Inhibition of mTOR mediates alleviation of intestinal fibrosis via suppression of myofibroblast proliferation

*Archana V. Patel, MD; *Jun Yang MD, PhD; James Cao, MD, PhD; David Jones, MD; David Conti, MD; Catherine Bartholomew, MD; Xinjun Zhu, MD

1Department of Medicine Division of Gastroenterology, 2Center for Cardiovascular Sciences, 3Department of Pathology, 4Department of Surgery, Albany Medical College, Albany, NY

Abstract:

Background: Intestinal fibrosis is irreversible which happens in response to prolonged injury or inflammation. The pathophysiology of intestinal fibrosis in inflammatory bowel disease (IBD) is not fully understood. Rapamycin, an inhibitor of mTOR, has been reported to alleviate IBD, presumably by improving the inflammatory response by down-regulating the immune system. The mTOR signaling pathway was reported to be involved in transforming growth factor β1 (TGF-β1) dependent fibrogenic processes in several organ systems. Low dose oral administration of rapamycin reduces fibrogenesis, improves liver function, and prolongs survival in mice with liver cirrhosis. We hypothesized that rapamycin ameliorates intestinal fibrosis via inhibition of submucosal myofibroblast proliferation.

Methods: Mice were divided into four groups including control, TNBS alone, rapamycin alone, and both rapamycin and TNBS. Rapamycin (5mg/kg/day) was administered intraperitoneally for 6 weeks and TNBS enema weekly for 6 weeks to induce intestinal fibrosis. Mouse colon samples were collected for examination of submucosal collagen deposition by masson's trichrome blue and myofibroblast proliferation by alpha-SMA or HIC-5 (focal adhesion protein hydrogen peroxide inducible clone 5). Isolated primary intestinal cells or smooth muscle cell line were treated with TGF-β1 for 72 hours either with or without rapamycin.

Results: Colon samples from TNBS-treated mice displayed severe stricture over the distal colon while only mild, patchy erythema and edema observed in the rectum from rapamycin treated TNBS group. While being completely distorted in vehicle treated TNBS group, mucosal crypt architecture was largely preserved in rapamycin treated TNBS group. Colon thickness is an indirect measure of intestinal inflammation and fibrosis. TNBS treatment induced overall thickening of the colon tissue, especially in the submucosa and muscularis layers, which was markedly prevented by rapamycin. Furthermore, we observed the presence of hyperproliferation of myofibroblasts along with significant collagen deposition in the intestine from TNBS treated group. In contrast, intestine from rapamycin group exhibited very mild proliferation of myofibroblasts and deposition of collagen. Direct effect of rapamycin on myofibroblast proliferation was also examined in primary intestinal cells and smooth muscle cell line. We observed that rapamycin markedly reduced the number of alpha-SMA and Hic-5 positive cells in primary intestinal cell culture treated with TGF-β1.

Conclusion: Rapamycin ameliorates intestinal fibrosis in TNBS mouse model. Our data suggest that Rapamycin exerts anti-fibrotic effect in part via direct suppression of myofibroblast proliferation. These findings may have preventive or therapeutic applications in fibrotic Crohn’s disease.

Introduction:

Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. Scarring is confluent fibrosis that obliterates the architecture of the underlying organ or tissue.

Hypothesis: Rapamycin, a bona fide inhibitor of intestinal fibrosis?

Methods:

I. Small bowel specimens were obtained from retrospective chart review of newly diagnosed Crohn’s patients and patients with renal transplant received rapamycin at Albany Medical College. The specimens were then used in immunohistochemistry assay for monitoring myofibroblast proliferation in the submucosa.

II. Mice were divided into four groups including control, TNBS alone, rapamycin alone, and both rapamycin and TNBS. Rapamycin (5mg/kg/day) was administered intraperitoneally for 6 weeks and TNBS enema weekly for 6 weeks to induce intestinal fibrosis. Mouse colon samples were collected for examination of submucosal collagen deposition by masson’s trichrome blue and myofibroblast proliferation by alpha-SMA or HIC-5.

III. Isolated primary intestinal cells or smooth muscle cell line was treated with TGF-β1 for 72 hours either with or without rapamycin treatment.

Results:

I. Increased myofibroblasts in the submuosa of small intestine from patients with newly diagnosed Crohn’s disease.

Methods: Mice were divided into four groups including control, TNBS alone, rapamycin alone, and both rapamycin and TNBS. Rapamycin (5mg/kg/day) was administered intraperitoneally for 6 weeks and TNBS enema weekly for 6 weeks to induce intestinal fibrosis. Mouse colon samples were collected for examination of submucosal collagen deposition by masson’s trichrome blue and myofibroblast proliferation by alpha-SMA or HIC-5. Isolated primary intestinal cells or smooth muscle cell line was treated with TGF-β1 for 72 hours either with or without rapamycin.

Conclusions:

1. Hyperproliferation of myofibroblasts occurs in active, but yet stricturing, early Crohn’s disease.

2. Rapamycin suppresses proliferation of myofibroblasts in small intestine from mice and humans.

3. Rapamycin ameliorates intestinal fibrosis in TNBS mouse model.

4. Our data suggest that rapamycin exerts anti-fibrotic effect in part via direct suppression of myofibroblast proliferation.

5. These findings may have preventive or therapeutic applications in fibrotic Crohn’s disease.

Clinical Relevance:

1. Once hyperproliferation of myofibroblasts is detected on small intestinal biopsy, it can be used as a tool to identify patients at risk for developing fibrosing Crohn’s disease.

2. Rapamycin can be used as a novel therapy to prevent Crohn’s induced intestinal fibrosis.

This work was supported in whole by National Institutes of Health Grants GM080459 and The G IBC Research Fund by Michael Ferrara

DISCLOSURE: There are no disclosures to report