Gastrointestinal Stability and Tissue Penetration of V565: a Novel Orally Administered Anti-TNFα Vorabody™

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Introduction

- VHsquared Ltd is a biotechnology company based in Cambridge, UK, using proprietary technology to engineer llama single domain antibodies for oral delivery (Vorabodies) for the treatment of inflammatory diseases of the intestine.
- V565, the lead Vorabody product, potently neutralises TNFα and has been engineered to be resistant to intestinal and inflammatory proteases.
- Crohn's disease (CD) is an incurable life-long inflammatory disease of the intestine, although selective neutralisation of TNFα has already been established as an effective therapeutic strategy.
- Conventional anti-TNFα antibodies must be injected or intravenously infused, which is inconvenient and painful for the patient and results in systemic suppression of TNFα.
- V565 is administered in a convenient oral formulation to enhance delivery direct to the disease site where there is overproduction of TNFα, while limiting systemic exposure and potential associated side-effects.

Development

- VHH domain antibodies with potent TNFα-neutralising activity were isolated from a llama VHH phage display library.
- V565 was engineered by altering its amino acid sequence to better resist proteolytic inactivation. Variants with improved stability that retained potency were modified further resulting in the fully optimised Vorabody V565.
- PK studies were performed in mice to assess the stability of V565 in transit through the GI tract and to determine luminal concentrations in different regions of the intestines following oral administration of the Vorabody.
- The penetration of V565 into inflamed regions of the intestinal tract was investigated in mice with DSS-induced colitis using immunocytochemistry.

Results

In vitro stability of V565 in Intestinal & Faecal Extracts

- Vorabodies are formulated for release at the site of inflamed mucosal tissues. In this case, the ileum and colon.
- V565 strongly resisted degradation in different regions of the GI tract: (i) mouse small intestine (ii) the human ileum and (iii) pooled human faeces (Fig. 1).

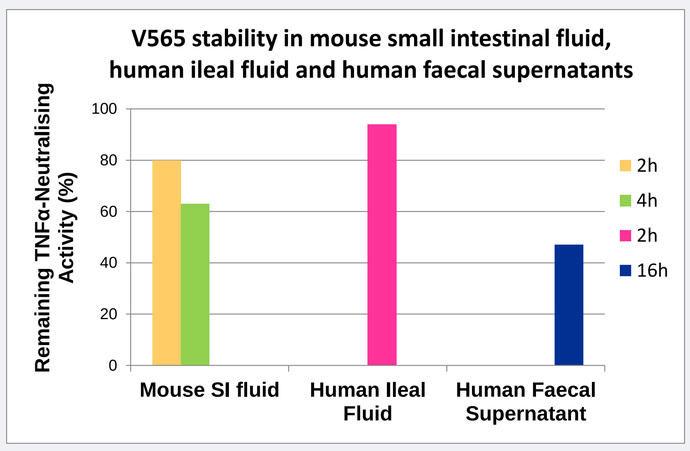


Figure 1: V565 was incubated in pooled mouse small intestinal fluid for 2 or 4h, with human ileal fluid for 2h, and human faecal extract for 16h at 37°C. The remaining TNFα-binding activity in each treated sample was measured by a TNFα ELISA, and results were compared with recoveries at 0 hours.

Resistance of V565 to disease-associated MMPs

- Matrix metalloproteinases (MMPs) are increased in the inflamed mucosa of patients with CD and can digest native human IgG and therapeutic agents that contain a human IgG scaffold (Biancheri *et al*, 2015).
- V565 was highly resistant to digestion by MMPs, compared to the IgG-based anti-TNFα therapy Enbrel (Fig. 2).

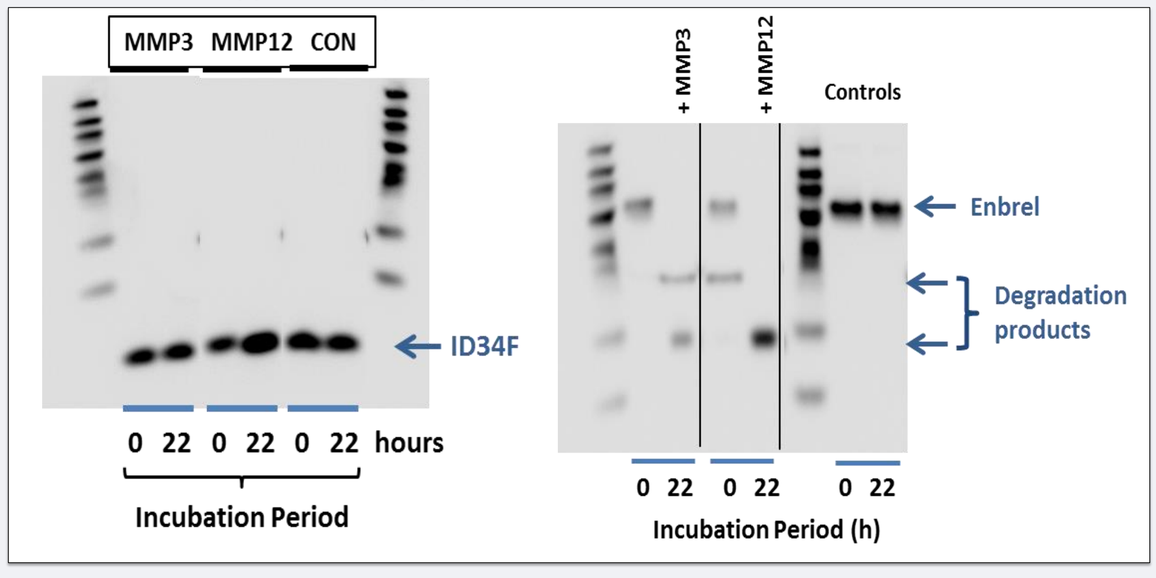


Figure 2: A close analogue of V565 (ID34F) was incubated for 22 h with either human MMP3 or MMP12 and the products were analysed by Western blotting using either anti-VHH or anti-IgG antibodies.

In vivo GI stability and distribution of V565 in the mouse

Local Delivery to the Intestinal Tract following Oral Administration

- Oral dosing of mice with V565 resulted in rapid transit of the Vorabody through the GI tract and by 3h V565 in the stomach and SI was below measurable levels.
- Meanwhile, high concentrations were present in both the caecum and colon.
- V565 levels in faeces varied between animals, but a peak concentration was detected at 3h post-dosing in all mice (Fig. 3B).
- Overall, V565 was shown to transit through the mouse GI tract delivering high concentrations in the lower GI tract and faeces up to 7h post-dosing.

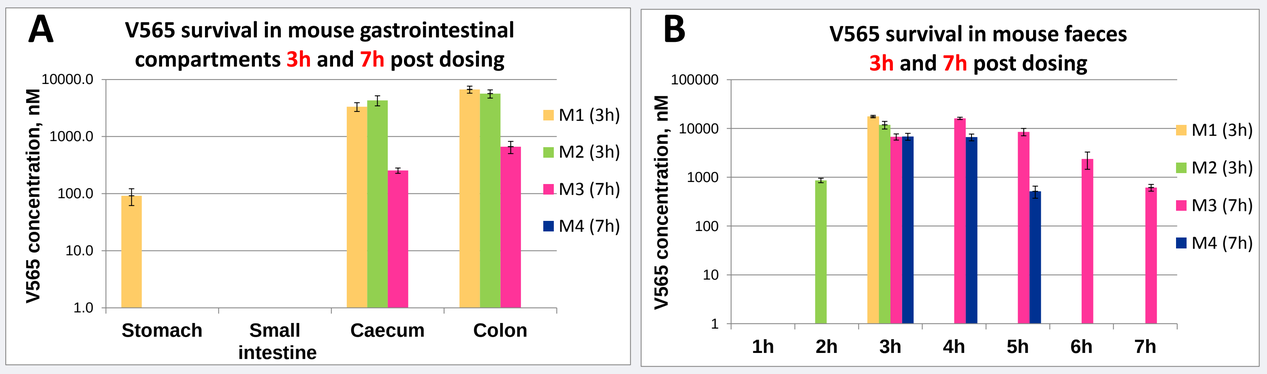
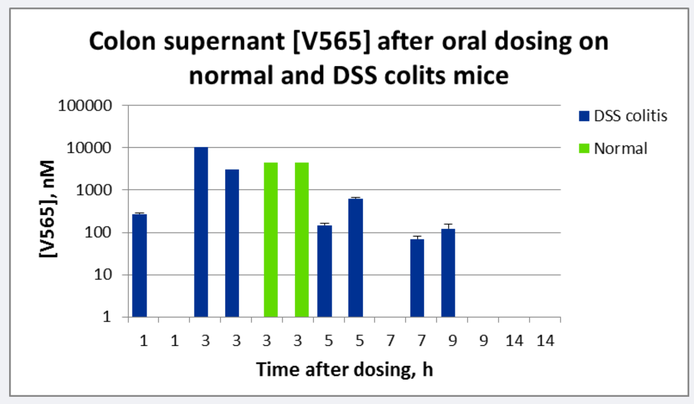


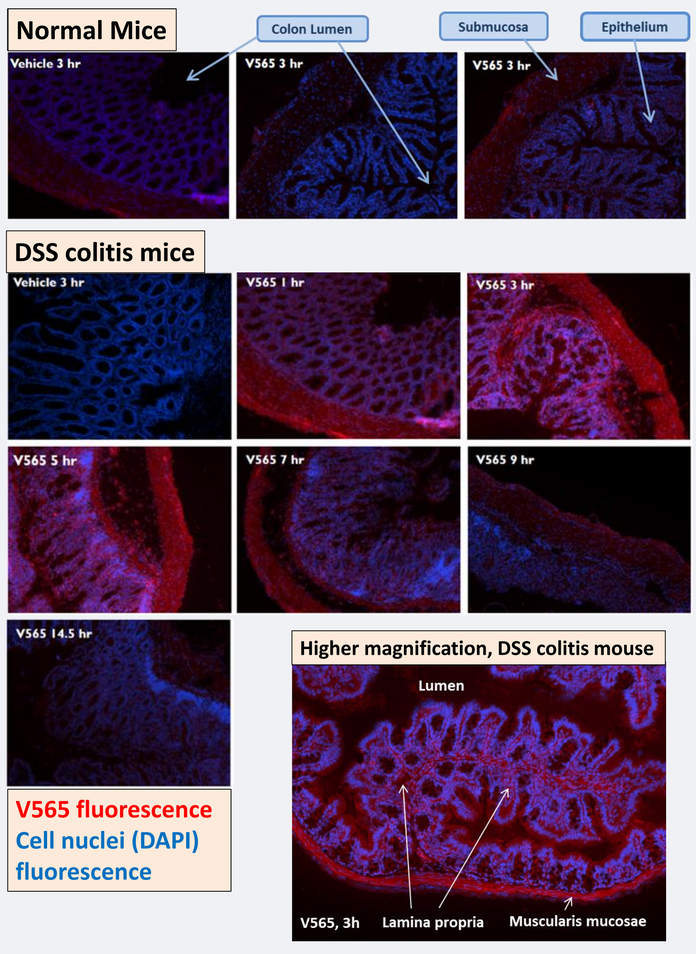
Figure 3: 140 µg V565 was dosed in milk and bicarbonate (to neutralise stomach acid) to mice by oral gavage. Concentrations of V565 in GI compartments at 3 and 7h post-dosing (A) and in faecal pellets collected at hourly intervals (B) were measured by ELISA.

Penetration of V565 into colonic mucosal and sub-mucosal tissue in DSS-colitis mice following oral administration.

- For V565 to be effective in patients with CD it must be able to access the lamina propria to neutralise the activity of TNFα at its site of production.
- Concentrations of V565 in the colons of normal and DSS colitis mice were maximal 3h after oral dosing, dropped after 5h and was no longer detectable in the colon after 14h (Fig. 4).

Figure 4: DSS colitis was induced in mice and 140 µg V565 (in milk and bicarbonate) was dosed by oral gavage to normal mice and mice with DSS colitis. Two DSS colitis mice were sacrificed at 1, 3, 5, 7, 9 and 14h post-dosing. Normal mice receiving vehicle or V565 were sacrificed at 3h. Colon contents were analysed by ELISA and colon segments processed for immunostaining (Fig. 5).





- Based on immunostaining there was very little penetration of V565 into normal colonic mucosal tissue, but immuno-staining for V565 in colonic mucosal tissue was markedly increased in mice with DSS colitis.
- V565 can be seen throughout the sub-mucosa as early as 1 hour post-dosing (Fig. 5).
- V565 staining in the colon tissue was maximal at 3-5h, corresponding to the peak concentrations of V565 in the lumen of the colon.
- V565 does not bind to murine TNFα and tissue staining decreased in parallel with luminal concentrations of the VHH, consistent with murine intestinal transit time

Figure 5: Colon segments were fixed and stained with DAPI to visualise cell architecture, and an anti-VHH antibody to visualise V565.

Conclusions

- V565 is highly resistant to proteases found in the lumen and mucosa of the gut.
- Recovery of high concentrations of V565 in the colons of normal and colitis mice following oral administration demonstrates the feasibility of delivering active Vorabody to the site of inflammation.
- Results showing that V565 can penetrate into inflamed colonic mucosal tissue from the gut lumen provides *in vivo* confirmation that orally delivered V565 can reach and potentially neutralise TNFα activity in sub-mucosal tissues.

References

Biancheri *et al* (2015) Proteolytic Cleavage and Loss of Function of Biologic Agents That Neutralize Tumor Necrosis Factor in the Mucosa of Patients With Inflammatory Bowel Disease. Gastroenterology 149:1564–1574.