Targeted topical anti-α4β7 integrin antibody results in reduced accumulation of α4β7 memory T-cells in gut tissue in DSS-induced colitis mice

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Introduction

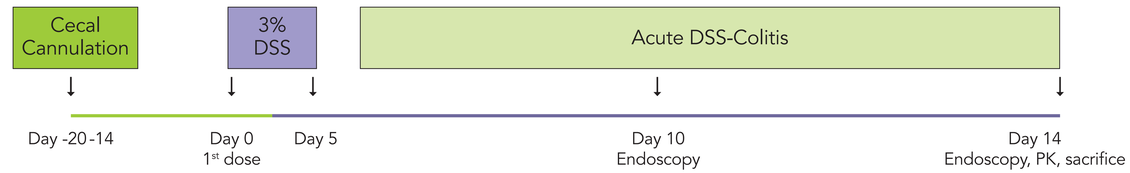
Systemically administered anti-α4β7 integrin antibodies such as Vedolizumab have been approved for the treatment of Crohn's disease (CD) and ulcerative colitis (UC). The objective of this study was to assess whether intracecally (IC) delivered anti-mouse α4β7 integrin antibody (DATK32) might penetrate disrupted mucosa and confer efficacy when compared with systemic intraperitoneal (IP) injection in DSS-induced colitis mice.

Methodology

Prior to the experiment (14-20 days), animals in IC treatment groups underwent surgical implantation of a cecal cannula for ease of bolus delivery to the cecum. Mice were treated with anti-mouse α4β7 integrin antibody (DATK32) during the acute phase of colitis. The test article was dosed at a volume of 0.1mL/20g days 0 to 14. DATK32 was administered IP (25 mg/kg) every 3 days (Q3D), and IC (25 mg/kg) Q3D or every day (QD). A lower dose (5 mg/kg) was also given IC QD.

At termination, mice were euthanized by CO2 inhalation immediately following endoscopy on day 14. Blood was collected via cardiac puncture into K2-EDTA-coated tubes and centrifuged at 4000xg for 10 min. Peyer's Patches (PP), mesenteric Lymph Nodes (mLN), and 100µL of whole blood were collected from all animals and processed for FACS analysis of α4 and β7 expression on memory helper T-cells. Tissue and blood were immediately placed in FACS Buffer (1x PBS containing 2.5 FCS) and analyzed.

Figure 1. Study Design



Results

PHARMACOKINETICS

- IC administration of anti-α4β7 integrin antibody (DATK32) resulted in a significantly lower mean drug concentration in plasma as compared to IP administration (**Figure 2**).
- IC administration of DATK32 resulted in a significantly higher mean drug concentrations in both colon contents and colon tissues as compared to IP (**Figure 3**).
- Drug levels remained elevated, above levels observed in systemic circulation, in colon contents and tissues for up to 48h after dosing where values were significantly elevated for up to 8h and 24h respectively (**Figure 4**).

DRUG DISPOSITION – IMMUNOHISTOCHEMISTRY

- The intensity and extent of labeling were generally higher in the proximal portion of colon and intensity DATK32-treated animals (**Figure 5**).
- The location of staining was most prominent at the luminal surface but was measurable even as deep as the tunica muscularis (**Figure 6**).

PHARMACODYNAMICS – T-CELL TRAFFICKING

- The mean number of α4β7 memory T-cells were significantly reduced in mLN in all groups receiving IC anti-α4β7 integrin antibody over vehicle control and animals treated with the drug systemically (**Figure 7**).
- Similar results were seen in Payer's Patches (PP) with QD but not Q3D high dose groups and the difference reached significance over both vehicle and systemically treated animals (**Figure 7**).
- The mean number of α4β7 memory T-cells were significantly increased in blood of groups receiving IC delivery over both vehicle control and systemically treated animals and reached significance for all groups except for Q3D dosing (**Figure 7**).

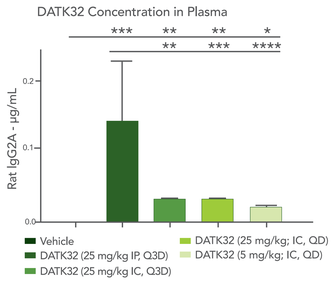
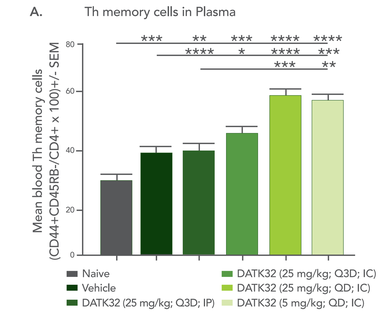
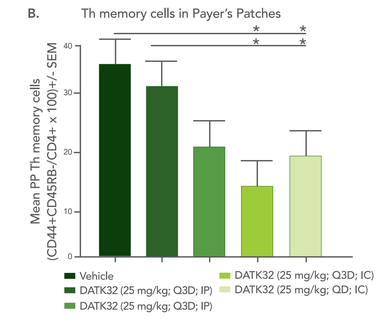


Figure 2. Shows concentration of DATK32 (µg/mL) in plasma of IP (25mg/kg) and IC (25 mg/kg and 5 mg/kg) treatment groups, given daily (QD) or every third day (Q3D). Pair-wise comparisons by two-tailed Mann-Whitney U-Test was done for the comparison of treatment effects; p<0.05*; p<0.01** and p<0.001***.





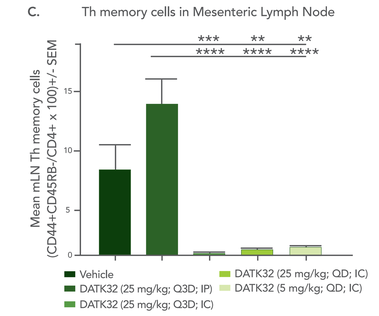


Figure 7. Shows mean number of Th memory cells (mean ± SEM) measured by FACS analysis, for IP (25mg/kg) and IC (25 mg/kg and 5 mg/kg) treatment groups given daily (QD) or every third day (Q3D) when compared to vehicle control (Vehicle) and when IP is compared to IC. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; p<0.05*; p<0.01** and p<0.001***.

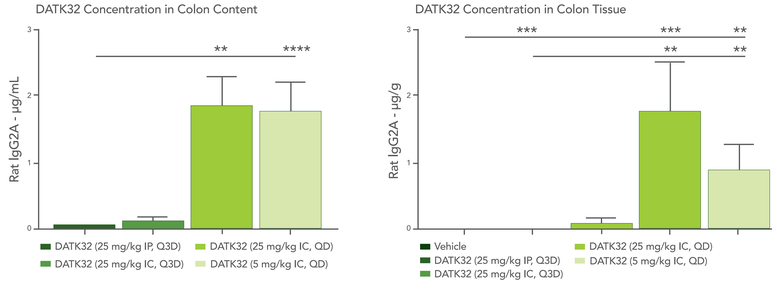


Figure 3. Shows mean concentrations of DATK32 (µg/mL) in colon contents (left) and colon tissues (right) of IP (25mg/kg) and IC (25 mg/kg and 5 mg/kg) treatment groups given daily (QD) or every third day (Q3D) where IP is compared to IC. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; p<0.05*; p<0.01** and p<0.001***.

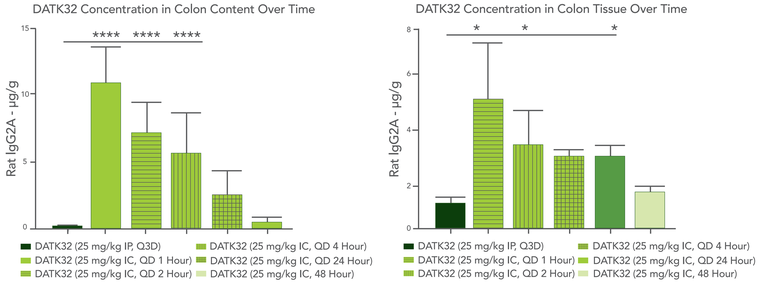
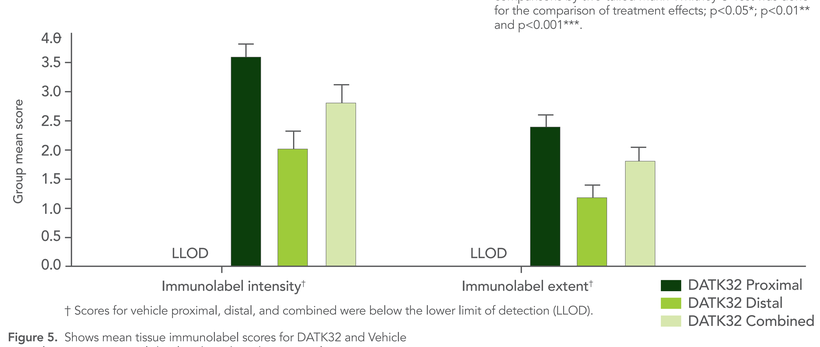
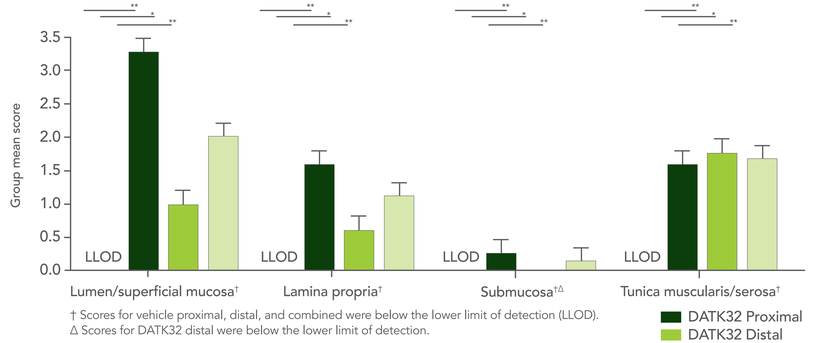


Figure 4. Concentration of DATK32 (µg/mL) in colon contents (left) and colon tissue (right) of IP (25mg/kg) and IC (25 mg/kg) treatment groups given daily (QD) over time (1, 2 ,4, 24, and 48 hours) where IP is compared to IC. Pair-wise comparisons by two-tailed Mann-Whitney U-Test for treatment effects; p<0.05*; p<0.01** and p<0.001***.



† Scores for vehicle proximal, distal, and combined were below the lower limit of detection (LLOD).

Figure 5. Shows mean tissue immunolabel scores for DATK32 and Vehicle control groups in proximal distal and combined anatomical compartments.



† Scores for vehicle proximal, distal, and combined were below the lower limit of detection (LLOD).
Δ Scores for DATK32 distal were below the lower limit of detection.

Figure 6. Shows mean location-specific tissue immunolabel scores for DATK32 and Vehicle control groups. Pair-wise comparisons by wo-tailed Mann-Whitney U-Test for treatment effects; p<0.05*; p<0.01** and p<0.001***.

Conclusions

- Targeted IC DATK32 treatment lead to significantly higher drug exposure in colon contents and tissues with limited blood exposure when compared with IP in an acute colitis model.
- Targeted IC DATK32 treatment showed a significantly reduced number of α4β7 memory T-cells in PP and mLN each representing populations within inflamed jejunal and colon tissues in an acute colitis model.
- An increased number of α4β7 memory T-cells in blood following IC, relative to IP, DATK32.

These data suggest that cells trafficked from blood to tissue can undergo reverse trafficking. Recent publications have reported a dose dependent increase in efficacy with systemically delivered anti-α4β7 integrin antibody despite full T-cell receptor occupancy in blood.

Our results point to the potential for increased efficacy with high concentrations in local inflamed tissues. Although the mechanism of action is unclear, this study suggests topically delivered anti-α4β7 integrin antibody may be an efficacious treatment in IBD.

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