EVO756 is a novel MRGPRX2 antagonist that potently inhibits human mast cell degranulation in response to multiple agonists – a potential treatment for CSU and beyond

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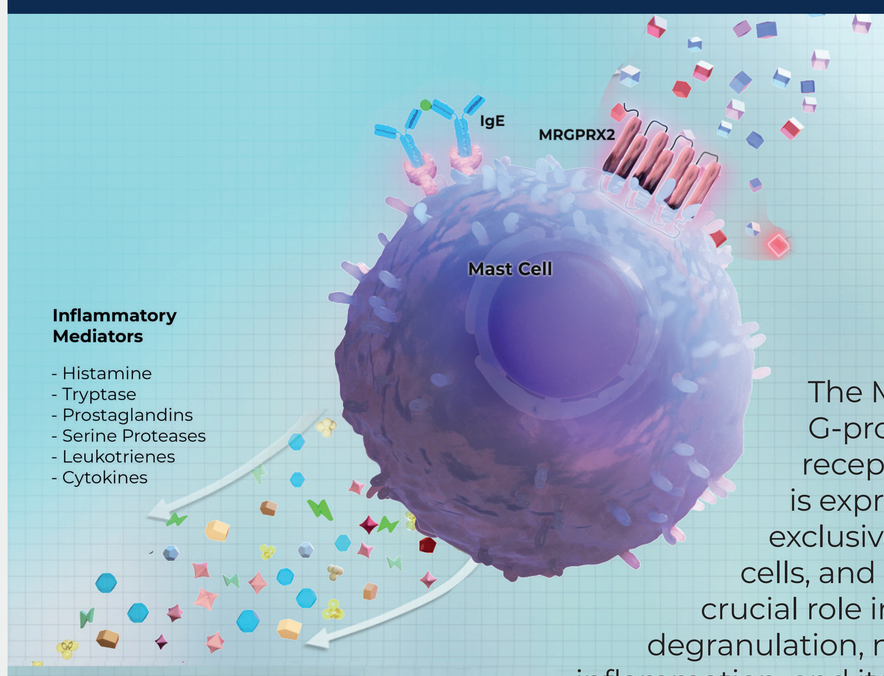
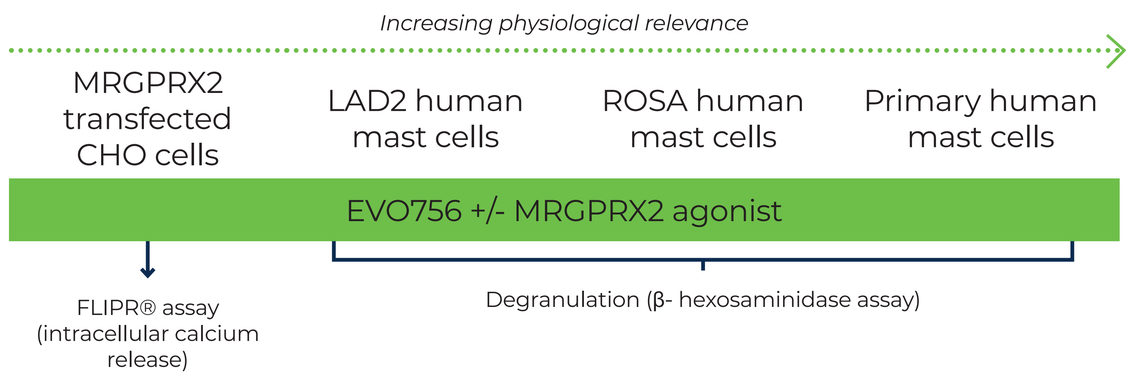
Background



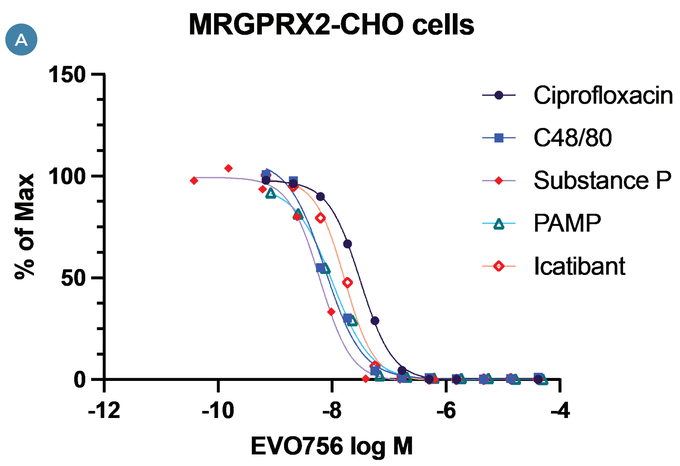
The Mas-related G-protein coupled receptor (MRGPRX2) is expressed almost exclusively on mast cells, and plays a crucial role in mast cell degranulation, neurogenic inflammation, and itch. This receptor is highly promiscuous and activated by a variety of endogenous and exogenous agonists including neuropeptides (e.g., substance P) and pharmaceuticals (e.g., codeine). Activation of MRGPRX2 on mast cells results in rapid degranulation and release of inflammatory mediators such as histamine and tryptase, and is considered a key pathway in non-IgE mediated mast cell degranulation. EVO756 is a novel, orally administered, small molecule antagonist of MRGPRX2. **Here, we describe the in-vitro pharmacological characterization of EVO756 in a variety of MRGPRX2-expressing cells, including MRGPRX2-transfected cell lines, transformed human mast cell lines, and primary human mast cells.**

Materials and Methods



Results

Figure 1: EVO756 potently and dose-dependently inhibits the response of MRGPRX2 transfected CHO cells to multiple MRGPRX2 ligands

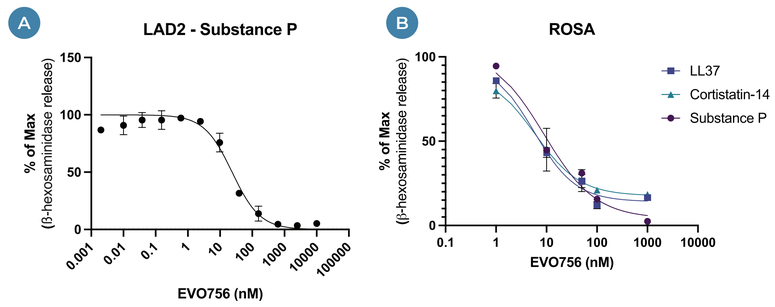


B

Agonist	EVO756 IC50
Ciprofloxacin	16.6 nM
C48/80	6.67 nM
Substance P	5.25 nM
PAMP	9.2 nM
Icatibant	12.9 nM

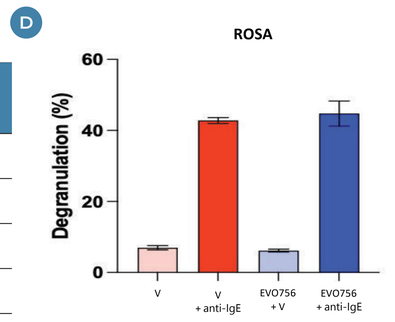
(A) MRGPRX2 agonists at EC_{80} (0.1 μ M substance P, 25 μM ciprofloxacin, 15 nM PAMP, 0.2 μM C48/80, 5 μM icatibant) were used to evaluate the ability of EVO756 to inhibit calcium flux (FLIPR® assay) in MRGPRX2-CHO cells, data are plotted as a percent of maximal response of the MRGPRX2-CHO cells to vehicle + agonist. (B) Calculated IC_{50} values for EVO756 for inhibition of MRGPRX2 activation in MRGPRX2-CHO cells

Figure 2: EVO756 potently inhibits degranulation of human mast cells lines (LAD2 and ROSA) to multiple MRGPRX2 ligands, but not to IgE mediated degranulation



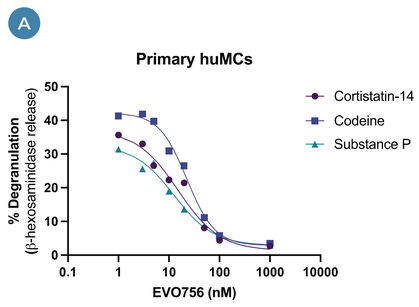
C

Cell line	Agonist	EVO756 IC50
LAD2	Substance P	27 nM
ROSA	Substance P	3.6 nM
ROSA	LL37	7 nM
ROSA	Cortistatin-14	7.6 nM



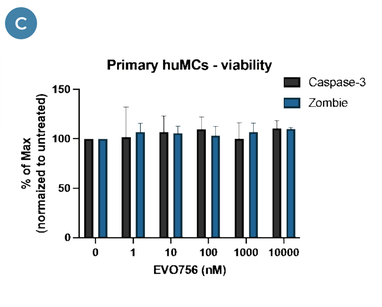
(A) LAD2 cells were treated with EVO756 prior to stimulation with 0.4 μM of substance P. (B) ROSA cells were treated with EVO756 prior to stimulation with 20 μM LL37, 40 μM cortistatin-14, and 10 μM substance P. The percent of maximal response (degranulation as measured by % of β-hexosaminase release based on agonist + vehicle) and normalized to baseline, is presented. (C) Calculated EVO756 IC_{50} values for inhibition of MRGPRX2 agonists in human mast cell lines. (D) ROSA cells were incubated with human IgE for 1 hour prior to treatment with 1 μM EVO756. IgE primed ROSA cells were stimulated with rabbit anti-human IgE. The percent of degranulation versus total is presented.

Figure 3: EVO756 potently and dose-dependently inhibits the response of primary skin mast cells to multiple MRGPRX2 ligands, without affecting cell viability



B

Agonist	EVO756 IC_{50}
Cortistatin-14	15.1 nM
Substance P	12.5 nM
Codeine	22.7 nM



Primary human mast cells isolated from human skin were treated with EVO756 at increasing concentrations in conjunction with stimulation with (A) 0.3 uM cortistatin-14, 10 μg/mL codeine, or 1 μM substance P. Percent of degranulation was determined by β-hexosaminase release. (B). Calculated IC_{50} values for EVO756 at inhibition of MRGPRX2 agonists in primary human mast cells (C) Primary human mast cells were treated with EVO756 in the absence of stimulation. Normalized (to no EVO756 treatment) caspase-3 and zombie staining data is shown.

Conclusions

- EVO756 is a novel, small molecule antagonist of MRGPRX2
- MRGPRX2 is a highly promiscuous receptor that can respond to many endogenous and exogenous ligands, resulting in non-IgE mediated degranulation in mast cells
- EVO756 demonstrates potent and dose-dependent inhibition of MRGPRX2 activation by multiple stimuli in several in vitro settings, including MRGPRX2-transfected CHO-cells, human mast cells lines (LAD2 and ROSA), and primary human mast cells isolated from human skin
- EVO756 does not inhibit IgE mediated degranulation of ROSA human mast cells
- EVO756 is not cytotoxic, nor does it induce apoptosis of primary human mast cells
- In conclusion, EVO756 may provide a novel oral approach for the treatment of multiple diseases with mast cell activation, such as chronic spontaneous urticaria.

Disclosures

JLH, HH, and JP are employees of and hold stock in Evommune SJG is a Scientific Advisor of, and holds stock in, Evommune.