Eur Arch Psychiatry Clin Neurosci (2004) 254 : 143–147 DOI 10.1007/s00406-004-0456-7

ORIGINAL PAPER

S. Salih Zoroglu · Ferah Armutcu · Sakir Ozen · Ahmet Gurel · Ercan Sivasli · Ozer Yetkin · Iclal Meram

Increased oxidative stress and altered activities of erythrocyte free radical scavenging enzymes in autism

Received: 6 August 2002 / Accepted: 5 September 2003

Abstract There is great evidence in recent years that oxygen free radicals play an important role in the pathophysiology of many neuropsychiatric disorders. The present study was performed to assess the changes in red blood cells thiobarbituric acid-reactive substances (TBARS) levels, and superoxide dismutase (SOD), catalase (CAT), adenosine deaminase (ADA) and xanthine oxidase (XO) activities in patients with autism (n = 27) compared to age- and sex-matched normal controls (n = 26). In the autistic group, increased TBARS levels ($p < 0.001$) and XO ($p < 0.001$) and SOD ($p < 0.001$) activity, decreased CAT ($p < 0.001$) activity and unchanged ADA activity were detected. It is proposed that antioxidant status may be changed in autism and this new situation may induce lipid peroxidation. These findings indicated a possible role of increased oxidative stress and altered enzymatic antioxidants, both of which may be relevant to the pathophysiology of autism.

Key words autism · thiobarbituric acid-reactive substances (TBARS) · superoxide dismutase (SOD) · catalase (CAT) · xanthine oxidase (XO) · adenosine deaminase (ADA) · free radicals

Dr. Suleyman Salih Zoroglu (✉) · O. Yetkin
Gaziantep Typ Fakultesi, Kolejtepe Binasi
Cocuk ve Ergen Psikiyatrisi AD
Kolejtepe
27200 Gaziantep, Turkey
Tel.: +90-342/3365-400 or -367
Fax: +90-342/3365-505
E-Mail: zoroglus@hotmail.com

F. Armutcu · A. Gurel
Dept. of Biochemistry at Faculty of Medicine
Zonguldak Karaelmas University
Zonguldak, Turkey

S. Ozen
Dept. of Psychiatry at Faculty of Medicine
Dicle University
Diyarbakyr, Turkey

E. Sivasli
Dept. of Pediatrics
Faculty of Medicine
Gaziantep University, Gaziantep

I. Meram
Dept. of Biochemistry
Faculty of Medicine
Zonguldak Karaelmas University
Zonguldak, Turkey

Introduction

Autism is a neurodevelopmental disorder that is characterized by impairments in socialization, and by abnormalities of verbal and non-verbal communication, and restricted, stereotyped interests and behaviors (APA 1994). Approximately three-quarters of persons with autism also have mental retardation and about one-third develops epilepsy. The underlying etiology of autism is unknown. Although it had initially been thought to be a consequence of defective parenting, nowadays autism is generally accepted as a biological disorder.

Free oxygen radicals as known reactive oxygen species (ROS) are reactive chemical species with an unpaired electron that are produced through a variety of physiologic and pathologic processes. Free radicals have been implicated in a variety of neuropsychiatric conditions, many of which are marked by the gradual development of psychopathologic symptoms and movement disorder. There is evidence that radical-induced damage may be important in Parkinson's disease, tardive dyskinesia, metal intoxication syndromes, Down's syndrome, and possibly also in schizophrenia, Huntington's disease, and Alzheimer's disease. Although some of this evidence is highly speculative, it may offer an avenue for further understanding and treatment of these conditions (Lohr 1991).

The hypothesis that ROS play an important role in autism remains speculative and there have been no detailed studies to test this hypothesis (Lombard 1998). The radicals originated from molecular oxygen are gen-

erally named as ROS. Reactive oxygen species including superoxide ($O_2^{\cdot-}$), hydroxyl ($^{\cdot}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and nitric oxide ($NO^{\bullet}$) can cause cellular injury when they are generated excessively or the enzymatic and nonenzymatic antioxidant defence systems are impaired. A number of oxygenated compounds, particularly aldehydes including malondialdehyde are produced during the attack of free radicals to membrane lipoproteins and polyunsaturated fatty acids (PUFAs). Therefore, assessment of thiobarbituric acid-reactive substances (TBARS) is probably the most commonly applied method for the measurement of lipid peroxidation (Esterbauer 1993). Because of its high lipid content, the central nervous system (CNS) is more sensitive to ROS attack and lipid peroxidation than other body compartments and organs.

One of the most important enzymatic sources of superoxide anion radical ($O_2^{\cdot-}$) is xanthine oxidase (XO). This enzyme is located in all of the nucleated cells and catalyses the conversion of hypoxanthine and xanthine to uric acid, the rate limiting step in purine nucleotide catabolism. Current interest in XO stems from its proposed role in postischemic reperfusion injury (Krenitsky et al. 1986); in such cases, the activity of this $O_2^{\cdot-}$ producer enzyme may increase through the proteolytic conversion of xanthine dehydrogenase to XO and produce enormous amounts of $O_2^{\cdot-}$. Adenosine deaminase (ADA), however, is an important enzyme that plays a relevant role in purine and DNA metabolism, immune responses, and peptidase activity. Several studies reported the altered activities of ADA in some neuropsychiatric disorders including autism (Stubbs et al. 1982). Recently, two genetic association studies related to ADA gen polimorphism (ADA1 and ADA2) indicated a significantly elevated frequency of the low-activity ADA2 allele in autistic cases (Persico et al. 2000; Bottini et al. 2001).

Superoxide dismutase is a potent protective enzyme that can selectively scavenge the radical $O_2^{\cdot-}$ by catalyzing its dismutation to hydrogen peroxide (H_2O_2). The other antioxidant enzyme, CAT, acts to decompose H_2O_2 to water and molecular oxygen (Fridovich 1983). CAT also oxidizes electron donors such as ethanol, methanol, or phenols (peroxidative activity). Although it has not yet been investigated, several recent studies proposed that altered activities of antioxidant system may have a pathophysiological role in autism (Lombard 1998; Johnson 2001). However, there has been only one study in which Golse et al. (1978) found that SOD I and glutathione peroxidase (GSH-Px) activities seem to be abnormal in the erythrocytes, whereas only SOD I activity appears to be abnormal in the platelets. As far as we know, there is no study about CAT, XO and TBARS in autism. In addition, there has been no study evaluating both on erythrocyte antioxidant enzymes and lipid peroxidation in the same autistic patient groups. Assessment of the activities of these free radical scavenging enzymes in red blood cells may help to better understand the changes in antioxidative status in autism. Therefore, the aim of the present study was to determine the activities of purine catabolizing and antioxidant enzymes, and the lipid peroxidation level in red blood cells of autistic patients and healthy controls.

Materials and methods

Patients

Twenty-seven patients and 26 healthy controls were included in this study. The autistic children were referred as outpatients to the Department of Child and Adolescent Psychiatry in Gaziantep University Medical School during 1999–2001. The children in the control group were selected from among the students of a kindergarten and a primary school in the City of Gaziantep. All of the patients and controls were screened for psychiatric disorders by obtaining historical information, performing clinical interviews, and using symptom ratings according to DSM-IV (APA 1994). They were also examined by the pediatrician (E. Sivaslı) for medical problems including the measurement of blood pressure and for their dietary patterns. Among all the patients and controls, no abnormal blood pressure level was found and there was no considerable difference between the patients and the controls with regard to the diet.

The child and adolescent psychiatry clinic of Gaziantep University is unique in the South Eastern region of Turkey as a university research hospital with a large regional catchment area for children and adolescents with a wide range of neuropsychiatric disorders. In addition to our patients, the children were referred by psychiatrists at City of Sanlıurfa and Diyarbakır. The consecutive sample of 27 children received the diagnosis of autism, according to DSM-IV diagnostic criteria and Childhood Autism Rating Scale (CARS) (Schopler et al. 1980) score, greater than 30. All diagnoses were made by a child psychiatrist (S. S. Zoroglu) and psychologist (O.Yetkin) independently. No subject had any diagnosed genetic, metabolic, or neurological etiology for autistic disorder. Patients who had a history of chronic systemic disease, acute and chronic inflammatory disease, or severe head injury were excluded from the study. The patients with autistic disorder were 16 boys and 11 girls. The mean age (± sd) was 4.7 (± 2.7) with an age range of 2–12 years. All subjects participated in the study after written informed parental consent had been obtained. All autistic subjects had evidence of mental retardation and all of the children in the patient and control groups were free of any medication. The study was approved by the ethical committee of Gaziantep University Medical School.

Sample collection and preparation

Blood from forearm vein was collected into 5 ml vacutainer tubes containing potassium EDTA. Some hematological parameters were carried out by routine laboratory techniques using an autoanalyzer (Coulter STKS, Coulter Electronics Ltd., UK). The blood samples were centrifuged at 1000 x g for 10 min at +4 °C to remove plasma. The buffy coat on the erythrocyte sediment was separated carefully after plasma was removed. Erythrocyte sediment was washed three times with 10-fold isotonic NaCl solution to remove plasma remnant. After each procedure, erythrocyte-saline mixture was centrifuged at 1000 x g for 10 minutes at + 4 °C. Aliquots of the samples were transferred into polyethylene tubes to be used in the assay of free radical scavenging enzymes and TBARS levels. Erythrocyte sediment samples were stored at –80 °C until analyses. After they were thawed, erythrocyte sediments were treated with 4-fold ice-cold deionized water to obtaine hemolyzate.

Enzymes, chemicals and instruments

Xanthine oxidase, xanthine, nitroblue tetrazolium (NBT), thiobarbituric acid, 1.1,3.3-tetramethoxy propane, adenosine, phenol, Na nitroprusside, and uric acid were purchased from Sigma Chemical Co. (St. Louis, MO) and $CuCl_2$, bovine serum albumine, H_2O_2, EDTA,

Na_2CO_3, $(NH_4)_2SO_4$, chloroform, ethanol, NaCl, KH_2PO_4, and $Na_2HPO_4{\cdot}2H_2O$ from Merck (Germany). LKB Biochrom Ultraspec Plus uv/visible spectrophotometer (Cambridge, England) was used to measure all the parameters studied.

CAT activity determination Catalase (EC 1.11.1.6) activity was determined by the method of Aebi (1974). The principle of the assay is based on the determination of the rate constant (s^{-1}, k) of the hydrogen peroxide decomposition. By measuring the absorbance changes per minute, the rate constant of the enzyme was determined. Activities were expressed as rate constant per gram hemoglobin (k/g Hb).

SOD activity determination Total (Cu-Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun et al. (1988) and a slightly modified method by Durak et al. (1993). The principle of the method is based on the inhibition of NBT reduction by the xanthine-xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the lyzate after 1.0 ml of an ethanol/chloroform mixture (5/3, v/v) was added to the same volume of the hemolyzate and centrifuged (McCord and Fridovich 1969). One unit of SOD was defined as the enzyme amount causing 50 % inhibition in the NBT reduction rate. SOD activity was also expressed as units per gram hemoglobin (U/g Hb).

XO activity determination Erythrocyte XO (EC 1.2.3.2) activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293 nm (Prajda and Weber 1975). A calibration curve was constructed by using 10–50 milliunits/mL concentrations of standard XO solutions (Sigma X-1875). One unit of activity was defined as 1 µmol uric acid formed per minute at 37 °C, pH 7.5. Results were expressed in units per gram hemoglobin (U/g Hb).

ADA activity determination ADA activity was estimated spectrophotometrically by the method of Giuisti (Giuisti 1974), which is based on the indirect measurements of the formation of ammonia that is produced when adenosine deaminase acts in excess of adenosine. Results were expressed as units per gram hemoglobin (U/g Hb).

TBARS levels determination The plasma thiobarbituric acid reactive substances (TBARS) level was determined by a method based on the reaction with thiobarbituric acid (TBA) at 90–100 °C (Esterbauer and Cheeseman 1990). In the TBA test reaction, malondialdehyde (MDA) or MDA-like substances and TBA react together to produce a pink pigment having an absorption maximum at 532 nm. The reaction was performed at pH 2–3 at 90 °C for 15 min. The sample was mixed with 2 volumes of cold 10 % (w/v) trichloroacetic acid to precipitate protein. The precipitate was pelleted by centrifugation, and an aliquot of the supernatant was reacted with an equal volume of 0.67 % (w/v) TBA in a boiling water bath for 10 min. After cooling, the absorbance was read at 532 nm. The results were expressed as micromole per gram hemoglobin (µmol/g Hb) according to a standard graphic, which was prepared with serial dilutions of standard chemical 1.1,3.3-tetramethoxypropane.

Statistical analysis

Data were analyzed using the SPSS® for Windows (Version 10.0) computing program. Parametric statistical methods were used to analyze the data. Categorical variables were compared by Chi-square test and continuous variables were compared by the Student – t tests. Two-tailed significance values were used. A p value less than 0.05 was accepted as significant.

Results

As to the demographic data (age, sex, etc.), patients and their controls showed homogeneity and there was no significant difference between the groups ($p > 0.05$). Results are summarized in the Table 1. There was a statistically significant increase in SOD (% 118, $p < 0.001$), XO (%154, $p < 0.001$) activities and TBARS levels (% 113, $p < 0.001$) and a decrease in CAT activities (%59, $p < 0.001$) in autistic patients compared to the control group. However, there was no difference in ADA activities between patients and control groups ($p = 0.52$).

Discussion

In the present study, we found an increased activity of SOD and XO, decreased activity of CAT, unchanged activity of ADA and elevated levels of TBARS in red blood cells of autistic patients. This is the first study which investigates both oxidant and antioxidant system in the same erythrocyte samples from autistic patients and interprets the results in the aspect of pathogenesis of autism.

We have preferred red blood cell as a cell pattern to estimate the activities of the free radical scavenging enzymes in autistic patients because these enzymes are constitutively expressed in the first stages of maturation process and erythrocytes are easy to obtain. On the other hand, blood can reflect the lability of the whole body to oxidative conditions, although some authors have not accepted this idea. They suggest that, because antioxidant enzyme activities vary among different tissues, and environmental factors might affect the enzyme activities only in susceptible organs, the activities found in erythrocytes do not necessarily reflect the antioxida-

Table 1 Characteristics and the activities of red blood cell antioxidant enzymes, ADA, and TBARS levels in patients with autism and controls included in this study

	Autism	Control	Statistics
N	27	26	–
Age years (mean ± SD)	4.7 ± 2.7	4.8 ± 2.5	$t^a = -0.85$; $p = 0.39$
Sex	16 M/11 F	14 M/12 F	$^b\chi^2 = 0.16$; $p = 0.74$
CAT (k/g Hb) (mean ± SD)	209.31 ± 61.92	515.77 ± 127.9	$t^a = 11.2$; $p < 0.001$
SOD (U/g Hb) (mean ± SD)	2123.59 ± 543.53	971.31 ± 239.14	$t^a = -10.1$; $p < 0.001$
TBARS (mmol/g Hb)(mean ± SD)	0.032 ± 0.0077	0.015 ± 0.0033	$t^a = -10.8$; $p < 0.001$
XO (U/g Hb) (mean ± SD)	143.94 ± 35.68	56.64 ± 17.37	$t^a = -11.4$; $p < 0.001$
ADA (U/g Hb) (mean ± SD)	0. 55 ± 0.13	0.53 ± 0.15	$t^a = -0.7$; $p = 0.52$

[a] Student- t test; [b] Chi-square test with Pearson value

tive defense of the whole organism (Andersen 1997). In the experiments in which oxidative stress is evaluated, erythrocytes appear to be excellent material because of their simple structure and the relatively large amounts of polyunsaturated fatty acids in their membranes (Akyol et al. 2001). Particularly, the lability of erythrocyte membranes to oxidative stress in vitro may reflect the lability of other cell membranes such as the central nervous system to oxidative damage *in vivo*. The altered activities of RBC antioxidant enzymes in autism might be a peripheral response of the organism to increased free oxygen radical (such as $O_2^{\cdot -}$, $\cdot OH$ and H_2O_2) production in the CNS. Many research groups have reported elevated RBC SOD activity in some neuropsychiatric disorders such as schizophrenia (Mahadik and Mukherjee 1996; Yao et al. 1998; Herken et al. 2001), Alzheimer's disease (Durany et al. 1999; Omar and Pappolla 1993), bipolar disorder (Abdalla et al. 1986), Down syndrome (Turrens 2001), and autism (Golse et al. 1978) compared to normal controls. We confirmed the results of many of the studies that SOD and CAT have complementary activities in antioxidative defence mechanism. For example, H_2O_2, the product of reaction catalyzed by SOD, is also substrate for CAT and GSH-Px. Increased antioxidant enzyme activities may reflect a preceding cellular oxidative stress or serve as a compensatory mechanism. The increased activity of erythrocyte SOD may result in over-production of H_2O_2 in erythrocytes. On the other hand, the decreased CAT activity may show the increased H_2O_2 level in erythrocytes in addition to the reason mentioned above. Since H_2O_2 is a neutral and highly liposoluble compound that can easily pass through the cytoplasmic membranes, an excess amount of H_2O_2 found in or outside the cell membrane can pass through the erythrocyte membrane into the cytosol.

It has previously been suggested that an unknown defect in some enzymes of purine metabolism could be involved in the pathogenesis of autistic disorder. Stubbs et al. (1982) investigated serum activity of enzymes of purine metabolism and found that only ADA activity was reduced in the sera of autistic children compared with normal controls. In addition, two genetic association studies (Persico et al. 2000; Bottini et al. 2001) with a case-control design suggest a proposed role for a genotype-dependent reduction in ADA enzymatic activity in the pathogenesis of autism. However, the result of family based tests – more sensitive and reliable than case control design to reduce racial and ethnical effects – involving 91 singleton families, as well as 44 additional Caucasian-American trios, did not support significant linkage/association (Persico et al. 2000). This observation suggests that racial and ethnic differences in AA allelic distributions may play a role in these statistical differences. In fact, it should be underscored that the importance of assessing and controlling for race effects is a procedure that has been widely neglected in biological psychiatry. For example, a recent study (McBride et al. 1998) related with autism indicated that the prevalence of hyperserotonemia in autistic individuals may have been overestimated because of a failure to control for these variables. This study highlights the importance of identifying and eliminating confounding factors before using a biological variable as a diagnostic test or correlate of pathology. In another example (Gelertner et al. 1993), the description of substantial differences in dopamine receptor allele frequencies across ethnic groups has also provided a dramatic demonstration of the need for caution in interpreting data from racially/ethnically heterogeneous populations. Our results indicated that ADA activity was not changed in autism. This discrepancy between our result and that of the previous study (Stubbs et al.1982) originated first from ethnic and race differences and second from that our study has been done in red blood cells, whereas the previous study in serum.

Since XO is the rate-limiting enzyme and ADA an important enzyme of purine catabolism, it was of interest to examine the relationship of these two enzymes and association of oxidant/antioxidant system with purine catabolic pathway in the erythrocytes from patients with autism. Our findings on these enzymes suggest that an isolated increase of XO activity instead of an increase in both of purine catabolyzing enzyme, XO and ADA, activities in autistic patients may show isolated superoxide production rather than the increase of purine catabolism in this disease.

TBARS is the end product of lipid peroxidation in the body. It is abundantly produced from PUFAs in the environment of oxidative stress. In healthy people, lipid peroxidation is normally controlled by the combined activity of various antioxidant enzymes present in the erythrocytes. In case of excessive ROS production, as in autism, this protection may not be sufficient. The fact that erythrocyte TBARS levels in patient groups were increased 2- to 3-fold compared to controls suggests increased lipid peroxidation likely in the membrane structures of autistic patients and thus oxidative stress still existed despite increased activities of the antioxidant enzyme SOD. Lowered erythrocyte CAT activity together with elevated lipid peroxides in autistic patients suggest that elevated lipid peroxidation may be closely associated with decreased CAT and increased XO activities. These data are in good agreement with the previous studies related to neuropsychiatric disorders (Horrobin et al. 1991; Keshavan et al. 1993). The possible explanation for the increased TBARS levels in red blood cells from patients with autism is increased catecholamine metabolism and a resultant overproduction of ROS.

Our findings suggest the following conclusions: i) decreased CAT activity in patients with autism may indicate a degradation process in which CAT is degraded by ROS during the detoxifying process. ii) Other enzymatic and nonenzymatic antioxidant defence systems and parameters not evaluated in this study, such as GSH-Px, glutathione reductase and β-glutamyl systeinil glycine (glutathione), may have been increased to protect the body in compensatory meaning from oxidative stress.

iii) Oxidative stress may have a pathophysiological role in autism. iv) Administration of antioxidants has been successful in some cases in protecting against the physiological impairment related to some disease states. Adding nonenzymatic antioxidant compounds including vitamin E, C, and β-carotenes to the treatment of autism may protect membranous structures from lipid peroxidation. Besides the antioxidant properties, vitamin C and E have been shown to interact with the brain's catecholamines (Mahadik and Scheffer 1996). By these two different routes, it might be possible to produce a remarkable improvement of some symptoms of autism. Further investigations in a larger cohort of autistic patients are needed to provide definitive data about antioxidant therapy.

References

1. Abdalla DS, Monteiro HP, Oliveira JA, Bechara EJ (1986) Activities of superoxide dismutase and glutathione peroxidase in schizophrenic and manic-depressive patients. Clin Chem 32: 805–807
2. Aebi H (1974) Catalase. In: Bergmeyer HU (ed) Methods of Enzymatic Analysis. Academic Press, Inc. New York and London, pp 673–677
3. Akyol O, Isci N, Temel I, Ozgocmen S, Uz E, Murat M, Buyukberber S (2001) The relationships between plasma and erythrocyte antioxidant enzymes and lipid peroxidation in patients with rheumatoid arthritis. Joint Bone Spine 68:311–317
4. American Psychiatric Association (APA) (1994) Diagnostic and Statistical Manual of Mental Disorders, 4th Edition. American Psychiatric Association, Washington, DC
5. Andersen HR, Nielsen JB, Nielsen F, Grandjean P (1997). Antioxidative enzyme activities in human erythrocytes. Clin Chem 43:562–568
6. Bottini N, De Luca D, Saccucci P, Fiumara A, Elia M, Porfirio MC, Lucarelli P, Curatolo P (2001) Autism: evidence of association with adenosine deaminase genetic polymorphism. Neurogenetics 3:111–113
7. Durak I, Yurtarslany Z, Canbolat O, Akyol O (1993) A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clin Chim Acta 214:103–104
8. Durany N, Munch G, Michel T, Riederer P (1999) Investigations on oxidative stress and therapeutical implications in dementia. Eur Arch Psychiatry Clin Neurosci 249(Suppl 3):68–73
9. Esterbauer H, Cheeseman KH (1990) Determination of aldehydic lipid peroxidation products: Malonaldehyde and 4-hydroxynonenal. In: Packer L, Glazer AN (eds) Oxygen radicals in biological systems, In Methods in Enzymology. Academic Press, California, vol 186, pp 407–421
10. Esterbauer H (1993) Cytotoxicity and genotoxicity of lipid-oxidation products. Am J Clin Nutr 57(5 Suppl):779–785
11. Fridovich I (1983) Superoxide radical: an endogenous toxicant. Annu Rev Pharmacol Toxicol 23:239–257
12. Gelertner J, Goldman D, Risch N (1993) The A2 allele at the D2 dopamine receptor gene and alcoholism: a reappraisal. JAMA 269:1673–1677
13. Giusti G (1974) Adenosine deaminase. In: Bergmeyer MV (ed) Methods of Enzymatic Analysis. Academic Press, New York, (2nd ed) pp. 1092–1098
14. Golse B, Debray-Ritzen P, Durosay P, Puget K, Michelson AM (1978) Alterations in two enzymes: superoxide dismutase and glutathion peroxidase in developmental infantile psychosis (infantile autism) Rev Neurol 134:699–705
15. Herken H, Uz E, Ozyurt H, Sogut S, Vyryt O, Akyol O (2001) Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. Mol Psychiatry 6:66–73
16. Horrobin DF, Manku MS, Hillman S, Iain A, Glen M (1991) Fatty acid levels in brains of schizophrenics and normal controls. Biol Psychiatry 30:795–805
17. Johnson S (2001) Micronutrient accumulation and depletion in schizophrenia, epilepsy, autism and Parkinson's disease? Med Hypotheses 56:641–645
18. Keshavan MS, Mallinger AG, Pettegrew JW, Dippold C (1993) Erythrocyte membrane phospholipids in psychotic patients. Psychiatry Res 49:89–95
19. Krenitsky TA, Spector T, Hall WW (1986) Xanthine oxidase from human liver: purification and characterization. Arch Biochem Biophys 247:108–119
20. Lohr JB (1991) Oxygen radicals and neuropsychiatric illness. Some speculations. Arch Gen Psychiatry 48:1097–1106
21. Lombard J (1998) Autism: a mitochondrial disorder? Med Hypotheses 50:497–500
22. Mahadik SP, Mukherjee S (1996) Free radical pathology and antioxidant defense in schizophrenia: a review. Schizophr Res 19: 1–17
23. Mahadik SP, Scheffer RE (1996) Oxidative injury and potential use of antioxidants in shizophrenia. Prostaglandins Leukot Essent Fatty Acids 55:45–54
24. McBride PA, Anderson GM, Hertzig ME, Snow ME, Thompson SM, Khait VD, Shapiro T, Cohen DJ (1998) Effects of diagnosis, race, and puberty on platelet serotonin levels in autism and mental retardation. J Am Acad Child Adolesc Psychiatry 37:767–776
25. McCord JM, Fridovich I (1969) Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 244:6049–6055
26. Omar R, Pappolla M (1993) Oxygen free radicals as inducers of heat shock protein synthesis in cultured human neuroblastoma cells: relevance to neurodegenerative disease. Eur Arch Psychiatry Clin Neurosci 242:262–267
27. Persico AM, Militerni R, Bravaccio C, Schneider C, Melmed R, Trillo S, Montecchi F, Palermo MT, Pascucci T, Puglisi-Allegra S, Reichelt KL, Conciatori M, Baldi A, Keller (2000) Adenosine deaminase alleles and autistic disorder: case-control and family-based association studies. Am J Med Genet 96:784–790
28. Prajda N, Weber G (1975) Malign transformation-linked imbalance: decreased XO activity in hepatomas. FEBS Lett 59:245–249
29. Schopler E, Reichler RJ, DeVellis RF, Daly K (1980) Toward objective classification of childhood autism: Childhood Autism Rating Scale. J Autism Dev Disord 10:91–103
30. Stubbs G, Litt M, Lis E, Jackson R, Voth W, Lindberg A, Litt R (1982) Adenosine deaminase activity decreased in autism. J Am Acad Child Psychiatry 21:71–74
31. Sun Y, Oberley LW, Li Y (1988) A simple method for clinical assay of superoxide dismutase. Clin Chem 34:497–500
32. Turrens JF (2001) Increased superoxide dismutase and Down's syndrome. Med Hypotheses 56:617–619
33. Yao JK, Reddy R, McElhinny LG, van Kammen DP (1998) Effects of haloperidol on antioxidant defense system enzymes in schizophrenia. J Psychiatr Res 32:385–391