EVO756 potently blocks MRGPRX2-mediated mast cell activation as well as MRGPRX2 and FcεR1-dependent synergistic responses

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Abstract

Mas-related G-protein coupled receptor X2 (MRGPRX2) is a GPCR expressed predominantly by mast cells and has been implicated in playing an important role in diseases such as chronic spontaneous urticaria, atopic dermatitis, asthma and ulcerative colitis. Cationic ligands, either endogenous or exogenous, can activate the receptor and induce IgE-independent mast cell activation, resulting in allergic responses and non-histaminergic itch. Therefore, blocking MRGPRX2 represents a promising therapeutic modality for a plethora of autoimmune conditions. Here we show that EVO756, a novel small molecule antagonist of MRGPRX2, demonstrates potent, concentration dependent inhibition of MRGPRX2 mediated activation in MRGPRX2 transfectants and LAD2 cells. Furthermore, EVO756 inhibits primary humans mast cell activity at the transcriptional and proteomic levels, as determined by the release of tryptase and surface expression of mast cell granules. We also demonstrate synergism between MRGPRX2-mediated and IgE-dependent primary human mast cell activation, which is inhibited by EVO756. These studies establish EVO756 as a potent inhibitor of MRGPRX2-mediated mast cell activation and therefore, a promising therapeutic for the treatment of mast cell driven diseases.

Background

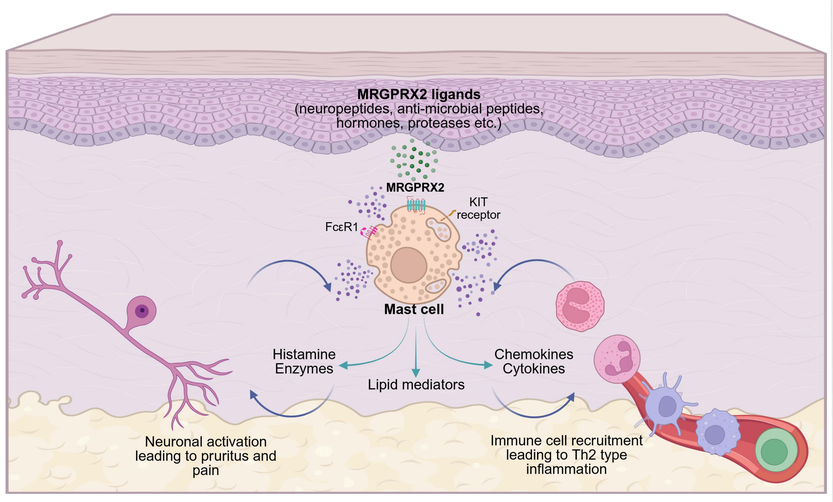


Figure 1. MRGPRX2 mediated activation of mast cells.

EVO756 blocks MRGPRX2 mediated signaling in the presence of various agonists

	Ligand category	Ligand	EC_{50} (μM)	IC_{50} (μM)
Endogenous	Neuropeptides	Cortistatin-14	0.02	0.06
		Substance P	0.05	0.10
		PACAP 1-27	0.08	0.10
		PACAP 1-38	0.33	0.25
		Dynorphin A	2.05	0.13
		Neuropeptide FF	6.18	0.10
	Antimicrobial peptides	LL-37	0.18	0.17
		Catestatin	3.29	0.10
		β-defensin 2	3.82	0.04
	Peptide hormones	PAMP-12	0.04	0.15
		VIP	0.45	0.07
		Vasopressin	6.04	0.09
	Protein fragments	Eosinophil cationic protein	9.32	0.15
		Hemokinin-1	1.10	0.04
Exogenous		Dextromethorphan	2.31	0.12
		C48/80	3.72	0.08
		Icatibant	6.18	0.05
		Chlorpromazine	13.53	0.20
		Ciprofloxacin	29.95	0.04

Table 1. IC50 values (μM) for EVO756 in the presence of various MRGPRX2 agonists

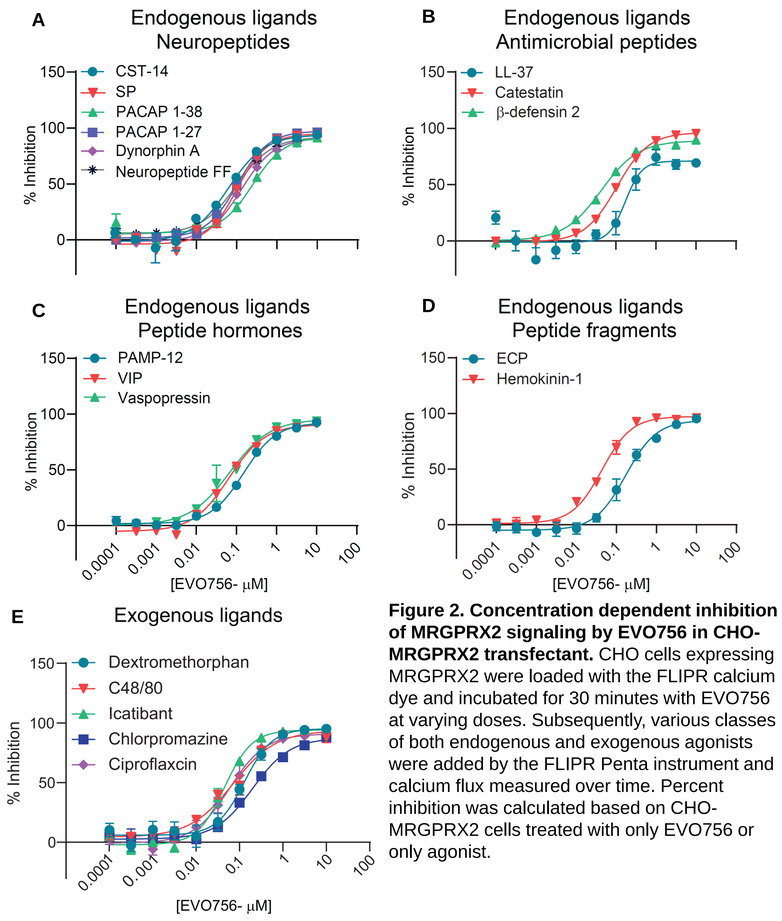


Figure 2. Concentration dependent inhibition of MRGPRX2 signaling by EVO756 in CHO-MRGPRX2 transfectant. CHO cells expressing MRGPRX2 were loaded with the FLIPR calcium dye and incubated for 30 minutes with EVO756 at varying doses. Subsequently, various classes of both endogenous and exogenous agonists were added by the FLIPR Penta instrument and calcium flux measured over time. Percent inhibition was calculated based on CHO-MRGPRX2 cells treated with only EVO756 or only agonist.

EVO756 blocks MRGPRX2 mediated mast cell degranulation in the presence of various agonists

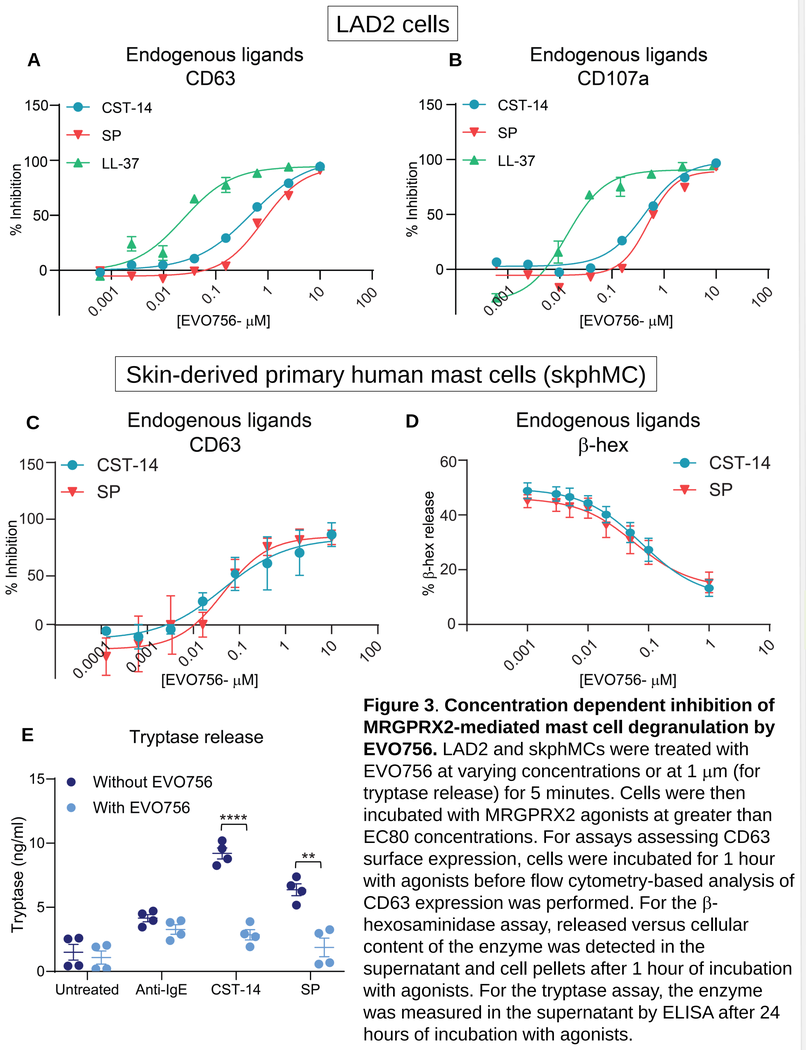
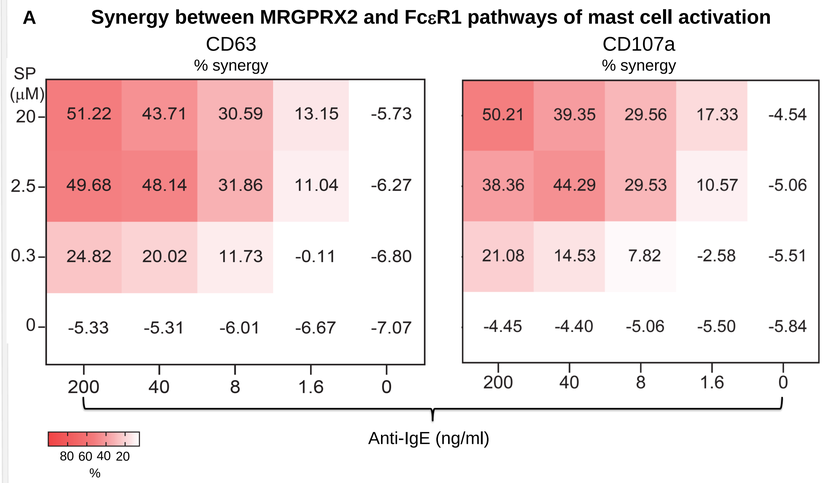
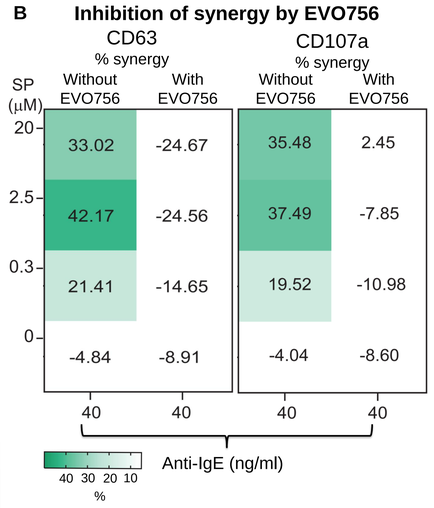


Figure 3. Concentration dependent inhibition of MRGPRX2-mediated mast cell degranulation by EVO756. LAD2 and skphMCs were treated with EVO756 at varying concentrations or at 1 μm (for tryptase release) for 5 minutes. Cells were then incubated with MRGPRX2 agonists at greater than EC80 concentrations. For assays assessing CD63 surface expression, cells were incubated for 1 hour with agonists before flow cytometry-based analysis of CD63 expression was performed. For the β-hexosaminidase assay, released versus cellular content of the enzyme was detected in the supernatant and cell pellets after 1 hour of incubation with agonists. For the tryptase assay, the enzyme was measured in the supernatant by ELISA after 24 hours of incubation with agonists.

EVO756 inhibits synergistic responses elicited by MRGPRX2 and FcεR1 mediated mast cell activation





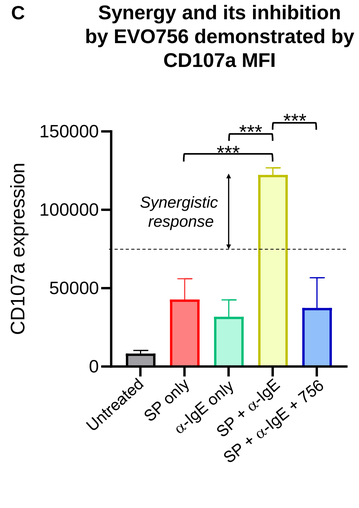


Figure 4. EVO756 blocks MRGPRX2 and FceR1 mediated synergy in skphMCs. skphMCs were treated with different concentrations of SP and/or IgE/anti-IgE in the presence or absence of EVO756 (5 μM). For CD63 and CD107a analysis, cells were incubated for 1 hour before flow cytometry was performed. Percent synergy was calculated using the Bliss model of independence.

Conclusions

- EVO756 potently inhibits MRGPRX2 responses to various endogenous and exogenous ligands *in vitro*
- EVO756 effectively reduced LAD2 and donor-derived mast cell activation upon treatment with different MRGPRX2 ligands
- EVO756 inhibited synergistic responses upon activation of mast cells through concomitant activation through MRGPRX2 and FcεR1.

Acknowledgements

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Disclosures

SB, CMA, AP, JL, JP, LRB and JLH are employees of, and hold stock in, Evommune.