Inflammatory bowel disease (IBD) is a chronic, relapsing-remitting systemic disease of the gastrointestinal tract, triggered by a complex interplay of genetic variability, the host immune system, and environmental factors. Previous studies have shown that altered gut flora, essential for maintaining intestinal homeostasis, is important in triggering chronic inflammation, including IBD. In this study, the Chinese authors investigated the protective effect of rosmarinic acid on the intestinal tract in an animal model of inflammatory bowel disease.

The human gastrointestinal tract contains around 100 trillion microorganisms that form a complex microbial ecosystem. This symbiotic relationship between the gut microbiota and the host is mutually beneficial.

Rosmarinic acid is a natural phenolic acid compound, isolated for the first time from leaves of *Rosmarinus officinalis* L. and later found in other species of Labiatae and Boraginaceae. Rosemary (*Rosmarinus officinalis* L.) is a plant of the Lamiaceae family that originates from the Mediterranean area. Over the past decades, experimental research has confirmed the pharmacological potential of rosemary and some of its primary compounds like diterpenes, carnosic acid, and carnosol. Numerous studies reported that rosemary extracts exhibit chemoprotective effects against hepatotoxicity and gastric ulcerative lesions, as well as anticancer, antimicrobial, antioxidant, and antidiabetic effects, both *in vitro* and *in vivo*. Rosemary and its derivatives can act on free radicals and defend DNA, proteins, and lipids from the oxidative damage of free radicals. Rosemary extracts have also been found to stimulate the innate immune response due to the increased activity of natural killer cells and create an anti-inflammatory cytokine profile. For more details see Luo C, Zou L. Front. Pharmacol. 28 February 2020. https://doi.org/10.3389/fphar.2020.00153

Rosmarinus officinalis L

About the study

A total of 40 mice of both sexes were randomly assigned to four groups. The first group was treated with rosmarinic acid every 24 hours, the second group was treated with rosmarinic acid and 5% dextran sulfate sodium salt every 24 hours, the third group was treated with 5% dextran sulfate sodium salt alone, and the fourth control group was fed with normal food and water. The administration of dextran sulfate sodium salt produced obvious signs of IBD in mice.

To evaluate the effects of rosmarinic acid, the authors used several methodologies, 16S rRNA sequencing, a quantitative polymerase chain reaction (PCR), Western blot, metagenomic technology, and histologic staining.

Results

The abundance of the bacterial genera in four groups of animals

The first group of animals treated only with rosmarinic acid had the highest abundance of the bacterial genera *Lactobacillus* (59.4%), *Dubosiella* (10.2%), and *Candidatus Arthromitus* (6.9%). The abundance of *Dubosiella* and *Lactobacillus* was higher in this group than in other groups.

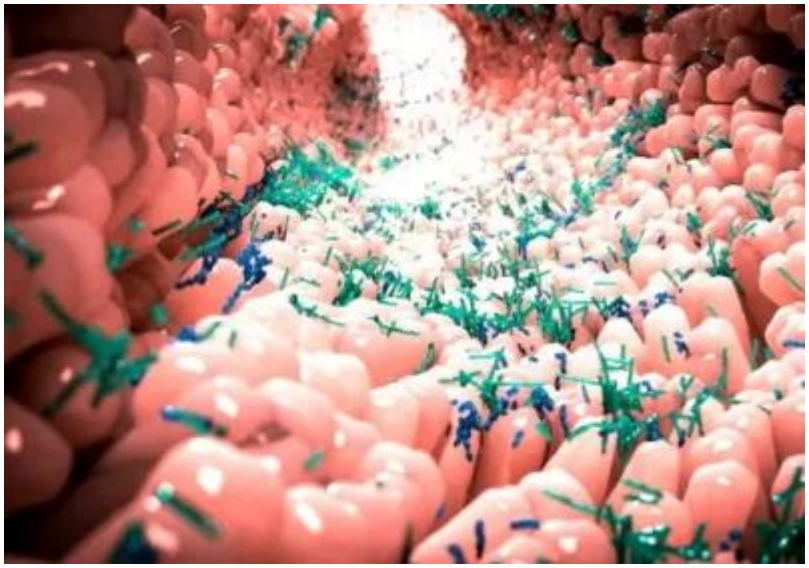
The second group treated with rosmarinic acid and dextran sulfate sodium salt, had the

highest abundance of the genera *Turicibacter* (21.4%), *Streptococcus* (16.9%), and *Clostridium sensu stricto1* (9.9%). The abundance of *Clostridium sensu stricto1*, *Sarcina*, *Streptococcus*, and *Turicibacter* was higher in this group than in other groups.

In the third group of animals treated with dextran sulfate sodium salt, the highest abundance had the genera *Bifidobacterium* (17.6%), *Faecalibaculum* (17.4%), and *Turicibacter* (7.9%). Compared to other groups, the abundance of *Bifidobacterium* and *Faecalibaculum* was higher, whereas the abundance values of *Lactobacillus* were lower.

The control group had the highest abundance of *Lactobacillus* (56.3%), *Limosilactobacillus* (14.7%), and *Candidatus Arthromitus* (10%). The abundance of *Bifidobacterium pseudolongum*, *Escherichia coli*, and *Romboutsia ilealis* was higher in this group than in other groups.

These results have shown that rosmarinic acid, at the species level, could maintain the balance of intestinal flora by reducing the abundance of *Bifidobacterium pseudolongum*, *Escherichia coli*, and *Romboutsia ilealis*, and increasing the abundance of *Lactobacillus johnsonii,* which was shown to reduce inflammation and endoplasmic reticulum stress in mice.



Intestinal microbiota

The effect of rosmarinic acid on damage to the small intestine tissue

The administration of dextran sulfate sodium salt produced obvious signs of IBD in mice. Histological examination demonstrated reduced height or even breakage of the small intestine villi, a severe deformation of cup cells, the absence of muscle-arranged cells, and inflammatory infiltration. Rosmarinic acid reduced damage to the small intestine tissue caused by dextran sulfate sodium salt, and alleviated the severe weight loss, diarrhea, and blood in the stools. The analysis of tight junction damage and inflammation confirmed a protective effect of rosmarinic acid.

Animals treated with dextran sulfate sodium salt had higher mRNA levels of E-cadherin, occlindin, ZEB, and genes encoding tight junction proteins ZO-1 and ZO-2. According to the authors, the upregulation of E-cadherin, ZEB, occlindin at the gene level, and tight junction-related genes suggest intestinal barrier disruption. Mice treated with rosmarinic acid and dextran sulfate sodium had lower mRNA levels of E-cadherin, occlindin, ZO-1, ZO-2, and ZEB than mice treated with dextran sulfate sodium salt alone.

Mice treated with dextran sulfate sodium alone had increased expression of (IL)-6, IL-10, IL-1β, and tumor necrosis factor (TNF)-α, and decreased expression of anti-inflammatory factor IL-10 compared to mice treated with rosmarinic acid and dextran sulfate sodium salt. In addition, mice treated with dextran sulfate sodium salt alone had increased protein levels of caspase3, caspase8, caspase9, caspase12, *p*-RIPK1, *p*-RIPK3, *p*-MLKL, and Bcl-2, which indicates cell necrosis, apoptosis of the mitochondrial pathway and the endoplasmic

reticulum stress pathway. The levels of these proteins were significantly lower in mice treated with rosmarinic acid and dextran sulfate sodium salt.

Mice treated with rosmarinic acid and dextran sulfate sodium salt also had decreased expression of *p*-MLC, RhoA, and ROCK. This suggests that rosmarinic acid could inhibit the abnormal smooth muscle contraction in the mouse intestine induced by dextran sulfate sodium salt.

Conclusion

This study demonstrated that rosmarinic acid, which acts as a natural antioxidant, displayed a protective effect on inflammatory bowel disease. The rosmarinic acid effectively reduces intestinal inflammation, flora dysbiosis, endoplasmic reticulum stress, cell death, and intestinal smooth muscle contraction abnormalities. These findings provide new insights into the potential treatment of IBD with rosmarinic acid.

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