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IRL201104, A Novel Immunomodulatory Peptide, Shows A Wide Spectrum Long Lasting Effect On Inflammatory Endpoints Through The Sublingual Route In A Model Of Allergic Inflammation

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REVOLO BIOTHERAPEUTICS

Introduction

- Epidemiological data suggests that *M. tuberculosis* (mTB) infection prevents asthma development (1).
- IRL201104 (1104) is a novel immunomodulatory peptide derived from an mTB chaperonin currently in development as an intravenous formulation for Eosinophilic Esophagitis and other allergic indications (2).
- We have previously shown that IRL201104, despite a short half-life (~10-15min) shows a long anti-inflammatory effect in allergic inflammation models through the intravenous, subcutaneous, and intranasal routes (1,3-6).
- Our aim was to test two new potential clinical sublingual IRL201104 formulations, using an oral disintegrating tablet, in comparison with the intravenous route in an ovalbumin (OVA) re-challenge model of allergic inflammation.

Methods

Clinical formulations

- Two sublingual (SL) oral disintegrating tablet formulations of IRL201104, in development for clinical applications, were tested in this study vs our preclinical Intravenous formulation (IV): prototype I (SL1) and prototype II (SL2).

Model protocol

- Female Balb/C mice (~25g) were sensitised with ovalbumin (15 µg, SC) and Imject Alum as an adjuvant on days 1 and 7. In order to elicit a local inflammatory response in the lungs, mice were challenged on day 15, 16 and 17 with an aerosol of either 1% w/v ovalbumin in phosphate buffered saline (PBS) or just PBS, generated with an ultrasonic nebuliser (Aerogen) for 20 min.
- IRL201104 or vehicle/placebo were administered sublingually (SL; 80µg/Kg) or intravenously (IV; 80 µg/Kg) 15 minutes prior to the OVA or PBS challenges on days 15, 16 and 17. 24 hours after the final challenge to OVA or PBS (day 18), samples were collected.
- In a separate cohort of animals, a second series of challenges on days 27, 28 and 29 with an aerosol of 1% w/v ovalbumin or PBS were carried out without further drug treatment. Samples were collected from these animals 24 hours after the final challenge (day 30).

Bronchoalveolar lavage fluid (BALF), serum collection and differential cell counts

- 24 hours after the last challenge (day 18 or 30) a blood sample was taken by venepuncture (via the lateral tail vein), processed to serum and stored at -80ºC for OVA specific IgE analysis.
- Animals were then overdosed with pentobarbitone and bronchoalveolar lavage was carried out using phosphate buffered saline. The isolated BALF was then centrifuged at 1500 rpm for 10mins at 4ºC and the supernatant was aliquoted and stored at -80ºC for cytokine analysis.
- BALF cells were analysed for total and differential cell numbers using a XT-2000iV analyser (Sysmex). Results were expressed as cells/mL.

OVA specific IgE level measurement

- OVA specific IgE level in serum was measured using an ELISA kit (AssayGenie) as per the manufacturer's instructions. Results were expressed as concentration in pg/mL.

Cytokine/chemokine measurement

- A 12-Plex cytokine/chemokine panel (IL-4, IL-5, IL-10, IL-12p70, IL-13, IL-17, KC, Eotaxin, G-CSF, GM-CSF, MCP-1, RANTES and Periostin) was run in BALF supernatant using a magnetic multiplex assay as per the manufacturer's instructions. Results were expressed as concentration in pg/mL.

Statistical analysis

- Data are shown as mean ± S.E.M. (standard error of the mean). Inter-group deviations were statistically analysed by a one-way analysis of variance (ANOVA) followed by a Dunnett's test. $p < 0.05$ was considered statistically significant.

Results (I)

Effect of treatment on OVA induced cell infiltration

24 hours post-challenge at day 18, exposure to OVA aerosol triggered inflammatory infiltration in the lung as measured by BALF differential cell counts. IRL201104 significantly reduced OVA-induced lung infiltration of eosinophils, neutrophils, lymphocytes and macrophages (Fig1A,B,C,D) through both routes of administration. The effect of IRL201104 was maintained upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig1E,F,G,H).

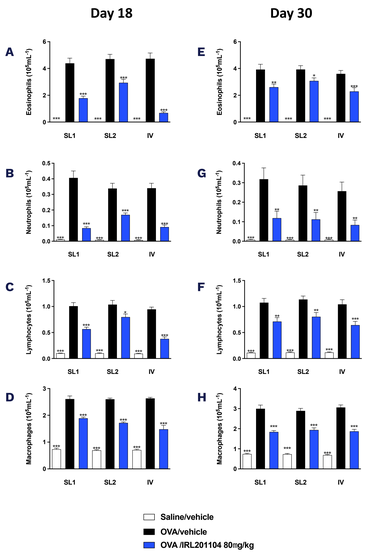

Figure 1 – Effect of IRL201104 on OVA-induced cellular infiltration. *Effect of IRL201104 on (A,E) eosinophils, (B,G) neutrophils, (C,F) lymphocytes and (D,H) macrophages. Data are expressed as cells per mL of BALF, mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01, ***P<0.001; n=8.*

Results (II)

Effect of treatment on OVA specific IgE levels

OVA challenge elicited an increase in OVA specific IgE (Fig2). IRL201104 treatment through both routes of administration significantly reduced OVA specific IgE in serum at day 18 (Fig2A) and the effect was maintained upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig2B)

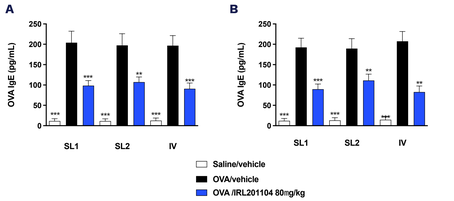

Figure 2 – Effect of IRL201104 on serum OVA specific IgE. *Effect at day 18 (A) and day 30 (B) on OVA specific IgE serum levels. Data are expressed as pg/mL of serum, mean ± SEM. Groups were compared to OVA/vehicle group using a one-way ANOVA, followed by a Dunnett's test; **P<0.01, ***P<0.001; n=8.*

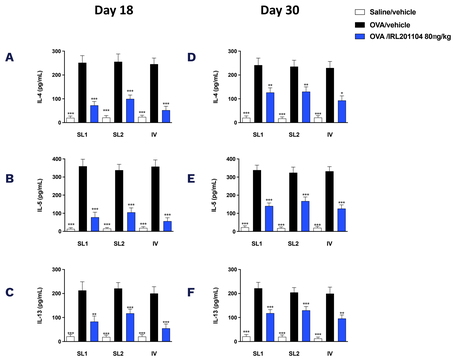

Figure 3 – Effect of IRL201104 on T2 cytokines in BALF after OVA challenge. *Effect on IL-4 (A,D), IL-5 (B,E) and IL-13 (C,F). Data are expressed as picograms per mL of BALF, mean ±SEM. Groups were compared to their respective OVA/vehicle groups using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01,***P<0.001; n=8.*

Results (III)

Effect of treatment on cytokine/chemokine release in BALF

24 hours post-challenge at day 18, exposure to OVA aerosol elicited the release of IL-4, IL-5, IL-13 (Fig3) as well as IL-10, IL-12(p70), IL-17, Eotaxin, GM-CSF, MCP-1, RANTES and Periostin in BALF supernatant (Table 1). IRL201104 significantly reduced OVA-induced cytokines/chemokine release through both routes of administration. This effect of IRL201104 was maintained through both routes of administration upon OVA re-challenge (day 30), 13 days after the last dose of the compound (Fig3; Table 1).

Day 18	BALF concentration (pg/ml)	Saline/vehicle SL1	OVA/vehicle SL1	OVA/1104 80µg/Kg SL1	Saline/vehicle SL2	OVA/vehicle SL2	OVA/1104 80µg/Kg SL2	Saline/vehicle IV	OVA/vehicle IV	OVA/1104 80µg/Kg IV
	IL-10	20±8***	202.5±29	104±14**	14±7***	198±22	111±14**	16±8***	216±21	80±13***
	IL-12p70	20±8***	133±18	70±13**	14±7***	124±14	87±8*	21±8***	126±14	47±15***
	IL-17	14.5±7***	168±19	87±12***	22±9***	161±18	102±13*	14±7***	162±21	44±14***
	KC	14±7	41±13	33±11	19±7	44±14	36±11	19±7	39±12	31±11
	Eotaxin	21±8***	160±19	66±8***	19±8***	170±18	97±11**	15±8***	151±16	39±12***
	GM-CSF	14±7***	132±19	59.5±13**	11±7***	118±15	73±8*	13±6***	134.5±18	50±16***
	G-CSF	15±8	44±14	24±10	12±6	34±12	29±11	14±7	38±11	24±9
	MCP-1	15±8***	137±20	50±13***	16±8***	128±17	67±16*	19±7***	147±17	34±12***
	RANTES	22±8***	104±11	60±12*	21±8***	111±12	75±11*	23±9***	107±10	44±17**
	Periostin	14±7***	179±25	87±10***	15±7***	173±19	113±16*	13±6***	185±22	74±9***
Day 30	**BALF concentration (pg/ml)**	**Saline/vehicle SL1**	**OVA/vehicle SL1**	**OVA/1104 80µg/Kg SL1**	**Saline/vehicle SL2**	**OVA/vehicle SL2**	**OVA/1104 80µg/Kg SL2**	**Saline/vehicle IV**	**OVA/vehicle IV**	**OVA/1104 80µg/Kg IV**
	IL-10	13±6***	174±23	108±14*	16±8***	173±25	119±12	16±6***	195.5±21	94±15***
	IL-12p70	14±7***	137±16	91±9*	13±7***	128±18	96±9	17±8***	115±18	87±15
	IL-17	14±7***	112±12	72±13*	13±6***	117±14	82±10*	14±7***	117±12	38±15***
	KC	20±8	33±13	24±10	14±7	38±15	28±11	13±6	41±13	23±9
	Eotaxin	20±8***	162±19	90±7**	15±7***	153.5±14	107±9**	14±7***	150±16	62±15***
	GM-CSF	15±7***	110±12	44±16**	14±7***	120±14	59±10**	15±7***	97±10	37±14**
	G-CSF	14±7	31±12	24±10	13±6	32±10	24±9	13±6	33±10	20±8
	MCP-1	20±8***	128±15	70±17*	15±7***	134±16	77.5±16*	15±7***	121±15	66±21*
	RANTES	15±7***	108±12	90±11	16±8***	101±11	89±12	20±8***	113.5±11	94±10
	Periostin	14±7***	179±23	98±13**	13±6***	180±24	108±15*	13±7***	156.5±20	82±13**

Table 1– Effect of IRL201104 (1104) on cytokines/chemokines in BALF after OVA challenge. *Effect on IL-10, IL-12(p70), IL-17, KC, Eotaxin, GM-CSF, G-CSF, MCP-1, RANTES and Periostin. Data are expressed as picograms per mL of BALF, mean ±SEM. Groups were compared to their respective OVA/vehicle groups using a one-way ANOVA, followed by a Dunnett's test; *P<0.05, **P<0.01,***P<0.001; n=8.*

Discussion

- **As seen previously with other routes of administration, IRL201104 also shows a similar wide spectrum long-lasting immunomodulatory effect when administered through an orally disintegrating tablet.**
- **Administration of the same dose of IRL201104 using two new sublingual clinical prototypes achieved remarkable efficacy, particularly SL1, quite similar to the intravenous route, on OVA induced lung infiltration, serum OVA specific IgE and BALF cytokines/chemokines.**
- **The present work shows the potential of the sublingual route when administering 1104 in the treatment of asthma and other allergic and inflammatory diseases. The study also validates our clinical prototypes, supporting transition to this route of administration for future clinical trials.**

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