Treatment with Apraglutide Preserves the Global Homeostatic Environment of Intestinal Microbiota During Chemotherapy in Mice

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INTRODUCTION

- Patients who undergo hematopoietic stem cell transplantation (HSCT) experience profoundly altered gut microbiota composition due to dysregulation of intestinal homeostasis by conditioning regimens, broad-spectrum antibiotics, immunosuppressants, and the introduction of foreign lymphocytes from the donor.[1]
- A growing body of evidence shows reduced microbiome diversity increases the incidence and severity of graft versus host disease (GvHD) and bacteremia.[1,2]
- Glucagon-like peptide-2 (GLP-2) is a peptide naturally secreted by L cells in the intestine to maintain gastrointestinal (GI) health and integrity.[3]
- Apraglutide, a novel long-acting synthetic GLP-2, has been shown to protect the GI epithelium structure from chemotherapy-induced injury, improve survival, and allowed better body weight maintenance in mice undergoing chemotherapy.
- In animal models of chemotherapy-induced intestinal mucosal injury, where the intestinal mucosa was severely injured by cytotoxic agents such as cytarabine, apraglutide promotes mucosal healing and preserves the physical integrity and barrier function of the small intestine.
- The objective of this study is to evaluate the protective effect of apraglutide on gut microbiota during chemotherapy with cytarabine.

METHODS

- Three treatment groups of Balb/c mice were included:
  A. Control: vehicle on Days 0-12
  B. Cytarabine-only: Cytarabine 30 mg/kg on Days 0-4
  C. Cytarabine + Apraglutide: Cytarabine 30 mg/kg on Days 0-4 with apraglutide 3.3 mg/kg on Days -4 to +12
- Fecal samples were collected over 24 hours for bacterial phenotyping at pre-treatment (Day 0) and the day before scheduled termination (Day 18) and for found dead or pre-terminally euthanized animals.
- Microbiota composition was determined by 16S taxonomical meta-sequencing.

Statistics:

- Statistical evaluation was performed using SAS® software version 9.3 (SAS Institute Inc., Cary, NC, USA) and XLSTAT software version 2014.6.01 (Addinsoft, France)
- All statistical tests were two-sided at the 5% level of significance.
- Analysis of variance for repeated measurements (repeated ANOVA) including treatment, day and treatment by day interaction as fixed factors in the statistical model was conducted. In this statistical model, post-hoc tests were conducted to:
  – Perform pairwise comparisons of treatment groups on Day 0 (pre-treatment) and on Day 18 (post-treatment), using tukey adjustment for multiple testing.
  – Compare pre- and post-treatment values in each treatment group.

RESULTS

Apraglutide pre-treatment and co-administration reduces cytarabine-induced changes in fecal bacterial composition

- *Firmicutes* (Gram-, anaerobic bacteria) and *Bacteroidetes* (Gram+, aerobic and anaerobic bacteria) were the two leading bacterial phyla identified in the healthy mice intestine.
- *Bacteroidetes* is composed by several families of opportunistic pathogenic bacteria populations.
- Meta-sequencing analysis of stools collected before and after treatment demonstrated that cytarabine had a large impact on the homeostatic environment of the intestine with significant changes in the composition of bacterial species.
- Statistically significant decrease of the Firmicutes population and increase of the Bacteroidetes population after cytarabine treatment resulted in a change of the ratio between *Firmicutes* and *Bacteroidetes*.
- When apraglutide was administered as pre-treatment and during conditioning chemotherapy with cytarabine, the ratio between the two fecal bacterial populations tended to normalize.
- Similar changes were observed at the family and genus levels.

Figure 1. Fecal Microbiota Composition

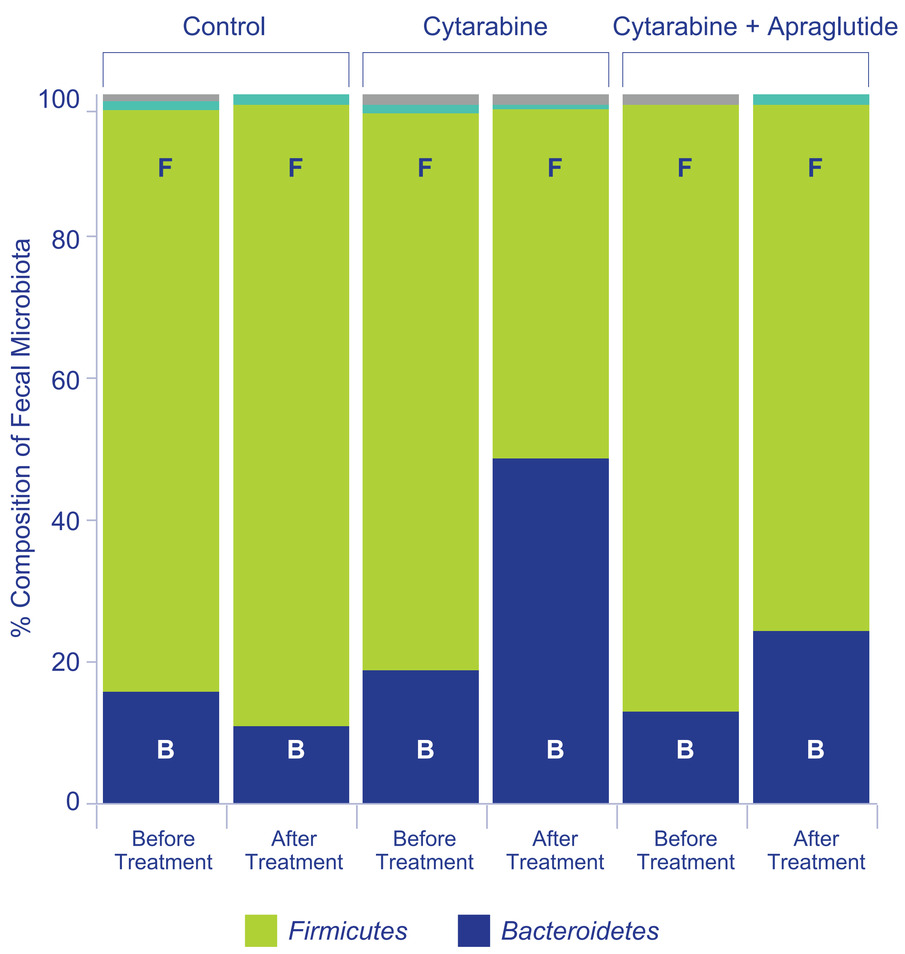

Table 1: Mean Percentage of Bacterial Phyla in the Feces

	Bacteroidetes			*Firmicutes*		
	Day 0 (%)	Day 18 (%)	*p*-value*	Day 0 (%)	Day 18 (%)	*p*-value*
Control	15.2	10.5	0.1723	82.6	88.2	0.1374
Cytarabine-only	18.3	48.7	<0.0001	78.7	49.5	<0.0001
Cytarabine + Apraglutide	12.2	24.0	0.0057	85.3	73.7	0.0107
Between Group Comparisons on Day 18	**Control vs. Cytarabine-only**	**Control vs. Cytarabine + Apraglutide**	**Cytarabine-only vs. Cytarabine + Apraglutide**	**Control vs. Cytarabine-only**	**Control vs. Cytarabine + Apraglutide**	**Cytarabine-only vs. Cytarabine + Apraglutide**
***p*-value**	<0.0001	0.0048	<0.0001	<0.0001	0.0031	<0.0001

* Change between Days 0 – 18

Table 2: Ratio Between Fecal Bacterial Populations

	Control		Cytarabine		Cytarabine + Apraglutide	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
***Firmucutes* (%)**	82.6	88.2	78.7	49.5	85.3	73.7
***Bacteroidetes* (%)**	15.2	10.5	18.3	48.7	12.2	24.0
***Firmicutes*-to-*Bacteroidetes* Ratio**	5.4	8.4	4.3	1.0	7.0	3.1

CONCLUSION

- Conditioning chemotherapy with cytarabine profoundly impacted bacterial homeostasis in the mouse intestine and significant changed the richness of the bacterial populations at the phylum level, with a notable increase in opportunistic pathogenic bacteria populations.
- The co-administration of apraglutide with conditioning chemotherapy maintained the bacterial homeostasis closer to physiological flora.
- The results demonstrated that treatment with apraglutide resulted in the preservation of a physiological intestinal microbiome.
- Prevention of intestinal dysbiosis may contribute to the improved outcomes (reduced body weight loss, increased survival) observed in mice when apraglutide was administered as pre-treatment and concomitantly with conditioning chemotherapy.

REFERENCES

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DISCLOSURES

The study was sponsored by VectivBio. V. Dimitriadou received fee for service as a consultant and oversaw the conduct of the study.