Preclinical evaluation of a novel non-steroidal glucocorticoid receptor agonist with low systemic exposure and reduced potential for skin atrophy

H. Norsgaard¹, M. Stahlhut¹, S. Eirefelt, N. Svitacheva, P. S. Johnson, M. Krogh-Madsen, T. Marstrand, D. A. Ewald, K. N. Dack and A. Ollerstam.
LEO Pharma, Ballerup, Denmark
¹Joint first authors

ABSTRACT

- With the aim to provide an effective and safe topical treatment of skin inflammation, we here describe a novel non-steroidal glucocorticoid receptor (GR) agonist, LEO 134310, with low systemic exposure and reduced potential for skin atrophy induction.
- The most well-described anti-inflammatory activity of GR agonists is the inhibition of transcriptional activation of pro-inflammatory genes by NF-κB and AP-1. Further, a number of genes (e.g., FKBP5, GILZ) is induced by activated GR.
- Major systemic adverse effects of GR activation relate to suppression of the hypothalamic-pituitary-adrenal (HPA) axis, whereas the main local adverse effect is skin atrophy. The structurally related mineralocorticoid receptor (MR) can be activated by steroidal GR agonists, and MR activation has been suggested to cause, at least partially, skin atrophy.
- With glucocorticoids adverse events are often observed and can limit the duration of treatment. Our data indicate that the non-steroidal GR agonist LEO 134310 retains anti-inflammatory activity, while it may offer improved safety for topical treatment of skin diseases such as atopic dermatitis and psoriasis.

MATERIALS AND METHODS

- GR binding assay** – Binding of GR agonists to human GR was evaluated in a fluorescence polarization assay to compete 2.5nM Fluormone™ GS1 (Thermo Fisher)
- TNFα inhibition assays** –PBMCs were from human buffy coats or animal whole blood (EDTA-treated). PBMCs in the presence of test compounds were stimulated with with 1μg/ml LPS (Sigma) and incubated overnight at 37°C. TNFα was measured by the use of a commercial AlphaLISA (Perkin Elmer).
- MR agonist assay** – This study was performed at Indigo Biosciences, PA, using their proprietary luciferase reporter cells expressing the native MR receptor. Luminescence was quantified after incubation at 37°C for 24 hr.
- Selectivity screening on nuclear receptors** – Studies were performed at CEREP, France, using their proprietary assays for nine nuclear hormone receptors employing specific, radiolabeled agonist ligands. Concentrations of LEO 134310 and LEO 134998 were 10μM.
- In vivo studies** – Animal experimental procedures as well as animal housing and husbandry were in compliance with Danish and EU legislation.
- TPA induced skin inflammation in mice** – Skin inflammation on female BALB/c mice (Taconic M&B, DK) was induced by repeated topical application of TPA in acetone on the inner and outer surface of the ear on study days 1, 3, 5, 8 and 10. Treatments were applied twice daily on days 8, 9, 10, and once on day 11 in a volume of 10 μl each on inner and outer ear surface. On day 11, animals were sacrificed 2 hours after the last dosing.
- Local tolerance study in minipigs** – Five male Göttingen SPF mini-pigs (Ellegaard Göttingen Minipigs A/S, Dalmose, DK) were treated for 28 days 6 hours daily with 25 μL of either topical formulation on an area of 2.5 cm x 2.5 cm: LEO vehicle (transcutol:acetone (1:9) with 10 % hydroxypropylcellulose); Clobetasol propionate 0.05% clinical formulation; CP 0.05% in LEO vehicle; BMV 0.1% clinical formulation; BMV0.1% in LEO vehicle; LEO 134310 0.5% in LEO vehicle; LEO 134310 2% in LEO vehicle. One test field in each animal was remained untreated. Skin reactions at the application sites were observed daily prior to dosing and on the day of necropsy. Skin biopsies were taken from anaesthetized animals 24 hours after the last treatment.
- Human skin explants** – The human full-thickness NativeSkin® model (NS002; GenoSkin, France) was used. Test compounds were applied in duplicates or triplicates on the skin surface (0.5 cm²) in a volume of 2 μL and incubated for 24 hrs. Quantitative real-time PCR was performed using the following Taqman® gene expression assays (*FKBP5*-Hs01561006_m1, *TSC22D3* (*GILZ*)-Hs00608272_m1, *ACTB*-Hs99999903_m1, *PPIA*-Hs99999904_m1, *GAPDH*-Hs99999905_m1). A geometric mean of the reference genes ACTB, PPIA and GAPDH was used for normalization.

ABBREVIATIONS

LEO 134310	non-steroidal GR agonist
LEO 134998	metabolite of LEO 134310
CP	clobetasol propionate
BMV	betamethasone valerate
TPA	phorbol ester
GR	glucocorticoid receptor
MR	mineralocorticoid receptor
PBMC	peripheral blood mononuclear cells
CL	Clearance
PD	pharmacodynamics
CV	coefficient of variation/relative standard deviation
log2FCH	fold change in log2 scale

RESULTS – compound performance and selectivity

- LEO 134310 behaved as a full GR agonist with high affinity for GR and nanomolar anti-inflammatory potency.

Compound	Binding to human GR (N=8-17), IC ₅₀ (nM)	Inhibition of human TNF-α (N=10-17), IC ₅₀ (nM)	Inhibition of mouse TNF-α (N=3), IC ₅₀ (nM)	Inhibition of Pig TNF-α (N=4-5), IC ₅₀ (nM)
LEO 134310	14	2.2	0.58	3.2
LEO 134998	150	260	35	210
CP	4.8	0.52	0.15	0.38
BMV	5.7	3.2	0.18	1.2

- LEO 134310 was selective for the GR and did not interact with the MR, a receptor which has been associated with induction of skin atrophy.

A) Human MR Agonist Assay, B) Inhibition of specific nuclear hormone receptor binding for LEO 134310 and LEO 134998 to nine nuclear hormone receptors. No significant interaction with any receptor was observed for LEO 134310 and its major metabolite, LEO 134998, in contrast to CP and BMV that activated the MR.

Pharmacological effects and clearance *in vivo*

- In a TPA-induced dermatitis mouse model LEO 134310 resolved inflammation with similar efficacy as BMV. No systemic adverse events were seen, in contrast to CP and BMV after topical treatment.

Compound	Emax (%)	Ear ID ₅₀ (nmol)	Spleen ID ₅₀ (nmol)	Safety ratio (Spleen ID ₅₀ / Ear ID ₅₀)
LEO 134310	62	3.0	4100	1366
CP	87	0.06	2.1	35
BMV	62	2.6*	69*	26*

* Relatively poor ID50 estimation due to the low number of dosing groups (n=3).

- Clearance of LEO 134310 in man was predicted to be >200 mL/min/kg, corresponding to >10-fold of the liver blood flow in man.

Allometric scaling of LEO 134310 from rat and dog Clearance data.

CONCLUSION

- LEO 134310 shows an improved safety profile in preclinical studies compared to traditional corticosteroids and may potentially offer an improved topical treatment solution for patients with atopic dermatitis and psoriasis.

Lack of atrophogenic effect in minipigs

- Minimal skin atrophy after 4-week application to minipigs was seen after treatment with LEO 134310, compared to treatment with CP and BMV.

Epidermal thickness in skin biopsies from mini-pigs after 4 weeks treatment.

Statistical significance was determined by one-way ANOVA analysis.

*: p<0.05; ***: p<0.001; ****: p<0.0001; ns: not significant. Error bars represent standard deviation.

- LEO 134310 had similar levels of target engagement as BMV, but less than CP.

GR target engagement using the PD marker FKBP5 measured by qRT-PCR in skin biopsies from mini-pigs treated with GR agonists for 4 weeks.

Statistical significance for the respective treatments was determined against vehicle using a paired t-test (*: P<0.05). Error bars represent standard error of the mean.

Pharmacological activity in skin explants

- Topical treatment with LEO 134310 in a human skin explant model resulted in full agonism, measured by GR target engagement, albeit at higher dosage strength than required for BMV and CP.

Response of GR PD markers FKBP5 and GILZ in human skin explants at 24h after dose. ▲ CP; ● BMV; ■ LEO 134310. Open symbols: untreated or vehicle treated controls. Lines represent best fits to a Sigmoid Emax model. Data from one study (one symbol represents one explant).

- Comparison of GR agonist strength on PD markers

Compound	FKBP5 ED ₅₀ (mg/mL)	Relative (fold) to CP	Relative (fold) to BMV	GILZ ED ₅₀ (mg/mL)	Relative (fold) to CP	Relative (fold) to BMV
LEO 134310	0.11 (87%)	360	31	0.066 (130%)	120	21
CP	0.00031 (40%)	-	-	0.00057 (37%)	-	-
BMV	0.0037 (100%)	12	-	0.0032 (80%)	5.6	-

ED₅₀ values (CV) calculated from curve fits were used to calculate relative strengths of LEO 134310, CP and BMV.