PERSPECTIVES

How do corticosteroids influence myelin genesis in the central nervous system?

Clinical use of corticosteroid (CS) therapy and links with adverse neurological effects

CS therapy is widely used in clinical practice worldwide, with administration of high and multiple doses prescribed for a range of disease and injury. Notably, CS have been used since the 1950's for the treatment of suspected respiratory distress syndrome, in order to accelerate lung maturation in premature babies, as also in antenatal therapy to pregnant women at risk of preterm birth (Haddad et al., 1956; Shinwell and Eventov-Friedman, 2009; Bonanno and Wapner, 2012). High dose immunosuppressive CS therapy is also widely used in the treatment of multiple sclerosis (MS) and spinal cord injury (Bracken, 2012; Burton et al., 2012). However, several reports have raised serious concerns regarding the adverse neurological consequences of CS use (including neurodevelopmental impairments and a suggested link with cerebral palsy) (Shinwell and Eventov-Friedman, 2009; Reynolds and Seckl, 2012). Data from experimental studies in rodents and sheep have demonstrated that CS treatment can significantly delay cerebral myelination in major white matter (WM) tracts including in areas such as the optic nerve and corpus callosum (Bohn and Friedrich, 1982; Antonow-Schlorke et al., 2009; Shields et al., 2012) as well as the spinal cord (**Figure 1**, our unpublished data). Axon loss has not been reported in conjunction with the impairment of myelination in the timeframes studied. Similarly, a handful of studies in recent years have shown that CS treatment can significantly delay the production of new myelin (re-myelination) around axons in adult animal models following induction of experimental myelin loss (demyelination) in the central nervous system (CNS) (Chari et al., 2006; Clarner et al., 2011). Remyelination is an important regenerative process that is likely to contribute to the remission of the clinical signs of the disease in conditions such as MS (Franklin and Gallo, 2014). Such regeneration delays have been suggested to render axons vulnerable to degeneration, particularly within the inflammatory environment of MS lesions (Chari et al., 2006). The mechanisms underlying CS-induced myelination delays during CNS development and during regenerative events are not known, and this remains a little researched area of experimental neurology.

The biological role of myelin and process of myelin genesis

CS are highly effective immunosuppressive agents–this is not a matter of debate. However, it is essential to elucidate the mechanisms that underpin the adverse effects of CS based immunotherapies on myelination, given our knowledge that an intimate relationship exists between myelin and axonal function/survival. For example, the ordered spatial arrangement of ion channels at the nodes of Ranvier (and therefore the conductive properties of axons) critically depends on correct myelination (Nie et al., 2006). Further, it has been demonstrated that axons can sprout aberrant branches in the absence of the inhibitory influence of myelin (Huang et al., 2005). In myelin mutants lacking compact myelin, axons display increased slow axonal transport, as well as abnormalities in cytoskeletal components, and metabolic function (Brady et al., 1999; Andrews et al., 2006). Oligodendrocytes are increasingly regarded to be critical for the survival and integrity of axons, possibly by providing trophic support or via axon-glia metabolic coupling mechanisms (Funfschilling et al., 2012; Oluich et al., 2012). This in turn has led to the widely held opinion in regenerative neurology, that myelin exerts an important neuroprotective role on axons (and by extension, has a critical impact on disease progression when the process is impaired). Indeed, deficiencies in myelin proteins have been demonstrated to lead to axonal degeneration (Lappe-Siefke et al., 2003). However, at least one recent long term study using mutant mice, that lack myelin due to defects in the gene encoding Myelin Basic Protein (or MBP, a major constituent of myelin), reports that no signs of axonal degeneration were observed despite aberrant myelination in these mutants (Smith et al., 2013).

From research in the past two decades, we know a considerable amount about the biology of myelination (Franklin and Gallo, 2014). Myelin genesis is a complex biological process, both during development and following myelin loss/damage, and is mediated by cells of the oligodendrocyte lineage–namely the oligodendrocytes and their parent cells, the oligodendrocyte precursor cells (OPCs). The OPCs are a major migratory and proliferative population of stem-like cells found in both the developing and adult CNS, which differentiate into oligodendrocytes during myelination. The latter engage with axons in developing WM tracts or lesions, and generate functional myelin around these axons (**Figure 2**). There is tight orchestration of events, both in terms of the timing of the stages of myelination and the molecular expression profiles that regulate these stages (Crawford et al., 2013). Besides cells of the oligodendrocyte lineage, other glial subpopulations such as the microglia and astrocytes are also implicated as having a major role in myelin genesis. For example, astrocytes play a key role as mediators of myelination, by influencing processes such as OPC migration and differentiation (Barnett and Linington, 2013). The secretion of a number of cytokines and other classes of soluble mediators by microglia has also been shown to influence the development of oligodendrocytes (Pang et al., 2013).

Potential mechanisms of CS effects on myelin production

Current CS administration regimens means that such treatment could delay myelination by influencing one or more of the above processes, but the effects of CS on this intricate process are far from clear. In respect of the mechanisms of drug action on neural cells, CS are highly lipophilic agents and can therefore cross cell membranes with relative ease; once in the cytosol, these drugs bind to their cognate cytosolic ligand-activated receptors (George et al., 2009). The drug-receptor complexes then translocate to the nucleus where these act on hormone response elements to regulate the associated genes, in other words acting like transcription factors. The actual cellular targets of CS action have not been resolved so far. It is not clear whether aberrations in myelination are underpinned by a '*direct*' CS action on cells involved in myelin genesis (the oligodendrocytes and OPCs) and/or an '*indirect*' action on other neural cell populations such as the astrocytes and/or microglia. Indirect CS effects *in vivo*, mediated *via* the endocrine system, for example via modifications

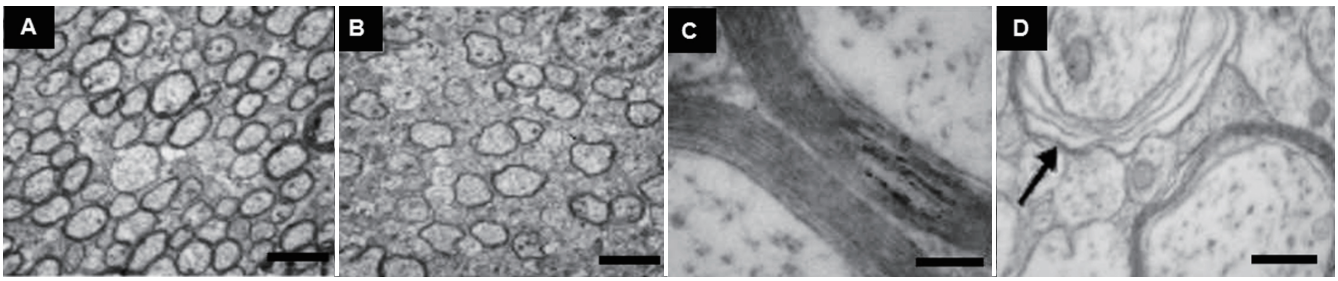

Figure 1 Electron micrographs of resin sections from saline (A–C) and corticosteroid-treated (B–D) rat pup spinal cord, taken from the dorsal funiculus, at 7 days post-corticosteroid treatment.

(A) shows a high proportion of myelinated axons, in contrast to (B) where a significant number of axons remain unmyelinated. (C) demonstrates the normal tight lamellar compaction of myelin, whilst in (D), the arrow indicates myelin lamellae that have either failed to associate, or failed to achieve a compact form around axons. Scale bars: A, C = 2 μm; B, D= 0.1 μm.

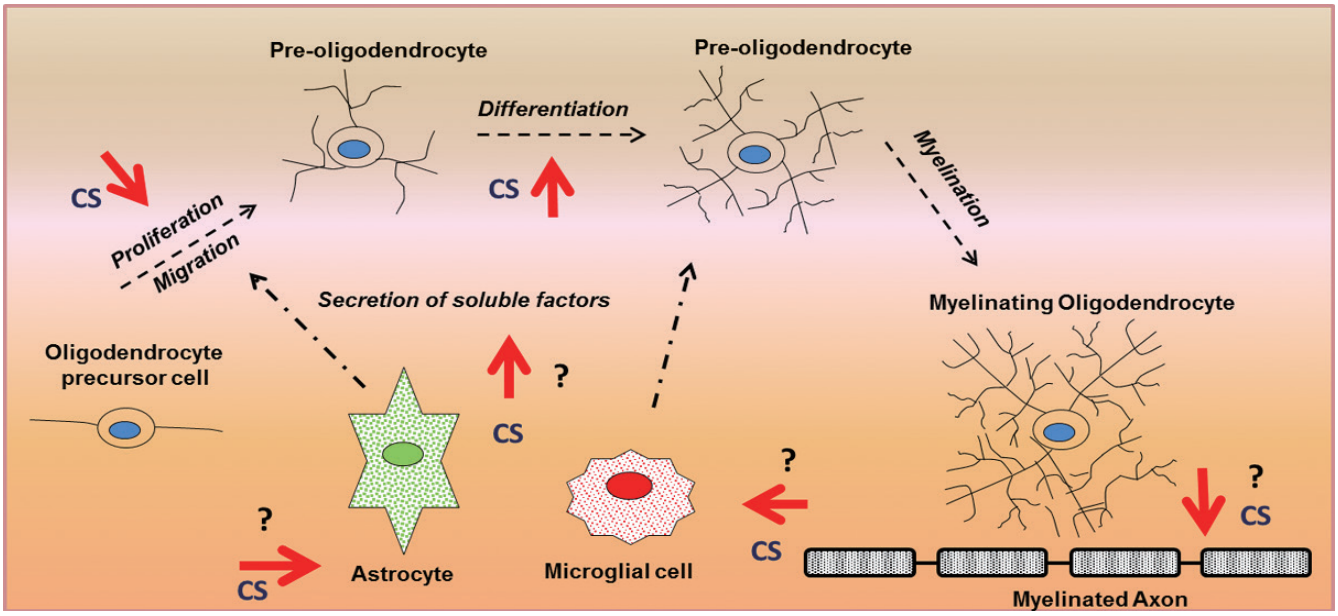

Figure 2 The biology of myelin genesis.
Schematic diagram depicting the various stages of myelin genesis, and highlighting the key cellular players in this process, along with some of the intercellular cross talk mechanisms that could impact myelin production. The red arrows indicate the phases where corticosteroids could potentially exert their influence.

in levels of circulating thyroid hormones, known to impact myelin genesis, can also not be ruled out. Studies so far that have aimed to establish the cellular targets of CS action during myelinogenesis and have yielded conflicting information. From a technical perspective, it is challenging to dissect issues of direct/indirect CS actions on neural cells within the complex, multicellular environment of CNS pathology foci in order to pinpoint the specific targets of drug action. Studies *in vivo* indicate that CS can both impair or enhance myelin genesis (Triarhou and Herndon, 1986; Pavelko et al., 1998). Similarly, *in vitro* studies report both drug effect and lack of effect on processes such as OPC proliferation and differentiation (Warringa et al., 1987; Halfpenny and Scolding, 2003). Complicating the situation further, the issue of whether myelinogenic cells express the glucocorticoid receptor (GR)–the mediator of drug effects–has also been equivocal. Oligodendroglial lineage cells reportedly express GR mRNA, suggesting that it is feasible for CS to act directly on these, but the evidence for GR expression in OPCs/oligodendrocytes has been far from definitive (Alonso, 2000; Matsusue et al., 2014).

Analysing CS effects on myelinogenic cells at the cellular and molecular level

Given the widespread clinical use of CS in neurological practice across the globe, the lack of consensus in experimental findings is of significant concern. This highlights the critical need for research focused on resolving key issues relating to the adverse neurological effects of CS use, in particular the mechanisms underpinning CS induced aberrations in myelin genesis. My group recently conducted a multidisciplinary study to investigate whether CS effects on myelin genesis are mediated *via* a direct action on OPCs and oligodendrocytes, using a two-pronged experimental strategy to identify the cellular and molecular targets of drug action (Jenkins et al., 2014). Histological assays were conducted to evaluate OPC proliferation/differentiation, and the extent of oligodendrocyte maturation, following CS application to isolated, purified cultures of these cells. As GRs essentially function as ligand activated transcription factors, complementary transcriptional analyses were simultaneously conducted using microarray and bioinformatics analysis. It was reasoned that the use of isolated, purified cells for these analyses would permit the examination of direct CS actions on myelinogenic cells- not possible within a multicellular lesion environment *in vivo*. Further, this work also took the first step towards assessing whether CS effects could potentially be mediated *via* indirect actions on other major neural cells types such as the astrocytes and/or microglia. Both cell types are typically activated and present in high numbers in sites of neural pathology, warranting examination of CS effects on these. The transcriptional changes in isolated astrocyte and microglial cultures were therefore evaluated in parallel, following CS treatment using identical protocols to those used for myelinogenic cells. To the best of our knowledge, this study has been the first of its kind to employ a dual and complementary technical approach in order to generate unbiased and corroborative data regarding CS effects on myelin genesis, with simultaneous experimental readouts at the morphological and molecular levels. We found CS treatment to be without discernible effect on the proliferation and differentiation of OPCs, or the extent of maturation of oligodendrocytes. We verified that the findings were not attributable to either an ineffective drug dose or the absence of GR expression- indeed we proved that all cell types studied in this work expressed GR, indicating that all are putative cellular targets for drug action. Our morphological evaluations were supported by the transcriptional element of the study, with modest gene expression changes detected in CS treated OPCs/oligodendrocytes. By contrast, CS effects were global and marked in drug treated microglia and astrocytes where a large number of genes were differentially expressed, including those related to cell migration and cytokine secretion. Of note, gene expression for the transcription factor Olig-1- "*a master regulator of myelination*" was greatly increased in microglia, a surprising finding in light of the prevailing view that Olig-1 expression is restricted to oligodendroglial cells (Arnett et al., 2004).

Future directions for research and the need for systems-based approaches

We believe the above data lend support to the idea that CS-induced myelination delays do not occur *via* a direct drug action on OPCs or oligodendrocytes. Instead, our findings suggest that these changes could occur secondary to altered behaviours of other major neural subtypes, with the immune component being a likely candidate, potentially *via* impaired clearance of myelin debris (during regeneration) or supernumerary oligodendrocytes (during development). These findings were unanticipated, given that OPCs and oligodendrocytes are key players in the process of myelin genesis. It should be pointed out that although we found global transcriptional changes in CS treated microglia, our investigations did not identify obvious mechanisms by which microglia may impact myelin genesis. However, it is important to note that correlations between alterations in transcript level and protein expression is generally poor, so mRNA results may not represent a reliable proxy for protein expression in the CS treated cells- some estimates suggest that only about 40% of variations in protein concentration are predictable based on mRNA abundance (Vogel

and Marcotte, 2012). It is reasonable to expect that altered molecular release profiles of microglia/astrocytes must mediate indirect influences on oligodendrocyte development and myelin genesis therefore our transcriptional analyses necessitate further systematic proteomic/secretomic studies on a range of neural cells, to shed further light on the potential mechanistic basis for indirect effects on myelin production. The ubiquitous expression of the GR in neural cells along with increasing evidence that CS can influence neural stem cell fate and behaviour (Yu et al., 2010) suggests that CS effects on neural development and regeneration is a multifaceted issue, with a number of potential contributory target mechanisms. The potential effects that CS have on neuronal function and neuron-glia interactions are also currently known and warrant further examination. We consider that studies of this nature have the potential to be of wide impact in the field of regenerative neurology. Information gained from such research can aid in the identification of factors underpinning remyelination failure and hence nerve fibre damage and repair. It can also assist in the identification of pharmacological targets to promote effective myelination for axon preservation, in turn leading to the development of better classes of therapeutic agents or to refinements to existing treatment regimens in neurological practice. Given the poorly researched nature of this area, it is clear that further work is required to elucidate the specific mechanisms underlying the adverse consequences of CS use, including histological and molecular analyses of CS treated human post-mortem tissue.

Divya M. Chari
Cellular and Neural Engineering Group, Institute for Science and Technology in Medicine, School of Medicine, Keele University, Keele, Staffordshire, ST5 5BG, UK
Corresponding author: *Divya M. Chari, Cellular and Neural Engineering Group, Institute for Science and Technology in Medicine, School of Medicine, Keele University, Keele, Staffordshire, ST5 5BG, UK, d.chari@keele.ac.uk.*
Funding: *This work was funded through grants from the British Neuropathological Society, North Staffordshire Medical Institute and University of Nottingham.*
Acknowledgments: *I am grateful to Dr Mark Pickard and Dr Alinda Fernandes, Keele University for their helpful comments on the manuscript.*
Conflicts of interest: *None declared.*
Accepted: *2014-05-13*
doi:*10.4103/1673-5374.133131* ***http****://www.nrronline.org/*
Chari DM. How do corticosteroids influence myelin genesis in the central nervous system? Neural Regen Res. 2014;9(9):909-911.

References

Alonso G (2000) Prolonged corticosterone treatment of adult rats inhibits the proliferation of oligodendrocyte progenitors present throughout white and gray matter regions of the brain. Glia 31:219-231.
Andrews H, White K, Thomson C, Edgar J, Bates D, Griffiths I, Turnbull D, Nichols P (2006) Increased axonal mitochondrial activity as an adaptation to myelin deficiency in the Shiverer mouse. J Neurosci Res 83:1533-1539.
Antonow-Schlorke I, Helgert A, Gey C, Coksaygan T, Schubert H, Nathanielsz PW, Witte OW, Schwab M (2009) Adverse effects of antenatal glucocorticoids on cerebral myelination in sheep. Obstet Gynecol 113:142-151.
Arnett HA, Fancy SP, Alberta JA, Zhao C, Plant SR, Kaing S, Raine CS, Rowitch DH, Franklin RJ, Stiles CD (2004) bHLH transcription factor Olig1 is required to repair demyelinated lesions in the CNS. Science 306:2111-2115.
Barnett SC, Linington C (2013) Myelination: do astrocytes play a role? Neuroscientist 19:442-450.
Bohn MC, Friedrich VL, Jr. (1982) Recovery of myelination in rat optic nerve after developmental retardation by cortisol. J Neurosci 2:1292-1298.
Bonanno C, Wapner RJ (2012) Antenatal corticosteroids in the management of preterm birth: are we back where we started? Obstet Gynecol Clin North Am 39:47-63.
Bracken MB (2012) Steroids for acute spinal cord injury. Cochrane Database Syst Rev 1:Cd001046.
Brady ST, Witt AS, Kirkpatrick LL, de Waegh SM, Readhead C, Tu PH, Lee VM (1999) Formation of compact myelin is required for maturation of the axonal cytoskeleton. J Neurosci 19:7278-7288.
Burton JM, O'Connor PW, Hohol M, Beyene J (2012) Oral versus intravenous steroids for treatment of relapses in multiple sclerosis. Cochrane Database Syst Rev 12:Cd006921.
Chari DM, Zhao C, Kotter MR, Blakemore WF, Franklin RJ (2006) Corticosteroids delay remyelination of experimental demyelination in the rodent central nervous system. J Neurosci Res 83:594-605.
Clarner T, Parabucki A, Beyer C, Kipp M (2011) Corticosteroids impair remyelination in the corpus callosum of cuprizone-treated mice. J Neuroendocrinol 23:601-611.
Crawford AH, Chambers C, Franklin RJ (2013) Remyelination: the true regeneration of the central nervous system. J Comp Pathol 149:242-254.
Franklin RJ, Gallo V (2014) The translational biology of remyelination: Past, present, and future. Glia doi: 10.1002/glia.22622.
Funfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Mobius W, Diaz F, Meijer D, Suter U, Hamprecht B, Sereda MW, Moraes CT, Frahm J, Goebbels S, Nave KA (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485:517-521.
George AA, Schiltz RL, Hager GL (2009) Dynamic access of the glucocorticoid receptor to response elements in chromatin. Int J Biochem Cell Biol 41:214-224.
Haddad HM, Hsia DY, Gellis SS (1956) Studies on respiratory rate in the newborn; its use in the evaluation of respiratory distress in infants of diabetic mothers. Pediatrics 17:204-213.
Halfpenny CA, Scolding NJ (2003) Immune-modifying agents do not impair the survival, migration or proliferation of oligodendrocyte progenitors (CG-4) in vitro. J Neuroimmunol 139:9-16.
Huang JK, Phillips GR, Roth AD, Pedraza L, Shan W, Belkaid W, Mi S, Fex-Svenningsen A, Florens L, Yates JR, 3rd, Colman DR (2005) Glial membranes at the node of Ranvier prevent neurite outgrowth. Science 310:1813-1817.
Jenkins SI, Pickard MR, Khong M, Smith HL, Mann CL, Emes RD, Chari DM (2014) Identifying the cellular targets of drug action in the central nervous system following corticosteroid therapy. ACS Chem Neurosci 5:51-63.
Lappe-Siefke C, Goebbels S, Gravel M, Nicksch E, Lee J, Braun PE, Griffiths IR, Nave KA (2003) Disruption of Cnp1 uncouples oligodendroglial functions in axonal support and myelination. Nat Genet 33:366-374.
Matsusue Y, Horii-Hayashi N, Kirita T, Nishi M (2014) Distribution of corticosteroid receptors in mature oligodendrocytes and oligodendrocyte progenitors of the adult mouse brain. J Histochem Cytochem 62:211-226.
Nie DY, Ma QH, Law JW, Chia CP, Dhingra NK, Shimoda Y, Yang WL, Gong N, Chen QW, Xu G, Hu QD, Chow PK, Ng YK, Ling EA, Watanabe K, Xu TL, Habib AA, Schachner M, Xiao ZC (2006) Oligodendrocytes regulate formation of nodes of Ranvier via the recognition molecule OMgp. Neuron Glia Biol 2:151-164.
Oluich LJ, Stratton JA, Xing YL, Ng SW, Cate HS, Sah P, Windels F, Kilpatrick TJ, Merson TD (2012) Targeted ablation of oligodendrocytes induces axonal pathology independent of overt demyelination. J Neurosci 32:8317-8330.
Pang Y, Fan LW, Tien LT, Dai X, Zheng B, Cai Z, Lin RC, Bhatt A (2013) Differential roles of astrocyte and microglia in supporting oligodendrocyte development and myelination in vitro. Brain Behav 3:503-514.
Pavelko KD, van Engelen BG, Rodriguez M (1998) Acceleration in the rate of CNS remyelination in lysolecithin-induced demyelination. J Neurosci 18: 2498-2505.
Reynolds RM, Seckl JR (2012) Antenatal glucocorticoid treatment: are we doing harm to term babies? J Clin Endocrinol Metab 97:3457-3459.
Shields A, Thomson M, Winter V, Coalson J, Rees S (2012) Repeated courses of antenatal corticosteroids have adverse effects on aspects of brain development in naturally delivered baboon infants. Pediatr Res 71:661-667.
Shinwell ES, Eventov-Friedman S (2009) Impact of perinatal corticosteroids on neuromotor development and outcome: review of the literature and new meta-analysis. Semin Fetal Neonatal Med 14:164-170.
Smith CM, Cooksey E, Duncan ID (2013) Myelin loss does not lead to axonal degeneration in a long-lived model of chronic demyelination. J Neurosci 33:2718-2727.
Triarhou LC, Herndon RM (1986) The effect of dexamethasone on L-alpha-lysophosphatidyl choline (lysolecithin)-induced demyelination of the rat spinal cord. Arch Neurol 43:121-125.
Vogel C, Marcotte EM (2012) Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 13: 227-232.
Warringa RA, Hoeben RC, Koper JW, Sykes JE, van Golde LM, Lopes-Cardozo M (1987) Hydrocortisone stimulates the development of oligodendrocytes in primary glial cultures and affects glucose metabolism and lipid synthesis in these cultures. Brain Res 431:79-86.
Yu S, Patchev AV, Wu Y, Lu J, Holsboer F, Zhang JZ, Sousa N, Almeida OF (2010) Depletion of the neural precursor cell pool by glucocorticoids. Ann Neurol 67:21-30.