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In Vitro and *in Vivo* Study of IP-1510, a Novel Interleukin-1 Receptor Antagonist, in the Management of Intestinal Inflammation

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Introduction:

Elevated levels of interleukin-1 (IL-1) and reduced levels of its natural antagonist IL-1ra have been observed in patients with inflammatory bowel disease [1, 2] . Polymorphisms of the genes for IL-1 and IL-1ra have been associated with the appearance of ulcerative colitis and resistance to treatment with steroids [3, 4]. Human recombinant IL-1ra (Anakinra, Kineret) is already used to treat rheumatoid arthritis. The IP-1510 is a new synthetic peptide receptor antagonist of interleukin-1 (IL-1) which inhibits the intracellular transport of the signal (Figure 1). Phase I/II studies of this peptide in the management of patients with advanced neoplastic disease and cancer-related cachexia have shown that it was well tolerated and safe in advanced cancer patients and it induced statistically significant improvement in anorexia, physical performance, and depression of patients with cancer-related cachexia [5].

Aim of the study:

The objective of this study was to determine *in vitro* the effect of IP-1510 on cell biology of the bowel mucosa during the intestinal inflammation and *in vivo* its effectiveness for prevention/treatment of colitis in a model of chemically induced murine colitis (DSS).

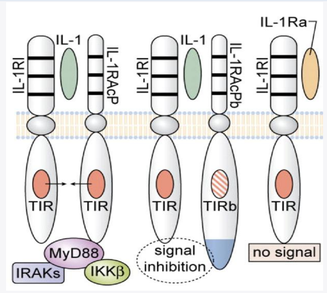


Figure 1: Representation of IL-1α/β receptor and its antagonist (IL-1ra)

Materials and Methods:

The human colonic epithelial carcinoma cell line HT-29, obtained from the European Collection of Animal Cell Cultures (ECACC) was used in *in vitro* experiments. Cells were cultured at 37°C and 5% CO2 as previously described [6]. Growth-arrested cells were stimulated with vehicle controls or 10 ng/ml of IL-1α added alone or in combination with 300U/ml of IFN-γ in the presence or not of various concentrations of IL-1ra (IP-1510L and IP-1510D). In addition, IL-1β added in the presence or not of various concentrations of IL-1ra (IP-1510L and IP-1510D). IL-8 and IL-17 production was measured using commercial available ELISA (Duoset® ELISA Development System, R&D SYSTEMS, UK). C57BL/6 female mice, six to eight week old, were purchased from the Institute of Molecular Biology and Biotechnology (IMBB) (Heraklion, Greece). All mice were housed at IMBB animal facility under specific pathogen free conditions. Colitis was induced by 3,5% (w/v) Dextran Sodium Sulphate (DSS) (molecular weight 35,000-50,000; MP Biomedicals, Solon, OH, United States) dissolved in autoclaved drinking water for 5 days as previously described [7]. 2.5μg IP-1510 (L or D) ή 0.4mg Anakinra were administered intraperitoneally (i.p.) to the treated group of mice every 48 or 24 hours (Figures). An age-sex matched group of mice received the vehicle (10% bicarbonate and 0.1% benzyl alcohol in water) at the same volume and time. Body weight was assessed to all groups throughout the experiment. Large bowel length and microscopic colitis were determined after euthanatizing mice.

Results (*in vitro*)

Figure 2

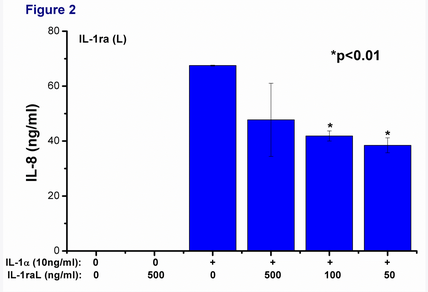


Figure 3

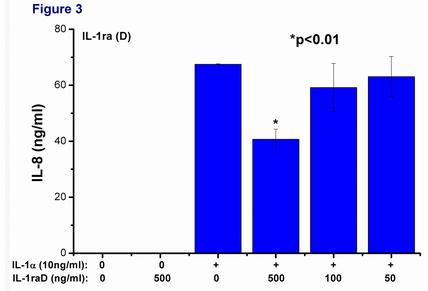


Figure 4

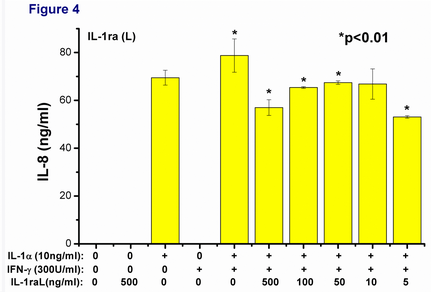


Figure 5

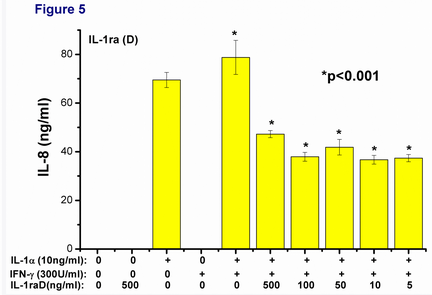


Figure 6

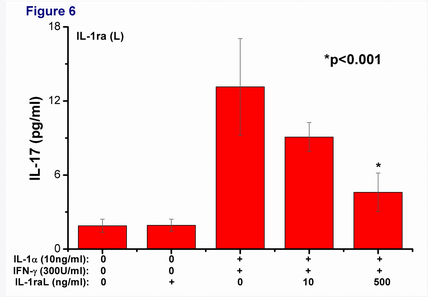


Figure 7

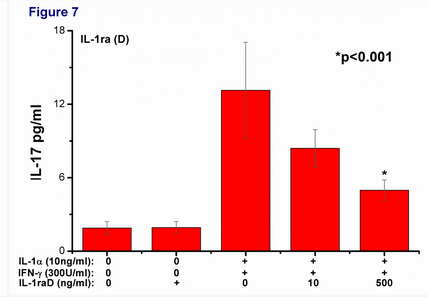


Figure 8

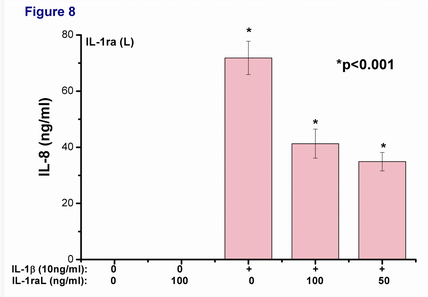


Figure 9

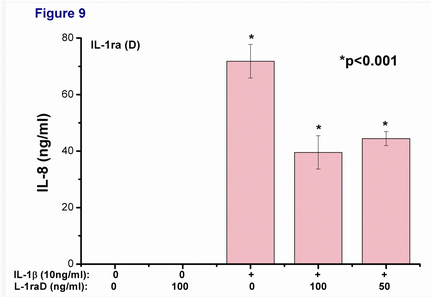


Figure 2 and 3: Both isomers of IP-1510 significantly (p<0.01) inhibited the IL-1α-induced IL-8 production by HT-29 colonic epithelial cells.

Figure 4 and 5: IP-1510 (D) significantly (p<0.001) inhibited the IL-1α+IFNγ-induced IL-8 production by HT-29 colonic epithelial cells at all concentration tested], while IP-1510 (L) demonstrated a lower inhibition (p<0.01).

Figure 6 and 7: Both isomers of IP-1510 significantly (p<0.001) inhibited the IL-1α+IFNγ-induced IL-17 production by HT-29 colonic epithelial cells.

Figure 8 and 9: Both isomers of IP-1510 significantly (p<0.001) inhibited the IL-1 β-induced IL-8 production by HT-29 colonic epithelial cells.

Both isomers of IP-1510 demonstrated a differential inhibition pattern on cytokine production by HT-29 cells.

The inhibition of IL-8 and IL-17 production by HT-29 colonic epithelial cells indicates that intestinal epithelial cells might be a potential cellular target of the anti-inflammatory action of IP-1510.

Results (*in vivo*)

Figure 10

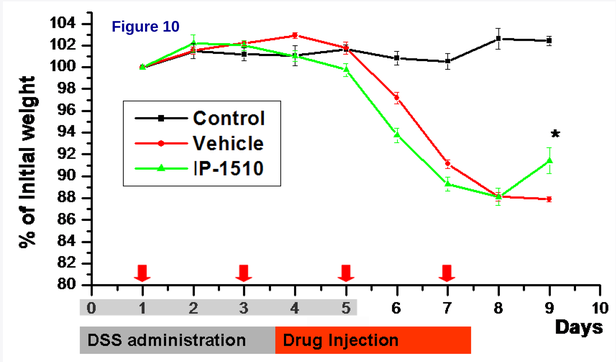


Figure 11

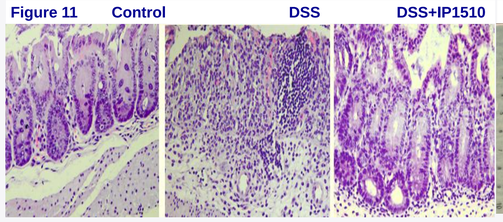


Figure 12 Large Bowel Length

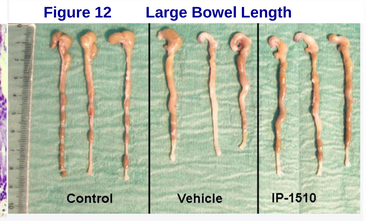


Figure 13

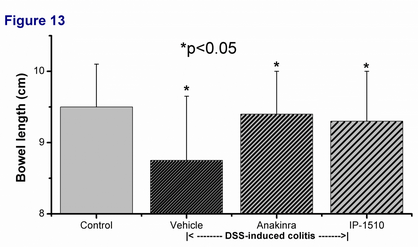
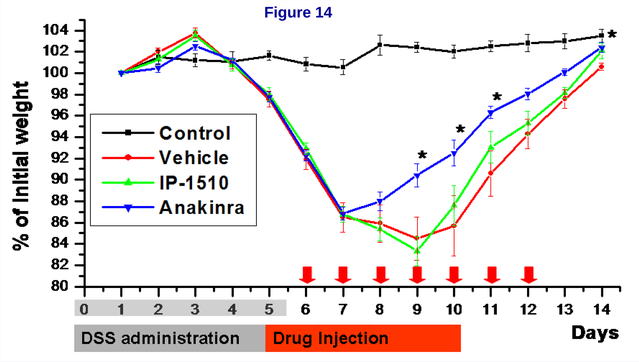


Figure 14



Figures 10, 11 and 12: The effect of IP-1510 on DSS-induced colitis i) Weight assessment ii) Hematoxylin eosin staining of proximal colon iii) Large bowel length assessment.
Figure 13 and 14. Comparison of IP-1510 with Anakinra in DSS colitis a) Study of bowel length, b) Study of body weight.

Conclusions:

- **These preliminary results from the effect of IP-1510 and Anakinra on the prevention and treatment of DSS colitis in mice suggest that the antagonists of IL-1 require further study on their possible therapeutic effect on IBD.**
- **Our data from the *in vitro* and *in vivo* study of IP-1510 demonstrate a possible beneficial effect of this peptide in intestinal inflammation.**

References:

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3. Tountas *et al.* ***Gastroenterology*** 1999; 117:806-813.
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7. Zhong *et al*. ***Inflamm Bowel Dis***. 2006; 12:790-796.