Red Bean Extracts Protect Rats against Intestinal Inflammatory Damage

Ki-Choon Choi¹, Seong-Wan Cho², and Jeong-Chae Lee*

Research Center of Bioactive Materials, Institute of Oral Biosciences and School of Dentistry, Chonbuk National University, Jeonju, Jeonbuk 54896, Korea

¹Grassland and Forage Division, National Institute of Animal Science, Rural Development Administration, Cheonan, Chungnam 31002, Korea

²Department of Pharmaceutical Engineering, Konyang University, Nonsan, Chungnam 32992, Korea

Received June 18, 2015
Revised September 28, 2015
Accepted October 6, 2015
Published online February 29, 2016

*Corresponding Author
Tel: +82-63-270-4049
Fax: +82-63-270-4004
E-mail: leejc88@jbnu.ac.kr

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Red beans comprise part of the diet in Korean culture. Anti-inflammatory and anti-septic activities of the butanol fraction of red bean ethanol extracts (BF-RBEE) are known. The intestinal anti-inflammatory activity of BF-RBEE and related mechanisms were studied using a rat model of acetic acid-induced colitis. Oral treatment with BF-RBEE (100 mg/kg of body weight) suppressed body weight loss and intestinal inflammatory damage in colitic rats. BF-RBEE treatment reduced the myeloperoxidase activity and the malondialdehyde level that were increased in colitic rats, and restored the reduced level of glutathione. BF-RBEE also inhibited acetic acid-mediated increases in mRNA levels of nitric oxide synthase, interleukin-1 β , and tumor necrosis factor- α . Similarly, treatment with 1 mg/kg of prednisolone protected rats against intestinal colitic damage with corresponding decreases in levels of these inflammatory indicators. Red beans can be useful for treatment of intestinal inflammatory diseases.

Keywords: red bean, colitis, intestinal damage, inflammatory cytokine, anti-inflammation

Introduction

Inflammatory bowel disease (IBD) is a chronic disease of the digestive tract caused by a dysfunctional epithelial immune response to intestinal microorganisms (1). Effective suppression of inflammatory responses is one of the primary strategies for treatment of IBD. Although immunosuppressant drugs are currently available for treatment of IBD (2), development of alternative medicines for use against IBD with reduced side effects has continued (3). In this regard, traditional foods and medicines that are known to have antioxidant and anti-inflammatory activities have been considered for effective treatment of IBD (4).

Adzuki beans are common legumes that comprise part of the daily diet in Korean culture (5). Seeds of *Phaseolus calcaratus* Roxburgh, known as red beans, have long been used as a traditional food and an anti-inflammatory medicine. Red bean extracts reportedly exerted antioxidant and anti-inflammatory activities in lipopolysaccharide-treated macrophages (6). Oral administration of a butanol fraction of red bean ethanol extracts (BF-RBEE) increased survival in a murine model of sepsis (7).

Catechin-7-O- β -D-glucopyranoside has been identified as an active component responsible for the antioxidant and anti-inflammatory effects of BF-RBEE (6,8). A possibility that BF-RBEE can be used as an

effective treatment for intestinal inflammatory diseases is suggested.

In this study, anti-inflammatory activities of BF-RBEE were studied using experimental rat models of acetic acid-induced colitis. Treatment efficacy was compared with a positive prednisolone control. The mechanism of BF-RBEE action against colitic damage was investigated based on measurement of mRNA and protein levels of tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , inducible nitric oxide synthase (iNOS), myeloperoxidase (MPO), reduced glutathione (GSH), and malondialdehyde (MDA) in inflamed colon tissue samples.

Materials and Methods

Animal use ethics This study was carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* as promulgated by the National Institute of Health. The experimental protocol was approved by the Commission of Ethics in the Laboratory Animal Center (Approved No. CBU 2010-0040) of the Chonbuk National University (Jeonju, Korea) and all institutional and national guidelines for the care and use of laboratory animals were followed.

Chemicals, laboratory ware, and plant material Unless otherwise specified, all chemicals and laboratory ware were purchased from

Sigma-Aldrich Co. (St. Louis, MO, USA) and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA), respectively. Preparation and separation of BF-RBEE was performed as described previously (6). Briefly, 50% ethanol extracts (680 g) obtained from 5 kg of dried red beans were subjected to additional serial extraction procedures in a stepwise manner with *n*-hexane, ethyl acetate, *n*-butanol, and distilled water. The butanol fraction was concentrated using rotary evaporator (N-1110KW; EYELA, Tokyo Rikakikai Co., Ltd., Tokyo, Japan) and lyophilized to yield 60 g of BF-RBEE. LC-MS analysis revealed that BF-RBEE contained approximately 1.5% catechin-7-*O*- β -D-glucopyranoside (data not shown). BF-RBEE was dissolved in distilled water prior to oral administration.

Colitis induction and treatment Male Sprague-Dawley rats (180–200 g) were obtained from Orient Co. (Seoul, Korea). Rats were randomly divided into 4 groups ($n=5$ /group) and deprived of food for 24 h prior to acetic acid treatment. Three groups received administration 1 mL of 7% acetic acid into the colon through the anus using a rubber cannula (8 cm in length with a 0.76 in diameter). Then, 24 h after acetic acid injection, 2 groups of rats were administered 2 mL of 10% PBS containing 100 mg/kg BF-RBEE or 1 mg/kg prednisolone daily for 4 days. An acetic acid control group received 1 mL of 10% PBS for the same time. Rats were checked daily for body weight, presence of diarrhea, and water and food intake throughout the experimental period. Animals were sacrificed using an overdose of halothane 24 h after the last administration of BF-RBEE or prednisolone.

Assessment of colitis Colons were removed from rats and washed briefly with cold PBS, followed by specimen photography. Macroscopic ulceration and inflammation scores on a 0–10 scale for colon tissues were assigned by 3 observers blind to the experimental design following previously reported criteria (9). In brief, scores were based on the appearance of ulceration and/or inflammation, as well as damaged sites and areas with inflammation. In addition, specimens from a region of inflamed colon tissue or non-inflamed colon tissue from non-colitic group rats were collected for analysis of histological and biochemical parameters, or for preparation of total mRNA extracts.

Histological observation Cross-sections of colon segments from each animal were fixed in 4% buffered paraformaldehyde and embedded in paraffin. Full-thickness sections of 5 μ m were hydrated and stained with hematoxylin and eosin followed by microscopic observation (ECLIPSE E6000; Nikon, Tokyo, Japan). Two pathologists blind to the study design evaluated histological damage on a 0–59 scale following previously described criteria (10). In brief, criteria consisted of 5 scoring scales from 0 to 4 for colon sections, such as mucosal epithelium and lamia propria, crypts, submucosa, and the muscular layer.

Table 1. Primer sequences used in real time RT-PCR assays

Primers		Sequences (5'-3')
IL-1 β	Forward	CAACCAACAAGTGATTATTCTCCATG
	Reverse	GATCCACACTCTCCAGCTGCA
TNF- α	Forward	CATCTTCTCAAATTCGAGTGACAA
	Reverse	TGGGAGTAGACAAGGTACAACCC
iNOS	Forward	CAGCTGGGCTGTACAAACCTT
	Reverse	CATTGGAAGTGAAGCGTTTCG
β -Actin	Forward	AGAGGGAAATCGTGCCTGAC
	Reverse	CAATAGTGATGACCTGGCCGT

Biochemical study of acetic acid colitic rats The MPO activity of colon tissue samples was determined following methods described elsewhere (11). Results were quantified as mU of MPO/g of wet tissue, where a unit of MPO activity corresponded to the amount of MPO needed to degrade 1 μ M of H₂O₂ per min at 25°C. The GSH content was determined using an ADI-900-160 kit (Enzolife Sciences, Plymouth Meeting, PA, USA) according to the manufacturer's instructions. The level of TNF- α in colon tissue samples was determined using a quantikine® ELISA kit (R&D Systems, Minneapolis, MN, USA) according to manufacturer instructions, and results were expressed as ng per mg of protein of wet tissue. To determine the level of MDA, tissue lysates (100 μ L per colon tissue sample) were mixed with 50 μ L of 8% sodium dodecyl sulfate, then with a buffer containing 20% acetic acid and 0.6% thiobarbituric acid. Mixtures were treated with a butanol:pyridine mixture (15:1, v/v) and MDA levels in supernatants were measured at 532 nm using 1,1,3,3-tetraethoxypropane as a standard. Results were expressed as μ mol per mg of protein of wet tissue.

Real time RT-PCR Total RNA was prepared from colonic tissue samples using the SV Total RNA Isolation System (Promega, Madison, WI, USA) and reverse-transcribed using an RNA PCR kit (Promega) according to the manufacturer's instructions. Real time RT-PCR amplification was performed using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR primer sequences used are shown in Table 1.

Statistical analysis Unless otherwise specified, all data were expressed as a mean $\pm$ standard deviation (SD). A one-way analysis of variance (ANOVA) (Version 12.0; SPSS Inc., Chicago, IL, USA) followed by Scheffe's test were used to determine significant differences between groups at $p<0.05$.

Results and Discussion

BF-RBEE prevented intestinal inflammatory damage in the acetic acid model of rat colitis Evaluation of medicinal efficacy via pre-clinical studies is a common step before clinical use with patients

Fig. 1. BF-RBEE treatment protects rats against colitic intestinal damage induced using acetic acid. Colonic tissue samples from non-colitic control, acetic acid control, and rats treated with either BF-RBEE (100 mg/kg of body weight) or prednisolone (1 mg/kg of body weight) daily for 4 days were collected 24 h after the last treatment. Colon tissues were photographed (A) then stained with hematoxylin and eosin (B). A representative result from 5 different tissue samples is shown. AA, acetic acid. B-R, BF-RBEE. PR, prednisolone.

Fig. 2. Acetic acid-induced increases in microscopic scores are inhibited by administration of BF-RBEE. Microscopic damage scores (0-59) were determined from the same tissue colon samples. ** $p < 0.01$ and *** $p < 0.001$ vs. acetic acid control rats ($n=5$ /group).

(12,13). In this study, rats were subjected to intracolonic treatment with acetic acid for production of a chemically-induced colitic model (14,15). Prednisolone was used as a positive control due to effective use against many immunological disorders, including ulcerative colitis. In addition, a BF-RBEE concentration of 100 mg/kg of body weight was applied to colitic animals with consideration of regulatory effects against sepsis and allergen sensitization and challenge (7). In comparison with the colon architecture in non-colitic control rats, acetic acid treatment disrupted colonic tissue (Fig. 1A). Microscopic evaluation of colonic tissue samples from acetic acid control rats also showed intense transmural disruption of colonic tissue (Fig. 1B). In contrast, supplementation with either BF-RBEE at 100 mg/kg of body weight or prednisolone at 1 mg/kg of body weight attenuated acetic acid-induced colonic damage.

With respect to microscopic scores from 0 to 59, acetic acid group rats presented severe grade lesions with a mean score value of 39 ± 7 (Fig. 2). Conversely, the histological appearance of colonic tissue

Fig. 3. BF-RBEE reduces macroscopic damage scores and prevents body weight loss in colitic rats. (A) Colonic tissue samples collected from rats of 4 different groups 24 h after the last treatment were processed for assignment of macroscopic damage scores (0-10). (B) Body weight changes of rats were measured prior to collection of colonic tissue samples. *** $p < 0.001$ vs. non-colitic control rats; ### $p < 0.001$ vs. acetic acid control rats ($n=5$).

specimens from rats treated with BF-RBEE revealed recovery of colonic tissue with significantly ($p < 0.01$) reduced scores of 12 ± 5.6 , compared with acetic acid group rats. Prednisolone treatment also provided protection against acetic acid-induced colonic damage as prednisolone-treated rats showed significantly ($p < 0.001$) lower scores than acid-induced colonic damage rats.

Macroscopic damage scores (0-10) indicated that acetic acid colitic rats showed a significantly ($p < 0.001$) 11.1x higher mean damage score than non-colitic control rats (0.71 ± 0.42) (Fig. 3A). However, this increase was significantly ($p < 0.001$) reduced in rats treated with either BF-RBEE or prednisolone to scores of 2.9 ± 0.51 and 3.8 ± 0.56 , respectively. Treatment with acetic acid also induced body weight loss, compared with non-colitic animals (Fig. 3B). The change in body weight in colitic animals was -2.1 ± 0.9 g 4 days after acetic acid treatment, which was different from non-colitic rats (3.6 ± 0.7 g).

Fig. 4. BF-RBEE restores colitis-mediated changes in levels of biochemical parameters to levels similar to non-colitic rats. Colonic tissue samples collected from 4 different rat treatment groups were processed for determination of MPO activity (A) and levels of TNF- α (B), MDA (C), and GSH (D). *** p <0.001 vs. non-colitic control rats; # p <0.05, ## p <0.01, and ### p <0.001 vs. the acetic acid control rats (n =5).

Acetic acid-induced body weight loss was recovered to levels similar to non-colitic control rats after treatment with either BF-RBEE or prednisolone. Thus, BF-RBEE is capable of reducing degrees of intestinal inflammation.

BF-RBEE recovers acetic acid-induced colonic damage via anti-inflammatory and antioxidant abilities Biochemical parameters in colon tissues were investigated to understand mechanisms by which BF-RBEE reduced the degree of acetic acid-mediated intestinal damage. The activity of MPO and levels of TNF- α and MDA in acetic acid colitic rats were increased up to 8.0x, 10.3x, and 18.9x, respectively, compared with non-colitic controls (Fig. 4). However, the increased MPO activity (281 ± 36 mU/g of tissue) in acetic acid colitic rats was significantly (p <0.001) attenuated to 82 ± 33 and 93 ± 42 mU/g of tissue in the rats treated with BF-RBEE and prednisolone, respectively (Fig. 4A).

Supplementation with either BF-RBEE or prednisolone also significantly (p <0.01) diminished acetic acid-induced increases in the levels of TNF- α (Fig. 4B) and MDA (Fig. 4C). Moreover, the decreased GSH level (0.41 ± 0.21 μ mol/g of tissue) in acetic acid control rats was significantly (p <0.05) restored to 1.11 ± 0.21 and 1.23 ± 0.23 μ mol/g tissue after treatment with prednisolone and BF-RBEE, respectively (Fig. 4D).

Changes in biochemical parameters, such as MPO, TNF- α , MDA,

Fig. 5. Administration of BF-RBEE reduces expression of inflammatory cytokines in colonic tissues of acetic acid-treated rats. Total RNA extracts were isolated from the same colon tissue samples for analysis of *iNOS*, *TNF- α* , and *IL-1 β* expressions using real time RT-PCR. ** p <0.01 and *** p <0.001 vs. the non-colitic control rats; # p <0.05 vs. the acetic acid control rats (n =5).

and GSH are commonly observed in IBD patients (16,17). Reactive oxygen species are also important mediators responsible for induction of intestinal disorders (16). This study revealed that the levels of all parameters tested were changed in acetic acid-treated rats, whereas such changes were inhibited after treatment with BF-RBEE. Thus, BF-RBEE plays a protective role against acetic acid-induced colitis both by inhibition of intestinal inflammatory responses and by stimulation of the antioxidant defense system.

BF-RBEE suppressed expression of inflammatory cytokines in inflamed colon tissues Activation of immune cells and enhanced production of inflammatory mediators are important inflammatory responses involved in IBD (4). Nitrogen metabolites also mediate induction of intestinal disorders (18,19). Therefore, mRNA levels of several inflammatory mediators were measured using real time RT-PCR analysis. Expressions of *iNOS*, *TNF- α* , and *IL-1 β* in inflamed colon tissues of acetic acid-treated rats were increased, compared with non-colitic control rats (Fig. 5). Treatment with BF-RBEE significantly (p <0.05) attenuated all acetic acid-induced increases, compared with controls. Similarly, prednisolone showed significant (p <0.05) inhibition of these increases, compared with controls.

Inflammatory cytokine stimulates expression of chemokines and subsequent monocyte adhesion (20). Supplementation with BF-RBEE reduced the serum TNF- α level in a murine sepsis model, eventually increasing the survival rate (7). A critical role for TNF- α is indicated in mediation of host immune responses to acute inflammatory stimuli. It is also important to consider that colitis induction in an acetic acid model is driven mainly by innate immune cells. Therefore, TNF- α is to be one of specific targets of BF-RBEE for treatment of inflammatory disorders, such as colitis and sepsis. In addition, intestinal anti-inflammation actions of BF-RBEE are associated with recovery of the intestinal epithelial barrier functions (21) because MUC2 and MUC3 are primary constituents of the mucus layer in the

colon and barrier dysfunction mediates intestinal inflammation, resulting in a strong immune response (22). Consequently, effective suppression of inflammatory cytokine production and restoration of epithelial barrier function are correlated with a property of BF-RBEE that protects rats against colitic damage.

On the other hand, BF-RBEE was reported to be the most active anti-inflammatory and antioxidant fraction among the first 4 serial fractions (6-8). BF-RBEE also contains 1.5% catechin-7-O- β -D-glucopyranoside, a standard active material in red beans. Furthermore, red bean extracts at 100 mg/kg of body weight showed intestinal anti-inflammatory activities similar to prednisolone at 1 mg/kg of body weight. Although the amount of BF-RBEE administered was 100x higher than the prednisolone, the beneficial effects of BF-RBEE on intestinal inflammatory diseases are important. Effective use of red beans for prevention and/or treatment of intestinal inflammatory damage is indicated.

In summary, this study demonstrated that BF-RBEE inhibits intestinal inflammatory responses in experimental colitis in rats. BF-RBEE and/or red beans can be used as functional foods of in an approach aimed at treating intestinal inflammatory disorders. However, an acetic acid model is an acute/self-limiting colitis model that exhibits limited applicability to human IBD. Future studies should focus on the cellular mechanisms by which BF-RBEE protects against colitic damage using more appropriate animal models of IBD, such as T-cell transfer and IL-10-deficient models (23).

Acknowledgment This work was supported by a grant from the Rural Development Administration, Ministry of Agriculture and Forestry, Republic of Korea (PJ010903032015).

Disclosure The authors declare no conflict of interest.

References

- Neuman MG. Immune dysfunction in inflammatory bowel disease. *Transl. Res.* 149: 173-186 (2007)
- Kho YH, Pool MO, Jansman FG, Harting JW. Pharmacotherapeutic options in inflammatory bowel disease: An update. *Pharm. World Sci.* 23: 17-21 (2001)
- Weizman AV, Ahn E, Thanabalan R, Leung W, Croitoru K, Silverberg MS, Steinhart AH, Nguyen GC. Characterization of complementary and alternative medicine use and its impact on medication adherence in inflammatory bowel disease. *Aliment Pharm. Ther.* 35: 342-349 (2012)
- Algieri F, Zorrilla P, Rodriguez-Nogales A, Garrido-Mesa N, Bañuelos O, González-Tejero MR, Casares-Porcel M, Molero-Mesa J, Zarzuelo A, Utrilla MP, Rodríguez-Cabezas ME, Galvez J. Intestinal anti-inflammatory activity of hydroalcoholic extracts of *Phlomis purpurea* L. and *Phlomis lychnitis* L. in the trinitrobenzenesulphonic acid model of rat colitis. *J. Ethnopharmacol.* 146: 750-759 (2013)
- Zhao QW, Huang X, Lou YJ, Weber N, Proksch P. Effects of ethanol extracts from Adzuki bean (*Phaseolus angularis* Wight.) and Lima bean (*Phaseolus lunatus* L.) on estrogen and progesterone receptor phenotypes of MCF-7/BOS cells. *Phytother. Res.* 21: 648-652 (2007)
- Fang M, Lee SY, Park SM, Choi KC, Lee YJ, Cho HK, Cho SW, Whang WK, Lee JC. Anti-inflammatory potential of *Phaseolus calcaratus* Roxburgh, an oriental medicine, on LPS-stimulated RAW 264.7 macrophages. *J. Pharm. Pharmacol.* 63: 120-128 (2011)
- Park S, Choi KC, Fang M, Lim YC, Jeon YM, Lee JC. Red bean extract reduces inflammation and increases survival in a murine sepsis model. *Food Sci. Biotechnol.* 20: 1125-1131 (2011)
- Baek JA, Son YO, Fang M, Lee YJ, Cho HK, Whang WK, Lee JC. Catechin-7-O- β -D-glucopyranoside scavenges free radicals and protects human B lymphoma BJAB cells on H₂O₂-mediated oxidative stress. *Food Sci. Biotechnol.* 20: 151-158 (2011)
- Camuesco D, Peran L, Comalada M, Nieto A, Di Stasi LC, Rodríguez-Cabezas ME, Concha A, Zarzuelo A, Galvez J. Preventative effects of lactulose in the trinitrobenzenesulphonic acid model of rat colitis. *Inflamm. Bowel Dis.* 11: 265-271 (2005)
- Arribas B, Suárez-Pereira E, Ortiz Mellet C, García Fernández JM, Buttersack C, Rodríguez-Cabezas ME, Garrido-Mesa N, Bailon E, Guerra-Hernández E, Zarzuelo A, Gálvez J. Di-D-fructose dianhydride-enriched caramels: Effect on colon microbiota, inflammation, and tissue damage in trinitrobenzenesulfonic acid-induced colitic rats. *J. Agr. Food Chem.* 58: 6476-6484 (2010)
- Krawisz JE, Sharon P, Stenson WF. Quantitative assay for acute intestinal inflammation based on myeloperoxidase activity assessment of inflammation in rat and hamster models. *Gastroenterology* 87: 1344-1350 (1984)
- Rosillo MA, Sanchez-Hidalgo M, Cárdeno A. Protective effect of ellagic acid, a natural polyphenolic compound, in a murine model of Crohn's disease. *Biochem. Pharmacol.* 82: 737-745 (2011)
- González-Mauraza H, Martín-Cordero C, Alarcón-de-la-Lastra C, Rosillo MA, León-González AJ, Sánchez-Hidalgo M. Anti-inflammatory effects of *Retama monosperma* in acute ulcerative colitis in rats. *J. Physiol. Biochem.* 70: 163-172 (2014)
- Kannan N, Guruvayoorappan C. Protective effect of *Bauhinia tomentosa* on acetic acid induced ulcerative colitis by regulating antioxidant and inflammatory mediators. *Int. Immunopharmacol.* 16: 57-66 (2013)
- V VP, Guruvayoorappan C. Protective effect of marine mangrove *Rhizophora apiculata* on acetic acid induced experimental colitis by regulating antioxidant enzymes, inflammatory mediators and nuclear factor-kappa B subunits. *Int. Immunopharmacol.* 18: 124-134 (2014)
- Nagib MM, Tadros MG, ElSayed MI, Khalifa AE. Anti-inflammatory and antioxidant activities of olmesartan medoxomil ameliorate experimental colitis in rats. *Toxicol. Appl. Pharm.* 271: 106-113 (2013)
- Peterson CG, Sangfelt P, Wagner M, Hansson T, Lettesjö H, Carlson M. Fecal levels of leukocyte markers reflect disease activity in patients with ulcerative colitis. *Scand. J. Clin. Lab. Inv.* 67: 810-820 (2007)
- Strober W, Fuss IJ. Proinflammatory cytokines in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 140: 1756-1767 (2011)
- Hasnat MA, Pervin M, Park SR, Jang YC, Lim JH, Kim YJ, Kim YO, Kweon HJ, Lim BO. Anti-inflammatory, counteractive, and preventive effects of orally administrated oat extracts on dextran sulfate sodium-induced colitis in mice. *Food Sci. Biotechnol.* 24: 619-628 (2015)
- Youn GS, Kwon DJ, Ju SM, Choi SY, Park J. Curcumin ameliorates TNF- α -induced ICAM-1 expression and subsequent THP-1 adhesiveness via the induction of heme oxygenase-1 in the HaCa T cells. *BMB Rep.* 46: 410-415 (2013)
- Tytgat KM, Bovelland FJ, Opdam FJ, Einerhand AW, Buller HA, Dekker J. Biosynthesis of rat MUC2 in colon and its analogy with human MUC2. *Biochem. J.* 309: 221-229 (1995)
- Su L, Shen L, Clayburgh DR, Nalle SC, Sullivan EA, Meddings JB, Abraham C, Turner JR. Targeted epithelial tight junction dysfunction causes immune activation and contributes to development of experimental colitis. *Gastroenterology* 136: 551-563 (2009)
- Koboziev I, Karlsson F, Zhang S, Grisham MB. Pharmacological intervention studies using mouse models of the inflammatory bowel diseases: Translating preclinical data into new drug therapies. *Inflamm. Bowel Dis.* 17:1229-1245 (2011)