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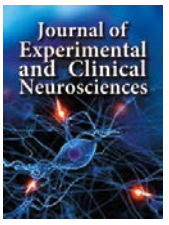

Review Article

Steroids Impact on Myelin Repair, Neurogenesis and Visual Pathway in Multiple Sclerosis: Preclinical Perspectives

M. Reza Sadaie [a,*], Nasi Samiy [b], Robert E. Rosenbaum [c], Gary A. Thomas [d]

a: NovoMed Consulting, Silver Spring, Maryland, USA

b: Retina Institute of the Carolinas, Charlotte, NC, USA

c: Department of Neurosurgery, NNMC Bethesda Naval Hospital Bethesda, MD, USA

d: Penn State Hershey Neurology, Penn State University, PA, USA

Correspondence

Dr M. Reza Sadaie, NovoMed Consulting, 12214 Plum Orchard Dr, Silver Spring, MD 20904.
Tel: +1 240 489 6255, ext. 541;
Fax: +1 860 540 8291
Email: rsadaie@novomedconsulting.com

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Abstract

Even though steroids have drawn significant therapeutic interests for decades, data about their benefits for neural regeneration are missing as current observational studies unfold progressive abnormalities in cerebral gray matter and cerebellum compartments, apart from demyelination lesions in white matter of the central nervous system (CNS) in multiple sclerosis (MS). This medical-scientific appraisal is based on a series of structured questions in part addressed in our investigative clinical review on the benefits and risks of glucocorticosteroids (GC), which highlights that steroids can intensify the disease progression, aside from other global side effects recognized in MS and associated optic neuritis (MS-ON). Corticosteroids treatments, whilst temporally and selectively suppress immune reactions, can interfere with the clearance of myelin debris and inhibit proliferation-migration of the myelinating cells, affecting the axonal repair and CNS functions. This review compiles and summarizes datasets extracted largely from complementary laboratory studies published in Medline, It discusses related pharmacologic and disease mechanisms and relevancies of the distinct MS disease models in rodents, with emphasis on the strengths and weaknesses of the associations of GCs use, glucocorticoid receptors sensitivity, and clinical outcomes. Based on these assessments, we conclude that steroids can suppress inflammation to the determent of neuronal remodeling in a mutually exclusive manner. Excess steroids can contribute to neuronal loss and retinal damage in optic pathology, and thus may expand cerebral atrophy and disease burden in multiple sclerosis.

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Introduction

Despite availability of many disease-modifying-therapies (DMT), neurodegeneration continues to spread to different regions of brain in multiple sclerosis (MS), manifesting as the marked decreases in whole-brain volume and gray matter (GM) in addition to increases in cerebral white matter (reviewed in [1]) and extensive spinal cord lesions [2, 3].

Aside from intent-to-treat MS attacks with glucocorticosteroids (GC) as standalone medications, steroids remain in the forefront of pharmacotherapy for MS and MS-associated optic neuritis (MS-ON). Steroids are also given in cyclical regimens as a prerequisite to boost the effectiveness of other DMT, such as interferon beta. Corticosteroid effects are highly nonspecific and ubiquitous; can pass blood brain barrier, bind to both glucocorticoid (GR) and mineralocorticoid (MR) receptors in neurons and glial cells in addition to many other cells [4].

Corticosteroids are given largely to control upstream and subsequent episodes of MS relapses. Frequent use of steroids may affect both paraclinical and clinical outcomes. Corticosteroids can be neuroprotective or neurodegenerative in a concentration/dose-response-dependent manner [4].

Conceivably, increased brain lesions may be related to intrinsic neurotoxicity of the steroids. The risks can also persist

during and after steroids treatments of optic neuritis, or clinically-isolated demyelinating syndrome (CIS) that turns to clinically-definite multiple sclerosis (CDMS). These concerns are reasonable based on paraclinical, clinical and basic science principals, though difficult to discern amongst confounding variables. Correlation is not a causation; however, the worsening neurodegeneration can possibly be due to excess steroids.

Corticosteroids are not publically approved by US-FDA for multiple sclerosis indication; these products are used off-label aside from investigational because of their well-known anti-inflammatory effects. Their use in clinical practice appear largely promoted in industry funded publications, although their use for MS indication is left uncertain because of insufficient proofs based on the AAN evidence classifications and outdated assessments [5] (Natl. MS Soc.; US Neurology, 2008).

For instance, a high-dose, but not a low dose, methylprednisolone/MP can significantly reduce contrast-enhanced T2 lesions [6], although the importance of T2 lesions as surrogate markers to assess treatment efficacy is controversial [7].

In this article, the neuroactive mechanisms of steroids that may be operative in new lesions environment, and complementary experimental evidence on relevancies of different disease models of MS and MS-ON in rodents are discussed, with a particular focus as follows:

- Analyses of the mechanisms-of-action of GCs in effects site or cerebral lesions environment ex vivo and in cells
- Evaluations of the molecular characteristics and pathogenic mechanisms in rodents with distinct etiopathological features

Biological Observations, Publications, and Assessments

PubMed and online databases were searched and the selected articles reviewed. Evidence-based articles were evaluated on the principals of quality of the methodology and relevant data were critically analyzed. Several key review articles were also included. Some citations were excluded because of scope constraints. Selected findings on preclinical studies, animal test systems and cell-culture models are discussed.

Chemistry, and Mechanisms of Action

Pharmacologic corticosteroids are short-to-medium-acting synthetic hydrocortisone analogs that belong to naturally produced glucocorticosteroid/GC class of steroid hormones. Corticosteroids bind to glucocorticoid receptors (GR) in the cytoplasm and translocate to the nucleus wherein they can bind to the GR elements in the promoter regions of the steroid sensitive genes, resulting in the genome modifications, and/or increases or decreases of gene expressions. Corticosteroids are among the most commonly utilized therapeutic agents for a wide variety of diseases, including autoimmune disease, allergic and hypersensitivity, and inflammations. GC use can temporally and selectively suppress the immune reactions, but it can also stimulate to develop tolerance, induce numerous detrimental side-effects, as GR is expressed in almost every cell in the body.

Recent insights into the molecular mechanisms and cellular resistance to glucocorticosteroids' control of inflammation in other autoimmune disease have been reviewed in detail elsewhere, e.g. [8, 9]. Steroids can inhibit certain enzymes (such as calcium-dependent, mitogen-activated protein kinase/MAPK phosphorylation) essential for neuroprotection and hence hinder survival of neuronal cells during an inflammation [10]. However, the dual (beneficial-detrimental) effect of steroids may be coupled or differentially active in effect site and other tissue regions, and this can depend upon both the dose and multiple interplaying molecular signatures in multiple sclerosis. It's been challenging to decipher these signatures based on the contradictory outcomes attributable to the pharmacologic GCs in MS care.

Corticosteroids exhibit modest therapeutic benefit in MS, probably in part because of their inactivation in the 'effect site' and/or suboptimal functionality or unresponsiveness of GR in target cells. Interestingly, the binding affinity and sensitivity of GR in multiple sclerosis [11], as well as certain other autoimmune disease are impaired, which can lead to local resistance to GCs [8, 12]. Expression of the three GR isoforms were inhibited in lymphocytes of all MS patients when the blood leukocytes were treated with dexamethasone—especially GRα and GRβ decreased two-fold with a concomitant increase in the heat-shock protein 90 to GR ratio, and consistently less inhibition of the lymphocytes proliferation in steroid resistant multiple sclerosis [13]. These data suggest that high-dose steroids downmodulate GR and this probably involves compensatory changes in other functions such as elevating GR stabilizer-transporter heat-shock proteins. These data can also provide a sense to interpret the elevated autoantibodies (38%) against heat-shock proteins in certain MS patients [14]. Whether additional parameters such as drug efflux and concentrations, in particular in CNS compartments, are involved cannot be ruled out.

Considerable other variables may affect the pharmacodynamic profiles of GCs in multiple sclerosis. Of note, erratic or ineffective clinical responses to the exogenously administered GCs may be due to inactivation of the drug in the effect site [15]. This probably occurs within (intralesional) injurious neurons, presumably including demyelinating oligodendrocytes, structural and functional neurons and auxiliary astrocytes/glial cells. Original lesion grows and spreads to interlesional spaces from mostly perivenular (73%) regions of a central vein from where additional small lesions sprout at both white matter and gray matter [16]. Theoretically the pharmacologic steroids, unlike interferon beta and most biologics, can diffuse from endothelial cell layers of vessels to intralesional and perilesional vascular cuffs and surrounding tissue.

A compelling evidence indicates that resistance to GCs may stem from their inactivation by the 11bHSD2 enzyme (11beta-hydroxysteroid dehydrogenase type 2) in the lesions [15]. This self-limiting reaction at best can compromise the purported antioxidant or neuroprotective role of GCs as well as the immunomodulatory functions of the active pharmaceutical-grade corticosteroids. As a result, a continued treatment might simply increase the cumulative (inactivated) intracellular concentrations of GCs (iGC) at the effect site, as well as peripheral cerebral and systemic tissues. This can allow lesions enlarge or grow in numbers unopposed, and hence sustain or accumulate disability. A part from suboptimal efficacy, in theory this can also account for high rates of side-effects even in seemingly GC-sensitive (GR-functional) multiple sclerosis, in particular when an intense long-term steroid therapy is implemented. Schematic representations of some these ideas are illustrated in Figure 1.

Sufficiency of experimental studies

In experimental autoimmune encephalomyelitis (EAE) model, mice given methylprednisolone/MP induces reversible remission of the pathological features including inflammatory infiltrates [17]. Corticosteroids are recognized for broad cytotoxic effects on lymphocytes, but in EAE model in C57BL/6 mice, T cells are resistant to GC-induced apoptosis and this appears to correlate with the disease severity [18]. In the absence of overt inflammation, immunosuppression by methylprednisolone/MP depletes T cells and macrophages in healthy mice and contributes to retinal damage, which deteriorates after ocular infection with murine cytomegalovirus (MCMV) [19, 20]. Some retinal cells can undergo apoptosis upon frequent treatments with corticosteroids, and prior to the onset of a disease, such as ocular infections. Such problem worsens, for instance, during reactivation of the MCMV retinitis in methylprednisolone/MP-treated mice [21]. The mechanism of cell death in the retina involves TNFalpha, which also is released by activated microglial cells. TNFalpha is among key neurotoxin inducers; it can exert opposing effects by activating both antiapoptotic and proapoptotic genes. Of note, its absence (in TNF-$\alpha^{-/-}$ mice) promotes apoptosis and necrosis in the retina of immune

suppressed mice [20]. The disease burden during neural injury may increase as a result of immunosuppression with corticosteroids.

In spinal cord injury model in rats and cells in culture, methylprednisolone/MP protects oligodendrocytes but not cortical neurons from excitotoxicity of either the neurotransmitter glutamate receptor agonist AMPA or the protein biosynthesis inhibitor staurosporine. This suggests that GCs can in part be neuroprotective [22]. In addition, dexamethasone/DEX promotes chemical-induced nuero-injury and inflammation in healthy mice, presumably through increasing proinflammatory cytokines such as TNFalpha [23].

Persuasive evidence is lacking for steroids-mediated upregulation of the ciliary neurotrophic factor (CNTF) which was thought to counteract neurotoxicity of TNFalpha [24]. CNTF administrations in EAE model to give trophic support to neurons and protect from lesion activity have been unsuccessful. Therefore, the enthusiasm to explore its effectiveness in clinical trials have been fading [25]. A recent study however demonstrates that subcutaneous injection of CNTF increases the expression of myelin oligodendrocyte protein (MOG) in cerebral cortex of cuprizone-induced demyelination in mice [26]. It is still premature to base therapy choice decisions for corticosteroids in multiple sclerosis based on evidence that GCs can increase expression of CNTF (Table 1).

Can steroid treatment help or hinder neurogenesis, in particular myelin repair? Conflicting recent observations demonstrate that GCs interfere with proliferation and/or maturations of oligodendrocytes and biosynthesis of growth factors in astrocytes. This effect might suppress the differentiation of oligodendrocyte progenitor cells (OPC), inhibit spontaneous myelin repair in non-immune cuprizone-induced lesion model in animals. Moreover, in addition to immune suppression, GCs can alter biosynthesis and release of growth/differentiation factors and impede myelin repair [24] (Table 1). Consistency-of-association of these findings have also relevancy to the plausibility of GC treatment-related perturbation of HPA axis, down-modulation of GR expression in astrocytes, inhibition of proliferation and reduction of astrocyte numbers in cortex—supported in an animal model as well as cells in culture [27]. These findings might also have relevancy to gray matter lesions in GC-treated MS patients.

Corticosteroids reduction of astrocyte numbers can be in favor of reactive gliosis or glial scar, though perhaps also at the expense of curtailing beneficial role of astrocytes in clearing myelin debris [28]. In MS patients with evidence of ongoing inflammation, what is not surprising though lymphocytes can acquire resistance to GCs [18]. Whether the pharmacologic glucocorticosteroids also affect the proliferation/migration of oligodendrocyte progenitor cells, and interfere with functional recovery of injurious neurons in the lesion environment is experimentally evidence-based safety concern —a sensible hypothesis (Figure 2).

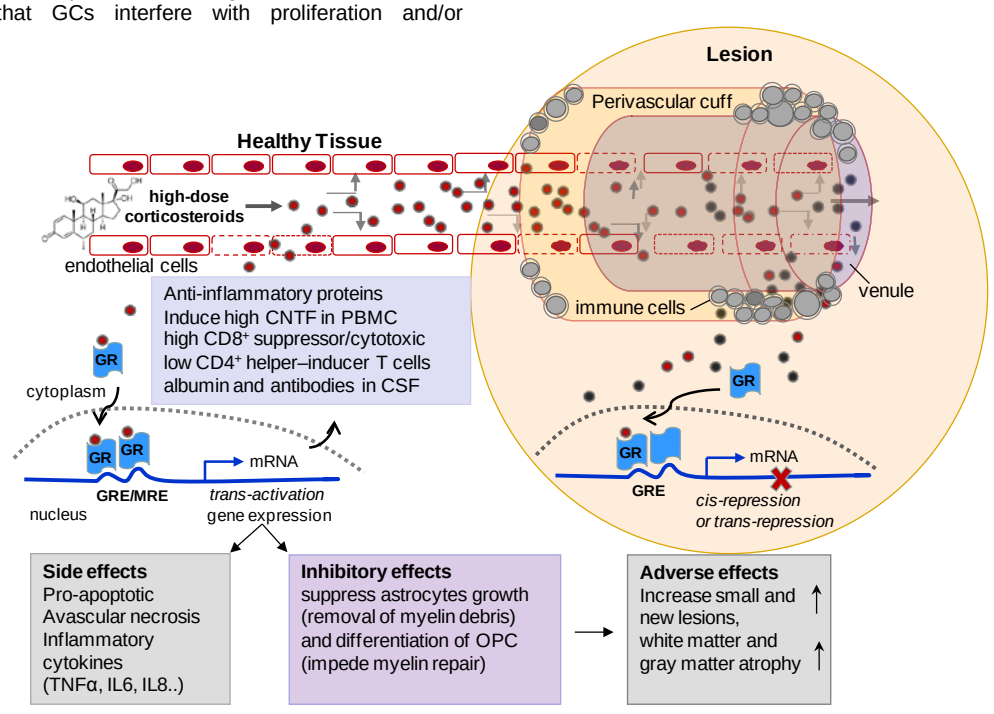

Figure 1. Dynamics of newly forming lesions in MS treated with corticosteroids. Extravasation of immune cells into the CNS is an ongoing process during MS attacks, when largely sensitized immune cells congregate around demyelinating neurons forming perivascular cuffs, hence inducing inflammation and edema in perilesional and intralesional cerebral tissue. Infused corticosteroids (red dots) initially shrink the lesions volume, wistfully thought to suppress the proliferation and infiltration of the immune cells, stabilize blood nerve barrier, reduce cell-trafficking and inflammatory cytokines, mitigate inflammation and edema, and enhance myelin repair through neuroprotective actions of peptides such as the neurotrophins ciliary neurotrophic factor (CNTF). Healthy cerebral tissue, including both gray matter and white matter, and possibly the dendrites density, as well as the recovery of injurious neurons are vulnerable to pharmacologic corticosteroids. These effects may further be compounded by steroid-resistance of the immune cells, which can occur by downregulation of GR and anti-apoptotic genes or selective inactivation of the steroids (black dots) by enzymes within the lesions or both and/or the drug efflux by multidrug resistant genes. Although the exact molecular mechanisms are unknown, an interlocking set of negative feedback reactions and compensatory gene expressions—including GR chaperons, engage cisacting repressor and transactivation glucocorticoid (GRE) and mineralocorticoid (MRE) response elements located in the promoters of steroid-responsive genes—are involved. GRE-directed gene expressions can cause assorted intra-cerebral AEs and SAEs, whilst MRE can induce extra-cerebral side effects such as osteonecrosis. The paraclinical benefits captured by MRI are transient and steadily change to increased constellations of T2-weighted small new lesions that ultimately may coalesce or enlarge to form expanded chronic lesions and neurodegeneration. MS patients are at high risk of developing cerebral atrophy after persistent cyclical treatments with mega-dose corticosteroids.

Timing of the steroid may influence its overall efficacy. Early experimental animal studies involving spinal cord injury suggested a possible role for systemic steroids in protecting injured nerves [29]. In EAE model, rats given steroids at the onset of induced encephalomyelitis had lower chance of developing ON; and when ON did occur, there was relative preservation of retinal ganglion cells (RGC) [30]. The authors argued that high-dose steroids given very early in the course of ON may produce a favorable outcome [30, 31] and "less effective after inflammation begins" [30], consistent with a previous idea that a neuroprotective therapy to lower the consequences of ON should be initiated prior to axonal injury which precedes RGC death [32].

Others have shown that steroids increase axonal damage and neuronal death in myelin oligodendrocyte glycoprotein (MOG)-EAE animals, but healthy controls, regardless of the treatment time; methylprednisolone/MP (at doses comparable to methylprednisolone/MP pulse therapy of patients with MS) reduces RGC density and impairs neural conduction and visuals functions significantly [10], despite an effective control of inflammation (Table 1) [33]. Notably, the steroid-induced RGC loss was neutralized by intravitreal injection of a blocker of voltage-gated calcium channels, suggesting that such outcomes partly occur non-specifically through non-genomic effects [10].

Translational reliability of the disease models

Accumulating evidence on experimental virus-induced ON and transgenic models of the axonal/demyelinating disease and EAE suggest that various etiopathogenic mechanisms can trigger MS- and/or ON-like disease in rodents [34- 36]. Axonal loss/dysfunction can occur because of a variety of nonviral causes: most notably, neurotoxins and associated downstream or intermediary products, including free radicals/oxidative damage, risk genes, and an array of other predisposing elements such as channel abnormalities/calcium elevations—all of which can lead to destruction of neurons [37- 39] and contribute to lesions pathogenesis in multiple sclerosis [40- 42]. Certain risk factors may additionally involve mutated myelin proteins such as proteolipid protein (PLP) with altered biochemical characteristics [43] and/or dysmyelination (reduced myelination) not demyelination, as demonstrated in murine systems in vivo [36, 44]. Or both, a complete demyelination in the absence and moderate inflammation [36].

These alternative MS/ON-like models in animals are testable resources to sufficiently evaluate new (anti-demyelination/neurodegenerative) agents in the central nervous system, nonetheless, and provide relevant preclinical cues. Although their values separately have little clinical relevancy, given that the etiopathogenesis of MS and ON are multilayered and complicated, for example, the same mutation(s) in PLP as the cause of neuro-injury is undocumented in multiple sclerosis.

It should be mentioned that rodents harbor various endogenous viruses, which might mimic endogenous/exogenous retroviruses or neurotropic viruses associated with neuro-inflammatory disease, including MS and MSON in humans. Although contributory roles of infections in MS etiopathogenesis have been debated for decades and are unreconciled, some may worsen demyelination outcomes through neuro-inflammation, and complicate the disease. On the other hand, steroids can promote infections—partly indirectly through immunosuppression and partly by stimulating steroid-responsive elements in viral/ancillary apoptotic genomes in the nervous system—complicate the disease and potentially mislead the telltale signs of biomarkers in neuro-injury.

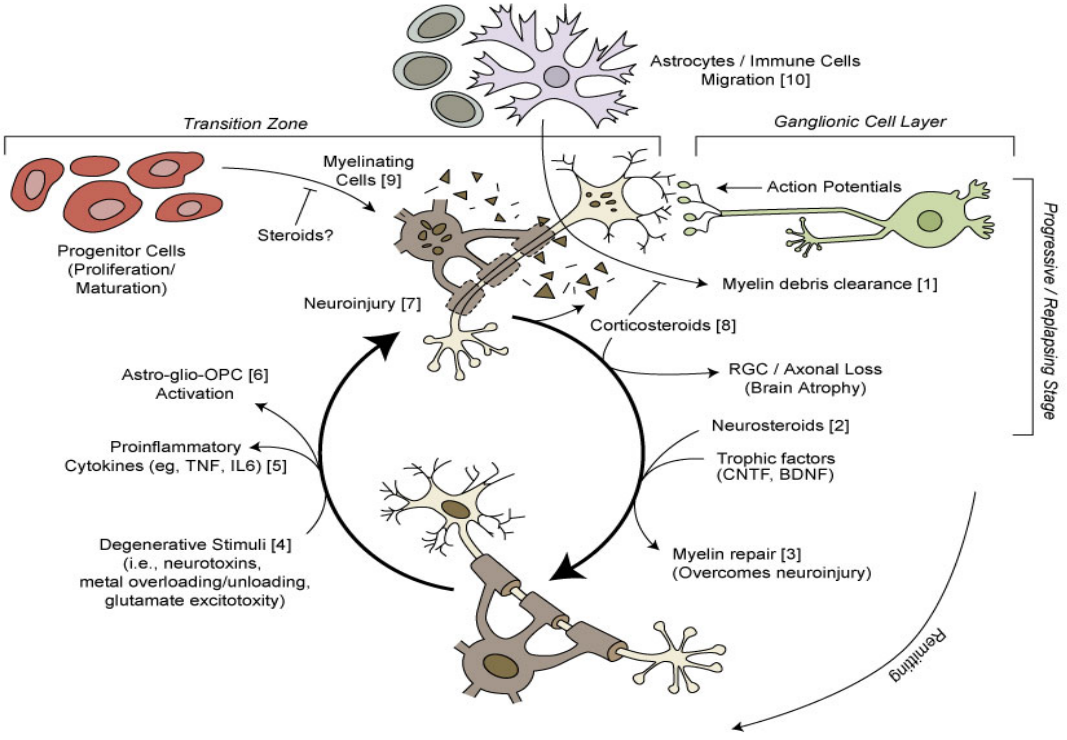

Figure 2. Steroids impact on de/remyelination cycle is illustrated with respect to disease mechanisms in RRMS and MS-ON. The relationships between injurious neurons and ganglionic cells and regenerative response may involve multiple cell types and endogenous cellular modulators in CNS; the highlighted elements including opposing beneficial (1,2,3,6,9,10) and perilous (4,5,7,8) steps during neural injury, and effects of endogenous and exogenous steroids. While pharmacologic steroids can recede infiltrated immune cells and damaging inflammation, can also trigger inhibitory/pro-apoptotic effects leading to profound changes in neuritis and dendrites densities, reparative axonal loss and brain atrophy.

In addition, spontaneous neural regenerations (remyelination) occur differentially, and regional differences exist between gray and white matter in the cuprizone-induced demyelination model [45, 46]. To dissect the impacts of steroids use on neuro-injury, the preclinical/translational studies should involve head-to-head analyses of at least two distinct MS disease models.

Steroids use may exacerbate the disease presumably through: 1. Inhibiting proliferation-differentiation of OPC; 2. Inducing cell death in retina ganglion cells and possibly in visual pathway; 3. Reducing glia trophic support; 4. Impairing axonal signal propagations/transport; 5. Suppressing neurogenesis/remyelination, in addition to weakening the immune system, as expected.

Importantly, corticosteroids can affect expressions of growth factors, astrocytes functions, and interfere with HPA axis homeostasis. Compelling evidence suggests that GC-induced differentiation/maturation of OPC has a trans-relation with myelin repair [24] (Table 1). Conceivably, complementary treatment with corticosteroids in addition to other immunosuppressants (e.g., type II topoisomerase inhibitor mitoxantrone and purine analog azathioprine) can interfere with OPC/glial cells and undermine the myelin repair. A suboptimal clearance of myelin debris, as well as purging cumulative GC concentrations in lesion environment, might leave insufficient time for the cell repair mechanisms to stay switched on for injurious neurons to recuperate. GCs can then perpetuate neurodegeneration and sabotage the intended therapy objectives.

To sum up, experimental studies performed only on one animal test system is exceedingly questionable—for instance, pharmacological manipulations and outcome measures based on only EAE model may insufficiently validate the next steps towards clinical investigations of new pharmacotherapies. The weight-of-evidence points at GCs being inefficacious in favor of both inflammation and myelin repair.

Discussion

Although natural molecular analogs of glucocorticosteroids/GC, so-called neuroactive steroids (such as metabolites of progesterone and gonadal steroid 17β-estradiol or E2) can show neuroprotection [47], cytoprotective and synaptogenic effects on cerebral cells, these particular molecules act primarily through non-transcriptional signaling mechanisms via interactions with neurotransmitter receptors. Alas, their development for therapeutic use thus far have been challenging. In contrast, readily available pharmacologic steroids act mainly via transcriptional machinery and cause profound changes in expressions and repressions of steroid responsive genes (Figure 1). Moreover, such steroids homologues or corticosteroids can arrest growth/proliferation of cells, enhance demyelination and neuronal loss, in particular in supra-physiological concentrations [4] (Figure 2). An ensuing injurious effects can permeate to normal white matter and gray matter tissue, and expand the atrophy to subcortical deep gray matter (SDGM) and cerebellar compartments, and may even cause a spinal cord damage, within 3 to 5 years in progressive multiple MS [2, 48-51], thus probably affecting motor functions as well as innermost workings of the brain. Some of these changes may be invariable and distinct in steroid-sensitive and steroid-resistant cells.

The relevancy of EAE model in animals—which is generated in response to immunization with myelin proteins and exhibit RRMS-like symptoms— is controversial; the EAE model does not produce the underlying pathology in MS of axonal injuries and neuronal loss. "EAE is mainly a lymphocyte driven model, which makes it difficult to distinguish between direct effects of corticosteroids and indirect effect as a result of corticosteroid immunomodulation" [24].

Table 1. Glucocorticoids effects on MS and ON models in animals and cells.

Test drug	Test system	Study design	Dosage (high dose)	Treatment endpoint	Outcome measures	Outcomes*	References
Methylprednisolone or Dexamethasone							
	Cuprizone-C57BL/6 mice	in-vivo	15 mg/kg/ day, IP	5 weeks	PLP biosynthesis	Decreases PLP (75%) (inhibits myelin repair)	[24]
	Neonatal rats OPC	in culture	0.01-1 µM	5 days	PLP, MBP mRNA expressions, histology	Increases: PLP (100%) MP,(200%) Dex MBP (50%) MP, (100%) Dex Accelerate OPC differentiation	
	Balb/c mice Astrocytes	in culture	0.01-1 µM	5 days	Growth factors-mRNA/proteins	Increases FGF2 (197%) Increases PDGF (82%) Decreases IGF-1 (33%)	
Dexamethasone							
	EAE C57BL/6 mice	ex-vivo	0.01-1 µM	15 days	GRα expression, Splenocytes/PBMC T-cell apoptosis	Decreased GRα (50%) in CD4+/CD8+ T cells (exhibits GC resistance)	[18]
Methylprednisolone							
	EAE (MOG-induced) rats	in/ex-vivo	(20 mg/kg) day/IP	8 days	biochemistry histopathology VEP/ERG	Inhibits MAPK-p42 activity Decreases RGC density (60%) Increases neurons death (108%) Impairs visual pathway	[10, 33]
Interpretation							
■ Downregulated proteins (PLP, GR, MAPK) disrupt remyelination, neuro-protection/-regeneration							
■ Steroid-induced GR resistance can limit effective anti-inflammation							
■ Steroids can aggravate visual dysfunctions in autoimmune optic neuritis							

***** Attributable percentage values were calculated as described [37], from the mean numerical fold changes after treatments with test agents relative to the base, controls/placebo, significant at ≤0.05 level

EAE = experimental autoimmune encephalomyelitis; **ERG** = electroretinograms; **FGF-2** = fibroblasts growth factor 2, known to promote OPC proliferation; **GRα** = glucocorticoid receptor alpha; **IGF-1** = insulin-like growth factor-1, known to promote OPC maturation; **IP** = intraperitoneal injection; **MAPK** = mitogen activated protein kinase-2 phosphorylation; **MBP** = myelin basic protein; **MOG** = myelin oligodendrocyte glycoprotein; **PDGFαα** = platelet-derived growth factor-αα, promotes OPC proliferation; **PLP** = proteolipid protein; **OPC** = oligodendrocyte progenitor cells; **RGC** = retinal ganglion cell; **VEP** = visual evoked potential

Second, recent experimental studies indicate that loss of oligodendrocytes and neurons begins in the earliest stages of the disease, and the disease progression is not associated with blood-driven inflammatory cells [52]. Other demyelinating disease models such as cuprizone-treated mice [52, 53] and lysophosphatidyl choline [54] have been largely excluded in preclinical and translational studies involving GCs —probably because these animals which lack overt inflammations are unreasonably deemed inapposite model. Third, the consistency-of-association of GC-induced lesion repair, which is evident to a considerable degree in early periods after treatments of MS, has presumably promoted continued use of intense GCs. Little attentions seem given to plausibility of GC-resistance (at least in a subset of MS), ineffectiveness of GCs in reducing the disability progression, and GC-induced degeneration of CNS and other cells [1].

Many underlying/risk factors—such as impaired metabolic rate, compounded by iron overload in SDGM compartments, chronic hypoxia, and decreased mitochondrial energy —presumably create axonal vulnerability, leading to an 'accelerating pathology' in the form of a progressing atrophy in SDGM and cerebellum structures, as seen over 5 years in multiple sclerosis with a simultaneous progression of disability [42, 51].

The predictive diagnostic value of the recently documented biomarkers such as SDGM abnormalities [49], as a non-treatment related actual disease markers is unclear. It is quite possible for a risk factor to operate in one community but not in another, in particular in relations to specific medications utilized. Nevertheless, abnormal phase changes seen in the images call for questions and monitoring other measures as well, such as endogenous and treatment-related cortisol AUC levels, and underlying infection outcome measures. In the new MRI studies depicting SDGM abnormalities, most patients were on DMT, and exposed to excess steroids prior to or during the study entry. Whether the cohorts with a progressive disease course were either responders or non-responders to the steroids are unclear (see further; [1]).

The most recent paraclinical studies are hypotheses driven and exploratory, and previous clinical studies fall short of excluding whether steroids are ancillary/contributory or sufficient cause of the deteriorating lesions, or development of new lesions, found in a fairly large proportion of the patients treated with DMTs, including methylprednisolone (1-3 g). Importantly, the relationships between steroids treatments and degenerative SDGM patterns as 'biomarkers' are unaddressed in most recent studies [50], although the analyses have uncovered further paraclinical areas of perplexity in multiple sclerosis.

It is noteworthy that the advanced MRI analytical models are currently unavailable in MS clinical practice. Independent evaluations can uncover the homogeneity of the subgroups and consistency-of-associations to validate the new models of data analyses tools. The new trials can shed more light when all study end-points are measured prospectively from the onset of the pathogenesis, with predetermined criteria, to rule-in or rule-out the suspected risk factors in treatment arms in comparison to the reference risk group.

There are probably so many other important issues related to etiologies of the 'progressive neurodegeneration' in multiple sclerosis. It is tempting to subscribe to the sense that MS is a neurodegenerative CNS disease with a post-activated immune system characteristics, rather than a preset autoimmunity that sets off an inflammatory cascade of disease (MS/ON) with neurodegenerative characteristics. Although inflammation may partly be involved, it is sensible that it may be a compensatory immune reaction primarily directed to clean up the myelin debris, rather than inherently poised injuring neurons, as a possible mechanism for recovery from the beginning of a demyelination trigger point. In contrast, inhibition of this repair mechanism can precipitate brain atrophy and frequent relapses.

Pathogenic mechanisms of MS involve biochemical modifications of various proteins embedded in axonal layers of oligodendrocytes, such as lipid peroxidation and protein lipoxidation [55]. Moreover, the MS pathology affects white matter and gray matter and the debilitating clinical outcomes correlate with the overall neurodegenerative process [52]. These observations call for a departure point from initiating clinical investigations on MS and ON based on scientific rationales and inferences from traditional immune-based preclinical models.

Conclusion

Treatments based on the assumption that an inflammation-driven degeneration is the sole mechanism for the disease progression is unconvincing. Importantly, inferences made from the EAE disease model as scientific rationales to base relevant to treatments in humans may be flawed. Ascribing causality of the worsening lesions to a steroid-induced progressive atrophy in gray matter and cerebellum compartments is difficult to rule out based upon the available data. Furthermore, the evidence to support the benefits of steroids to reduce inflammation to improve retina ganglion cells in animal models of optic neuritis is equally unconvincing. Modified approaches that are evidence-based, rather than extrapolation-based beyond the range of data, are needed to: i) prevent or reduce the spread of atrophy in CNS, ii) effectively cure the relapses with innovative measures without steroids, and iii) stimulate neurogenesis to recover normal cerebral functions in multiple sclerosis.

Conflict of interests: The authors have no conflicting commitments with respect to the reviewed technologies, products or competitor products, and used no external funds for this research.

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