Letters to the editor

‘Erythrocyte glutathione system and children with Down syndrome’

Sir—Down syndrome (DS) is one of the most common congenital birth defects. It is associated with several neurobiological abnormalities and is caused by duplication of the genetic material on chromosome 21.

Superoxide dismutase catalyses dismutation of the superoxide anion and produces hydrogen peroxide (H$_2$O$_2$). An increase in activity of cytosolic superoxide dismutase has been reported in the red cells of individuals with DS. Studies have also shown that the cytosolic superoxide dismutase gene is located on the subband 21q22.1 of chromosome 21.

Glutathione (GSH) is found in all living cells and is the most important intracellular thiol: it keeps the sulphydryl-reduction of H$_2$O$_2$ and organic hydroperoxides by oxidizing GSH are more susceptible to chemical and oxidative stress.

Glutathione peroxidase is a selenium protein and an enzyme that prevents oxidative stress. It catalyses the reduction of H$_2$O$_2$ and organic hydroperoxides by oxidizing GSH to glutathione disulfide. Its activity is linked to the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) by glutathione reductase, which is a flavoprotein, causing the production of reduced glutathione in the presence of NADPH.

Fourteen children with DS (male:female 0.8) aged between 4 and 15 years (mean 8.45 ± 4.3 SD) were studied. These children were participating in a programme for children with learning disabilities in Integration High School for Exceptional Children in Eskişehir. Clinical features of DS were observed in all of these children. The control group comprised 14 healthy children of the same age. Venous blood samples were taken and stored in heparinized tubes at −20°C until analysis. Plasma was removed by centrifugation and the red cells were washed three times with isotonic saline to separate the sedimented erythrocytes from the supernate-suspended leukocytes.

The level of reduced glutathione in red cells was determined with the use of glutathione reductase 2-nitrobenzoic acid recalculating assay. Glutathione peroxidase activity was assayed using the method described by Paglia and Valentine. The conversion of NADPH to NADP$^+$ was monitored by recording optical density changes at 340 nm. Glutathione reductase activity was assayed according to the method described by Racker. Briefly, 1 mol Tris-HCl and 0.2 mol ethylidene diamine tetraacetatic acid (pH 8.0) were added to the lysate and incubated at 37°C. After 0.033 mol oxidize glutathione (GSSG) was added to the mixture, the reaction was initiated by adding 2 mol NADPH. The conversion of NADPH to NADP$^+$ was monitored by optical density changes at 340 nm.

The Student's t test was used to analyse the data, P values <0.05 were considered significant. All results were expressed as mean ± SEM.

In the 14 children studied, higher GSH levels were found in children with DS (126.910 ± 5.632 mg/dl) than in the control children (77.079 ± 3.148 mg/dl). This difference was significant (P<0.01).

Glutathione peroxidase and reductase activities have been determined in the present study. Glutathione peroxidase activity was significantly higher in the erythrocytes of the children with DS than in the control children (151.024 ± 8.089 U/mg Hb; 247.145 ± 14.913 U/mg Hb; P<0.05). Glutathione reductase activity was also significantly higher in the children with DS (73.639 ± 3.024 U/mg Hb) than in the control children (118.724 ± 6.444 U/mg Hb; P<0.05).

Overproduction of cytosolic superoxide dismutase in the cells of individuals with DS led to an increased production of H$_2$O$_2$, which may decompose to a hydroxyl radical and possibly lead to the initiation of lipid peroxidation. In vitro studies showed that increased superoxide dismutase activity results in increased lipid peroxidation. Because cell membranes of individuals with DS have different compositions of polyunsaturated fatty acids, these individuals were more prone to lipoperoxidation. Increased lipid peroxidation has been shown to cause brain damage. Lipofuscin accumulation, observed in the brain of individuals with DS, may originate from lipid peroxidation. Neurotransmitter uptake might become impaired due to alterations in the membrane properties of chromaffin granules as a result of lipid peroxidation. Electric membrane properties in the nerve cells of individuals with DS may be disturbed.

H$_2$O$_2$ is removed by glutathione peroxidase. In this study, glutathione peroxidase activity was significantly increased in the red cells. This may be secondary to the increased levels of cytosolic superoxide dismutase due to the acceleration of the peroxide formation processes. This result is in agreement with previous studies.

Glutathione peroxidase plays a part in preventing peroxide accumulation in cells, and in doing so prevents lipid peroxide formation. Glutathione peroxidase is particularly important in brain cells with low levels of catalase and those with high levels of unsaturated fatty acids.

Glutathione has been implicated in many cellular functions including detoxification, regulation of cell-cycle and gene expression, synthesis, protection of proteins and other cellular components, and acting as an antioxidant. The status of GSH mainly effects protein and DNA synthesis. It can detoxify xenobiotics via glutathione-S-transferase, protect thiol groups against reactive oxygen species which are important for the function of many proteins, and can scavenge free radicals non-enzymatically. It reduces H$_2$O$_2$ and lipid peroxides via the glutathione peroxidase reaction.

* Non-UK usage – mental retardation.
2GSH + ROOH → GSSG + ROH + H₂O, preventing propagation of lipid peroxidation. 5,7 Glutathione reduces chromanoxyl radicals and causes regeneration of α-tocopherol which is a chain-breaking antioxidant.

GSH is a tripeptide formed from glutamic acid, cysteine, and glycine, and catalyzed by γ-glutamylcystein synthetase and glutathione synthetase in the presence of ATP. 4,5,8 Oxidation converts it to the disulphide form (GSSG). The total amount of GSH in the cell depends on either recycling of GSSG by glutathione reductase or de novo synthesis. 8

We have found that levels of GSH in children with DS are significantly higher than those in the control children. The increase can be explained by a reduction of GSSG, which is the result of an increased peroxidative process within the cells of individuals with DS. Our result is correlated with an increase in glutathione reductase activity. Increased levels of GSH may be a protective factor in the red cells of individuals with DS because of the role it has in detoxifying reactive oxygen species. These increased levels may also play an important role in protecting the formation and progression of neurobiological abnormalities due to less oxidative damage within the cells of children with DS.

An increase in glutathione reductase activity has been observed. This enzyme maintains glutathione in steady state levels in the cell. In red cells, bezox monophosphate shunt is the only source of NADPH, as a cofactor for glutathione reductase. It has been reported that the bezox monophosphate shunt activity in red cells was increased in individuals with DS. 16 Studies have shown an increase in 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase activities in red cells. 19–21 Increased activity of glutathione reductase could be explained by increased peroxide production which is catalyzed by glutathione peroxidase. It is correlated with increased glutathione peroxidase activity in the red cells of individuals with DS.

In conclusion, cytoplasmic glutathione, glutathione peroxidase, and glutathione reductase might form a powerful antioxidant system against hydrogen peroxide radicals produced in DS red cells and increased GSH may play a pivotal role in the prevention of oxidative damage.

Inal Erdem Mine PhD
Oytun Portakal MD
Filiz Özdemir, PhD student; Department of Biochemistry
Osmangazi University
Eskişehir, Turkey

References
These exclusions may well have been deliberate but should not hide the probability that language disorders, particularly those affecting comprehension and associated with paroxysmal EEG activity especially in sleep, are seen clinically in a spectrum of disorders including pure Landau–Kleffner syndrome, pure developmental dysphasia, some children with benign rolandic epilepsy, children with severe multifocal epilepsy with developmental delay or regression and autistic features, and children with autistic spectrum disorder with little or no obvious epilepsy, but paroxysmal EEG activity.

I am sure that the authors are right to suggest that children with developmental dysphasia should be investigated with sleep EEG studies. And I feel that it is vital for the authors to publish the data they refer to, which demonstrate effective treatment of developmental dysphasia with sodium valproate or clobazam.

The publication of such an intervention study would have a big impact on clinical practice and more importantly on the welfare of children with developmental language disorders.

Dr W Whitehouse
Consultant Paediatric Neurologist
The Birmingham Children’s Hospital
Birmingham, UK

Reference

Alain Picard replies:
SIR—I thank Dr Whitehouse for his comments on our paper ‘Sleep EEG and developmental dysphasia’. First, the purpose of our study was to ascertain the frequency of paroxysmal abnormalities in subjects with developmental dysphasia, as studies already published on this subject have divergent results.

Second, Dr Whitehouse questions whether treatment of paroxysmal abnormalities (sodium valproate or clobazam) improves developmental dysphasia. Paroxysmal abnormalities were not considered to be the cause of language impairment, but they may aggravate the outcome. We have treated the children who have language impairment and verified that paroxysmal abnormalities have disappeared; treatment has been continued if children appeared to be making progress. To demonstrate the efficacy of this practice, analysis additional to the longitudinal analysis of progress in language of children treated needs to be performed. A double-blind study, comparing treated and non-treated children will prove the efficacy of treatment. A study of this kind requires a larger group of children with multicentre involvement. We are ready to participate in such a study.

Dr Alain Picard
Service de Neurologie et de Rééducation Infantiel
Hôpital Raymond Poincarè
104 Boulevard Raymond Poincarè
92380 Garches
France