showed a significant increase. It is interpreted as one of the reason for the greater longevity in females[13].

Table II. SOD activity in children and young Spanish people according to age and sex

	Sex	Total no.	SOD activity (mean ± SD) (units/ml blood)
Newborns	males	61	4.68 ± 0.87
	females	64	4.85 ± 0.83
0-1 yr	males	71	5.5 ± 0.85.
	females	60	5.4 ± 0.88
≥5 yr	males	136	5.09 ± 0.75
	females	119	5.2 ± 0.34
≥9 yr	males	110	4.71 ± 0.78
	females	96	4.87 ± 0.97
≥14 yr	males	66	4.6 ± 0.83
	females	60	4.74 ± 0.88
≥18 yr	males	213	4.25 ± 0.8*
	females	156	4.07 ± 0.78

*P<0.05 compared to females

However, our present results showed differences in specific activities when compared to those obtained by Gaeta *et al*[26] who determined SOD and glutathion peroxidase (GPx) activities in 45 healthy paediatric subjects between 0 and 14 yr of age. These discrepancies may depend on the different methods used to determine the SOD activity or can be caused by the different number of subjects analysed or by the geographical location. Significant racial inequalities have been reported by Glauser *et al*[27] between Africans-Americans and Caucasian children.

We previously detected that SOD activity was different in rural and urban Spanish areas, being 10 per cent higher than in urban Spanish population than in the rural Spanish one[28]. However, in the present study we have found no differences between subjects from rural or urban Spanish areas, probably because the life style in both areas is very similar nowadays.

In conclusion, our findings show that SOD activity in newborns, children and young in Spain is affected by age but not by gender (except from 14-18 yr) or origin.

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References

1. Halliwell B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 1984; *219* : 1-14.

2. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. Oxford: Claredon Press; 1985.

3. Kelly F. Free radical disorders of preterm infants. *Br Med Bull* 1993; *49* : 668-78.

4. Kinght JA. Reactive oxygen species and the neurodegenerative disorders. *Ann Clin Lab Sci* 1997; *27* : 11-25.

5. Weber GF, Maertens P, Xiangxiong M, Pippenger CE. Glutathione peroxidase deficiency and childhood seizures. *Lancet* 1991; *337* : 1443-4.

6. Michel C, Raes M, Toussaint O, Remacle J. Importance of Se-glutathione peroxidase, catalase and Cu/Zn SOD for cell survival against oxidative stress. *Free Radic Biol Med* 1994; *17* : 235-48.

7. Sun AY, Chen YM. Oxidative stress and neurodegenerative disorders. *J Biomed Sci* 1985; *5* : 401-14.

8. Mc Cord JM, Fridovich I. Superoxide dismutase: an enzymatic function for erythrocuprein (hemocuprein). *J Biol Chem* 1969; *244* : 6049-55.

9. Bannister JV, Bannister WH, Rotilio G. Aspects of the structure, function, and applications of superoxide dismutase. *CRC Crit Rev Biochem* 1987; *22* : 111-80.

10. Beckman G, Lundgren E, Tarnvik A. Superoxide dismutase isozymes in different human tissues, their genetic control and intracellular localization. *Human Hered* 1973; *23* : 338-45.

11. Misra HP, Fridovich I. The generation of superoxide radical during the autooxidation of haemoglobin. *J Biol Chem* 1972; *247* : 6960-2.

12. De la Torre R, Casado A, López-Fernández ME. Superoxide dismutase activity in Spanish population. *Experientia* 1990; *46* : 854-6.

13. De la Torre R, Casado A, López-Fernández ME, Carrascosa D, Venarucci D. Superoxide dismutase activity levels in a Spanish population 50-93 years. *Am J Hum Biol* 1999; *11* : 45-7.

14. Minami M, Yoshikawa H. A simplified assay method of superoxide dismutase activity for clinical use. *Clin Chim Acta* 1979; *92* : 337-42.

15. Aliakbar S, Brown PR, Bidwell D, Nicolaides KH. Human erythrocyte superoxide dismutase in adults, neonates and normal, hypoxaemic, anaemic and chromosomally abnormal fetuses. *Clin Biochem* 1993; *26* : 109-15.

16. Huston RK, Shearer TR, Jelen BJ, Whall PD, Reinolds JW. Relationship of antioxidant enzymes to trace metals in premature infants. *J Parent Ent Nutr* 1987; *11* : 163-8.

17. Robles R, Palomino N, Robles A. Oxidative stress in neonate. *Early Hum Develop* 2001; *65* (Suppl): S75-S81.

18. Kigawa J. Studies on the levels of pO_2 and pCO_2 in the uterine cavity and uterine tissue. *Acta Obstet Gynaecol Jpn* 1981; *33* : 1646-54.

19. Zhao J, Lui XJ, Ma JW, Zheng RL. DNA damage in healthy term neonate. *Early Hum Develop* 2004; *77* : 89-98.

20. Frank L, Price LT, Whitney PL. Possible mechanism for late gestational development of the antioxidant enzymes in the fetal rat lung. *Biol Neonate* 1996; *70* : 116-27.

21. Casado A, López-Fernández ME. Age-correlated changes of the erythrocyte catalase activity in the Spanish population. *Gerontology* 2003; *49* : 251-4.

22. McElroy MC, Postle AD, Kelly FJ. Catalase, superoxide dismutase and glutathione peroxidase activities of lung and liver during human development. *Biochem Biophys Acta* 1992; *117* : 153-8.

23. Inal ME, Kambak G, Sunal E. Antioxidant enzyme activities and malondialdehyde related to aging. *Clin Chim Acta* 2001; *305* : 75-80.

24. Andersen HR, Nielsen YB, Nielsen F, Grandjean P. Antioxidative enzyme activities in human erythrocytes. *Clin Chem* 1997; *43* : 562-8.

25. Guemori L, Artur Y, Herberth B, Jaendel C, Cuny G, Siest G. Biological variability of superoxide dismutase, glutathione peroxidase and catalase in blood. *Clin Chem* 1981; *37* : 1932-7.

26. Gaeta LM, Tozzi G, Pastore A, Federici G, Bertini E, Piemonte F. Determination of superoxide dismutase and glutathione peroxidase activities in blood of healthy pediatrics subjects. *Clin Chim Acta* 2002; *322* : 117-20.

27. Glauser TA, Titanic-Schefft M, Pippenger CE. Racial differences in free radical scavenging enzyme activity in children. *J Chlid Neurol* 1999; *305* : 75-80.

28. De la Torre R, Casado A, López-Fernández ME. Actividad de superóxido dismutasa en poblaciones rurales y urbanas españolas. *Genét Ibér* 1988; *40* : 39-4.

Reprint requests: Dra. Ángela Casado, Departamento de Fisiopatología y Genética Molecular Humana
Centro de Investigaciones Biológicas (CSIC), c/ Ramiro de Maeztu, 9, 28040 MADRID (Spain)
e-mail: acasado@cib.csic.es