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Novel Approaches to Corticosteroid Treatment in Duchenne Muscular Dystrophy

Eric P. Hoffman, Ph.D.^{a,b,*}, Erica Reeves, Ph.D.^b, Jesse Damsker, Ph.D.^b, Kanneboyina Nagaraju, D.V.M., Ph.D.^{a,b}, John M. McCall, Ph.D.^b, Edward M. Connor, M.D.^{a,b}, and Kate Bushby, M.D.^c for the FOR DMD investigators of the MSG and Treat NMD

^aCenter for Genetic Medicine Research, Children's National Medical Center, 111 Michigan Ave. NW, Washington, DC, 20010, USA

^bReveraGen Biopharma, 9700 Great Seneca Hwy, Rockville, MD 20850, USA

^cInstitute of Human Genetics, International Centre for Life, Central Parkway, Newcastle upon Tyne NE1 3BZ, UK

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INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked progressive muscular dystrophy, caused by loss of the dystrophin protein at the myofiber membrane.^{1,2} Pharmacologic treatment of DMD patients with glucocorticoids can improve patient strength and prolong ambulation, with concomitant improvements in quality-of-life scales.^{3–9} As such, glucocorticoid treatment for DMD is recommended in standard-of-care guidelines, and as an American Academy of Neurology practice parameter.^{10–12} The majority of trials and treatment recommendations have used an oral dose of prednisone at 0.75 mg/kg/d. However, alternative dosing regimens have been reported as changing the efficacy versus side-effect profiles, including weekend dosing,^{13,14} lower doses,¹⁵ and alternative-day doses (10 mg/kg/wk divided over 2 weekend days).^{16–18} In each study, a goal was to achieve a better balance of efficacy (increased strength and delay of disease progression) with fewer side effects (bone fragility, weight gain, mood changes).^{19–21} It is pertinent to note that muscle weakness and wasting is an acknowledged side effect of chronic glucocorticoid administration in many indications, such as critical care medicine, and is the most common drug-induced form of muscle weakness.²² Glucocorticoids have a direct molecular effect on myofibers, stimulating the catabolic AKT1/FOXO1 pathway, decreasing protein synthesis and increasing the rate of protein catabolism, resulting in weakness and atrophy.²³ Thus it is likely that DMD patients treated with glucocorticoids show the clinical outcome of increased muscle strength mitigated to some extent by the side effect of muscle catabolism. Clearly any effort to reduce side effects such as weight gain and short stature may also lead to lessening of the side effect of muscle weakness, whereby the balance would then be tipped to greater efficacy.

Fluorinated glucocorticoids, such as dexamethasone, are considerably more potent, with higher affinity to the glucocorticoid receptor (Fig. 1). However, these tend to also exacerbate side effects, and are generally avoided in indications of chronic use, such as muscular dystrophy. On the other hand, less potent nonfluorinated varieties of glucocorticoids have been tried, such as deflazacort. Deflazacort trials in DMD have suggested similar efficacy to that of prednisone (albeit at a higher dose), with an improvement in some side-effect profiles.^{3,24–26}

FINDING THE OPTIMUM REGIMEN OF CORTICOSTEROIDS FOR DMD (FOR-DMD) CLINICAL TRIAL

To study the balance of efficacy and side effects, depending on steroid type (prednisone vs deflazacort) and dosing regimen (daily vs 10 days on, 10 days off), the FOR-DMD trial was designed and implemented. FOR-DMD is a multicenter, double-blind, parallel-group, 36- to 60-month study, comparing 3 corticosteroid regimens in wide use in DMD:

- Daily prednisone (0.75 mg/kg/d)
- Intermittent prednisone (0.75 mg/kg/d, 10 days on, 10 days off)
- Daily deflazacort (0.9 mg/kg/d).

The hypothesis being tested is that daily corticosteroids (prednisone or deflazacort) will be of greater benefit than intermittent corticosteroids (prednisone) in terms of function and subject/parent satisfaction. A secondary outcome is to study whether daily deflazacort will be associated with a better side-effect profile than daily prednisone.

The primary outcome variable will be a 3-dimensional (multivariate) outcome consisting of the following 3 components (each averaged over all postbaseline follow-up visits through month 36): (1) time to stand from lying (log-transformed), (2) forced vital capacity, and (3) subject/parent global satisfaction with treatment, as measured by the Treatment Satisfaction Questionnaire for medication.

Secondary outcome variables will include regimen tolerance, adverse event profile, and secondary functional outcomes including the 6-minute walk test, quality of life, and cardiac function. The analyses will be adjusted for covariates, namely country/ region, baseline time to stand from lying, baseline forced vital capacity (FVC), and initial weight band. A sample size of 100 subjects per group (300 in total) will provide adequate power to detect differences that are thought to be of minimal clinical significance between any 2 of the 3 treatment groups, assuming a 10% rate of subject withdrawal.

The trial will randomize 300 boys aged 4 to 7 years to 0.75 mg/kg/d prednisone; 0.75 mg/kg/d prednisone for 10 days alternating with 10 days off; or 0.9 mg/kg/d deflazacort. All boys will complete a minimum 3 years (36 months) treatment period. All boys entering the trial will remain on the study drug until the last boy completes the 36 months of study; this may be up to 60 months.

Eligible boys will be those with confirmed DMD (defined as male with clinical signs compatible with DMD and confirmed DMD mutation in the dystrophin gene [out-of-frame deletion or point mutation or duplication] or absent/<3% dystrophin on muscle biopsy); age at least 4 years and under 8 years; ability to rise independently from the floor; willingness and ability of parent or legal guardian to give informed consent; willingness and ability to comply with scheduled visits, drug administration plan, and study procedures; and ability to maintain reproducible FVC measurements.

The study is funded by the National Institutes of Health (Kate Bushby and Robert Griggs, study Chairs), and will begin enrollment in 2012.

DEVELOPMENT OF DISSOCIATIVE STEROIDS FOR DMD

An alternative approach to optimizing dosing regimens of traditional glucocorticoid drugs is to change the chemistry of the drug, with the goal of broadening the therapeutic window (increasing efficacy while decreasing side effects). Glucocorticoid drugs are recognized to have 2 subactivities: serving as a ligand for steroid hormone receptors, and nonreceptor-mediated effects on plasma membranes. The ligand/receptor complex has 2 further subactivities: transactivation and transrepression properties. Transactivation (also termed *cis*-regulation) is the best characterized molecular response, whereby ligand/glucocorticoid receptor complexes translocate from the cytoplasm to the nucleus and then interact directly with DNA and gene promoters (Fig. 2). With transactivation, the ligand/receptor dimers typically bind to a DNA sequence motif (glucocorticoid response elements [GRE]), and activate transcription of the nearby gene (hence the designation “transactivation”). Of importance, there is increasing evidence that the transactivation subactivity is associated more with side effects rather than with drug efficacy.²⁷

Clinical efficacy, on the other hand, is increasingly associated with the second, transrepression subactivity. Transrepression involves ligand/receptor interactions with other cellular signaling proteins, such as nuclear factor (NF)- κ B, activator protein 1, and STAT5 complexes, with downstream changes in cell signaling, and more indirect effects on gene transcription (non-GRE-mediated).^{27,28} Transrepression has been associated with anti-inflammatory activity and clinical efficacy.

All steroid hormones, including glucocorticoids, are lipophilic, and readily traverse lipid bilayers (cell membranes). Some steroid drugs have been optimized for membrane activities, such as the lazaroids (see Fig. 1). Lazaroids, including the Δ -9,11 modification thought to block binding of the drug to the receptor, were optimized for effects on cell membranes (prevention of lipid peroxidation), and tested clinically for neuroprotection.^{29–31} In DMD, there are well-documented changes in myofiber membrane function and integrity, and steroids are likely to modify this defect (for better or worse). Consistent with this, recent studies of lazaroids in myogenic cells in culture³² and ischemia/reperfusion injury in vivo have shown benefit of lazaroid drugs.³³

In an effort to improve upon glucocorticoid therapy for DMD, the authors studied drugs with the Δ -9,11 chemistry (see Fig. 1). The goal was to determine whether this chemistry represented a dissociative steroid (eg, separation of the transactivation [side effects] and transrepression [efficacy]) (see Fig. 2). A Δ -9,11 drug, anecortave, did in fact bind the glucocorticoid receptor, albeit at lower affinity than pharmacologic glucocorticoids.³⁴ Of importance, the ligand/glucocorticoid receptor complex was found to translocate to the nucleus, but showed no activity in binding to GRE elements and activating GRE-mediated gene transcription. Thus, the Δ -9,11 drug appeared to have lost the transactivation subactivity associated with many deleterious side effects (see Fig. 2).

To determine whether the Δ -9,11 drug retained transrepression (the subactivity associated with glucocorticoid efficacy), the authors studied anti-inflammatory effects using NF- κ B reporter assays.³⁵ NF- κ B inhibitory activity was found to be retained by the Δ -9,11 drug, at a potency similar to that of prednisone.³⁴ We were also interested in the effects of the drugs on the phospholipids that make up the membrane. Phospholipid bilayers have a hydrophilic head on the inner and outer walls of the membrane and hydrophobic tails. Lipids such as cholesterol are known to compress head groups, strengthen the bilayer, and decrease permeability when incorporated into a lipid bilayer. Indeed, our delta 9,11 steroids exert a

similar and more profound effect on phospholipid bilayers than either prednisolone or cholesterol. The D-ring functionality (17-hydroxy-20-keto-21-hydroxy) orients within the phospholipid head groups while the hydrophobic ABC and most of the D ring orients in the lipid core. Since the C-17 C-20 bond can rotate, our compounds are operationally cone-like wedges in the phospholipid. They compress the head groups and decrease permeability while disordering the hydrophobic core which, among other things, protects against lipid peroxidation by decreasing the repeat number in lipid peroxidation chain reactions. This phenomenon for the Δ 9,11 steroids has been described.³⁶ These results encouraged the authors to conduct a preclinical study of the dystrophin-deficient *mdx* mouse model of DMD. Evidence of efficacy in vivo was found, whereby daily oral delivery of Δ -9,11 analogue reduced muscle inflammation and improved multiple functional assays. Of note, no side effects of reductions in body weight or spleen size seen with prednisone treatment were observed, suggesting that the Δ -9,11 drug had indeed lost side effects. These data suggest that the Δ -9,11 chemistry holds promise as a dissociative steroid, with retention of efficacy via transrepression, and loss of side effects via reductions in transactivation subactivities. Current studies are focused on testing a series of Δ -9,11 compounds to optimize the potency, bioavailability, and toxicity profiles (lead compound selection), as well as testing of the optimized lead compound in animal models of multiple chronic inflammatory conditions, including other types of muscular dystrophy.

SUMMARY

DMD is among the most common of the muscular dystrophies, leading to shortened life span and considerable disability. Glucocorticoids are considered the standard of care, yet dose regimens have not been optimized, and the balance of efficacy and side effects for specific types of glucocorticoids requires further study. The FOR-DMD trial promises to shed light on dose optimization, as well as the therapeutic window of prednisone versus deflazacort. An alternative approach to optimizing currently available steroid regimens is to develop new drugs that are able to broaden the therapeutic window (increased efficacy with decreased side effects). Initial studies of Δ -9,11 modifications of the steroid backbone suggests that this chemistry produces a dissociative steroid, whereby anti-inflammatory activity is retained (transrepression) and membrane stabilization properties enhanced, while side effects are mitigated (loss of transactivation subactivity). Current studies are focusing on lead compound optimization using transactivation and membrane stability assays.

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KEY POINTS

- Current standard of care of Duchenne muscular dystrophy (DMD) includes pharmacologic treatment with oral glucocorticoids.
- Gains in strength and slowed progression of disease afforded by glucocorticoids are offset, in part, by the wide range of side effects of drug treatment.
- Dose optimization studies are limited, and new larger clinical studies are needed to best balance efficacy and side effects (therapeutic window), as are studies of glucocorticoid alternatives to prednisone.
- The FOR-DMD trial funded by the National Institutes of Health is under way to compare different dose regimens and types of glucocorticoids (prednisone, deflazacort).
- A novel dissociative steroid, a Δ -9,11 drug, is under clinical development for DMD. This drug promises to broaden the therapeutic window and reduce side-effect profiles.

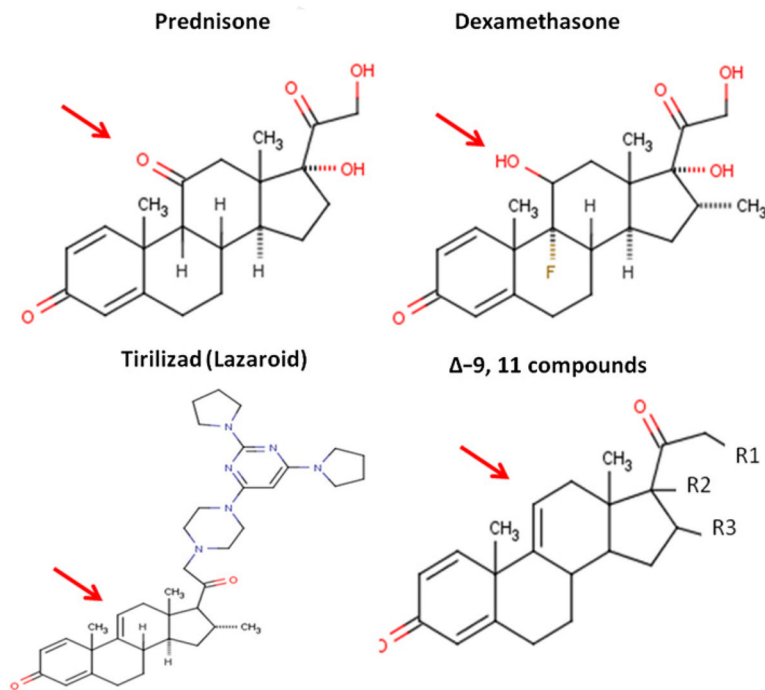


Fig. 1. Chemical structures of glucocorticoids and dissociative steroids. The arrow indicates the position of the key 9,11 alterations distinguishing classic glucocorticoids (prednisone, dexamethasone) from dissociative steroids (Δ -9,11 analogues).

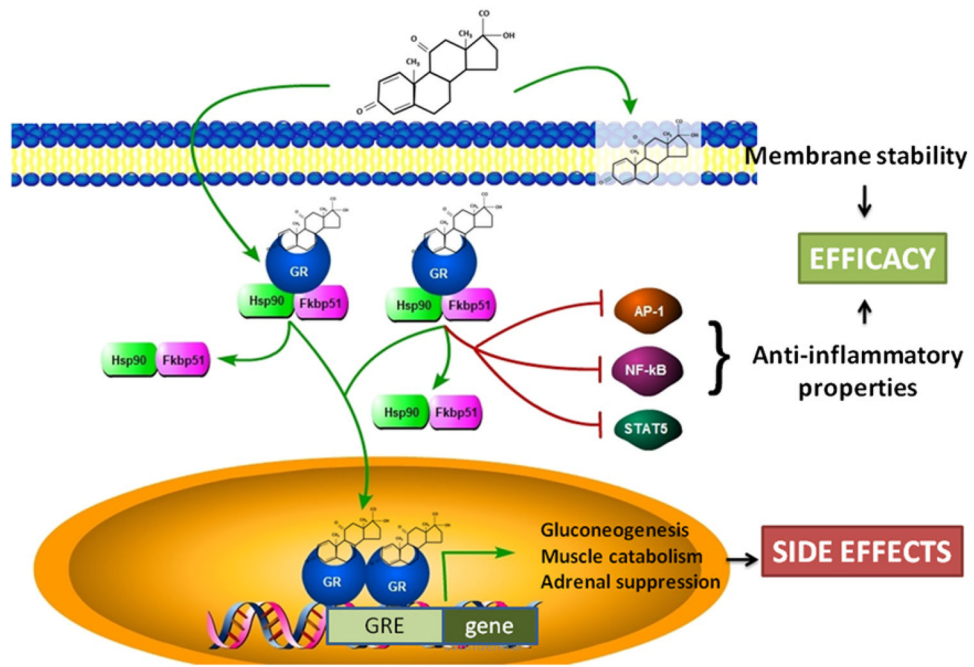


Fig. 2. Molecular action of glucocorticoids and dissociative steroids. Classic pharmacologic glucocorticoids have anti-inflammatory, membrane fluidity, and glucocorticoid response element (GRE)-mediated transcriptional activities. Dissociative steroids retain membrane and anti-inflammatory subactivities associated with efficacy, but do not retain the GRE-mediated transcriptional activities associated with side-effect profiles. GR, glucocorticoid receptor.