# Monitoring the Inflammatory Process in the Colon of Carrageenan-fed Rats by 1-H MRS

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## Synopsis

In the investigation of the increased risk of colon cancer associated with inflammatory bowel disease, rodent models are becoming increasingly utilized. Among various agents which induce inflammation in the colon of animals, is the degraded form of Carrageenan. Proton magnetic resonance spectroscopy was used herein to assess the inflammatory process in the colon of rats fed with a diet containing Carrageenan. Our results show the presence of significantly elevated ($p < 0.05$) levels of choline and taurine, and a significant reduction ($p < 0.05$) in the methylene to methyl spectral resonance intensity ratio in the Carrageenan-fed rats.

## Introduction

Rodent models are increasingly utilized to investigate management and treatment of inflammatory bowel disease and reduction in the heightened risk of colon cancer. Among various agents which induce inflammation in the colons of animals, is the degraded form of Carrageenan. Degraded Carrageenan is highly toxic and also carcinogenic. Recently, undegraded Carrageenan has been found to increase the risk of colon cancer and induce a number of cytokines and enzymes known to be involved in inflammation. Undegraded Carrageenan is non-carcinogenic and when administered in the diet of the animals induce sustained inflammation without bleeding and ulceration. Therefore, undegraded Carrageenan may serve to be a superior agent to use to induce inflammatory responses in a long term animal studies. Recent advances in MRS technology to assess cellular changes in a small tissue sample may provide a sensitive approach to investigate the presence or absence of inflammation. The main objective of the present study was to determine if 2 or 4% Carrageenan in the diet would elicit responses in the colon within a short time.

## Materials and Methods

Eleven male Sprague Dawley rats were fed a semi-synthetic diet with or without Carrageenan (2% or 4% by weight) for four days. Colonic mucosal samples (6 from control, and 15 from Carrageenan fed rats) were scraped and kept frozen at -70 $^0$C. Specimens were mounted in small capillary tubes, and subjected to $^1$H MRS (360 MHz) at 25 $^0$C with presaturation of the water signal. Acquisition parameters included: 90$^0$ pulse of 8 μs, number of scans = 256, spectral width = 5000 Hz, recycle delay = 2.41s, and time domain data points = 4K. A solution of para-amino benzoic acid (PABA) in $D_2O$-PBS placed in the NMR tube was used as a reference (6.81 ppm). Immediately following the MRS experiments, all samples were fixed in formalin for histopathological assessment. The resonance areas (intensities) were determined for the following ten spectral regions using standard Bruker integration routines: 4.39 – 4.18, 4.18 – 4.01, 3.46–3.36, 3.36-3.14, 3.14-2.88, 2.88-2.48, 1.79-1.55, 1.55-1.11, and 1.11-0.71 ppm. The ratios of these values for the two groups were determined and their means compared using the Student t-test ($p < 0.05$; EXCEL 4.0).

## Results and Discussion

Figure 1 shows representative spectra from a control rat colon, and one that had 4% Carrageenan in the diet. As can be seen in the figure, there are marked differences between the two MR spectra. The spectrum in A looks very similar to those from biopsies obtained from patients with inflammatory bowel disease (1). The mean levels of choline-containing metabolites (3.2 ppm), and taurine (3.4 ppm) were found to be significantly elevated ($p < 0.05$) in the study group. The presence of elevated choline, associated with inflammation, is consistent with other findings in the literature (2). Taurine is believed to play a role in the modulation of inflammatory processes (3) and hence, its presence at elevated levels is consistent with that role. Interestingly, the intensity ratio of the methylene ($-CH_2-$) resonance to methyl ($-CH_3$) resonance was also significantly higher in the control rats. The underlying mechanism leading to these changes remains elusive. Although some rats in the study group were fed with 2% Carrageenan and some with 4%, no statistically significant spectral differences were observed between the two groups, suggesting that the 2% level might be sufficient to induce significant inflammation in the colon.

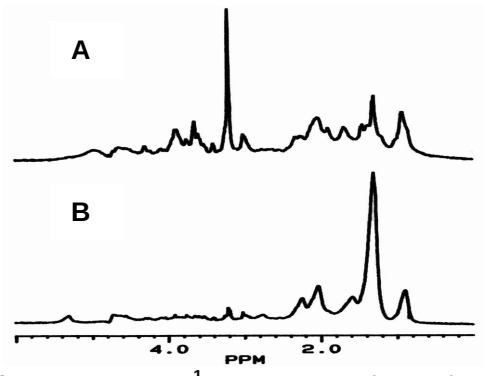


**Figure 1**. 360 MHz $^1$H MR spectra of rat colon: (A) Carrageenan-fed, and (B) Control

## Conclusion

Our results show the presence of significantly elevated ($p < 0.05$) levels of choline and taurine, and a significant reduction ($p < 0.05$) in the methylene to methyl spectral resonance intensity ratio in the Carrageenan-fed rats. This finding suggests that MRS could serve as a tool in the study of inflammation in the animal model, and modulation of inflammatory responses by therapeutic agents.

## References

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