Integrin α4β7: discovery of gut-restrictive oral peptide antagonists that are active in murine models of inflammatory bowel disease

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ABSTRACT

Background

The α4β7 integrin is a clinically validated target for inflammatory bowel disease (IBD). The anti-α4β7 antibody vedolizumab is approved by the FDA for treating moderate-to-severe ulcerative colitis and Crohn's disease. Vedolizumab binds to α4β7 on circulating memory/effector T cells in the blood and blocks their homing to intestinal tissues expressing the ligand MAdCAM-1. The aim of this study is to develop orally stable α4β7 antagonist peptides that act locally in the intestinal tissue. These peptides have minimal systemic exposure, yet are effective in blocking T cell homing and are efficacious in murine models of IBD.

Methods

Potent, selective and orally stable peptide antagonists of α4β7 integrin were identified through Protagonist's peptide and peptidomimetic technology platform. To evaluate oral stability, the peptides were incubated in a variety of ex vivo intestinal/colonic washes or simulated gastric/intestinal fluids, and half-lives determined by mass spectrometry. Potency and selectivity assays used transformed cell lines or primary cells from PBMC donors. Pharmacokinetic (PK), pharmacodynamic (PD) and chronic colitis studies were conducted in mice.

Results

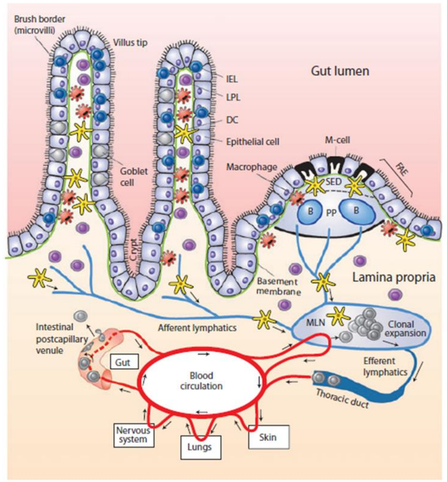
The peptides are potent against α4β7, but not α4β1 and αLβ2 as measured in biochemical and cell adhesion assays. In α4β7 specific cell adhesion assays, the peptides block adhesion of the human RPMI 8866 cell line (B cell lymphoblastoid) or mouse TK-1 (T cell lymphoblast) to immobilized MAdCAM-1 (IC_{50} < 20 nM). In the α4β1 or αLβ2 specific cell adhesion assay using human Jurkat cells, they are inactive up to the highest tested concentration, 100 μM. To facilitate oral delivery, we chemically engineered the peptides to be resistant to chemical and proteolytic degradation in a variety of gastric and intestinal fluids, while maintaining their potency and selectivity. PK studies in normal or dextran sodium sulfate (DSS) treated mice and rats showed that oral dosing results in exposure in the small intestine, colon and mesenteric lymph nodes (MLN), but no significant measurable levels in the blood and urine. A PD assay was used to assess the effect of oral dosing on trafficking of endogenous memory T cells in the mouse. Mice treated with DSS were orally dosed daily with peptides for 9 or 13 days, and harvested tissues were analyzed by FACS. FACS analysis of tissues from animals dosed with peptides showed that there is a reduction of CD4+ $CD44^{High}$ $CD45RB^{Low}$ α4β7+ T cells in the MLN and Peyer's Patches, but not in the spleen or blood. There was also marked reduction of clinical disease symptoms. We also evaluated these peptides in a T cell adoptive transfer chronic colitis model. Daily oral dosing with peptides reduced the severity of disease as measured by colon weight length ratio and histology.

Conclusions

Potent, selective and orally stable peptide antagonists of α4β7 integrin were shown in oral PK studies to have significant exposure in intestinal tissues, but not blood and urine. Despite low blood exposure, these peptides block T cell homing to gut associated lymphoid tissue and attenuate disease in murine models of IBD. These results support the therapeutic potential of locally blocking T cell homing while minimizing immunogenicity and the risk of opportunistic infections associated with systemically delivered immunosuppressants and biologics.

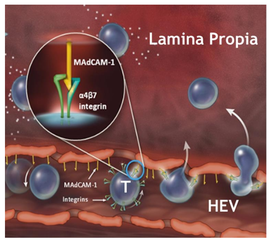
BACKGROUND

Figure 1. Lymphocyte trafficking.



Marsal and Agace, 2012

Figure 2. Gut-homing T cell binding to MAdCAM-1 and extravasation.



RESULTS

In vitro assays

Table 1. PTG-100 is potent and selective for α4β7 integrin.

Integrin	α4β7		α4β1	αLβ2
Ligand	MAdCAM-1		VCAM-1	ICAM-1
Cell Line	RPMI8866 (Hu)	TK1 (Mu)	Jurkat (Hu)	Jurkat (Hu)
IC_{50} (nM)	0.72	0.50	>100,000	>100,000

Cell adhesion assay for indicated ligand

Table 2. PTG-100 is selective for human circulating α4β7+ memory T cells.

Integrin	α4β7	α4β1	αLβ2
Ligand	MAdCAM-1	VCAM-1	ICAM-1
IC_{50} (nM)	1.3	>100,000	>100,000

PBMC cell adhesion assay for indicated ligand

Table 3. Binding kinetics to α4β7 by SPR. Tool peptide PN-10742 has a slower dissociation rate compared to vedolizumab

	k_a (M^{-1} sec^{-1})	k_d (sec^{-1})	K_d (nM)
PN-10742	793	5.3×10^{-5}	67
Vedolizumab	6460	4.1×10^{-4}	64

Biotinylated peptide or Ab was immobilized to chip for binding of integrin in solution

Table 4. PTG-100 is stable in a variety of gastric and intestinal fluids, and S9 fraction homogenates

	Intestinal fluids			Gastric fluid	Plasma	S9 Fraction	
	HIF	RIF	SIF	SGF	Rat	Liver	Intestine
PTG-100	>24	>5	11	>6	>6	>6	>1

Values expressed as half-life (h) after incubation of peptide (20 μM).
HIF- human intestinal fluid; RIF-rat intestinal fluid; SIF- simulated intestinal fluid (porcine); SGF- simulated gastric fluid (porcine).

PK study

Table 5. PTG-100 exposure is restricted to intestinal tissues, not blood.

Small intestine		MLN		Peyer's Patches		Colon	
C_{max} (nM)	AUC (μg h/g)	C_{max} (nM)	AUC (μg h/g)	C_{max} (nM)	AUC (μg h/g)	C_{max} (nM)	AUC (μg h/g)
2417	22	150	1	4563	49	4027	30

30 mg/kg PO C57BL/6 mice pretreated with 3% DSS for 6 days

Plasma	
C_{max} (nM)	AUC (μg h/ml)
BLQ	BLQ

BLQ- Below limit of quantitation

PD Study- Trafficking of T cells in DSS colitis mice

Figure 3. PD study design.

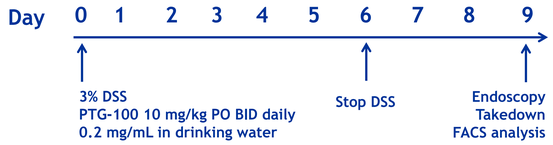


Figure 4. PTG-100 significantly reduces endoscopy score.

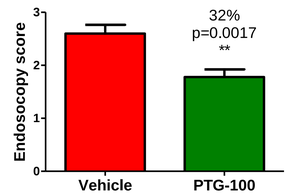
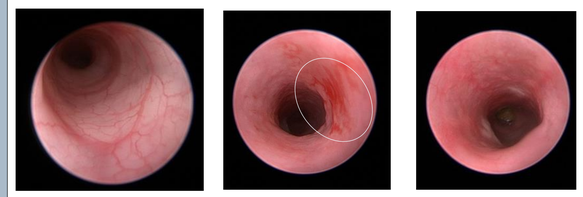
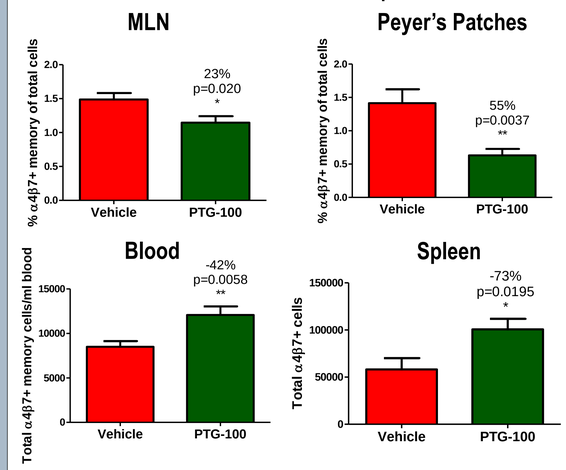


Figure 5. PTG-100 reduces colonic friability and mucosal injury.



Normal Score=0 | Vehicle Score=3 | PTG-100 Score=1

Figure 6. PTG-100 reduces α4β7+ T cells in gut lymphoid tissues and redirects them to blood and spleen.



CONCLUSIONS

- The α4β7 integrin is a specific IBD target that is clinically validated by the intravenous antibody vedolizumab.
- PTG-100 is an oral α4β7 antagonist peptide that has low systemic exposure, and it is effective in blocking T cell homing and preventing mucosal injury in a murine model of IBD.
- PTG-100 has the potential to be an oral therapeutic for the treatment of IBD while minimizing the risk of immunogenicity associated with systemically delivered biologics.

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DISCLOSURE

Dr. Aida Habtezion's contribution to this publication was as a paid consultant, and was not part of her Stanford University duties or responsibilities