Dear Healthcare Professional,

Thank you for your unsolicited request for information. Accompanying this letter is the following information you requested on Purified Cortrophin® Gel. If we can be of any further assistance, please contact our Medical Information department at (844) CORT-GEL (844-267-8435) between the hours of 9:00 AM to 7:00 PM ET (6:00 AM to 4:00 PM PT), Monday through Friday or via email at cortrophinmedinfo@anipharmaceuticals.com.

Purified Cortrophin Gel is indicated in the following disorders:

1. Rheumatic disorders:
As adjunctive therapy for short-term administration (to tide the patient over an acute episode or exacerbation) in:
- Psoriatic arthritis.
- Rheumatoid arthritis, including juvenile rheumatoid arthritis (selected cases may require low-dose maintenance therapy).
- Ankylosing spondylitis.
- Acute gouty arthritis.

2. Collagen diseases:
During an exacerbation or as maintenance therapy in selected cases of:
- Systemic lupus erythematosus.
- Systemic dermatomyositis (polymyositis).

3. Dermatologic diseases:
Severe erythema multiforme (Stevens-Johnson syndrome).
Severe psoriasis.

4. Allergic states:
Atopic dermatitis
Serum sickness.

5. Ophthalmic diseases:
Severe acute and chronic allergic and inflammatory processes involving the eye and its adnexa such as:
- Allergic conjunctivitis.
- Keratitis.
- Iritis and iridocyclitis.
- Diffuse posterior uveitis and choroiditis.
- Optic neuritis.
- Chorioretinitis.
- Anterior segment inflammation.

6. Respiratory diseases:
Symptomatic sarcoidosis.

7. Edematous states:
To induce a diuresis or a remission of proteinuria in the nephrotic syndrome without uremia of the idiopathic type or that due to lupus erythematosus.

8. Nervous system:
Acute exacerbations of multiple sclerosis.

Purified Cortrophin Gel is contraindicated for intravenous administration.

Purified Cortrophin Gel is contraindicated in patients with scleroderma, osteoporosis, systemic fungal infections, ocular herpes simplex, recent surgery, history of or the presence of a peptic ulcer, congestive heart failure, hypertension, or sensitivity to proteins derived from porcine sources.

Purified Cortrophin Gel is contraindicated in patients with primary adrenocortical insufficiency or adrenocortical hyperfunction.

Please see the enclosed Purified Cortrophin Gel Prescribing Information (PI) for detailed information including Warnings and Precautions and Adverse Reactions as well as the appropriate use of Purified Cortrophin Gel.

Thank you for your inquiry.

Sincerely,

Steve Wu, PharmD
ANI Pharmaceuticals Medical Information

Mechanism of Action of Purified Cortrophin® Gel (Repository Corticotropin Injection USP) 80 U/mL in Adults With Systemic Lupus Erythematosus

Abstract

This document provides information pertaining to select available evidence supporting the mechanism of action of Cortrophin Gel (Repository Corticotropin Injection USP) in systemic lupus erythematosus (SLE). The active agent in Cortrophin Gel is porcine-derived adrenocorticotropic hormone (ACTH peptide, amino acids 1-39).[1] Cortrophin Gel is of the same class as other, FDA-approved natural-product (Repository Corticotropin Injection)[2] and synthetic corticotropins[3] and is chemically similar to endogenous, human ACTH.[4]

Note that this document is for information purposes only. This document may contain information not included in the prescribing information for Cortrophin Gel. The usage of corticotropin being discussed may not have been approved by the FDA as safe and effective. ANI Pharmaceuticals does not recommend the use of its products in any manner inconsistent with the FDA-approved labeling.

To report an Adverse Event for any ANI Pharmaceuticals product, please call 1-800-308-6755 or contact the FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

Email: drugsafety@anipharmaceuticals.com.

Background

Purified Cortrophin Gel (Repository Corticotropin Injection USP) is approved by the FDA for the treatment of collagen diseases, such as SLE.[1]

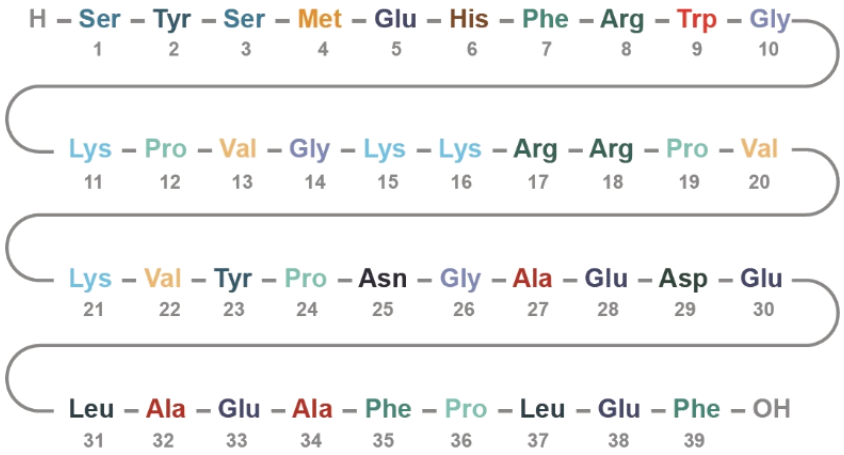
- The active agent in Cortrophin Gel is porcine-derived adrenocorticotropic hormone (ACTH peptide, amino acids 1-39).[1] Cortrophin Gel is of the same class as other, FDA-approved natural-product (Repository Corticotropin Injection)[2] and synthetic corticotropins[3] and is chemically similar to endogenous, human ACTH.[4]

Composition of Cortrophin Gel

Cortrophin Gel is a porcine-derived purified corticotropin (ACTH) in a sterile solution of gelatin. It is made up of a complex mixture of ACTH, ACTH-related peptides, and other porcine pituitary-derived peptides.[1]

The drug product is a sterile preparation containing 80 USP units per mL and it contains 0.5% phenol (as preservative), 15.0% gelatin (for prolonged activity), water for injection, and the pH is adjusted with hydrochloric acid and sodium hydroxide.[1]

Cortrophin Gel contains the porcine-derived ACTH (1-39) with the following amino acid sequence[1]:



ANI conducted a study on the pharmacodynamic effect of Cortrophin Gel, including E_{max}, $AUEC_{0-24}$, and TE_{max}, and compared it with the response of the same or similar depot structures from published literature for bioequivalence.[5]

Mechanism of Action of Cortrophin Gel in SLE

Overview of ACTH Mechanism of Action

While the precise mechanism of action of Cortrophin Gel is not fully understood, its ability to stimulate glucocorticoid production in the adrenal glands has been well characterized. Some preclinical studies suggest that ACTH may have a nonsteroidogenic component to its mechanism of action.

ACTH is a member of the melanocortin family of peptides that are ligands for the melanocortin receptors, or MCRs, of which there are 5 known isoforms (MC1R-MC5R).[6] These endogenous receptors for ACTH are broadly expressed on cells that may play a role in the pathophysiology of SLE.[7]

The following sections provide a summary of clinical observations that have led to the exploration of the potential nonsteroidogenic effects of ACTH in SLE, as well as the preclinical data that help characterize the role of MCRs in potentially mediating these effects.

Clinical Observations in SLE Related to Mechanism of Action

Although ACTH primarily stimulates steroidogenesis through the adrenal cortex by binding to MC2R, some studies have found that it can provide anti-inflammatory effects through nonsteroidogenic mechanisms.[8,9] Previous reports have observed that patients with SLE already receiving corticosteroids experience increased clinical benefit from the addition of ACTH.[7,8,10–12] Furthermore, some patients were

able to reduce their intake of corticosteroids when treated with ACTH.[10] These findings suggest that ACTH may possess additional activity independent of steroidogenesis via the adrenal cortex.

Use of α-MSH as an Analog of ACTH in Preclinical Studies

Preclinical studies interrogating the function of MCRs often use the melanocortin α-melanocyte stimulating hormone (α-MSH), a modified cleavage product of ACTH. With the exception of MC2R, which is selective for ACTH within the adrenal cortex, α-MSH binds and activates all other MCR family members (MC1R, MC3R, MC4R, and MC5R) analogously to ACTH.[6,13] In light of limited preclinical evidence in the context of SLE, experiments using α-MSH as well as ACTH are used to highlight the potential nonsteroidogenic action of ACTH.

Preclinical Evidence of Nonsteroidogenic Mechanisms of ACTH

While the exact cause of SLE is unknown, abnormal innate and adaptive immune responses, including the production of autoantibodies, are commonly involved in the pathology of SLE.[14,15] Immune cells that are purportedly driving these abnormal responses, including B cells, T cells, macrophages, and other inflammatory cells, are known to express MCRs, suggesting there is potential for action of ACTH on these various cell types.[16,17] MCRs are also expressed on immune cells with anti-inflammatory effects in SLE, such as dendritic cells (DCs) and regulatory T cells (Tregs).[18,19] The following paragraphs describe data suggesting the potential nonsteroidogenic actions of ACTH on the pathology of SLE.

B cells play a major role in the pathophysiology of SLE as they have been implicated in the production of autoantibodies.[20] In isolated peripheral B-cell cultures, ACTH was shown to inhibit B-cell proliferation and immunoglobulin G production without affecting their viability, which may suggest the possibility of ACTH acting directly on B cells.[21] In another study to evaluate the effects of ACTH on B-cell gene expression, ACTH treatment resulted in the downregulation of mRNAs critical for B-cell proliferation under activated conditions.[22]

The NF-κB pathway is often activated in immune cells in inflammatory diseases such as SLE.[23] NF-κB is a transcription factor that is an important inflammatory mediator associated with pro-inflammatory cytokine release and other immune processes.[24] In a study of isolated human monocytes, α-MSH inhibited NF-kB activation induced by various inflammatory stimuli.[25] In another preclinical study using a cell line of human keratinocytes, which are the most common cell type in the outermost layer of the skin, NF-κB activation was downregulated by both α-MSH and ACTH (1-39).[26]

In synovial tissue, macrophages are typically inactive, but they are activated in inflamed joints and have been implicated in the development of SLE. Macrophages play an essential role in regulating the secretion of pro-inflammatory cytokines and enzymes responsible for driving inflammatory response and joint damage.[27] In patients with different types of arthritis, including those with rheumatic diseases associated with joint inflammation, α-MSH has been found in the synovial fluid of knee joints, suggesting local production of the peptide may occur at sites of inflammation.[28] There is also support for the expression of POMC and secretion of α-MSH by cultured murine macrophages, with α-MSH increased in the presence of a pro-inflammatory stimulus.[29] In another in vitro study using human cultured macrophages, α-MSH inhibited lipopolysaccharide-induced NF-κB activation.[30]

Both DCs and Tregs have been implicated in SLE pathophysiology, with tolerogenic DCs promoting immune tolerance by inducing Tregs. This aids in preventing the occurrence of autoimmune conditions and pathological inflammation.[31] Previous studies reported a decrease in the frequency of Tregs in patients with SLE.[32,33] A preclinical study demonstrated that α-MSH, through binding to MC1R, induced tolerogenic DCs, which increased Tregs both in vitro and in vivo. In vitro, α-MSH-treated Tregs were shown to prevent cutaneous contact allergy. The study also found that α-MSH may induce tolerogenic DCs capable of generating functional Tregs in human blood. These Tregs suppressed the proliferation and cytokine secretion of pathogenic Th17 cells.[34]

Citations

1. Purified Cortrophin™ Gel. Package insert. ANI Pharmaceuticals, Inc. Published online 2022.

2. Acthar® Gel. Package insert. Mallinckrodt ARD LLC. Published online February 2021. Accessed September 16, 2021. https://acthar.com/Static/pdf/Acthar-PI.pdf

3. Synacthen® Depot Ampoules 1 mg/ml - Summary of product characteristics (SmPC) - (emc). Published online October 4, 2021. Accessed October 4, 2021. https://www.medicines.org.uk/emc/product/10823/smpc/

4. Riniker B, Sieber P, Rittel W, Zuber H. Revised amino-acid sequences for porcine and human adrenocorticotrophic hormone. *Nature New Biol*. 1972;235(56):114-115. doi:10.1038/newbio235114b0

5. ANI Pharmaceuticals, Inc. Data on file.

6. Wang W, Guo DY, Lin YJ, Tao YX. Melanocortin regulation of inflammation. *Front Endocrinol*. 2019;10:683. doi:10.3389/fendo.2019.00683

7. Fiechtner J, Montroy T. Treatment of moderately to severely active systemic lupus erythematosus with adrenocorticotropic hormone: a single-site, open-label trial. *Lupus*. 2014;23(9):905-912.

8. Furie R, Mitrane M, Zhao E, Das M, Li D, Becker PM. Efficacy and tolerability of repository corticotropin injection in patients with persistently active SLE: results of a phase 4, randomised, controlled pilot study. *Lupus Sci Med*. 2016;3(1):e000180.

9. Novoselova TV, King PJ, Guasti L, Metherell LA, Clark AJL, Chan LF. ACTH signalling and adrenal development: lessons from mouse models. *Endocr Connect*. 2019;8(7):R122-R130. doi:10.1530/EC-19-0190

10. Furie RA, Mitrane M, Zhao E, Becker PM. Repository corticotropin injection in patients with persistently active SLE requiring corticosteroids: post hoc analysis of results from a two-part, 52-week pilot study. *Lupus Sci Med*. 2017;4(1):e000240.

11. Askanase AD, Zhao E, Zhu J, Bilyk R, Furie RA. Repository corticotropin injection for persistently active systemic lupus erythematosus: results from a phase 4, multicenter, randomized, double-blind, placebo-controlled trial. *Rheumatol Ther*. 2020;7:893-908.

12. Askanase AD, Wan GJ, Panaccio MP, et al. Patient-reported outcomes from a phase 4, multicenter, randomized, double-blind, placebo-controlled trial of repository corticotropin injection (Acthar® Gel) for persistently active systemic lupus erythematosus. *Rheumatol Ther*. 2021;8:573-584.

13. Brzoska T, Luger TA, Maaser C, Abels C, Böhm M. Alpha-melanocyte-stimulating hormone and related tripeptides: biochemistry, antiinflammatory and protective effects in vitro and in vivo, and future perspectives for the treatment of immune-mediated inflammatory diseases. *Endocr Rev*. 2008;29(5):581-602. doi:10.1210/er.2007-0027

14. Illescas-Montes R, Corona-Castro CC, Melguizo-Rodríguez L, Ruiz C, Costela-Ruiz VJ. Infectious processes and systemic lupus erythematosus. *Immunology*. 2019;158(3):153-160. doi:10.1111/imm.13103

15. Fernandez D, Kirou KA. What causes lupus flares? *Curr Rheumatol Rep*. 2016;18(3):14. doi:10.1007/s11926-016-0562-3

16. Andersen GN, Hägglund M, Nagaeva O, et al. Quantitative measurement of the levels of melanocortin receptor subtype 1, 2, 3 and 5 and pro-opio-melanocortin peptide gene expression in subsets of human peripheral blood leucocytes. *Scand J Immunol*. 2005;61(3):279-284. doi:10.1111/j.1365-3083.2005.01565.x

17. Ma C, Xia Y, Yang Q, Zhao Y. The contribution of macrophages to systemic lupus erythematosus. *Clin Immunol*. 2019;207:1-9. doi:10.1016/j.clim.2019.06.009

18. Rennalls LP, Seidl T, Larkin JM, et al. The melanocortin receptor agonist NDP-MSH impairs the allostimulatory function of dendritic cells. *Immunology*. 2010;129(4):610-619.

19. Zhao J, Jiang L, Uehara M, et al. ACTH treatment promotes murine cardiac allograft acceptance. *JCI Insight*. 2021;6(13). doi:10.1172/jci.insight.143385

20. Loram LC, Culp ME, Connolly-Strong EC, Sturgill-Koszycki S. Melanocortin peptides: potential targets in systemic lupus erythematosus. *Inflammation*. 2015;38(1):260-271.

21. Olsen NJ, Decker DA, Higgins P, et al. Direct effects of HP Acthar Gel on human B lymphocyte activation in vitro. *Arthritis Res Ther*. 2015;17:300. doi:10.1186/s13075-015-0823-y

22. Benko AL, McAloose CA, Becker PM, et al. Repository corticotrophin injection exerts direct acute effects on human B cell gene expression distinct from the actions of glucocorticoids. *Clin Exp Immunol*. 2018;192(1):68-81. doi:10.1111/cei.13089

23. Álvarez K, Villar-Vesga J, Ortiz-Reyes B, et al. Induction of NF-κB inflammatory pathway in monocytes by microparticles from patients with systemic lupus erythematosus. *Heliyon*. 2020;6(12):e05815.

24. Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. *Signal Transduct Target Ther*. 2017;2(1):1-9. doi:10.1038/sigtrans.2017.23

25. Manna SK, Aggarwal BB. Alpha-melanocyte-stimulating hormone inhibits the nuclear transcription factor NF-kappa B activation induced by various inflammatory agents. *J Immunol Baltim Md 1950*. 1998;161(6):2873-2880.

26. Moustafa M, Kemp EH, MacNeil S, et al. Inhibition of tumor necrosis factor-α stimulated NFκB/p65 in human keratinocytes by α-melanocyte stimulating hormone and adrenocorticotropic hormone peptides. *J Invest Dermatol*. 2002;119(6):1244-1253.

27. Kennedy A, Fearon U, Veale DJ, Godson C. Macrophages in synovial inflammation. *Front Immunol*. 2011;2:52.

28. Catania A, Gerloni V, Procaccia S, et al. The anticytokine neuropeptide alpha-melanocyte-stimulating hormone in synovial fluid of patients with rheumatic diseases: comparisons with other anticytokine molecules. *Neuroimmunomodulation*. 1994;1(5):321-328. doi:10.1159/000097183

29. Star RA, Rajora N, Huang J, Stock RC, Catania A, Lipton JM. Evidence of autocrine modulation of macrophage nitric oxide synthase by alpha-melanocyte-stimulating hormone. *Proc Natl Acad Sci U S A*. 1995;92(17):8016-8020. doi:10.1073/pnas.92.17.8016

30. Yoon SW, Goh SH, Chun JS, et al. alpha-melanocyte-stimulating hormone inhibits lipopolysaccharide-induced tumor necrosis factor-alpha production in leukocytes by modulating protein kinase A, p38 kinase, and nuclear factor kappa B signaling pathways. *J Biol Chem*. 2003;278(35):32914-32920. doi:10.1074/jbc.M302444200

31. Liu J, Zhang X, Cao X. Dendritic cells in systemic lupus erythematosus: From pathogenesis to therapeutic applications. *J Autoimmun*. 2022;132:102856. doi:10.1016/j.jaut.2022.102856

32. Miyara M, Amoura Z, Parizot C, et al. Global natural regulatory T cell depletion in active systemic lupus erythematosus. *J Immunol*. 2005;175(12):8392-8400. doi:10.4049/jimmunol.175.12.8392

33. Crispin JC, Martínez A, Alcocer-Varela J. Quantification of regulatory T cells in patients with systemic lupus erythematosus. *Meet Fed Clin Immunol Soc*. 2003;21(3):273-276. doi:10.1016/S0896-8411(03)00121-5

34. Auriemma M, Brzoska T, Klenner L, et al. α-MSH-stimulated tolerogenic dendritic cells induce functional regulatory T cells and ameliorate ongoing skin inflammation. *J Invest Dermatol*. 2012;132(7):1814-1824.