Oral delivery of a new class of non-antibody protein scaffold Nanofitins targeting TNF-α shows a strong preventive and curative anti-inflammatory effect in models of IBD

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ABSTRACT

Introduction

Despite a remarkable efficacy, treatment of inflammatory bowel diseases (IBD) using systemic administration of anti-TNF-alpha antibodies remains associated with serious adverse effects. Oral administration of such therapeutics would benefit from a better targeting to the site of inflammation in the gut while decreasing their systemic exposure and related side effects. To this aim, the SADEL FP7 European project has been developing oral formulation of anti-TNF-alpha Nanofitins (NF), a novel alternative scaffold derived from the sac7d protein found in an extremophilic archeabacterium and stable enough to survive the hostile environment of the gut.

Methods

Screening of anti-TNF-alpha NF hits was done in vitro using surface plasmon resonance and in vivo by evaluating their anti-inflammatory effects in preventive mode after intrarectal instillation (10 mg/kg) in TNBS-induced model of colitis (C57Bl6 mice), using 5-ASA at optimal dosage (30 mM) for comparison. NFs providing reduction of inflammation similar or superior to 5-ASA were engaged in a dose-range finding study and further confirmed at the optimal dose in DSS-induced model of colitis (Balb/C mice). Oral efficacy was performed in TNBS-induced model of colitis by oral gavage of the NF leads, in preventive mode with a dose escalation (10, 100 and 400 mg/kg) and/or in curative mode at a single dose (100 mg/kg).

Results

3 out of the 9 anti-TNF-alpha NFs screened at 10 mg/kg by intrarectal instillation in TNBS-induced model of colitis have demonstrated remarkable anti-inflammatory effects, with the lead NF candidate decreasing 30 % (p = 0.0008) of the lesions (5-ASA, 35 %) and 62 % of TNF-alpha expression (5-ASA, 46 %). The preventive effect of the NFs appeared to be directly correlated with their respective binding characteristics to TNF-alpha. The therapeutic efficacy of these 3 NFs in TNBS model has been improved again at the optimal dosage of 100 mg/kg, and has been further confirmed in DSS-induced model of colitis. Their anti-inflammatory efficacy was fully retained upon oral delivery without the need for a specific formulation as for the lead NF at 100 mg/kg decreasing 65 % (preventive, p < 0.00001) or 37 % (curative, p = 0.047) of intestinal inflammation.

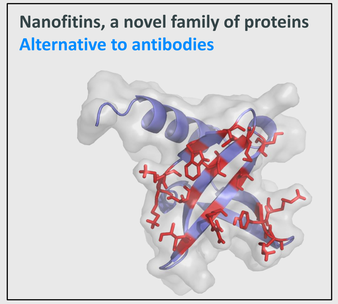
Conclusion

The extreme stability of the NF scaffold allowed the generation of anti-TNF-alpha therapeutics with a powerful preventive and curative anti-inflammatory action after oral administration. The use of the oral route is expected to prevent from systemic-related side effects; making anti-TNF-alpha NFs a promising new avenue for the treatment of IBD. The drug development of the lead NFs is pursued in collaboration with a pharmaceutical partner within the SADEL project.

Introduction

Anti-TNFα antibodies remains the most efficient treatment for IBD patients. However, there are some side effects induced by the injection of these expensive antibodies such as reaction to the injection, systemic side effects (infection, immunogenicity, …

Nanofitins (Nfs) derive from bacterial proteins from Sac7d family, natural DNA binders to protect the genome from fusion:
-Single chain, small protein (7kDa)
-No disulfide bond
-With extreme resistance to pH (0-12) and temperature (>70°C)
-Highly resistant to human intestinal fluids
-Long half-life in digestive track
-With specific high binding to target
-Simple and cost-effective manufacturing by *E.coli* fermentation / chemical synthesis



Aim: To develop anti-TNFα compounds orally delivered without side effects.

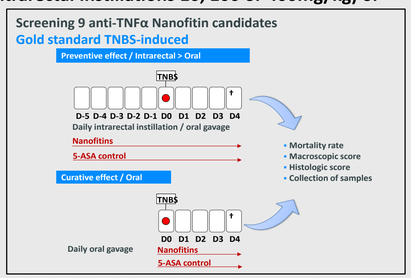
Methods

Compound and route of administration

9 NFs were first administered locally by intrarectal instillations 10, 100 or 400mg/kg) or by oral gavage (100mg/kg)
5-ASA (30mM) by intrrectal instillations

Preventive treatment mode:
5 days before the colitis induction until euthanasia

Curative treatment mode:
From the day of colitis induction (d0) Until euthanasia (d4)



Model of colitis induced by TNBS

Nfs were tested in the Gold standard model of colitis induced by an intrarectal injection of TNBS (150mg/kg) in C57bl6 mice. Intensity of inflammation was evaluated at macroscopic and histological levels using the multiparametric validated scores of Wallace and Ameho, respectively.

Results

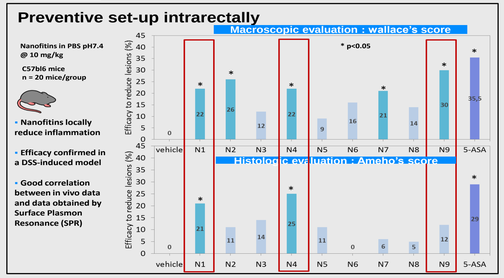


Figure 1. 9 Nfs were screened for their preventive anti-inflammatory properties at macroscopic and histological levels after a topic administration by intrarectal instillation. 3 Nfs were selected for their anti-inflammatory properties similar to those induced by 5-ASA used at its optimal dosage

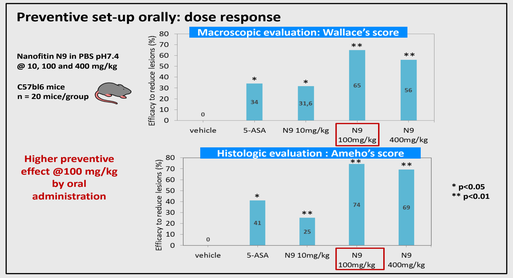


Figure 2. A dose effect for N9 (10, 100 & 400mg/kg) was performed to determine the optimal dosage of topic administration of the Nf in the model of colitis induced by TNBS. N9 at 100mg/kg exerts higher anti-inflammatory effects compared to 5-ASA used at its optimal dosage (30mM)

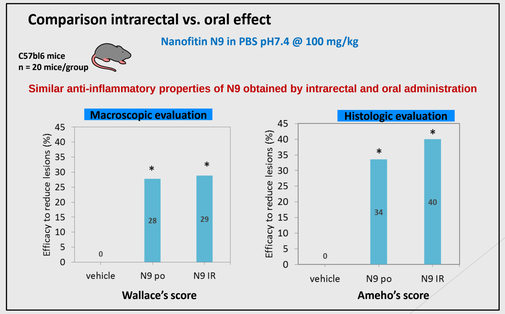


Figure 3. Efficacy of preventive oral administration vs intrarectal instillation was tested in the same model of colitis induced by TNBS using the optimal dosage (100mg/kg). Oral administration exerts similar anti-inflammatory properties to topic administration of the Nfs.

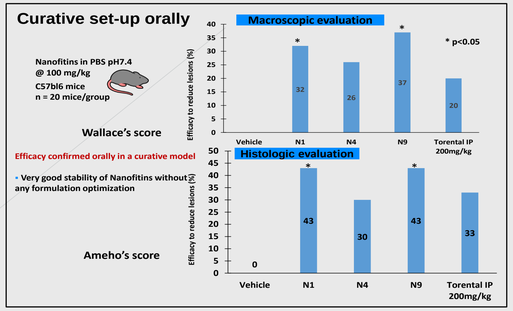


Figure 4. Efficacy of the 3 selected Nfs in a curative treatment and oral administration was evaluated in the model of colitis induced by TNBS using the optimal dosage (100mg/kg). We observed significant anti-inflammatory effects of curative administration of N1 & N9 at macroscopic and histological levels wit a higher efficacy compared to Torental administered by IP at 200mg/kg.

Conclusion & perspectives

→ Extreme stability of the Nanofitin scaffold allowed the generation of anti-TNF-α therapeutics with a powerful preventive and curative anti-inflammatory action after oral administration in mice.
→Drug development of the lead Nanofitin is pursued in collaboration with Ferring Pharmaceuticals within the SADEL FP7 European project.

SADEL is a project supported by the European Commission through the Seventh Framework Programme (FP7)