Arch. Biol. Sci., Belgrade, 67(4), 1297-1302, 2015 DOI:10.2298/ABS150909106C

IDIOPATHIC SUDDEN HEARING LOSS: OXIDATIVE STATUS BEFORE AND AFTER CORTICOID TREATMENT

Bogdan D. Cavaleriu[1,*], Dan V. Mârțu[1], Lucian Hrițcu[2], Oana R. Manolache1 and Luminița M. Rădulescu[1]

[1] *Department of ENT "Grigore T. Popa" University of Medicine and Pharmacy*, Iasi, Romania

[2] *Department of Biology, "Alexandru Ioan Cuza" University*, Iasi, Romania

*__Corresponding author__: cavaleriu@yahoo.com

Abstract: The aim of this study was to investigate antioxidant enzyme activities and lipid peroxidation levels after systemic corticoid therapy (Solu-Medrol, 250mg/day, for 7 days). The effects of corticoid treatment on superoxide dismutase (SOD) and glutathione peroxidase (GPX) activity were investigated. Fifteen patients diagnosed with sudden sensorineural hearing loss were enrolled. Serum markers of oxidative stress were measured using spectrophotometric methods. In ten cases, the SOD and GPX activities and malondialdehyde (MDA) serum levels before and after corticoid treatment were investigated. Corticoid treatment enhanced antioxidant activity by increasing SOD and GPX activities and decreasing MDA serum levels.

Key words: idiopathic; sudden hearing loss; stress; corticoid

Received: September 9, 2015; **Revised**: January 12, 2015; **Accepted**: July 8, 2015

INTRODUCTION

Sudden sensorineural hearing loss (SSNHL) is an emergency in otolaryngology. It has been defined as 30 dB or more sensorineural hearing loss over at least three contiguous audiometric frequencies occurring within 3 days or less. The specific cause is identified in about 10-15% of cases (Cavaleriu, 2013). Various infective (especially viral) (Stokroos et al., 1998), vascular, and immune causes (Cogan's syndrome) (Berrocal and Ramirez-Camacho, 2002) have been proposed, with vascular disorders in cochlear terminal vascularization playing an important role (Tran, 2002).

There are ototoxic drugs that can damage hearing, such as antibiotics (aminoglycosides), diuretics and certain anticancer drugs. Acoustic trauma or trauma such as head injuries and temporal bone fractures can cause SSNHL. About 10% of people with Meniere's disease experience SSNHL. In addition, tumors such a vestibular schwannoma or cerebellopontine angle (CPA) tumors can cause SSNHL. There are many potential causes of SSNHL, but despite extensive evaluation, the majority of cases remain idiopathic.

An increase in reactive oxygen species (ROS) production is assumed to play an important role in sudden hearing loss (Halliwell et al., 1992). Recent studies have shown the importance of oxidative stress, defined as an excess of pro-oxidant species not counterbalanced by an adequate endogenous and exogenous antioxidant defense system (Campise et al., 2003; Sachdev and Davies, 2008), as a risk factor for microvascular damage in different vascular disorders (Halliwell and Gutteridge, 1984; Son, 2007).

The most common ROS are superoxide anion (O_2^-), hydroxyl radical (OH^-), hypochlorite (OCl^-) and nitric oxide (NO^-). ROS are converted to non-reactive molecules by endogenous cellular enzymes, such as copper/zinc superoxide dismutase (SOD1), manganese superoxide dismutase (SOD2), catalase and peroxidase. They are generated *in vivo* as a by-product of mitochondrial respiration, and are also produced via autooxidation of chemical and biological molecules (Seidman et al., 1997).

The imbalance between ROS and total antioxidant capacity (TAC) is thought to be a potential pathoge-

netic mechanism leading to endothelial dysfunction. ROS derived from various oxidation pathways can generate products leading to cellular deregulation (Capaccio et al., 2012). ROS directly damages subcellular structures, such as mitochondrial DNA (mtDNA), creating deletions and mutations, lipid peroxidation, polysaccharide depolymerization, nucleic acid disruption and oxidation of sulfhydryl groups, leading to enzyme inactivation and subsequently producing bioenergetically deficient cells. This cascade of events is responsible for the reduction in the mitochondrial membrane potential and the loss of cochlear hair cells, with an attendant increase in the auditory threshold leads to hearing loss (Darrat et al., 2007).

Regarding the treatment of sudden hearing loss, an important number of clinical studies have noted that the best results in terms of hearing recovery were obtained after corticosteroid administration. Glucocorticoids have been widely used as a therapeutic drug for sudden sensorineural hearing loss (Wilson et al., 1980). However, very little is known about the mechanism(s) underlying the protective effect of glucocorticoids against hearing loss (Nagashima and Ogita, 2006). As an approach toward elucidating the mechanism(s), we evaluated the effects of steroid treatment on superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities and malondialdehyde (MDA) concentration by measuring blood serum-level changes after administration in patients diagnosed with idiopathic sudden sensorineural hearing loss.

MATERIALS AND METHODS

This study included 15 patients with sudden sensorineural hearing loss, according to American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) guidelines of idiopathic sudden sensorineural hearing loss (ISSNHL), and were enrolled at the Otolaryngology Clinic of the Recuperare Hospital between September 2013 and July 2014.

Inclusion criteria comprised patients with sudden sensorineural hearing loss of more than 30 dB over at least three contiguous frequencies with pure tone audiometry observed within 72 h of onset. Exclusion criteria included other etiologies than idiopathic hearing loss (e.g. autoimmune diseases, history of cardiovascular disease or renal insufficiency and diabetes mellitus, head trauma with rupture of round windows membrane, and tumoral cerebral mass –vestibular schwannoma or cerebellopontine angle tumors).

This study was conducted according to the provisions of the Helsinki Declaration and all patients signed a written consent for participation in this study. A careful history and detailed medical examination was made with special attention directed toward the onset time, possible causes and associated symptoms.

The 15 patients underwent intravenous systemic administration of 250mg/day of the corticosteroid, methylprednisolone sodium succinate (Solu-Medrol(r), Pharmacia Enterprises S.A. Belgium), given for 7 days, with audiological and clinical follow-up at the end of the therapy, and one month post-event.

Sample collection and laboratory methods

Blood samples were drawn in the morning from each patient before corticoid treatment and 12 h after the last corticoid administration, and were collected in light-protected tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Serum aliquots were frozen after separation and stored at -80°C until assayed. After treatment, only ten patients accepted blood sampling.

Determination of SOD activity

The activity of superoxide dismutase (SOD) was assayed by monitoring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). Each 1.5 mL reaction mixture contained 100 mM TRIS/HCl (pH 7.8), 75 mM NBT, 2 μM riboflavin, 6 mM EDTA, and 200 μL of blood serum. One unit of SOD is defined as the quantity required to inhibit the rate of NBT reduction by 50% as previously described

by Winterbourn et al. (1975). The enzyme activity is expressed as units/mg protein.

Determination of GPX activity

Glutathione peroxidase (GPX) activity was analyzed by spectrophotometric assay. A reaction mixture consisting of 1 ml of 0.4 M phosphate buffer (pH 7.0) containing 0.4 mM EDTA, 1 mL of 5mM NaN_3, 1 mL of 4 mM glutathione (GSH), and 200 µL of blood serum was pre-incubated at 37°C for 5 min. Then 1 mL of 4 mM H_2O_2 was added and incubated at 37°C for a further 5 min. The excess amount of GSH was quantified by the DTNB method as previously described by Sharma and Gupta (2002). One unit of GPX is defined as the amount of enzyme required to oxidize 1 nmol GSH/min. The enzyme activity is expressed as units/mg protein.

Determination of MDA level

Malondialdehyde (MDA), which is an indicator of lipid peroxidation, was spectrophotometrically measured using the thiobarbituric acid assay as previously described by Ohkawa et al. (1979). Two hundred µL of blood serum was added and briefly mixed with 1 mL of 50% trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. After vortex mixing, samples were maintained at 95°C for 20 min. Samples were centrifuged at 960 x g for 10 min and supernatants were read at 532 nm. A calibration curve was constructed using MDA as standard and the results were expressed as nmol/mg protein.

Estimation of protein concentration

Estimation of protein was done using a BCA protein assay kit (Sigma-Aldrich, Germany). The BCA protein assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantification of total protein, as previously described by Smith et al. (1985).

Statistical analysis

Descriptive statistics were calculated for the continuous (mean ± SEM) variables and comparisons were made using the Student t-test for paired data. *P* values less than 0.05 were considered statistically significant.

RESULTS

Experimental data were registered after 7 days of corticoid systemic administration. The corticoid treatment significantly enhanced the SOD ($p<0.002$) (Fig. 1) and GPX activities ($p<0.0001$) (Fig.2) compared to initial levels (before corticoid treatments).

Oxidative stress generates free radicals that induced peroxidation of the membrane lipids resulting in the formation of MDA. The corticoid treatment significantly decreased the MDA concentration ($p<0.0003$) (Fig. 3).

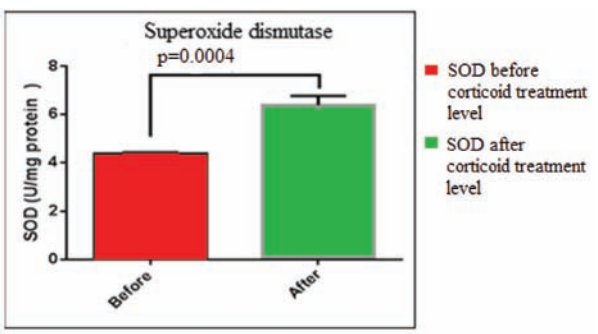

Fig. 1. Effect of corticoid treatment on SOD activity. Data are presented as the mean ± SEM; *p<0.05 vs. initial concentration.

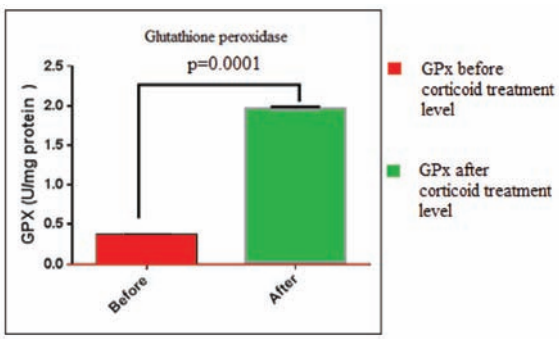

Fig. 2. Effect of corticoid treatment on GPX activity. Data are presented as the mean ± SEM; *p<0.05 vs. initial concentration.

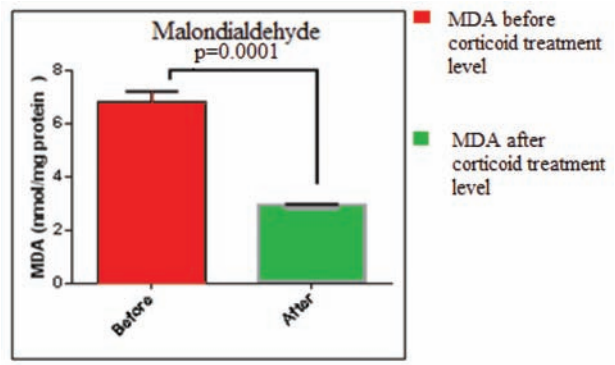

Fig. 3. Effect of corticoid treatment on MDA activity. Data are presented as the mean ± SEM; *p<0.0001 vs. initial concentration.

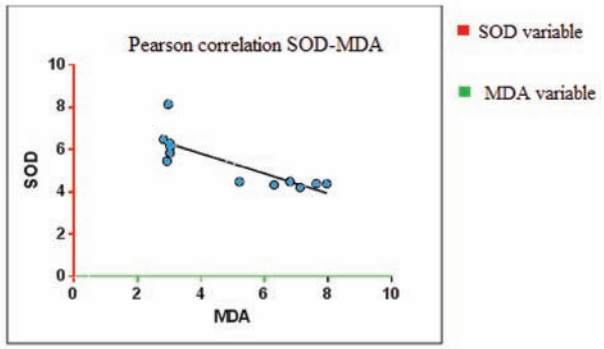

Fig. 4. Pearson correlation SOD-MDA.

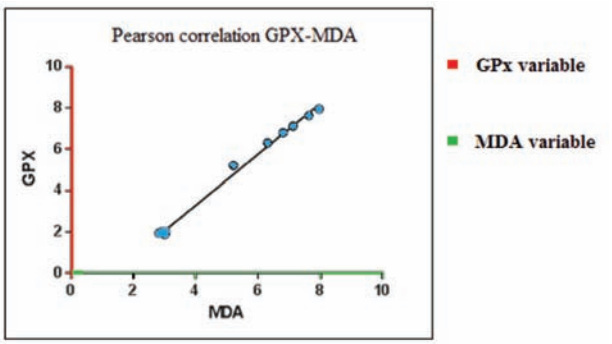

Fig. 5. Pearson correlation GPX-MDA.

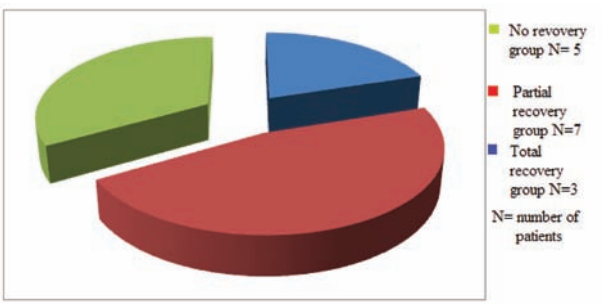

Fig. 6. Patients distrubution by hearing recovery level after corticoid treatment.

Steroid treatment significantly increased the endogenous antioxidant status in SOD and GPX activities. A decrease in the activities of these free radical scavenging enzymes could result in the generation of superoxide anions and hydrogen peroxide, which in turn produce hydroxyl free radicals, the cause of many toxic reactions.

When linear regression was calculated using post-corticoid treatment values, a significant correlation between SOD and MDA (r = -0.8214) (Fig.4) and GPX vs. MDA (r = 0.9977) (Fig.5) was observed. These results suggest that of the increase in antioxidant defense was related to a significant decrease in the lipid peroxidation (MDA) level.

Total hearing recovery was observed in 3 patients, partial recovery in 7 and no recovery in 5 (Fig.6). A statistically significant difference was found between initial audiometry values and after corticoid treatment, p = 0.0002 (p<0.005) for the recovery group. No side effects or complications were noted following treatment.

DISCUSSION

Recent studies have reported that the impaired microvascular perfusion occurring during an ischemic event in vascular disorders, including SSNHL, may be related not only to traditional vascular risk factors such as hypercholesterolemia, hyperfibrinogenemia, hyperhomocysteinemia and microembolisms, but also to oxidative stress, which may be synergically responsible for endothelial damage, especially in terminal microvascular systems (Capaccio et al., 2012).

We analyzed the influence of corticoid treatment on oxidative stress by measuring the activities of SOD and GPX before and after treatment. The beneficial role of corticosteroid therapy in stimulating the ac-

tivation of SOD and GPX, antioxidant enzymes that neutralize $NO^{\cdot}$ was obvious.

In our study, corticoid treatment significantly decreased the MDA concentration. Early and efficient adminitration of corticoid treatment on patients diagnosed with SBI could block or at least ameliorate oxidative injury in the cochlear stria vascularis and thus achieve a satisfactory auditory rehabilitation.

The role of antioxidants in the prevention and management of hearing loss is still under debate. Targeting the oxidant response by antioxidants and modulating specific enzymes (e.g. NO synthases, NADPH oxidase) represent a potential therapeutic strategy; different clinical trials of antioxidant therapy (such as vitamin E, C, beta carotene, coenzyme Q10) have been proposed for main cardiovascular diseases but failed to show any convincing benefits (Mak and Newton, 2001). However, antioxidant therapy is often given not only for traditional cardiovascular diseases but also for otological diseases such as tinnitus and slowly progressive sensorineural hearing loss (Hatano et al., 2008).

Medication with steroids and antioxidants may help in reducing the oxidative stress in the cochlea in SSNHL, implying a new direction in the treatment of this disease.

The small size of our sample may be a limitation on the results statistical significance value. A larger sample was not possible because sudden deafness is a rare condition, and, on the other hand, some patients refused to participate in a study whose results will not affect their immediate hearing recovery. Further studies on a larger population could assist in confirming whether corticoids are implicated in mechanisms underlying the effect in hearing rehabilitation after sudden deafness.

CONCLUSIONS

In this study we describe the increase in antioxidant enzyme (SOD and GPX) activities and decrease in MDA concentration in ISSNHL patients after systemic corticoid treatment. Our results contribute towards the clarification of mechanisms which underlie the protective effect of glucocorticoids against SSNHL.

Acknowledgments: The research was supported by the "Invest in people!" Project co-financed by the European Social Fund Operational Program Human Resources Development 2007 – 2013 Priority Axis 1 "Education and training in support of growth and development of knowledge-based society" KEY AREA OF INTERVENTION 1.5 "Doctoral and postdoctoral programs in support of research" Project Title: "Inter-university partnership to improvement quality and interdisciplinary doctoral medical research by granting doctoral scholarships -DocMed.net" Contract Code: POSDRU/107/1.5/S/78702 Partner 1: "Gr T. Popa" University of Medicine and Pharmacy Iasi.

Authors' contributions: Study conception and design: Bogdan Cavaleriu; acquisition of data: Bogdan Cavaleriu, Oana Manolache; analysis and interpretation of data: Lucian Hrițcu; drafting of manuscript: Bogdan Cavaleriu; critical revision: Prof. Dr. Dan Mârțu, Dr. Luminița Rădulescu

Conflict of intereset disclosure: The authors declare that there is no conflict of interest.

REFERENCES

Berrocal, J.R. and *R. Ramirez-Camacho* (2002). Sudden sensorineural hearing loss: supporting the immunologic theory. *Ann. Otol. Rhinol. Laryngol.* **111**, 989-997.

Campise, M., Bamonti, F., Novembrino, C., Ippolito., S., Tarantino, A., Cornelli, U., Lonati, S., Cesana, B.M. and *C. Ponticelli* (2003). Oxidative stress in kidney transplant patients. *Transplantation.* **76**, 1474-1478.

Capaccio, P., Pignataro, L., Gaini, L., Sigismund, P., Novembrino, C., Giuseppe, R., Uva, V., Tripodi, A. and *Bamonti, F.* (2012). Unbalanced oxidative status in idiopathic sudden sensorineural hearing loss. *Eur. Arch. Otorhinolaryngol.* **269**, 449-453.

Cavaleriu, B., Rădulescu, L. and *D. Mârțu* (2013). Systemic corticosteroids associated with intratympanic corticosteroid treatment for idiopathic sudden hearing loss *Romanian Journal of Oral Rehabilitation.* **5**, 33-38.

Darrat, I., Ahmad, N., Seidman, K., and Seidman, M. D. (2007). Auditory research involving antioxidants. *Curr. Opin. Otolaryngol. Head Neck Surg.* **15**(5), 358-363.

Halliwell, B., Gutteridge, J.M. and *C.E. Cross* (1992). Free radicals, antioxidants, and human disease: where are we now? *J. Lab. Clin. Med.* **119**, 589-620.

Halliwell, B. and *J.M.C. Gutteridge* (1984). Lipid peroxidation, oxygen radicals, cell damage, and antioxidant therapy. *Lancet.* **1**, 1396-1397.

Hatano, M., Uramoto, N., Okabe, Y., Furukawa, M. and *I. Makaoto* (2008). Vitamin E and vitamin C in the treatment of idiopathic sudden sensorineural hearing loss. *Acta Otolaryngol.* **128**, 116-121.

Mak, S. and *G.E. Newton* (2001). The oxidative stress hypothesis of congestive heart failure: radical thoughts. *Chest.* **120**, 2035-2046.

Ohkawa, H., Ohishi, N. and *K. Yagi* (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **95**, 351-358.

Reiko, N. and O. Kiyokazu (2006). Enhanced biosynthesis of glutathione in the spiral ganglion of the cochlea after in vivo treatment with dexamethasone in mice. *Brain Research.* **1**, 101-108.

Sachdev, S. and *K.J. Davies* (2008). Production, detection, and adaptive responses to free radicals in exercise. *Free Radic. Biol. Med.* **44**, 215-223.

Seidman, M.D., Bai, U., Khan, M.J. and *W.S. Quirk,* (1997). Mitochondrial deletions associated with aging and presbyacusis. *Arch. Otolaryngol. Head Neck Surg.***123**, 1039-1045.

Sharma, M. and *Y.K. Gupta* (2002). Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. *Life Sci.* **7**, 2489-2498.

Smith, P.K., Krohn, R.I,, Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and *D.C. Klenk* (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem.* **150**, 76-85.

Son, S.M. (2007). Role of vascular reactive oxygen species in development of vascular abnormalities in diabetes. *Diabetes Res. Clin. Pract*. **77**(suppl. 1), 65-70.

Stokroos, R.J., Albers, F.W. and *J. Schirm* (1998). The etiology of idiopathic sudden sensorineural hearing loss. Experimental herpes simplex virus infection of the inner ear. *Am. J. Otol.* **19**, 447-452.

Tran, B.H. (2002). Endolymphatic deafness: a particular variety of cochlear disorder. *ORL J. Otorhinolaryngol. Relat. Spec.* **64**, 120-124.

Winterbourn, C., Hawkins, R., Brian, M. and *R. Carrell* (1975). The estimation of red cell superoxide dismutase activity. *J. Lab. Clin. Med.* **85**, 337.

Wilson, W.R., Byl, F.M. and *N. Laird* (1980). The efficacy of steroids in the treatment of idiopathic sudden hearing loss. A double-blind clinical study. *Arch. Otolaryngol.* **106(**12), 772-776.