An Investigation into the Potential Use of Chitin Derivatives in the Therapy of Inflammatory Bowel Diseases
H. D. Jhundoo\textsuperscript{1}, C. C. Larsen\textsuperscript{2}, C. Schmidt\textsuperscript{3}, A. Lamprecht\textsuperscript{1}
\textsuperscript{1}University Bonn, \textsuperscript{2}Ferring Pharmaceuticals Inc., \textsuperscript{3}Ferring Pharmaceuticals A/S

**Purpose**
Inflammatory bowel diseases (IBD) are chronic and disabling disorders of the gastrointestinal tract. The use of natural polymers in the treatment of a number of conditions and drug delivery is appealing as these are known to be biocompatible, biodegradable, stable and non-toxic. However, the potential use of chitin derivatives in IBD has not been established. The aim of this study was to investigate the potential therapeutic effect of the approved pharmaceutical excipient, chitosan, a chitin derivative on LPS-stimulated RAW 264.7 macrophage cells and on the extent and severity of IBD in a mouse colitis model.

**Methods**
Both untreated and LPS-activated RAW 264.7 macrophage cells were incubated with increasing concentrations of chitosan for 24 hours prior to the evaluation of the cell viability using the MTT assay. The concentration of TNF-\(\alpha\), IL-6 and IL-1\(\beta\) in the supernatant was determined using a commercial ELISA kit. 2,4,6-trinitrobenzenesulfonic acid (TNBS) was used to induce colitis in 6-weeks old Swiss mice at a dose of 90 mg/kg. The mice were treated with 30 mg/kg of chitosan administered intracolonically for three consecutive days prior to the assessment of the inflammatory response. The levels of myeloperoxidase (MPO) and alkaline phosphatase (ALP) activity, TNF-\(\alpha\), IL-6 and IL-1\(\beta\) were determined from the colonic tissue. The control group received saline solution after induction of colitis with TNBS.

**Results**
The viability of RAW 264.7 cells was not significantly reduced up to a concentration of 1000 \(\mu\)g/ml chitosan, which confirms that chitosan is not toxic in vitro. A significant decrease in TNF-\(\alpha\) levels was observed after incubation of LPS-activated RAW 264.7 cells with 10-600 \(\mu\)g/ml chitosan as depicted in Figure 1. However, an increase in the secretion of TNF-\(\alpha\) was noted after incubation of LPS-stimulated RAW 264.7 cells with higher concentrations of chitosan. The secretion of IL-6 and IL-1\(\beta\) did not change significantly after incubation of LPS-activated RAW 264.7 cells with 10-2000 \(\mu\)g/ml chitosan. Intracolonic treatment of colitis with chitosan at a dose of 30 mg/kg for 3 days led to a significant decrease in colonic MPO activity, TNF-\(\alpha\), IL-6 and IL-1\(\beta\) and ALP in colitis mice compared to untreated animals. Furthermore, chitosan attenuated the extent of macroscopic colonic damage induced by TNBS.

**Conclusion**
Chitosan may demonstrate potential anti-inflammatory effects via the stimulus of TNF-\(\alpha\) pathway in RAW 264.7 cells. Furthermore, chitosan may be effective in the treatment of IBD as it effectively suppressed the inflammatory response in a murine colitis model.

![Figure 1. TNF-\(\alpha\) levels after incubation of RAW 264.7 cells with increasing concentrations of chitosan for 24 h](image1.png)

![Figure 2. MPO activity after intracolonic treatment with chitosan for 3 consecutive days](image2.png)