TOPICAL ROR γ t INVERSE AGONIST ALLEVIATES PRECLINICAL MODELS OF PSORIASIS

Catherine Hervouet, Franck Bihl, Patricia Rossio, David Froude, Coralie Pascau, Tony Minier, Branislav Musicki, Gilles Ouvry, Claire Bouix-Peter, Jonathan Pascau, Claire Chaussade, David Piwnica, Sophie Deret, Valérie Julia, Laurent Hennequin, Emmanuel Vial and Ferial Hacini-Rachinel

NESTLE SKIN HEALTH, GALDERMA R&D, SOPHIA ANTIPOLIS, 06410 BIOT, FRANCE

INTRODUCTION

Psoriasis is a common chronic inflammatory disease involving dynamic interactions between the immune system and the skin. Histologically, psoriatic lesions are characterized by epidermal hyperplasia, immune cell infiltration in the dermis and hypervascularization. Recently developed IL-17-targeting monoclonal antibodies have proven to be successful in reducing the disease burden of moderate-to-severe psoriasis patients. Within psoriatic lesions, IL-17 can be produced by several cell types, including Th17

cells, $\gamma\delta$ T cells, CD8 T cells, innate lymphoid cells (ILC3s), neutrophils and possibly mast cells. Although several transcription factors may be important, the development and maintenance of IL-17 producing cells is controlled by the nuclear receptor retinoid related orphan receptor gamma t (ROR γ t). ROR γ t is both necessary and sufficient for IL-17 expression and Th17 lineage differentiation in both humans and mice. In addition, imiquimod-induced psoriasisform-skin inflammation in mice is attenuated by

ROR γ t gene deficiency as well as by oral treatment with ROR γ small molecule inverse agonists. Here we describe compound A, a potent and selective inverse agonist of the human ROR γ receptor specifically designed for the topical administration, that markedly inhibits Th17-type cytokine production and the hallmarks of psoriasis in multiple assays systems, including (i) human and mouse cellular assays, (ii) the imiquimod mouse model, and (iii) a human skin transplant mouse model.

RESULTS

IN VITRO ACTIVITY

COMPOUND A IS A POTENT AND SELECTIVE ROR γ t INVERSE AGONIST

A Potency on ROR γ t transcriptional activity and others nuclear receptors							B Potency on IL17-A secretion (CD4 $^{+}$ T cells)
IC50 (μ M)							
ROR γ t	ROR α	RaR γ	PPAR γ	LXR β	VRD	GR	
0.019	>10	>10	>10	>10	>10	>10	IL-17A secretion
							IC50 (nM) 9.6 ($\pm$ 9, n=6)

(A) Activity of compound A was evaluated in a luciferase reporter assay. HG5LN cells were transfected with nuclear receptors fused with GAL4 DNA binding domain. After incubation with compound A for 18 hours, relative luminescence units were measured. Compound A was dissolved in DMSO and serial dilutions (10000-0.5nM) were tested.

(B) Cryopreserved CD4⁺ T cells from peripheral human blood were cultured for 4 days with anti-CD3/CD28 antibodies in the presence or absence of serially diluted compound A. Secreted IL-17A was then quantified in the cell culture supernatants by HTRF assay.

IC50: half maximal inhibitory concentration; was calculated from a 4-parameter logistic model with the XLFit software (IDBS).

COMPOUND A POTENTLY INHIBITS HUMAN AND MOUSE TH17 DIFFERENTIATION WITH NO IMPACT ON TREG DIFFERENTIATION

Cryopreserved human CD4⁺ T cells were stimulated for 5 days with anti-CD3/CD28 antibodies and polarized toward Th17 (with rIL-1 β , rIL-6, rIL-21, rIL-23, rTGF- β) phenotype. CD4⁺ T cells were cultured in absence (DMSO control) or presence of 0.15 μ M compound A for 5 days. Data represent 3 donors and are expressed as % of IL17⁺ cells (Th17) as determined by flow cytometry. Dot plots are representative for the donor 2.

Naïve CD4⁺ enriched mouse splenocytes were stimulated for 5 days with anti-CD3/CD28 antibodies and polarized toward Th17 (with anti-IL4, anti-IFN γ , rIL-6, rIL-23, rTGF- β ,) or Treg (with anti-IL4, anti-IFN γ , rTGF- β) phenotypes. CD4⁺ T cells were cultured in absence (DMSO control) or presence of compound A at the indicated concentrations for 5 days.

Data are expressed as % of IL17⁺ cells (Th17) or Foxp3⁺ cells (Treg) as determined by flow cytometry. IC50: half maximal inhibitory concentration was estimated between 0.01 and 0.1 μ M.

IN VIVO EFFICACY

COMPOUND A ATTENUATES IMIQUIMOD-INDUCED PSORIASIS-LIKE INFLAMMATION

(A) Study design AldaraTM cream (14mg) was applied daily on shaved dorsal skin of female Balb/c mice. Compound A was topically applied at different doses twice daily 2 hrs before and 6 hrs after AldaraTM application on the same dorsal site. Corticosteroid (Betamethasone valerate) was applied daily 2 hrs before AldaraTM application.

Clinical scores were evaluated daily. Blood samples were collected at different times during the protocol. At days 3 and 7, skin biopsies were collected: • H&E staining was performed to measure epidermis thickness at day 7 • Real-time qPCR was performed to measure mRNA at day 3 • Single cells suspensions were achieved by collagenase treatment for flow cytometry analysis of effector T cells at day 7

(B) Plasmatic exposure of compound A applied topically throughout the study. Plasma levels corrected by plasma protein binding were well below the IC50. Plasma were collected at early and late phases of AldaraTM treatment and dosed for compound A. Each dot represents an individual mouse.

(C) Attenuation of imiquimod-induced inflammation with compound A. The clinical score is a global grading consisting of the sum of erythema, edema and desquamation scores.

(D) Significant reduction of epidermis thickness at day 7 following topical treatment with compound A.

(F) Gating strategy for the quantification of IL17-producing $\gamma\delta$ T cells and Treg cells in the skin (G) Significant reduction of IL17-producing $\gamma\delta$ T cells in the skin at day 7 following topical treatment with compound A (H) No effect of topical treatment with compound A on Treg cell content at day 7

Blue numbers: % inhibition (vs mean of vehicle group) Statistical analysis: data are mean, vs vehicle using One-way ANOVA followed by a Dunnett's multiple comparisons test Individual data are represented

vs	Naïve		Corticosteroid		Compound A 0.3%		Compound A 1%		Compound A 3%	
	FC	Adj_pval	FC	Adj_pval	FC	Adj_pval	FC	Adj_pval	FC	Adj_pval
Vehicle										
IL23r	1.36	0.007	-1.18	0.158	1.10	0.577	-1.00	0.978	1.05	0.837
IL23a	-2.96	0.000	-2.28	0.002	1.53	0.379	1.58	0.419	1.24	0.626
Ccl20	-5.55	0.000	2.06	0.000	1.56	0.179	1.71	0.216	2.03	0.024
Ccr6	1.78	0.011	-1.90	0.005	-1.70	0.179	-1.81	0.216	-1.76	0.123
IL10	-1.48	0.041	-2.01	0.001	-1.27	0.456	1.18	0.657	1.32	0.371
IL1b	-4.49	0.000	-3.93	0.000	-2.46	0.130	-1.97	0.311	-3.29	0.027
IL6	-7.56	0.000	-2.70	0.000	-1.85	0.179	-1.54	0.446	-3.44	0.007
Tnf	1.07	0.775	-1.99	0.008	1.02	0.949	1.05	0.949	1.23	0.626
Ifng	1.73	0.003	-1.28	0.194	1.87	0.053	2.36	0.017	2.50	0.007
Cxcl9	1.49	0.038	-2.69	0.000	1.78	0.094	2.20	0.032	1.83	0.055
Ccl2	-3.70	0.000	-2.23	0.000	-1.30	0.382	-1.09	0.831	-1.41	0.243
Ccl3	-3.53	0.000	-1.62	0.054	-1.71	0.193	-1.65	0.311	-1.58	0.243
Ccl5	-1.40	0.058	-1.31	0.158	1.33	0.382	1.32	0.449	1.78	0.055
Csf3	-9.09	0.000	-3.93	0.001	-2.55	0.179	-1.38	0.667	-3.23	0.081
S100a7a	-6.07	0.000	-3.01	0.000	-3.47	0.029	-1.94	0.285	-3.64	0.008
Cone1	-2.33	0.000	1.07	0.336	-1.04	0.709	-1.10	0.504	-1.15	0.237
IL19	-27.64	0.000	-10.47	0.000	-1.73	0.473	-1.08	0.949	-2.16	0.330
IL12b	-3.46	0.000	-6.79	0.000	-2.89	0.042	-2.13	0.216	-2.14	0.090

(E) Downregulation of *IL17a*, *IL17f* and *IL22* transcript levels at day 3 following topical treatment with compound A. The table represents the off-target effects. *Actb*, *Hprt* and *18S* genes were used as housekeeping genes. Fold changes (FC) and adjusted p-values were calculated using the vehicle group as the reference.

COMPOUND A DECREASES THE HALLMARKS OF PSORIASIS IN A HUMAN SKIN TRANSPLANT MOUSE MODEL

(A) Study design Based on the xenograft model described by Caspari F, BrJ Dermatol. 2005 Nov;153(5):937-44. Non-lesional skin biopsies (Ø 6mm) and blood were obtained from psoriasis patients. Within 24 hours the skin biopsies were transplanted onto immuno-deficient BNX mice. In parallel peripheral blood mononuclear cells (PBMC) were isolated from the donor's blood and stored in liquid nitrogen. After 3 weeks, the skin graft was intra-dermally injected with the PBMC which were activated 2 days earlier with rIL-2 and Staphylococcus Enterotoxin B (SEB) in order to induce the proliferation of T-cells. The treatments were performed either daily by topical, or oral route or twice a week by IP injection. The treatments started one week after PBMC injection and lasted 3 weeks. Diprolene[®] Salve 0,05% (Betamethasone dipropionate) was used as positive control and applied twice a day. At the end of the study, 24 hours after the last treatment, the psoriasis-like transplant was harvested and analyzed for histology: measure of epidermal ridges thickness and Ki67 immunostaining to determine the epidermal proliferation index (number of Ki67 positive cells per unit of epidermis length).

(B) and (C) Efficacy of different reference compounds (B) and compound A (C) on epidermal ridges thickness and skin proliferation index Blue numbers: % inhibition (vs mean of vehicle group) Statistical analysis: data are mean, vs vehicle using One-way ANOVA followed by a Dunnett's multiple comparisons test Individual data are represented

CONCLUSION

Here we describe the pharmacological properties of compound A, a potent and selective inverse agonist of the human ROR γ receptor specifically designed for topical administration. Compound A potentially inhibits IL-17A release from human and murine CD4⁺ T cells with no impact on regulatory T cell polarization. In the imiquimod-induced psoriasisform skin inflammation model, compound A applied topically dose-dependently downregulates the expression of skin IL-17 mRNA; decreases the frequency of skin IL17-producing $\gamma\delta$ T cells as well as the epidermis thickness. Finally, similarly to topical corticosteroid, systemic anti-IL-17 mAb, and oral ROR γ inhibitor, topical treatment with compound A decreases the hallmarks of psoriasis in a human skin transplant model.

Altogether these results suggest that compound A, our topical small molecule inhibitor targeting ROR γ , may represent an alternative topical medicine for the treatment of psoriasis patients where the topical route is the most appropriate route of administration.