SUMMARY OF PhD THESIS

Morphofunctional evaluation of mucin-producing cells in acute intestinal inflammation

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INTRODUCTION

The mammalian gastrointestinal tract is a complex system in which the interaction of different cell types and molecules plays a crucial role in maintaining intestinal homeostasis. Among these cell types, those involved in the synthesis and secretion of mucins, especially goblet cells, are essential for protecting the intestinal mucosa.

Inflammation at the intestinal level can lead to increased epithelial permeability, damage to the mucus barrier and changes in the composition of the intestinal microbiota, thus exacerbating the imbalance that has arisen in the body. At the same time, inflammation at the level of the epithelium of the digestive tract organs can cause a reduction in the number of cells involved in the synthesis of mucins, can influence their ability to produce the respective glycoproteins, or can change the type of secretion of the cells and implicitly the ratios between the different types of mucins.

On a large scale, there is talk of a potential therapeutic approach to support intestinal health, namely the use of prebiotics, such as inulin.

The interaction between mucin-producing cells, intestinal inflammation, and prebiotics offers a promising area for research aimed at managing and treating gastrointestinal disorders. *In vivo* experiments in laboratory animals provide essential information in this direction.

DOCTORAL THESIS STRUCTURE

The doctoral thesis entitled "*Morphofunctional evaluation of mucin-producing cells in acute intestinal inflammation*" is structured in two parts: Part I – Literature review and Part II - Personal contributions, comprising a total of 117 pages, 37 figures, 12 tables and 139 references.

PART I – Literarture review

The first part of the doctoral thesis is structured in 3 chapters and provides a synthesis of information on the protective barriers in the digestive system, summarizing aspects regarding the physical protective barriers in the stomach, small intestine and large intestine, as well as details about the secretory function of gastrointestinal epithelial cells. At the same time, the pathophysiological mechanisms involved in inflammatory processes, especially in the intestinal ones, are described. In this part of the thesis, references are made to the use and protective potential of inulin, arguments being made for its use in medicine but also in relation to its use in other fields.

PART II – Personal contributions

Part II is structured in 6 chapters and has a total of 63 pages. This part presents the working hypothesis and general objectives of the research, the materials and methods used, the results of the studies performed, the general conclusions, the recommendations formulated based on the results obtained, the originality and innovative contributions of the thesis.

WORKING HYPOTHESIS AND RESEARCH OBJECTIVES

Morphological and functional disorders of the digestive tract, both in humans and animals, represent a frequently encountered class of pathologies. Moreover, the presence of acute inflammation, not properly treated, significantly increases the risk of chronic diseases, both locally and systemically.

Experimental studies on animals, which consist of inducing intestinal inflammation, are crucial in understanding the multiple pathologies that occur at this level, and can subsequently be used in human medicine. In this way, research can be carried out, including in terms of new methods of approaching and treating these disorders.

Of particular interest is the manipulation and modeling of the microbiome, being one of the main elements that contribute to maintaining intestinal homeostasis. Thus, interest in research related to the impact of antibiotics, pro- and prebiotics, or even fecal transfer has increased significantly.

Considering these aspects, the working hypothesis of the present study was the analysis of mucin-producing cells in an acute inflammatory bowel disorder, as well as *in vivo* monitoring of the effects of inulin in this situation.

To achieve the main objective of the study, the following secondary objectives were taken into account:

1. Screening of mucin-producing structures in the digestive system of rats, for a better understanding of their morphology;

2. Establishing an experimental protocol for inducing acute intestinal inflammation, by using a chemical reagent and histological analysis of the lesions that occurred, as well as histochemical observation of the effects of inulin;

3. Induction of acute intestinal inflammation, as well as the concomitant administration of inulin, to observe potential improvements in pathological processes.

RESEARCH STUDIES

1. Histological and functional analysis of mucin-producing cells in the digestive tract in rats

Research activities of the study:

- ✓ Preparation of histological samples from the organs of the digestive tract where there are mucin-producing structures;
- ✓ Staining of smears by the Trichrome Goldner (TG) method;
- ✓ Morphometric analysis of mucin-producing cells.

Results of the study

Morphometric analysis of mucin-producing structures

Following the histological processing of the segments of the stomach, small intestine and large intestine, and their staining by the TG method, the characteristic elements of each organ were observed.

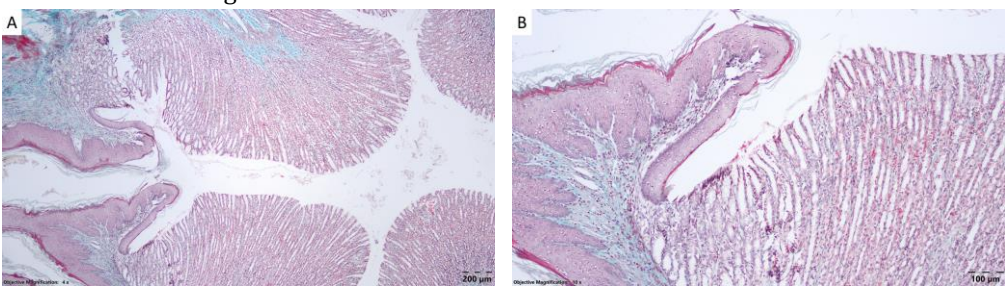


Fig. 1 Histological appearance of the gastric mucosa in Wistar rats, TG staining
A – overall appearance with 4X objective; B - more detailed appearance with 10X objective

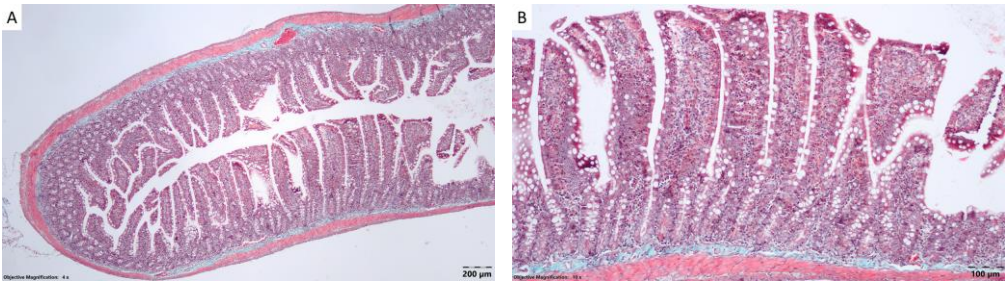


Fig. 2 Histological appearance of the jejunal mucosa in Wistar rats, TG staining
A – overall appearance with 4X objective; B - more detailed appearance with 10X objective

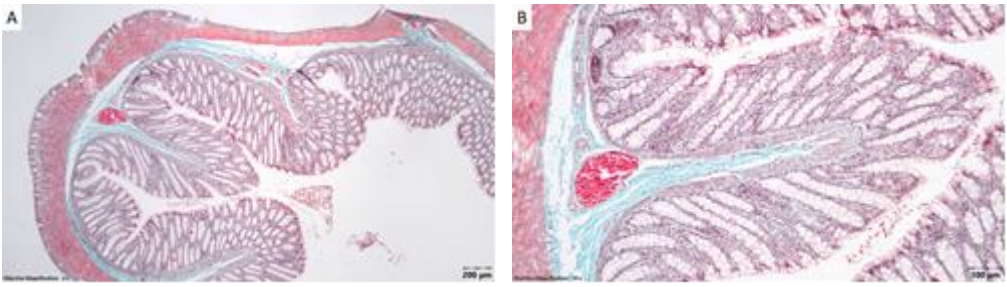


Fig. 3 Histological appearance of the colon mucosa in Wistar rats, TG staining
A – overall appearance with 4X objective; B - more detailed appearance with 10X objective

The results of the test for normal distribution of the data revealed that they are normally distributed, therefore no correction was applied to the data.

Comparative analysis of mucin-producing structures

Statistical comparison between cell surfaces was performed using One way ANOVA tests, which suggest a significant difference between means with $p<0.0001$, and Tukey's post hoc test.

Table 1

Statistical differences between the average areas (μm²) of mucin-producing cells (Tukey's post hoc test)

Anatomical structure	Mean (μm²) ± Standard deviation (μm²)
Stomach	373,5 ± 79,09 [b, c, d, e]
Goblet cells small intestine	618,4 ± 125,6 [a, c, e]
DCSc small intestine	780,3 ± 177,1 [a, b, e]
Goblet cells large intestine	765,5 ± 155,1 [a, e]
DCSc large intestine	1020 ± 217,3 [b, c, d]

Note: values represent the mean ± standard deviation for 19 measurements of mucin-producing cells. a–e Means marked with letters in the column indicate statistical differences $\leqslant 0.05$

2. Experimental model of induction of acute intestinal inflammation with DSS and quantification/histochemical evaluation of the effect of inulin.

Research activities of the study:

- ✓ Identification of morphological and morphometric changes in mucin-producing cells, from the digestive tract, in induced intestinal inflammation, in rats.
- ✓ Evaluation of changes induced by oral administration of inulin by quantifying goblet cells according to their type of secretion, based on histochemical preparations.

Study results

Morphological changes in mucin-producing cells

Inflammatory lesions of varying intensity were identified on histological slides obtained after sampling and processing of small intestine (jejunum) sections from the experimental group. These ranged from epithelial desquamation accompanied by cell apoptosis at the level of the surface epithelium, to coagulation necrosis with a shadowy appearance of the intestinal epithelium and abundant inflammatory cellular infiltrate.

Morphometric analysis of the small intestine indicated the presence of 21.78 goblet cells in 235721.97 μm² of crypt surface in the control group and 12.22 cells in 195643.76 μm² in the experimental group.

Histological analysis of the colon of the animals in the experimental group allowed the identification of certain inflammatory lesions such as epithelial

desquamation with a high level of cell apoptosis compared to the control group, discrete edema of the lamina propria and subepithelial spaces with multifocal denudation.

Following the morphometric analysis, a number of 46 goblet cells were observed on a surface of 735627.1 μm^2 in the rats in the control groups and a number of 16.04 cells on 40718.7 μm^2 in the animals that consumed 5% DSS solution.

Comparative analysis of mucin-producing structures: morphometric and histochemical quantification

The number of mixed-secretion goblet cells was lower compared to those with acid secretion, regardless of the segment analyzed, the amount of inulin in the administered solutions or the period of their administration, and the duration of the experimental intervention did not influence the number of cells, except for those producing acidic mucins in the large intestine, where there are statistically significant differences. When comparing mixed goblet cells in the small intestine at 28 days between groups, and acidic ones, also at 28 days, from both intestinal segments, the differences are statistically significant.

3. The effect of inulin in DSS-induced intestinal inflammation in rats.

Research activities of the study:

- ✓ Induction of acute intestinal inflammation and concomitant administration of prebiotic;
- ✓ Evaluation of weight gain and disease activity index in rats;
- ✓ Evaluation of microbiological examinations;
- ✓ Preparation of histological, histochemical and immunohistochemical preparations from small and large intestine segments;
- ✓ Histopathological analysis of tissues;
- ✓ Quantification of mucin-producing cells according to their type of secretion on histochemical preparations;
- ✓ Evaluation of the expression of the MUC2 marker at the level of the analyzed segments, by immunohistochemical techniques.

Study results

Evaluation of weight gain and disease activity index

The feed efficiency ratio values indicate that animals in the DSS groups lost weight, unlike rats in the inulin group and those in the control group who gained weight during the experiment.

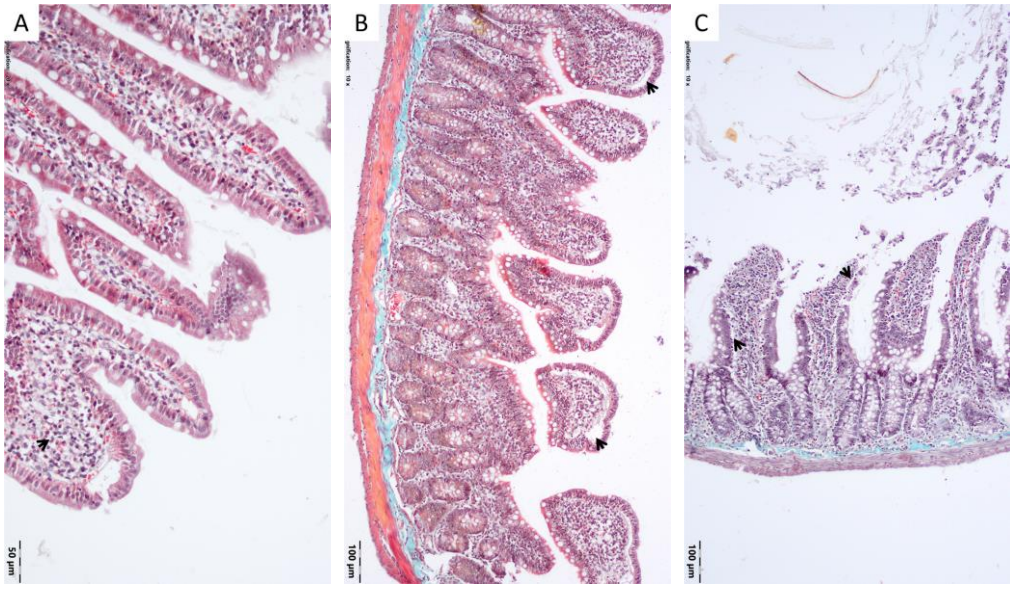
In the case of groups 3 (5% inulin solution) and 4 (control) the DAI score is 0, which denotes that rats in these groups did not show clinical signs of enterocolitis during the experiment. In rats that consumed the 5% DSS solutions (group 1 and group 2), the onset of symptoms was observed starting on day 3 of treatment, with their worsening, DAI scores at the end of treatment varying between 8 and 9.

Microbiological examination

Following the microbiological examination, it was observed that the dominant bacterial flora was represented by intestinal coliform bacteria, bacteria of the genus *Enterococcus*, and sporadically colonies of staphylococci were isolated. *Escherichia coli* had a prevalence of 100%, being identified in all experimental groups, quantitatively higher in the groups that consumed the DSS solutions, while bacteria of the genus *Enterococcus* and *Staphylococcus epidermidis* had a prevalence of 75%, missing in the animals belonging to the group that received only the 5% inulin solution.

Histopathological analysis

Following the histopathological examination of the small intestine from the experimental groups, inflammatory lesions of varying severity were observed (Fig. 4). The lesion score was 14 for the group with the 5% DSS solution, 13 for the group with the 5% inulin treatment, both having the moderate indicator, and the severe indicator for the group with the 5% DSS solution and 5% inulin, with a total score of 21. Inulin aggravated the intensity of inflammatory lesions when administered concomitantly with the DSS solution; administered alone, even if no clinical symptoms or weight loss of the animals were reported, inflammatory signs were observed histologically.



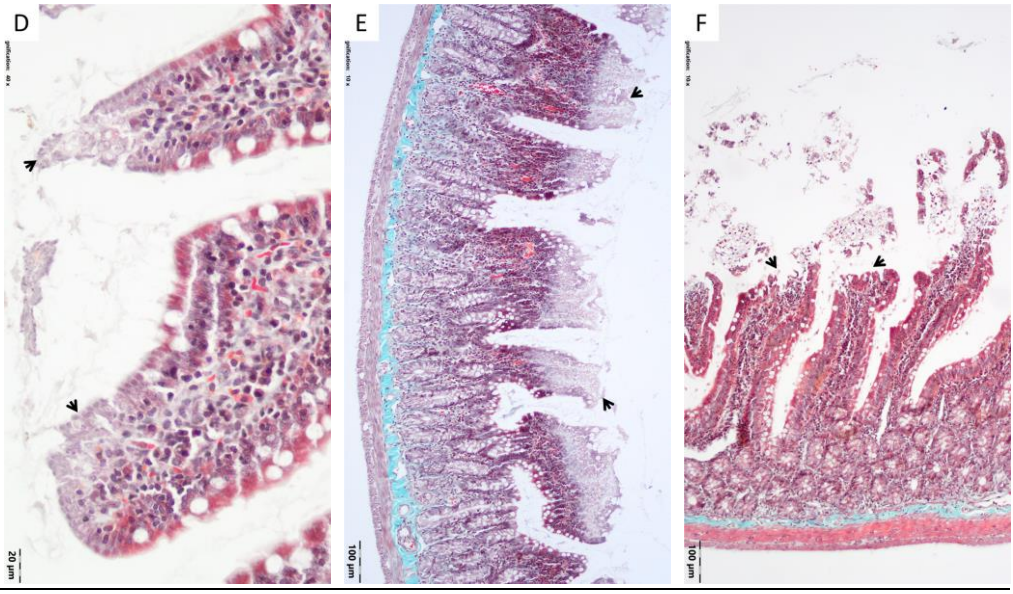


Fig. 4 Histopathological examination, jejunum, Wistar rats experimental groups, TG staining
A: congestion and edema in the lamina propria (ob. 20X); B: subepithelial spaces (ob. 10X); C: desquamative enteritis (ob. 10X); D: necrotic microfoci (ob. 40X); E: coagulation necrosis with shadow aspect (ob. 10X); F: necrosis of the surface epithelium and lamina propria, with shaved aspect of the villi (ob. 10x)

Histological analysis of the segments belonging to the large intestine did not reveal structural changes or the appearance of inflammatory lesions in any of the experimental groups, thus no differences appeared between the groups in the analysis of histochemical and immunohistochemical examinations.

Histochemical examination

During the histochemical examination, it was observed that the goblet cells in the small and large intestine have positive reactions to both the PAS method and the AB staining, in all experimental groups. This attests to the fact that this cell type synthesizes and secretes both neutral and acidic mucins. Following the PAS-AB histochemical staining of the small intestine samples from the experimental groups, the quantification of cells with a role in the secretion of neutral mucins and those with mixed mucin secretion was performed (Fig. 5)

The control group presented significantly more cells with mixed mucin secretion (34.8) and very few cells with neutral secretion (1.834), which differentiates it from the other groups. In the experimental groups, compared to the control group, an increase in both the number and percentage of cells with neutral mucin secretion was observed relative to a total surface area of 100,000 μm^2, especially in those with the administration of DSS solution.

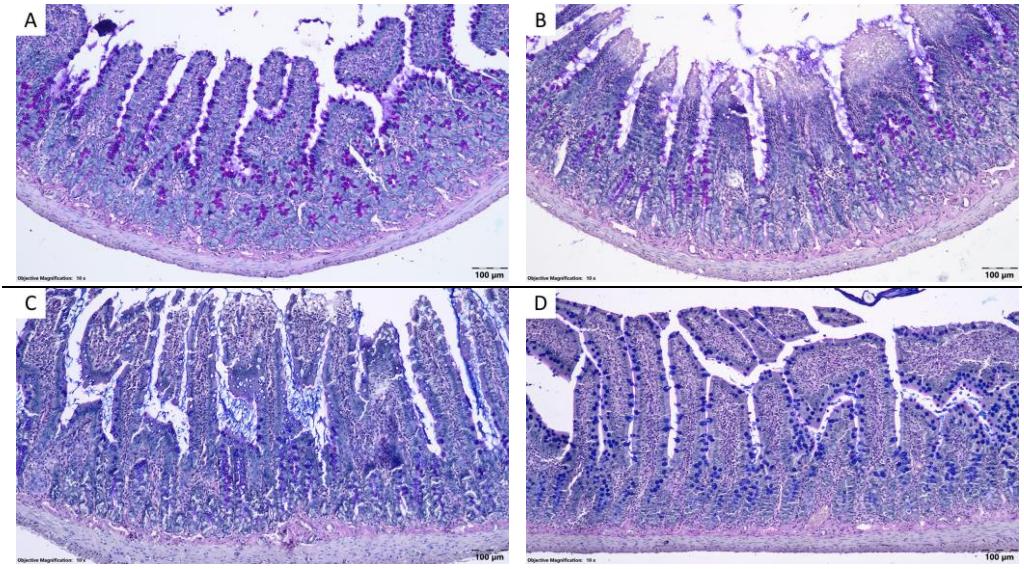


Fig. 5 PAS-AB staining, small intestine, Wistar rats, 10X objective
A: Lot 1 – DSS; B: Lot 2 – DSS + inulin; C: Lot 3 – inulin; D: Lot 4 – control

Immunohistochemical examination

From the point of view of the distribution of immunoreactivity among the three experimental groups, excluding the control group, we consider that the group with the administration of the 5% DSS solution has the highest proportion of cells with slightly positive " + " and intensely positive " ++ " immunoreactivity. In the case of the control group, the lack of cells with absent immunoreactivity " - " and a large number of cells with a " ++ " reaction is observed, which suggests a significant difference compared to the other groups. The groups with the administration of the 5% inulin solution (groups 2 and 3) have a more balanced distribution between the categories " - ", " + " and " ++ ", but with statistical differences between them. Analyzing the immunoreactivity comparatively, we observe that the control group has the lowest slightly positive immunoreactivity " + ", but the highest intensely positive immunoreactivity " ++ ", which may indicate the specific functional differentiation of the cells, by marking an increased expression of the MUC2 marker. In the groups treated with 5% DSS and 5% inulin solutions, a decrease in the expression of this marker was observed, which means a reduction in the synthesis of MUC2 mucin by specialized cells in the small intestine. According to the Bonferroni test, the group with the administration of the DSS solution and the control group differ significantly in the distribution of " - " and " ++ " cells.

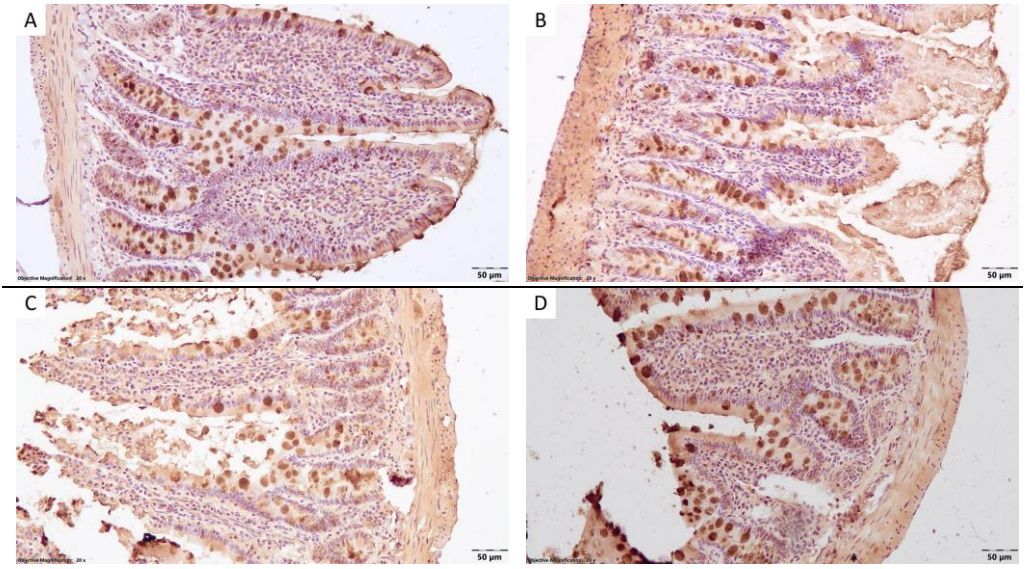


Fig. 6 Immunohistochemical reaction for MUC2, small intestine, Wistar rats, 20X objective
A: Lot 1 – DSS; B: Lot 2 – DSS + inulin; C: Lot 3 – inulin; D: Lot 4 – control

RECOMMENDATIONS

1. Avoiding the concomitant administration of inulin and DSS, as this may aggravate intestinal inflammation and epithelial damage.
2. Close monitoring of the intestinal microbiota in case of exposure to DSS, considering the quantitative increase of *Escherichia coli* and *Enterococcus* spp.
3. Further, long-term investigations on the effects of inulin, to determine the mechanisms by which it contributes to intestinal histological changes and to clarify the safety of its administration.
4. Use of intestinal epithelial protective therapies, to limit the toxic effects of DSS-induced acute intestinal inflammation on mucin-producing cells.
5. Correlating histological, histochemical and immunohistochemical changes with clinical parameters, for a better understanding of the functional effects on the body.
6. Evaluation of probiotic and/or prebiotic administration strategies, which would attenuate the microbial imbalances induced by DSS, respectively of acute intestinal inflammation.
7. Development of complex therapeutic protocols focused on protecting mucin-producing cells in inflammatory bowel diseases.
8. Extension of studies to other animal models or cell cultures to validate our conclusions.