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REVIEW

Vamorolone as a New Dissociative Steroid and Its Effects on Bone

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ABSTRACT

Conventional steroids continue to be extremely valuable medications today; however, their side-effects, specifically in chronic administration, are well appreciated, including those on the musculoskeletal system. Conventional steroids exert their activity via the transcriptional changes mediated by the glucocorticosteroid receptor in the cell nucleus. To reduce the adverse effects of conventional steroids, a new class of dissociative steroids has been developed, with vamorolone as a most known member. Similarly, vamorolone binds to the nuclear glucocorticosteroid receptor and renders anti-inflammatory effects by inhibiting pro-inflammatory NF-κB signaling; however, while retaining its anti-inflammation potential, vamorolone has ability to cause less adverse effects with chronic use. The objective of this paper is to review the current status of initial clinical experience with vamorolone, address its indications, dosage, side-effects, specifically on bone, and discuss whether its theoretical expectations could be corroborated by examining recently conducted pertinent clinical trials.
Level of Evidence: V; Expert opinion.
Keywords: Steroids; Dissociative steroids; Steroid side-effects; Vamorolone.

INTRODUCTION

Conventional glucocorticoids are widely used medications for a broad range of autoimmune and anti-inflammatory therapies in orthopedics, some of which include rheumatoid arthritis, systemic lupus erythematosus, scleroderma, multiple sclerosis, myasthenia gravis, poliomyelitis, and Duchenne muscular dystrophy (DMD). Glucocorticoids, such as prednisone, are utilized for a range of therapeutic effects, from mild pain relief to strong immunosuppressive and anti-inflammatory actions. For instance, prednisone is a helpful therapy in reducing joint tenderness and painful flareups while improving grip strength in patients with rheumatoid arthritis. In certain incurable diseases, such as DMD, prednisone is prescribed in order to slow the disease's progressive course. Studies have indicated that prednisone is a first-line agent for symptom control in DMD by preserving muscle mass and function, delaying the loss of ambulation, and reducing

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the risk of scoliosis while simultaneously providing anti-inflammatory effects and improving overall survival rate. There are minimal alternative treatments for disorders, such as DMD, where glucocorticoids are highly indicated and constitute the primary form of therapy that can control symptoms and disease progression.

Molecular effects of conventional steroids

Certain doses and chronic use of glucocorticoids raise the risk of adverse effects, including Cushingoid features, cataracts, hypertension, insulin resistance, adrenal suppression, mood disorders, and especially glucocorticoid-induced osteoporosis. Prednisone doses as low as 0.2mg/kg daily can increase the risk of bone loss and vertebral fractures. Glucocorticoid-induced osteoporosis is the most common form of secondary osteoporosis and the first cause in young people. Glucocorticoid-induced bone destruction is promoted via the apoptosis of osteoblasts and intensifying osteoclast activity. These steroids are able to stimulate osteoclastogenesis by altering the expression of osteoprotegerin (OPG), nuclear factor kappa B subunit (NF-κB) ligand, and colony-stimulating factor-1 [1]. Essentially, glucocorticoids inhibit the synthesis of OPG and upregulate the expression of macrophage colony-stimulating factor (M-CSF) and RANKL (receptor activator of nuclear factor kappa-B ligand) by osteocytes. In turn, this induces the production of osteoclasts and leads to accelarated bone resorption [2,3]. The reduction of bone formation, and increase in bone resorption depict the risk of vertebral fractures and prevails as one of the critical adverse effects of chronic conventional steroid use.

Molecular effects of dissociative steroids

The severe side-effect profiles of conventional glucocorticoids are linked to the transcriptional changes associated with the activities of the glucocorticosteroid receptor (GR)/ligand dimer complex on nuclear DNA glucocorticoid response elements (GRE) [5,6]. Vamorolone is a new class of dissociative steroidal drugs that is similar to conventional glucocorticoids as it also binds to the GR. Dissociative steroids also maintain the GR/ligand monomers that mediate anti-inflammatory effects by inhibiting pro-inflammatory signaling cascades and genes under NF-κB control [5,7]. However, vamorolone retains the monomeric activity of anti-inflammation while also minimizing the adverse effects associated with GR/ligand dimer formation [8]. Generally speaking, this mechanism is termed the "dissociation" of steroid benefits from adversities as the significant complications of long-term conventional glucocorticoid use can be avoided with the use of dissociative steroids [5,9].

Stuctural Differences

Vamorolone is structurally similar to certain conventional steroids, such as prednisone. Prednisone possesses a 11β-hydroxyl group on the C-ring as opposed to vamorolone which occupies a carbon-carbon double bond at the 11β-position. The Δ9,11 modification of vamorolone (**Figure 1 and 2**) allows the steroid to avoid interactions with a conserved receptor residue, N770/N564, in both mineralocorticoid and glucocorticoid receptors [10,12]. Conventional steroids, such as prednisone, are able to interact with the receptor residue N770/N564 in mineralocorticoid and glucocorticoid receptors so that it

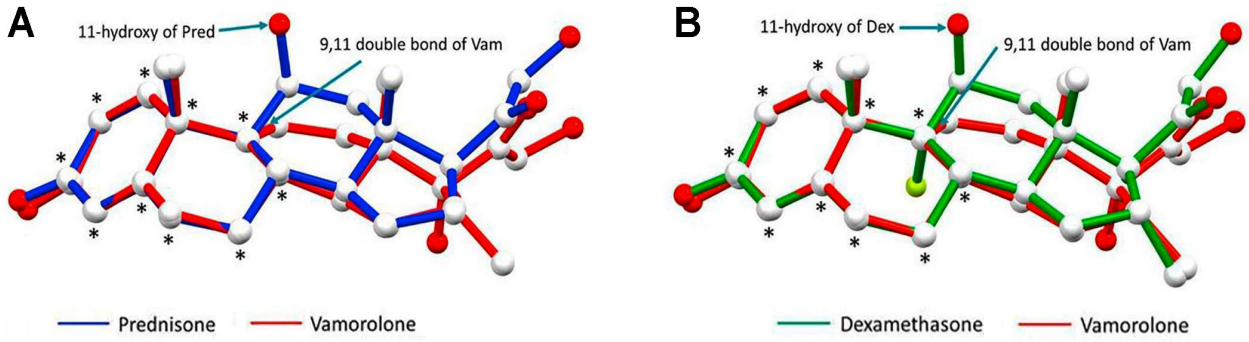

Figure 1. Overlay comparisons of crystal structures of predison versus vamorolone (**A**) and dexamethasone versus vamorolone (**B**), in which the 11-hydroxy groups of prednisone and dexamethasone as well as 9,11 double bond of vamorolone are depicted.

modulates receptor transactivation. When glucocorticoids diffuse across the cell membrane and bind to the GR in the cytoplasm, the GR is displaced from a complex of chaperone proteins and migrates to the cell nucleus where transactivation of genes regulated by GR-bound promoter elements occurs. In many cases, transactivation is dependent on the homodimerization of GR [11]. Due to vamorolone's structural difference, homodimerization of the GR does not occur, preventing transactivation. Transactivation of GRE-regulated genes mediate the majority of adverse effects associated with conventional steroids, such as a decrease in bone mineral density. Therefore, vamorolone's ability to avoid off-target transactivation of GRE-regulated genes prevails as one potential mechanism of the drug's reduced adverse effects and improved safety.

Diversely, GRs participate in the transrepression of inflammatory genes. Transcription factors, such as activating protein 1 (AP-1) and NFκB, are involved in the expression of pro-inflammatory genes. GRs are present at AP-1 and NFκB binding sites, altering the recruitment of co-activators and repressors [11]. This property of glucocorticoids allows the indirect repression of inflammatory genes. Therefore, in addition to vamorolone's ability to avoid off-target transactivation, it is able to maintain the transrepression of inflammatory genes by preserving the inhibitory GR-NFκB protein-protein interaction. Compared to prednisone, deflazacort, and eplerenone

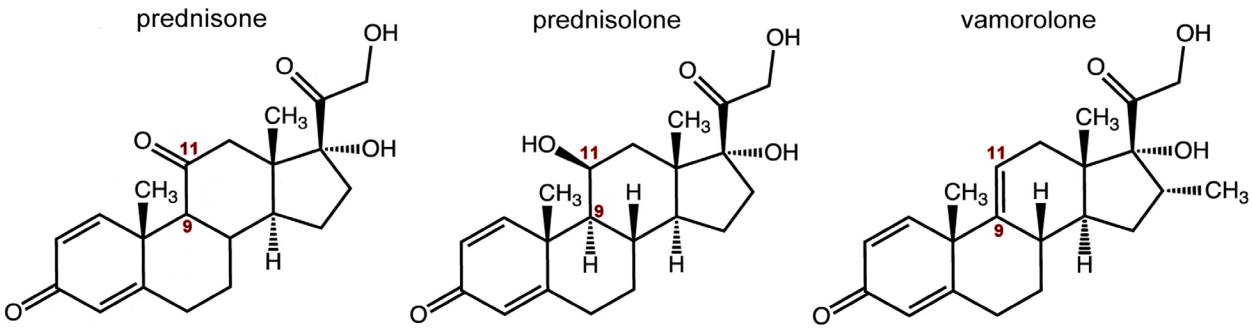

Figure 2. Organic structures of prednisone, prednisolone, and vamorolone, demonstrating the delta 9,11 carbon-carbon double bond modification of vamorolone.

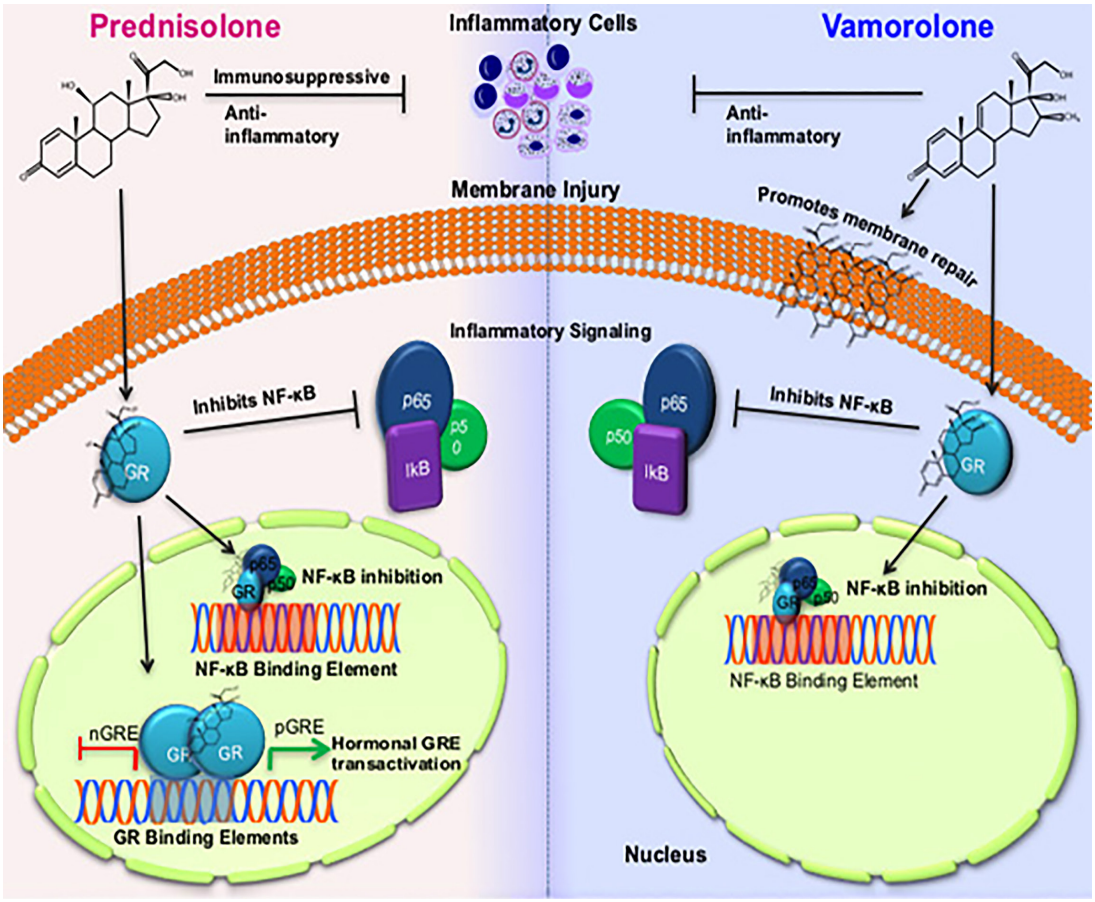

Figure 3. Comparative mechanisms of actions of prednisolone versus vamorlone. The apparent distictions include transrepression, transactivation, physicochemical membrane effects, synchronization of tissue remodeling, and mineralocorticoid receptor antagonism. Prednisolone exhibits a dose-dependent increase in GRE-mediated transactivation activity, whereas vamorolone does not, indicating that vamorolone has potentially a reduced GRE-mediated side effect profile (14).

used for DMD, vamorolone is capable of anti-inflammatory effects by interacting with the GR-dependent NF-κB promoter, while simultaneously maintaining the inactivation of the GR-dependent GRE promoter, which is responsible for many adverse effects [10,15]. The evasion of adverse effects demonstrates vamorolone's unique characteristics that make it a desirable alternative to conventional glucocorticoids (**Figure 3**).

Glucocorticoid Effects on Muscle and Oxidative Stress

In addition to bone loss, glucocorticoids also lead to muscle cell atrophy via a catabolic manner that is mediated by transcription factors, one being forkhead box O (FOXO). FOXO is a protein found within the Forkhead family of transcription factors. Studies show that glucocorticoids enhance FOXO-1 and FOXO-3 gene expression in myotubes. Similar to bone, glucocorticoid-induced muscle atrophy is thought to be related to the transactivation of GR-dependent genes that result in increased protein breakdown and a decrease in protein synthesis. Glucocorticoid-induced muscle atrophy involves various degradation pathways, including the ubiquitin-proteasome degradation pathway, the autophagy system, and the calcium-dependent calpains.

Studies demonstrate vamorolone's ability to avoid the induction of the protease calpain 1 involved in myocyte degradation, unlike prednisone, allowing it to preserve glucocorticoid-induced ergogenic effects in muscle metabolism [16]. Furthermore, the ATP-dependent ubiquitin-proteasome degradation pathway is regulated during steroid-induced atrophy by several genes that are controlled by the FOXO transcription factors. The genes involved in myocyte atrophy include atrogenes, such as MuRF-1 (muscle RING-finger protein-1), cathepsin L, and atrogin-1, which is specifically activated by FOXO [17]. These genes encode ubiquitin ligases which conjugate ubiquitin to other proteins. Glucocorticoid mediated overexpression of FOXO and subsequent activation of atrogenes demonstrate the role of glucocorticoids in muscle cell atrophy.

Conversely, glucocorticoids also possess ergogenic effects. In muscle wasting diseases, such as DMD, the ergogenic properties of glucocorticoids are used to blunt muscle degeneration. Glucocorticoids are involved in specific regulatory pathways in ergogenic metabolism that are distinct from those involving muscle atrophy and degradation. KLF15 (Kruppel-like factor 15) is a metabolic transcription factor involved in the metabolic and ergogenic pathways in muscle. Studies show that DMD expresses a state of relative KLF15 deficiency, contributing to the atrophic and dystrophic effects of the disease. Glucocorticoids play a role in ameliorating muscle atrophy in DMD through the induction of KLF15. This demonstrates the therapeutic effects of chronic glucocorticoids in slowing myocyte degradation in muscle-wasting diseases, such as DMD [18].

Vamorolone's Effects on Bone

Although the dissociation model is simplified, in vitro and in vivo studies of cytokine profiles, bone health markers, and genetic activity associated with osteoporosis show initial promise for vamorolone attenuating adverse effects of steroids on bone. Investigators have observed the preservation of bone health when using vamorolone in animal models and in early phase 1 and 2 human clinical trials, making vamorolone a steroidal drug of great promise for even further inquiry [8,12,13,19-21].

Conklin et al [19] conducted a Phase 2 open-label clinical trial of 48 DMD children (in 4 cohorts of 12) who received varying ascending doses of vamorolone over 14 days. Osteocalcin, a biomarker indicative of bone formation, and C-terminal telopeptide (CTX), a biomarker indicative of bone resorption, were both compared at specific doses of vamorolone (6.0 mg/kg) and prednisone (0.75mg/kg). Comparing published data of boys with DMD treated with glucocorticoids and boys with DMD treated with vamorolone, the latter significantly reduced bone resorption at 2.0 mg/kg and 6.0 mg/kg daily doses and a mild reduction in osteocalcin at a daily dose of 6.0 mg/kg and above, whereas prednisone induced reductions in osteocalcin at a daily dose as low as 0.75 mg/kg (~10-fold difference). Additionally, Conklin et al [19] explored the dose-responsive anti-inflammatory effects of vamorolone by measuring serum CD23, macrophage-derived chemokine (MDC), and IL-22 Binding Protein (IL22BP) at incremental vamorolone doses. CD23 (Fc epsilon RII) is present on B lymphocytes and various antigen presenting cells. The stimulation of pro-inflammatory cytokine production and macrophage inflammatory reactions is mediated by the cross-linking

of CD23. Therefore, its levels are indicative of inflammatory processes. After 2 weeks of treatment with vamorolone at daily doses of 2.0 mg/kg and 6.0 mg/kg in boys with DMD, CD23 levels decreased in a dose-responsive pattern. MDC helps mediate the movement of activated T-lymphocytes to sites of inflammation. Treatment with 6.0 mg/kg of vamorolone for 2 weeks demonstrated significant reduction in serum MDC. IL-22BP levels are correlated with IL-22, a pro-inflammatory cytokine produced by various immunologic cells that binds to its receptor IL-22BP. After treatment with 2.0 mg/kg and 6.0 mg/kg of vamorolone, IL-22BP serum levels were significantly reduced in a dose-dependent manner. The reduction of serum CD23, MDC, and IL-22BP demonstrates vamorolone's significant anti-inflammatory effects. Furthermore, the reduced potency of vamorolone in decreasing bone formation markers relative to prednisone, coupled with the opposite effect of the 2 drugs on bone resorption markers, and the lack of any changes of these markers in adult volunteers through 20 mg/kg of daily dosing [12] collectively suggest the potential for improvement in bone safety with vamorolone compared to traditional glucocorticoids.

Hoffman et al [12] conducted a phase 1 clinical trial involving 86 healthy adult males who received ascending doses of vamorolone over 14 days. Consistent with bone findings in pre-clinical models, where prednisone (5 mg/kg/day) induced severe stunting of growth and osteopenia, yet vamorolone (30 mg/kg/day) did not [8]. Bone turnover markers bridged to later bone morbidities include osteocalcin for bone formation, and CTX1 (C-terminal telopeptide of collagen 1) for bone absorption; furthermore, Hoffman et al [12] found no alterations of these bone turnover markers at any dose of vamorolone in the adult volunteers. Bone morbidity due to chronic treatment of glucocorticoids are among the chief safety concerns of patients and their physicians [22,23], and prednisone causes both acute and chronic changes of bone turnover markers at doses of 0.2 mg/kg/day [6,24]. The lack of any changes in bone turnover markers in vamorolone-treated volunteers through 20 mg/kg/day is particularly promising regarding potential improved bone safety of this dissociative steroid.

Dillingham et al [13] studied the effectiveness of vamorolone in inhibiting inflammation and disease progression in experimental autoimmune encephalomyelitis (EAE), a widely used mouse model of multiple sclerosis. Damsker at al [21] also conducted pre-clinical in vitro and vivo studies with vamorolone to measure inflammatory cytokine profiles and effects on a mice model of inflammatory bowel disease. Both studies examined the bone resorption effects of vamorolone compared to prednisone in vitro. Prednisone causes deleterious bone effects as it induces GRE-mediated transcription of RANKL in osteoblasts which in turn activate osteoclasts and their bone resorptive properties. MG-63 cells (osteoblast-like cells) were utilized to estimate RANKL expression in vivo after treatment of vamorolone and prednisone after 24 hours. Dillingham et al [8] found a significant 2.5-fold increase in RANKL in prednisone treatment compared to vamorolone, demonstrating vamorolone's improved bone safety. Treatment with prednisone, but not vamorolone, increased expression of genes associated with bone loss and muscle atrophy as vamorolone is able to dissociate glucocorticosteroid mechanisms that control bone loss and muscle atrophy, suggesting the lack of side effects of vamorolone.

Damsker et al [25] conducted preclinical research to assess the effect of vamorolone, a first-in-class dissociative steroidal compound, to inhibit inflammation when administered after disease onset in the murine collagen antibody-induced arthritis (CAIA) model of arthritis. In their studies, vamorolone and prednisolone (10, 20, 40, mg/kg/day) were administered in mice models after full induction of CAIA. They found that both vamorolone and prednisolone effectively reduced disease score and joint inflammation as measured by cathepsin B activity, a proteinase that plays a role in joint destruction in rheumatoid arthritis. Furthermore, they assessed the presence of pro-inflammatory mediators in CAIA joints following vamorolone and prednisolone treatment. Three cytokines (IL-6, IL-1β, and IL-4) that have been previously implicated in promoting the pathogenesis of rheumatoid arthritis were shown to be reduced by both vamorolone and prednisolone at all doses (10, 20, 40 mg/kg/day) tested. As IL-6 and IL-1β have been previously shown to be regulated by NF-κB, the reductions of these cytokines are consistent with the well-described transrepression (eg, NF-κB inhibition) property of steroidal compounds [26,27].

One of the most well-described adverse effects associated with long-term glucocorticoid use is bone loss [28]. Histopathological analysis of affected joints demonstrated that vamorolone treatment of 20 mg/kg/day resulted in a significant dose–responsive decrease in bone erosion, whereas prednisolone treatment did not result in such a reduction pattern. The deleterious effects of prednisolone on bone health were further demonstrated by a reduction in intra-articular space and bone adhesions at all 3 prednisolone doses (10, 20, 40 mg/kg/day) tested in this study. These findings are consistent with previous work demonstrating that vamorolone possesses bone-sparing properties in vivo when compared to prednisolone [8].

Hoffman et al [29] conducted an open-label multiple-ascending-dose study of vamorolone in 48 boys with DMD over 24 weeks. A biomarker of bone formation, osteocalcin, increased in vamorolone-treated boys, suggesting possible loss of bone morbidities seen with glucocorticoids. Over 24 weeks, treatment with vamorolone doses of 0.75 mg/kg/day up to 2.0 mg/kg/day demonstrated a statistically significant rise in osteocalcin levels while a vamorolone dose of 6.0 mg/kg/day did not significantly increase osteocalcin levels over 24 weeks. Increases in serum osteocalcin is indicative of the improvement of bone density and the loss of deleterious bone effects that prednisone can precipitate at daily doses (0.75 mg/kg) approximately 3 times lower than that of vamorolone (2 mg/kg/day). Studies also demonstrated that vamorolone treatment led to a reduction of several disease parameters, including disease score, joint inflammation, and the presence of pro-inflammatory mediators to a degree similar to that observed with prednisone treatment. More importantly, histopathological analysis of affected joints showed that vamorolone treatment significantly reduced the degree of bone erosion while this bone-sparing property was not observed with prednisolone treatment at any of the tested doses.

CONCLUSIONS

Vamorolone, a new dissociative steroid, has been developed as an alternative glucocorticoid therapy for several autoimmune and chronic inflammatory conditions, such as

DMD, multiple sclerosis, rheumatoid arthritis, autoimmune encephalomyelitis, lung disease, and inflammatory bowel disease, all of which prednisone is typically indicated. Animal studies demonstrating the reduction of asthma-associated lung fibrosis and inflammation with vamorolone therapy have been reported [20]. Additionally, chronic therapy with glucocorticoids has proven to induce remission and prevent relapse of ANCA-associated vasculitis (AAV), a group of autoimmune diseases characterized by small vessel inflammation. Similarly, glucocorticoids are indicated for juvenile dermatomyositis (JDM), a pediatric inflammatory myopathy that induces muscle weakness and skin rashes over joints and eyelids. Due to the deleterious systemic adverse effects of chronic steroid use in AAV and JDM patients, clinical studies of vamorolone therapy for AAV and JDM are being developed. Dissociative steroids are able to replace conventional glucocorticoid therapies as they share similar chemical structures and mechanisms, although vamorolone possesses a carbon-carbon double bond at the 11-β position on the C-ring. This structural difference prevents vamorolone from interacting with residues that bind gene-activating ligands, thus hindering off-target transactivation while maintaining the ability to repress inflammatory genes. Vamorolone's ability to sustain anti-inflammatory properties while avoiding adverse effects designates it as a desirable replacement for prednisone and other conventional glucocorticoids. Specifically, prolonged use of conventional steroids, such as prednisone, can precipitate systemic adverse effects where bone morbidity prevails as a chief concern of patients and physicians. Compared to prednisone, vamorolone demonstrated advanced bone safety profiles as it reduced the risk of suppressing bone growth and the development of osteoporosis with continuous use. Studies have established that doses as low as 0.2mg/kg/day of prednisone can generate acute and chronic changes of bone turnover markers while 20mg/kg/day of vamorolone did not induce changes in bone turnover markers, indicating vamorolone's reformed bone safety and potential as a sustainable therapy for chronic inflammatory and auto-immune diseases. A recent 6-month GLP chronic toxicology study of vamorolone tested on mice has determined that doses as high as 45 mg/kg/day can be administered with minimal adverse effects [25]. The reduction in adverse effects at higher doses of vamorolone compared to prednisone allows for improved efficacy of treatment for chronic conditions, such as rheumatoid arthritis. Additionally, vamorolone is capable of antagonizing the mineralocorticoid receptor while other glucocorticoids, such as prednisone, are agonists of this receptor that induce adverse effects such as hypertension and steroid-induced diabetes mellitus. Future directions can potentially study whether vamorolone's ability to antagonize the mineralocorticoid receptor can improve cardiac and skeletal muscle function in DMD and other chronic inflammatory conditions.

In correspondence to DMD, limb-girdle muscular dystrophy type 2B is characterized by a deficiency of dysferlin, a protein, which is vital for muscle membrane repair. Recent animal studies have demonstrated unique membrane-stabilizing properties of vamorolone which may be helpful in treating this particular dystrophy [30]. For DMD, glucocorticoids maintain crucial anti-inflammatory effects in order to slow the progression of muscle wasting. Deflazacort is currently the only FDA-approved therapy for DMD, although predni-

sone is commonly prescribed off-label. As of September 2020, vamorolone is expected to have an NDA submission to the US FDA for DMD by early 2021. Several studies have demonstrated vamorolone as a superior replacement for conventional glucocorticoids due to its improved bone safety and significant anti-inflammatory effects at lower doses than prednisone. There is compelling evidence that the anti-inflammatory effects of vamorolone not only improve muscle pathology, but also improve dystrophin markers in Becker muscular dystrophy, an intriguing implication for future studies [31].

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