Published in final edited form as:

Neurosci Lett. 2011 September 26; 503(1): 10–14. doi:10.1016/j.neulet.2011.07.053.

SOD1 overexpression improves features of the oligodendrocyte precursor response in vitro

S. Veiga^a, J. Ly^a, P.H. Chan^b, J.C. Bresnahan^a, and M.S. Beattie^{a,*}

^aBrain and Spinal Injury Center, Dept. of Neurological Surgery, University of California, San Francisco

^bDept. of Neurological Surgery, Stanford University

Abstract

Spinal cord injury (SCI) produces a significant loss of oligodendrocytes (OL) and demyelination. The oligodendrocyte precursor cells (OPCs) response includes a group of cellular changes in OPCs that are directed to replenish OL loss from the injury. However, this adaptive response is hampered and OPCs eventually die or fail to differentiate to mature and functional OL. In this study, we wanted to evaluate if overexpression of human superoxide dismutase 1 (hSOD1) in OPCs from the SOD1 transgenic rat could improve some of the features of the OPC response *in vitro*. We found that hSOD1 overexpression increases the proliferation of OPCs and accelerates their differentiation to mature OL *in vitro*. Furthermore, hSOD1 overexpression reduces oxidative stress-mediated death in OPCs. These results suggest hSOD1 as a therapeutic target to increase OPC response success and potentially, OL replacement and remyelination after SCI.

Keywords

oligodendrocyte differentiation; OPC proliferation; oxidative stress; OPC response; superoxide dismutase

Introduction

Oligodendrocyte (OL) death and demyelination have been postulated to contribute to functional deficits after spinal cord injury (SCI) [20]. Oligodendrocyte precursor cells (OPCs) respond to injury adopting a reactive morphology, which includes a hypertrophy of the cell and the overexpression of NG2 proteoglycan, and increasing their proliferation rate in an attempt to replenish the OL population lost after SCI [12]. However, several reports show that this endogenous adaptive response fails and that OPCs die or are unable to differentiate to mature and functional OL [9] [19]. Therapies that might prevent OL loss and promote their replacement are considered as potential therapeutic targets for SCI treatment, and understanding the mechanisms by which OL die and OPC response fail to replace OL loss has become critical.

© 2011 Elsevier Ireland Ltd. All rights reserved.

*Corresponding author: Michael S. Beattie, Ph.D. Brain and Spinal Injury Center, University of California, San Francisco, 1001 Potrero Ave, Bldg 1, Rm 101, San Francisco, CA 94110, michael.beattie@ucsf.edu 415-206-3859.

Declaration: The authors declare no competing interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Recently, it has been proposed that oxidative stress might be involved in the failure of the OPC response after SCI [19]. Radical superoxide is one of the main contributors to cellular oxidative stress and has been reported to reduce OPC survival both directly [1] and indirectly, through the formation of other reactive oxygen species such as peroxynitrite [10]. Superoxide dismutase (SOD) constitutes the natural defense of cells against radical superoxide-mediated oxidative stress and increasing SOD activity by overexpression or exogenous treatment protects OPCs in some *in vitro* paradigms of oxidative stress [3, 7]. However, whether increasing SOD activity may have any effect on the OPC response has never been tested.

Here we have isolated OPCs from the cerebral cortices of the overexpressing-hSOD1 transgenic rat and have used them to test if decreasing radical superoxide affects OPC proliferation, differentiation to mature OL and OPC survival to oxidative stress *in vitro*, all of which are crucial events in the OPC response after SCI.

Material and Methods

SOD1 genotyping

Transgenic rats over-expressing the human SOD1 gene (hSOD1) [18] were used in this study. Amplification of the hSOD1 gene by PCR was performed to identify hSOD1-overexpressing rats. Briefly, DNA was extracted from the tail of adult or newborn Sprague-Dawley rats with the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA, USA) following manufacturer instructions. The PCR reaction was performed in a thermo cycler (PTC 100, MJ Research Inc., Massachusetts, USA) on 2.5 μ l of genomic DNA with 1.25U of Taq polymerase (Invitrogen, Eugene, Oregon, USA) and 0.5 μ M of hSOD1 primers in a total volume of 25 μ l. The amplification protocol followed was: 1 cycle of 10 min at 95°C; 35 cycles of 30 sec at 95°C, 30 sec of annealing at 60°C and 40 sec of elongation at 72°C; 1 cycle of final elongation at 72 °C for 7 min. The primers used for hSOD1 amplification were: *forward* CCA TCT CCC TTT TGA GGA CA and *reverse* AGG CAT GAG GAT CAA TGG AG and the resulting amplification fragment was 507 bp in length. The PCR products were resolved onto a 1 % agarose gel at 70mV. This technique was verified to produce the same genotyping results than isoelectric focusing gel electrophoresis method used previously to genotype SOD transgenic rats.

Cell culture

All procedures were approved by the IACUC at UCSF. OPCs were isolated from postnatal day 1–2 Sprague-Dawley rat pups as described in detail by Miller et al. [13] with the addition of a final purification step where depletion of contaminating microglia was further guaranteed through positive isolation using goat anti-mouse IgG magnetic beads (Invitrogen) pre-coated with anti-CD11b antibody (Serotec, Raleigh, NC, USA). The resulting cell suspension was plated at a 20000 cells/well. These OPC/OL cultures were >98% pure with only 2% of contaminating microglia as verified by immunocytochemistry against olig2 (1:2000, Millipore, Temecula, CA, USA) and CD11b (Serotec, 1:300). OL cultures were prepared using the same procedure as OPC cultures but replacing PDGF alpha and bFGF with 0.33 μ g/ml L-thyronine (T3) and 0.40 μ g/ml L-thyroxine in the culture media. Serum was not present at any time during OPC/OL isolation to prevent serum mediated-OPC to OL differentiation.

Immunocytochemistry

A2B5 has been widely used as a marker for OPCs in culture [13]. Furthermore, A2B5 surface expression is used for immunopanning to selectively isolate OPCs from other contaminating cell types or more mature OLs [17]. For these reasons, we considered A2B5

as the best marker to detect OPCs in our experimental setting. Pilot studies showed that under our experimental conditions all cells co-stained A2B5 with NG2 or with olig 2 after 1 or 2 days in vitro, showing that all cells reported in our experiments were from the oligodendrocyte-cell lineage and from an initial immature state.

Cells were fixed with 4% PFA in PBS 0.01 M with 4% sucrose for 10 minutes and rinsed with PBS. When A2B5 staining was needed, cells were incubated with A2B5 antibody (1:5, ATCC, Manassas, VA, USA) at 37°C and 5% CO₂ for 15 minutes before fixing. Cells were then washed three times with washing buffer (PBS with 0.3% BSA) and incubated overnight with primaries antibodies in washing buffer containing 5% normal goat serum (NGS). For MBP (1:500, Millipore), O_x42 (Serotec, 1:300) and Olig2 (Millipore, 1:2000) staining, the washing buffer and primary incubation solution contained 0.3% Triton. Cells were rinsed later three times with washing buffer during 10 minutes and incubated with respective alexa Fluor 594 or alexa 488 goat secondary antibodies (Invitrogen, 1:200) for 1 hour and 30 minutes. Three additional washes of 10 minutes with PBS were performed before mounting with Vectashield with DAPI (Vector, Burlingame, CA, USA).

To assess OPC proliferation, double immunocytochemistry with A2B5 and BrdU was performed. BrdU (20uM, Roche, Mannheim, Germany) was added to OPC cultures 2 hours after plating and maintained in culture until end of the experiment (approximately 24 or 48 hrs after plating). After fixation, cells were rinsed three times during 10 minutes with PBS with 0.3% BSA and incubated with alexa Fluor 594 goat anti mouse IgM (Invitrogen, 1:200) for 1 hour and 30 minutes. Coverslips were rinsed with PBS and post-fixed with methanol for 10 min at -20°C, followed by DNA denaturation with 2N hydrochloric acid for 30 min. Acid was neutralized with borate buffer 0.1M pH 8.5 for 10 minutes and coverslips were incubated overnight with anti-BrdU (1:50, G3G4, developed by Stephen J Kaufman, Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, USA) in washing buffer containing 0.3% Triton and 5% NGS. After three washes of 10 minutes with washing buffer with 0.3% Triton, coverslips were incubated with alexa Fluor 488 goat anti mouse IgG1 (1:200, Invitrogen) for 1 hour 30 minutes, rinsed with PBS and mounted with Vectashield with DAPI (Vector).

Cell counts

A similar protocol as described previously [13] was used to determine cell number. Cell counts were performed in a blinded-fashion using a Nikon Microphot-FXA microscope with a 25X objective and a false magnification of 1.5X. Briefly, continuous, non-overlapping fields were evaluated across the entire diameter of the coverslip, using the eyepiece reticule as the counting frame (area 0.044 mm²) and considering both top and right line of the reticule as inclusion lines. Live cells were defined as cells with intact, non-pyknotic and/or non-fragmented nuclei and with intact staining of any of the oligodendrocyte lineage surface markers used in this study (A2B5 or MBP). Results are expressed as cells per mm².

Cytotoxicity assay

In cytotoxicity experiments, OPCs were allowed to attach to the culture dish for 2 hours before the addition of t-BuOOH. Cytotoxicity was measured in cell culture supernatants using the LDH cytotoxicity detection kit (Roche) following manufacturer instructions. Data is expressed as % cytotoxicity, which was calculated using WT cultures treated with 2% Triton as a maximum LDH release control and cell culture media alone as the minimum LDH release control.

Results

SOD1 overexpression increases proliferation rate and OPC number *in vitro*

To evaluate if hSOD1 overexpression in OPCs affected their proliferation rate *in vitro*, OPCs from WT and hSOD1 overexpressors were plated at the same cell density (20,000 cells per well) and the percentage of proliferating OPCs as well as the total number of OPCs were assessed after 1 and 2 days *in vitro* (DIV). We found that the percentage of proliferating OPCs, defined as the percentage of A2B5 immunoreactive (ir) cells that were also immunostained with the proliferative marker BrdU, was significantly increased (t-test, $p=0.003$) in hSOD1-overexpressing OPCs after 1 DIV (Figure 1B). Consistent with this finding, the total number of A2B5 immunoreactive cells at 2 DIV was found to be significantly higher (t-test, $p=0.004$) in hSOD1-overexpressing OPCs than in WT OPCs (Figure 1C).

SOD1 overexpression in OPCs accelerates the acquisition of a mature oligodendrocyte phenotype *in vitro*

OPCs from WT and hSOD1-overexpressing animals were plated and treated with thyroid hormones to induce their differentiation to OL. OPCs go through different maturation stages *in vitro* until they reach a final maturation stage characterized by the expression of Myelin Basic Protein (MBP) [14]. In addition to MBP acquisition, OPCs also acquire a more complex morphology throughout the maturation process, going from bipolar or poorly ramified to highly branched cells, sometimes forming membrane sheets. The acquisition of a mature phenotype by OPCs was evaluated by determining the % of MBP immunoreactive (ir) oligodendrocytes with branched morphology at different maturation times (1, 3 and 5 DIV).

Two-way ANOVA showed no significant interaction of “genotype” (WT, SOD) by “days *in vitro*” (1, 3 and 5 days) on the percentage of MBP ir OL ($p=0.532$). As expected, we found a significant effect of the factor “days *in vitro*” on the percentage of MBP ir cells ($p<0.001$), indicating that the percentage of MBP ir OLs significantly increases over time in both genotypes (Figure 2B). However, a significant effect of genotype ($p=0.035$) was also detected suggesting that hSOD1 overexpression in OPCs accelerates the differentiation to mature OLs (Figure 2B). In agreement with this, we observed that hSOD1-overexpressing OL appeared more ramified than WT OLs at 5D IV (Figure 2A).

Overexpression of hSOD1 in OPCs reduces oxidative stress-mediated death in OPCs, but not in mature OL

We wanted to evaluate if OPCs from hSOD1 overexpressors were more resistant to oxidative stress than OPCs obtained from WT animals. With that purpose, we exposed OPCs from WT and SOD1 overexpressors to different doses of tert-butyl-hydroperoxide (t-BuOOH), a membrane-permeant oxidant that induce oxidative stress in OPC cultures [6] [17]. Two-way ANOVA revealed no interaction of genotype (WT, SOD) by dose of t-BuOOH (0, 0.5, 2.5, 5, 25, 50, 250). In agreement with this, t-BuOOH produced a significant dose-dependent increase of cytotoxicity in OPC cultures independently of their genotype (dose effect, $p<0.001$). However, we found a significant effect of genotype as well ($p=0.008$), showing that t-BuOOH-mediated cytotoxicity is reduced in OPCs from hSOD1 overexpressors compared to OPCs from WT animals (Figure 3A).

We also evaluated whether hSOD1 overexpression increased resistance to oxidative stress in mature OL cultures. With that purpose, we isolated OPCs from WT and hSOD1-overexpressing animals and differentiated them *in vitro* by culturing them in the presence of thyroid hormones for 5 days. In contrast to OPC cultures, t-BuOOH treatment did not

increase cellular death in mature OL cultures, even when they were exposed for 24 hours to the highest concentration of t-BuOOH used in the OPC cultures (250 μ M) (Figure 3B). No interaction was found between the factors “genotype” and “dose of t-BuOOH” ($p=0.581$) nor a significant effect of genotype ($p=0.274$) nor the dose of t-BuOOH ($p=0.577$). These results indicate that mature OL are more resistant than OPCs to oxidative stress and that hSOD1 overexpression in mature OL has no effect on their response to oxidative stress *in vitro*.

Discussion

The mechanisms that govern OL loss and their potential replacement by OPCs have received attention from spinal cord injury researchers in the last several years [20]). The present study demonstrates that hSOD1 overexpression in OPCs not only augments OPC resistance to oxidative stress *in vitro*, but also increases OPC proliferative rate and accelerates the acquisition of a mature OL phenotype, all of which are key features in the OPC response *in vivo*. These *in vitro* results suggest that oxidative stress in OPCs could be one of the mechanisms that prevent OPC response success after SCI by reducing OPC survival and through affecting OPC proliferation and differentiation.

Numerous studies show that oligodendrocyte-lineage cells, and particularly OPCs and immature OLs, are very sensitive to oxidative damage in different *in vitro* paradigms, including cystine deprivation-induced glutathione depletion [1, 3], hyperoxia [7] and treatment with oxidative agents [2] including t-BuOOH [17] [6]. Radical superoxide plays a key role in the production of oxidative stress in these paradigms as it can induce oxidative stress by itself or through the formation of other reactive oxygen species [1] [10]. In agreement with this, we found that overexpression of superoxide dismutase, the natural antioxidant defense against radical superoxide, reduces t-BuOOH-mediated death in OPC cultures. It is important to note that SOD1-mediated protection of OPCs is still observed even when we used high doses of t-BuOOH (250 μ M), that produced as much cytotoxicity as 2% of Triton.

On the other hand, t-BuOOH, even at the highest dose and exposed for a longer period of time, failed to produce significant death in mature OLs, indicating that mature OLs are more resistant to oxidative stress than OPCs. Increased resistance of mature OLs to oxidative stress compared to OPCs has been reported previously and it is explained by a developmental upregulation of free radical scavengers such as glutathione [5] and of antioxidant enzymes like glutathione peroxidase [2] and SOD [3]. Three different isoforms of SOD have been described in humans that differ in their cellular location as well as in their prosthetic group. SOD1 and SOD3 have a copper, zinc-containing prosthetic group (Cu,ZnSOD) and are localized in the cytoplasm and the extracellular space, respectively. On the other hand, the prosthetic group of SOD2 contains manganese (MnSOD) and this isoform is confined to the mitochondria [8]. In all of our experiments, we have used OPCs isolated from a transgenic rat that overexpresses the cytosolic CuZnSOD isoform or from their wildtype littermates. Cellular CuZnSOD enzymatic activity in transgenic hSOD1 - overexpressing rats increased four to six-fold compared with that in wild-type littermates in several regions of the nervous system, including hippocampus, striatum and spinal cord [18]. Previous reports have shown that MnSOD and not CuZnSOD is the isoform that is upregulated during OL development, suggesting that MnSOD is the isoform responsible for the protection against oxidative stress in mature OLs [3]. Furthermore, MnSOD overexpression protects OPCs against cystine deprivation-mediated oxidative stress, indicating that mitochondrial radical superoxide production might be what dictates oxidative stress in OPCs *in vitro* [3]. Although hSOD1 is a CuZnSOD and under normal condition is mainly expressed in the cytosol and not in the mitochondria, we found that hSOD1

overexpression in OPCs increased their resistance to t-BuOOH-mediated oxidative stress. This apparent contradiction with the results previously discussed, is easily explained by the finding that overexpressing-hSOD1 transgenic rats also express hSOD1 in the mitochondria in significant higher quantities than WT littermates [21].

Interestingly, some reports have shown a paradoxical increase in cytotoxicity by SOD1 overexpression in OPC cultures [7]. The authors explained this by an excess of intracellular hydrogen peroxide production caused by increased SOD1 activity that exceeded peroxidase activity in OPCs. However, the same authors show that exogenous treatment with SOD protects OPCs from oxidative stress [7], suggesting that even though increasing SOD activity is protective against oxidative stress, peroxidase activity needs to be accordingly increased to prevent further hydrogen peroxide toxicity. We did not find any increase in cytotoxicity in SOD1-overexpressing OPCs, suggesting that peroxidase activity has not been overwhelmed in our experiments. However, we did find a not significant trend to cytotoxicity in SOD1-overexpressing OL cultures, highlighting the importance of considering peroxidase activity when designing a treatment based in increasing SOD activity.

The effect of redox status in OPC proliferation has received special attention in the last years (for a review see [15]). It has been reported that a reduced state is associated with self-renewal [17]. It is tempting to hypothesize that hSOD1 overexpression might produce a more reduced state in OPCs by diminishing radical superoxide accumulation and that this would subsequently lead to an increase in the proliferation rate as described in [17]. However, hydrogen peroxide levels are also expected to be increased by hSOD1 overexpression and some reports also showed that hydrogen peroxide can induce proliferation in some cells types [11]. Additional experiments that directly measure redox state are needed to define if hSOD1 overexpression leads to a more reduced or oxidized state in our experiments. Nevertheless, it is reasonable to argue that the increase on OPC proliferation by hSOD1 overexpression that we observed in our experiments might be due to a modulation of redox status in OPCs.

Finally, we found that hSOD1 overexpression accelerates the acquisition of a mature phenotype, suggesting that radical superoxide might not only be involved in OPC death but also might affect directly OPC differentiation process. In agreement with this, it has been described that redox state dictates the self-renewal or maturation in OPC cultures [17] and that oxidative stress disrupts oligodendrocyte maturation through the modulation of key genes involved in OL differentiation and by preventing global histone deacetylation [6]. On the other hand, It is reported that high cell density induces OPC maturation [16] and we have described that the number of OPCs in hSOD1 cultures is significantly higher than their wild types at 2 DIV. One might think that it is possible that the acceleration of acquiring a mature phenotype in hSOD1-overexpressing OPC might be produced by a higher cell density in hSOD1 OPC cultures as compared to wild-type OPCs. In agreement with this, the total number of cells in hSOD1 overexpressing cultures was found to be significantly higher than that of WT cultures (30.35 ± 2.14 in WT vs 45.61 in SOD1 after 5 DIV, data are mean $\pm$ SEM). Nevertheless, either by affecting redox status or through increasing cell density, hSOD1 overexpression promotes OPC differentiation to mature OL. Acceleration of the acquisition of a mature phenotype would potentially increase OPC response success *in vivo*, because mature OL are more resistant not only to oxidative stress as commented above, but also to glutamate toxicity [4], another key factor that have been related to cell death after SCI.

These results suggest that reducing superoxide formation might be a useful therapeutic target to increase OPC survival, proliferation and differentiation after SCI. Currently, we are measuring OL survival and OPC proliferation and differentiation in hSOD1-overexpressing

rats after SCI, to see if these *in vitro* results translate into the more complex situation of the injured spinal cord.

Conclusions

Our findings indicate that SOD1 over-expression in OPCs is protective, and enhances the OPC response *in vitro*. These results encourage the use of SOD1mimetics or superoxide scavengers as a therapeutic tool to prevent OPC response failure after SCI and to reestablish OL function after SCI.

Acknowledgments

We thank Dr. Brandon Miller for expertise advice during OPC isolation and culture. This research is funded by the Craig H. Nielsen Foundation and the NIH grants NS- 31193 and NS-38079.

References

1. Back SA, Gan X, Li Y, Rosenberg PA, Volpe JJ. Maturation-dependent vulnerability of oligodendrocytes to oxidative stress-induced death caused by glutathione depletion. *J Neurosci*. 1998; 18:6241–6253. [PubMed: 9698317]
2. Baud O, Greene AE, Li J, Wang H, Volpe JJ, Rosenberg PA. Glutathione peroxidase-catalase cooperativity is required for resistance to hydrogen peroxide by mature rat oligodendrocytes. *J Neurosci*. 2004; 24:1531–1540. [PubMed: 14973232]
3. Baud O, Haynes RF, Wang H, Folkert RD, Li J, Volpe JJ, Rosenberg PA. Developmental up-regulation of MnSOD in rat oligodendrocytes confers protection against oxidative injury. *Eur J Neurosci*. 2004; 20:29–40. [PubMed: 15245476]
4. Deng W, Rosenberg PA, Volpe JJ, Jensen FE. Calcium-permeable AMPA/kainate receptors mediate toxicity and preconditioning by oxygen-glucose deprivation in oligodendrocyte precursors. *Proc Natl Acad Sci U S A*. 2003; 100:6801–6806. [PubMed: 12743362]
5. Frago G, Martinez-Bermudez AK, Liu HN, Khorchid A, Chemtob S, Mushynski WE, Almazan G. Developmental differences in HO-induced oligodendrocyte cell death: role of glutathione, mitogen-activated protein kinases and caspase 3. *J Neurochem*. 2004; 90:392–404. [PubMed: 15228596]
6. French HM, Reid M, Mamontov P, Simmons RA, Grinspan JB. Oxidative stress disrupts oligodendrocyte maturation. *J Neurosci Res*. 2009; 87:3076–3087. [PubMed: 19479983]
7. Gerstner B, DeSilva TM, Genz K, Armstrong A, Brehmer F, Neve RL, Felderhoff-Mueser U, Volpe JJ, Rosenberg PA. Hyperoxia causes maturation-dependent cell death in the developing white matter. *J Neurosci*. 2008; 28:1236–1245. [PubMed: 18234901]
8. Johnson F, Giulivi C. Superoxide dismutases and their impact upon human health. *Mol Aspects Med*. 2005; 26:340–352. [PubMed: 16099495]
9. Labombarda F, Gonzalez SL, Lima A, Roig P, Guennoun R, Schumacher M, de Nicola AF. Effects of progesterone on oligodendrocyte progenitors, oligodendrocyte transcription factors, and myelin proteins following spinal cord injury. *Glia*. 2009; 57:884–897. [PubMed: 19053058]
10. Li J, Baud O, Vartanian T, Volpe JJ, Rosenberg PA. Peroxynitrite generated by inducible nitric oxide synthase and NADPH oxidase mediates microglial toxicity to oligodendrocytes. *Proc Natl Acad Sci U S A*. 2005; 102:9936–9941. [PubMed: 15998743]
11. Mander PK, Jekabsone A, Brown GC. Microglia proliferation is regulated by hydrogen peroxide from NADPH oxidase. *J Immunol*. 2006; 176:1046–1052. [PubMed: 16393992]
12. McTigue DM, Wei P, Stokes BT. Proliferation of NG2-positive cells and altered oligodendrocyte numbers in the contused rat spinal cord. *J Neurosci*. 2001; 21:3392–3400. [PubMed: 11331369]
13. Miller BA, Crum JM, Tovar CA, Ferguson AR, Bresnahan JC, Beattie MS. Developmental stage of oligodendrocytes determines their response to activated microglia *in vitro*. *J Neuroinflammation*. 2007; 4:28. [PubMed: 18039385]
14. Nicolay DJ, Doucette JR, Nazarali AJ. Transcriptional control of oligodendrogenesis. *Glia*. 2007; 55:1287–1299. [PubMed: 17647291]

15. Noble M, Mayer-Proschel M, Proschel C. Redox regulation of precursor cell function: insights and paradoxes. *Antioxid Redox Signal*. 2005; 7:1456–1467. [PubMed: 16356108]
16. Rosenberg SS, Kelland EE, Tokar E, De la Torre AR, Chan JR. The geometric and spatial constraints of the microenvironment induce oligodendrocyte differentiation. *Proc Natl Acad Sci U S A*. 2008; 105:14662–14667. [PubMed: 18787118]
17. Smith J, Ladi E, Mayer-Proschel M, Noble M. Redox state is a central modulator of the balance between self-renewal and differentiation in a dividing glial precursor cell. *Proc Natl Acad Sci U S A*. 2000; 97:10032–10037. [PubMed: 10944195]
18. Sugawara T, Noshita N, Lewen A, Gasche Y, Ferrand-Drake M, Fujimura M, Morita-Fujimura Y, Chan PH. Overexpression of copper/zinc superoxide dismutase in transgenic rats protects vulnerable neurons against ischemic damage by blocking the mitochondrial pathway of caspase activation. *J Neurosci*. 2002; 22:209–217. [PubMed: 11756504]
19. Sun F, Lin CL, McTigue D, Shan X, Tovar CA, Bresnahan JC, Beattie MS. Effects of axon degeneration on oligodendrocyte lineage cells: Dorsal rhizotomy evokes a repair response while axon degeneration rostral to spinal contusion induces both repair and apoptosis. *Glia*. 2010; 58:1304–1319. [PubMed: 20607865]
20. Wu B, Ren X. Promoting axonal myelination for improving neurological recovery in spinal cord injury. *J Neurotrauma*. 2009; 26:1847–1856. [PubMed: 19785544]
21. Yu F, Sugawara T, Nishi T, Liu J, Chan PH. Overexpression of SOD1 in transgenic rats attenuates nuclear translocation of endonuclease G and apoptosis after spinal cord injury. *J Neurotrauma*. 2006; 23:595–603. [PubMed: 16689664]

Highlights

- Overexpression of hSOD1 increases proliferation rate and oligodendrocyte precursor cells (OPCs) *in vitro*.
- SOD1 overexpression in OPCs accelerates the acquisition of a mature oligodendrocyte (OL) phenotype *in vitro*.
- Overexpression of hSOD1 in OPCs, but not mature OLs, rescues cells from oxidative stress-mediated death.

Fig. 1. OPC proliferation and OPC number after 1 and 2 DIV

We evaluated the proliferation and total number of WT and hSOD1-overexpressing OPCs at 1 and 2 days *in vitro* (DIV). **(A)** Representative images of hSOD1 OPC cultures after 2DIV immunostained with the OPC marker A2B5 (left), the proliferation marker BrdU (middle), and merged (right). Arrows points to two proliferating OPCs defined as A2B5 and BrdU immunoreactive cells. Scale bar 20 μm **(B)** Percentage of proliferating OPCs after 1 DIV. Bars are mean ± SEM. Asterisks represent statistical significance $p < 0.05$. **(C)** Number of OPCs after 2 DIV. Bars are mean ± SEM. Asterisks represent statistical significance $p < 0.05$.

Fig. 2. Percentage of mature OL in WT and SOD1 OL culture

OPCs from WT and hSOD1 overexpressors were plated and the mature OL marker MBP was detected by immunocytochemistry at 1, 3 and 5 days after plating. **(A)** Representative pictures of wild type (WT) and hSOD1-overexpressing (SOD1) OL immunoreactive to the mature oligodendrocyte marker MBP after 5 days *in vitro* (DIV). Scale bar: 40 mm. **(B)** Percentage of MBP immunoreactive (ir) oligodendrocytes (OL) after 1, 3 or 5 days *in vitro* (DIV). Two Way ANOVA revealed no Interaction between the factors “days *in vitro*” (1, 3 and 5 days) and “genotype” (WT and SOD1). However a significant effect of “genotype” ($p=0.035$) on the % MBP immunoreactive cells was found, revealing that hSOD1 overexpression accelerate the maturation of OPCs *in vitro*. As expected we also found a significant effects of “days *in vitro*”, reflecting that OPCs from both genotypes differentiate into MBP-expressing OLs over time. Data are mean $\pm$ SEM, * shows “genotype” effect statistical significance, ($p < 0.05$) and ### shows statistical significance of the factor “days *in vitro*”, $p < 0.001$.

Fig. 3. Resistance to t-BuOOH-mediated oxidative stress in OPC and OL cultures

Evaluation of Tert-Butyl hydroperoxide (t-BuOOH)-mediated cytotoxicity in WT and hSOD1-overexpressing OPC cultures. OPCs from WT and hSOD1 overexpressors were plated and treated with different doses of the oxidant t-BuOOH and cytotoxicity was measured after 8 hours. Bars are mean $\pm$ SEM. Two way ANOVA revealed no interaction of “genotype”(WT, SOD1) by “t-BuOOH dose” (0, 0.5, 2.5, 5, 25, 50 and 250 μ M). However, a significant effect of genotype was found ($p=0.008$), showing that t-BuOOH-mediated cytotoxicity in hSOD1 overexpressing OPCs is reduced compared to OPCs-derived from WT animals. (Asterisks represent statistical significance $p<0.05$). A significant effect of t-BuOOH dose was also found (not shown) (**B**) Evaluation of Tert-Butyl hydroperoxide (t-BuOOH)-mediated cytotoxicity in WT and hSOD1-overexpressing OL cultures. Mature OLs were treated with different doses of the oxidant t-BuOOH and cytotoxicity was measured after 24 hours. Bars are mean $\pm$ SEM. Two way ANOVA revealed no interaction of “genotype”(WT, SOD1) by “t-BuOOH dose” (0, 5, and 250 μ M). No significant effect of “genotype” or “t-BuOOH dose” was found.