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 1950s 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cerebral palsy) (Shinwell and Eventov-Friedman, 2009; Shields et al., 2012) as well as the spinal cord (Figure 1, our unpublished data).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 central 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and Friedrich, 1982; Antonow-Schlorke et al., 2009; Shields et al., 2012) as well as the spinal cord (Figure 1, our unpublished data).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 central 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and Friedrich, 1982; Antonow-Schlorke et al., 2009; Shields et al., 2012) as well as the spinal cord (Figure 1, our unpublished data).

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 (Furushlining 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 sub-populations 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 compaction of myelin, whilst in (D), the arrow indicates myelin lamelae 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.
in levels of circulating thyroid hormones, known to impact myelin genesis, can also not be ruled out. Studies so far have aimed to establish the cellular targets of CS action during myelination 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 (Triathoul 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 myelogenic 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 myelogenic 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 myelogenic 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 cell 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 myelogenic 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.

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