Inflammation Research (2019) 68:969–980
https://doi.org/10.1007/s00011-019-01279-z

Inflammation Research

ORIGINAL RESEARCH PAPER

Vamorolone, a dissociative steroidal compound, reduces collagen antibody-induced joint damage and inflammation when administered after disease onset

Jesse M. Damsker[1] · Michaelyn R. Cornish[1] · Priya Kanneboyina[1] · Ila Kanneboyina[1] · Qing Yu[2] · Rachel Lipson[1] · Aditi Phadke[2] · Susan M. Knoblach[2] · Karuna Panchapakesan[2] · Melissa Morales[3] · Alyson A. Fiorillo[2] · Terence Partridge[2] · Kanneboyina Nagaraju[1,2,3]

Received: 10 January 2019 / Revised: 2 July 2019 / Accepted: 19 August 2019 / Published online: 24 August 2019

Abstract

Objective and design The objective of this study was 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 model of arthritis.

Animals 84 DBA1/J mice were used in this study ($n = 12$ per treatment group).

Treatment Vamorolone or prednisolone was administered orally after disease onset for a duration of 7 days.

Methods Disease score and bone erosion were assessed using previously described scoring systems. Cytokines were measured in joints via immunoassay, and joint cathepsin B activity (marker of inflammation) was assessed using optical imaging of joints on live mice.

Results We found 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 of that observed with prednisolone 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 While many intervention regimens in other studies are administered prior to disease onset in animal models, the current study involves delivery of the potential therapeutic after disease onset. Based on the findings, vamorolone may offer an efficacious, yet safer alternative to conventional steroidal compounds in the treatment of rheumatoid arthritis and other inflammatory diseases.

Keywords Rheumatoid arthritis · Glucocorticoids · Inflammation · Autoimmunity

Abbreviations

CAIA	Collagen antibody-induced arthritis
RA	Rheumatoid arthritis
GCs	Glucocorticoids
DMARDs	Disease-modifying anti-rheumatic drugs
GREs	Glucocorticoid response elements
MR	Mineralocorticoid receptor
i.p.	Intraperitoneal
LPS	Lipopolysaccharide
Vam.	Vamorolone
Pred.	Prednisolone
Pisi	Pisiform
NL	Navicular lunate
Tri	Triangular
mc	Metacarpal
cb	Cortical bone
ep	Epiphysis

Responsible Editor: John Di Battista.

✉ Jesse M. Damsker
Jesse.Damsker@reveragen.com

✉ Kanneboyina Nagaraju
knagaraju64@gmail.com

Extended author information available on the last page of the article

Introduction

Rheumatoid arthritis (RA) is a chronic disease characterized by inflammation of the lining of the synovial joints resulting in long-term joint damage, loss of function, and pain. It is estimated that RA affects over 24 million people worldwide while between 14.6 and 90% of patients are treated with synthetic glucocorticoids (GCs) [1, 2].

While the beneficial effects of GCs on disease have been well documented, the prescription of GCs in RA remains controversial partly due to significant adverse effects associated with chronic use including osteopenia with bone fragility, adrenal insufficiency, metabolic disturbance, endocrine imbalances, muscle wasting, growth stunting, and mood changes. Despite the advent of numerous classes of drugs for the treatment of RA including disease-modifying anti-rheumatic drugs (DMARDs) and biologics, many clinicians still prescribe GCs for the treatment of RA due to their proven effectiveness [3–5]. However, chronic use is discouraged due to the deleterious consequences of adverse effect profiles on patient quality of life [6–9].

While the physiological responses to GCs are well understood, molecular mechanisms remain less understood. Glucocorticoids bind cytoplasmic glucocorticoid receptors (NR3C1), form dimerized ligand/receptor complexes, which then enter the nucleus and bind to glucocorticoid response elements (GREs) altering gene transcription [10, 11]. Increasingly, this 'hormonal' or transactivation subactivity has been associated with adverse effect profiles. Efficacy is increasingly associated with transrepression, where ligand/receptor complexes inhibit the activity of pro-inflammatory transcription factors (NFκB) [12]. This process is generally independent of GRE-mediated gene transcription. Many of the beneficial, anti-inflammatory effects of GCs are attributed to transrepression, whereas adverse effects are attributed to GRE-mediated gene transcription.

Vamorolone (VBP15) is a first-in-class dissociative steroidal compound where the 11-ß-hydroxy or 11-keto group found on traditional GCs is replaced by a double bond between carbons 9 and 11. Because of this modification, vamorolone does not induce GRE-mediated gene transcription yet still retains anti-inflammatory activity by inhibiting NFκB activity [13, 14]. Interestingly, vamorolone has also been shown to act as a potent mineralocorticoid receptor (MR) antagonist [14, 15]. We have previously demonstrated efficacy that vamorolone reduces inflammation and improves function in mouse models of allergic asthma [16], muscular dystrophy [14], multiple sclerosis [17], and inflammatory bowel disease [18]. Additionally, vamorolone has been shown to possess a reduced adverse effect profile in mice compared to traditional GCs as it induces much less growth stunting and the transcription of genes associated with muscle atrophy [14, 16–18]. Furthermore, phase 1 and phase 2a clinical trials (14 days of treatment) assessing the safety and pharmacokinetics of vamorolone in healthy adult volunteers and in patients with Duchenne muscular dystrophy (DMD) demonstrated similar pharmacokinetic and metabolism profiles to prednisone as well as no drug-related serious adverse events through the highest doses tested in each trial (20 mg/kg/day in adults; 6 mg/kg/day in DMD patients). Biomarker studies in these trials revealed an improved safety profile of vamorolone versus GCs as demonstrated by beneficial changes in bone turnover, as well as, reductions of insulin resistance and adrenal suppression [19, 20]. Thus, these data suggest that vamorolone improves safety profiles compared to standard glucocorticoid regimens.

Due to the established anti-inflammatory capacity and reduced adverse effect profile associated with vamorolone, we were interested in assessing its effects within the context of rheumatoid arthritis. Importantly, previous studies have demonstrated that vamorolone has the capacity to reduce NFkB activity in macrophages and splenocytes—two cell types highly relevant to the pathogenesis of RA [14, 15, 17]. The current study utilizes a series of well-established preclinical endpoints in the widely used collagen antibody-induced mouse model of arthritis (CAIA). Furthermore, while many studies using this model initiate intervention regimens before disease induction, the current pilot study in this acute disease model evaluates the therapeutic effect(s) of vamorolone by initiating treatment after disease is initiated.

Materials and methods

Animals

Male DBA/1 J mice (aged 6–8 weeks) were purchased from Jackson Laboratories (Bar Harbor, ME). All studies were reviewed and approved by the Institutional Animal Care and Use Committee at Children's National Health System.

Collagen antibody-induced arthritis mouse model

On day 1, DBA1/J mice received an intraperitoneal (i.p.) injection of 2 mg of monoclonal antibody cocktail specific for collagen (CII) (MD Biosciences, St. Paul, MN). Two days later (day 3), mice received an i.p. injection of 100 µg of lipopolysaccharide (LPS). Mean clinical severity of CAIA was determined using a macroscopic scoring system on days 4, 7, 10, and 13 by two blinded individuals. The scoring criteria were performed as follows: 0: no signs of disease; 1: mild redness and/or swelling of one digit; 2: redness and/or

swelling of 2 digits; 3: redness and/or swelling of more than 2 digits; 4: redness and swelling of entire paw. For intervention studies, mice ($n = 12$) received oral doses of 10, 20, and 40 mg/kg of vamorolone or prednisolone suspended in cherry-flavored syrup (30 µl total volume) on day 7 through day 13. Additional groups of mice ($n = 12$) received cherry-flavored syrup as a negative control. On day 14, animals were killed via CO_2 exposure.

Optical imaging

On day 13, mice were anesthetized with isoflurane, and cathepsin-caged near-infrared imaging was subsequently performed on six mice per group as described previously [21]. Mice received i.p. injections of ProSense 680 (Perkin–Elmer) in PBS 24 h prior to imaging with an Optix MX2 Imager (ART). Optical imaging scans of uninjected mice were performed to obtain baseline optical intensity measurements. Forelimb measurements were made at 0.5-mm resolution and analyzed using Optiview software.

Histopathological assessments

Mouse forelimbs were removed and placed in 10% formalin and sent to Histoserv Inc. (Germantown, MD) for processing and staining with Safranin-O and hematoxylin and eosin (H and E). Subsequently, slides were imaged with an Olympus VS-120 virtual scanning microscope. The presence of cartilage was qualitatively assessed on Safranin-O-stained sections. Bone erosion was quantified on H and E-stained sections on six different joint interfaces comprising the front paw using a scoring system that was modified based on a previously described method [22]. The joint interfaces examined in a blinded manner were the following: pisiform–ulna, ulna–radius, radius–navicular lunate, triangular–navicular lunate, triangular–carpal, and navicular lunate–carpal. The scoring method is described as follows: 0; no erosion of either bone, 1: minimal erosion of either bone, 2: moderate bone loss at the edges of the bones; 3: major erosion on one of the bones; 4: complete erosion of both bones. An average score per paw was then generated from these individual scores.

Cytokine analysis

Following killing, mouse forelimbs were snap frozen in liquid nitrogen. Subsequently, limbs were homogenized in RIPA buffer with a HALT Protease Inhibitor Cocktail (ThermoFisher Scientific, Waltham, MA) and EDTA added. A multiplex sandwich immunoassay (V-PLEX; Meso Scale Discovery, Rockville, MD) was then performed on forelimb homogenates per manufacturer's instructions.

Statistical analysis

For all analyses except for bone erosion quantification, statistical significance was established using one-way ANOVA with post hoc Tukey's test. Statistical analysis on bone erosion quantification was performed using an unpaired two-tailed t test. All analyses were performed using Graphpad Prism software.

Results

Vamorolone reduces the disease score of collagen antibody-induced arthritis

To determine if vamorolone can reduce inflammation within the context of rheumatoid arthritis, we performed a study that utilized the well-described and widely used mouse collagen antibody-induced arthritis (CAIA) model (Fig. 1b). On day 1, DBA/1 male mice received an i.p. injection of a monoclonal antibody cocktail specific for collagen. Two days later (day 3), mice received an i.p. injection of LPS. Following disease induction, mice ($n = 12$/group) were orally administered vamorolone (10, 20, 40 mg/kg/day), prednisolone (10, 20, 40 mg/kg/day), or cherry syrup as a vehicle control starting 4 days after LPS injection (day 7) and continuing daily through day 13. Mean clinical severity of CAIA was determined using a macroscopic scoring system by two blinded individuals on days 4, 7, 10, and 13 until the end of the experiment (day 13). We found that vamorolone treatment at the highest dose of 40 mg/kg/day and prednisolone treatment at all doses (10, 20, 40 mg/kg/day) led to significant reductions in disease score on day 13 (Fig. 1c).

Vamorolone and prednisolone treatment reduces joint inflammation in arthritic mice

Cathepsin B is a proteinase that contributes to joint destruction in RA [23]. One day prior to killing, mice underwent live optical imaging as a means of testing cathepsin B activity in joints. ProSense 680, a substrate cleaved by cathepsin proteases was injected as reported previously [21]. Cathepsin B activity was elevated in the forelimb joints of vehicle-treated CAIA mice, but vamorolone (40 mg/kg/day) and prednisolone (10–40 mg/kg/day) treatment significantly reduced cathepsin activity in joint forelimbs (Fig. 2) suggesting a reduction in the presence of joint inflammation which is in line with the disease score findings.

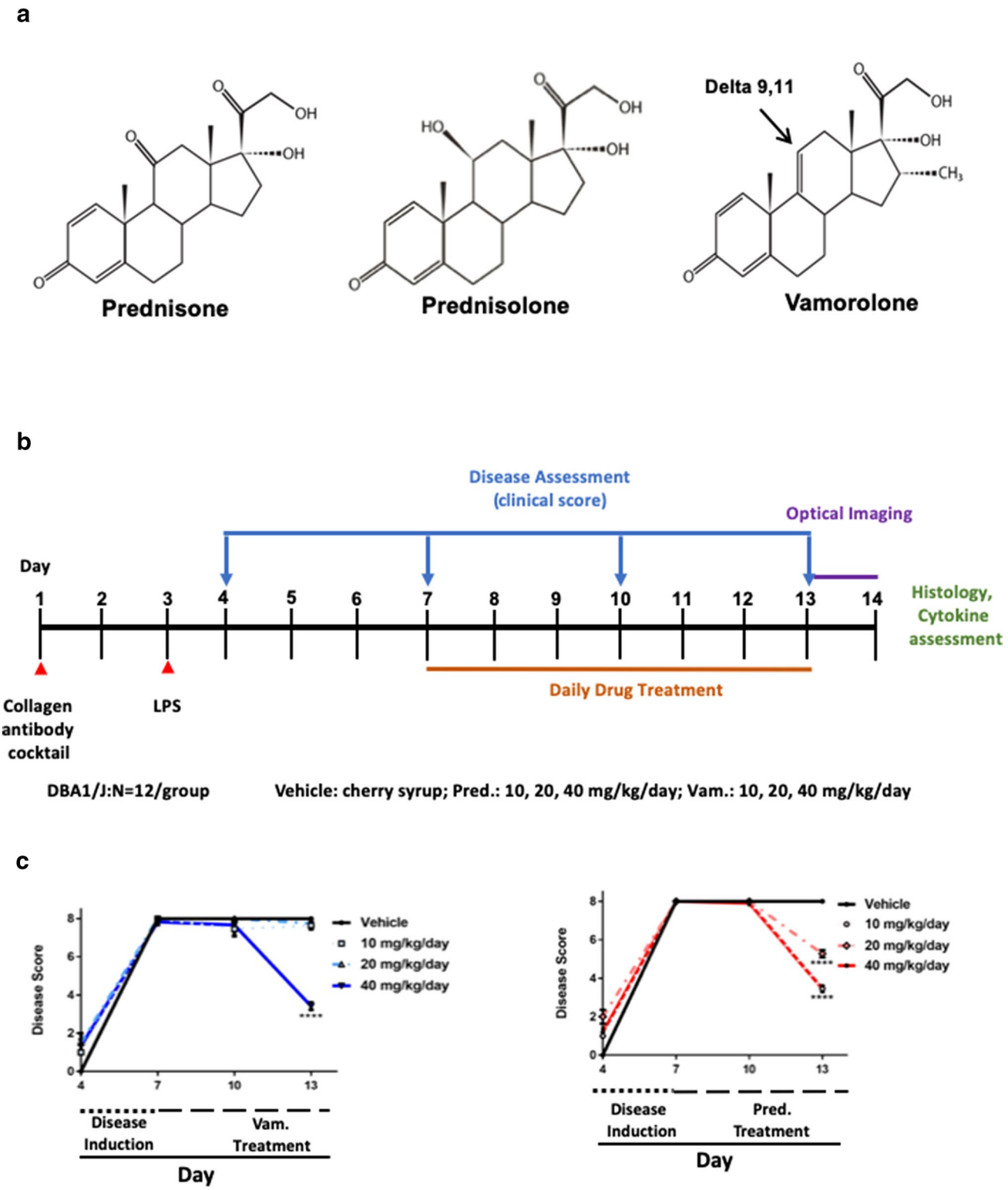


Fig. 1 Vamorolone treatment following disease induction reduces the severity of collagen antibody-induced arthritis. Structures of prednisone (left panel), prednisolone (mid panel), and vamorolone (right panel) are shown (**a**). Vamorolone (10, 20, 40 mg/kg/day), prednisolone (10, 20, 40 mg/kg/day), or vehicle control was administered on day 7 (**b**). Disease severity was assessed by calculating disease score. Disease score graphs (**c**) represent mean ± SE disease score for vamorolone (left)- or prednisolone (right)-treated mice ($n = 12$ mice/group). $^{****}p < 0.0001$ compared to vehicle-treated group

Vamorolone treatment reduces the presence of pro-inflammatory mediators in joints of arthritic mice

A pro-inflammatory role within the context of RA has been previously described for cytokines such as IL-6, IL-1β, and IL-4 [24–26]. Following killing, protein was extracted from excised joints. A subsequent multiplex immunoassay revealed that vamorolone and prednisolone treatment resulted in decreases of IL-6, IL-1β, and IL-4 at all three doses (Fig. 3).

Vamorolone treatment improves cartilage and bone health in CAIA mice

Following killing on day 14, mouse forelimbs were removed and processed for histology and stained with Safranin-O and hematoxylin and eosin. The presence of cartilage was qualitatively assessed on the front paws of vehicle and drug-treated mice (Fig. 4). Vamorolone treatment at all doses showed reduced cartilage destruction compared to vehicle-treated mice. On the contrary, prednisolone-treated mice at higher doses (20 mg/kg/day and 40 mg/kg/day) did not show this property of reduced cartilage destruction. A quantitative assessment of bone erosion was performed on paw sections stained with hematoxylin and eosin. Bone erosion was quantified on six different joint interfaces in the front paw, and an average score per paw was then generated. We found that vamorolone treatment resulted in a clear demarcation of intra-articular space (Fig. 5b, c (Inset)). Additionally, we observed that vamorolone treatment resulted in a dose–responsive decrease in bone erosion with significance reached at 20 mg/kg/day (Fig. 5d). In contrast, prednisolone treatment did not result in a dose–responsive reduction in bone erosion (Fig. 5e). More importantly, we noticed a reduction in intra-articular space and bone adhesions at all three prednisolone doses suggesting significant deleterious effects on bone health (Fig. 5a, b, c). It must be stated that while the vamorolone-induced reduction in bone erosion reached statistical significance at 20 mg/kg/day, the reduction at 40 mg/kg/day did not reach statistical significance because of small sample size ($n=4$) due to sectioning artifacts of joints.

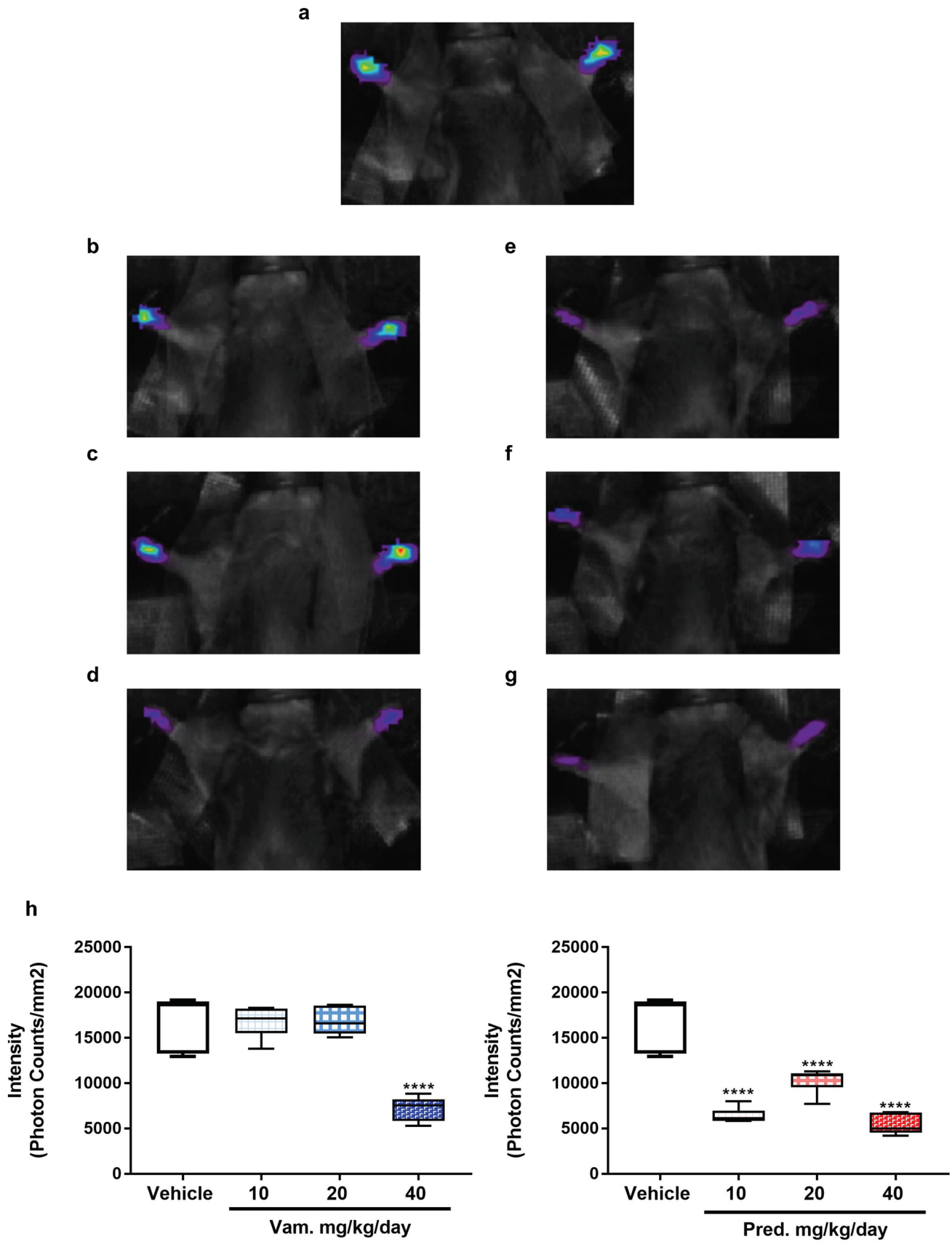
Discussion

Vamorolone is a dissociative steroidal compound that has been previously shown to effectively inhibit NFkB activity [13–18], behave as an MR antagonist, and effectively reduce inflammation in multiple acute and chronic mouse models of inflammatory disease. It has been previously demonstrated that vamorolone has the capacity to reduce leukocyte infiltration into lungs of mice undergoing allergic lung inflammation [16]. Vamorolone has been shown to increase muscle strength in mouse models of muscular dystrophy [14, 27]. In mice with experimental autoimmune encephalomyelitis (a mouse model for multiple sclerosis), vamorolone was shown to reduce the degree of limb paralysis [17]. Additionally, vamorolone has proven effective at reducing the disease score in a mouse model of inflammatory bowel disease [18]. Vamorolone does not induce GRE-mediated transcription leading to a much reduced glucocorticoid-associated adverse effect profile in vivo [14–17]. Phase 1 and Phase 2a clinical trials (14-day treatment) have shown that vamorolone is well tolerated in healthy adult volunteers and DMD patients at doses of up to 20 mg/kg/day and 6 mg/kg/day, respectively [19, 20].

Since GCs are still heavily prescribed for treating RA and since vamorolone has been previously shown to reduce NFkB activity in RA-relevant cell types (macrophages, splenocytes) [14, 15, 17], we proposed to assess the anti-inflammatory capacity of vamorolone within the context of RA by utilizing the widely used murine collagen antibody-induced model of arthritis. Vamorolone and prednisolone (10, 20, 40, mg/kg/day) were administered after full induction of CAIA. We found that both vamorolone and prednisolone effectively reduced disease score and joint inflammation as measured by cathepsin B activity.

Furthermore, we 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 RA [24–26] were shown to be reduced by both vamorolone and prednisolone at all doses tested. As IL-6 and IL-1β have been previously shown to be regulated by NFkB, the reductions of these cytokines are consistent with the well-described transrepression (e.g., NFkB inhibition) property of steroidal compounds [28, 29]. Furthermore, these findings are consistent with previous studies demonstrating that vamorolone has the capacity to reduce IL-6 and IL-1β production in macrophages [15].

One of the most well-described adverse effects associated with long-term glucocorticoid use is bone loss [9]. Histopathological analysis of affected joints demonstrated that vamorolone treatment resulted in a dose–responsive decrease in bone erosion, whereas prednisolone treatment did not result in such a dose–responsive reduction. The deleterious effects of prednisolone on bone health were further demonstrated by a reduction in intra-articular space and bone adhesions at all three prednisolone doses tested in our study. These findings are consistent with previous work demonstrating that vamorolone possesses bone-sparing properties in vivo when compared to prednisolone [14]. It must be stated that an additional study assessing bone density in a


a
b
c
d
e
f
g
h
Intensity (Photon Counts/mm2)
25000
20000
15000
10000
5000
0
****
Vehicle
10
20
40
Vam. mg/kg/day
Pred. mg/kg/day

◂**Fig. 2** Vamorolone treatment reduces cathepsin B activity in arthritic joints. Mice received i.p. injections of ProSense 680 24 h prior to imaging. Optical imaging assessing cathepsin B activity was performed on day 13 post-disease induction. Images are representative of each treatment group: vehicle (**a**), vamorolone 10 mg/kg/day (**b**), vamorolone 20 mg/kg/day (**c**), vamorolone 40 mg/kg/day (**d**), prednisolone 10 mg/kg/day (**e**), prednisolone 20 mg/kg/day (**f**), prednisolone 40 mg/kg/day (**g**). Whisker plots (H) show intensity (photon counts) in forelimb joints of vamorolone-treated (left panel) and prednisolone-treated (right panel) mice ($n=6$ mice/group). ****$p<0.0001$ compared to vehicle-treated group

chronic mouse model is planned to fully evaluate the effects of vamorolone vs. prednisolone on bone loss.

In addition to bone health, the current study also qualitatively analyzed the effect of both drugs on cartilage destruction. While vamorolone treatment at all three doses appeared to reduce the loss of cartilage compared to vehicle-treated mice, prednisolone treatment at higher doses did not result in reduced cartilage destruction. This is in line with previous reports describing the detrimental effects of traditional steroidal compounds on articular cartilage [30].

While the CAIA model is widely used to assess the anti-inflammatory capacity of potential therapeutics, there are some key limitations associated with using this model. The inflammation in CAIA is acute-in-nature and self-resolves [31]. Rheumatoid arthritis, on the other hand, is well described as being chronic and progressive. Moreover, RA is associated with extra-articular manifestations while the CAIA model is limited to the joints [32]. Chronic collagen-induced arthritis models have been described which may better mimic the chronic, progressive nature of RA. Additionally, some chronic models have been shown to display some of the extra-articular manifestations associated with RA [33]. Therefore, further work in chronic models may be warranted to greatly assess the therapeutic potential of vamorolone within the context of RA. Regardless, the results of the current CAIA study should help design upcoming studies in more chronic animal models.

Current treatments for RA involve the specific targeting of the inflammatory cytokine, TNF-α, since it is well known to play an important role in the pathogenesis of the disease. Indeed, previous studies have indicated that TNF-α is important for the pathogenesis of collagen-induced arthritis [34]. Thus, we assessed TNF-α in the joint homogenates of the current study. However, the levels detected were lower than the assay's limit of detection (data not shown). While this can be considered a limitation of the current study, this particular finding in our study has been corroborated in other studies assessing the presence of TNF-α in joint homogenates of mice with experimental arthritis [35]. Therefore, future studies may be necessary to assess the effect(s) of vamorolone on the presence of TNF-α within the context of rheumatoid arthritis.

Based on the data presented, vamorolone is effective at about four times the dose of prednisolone in reducing disease score and joint inflammation (cathepsin B activity). Indeed, previous studies have shown that it takes higher in vivo doses of vamorolone to achieve the prednisolone anti-inflammatory effect whenever mouse models that involve immunological adjuvants (LPS, PTX) are used [17]. On the contrary, whenever adjuvants are not used, vamorolone treatment reduces in vivo inflammation to a similar degree to prednisolone at the same dose tested [18]. It must also be pointed out that vamorolone and prednisolone show similar potencies in vitro in terms of their ability to inhibit NFkB activity [13, 14]. Importantly, previous studies have assessed the capacity of vamorolone and prednisolone to induce GRE-mediated transcription in vivo using doses comparable to the effective doses observed for both drugs in the current study. These studies demonstrated that chronic treatment of vamorolone did not induce transcription of genes with GREs in their promoter regions whereas prednisolone treatment did induce transcription of these genes in vivo [15, 17]. Additionally, these differences in GRE-mediated gene transcription were further reflected in our assessment of steroid-associated side effects as mice chronically treated with vamorolone did not show evidence of bone growth inhibition in contrast to mice receiving prednisolone [14, 16]. These previous findings are in line with the current study demonstrating that vamorolone appears to lose the deleterious effects on bone health associated with prednisolone treatment. Furthermore, a 6-month GLP chronic toxicology study of vamorolone performed in mice demonstrated a no-observed-adverse-effect level of 45 mg/kg/day which is higher than the effective vamorolone dose observed in the current study. Taken together, these findings demonstrate that vamorolone is better tolerated than conventional GCs in diseased mice. Thus, vamorolone may offer an efficacious, yet safer alternative to traditional steroids in the treatment of RA and other inflammatory diseases..

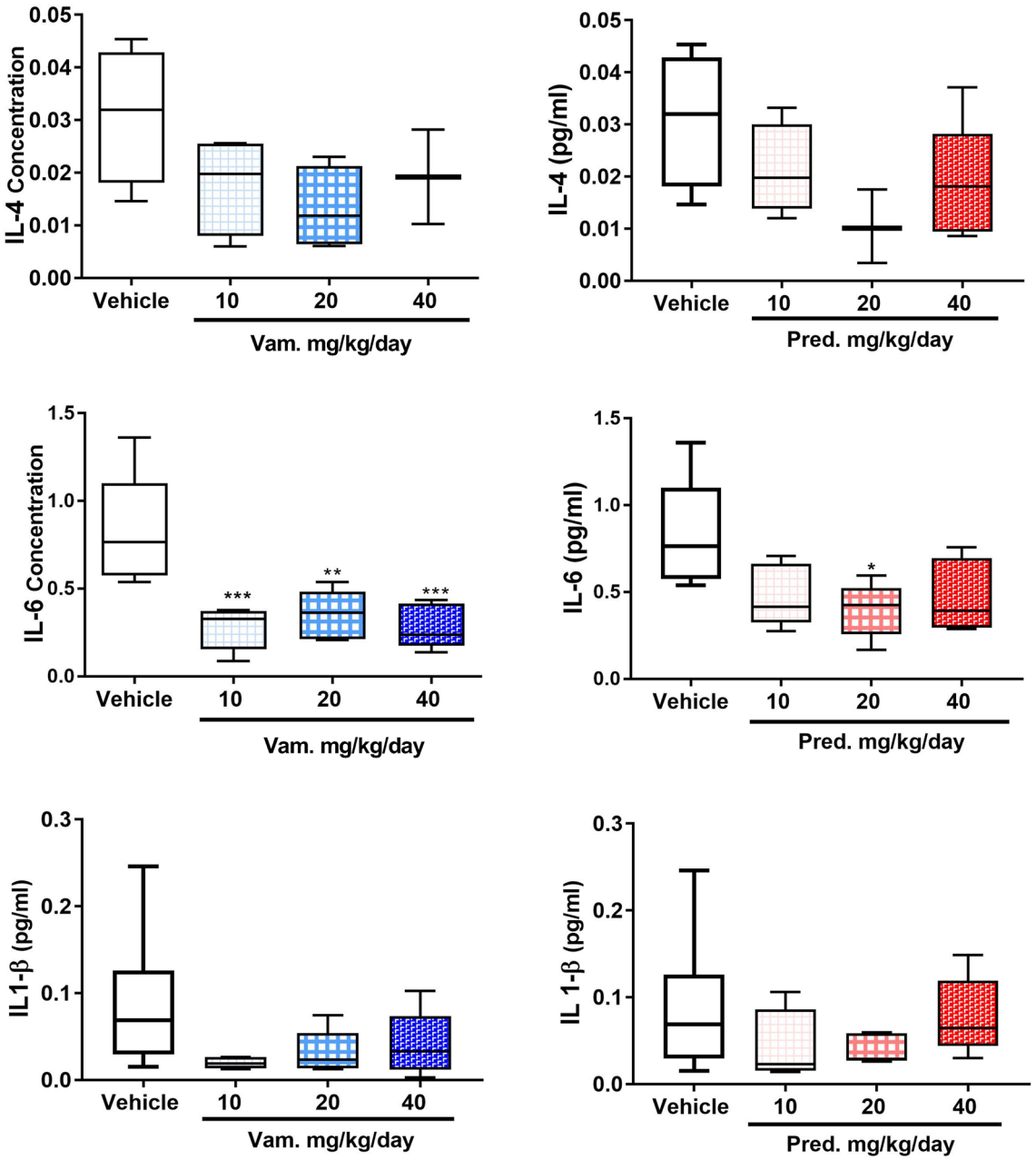


Fig. 3 Vamorolone treatment reduces the presence of inflammatory cytokines. A multiplex immunoassay measuring cytokines was performed on forelimb homogenates. Whisker plots show levels of IL-4, IL-6, and IL-1β in vamorolone-treated (left panels) and prednisolone-treated (right panels) mice ($n=6$ mice/group). $^{**}p<0.01$; $^{***}p<0.001$ compared to vehicle-treated group

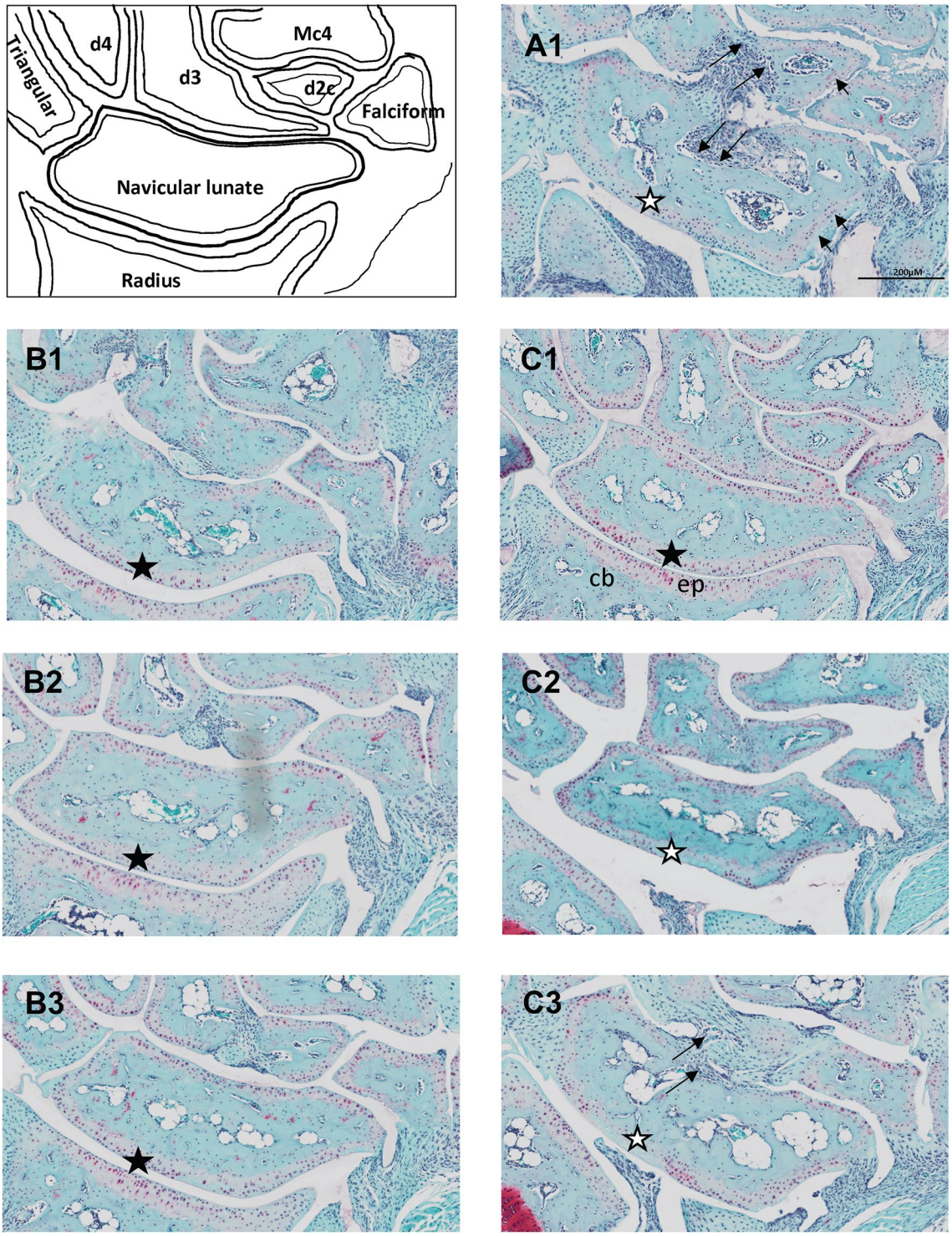
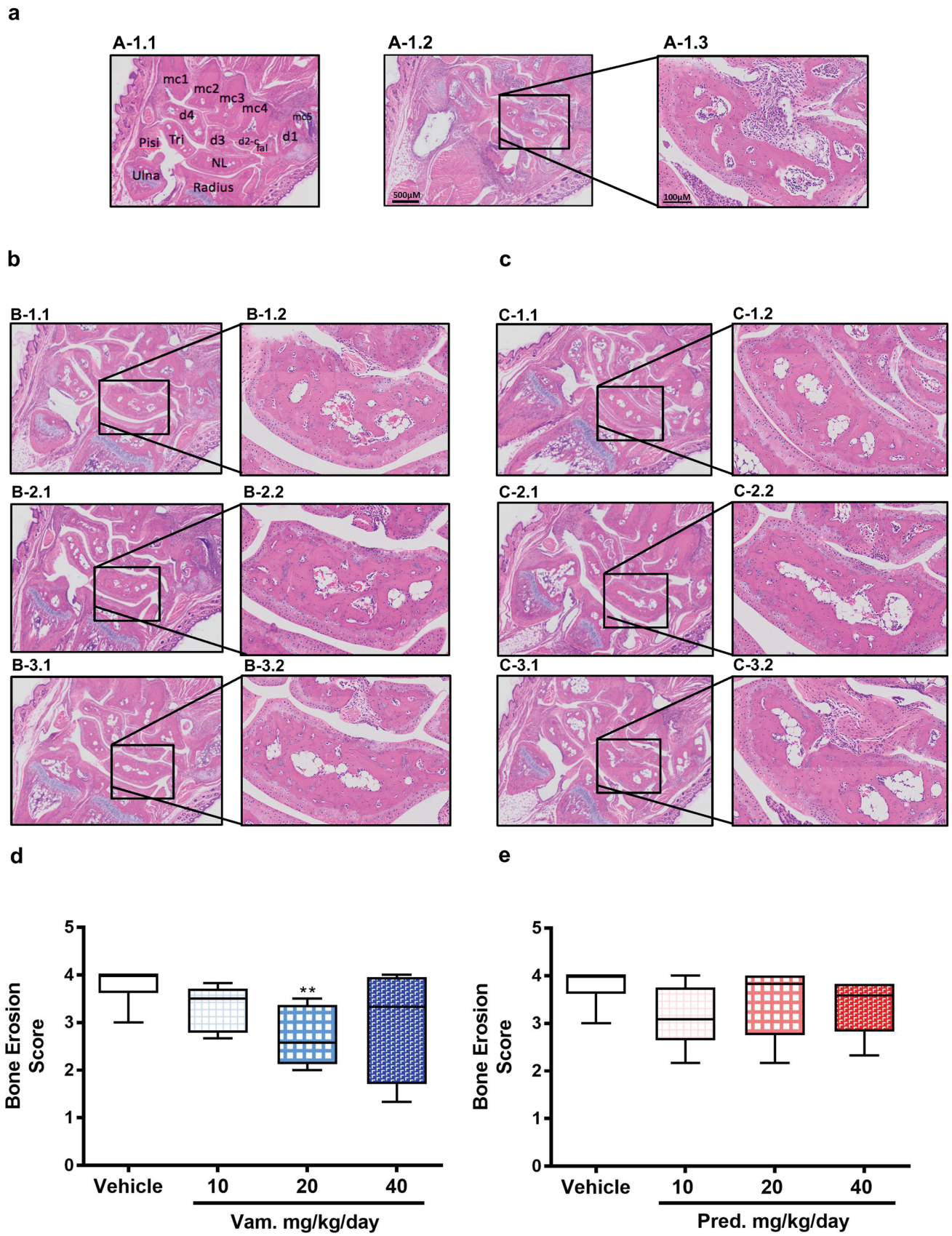


Fig. 4 Vamorolone treatment reduces cartilage destruction in CAIA mice. Top-left panel represents a diagram of different joint areas of the paw. The presence of cartilage was qualitatively assessed on the front paws of vehicle-treated mice (**A1**), vamorolone-treated mice at 10, 20, 40 mg/kg/day (**B1**, **B2**, **B3**, respectively), and prednisolone-treated mice at 10, 20, 40 mg/kg/day (**C1**, **C2**, **C3**, respectively). White stars on navicular lunate represent areas of cartilage destruction. Black stars on navicular lunate represent areas of reduced cartilage destruction. Long arrows represent pannus-driven destruction of the bone cortex. Short arrows in **A1** represent additional areas of cartilage destruction. *Cb* cortical bone, *ep* epiphysis. Scale bar = 200μM


a
A-1.1
mc1
mc2
mc3
mc4
mc5
d4
Pisi
Tri
d3
d2-c
fal
d1
NL
Ulna
Radius
A-1.2
500μM
A-1.3
100μM
b
B-1.1
B-1.2
B-2.1
B-2.2
B-3.1
B-3.2
c
C-1.1
C-1.2
C-2.1
C-2.2
C-3.1
C-3.2
d
Bone Erosion Score
0
1
2
3
4
5
**
Vehicle
10
20
40
Vam. mg/kg/day
e
Bone Erosion Score
Vehicle
10
20
40
Pred. mg/kg/day

◂**Fig. 5** Vamorolone treatment improves bone health in CAIA mice. Bone erosion was quantified on six different joint interfaces of within the front paw: pisiform (Pisi)–ulna, ulna–radius, radius–navicular lunate (NL), triangular (Tri)–navicular lunate, triangular–carpal (d4), and navicular lunate–carpal (d3) as labeled in **A-1.1**. Metacarpals (mc1, mc2, mc3, mc4, mc5) are labeled as well in **A-1.1**. A four-point scoring method was implemented on each joint interface, and an average score per paw was generated from these individual scores. Representative images from vehicle treated (**A-1.2**), vamorolone treated at 10, 20, 40 mg/kg/day (**B-1.1**, **B-2.1**, **B-3.1**, respectively), and prednisolone treated at 10, 20, 40 mg/kg/day (**C-1.1**, **C-2.1**, **C-3.1**, respectively) are shown. Corresponding magnified images are placed directly to the right of each image (**A-1.3**, **B-1.2**, **B-2.2**, **B-3.2**, **C-1.2**, **C-2.2**, **C-3.2**). Whisker plots show bone erosion of vamorolone-treated (**d**) and prednisolone-treated (**e**) mice ($n=6$ mice/group). $^{**}p<0.01$ compared to vehicle-treated group

Funding Supported by a small business technology transfer (STTR) Grant from the National Institutes of Health (1R41AR068816-01). This award supported all experiments described in this manuscript.

Compliance with ethical standards

Conflict of interest :JMD, MRC, RL, and KN are employed by ReveraGen BioPharma Inc. JMD has ReveraGen BioPharma Inc. stock options, and KN has ReveraGen BioPharma Inc. founder shares. AAF has a consulting agreement with ReveraGen BioPharma Inc.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the Institutional Animal Care and Use Committee at Children's National Health System.

References

1. Vos T, Allen C, Arora M, Barber RM, Bhutta ZA, Brown A, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the global burden of disease study 2015. Lancet. 2016;388:1545–602.
2. Sokka T, Toloza S, Cutolo M, Kautiainen H, Makinen H, Gogus F, et al. Women, men, and rheumatoid arthritis: analyses of disease activity, disease characteristics, and treatments in the QUEST-RA study. Arthritis Res Ther. 2009;11:R7.
3. Haraoui B, Jovaisas A, Bensen WG, Faraawi R, Kelsall J, Dixit S, et al. Use of corticosteroids in patients with rheumatoid arthritis treated with infliximab: treatment implications based on a real-world Canadian population. RMD Open. 2015;1:e000078.
4. Bijlsma JW, Jacobs JW. Glucocorticoid in the treatment of rheumatoid arthritis: still used after 65 years. Ann NY Acad Sci. 2014;1318:27–31.
5. Keystone EC, Smolen J, Riel PV. Developing an effective treatment algorithm for rheumatoid arthritis. Rheumatology (Oxford). 2012;51:v48–54.
6. Baschant U, Lane NE, Tuckerman J. The multiple facets of glucocorticoid action in rheumatoid arthritis. Nat Rev Rheumatol. 2012;8:645–55.
7. Ethgen O, Esteves FDL, Bruyere O, Reginster JY. What do we know about the safety of corticosteroids in rheumatoid arthritis? Curr Med Res Opin. 2013;29:1147–60.
8. Jacobs JW, Bijlsma JW, van Laar JM. Glucocorticoids in early rheumatoid arthritis: are the benefits of joint-sparing effects offset by the adverse effect of osteoporosis? the effects on bone in the Utrecht study and the CAMERA-II study. Neuroimmunomodulation. 2015;22:66–71.
9. Rehman Q, Lane NE. Effect of glucocorticoids on bone density. Med Pediatr Oncol. 2003;41:212–6.
10. Herrlich P. Cross-talk between glucocorticoid receptor and AP-1. Oncogene. 2001;20:2465–75.
11. Coghlan MJ, Elmore SW, Kym PR, Kort ME. The pursuit of differentiated ligands for the glucocorticoid receptor. Curr Top Med Chem. 2003;14:1617–35.
12. Hudson WH, Vera IMS, Nwachukwu JC, Weikum ER, Herbst AG, Yang Q, Bain DL, Nettles KW, Kojetin DJ, Ortlund EA. Cryptic glucocorticoid receptor-binding sites pervade genomic NF-κB response elements. Nat Commun. 2018;9:1337.
13. Reeves EK, Hoffman EP, Nagaraju K, Damsker JM, McCall JM. VBP15: preclinical characterization of a novel anti-inflammatory delta 9,11 steroid. Bioorganic Med Chem. 2013;21:2241–9.
14. Heier CR, Damsker JM, Yu Q, Dillingham BC, Huynh T, Van der Meulen JH, Sali A, et al. VBP15, a novel anti-inflammatory and membrane-stabilizer, improves muscular dystrophy without side effects. EMBO Mol Med. 2013;5:1569–85.
15. Heier CR, Yu Q, Fiorillo AA, Tully CB, Tucker A, Mazala DA, Uaesoontrachoon K, et al. Vamorolone targets dual nuclear receptors to treat inflammation and dystrophic cardiomyopathy. Life Sci Alliance. 2019;2(1):6.
16. Damsker JM, Dillingham BC, Rose MC, Balsley MA, Heier CR, Watson AM, et al. VBP15, a glucocorticoid analogue, is effective at reducing allergic lung inflammation in mice. PLoS One. 2013;8:e63871.
17. Dillingham BC, Knoblach SM, Many GM, Harmon BT, Mullen AM, Heier CR, et al. VBP15, a novel anti-inflammatory, is effective at reducing the severity of murine experimental autoimmune encephalomyelitis. Cell Mol Neurobiol. 2015;3:377–87.
18. Damsker JM, Conklin LS, Sadri S, Dillingham BC, Panchapakesan K, Heier CR, et al. VBP15, a novel dissociative steroid compound, reduces NFκB-induced expression of inflammatory cytokines in vitro and symptoms of murine trinitrobenzene sulfonic acid-induced colitis. Inflamm Res. 2016;65:737–43.
19. Hoffman EP, Riddle V, Siegler MA, Dickerson D, Backonia M, Kramer WG, et al. Phase 1 trial of vamorolone, a first-in-class steroid, shows improvements in side effects via biomarkers bridged to clinical outcomes. Steroids. 2018;134:43–52.
20. Conklin LS, Damsker JM, Hoffman EP, et al. Phase Iia trial in Duchenne muscular dystrophy shows vamorolone is a first-in-class dissociative steroidal anti-inflammatory drug. Elsevier Pharma Res. 2018;136:140–50.
21. Baudy AR, Sali A, Jordan S, Kesari A, Johnston HK, Hoffman EP, Nagaraju K. Non-invasive optical imaging of muscle pathology in mdx mice using cathepsin caged near-infrared imaging. Mol Imaging Biol. 2011;13:462–70.
22. Kachigian LM. Collagen antibody induced arthritis. Nat Protoc. 2006;1:2512–6.
23. Tong B, Wan B, Wei Z, Wang T, Zhao P, Dou Y, et al. Role of cathepsin B in regulating migration and invasion of fibroblast-like synoviocytes into inflamed tissue from patients with rheumatoid arthritis. Clin Exp Immunol. 2014;177:586–97.
24. Ohmura K, Nguyen LT, Locksley RM, Mathis D, Benoist C. Interleukin-4 can be a key positive regulator of inflammatory arthritis. Arthritis Rheum. 2005;52:1866–75.
25. Firestein GS, Alvaro-Gracia JM, Maki R, Alvaro-Garcia JM. Quantitative analysis of cytokine gene expression in rheumatoid arthritis. J Immunol. 1990;144:3347–53.
26. van Leeuwen MA, Westra J, Limburg PC, van Riel PL, van Rijswijk MH. Interleukin-6 in relation to other proinflammatory cytokines, chemotactic activity and neutrophil activation in rheumatoid synovial fluid. Ann Rheum Dis. 1995;54:33–8.

27. Sreetama SC, Chandra G, Van der Meulen JH, Ahmad MM, Suzuki P, et al. Membrane stabilization by modified steroid offers a potential therapy for muscular dystrophy due to dysferlin deficit. Mol Ther. 2018;26:2231–42.
28. Fujisawa K, Aono H, Hasunuma T, Yamamoto K, Mita S, Nishioka K. Activation of transcription factor nf-κb in human synovial cells in response to tumor necrosis factor α. Arthritis Rheum. 1996;39:197–203.
29. Miyazawa K, Mori A, Yamamoto K, Okudaira H. Constitutive transcription of the human interleukin-6 gene by rheumatoid synoviocytes: spontaneous activation of NF-kappaB and CBF1. Am J Pathol. 1998;152:793–803.
30. Wernecke C, Braun HJ, Dragoo JL. The effect of intra-articular corticosteroids on articular cartilage. Orthop J Sports Med. 2015;3:2325967115581163.
31. Bas DB, Su J, Sandor K, Agalave NM, Lundberg J, Codeluppi S, Baharpoor A, et al. Collagen antibody-induced arthritis evokes persistent pain with spinal glial involvement and transient prostaglandin dependency. Arthritis Rheum. 2012;64:3886–96.
32. Cojocaru M, Cojocaru IM, Silosi I, Vrabie CD, Tanasescu R. Extra-articular manifestations in rheumatoid arthritis. Maedica (Buchar). 2010;5:286–91.
33. Hansson AS, Lu S, Holmdahl R. Extra-articular cartilage affected in collagen-induced, but not pristane-induced, arthritis models. Clin Exp Immunol. 2002;127:37–42.
34. Marinova-Mutafchieva L, Williams RO, Mason LJ, Mauri C, Feldmann M, Maini RN. Dynamics of proinflammatory cytokine expression in the joints of mice with collagen-induced arthritis (CIA). Clin Exp Immunol. 1997;107:507–12.
35. Rioja I, Bush KA, Buckton JB, Dickson MC, Life PF. Joint cytokine quantification in two rodent arthritis models: kinetics of expression, correlation of mRNA and protein levels and response to prednisolone treatment. Clin Exp Immunol. 2004;137:65–73.

Affiliations

Jesse M. Damsker[1] · Michaelyn R. Cornish[1] · Priya Kanneboyina[1] · Ila Kanneboyina[1] · Qing Yu[2] · Rachel Lipson[1] · Aditi Phadke[2] · Susan M. Knoblach[2] · Karuna Panchapakesan[2] · Melissa Morales[3] · Alyson A. Fiorillo[2] · Terence Partridge[2] · Kanneboyina Nagaraju[1,2,3]

Michaelyn R. Cornish
cornmi01@gmail.com

Priya Kanneboyina
priya113k@gmail.com

Ila Kanneboyina
ila.k99992@gmail.com

Qing Yu
qyu@childrensnational.org

Rachel Lipson
rachellipson1222@hotmail.com

Aditi Phadke
aphadke@childrensnational.org

Susan M. Knoblach
sknoblach@childrensnational.org

Karuna Panchapakesan
kpanchapakesan@childrensnational.org

Melissa Morales
mmorales@binghamton.edu

Alyson A. Fiorillo
afiorillo@childrensnational.org

Terence Partridge
tpartridge@childrensnational.org

[1] ReveraGen BioPharma Inc., Rockville, MD 20850, USA

[2] Research Center for Genetic Medicine, Children's National Health System, Washington, DC 20010, USA

[3] School of Pharmacy and Pharmaceutical Sciences, Binghamton University, Binghamton, NY, USA