European Review for Medical and Pharmacological Sciences 2018; 22: 8839-8848

PPAR-γ with its anti-inflammatory and anti-fibrotic action could be an effective therapeutic target in IBD

A. VETUSCHI[1], S. POMPILI[1], E. GAUDIO[2], G. LATELLA[3], R. SFERRA[1]

[1]Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, L'Aquila, Italy
[2]Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Sapienza University of Rome, Rome, Italy
[3]Department of Life, Health and Environmental Sciences, Gastroenterology Unit, University of L'Aquila, L'Aquila, Italy

Abstract. – OBJECTIVE: Intestinal fibrosis is a process characterized by an excessive deposition of Extracellular Matrix (ECM) proteins by activated myofibroblasts and represents a consequence of a chronic inflammation that usually occurs during Inflammatory Bowel Disease (IBD). The relationship between inflammation and fibrosis in IBD remains still unclear and nevertheless the recent pharmacological progresses, currently the only resolutive therapeutic strategy is surgery, especially when complications (stricture, stenosis and obstruction of intestinal tracts) appear. As many different cellular types and molecular mechanisms are implicated in the pathogenesis of IBD, the identification of molecules able to counteract this process could be crucial.

MATERIALS AND METHODS: This is a literature review of several articles published on PubMed databases.

RESULTS: A number of researches suggest that Proliferator-Activated Receptor-gamma (PPAR-γ) has both anti-inflammatory and anti-fibrotic effects in many organs. PPAR-γ has been demonstrated to be able to downregulate pro-inflammatory cytokines production such as Interleukin (IL)-4,-5,-6 but also to interfere with pro-fibrotic molecules as Platelet-Derived Growth Factor (PDGF), IL-1 and Transforming Growth Factor Beta (TGF-β), the main promoter of fibrosis. In preliminary clinical trials and in experimental models of intestinal fibrosis, natural and chemical PPAR-γ ligands have ameliorated the fibrotic process.

CONCLUSIONS: Since PPAR-γ could play a crucial role in the development of the disease, the research of new molecules, capable of ameliorating both inflammation and fibrosis lesions, as PPAR-γ agonists, could represent a valid and effective therapeutic approach for the prevention and treatment of IBD and intestinal fibrosis.

Key Words:
PPAR-γ, TGF-β, Extracellular matrix, Fibrosis, Inflammatory Bowel Disease.

Introduction

In Inflammatory Bowel Disease (IBD), including Ulcerative Colitis (UC) and Crohn's Disease (CD), chronic damage occurring in intestinal wall leads to an excessive accumulation of fibrillary Extra Cellular Matrix (ECM) proteins, responsible for fibrosis, strictures, stenosis and obstructions[1-3]. Features and evolution of intestinal lesions are different between UC and CD: UC shows inflammatory lesions in the large bowel mucosa and submucosa, while in CD the inflammation is transmural and fibrosis can involve the whole intestinal wall of the gastrointestinal tract affected by the disease, specially the terminal ileum[4]. The current objective of medical treatment is to achieve not only clinical remission, but also healing of intestinal lesions. Available therapies, including aminosalicytes, steroids, immunomodulators and biologic drugs can relieve the inflammatory symptoms but they do not significantly improve the fibrosis and fibrostenosing lesions. Pathophysiology of chronic mucosal healing and late events of repair leading to intestinal fibrosis remain largely unknown[2,5,6]. To date efficient and well-tolerated antifibrotic drugs are not yet available and surgery represents the only therapeutic option once intestinal fibrostenonis has occurred[5,7-9]. The relationship between inflammation and fibrosis in IBD remains still unclear. At an early stage, intestinal lesion is

Corresponding Author: Simona Pompili BS, Ph.D; e-mail: pompili.simona@virgilio.it

followed by an acute inflammatory response and a healing of damaged tissue with the restoration of organ function. On the other hand, when the inflammatory process is prolonged over time, weeks or months from its onset, it can lead to fibrosis[10]. It is commonly accepted that chronic intestinal inflammation inevitably leads to fibrosis; however, this process does not occur in all chronic intestinal disorders. Celiac disease and lymphocytic colitis are not complicated by fibrotic processes and stenosis development, indicating the existence of distinct mechanisms between inflammation and fibrosis[11]. Administration of anti-inflammatory drugs in IBD, as well as in other fibroproliferative diseases associated with chronic inflammation, does not prevent the development of fibrosis after the extracellular matrix deposition has begun [12]. The lack of effective anti-fibrotic drugs is due to the fact that the main and specific cellular and molecular events leading to fibrosis still remain unclear[13,14].

Main Headings

Cellular and Molecular Mechanisms Involved in Intestinal Fibrosis

Once intestinal epithelium is injured by external factors, intraluminal bacteria and antigens cross the epithelial layer, trigger antigen presenting cells and transform naïve T cell into Th1, Th2, Th7, and natural killer T cells responsible for release of multiple type of proinflammatory citokines[15]. Persistent epithelial and endothelial damage leads to chronic inflammation and the release of inflammatory factors that promote activation of ECM producing cells[2,7] especially fibroblasts and myofibroblasts, activated by multiple pathways (autocrine factors, paracrine signals and microbe associated molecular patterns)[16-18]. During intestinal inflammation and remodelling process, the normal turnover of the extracellular matrix components is regulated by the delicate balance between proteolytic enzymes, like Metalloproteinases (MMPs), and theirs Tissue Inhibitors (TIMPs). Therefore, both the imbalance of the MMPs/TIMPs system and a failure in myofibroblast apoptosis and/or a lack in their reversion to a non-activated state result in an excessive deposition of ECM proteins[11,17]. Researches[11,19] have demonstrated that activated miofibroblasts can derive from several and distinct cellular sources such as resident mesenchymal cells (fibroblasts, subepithelial miofibroblasts, smooth muscle cells and interstitial cells of Cajal) as well as by Hepatic Stellate Cells (HSC), pericytes and bone marrow stem cells. In addition, activated miofibroblasts can also differentiate from non-mesenchymal cells, such as endothelial and epithelial cells[16,20]. Epithelial to Mesenchymal Transition (EMT) represents an important source of ECM producing cells[2,21,22]. Evidence demonstrated that epithelial cells play a crucial role on the development and progression of fibrosis comparable to that of the fibroblasts. Therefore, EMT may represent one of the pivotal mechanisms promoting fibro-proliferative processes. Epithelial phenotype is characterized by polarized cells, which interact with basal membrane and show a highly specialized cell-cell apical junctions, including Adherens Junctions (AJ), Tight Junctions (TJ) together with desmosomes and gap junctions necessary for maintaining the integrity of the epithelium and its barrier function[23]. The Apical Junctional Complex (AJC) is constituted by the tight junctions and adherens junctions and its key proteins are represented by Occludin, the Claudin protein family and Junctional Adhesion Molecules (JAM), all localized in the TJ, whereas E-cadherins are confined in the AJ. The extracellular region of E-cadherins is located along the lateral cell surface and binds to cadherins presented on adjacent cells[24] while its intracellular portion contains binding sites to interact with catenins. Thus, E-cadherin forms a complex with β-catenin that contributes to maintaining the epithelial stability. A typical feature of EMT is the disruption of intercellular junctions that leads to a downregulation of AJ and TJ proteins and in particular a loss of E-cadherin that promotes β-catenin release and its nuclear translocation faciliting EMT. In addition, a *de novo* synthesis of proteins associated with myofibroblasts including vimentin, α-Smooth Muscle Actin (α-SMA) and production of interstitial matrix components, as fibronectin and type 1 collagen, have been demonstrated[23,25,26]. This transformed cellular phenotype can be reversed to epithelial phenotype (Mesenchymal Epithelial Transition, MET) when the expression of E-cadherin normalizes.

EMT and Intestinal Fibrosis

Several extracellular mediators including Transforming Growth Factor-β (TGF-β), Connective Tissue Growth Factor (CTGF), Epidermal Growth Factor (EGF), Fibroblasts Growth Factors-2 (FGF-2), Interleukin-1 (IL-1) and Wnt

ligands are involved in inducing and maintaining EMT during the fibrosis process[27]. Several investigations[28-30] suggest that disruption of the TGF-β/Smads (Small mother against decapentaplegic) pathway, such as the loss of Smad3 or the overexpression Smad7, is able to prevent the development of tissue fibrosis in a large number of organs (skin, kidney, lunge, liver, intestine). Targeted deletion of Smad3 gene confers resistance to the development of both intestinal and liver fibrosis[30,31]. Experimental mouse model of IBD by administration of 2,4,5-Trinitrobenzene Sulphonic Acid (TNBS) showed that Smad3 knockout (KO) mice were protected from the development of colorectal fibrosis. Histological and immunohistochemistry evaluations indicated an increase in the expression of collagen I-III, α- SMA and TGF-β1 in the colonic wall of Wild type mice compared to KO mice supporting the key role of TGF-β/Smad3 signaling[30-31] that can be considered as a "core pathway" of intestinal fibrosis[6]. Once activated, TGF-β binds to specific membrane receptors, leading to phosphorylation of Smad2 and 3, which combine with Smad4 and translocate into the nucleus where regulate specific TGF-β target genes. TGF- β also represents the most powerful mediator *in vitro* and *in vivo* of EMT[27,32,33]. It was described as an inducer of EMT in mammary epithelial cells in a large number of different adult tissue (heart, eye, liver, kidney, lung) also including the intestine[6,22,27,34-39]. In CD patients, TGF-β1 expression was increased in intestinal submucosal layers, in particular in fibrotic areas[32]. In TNBS experimental induced colitis, intestinal epithelial cells are driven to EMT and express Fibroblast Specific Protein 1(FSP1)[21]. In animals receiving TNBS, the intestinal fibrotic tract showed an increased number of fibroblasts expressing α-SMA as well as E-cadherin and FSP1, suggesting the onset of EMT. The role of TGF-β/Smad pathway in intestinal EMT has been also confirmed by the observation that some molecules, like miR-200, Glycogen Synthase Kinase-3 beta (GSK-3β), and Peroxisome Proliferator-Activated-Receptor Gamma (PPAR-γ), are able to inhibit TGF-β1-induced EMT[40]. The members of miR-200 family have been proved to be able to maintain the epithelial phenotype through a downregulation of Zinc finger E-box-binding homeobox (ZEB) 1 and ZEB2 resulting in enhanced E-cadherin expression ameliorating intestinal epithelial barrier function. Indeed, it has been demonstrated that miR-200 repressing Smad2 protein, inhibits vimentin expression through TGFβ1/Smad2 signal pathway preventing TGF-β1 induced EMT[40]. GSK-3β seems to be able to negatively regulate EMT as it resulted not activated in the fibrotic intestinal condition, thus β-catenin is free to translocate into the nucleus and promote its pro-fibrotic signaling[22]. In the same study conducted in a mouse model of Dextran Sodium Sulphate (DSS)-induced intestinal fibrosis, the expression of proteins related to EMT has been investigated, showing a relationship between TGF-β, Smad3, E-cadherin, Zinc finger protein (Snail), ZEB1, β-catenin and GSK-3β. A marked increase in α-SMA, collagen I-III, fibronectin (main fibrosis markers) and a similar increased expression of IL-13, TGF-β and Smad3 (pro-fibrotic molecules) has been demonstrated in mice with DSS-induced chronic colitis compared to control mice. Furthermore, in DSS mice it has been observed β-catenin nuclear translocation and E-cadherin downregulation suggesting that PPAR-γ activation could be strongly related to the Smad dependent or Smad independent TGF-β signaling pathway and may attenuate fibrosis and TGF-β1 induced EMT[22]. PPARs are nuclear receptors related with tissue fibrogenesis acting on gene transcription by binding to retinoid X receptors. Three different isoforms of PPARs are involved in several processes including fibrosis and, in particular the PPAR-γ isoform, has been shown to be largely expressed in the colorectal mucosa; its stimulation, from specific ligands, antagonizes Smad3 or downregulates CTGF expression[41-43]. PPAR-γ has been identified as an endogenous factor involved in several metabolic and cellular functions like lipid and carboidrate metabolisms and homeostasis, carcinogenesis (cell cycle regulation, cell differentiation), inflammation and fibrosis[44,45] (Figure 1).

It appears to be able to regulate intestinal inflammation and fibrosis both in IBD patients and in DSS and TNBS experimental colitis, decreasing pro-inflammatory and pro-fibrotic cytokines and chemokines[44].

Material and Methods

This is a systematic review of several articles published on PubMed databases. It was set up following a preliminary meeting in which all the authors have identified and discussed the main scientific studies about the anti-inflammatory and anti-fibrotic effects of PPAR- γ.

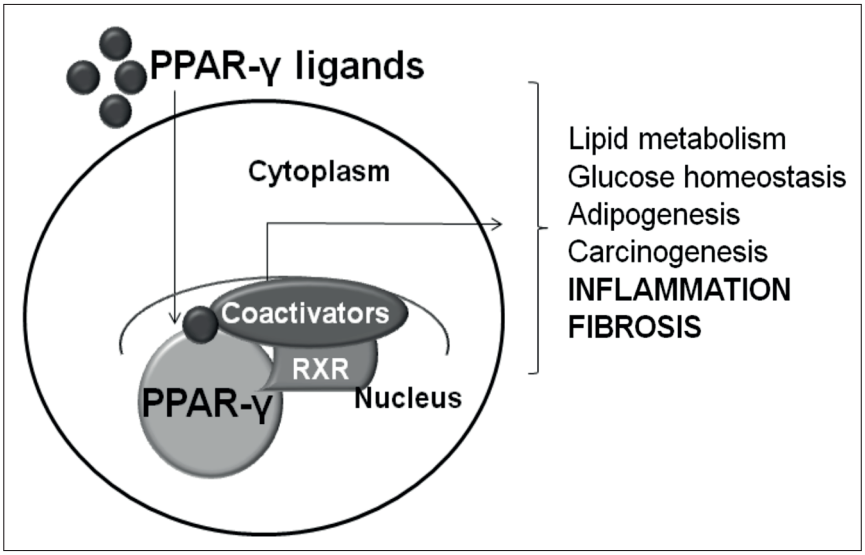

Figure 1. Main effects of PPAR-γ on metabolism and cellular functions.

Results

PPAR-γ in Intestinal Inflammation and Fibrosis

PPAR-γ plays a crucial role both in inflammation and fibrosis in several organs modulating the production of several mediators[46-50]. PPAR-γ activation decreases the production of the pro-inflammatory cytokines such as Tumor Necrosis Factor α (TNF-α) and IL-6, and inhibits transcription factors such as Nuclear Factor-kappa-light-chain-enhancer of activated B cells (NF-κB), Activator Protein-1 (AP-1), Signal Transducer, Activator of Transcription (STAT-1) and the expression of adhesion molecules, such as Intercellular Adhesion Molecule (ICAM-1), as well as MMP-9. In addition, PPAR-γ has been recognized as a key anti-inflammatory modulator regulating macrophage differentiation and cellular polarization. In 2004 Xiong et al[50] investigated the implication of PPAR-γ during inflammatory process in Human Mesangial Cells (HMCLs) stimulated by IL-1β. Xiong et al[50] demonstrated that pro-inflammatory cytokines as IL-6 and TNF-α were increased in HMCLs respect to untreated cells and there was a reduction of TNF-α and IL-6 when HMCLs were treated with PPAR-γ agonists such as troglitazone, rosiglitazone and 15deoxy-delta (12,14)-prosglandinJ2. Moreover, in cyclophosphamide-induced renal toxicity it was found that PPAR-γ agonists showed protective effects, downregulating pro-inflammatory cytokines and inhibiting apoptosis[51]. Anti-inflammatory effects of PPAR-γ were also reported in airways diseases. The Authors demonstrated that the administration of PPAR-γ agonists was able to reduce pro-inflammatory cytokines such as IL-4 and IL-5, eosinophilic inflammation and airway hyper-responsiveness. The inhibition of IL-10 activity partially reverted the inflammatory process, suggesting that PPAR-γ played a protective role in the physiopathology of asthma[52]. Investigations conducted in Chronic Obstructive Pulmonary Disease (COPD) have also highlighted the anti-inflammatory effect of PPAR-γ: in epithelial cells from COPD patients; PPAR-γ resulted reduced while NF-kB was increased. After treatment with PPAR-γ synthetic ligand as rosiglitazone and natural ligand as 10-nitro-oleic acid, epithelial cells showed an increase of PPAR-γ expression and an inhibition of secretion of inflammatory cytokines[53]. PPAR-γ has shown an anti-inflammatory activity also within pulmonary Vascular Endothelial (VE) cells, promoting transcription of genes for anti-inflammatory factors and inhibiting the activity of NF-κB, AP-1, and other proinflammatory transcription factors[54] (Figure 2).

The relevant role of PPAR-γ agonists in inflammatory diseases was also demonstrated in systemic lupus erythematous, renal disease, atherosclerosis, brain inflammation, pancreatitis and in experimental rat model of IBD[55-59]. In 1999, for the first time, Su et al[46] showed the involvement of PPAR-γ in the regulation of intestinal inflammation in DSS-induced colitis in mice. The administration of synthetic agonist of PPAR-γ ameliorated the clinical course of colitis compared to control mice[46-47]. In experimental TNBS-induced colitis in heterozygous PPAR-γ

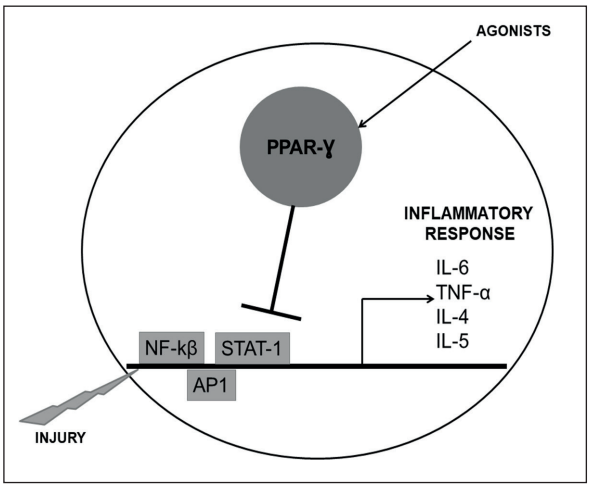

Figure 2. Schematic diagram representing the anti-inflammatory effect of PPAR-γ. After injury transcription factors such as NF-kβ, STAT-1, AP-1, induce the release of inflammatory cytokines. PPAR-γ is able to inhibit the activity of these molecules.

+/- mice and wild-type mice, Dubuquoy et al[47] showed that PPAR-γ was able to mimic the therapeutic anti-inflammatory action of 5-aminosalicyloic acid (5-ASA; Pentasa, Ferring Pharmaceuticals, Saint-Prex, Switzerland)[55]. Among side PPAR-γ anti-inflammatory action, there is evidence that it is an innate protector against fibrogenesis in several organs[5,43,45,60-62]. It has been shown[43] that in Hypertrophic Scar Fibroblasts (HSFs) a concomitant exposition to different concentrations of PPAR-γ natural ligands as 15-deoxy-D12,14-prostaglandin J2 and synthetic ligand as GW7845 (GlaxoSmithKline Pharmaceuticals, Brentford, London, UK), lead to a reduced expression of CTGF, collagens and fibronectin. PPAR-γ resulted also implicated in liver fibrosis showing the capacity to activate HSC. Both *in vitro* and *in vivo* experiments showed that HSC activation due to a reduction of PPAR-γ expression was reversed by PPAR-γ ligands[62-64] assuming that its agonists could have a therapeutic benefits[65-69]. In experimental model of bleomycin-induced skin fibrosis, using mice with a fibroblast selective depletion of PPAR-γ, Kapoor et al[60] demonstrated that fibroblasts resulted most susceptible to profibrotic effects of TGFβ1 and there was an increase of the main signs of fibrosis[60]. It is well known that during IBD there is an imbalance between pro-inflammatory and anti-inflammatory cytokines due to an anomalous activation of different subtypes of T-cell: Th1, Th2, Th17 and regulatory T cells (Treg)[6]. Th1 cells differentiation is induced by IL-12 with the input of a pro-inflammatory cytokines INF-γ that promotes macrophages differentiation and additional pro-inflammatory cytokine production (IL-1β, IL-6, IL-8, granulocyte-macrophage colony stimulating factor (GM-CSF) and TNF-α). Instead, Th2 cells are able to produce pro-inflammatory cytokines as IL-4, IL-5 and IL-13. Th17 cells are also implicated in the process of chronic inflammation, through the production of cytokines as IL-17, IL-21 and IL-22. T-cell responses are monitored by Treg cells, which produce cytokines with anti-inflammatory action namely IL-10 and TGF-β. In IBD an unbalanced toward cytokines with pro-inflammatory action and the activation of myofibroblasts leads to an excessive deposition of extracellular matrix proteins modulate by pro-fibrotic (TGF-β, activins, CTGF, PDGF, IL-1,-4,-6,-13,-17,-21,-22,-23,-33) and anti-fibrotic molecules (Interferon (IFN)-α, IFN-γ, IL-7, IL-10, IL-12, Smad7)[6]. Nevertheless, TGF-β/Smad appears to act as a driving force of fibrosis promoting the progression of damage, other several profibrogenic factors (integrins, mammalian Target of Rapamycin (mTOR), Wnt/β-catenin pathway, Hedgehog and Notch signaling and Serotonin) and antifibrotic factors (PPAR-γ, adiponectin, Hippo, Klotho, Bone Morphogenetic Protein-7 (BMP-7) and Sirtuin 1 (Sirt1), which can directly or indirectly interact with this pathway[6] (Figure 3).

Discussion

PPAR-γ As Potential Therapeutical Target

The anti-fibrotic action of PPAR-γ agonists could be a new therapeutical approach for the treatment of several diseases including IBD. Thiazolidinedione, tioglitazone, rosiglitazone and pioglitazone are PPAR-γ agonists currently used as anti-diabetic drugs, which have also shown anti-fibrogenic effects in many organs (lungs, skin, kidneys, eyes, heart)[48,70,71] including intestinal fibrosis[22,48,49,62].

Recently, Chen et al[40] demonstrated an improvement of TNBS-induced intestinal fibrosis by using curcumin, a natural PPAR-γ agonist, that was proved to be as effective as rosiglitazone in reverting fibrotic markers (TGF-β, α- SMA, E-cadherin)[72]. 5-hydroxytryptamine 3 ($5HT_3$) receptor antagonists such as tropisetron, granisetron and ondansetron, currently used for

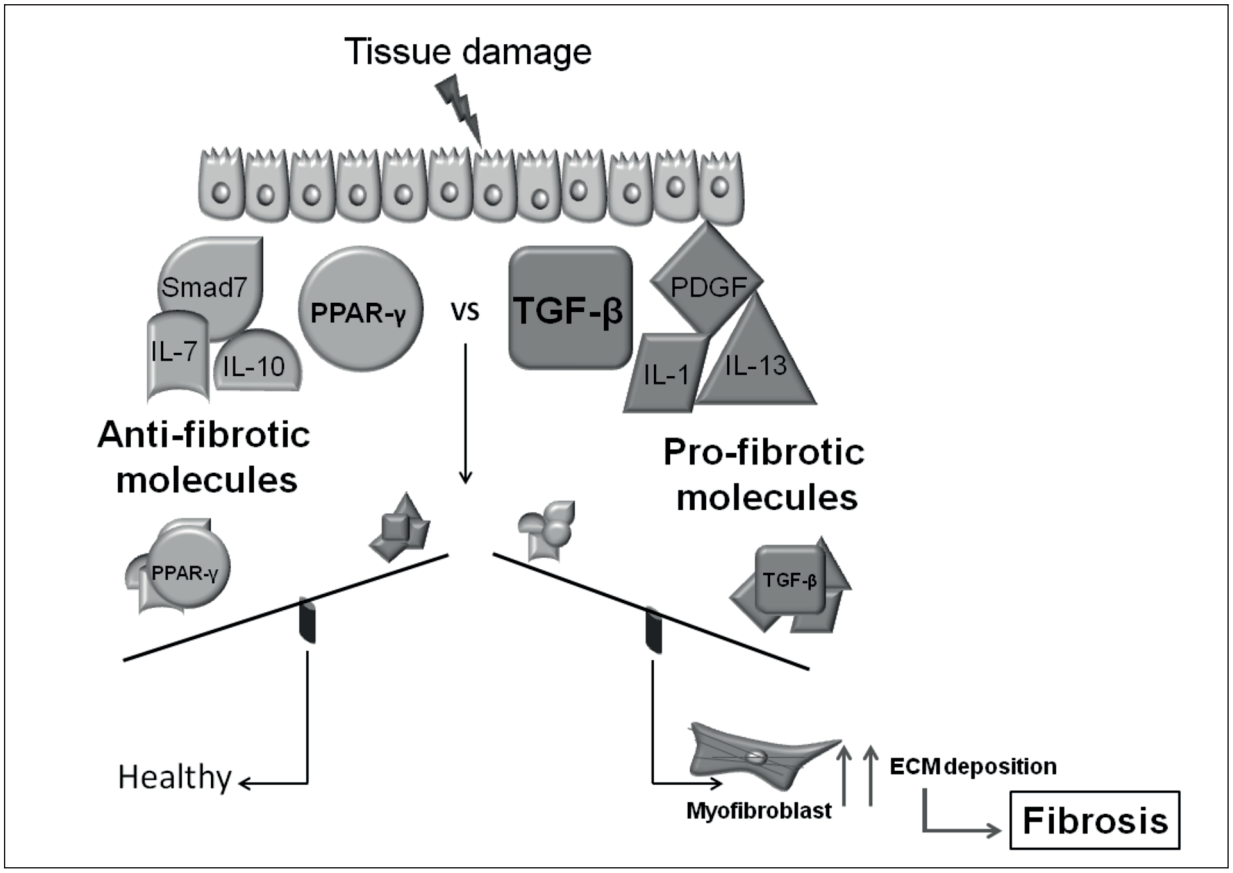

Figure 3. Cartoon showing the delicate balance between anti-fibrotic (PPAR-γ, Smad7, IL7, IL-10) and pro-fibrotic (TGF-β, IL-1, IL-6 and PDGF) molecules. Predominance of anti-fibrotic factors preserves the tissue integrity while overcoming of pro-fibrotic factors induces fibrosis.

ameliorate the chemotherapy-induced emesis, were able to reduce the levels of proinflammatory cytokines in experimental model of colitis[73] and also improve macroscopic and histological lesions of colonic wall during the course of colitis[74]. These molecules are PPAR-γ dependent since their effects are partially or completely reversed using a PPAR-γ antagonist such as GW9662 (Merck KGaA, Darmstadt, Germany)[75]. AL-1, an andrographolide-lipoic acid conjugate, has shown anti-inflammatory effects in mice with TNBS induced colitis in which it improved the clinical symptoms, macroscopic features and histological damage. AL-1 suppresses recruitment of immune inflammatory cells, down-modulating NF-κB pathway and the secretion of pro-inflammatory cytokines, and increasing the expression of PPAR-γ[76]. It is well known that both dysbiosis of microbiota, (especially a decrease in anaerobic bacteria including *Lactobacillus*, *Escherichia* and *Bacteroides*) and oxidative stress are responsible for the destruction of epithelial barrier[77]. Selenoproteins, especially GPx2 and SEPP1, act as antioxidants and show a protective role against oxidative stress being able to determine a down-regulation of NF-kB, which is highly activated in IBD. Selenium could inhibit the activation of NF-kB in intestinal epithelial cells, macrophages and dendritic cells via up-regulation and activation of PPAR-γ[78-81]. Moreover, it could be a promising candidate to ameliorate intestinal inflammation in IBD by creating a homeostatic environment in the gut and impacting commensal bacteria that regulate NF-kB and PPAR-γ[82-84]. It has also been confirmed that a probiotic mixture, known as VSL#3, is able to modulate gut local microbiota, decreasing colonic bacterial diversity and to favour local Conjugated Linoleic Acid (CLA) production. In the colon, CLA is implicated in PPAR-γ-dependent mechanisms of action that lead to the regulation of inflammatory reaction[85] through a mechanism involving epithelial TNF-α and NF-kB with the final result of resti-

tution of normal barrier function[86]. In DSS-induced colitis in mice, PPAR-γ levels could also be restored after administration of Portulaca extracts, a traditional Chinese herb containing multiminerals proteins, β carotene, vitamins and fatty acids [87], suggesting that Portulaca extracts could improve general symptoms of IBD decreasing the Disease Activity Index (DAI). Moreover, in DSS-induced colitis, the same herb extracts significantly reduced the expression of several cytokines (at both mRNA and protein levels) and increased the PPAR-γ expression[87]. It has been evaluated the activity of new PPAR-γ modulator, GED-0507-34 Levo (GED, Nogra Pharma Ltd., Dublin, Ireland), on fibrosis and EMT-associated mediators in DSS-induced colitis[22]. Di Gregorio et al[22] highlighted that GED was able to revert histological features of intestinal fibrosis, to normalize both the expression of main fibrosis markers and pro-fibrotic molecules and also to modulate the expression of other proteins (E-cadherin, ZEB1, Snail, β-catenin and GSK-3β) involved in fibrosis and EMT. All these effects, induced by daily oral administration of GED, were antagonized by simultaneous administration of the PPAR-γ inhibitor GW9662.

Conclusions

In the last twenty years a great deal of progress has been made in the study of the pathophysiology of intestinal fibrosis in IBD. However, to date surgery still remains the only resolutive treatment of the intestinal fibrostenosis, especially in Crohn's disease. Approximately 80% of patients with Crohn's disease undergo surgical resection within 10 years from diagnosis due to intestinal complications such as fibrostenotic lesions, alone or associated to intestinal fistulas and abscesses[88-90]. Pharmacological agents that induce PPAR-γ expression and its activation could be more extensively used in different experimental models of IBD and in clinical trials, in order to better evaluate and confirm both their anti-inflammatory and anti-fibrotic effectiveness. PPAR-γ agonists might open a new potential avenue for the treatment of IBD, specially for the prevention and treatment of intestinal fibrosis which is a common complication of these diseases.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Funding

This study was self-funded by the authors and their institutions (Department of Biotechnological and Applied Clinical Sciences and Department of Life, Health and Environmental Sciences, Gastroenterology Unit, University of L'Aquila, L'Aquila, Italy; Department of Anatomical, Histological, Forensic Medicine and Orthopedic Sciences, Sapienza University of Rome).

References

1) Rieder F, Brenmhoehl J, Leeb S, Scholmerich J, Rogler G. Wound healing and fibrosis in intestinal disease. Gut 2007; 56: 130-139.
2) Rieder F, Fiocchi C. Intestinal fibrosis in inflammatory bowel disease - Current knowledge and future prospective. J Crohns Colitis 2008; 2: 279-290.
3) Burke JP, Mulsow JJ, O'Keane C, Docherty NG, Watson RW, O' Connell PR. Fibrogenesis in Crohn's disease. Am J Gastroenterol 2007; 102: 439-448.
4) Fakhoury M, Negrulj R, Mooranian A, Al-Salami H. Inflammatory bowel disease: clinical aspects and treatments. J Inflamm Res 2014; 7: 113-120.
5) Latella G, Sferra R, Speca S, Vetuschi A, Gaudio E. Can we prevent, reduce or reverse intestinal fibrosis in IBD? Eur Rev Med Pharmacol Sci 2013; 17: 1283-1304.
6) Latella G, Di Gregorio J, Flati V, Rieder F, Lawrance IC. Mechanisms of initiation and progression of intestinal fibrosis in IBD. Scand J Gastroenterol 2015; 50: 53-65.
7) Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 2012; 18: 1028-1040.
8) Bettenworth D, Rieder F. Medical therapy of stricturing Crohn's disease: what the gut can learn from other organs - a systematic review. Fibrogenesis Tissue Repair 2014; 7: 5.
9) Rockey DC, Bell PD, Hill JA. Fibrosis--a common pathway to organ injury and failure. N Engl J Med 2015; 373: 96.
10) Wynn TA. Cellular and molecular mechanisms of fibrosis. J Phatol 2008; 214: 199-210.
11) Speca S, Giusti I, Rieder F, Latella G. Cellular and molecular mechanisms of intestinal fibrosis. World J Gastroenterol 2012; 18: 3635-3661.
12) Vetuschi A, Latella G, Pompili S, Gaudio E, Sferra R. Features of intestinal lesions in the clinical course of inflammatory bowel diseases. Ital J Anat Embriol 2014; 119: 286-303.
13) Toy LS, Scherl EJ, Kornbluth A, Marion JF, Greenstein AJ, Agus S, Greenstein SA, Charles G, Nathan F, Daniel H. Complete bowel obstruction following initial response to infliximab therapy for Chron's disease: a series of a newly described complication. Gastroenterology 2000; 118: 569.

14) Faubion WA, Loftus EV, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease. A population-based study. Gastroenterology 2001; 121: 255-260.

15) Degli-Esposti MA, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. Nat Rev Immunol 2005; 5: 112-124.

16) Rieder F. The gut microbiome in intestinal fibrosis:environmental protector or provocateur? Sci Transl Med 2013; 5: 190.

17) Lawrance IC, Rogler G, Bamias G, Breynaert C, Florholmen J, Pellino G, Reif S, Speca S, Latella G. Cellular and molecular mediators of intestinal fibrosis. J Crohns Colitis 2017; 11: 1491-1503.

18) Rieder F, Fiocchi C, Rogler G. Mechanisms, management and treatment of fibrosis in patients with inflammatory bowel diseases. Gastroenterology 2017; 152: 340-350.

19) Vetuschi A, Sferra R, Latella G, D'Angelo A, Catitti V, Zanninelli G, Continenza MA, Gaudio E. Smad3-null mice lack interstitial of Cajal in the colonic wall. Eur J Clin Invest 2006; 36: 41-48.

20) Fiocchi C. TGF-β/Smad signaling defects in inflammatory bowel disease: mechanisms and possible novel therapies for chronic inflammation. J Clin Invest 2001; 108: 523-526.

21) Flier SN, Tanjore H, Kokkotou EG, Sugumoto H, Zeisberg M. Identification of epithelial to mesenchymal transition as novel source of fibroblasts in intestinal fibrosis. J Biol Chem 2010; 285: 20202-20212.

22) Di Gregorio J, Sferra R, Speca S, Vetuschi A, Dubuquoy C, Desreumaux P, Pompili S, Cristiano L, Gaudio E, Flati V, Latella G. Role of glycogen synthase kinase-3β on ephitelial-to-mesenchymal transition in DSS-induced colorectal fibrosis. PLoS One 2017; 12: e0171093.

23) Zavadil J, Böttinger EP. TGF-beta and epithelial-to-mesenchymal transitions. Oncogene 2005; 2: 5764-5774.

24) Shapiro L, Weis WI. Structure and biochemistry of cadherins and catenins. Cold Spring Harb Perspect Biol 2009; 1: a003053.

25) Rastaldi MP. Epithelial-mesenchymal transition and its implications for the development of renal tubulointerstitial fibrosis. J Nephrol 2006; 19: 407-412.

26) Savagner P. Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. Bioessays 2001; 23: 912-923.

27) Willis BC, Borok Z. TGF-β induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol 2007; 293: 525-534.

28) Sferra R, Vetuschi A, Pompili S, Gaudio E, Speca S, Latella G. Expression of pro-fibrotic and anti-fibrotic molecules in dimethylnitrosamine-induced hepatic fibrosis. Phatol Res Pract 2017; 213: 58-65.

29) Inazaki K, Kanamaru Y, Kojima Y. Smad3 deficiency attenuates renal fibrosis, inflammation, and apoptosis after unilateral ureteral obustruction. Kidney Int 2004; 66: 597-604.

30) Latella G, Vetuschi A, Sferra R, Zanninelli G, D'Angelo A, Catitti V, Caprilli R, Flanders KC, Gaudio E. Smad3 loss confers resistance to the development of trinitrobenzene sulfonic acid-induced colorectal fibrosis. Eur J Clin Invest 2009; 39: 145-156.

31) Zanninelli G, Vetuschi A, Sferra R, D'Angelo A, Fratticci A, Continenza MA, Chiaramonte M, Gaudio E, Caprilli R, Latella G. Smad3 knock-out mice as a useful model to study intestinal fibrogenesis. World J Gastroenterol 2006; 12: 1211-1218.

32) Scharl M, Huber N, Lang S, Fürst A, Jehle E, Rogler G. Hallmarks of epithelial to mesenchymal transition are detectable in Crohn's disease associated intestinal fibrosis. Clin Transl Med 2015; 4: 1.

33) Greenburg G, Hay E.D. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J Cell Biol 1982; 95: 333-339.

34) Miettinen PJ, Ebner R, Lopez AR, Derynck R. TGF-beta inuced transdifferentation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol 1994; 127: 2021-2036.

35) Hales AM, Schulz MW, Chamberlain CG, McAvoy JW. TGF-beta 1 induces lens cells to accumulate alpha-smooth muscle actin, a marker for subcapsular cataracts. Curr Eye Res 1994; 13: 885-890.

36) Fan JM, Ng YY, Hill PA, Nikolic-Paterson DJ, Mu W, Atkins RC, Lan HY. Transforming growth factor-β regulates tubular epithelial-myofibroblast transdifferentiation in vitro. Kidney Int 1999; 56: 1455-1467.

37) Saika S, Kono-Saika S, Ohnishi Y, Sato M, Muragaki Y, Ooshima A, Sato M, Muragaki Y, Ooshima A, Yoo J, Flanders KC, Roberts AB. Smad3 signaling is required for epithelial-mesenchymal transition of lens epithelium after injury. Am J Pathol 2004; 164: 651-663.

38) Kasai H, Allen JT, Mason RM, Kamimura T, Zangh Z. TGF-β1 induces human alveolar epithelial to mesenchymal cell transition (EMT). Respir Res 2005; 6: 56.

39) Brighman CW, Borok Z. TGF-β induced EMT: mechanisms and implications for fibrotic lung disease. Am J Physiol Lung Cell Mol Physiol 2007; 293: 525-534.

40) Chen Y, Xiao Y, Ge W, Zhou K, Wen J, Yan W, Wang Y, Wang B, Qu C, Wu J, Xu L, Cai W. miR-200b inhibits TGF-β1-induced epithelial-mesenchymal transition and promotes growth of intestinal epithelial cells. Cell Death Dis 2013; 4: e541.

41) Zhao C, Chen W, Yang L, Chen S, Stimpson A, Diehl AM. PPARγ agonist prevent TGFβ1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 2006; 350: 385-391.

42) Houseknecht KL, Cole BM, Steele PJ. Peroxisome proliferator activated receptor gamma (PPARγ)

and its ligands: a review. Domest Anim Endocrinol 2002; 22: 1-23.

43) Zang G, Cheng T, Zheng M, Yi CG, Pan H, Li ZJ, Chen XL, Yu Q, Jiang LF, Zhou FY, Li XY, Yang JQ, Chu TG, Gao WY. Activation of peroxisome proliferator activated receptor gamma inhibits transforming growth factor beta1 induction of connective tissue growth factor and extracellular matrix in hypertrophic scar fibroblast in vitro. Arch Dermatol Res 2009; 301: 515-522.

44) Nakajiama A, Wada K, Miki H, Kubota N, Nakajima N, Terauchi Y. Endogenous PPARgamma mediates anti-inflammatory activity in murine ischemia reperfusion injury. Gastroenterology 2001; 120: 460-469.

45) Zhang F, Lu Y, Zheng S. Peroxisome proliferator-activated receptor-γ cross-regulation of signaling events implicated in liver fibrogenesis. Cell Signal 2012; 24: 596-605.

46) Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPAR-gamma ligands to inhibit the epithelial inflammatory response. J Clin Invest 1999; 104: 383-389.

47) Dubuquoy L, Rousseaux C, Thuru X, Peyrin-Biroulet L, Romano O, Chavatte P, Chamaillard M, Desreumaux P. PPARgamma as a new therapeutic target in inflammatory bowel diseases. Gut 2006; 55: 1341-1349.

48) Koo JB, Nam MO, Jung Y, Yoo J, Kim DH, Kim G, Sung PH, Kwang JL, Jun HY. Anti-fibrogenic effect of PPAR-γ agonists in human intestinal myofibroblasts. BMC Gastroenterol 2017; 17: 73.

49) Wei J, Ghosh AK, Sargent JL, Komura K, Wu M, Huang QQ, Jain M, Whitfield ML, Feghali-Bostwick C, Varga J. PPARγ downregulation by TGFβ in fibroblast and impaired expression and function in systemic sclerosis: a novel mechanism for progressive fibrogenesis. PLoS One 2010; 5: e13778.

50) Xiong Z, Huang H, Li J, Guan Y, Wang H. Anti-inflammatory effect of PPARgamma in cultured human mesangial cells. Ren Fail 2004; 26: 497-505.

51) Sharma S, Sharma P, Kulurkar P, Singh D, Kumar D, Patial V. Iridoid glycosides fraction from Picrorhiza kurroa attenuates cyclophosphamide-induced renal toxicity and peripheral neuropathy via PPAR-γ mediated inhibition of inflammation and apoptosis. Phytomedicine 2017; 36: 108-117.

52) Kim SR, Lee KS, Park SJ, Min KH, Jin SM, Lee YC. Involvement of IL-10 in peroxisome proliferator-activated receptor gamma-mediated anti-inflammatory response in asthma. Mol Pharmacol 2005; 68: 1568-1575.

53) Laksmi SP, Reddy AT, Zhang Y, Sciurba FC, Mallampalli RK, Duncan SR, Reddy RC. Down-regulated peroxisome proliferator-activated receptor γ (PPARγ) in lung epithelial cells promotes a PPARγ agonist-reversible proinflammatory phenotype in chronic obstructive pulmonary disease (COPD). J Biol Chem 2014; 289: 6383-6393.

54) Reddy AT, Lakshmi SP, Kleinhenz JM, Sutliff RL, Hart CM, Reddy RC. Endothelial cell peroxisome proliferator-activated receptor γ reduces endotoxemic pulmonary inflammation and injury. J Immunol 2012; 189: 5411-5420.

55) Rousseaux C, Lefebvre B, Dubuquoy L, Lefebvre P, Romano O, Auwerx J, Metzger D, Wahli W, Desvergne B, Naccari GC, Chavatte P, Farce A, Bulois P, Cortot A, Colombel JF, Desreumaux P. Intestinal antiinflammatory effect of 5-aminosalicylic acid is dependent on peroxisome proliferator-activated receptor-gamma. J Exp Med 2005; 201: 1205-1215.

56) Hyong A, Jadhav V, Lee S, Tong W, Rowe J, Zhang JH, Tang J. Rosiglitazone, a PPAR gamma agonist, attenuates inflammation after surgical brain injury in rodents. Brain Res 2008; 1215: 218-224.

57) Yu JH, Kim KH, Kim H. SOCS 3 and PPAR-gamma ligands inhibit the expression of IL-6 and TGF-beta1 by regulating JAK2/STAT3 signaling in pancreas. Int J Biochem Cell Biol 2008; 40: 677-688.

58) Aprahamian T, Bonegio RG, Richez C, Yasuda K, Chiang LK, Sato K, Walsh K, Rifkin IR. The peroxisome proliferator-activated receptor gamma agonist rosiglitazone ameliorates murine lupus by induction of adiponectin. J Immunol 2009; 182: 340-346.

59) Kawai T, Masaki T, Doi S, Arakawa T, Yokoyama Y, Doi T, Kohno N, Yorioka N. PPAR-gamma agonist attenuates renal interstitial fibrosis and inflammation through reduction of TGF-beta. Lab Invest 2009; 89: 47-58.

60) Kapoor M, McCann M, Liu S, Huh K, Denton CP, Abraham DJ, Andrew Leask. Loss of peroxisome proliferator-activated receptor gamma in mouse fibroblasts results in increased susceptibility to bleomycin-induced skin fibrosis. Arthritis Rheum 2009; 60: 2822-2829.

61) Pirat C, Farce A, Lebègue N, Renault N, Furman C, Millet R, Yous S, Speca S, Berthelot P, Desreumaux P, Chavatte P. Targeting peroxisome proliferator-activated receptors (PPARs): development of modulators. J Med Chem 2012; 55: 4027-4061.

62) Speca S, Rousseaux C, Dubuquoy C, Rieder F, Vetuschi A, Sferra R, Giusti I, Bertin B, Dubuquoy L, Gaudio E, Desreumaux P, Latella G. Novel PPARγ Modulator GED-0507-34 levo ameliorates inflammation-driven intestinal fibrosis. Inflamm Bowel Dis 2016; 22: 279-292.

63) Marra F, Efsen E, Romanelli RG, Caligiuri A, Pastacaldi S, Batignani G, Bonacchi A, Caporale R, Laffi G, Pinzani M, Gentilini P. Ligands of peroxisome proliferator-activated receptor gamma modulate profibrogenic and proinflammatory actions in hepatic stellate cells. Gastroenterology 2000; 119: 466-478.

64) Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF Jr, Motomura K, Anania FA, Willson TM, Tsukamoto H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000; 275: 35715-35722.

65) Hazra S, Miyahara T, Rippe RA, Tsukamoto H. PPAR Gamma and hepatic stellate cells. Comp Hepatol 2004; 3: S7.

66) Tomita K, Azuma T, Kitamura N, Nishida J, Tamiya G, Oka A, Inokuchi S, Nishimura T, Suematsu M, Ishii H. Pioglitazone prevents alcohol-induced fatty liver in rats through up-regulation of c-Met. Gastroenterology 2004; 126: 873-885.

67) Zhao C, Chen W, Yang L, Chen L, Stimpson SA, Diehl AM. PPARgamma agonists prevent TGF-beta1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 2006; 350: 385-391.

68) Bae MA, Rhee SD, Jung WH, Ahn JH, Song BJ, Cheon HG. Selective inhibition of activated stellate cells and protection from carbon tetrachloride-induced liver injury in rats by a new PPARgamma agonist KR62776. Arch Pharm Res 2010; 33: 433-442.

69) Yu J, Zhang Z, Li Z, Feng X, He L, Liu S, Mao J, Wang G, Wang X. Peroxisome proliferator-activated receptor-gamma (PPARgamma) agonist improves coronary artery endothelial function in diabetic patients with coronary artery disease. J Int Med Res 2010; 38: 86-94.

70) Lin Q, Fang LP, Zhou WW, Liu XM. Rosiglitazone inhibits migration, proliferation, and phenotypic differentiation in cultured human lung fibroblasts. Exp Lung Res 2010; 36: 120-128.

71) Guo N, Woeller CF, Feldon SE, Phipps RP. Peroxisome proliferator-activated receptor gamma ligands inhibit transforming growth factor-beta-induced, hyaluronan-dependent, T cell adhesion to orbital fibroblasts. J Biol Chem 2011; 27: 18856-18867.

72) Xu S, Jiang B, Wang H, Shen C, Chen H, Zeng L. Curcumin suppresses intestinal fibrosis by inhibition of PPARγ-mediated epithelial-mesenchymal transition. Evid Based Complement Alternat Med 2017; 7876064.

73) Mousavizadeh K, Rahimian R, Fakhfouri G, Aslani FS, Ghafourifar P. Anti-inflammatory effects of 5-HT receptor antagonist, tropisetron on experimental colitis in rats. Eur J Clin Invest 2009; 39: 375-383.

74) Fakhfouri G, Rahimian R, Daneshmand A, Bahremand A, Rasouli MR, Dehpour AR, Mehr SE, Mousavizadeh K. Granisetron ameliorates acetic acid-induced colitis in rats. Hum Exp Toxicol 2010; 29: 321-328.

75) Rahimian R, Zirak MR, Keshavarz M, Fakhraei N, Mohammadi-Farani A, Hamdi H, Mousavizadeh K. Involvement of PPARγ in the protective action of tropisetron in an experimental model of ulcerative colitis. Immunopharmacol Immunotoxicol 2016; 20: 1-9.

76) Yang Y, Yan H, Jing M, Zhang Z, Zhang G, Sun Y, Shan L, Yu P, Wang Y, Xu L. Andrographolide derivative AL-1 ameliorates TNBS-induced colitis in mice: involvement of NF-κB and PPAR-γ signaling pathways. Sci Rep 2016; 6: 29716.

77) Desreumaux P, Colombel JF. Intestinal flora and Crohn's disease. Ann Pharm Fr 2003; 61: 276-281.

78) Chu FF, Esworthy RS, Doroshow JH. Role of Se-dependent glutathione peroxidases in gastrointestinal inflammation and cancer. Free Radic Biol Med 2004; 36: 1481-1495.

79) Te Velde AA, Pronk I, de Kort F, Stokkers PC. Glutathione peroxidase 2 and aquaporin 8 as new markers for colonic inflammation in experimental colitis and inflammatory bowel diseases: an important role for H2O2? Eur J Gastroenterol Hepatol 2008; 20: 555-560.

80) Narayan V, Kudva AK, Prabhu KS. Reduction of tetrathionate by mammalian thioredoxin reductase. Biochemistry 2015; 54: 5121-5124.

81) Hoffmann PR. An emerging picture of the biological roles of selenoprotein K. In Selenium: its molecular biology and role in human health; Springer Science and Business Media: New York, NY, USA 2012; pp. 335-344.

82) Kaci G, Lakhdari O, Doré J, Ehrlich SD, Renault P, Blottière HM, Delorme C. Inhibition of the NF-kappaB pathway in human intestinal epithelial cells by commensal streptococcus salivarius. Appl Environ Microbiol 2011; 77: 4681-4684.

83) Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, Torres TP, Byndloss AJ, Faber F, Gao Y, Litvak Y, Lopez CA, Xu G, Napoli E, Giulivi C, Tsolis RM, Revzin A, Lebrilla CB, Bäumler AJ. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. Science 2017; 357: 570-575.

84) Kasaikina MV, Kravtsova MA, Lee BC, Seravalli J, Peterson DA, Walter J, Legge R, Benson AK, Hatfield DL, Gladyshev VN. Dietary selenium affects host selenoproteome expression by influencing the gut microbiota. FASEB J 2011; 25: 2492-2499.

85) Bassaganya-Riera J, Viladomiu M, Pedragosa M, De Simone C, Carbo A, Shaykhutdinov R, Jobin C, Arthur JC, Corl BA, Vogel H, Storr M, Hontecillas R. Probiotic bacteria produce conjugated linoleic acid locally in the gut that targets macrophage PPAR γ to suppress colitis. PLoS One 2012; 7: e31238.

86) Pagnini C, Saeed R, Bamias G, Arseneau KO, Pizarro TT, Cominelli F. Probiotics promote gut health through stimulation of epithelial innate immunity. Proc Natl Acad Sci U S A 2010; 107: 454-459.

87) Kong R, Luo H, Wang N, Li J, Xu S, Chen K, Feng J, Wu L, Li S, Liu T, Lu X, Xia Y, Shi Y, Zhou Y, He W, Dai Q, Zheng Y, Lu J. Portulaca extract attenuates development of dextran sulfate sodium induced colitis in mice through activation of PPARγ. PPAR Res 2018; 6079101.

88) Bernell O, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. Ann Surg 2000; 231: 38-45.

89) Francis H, Hanan K. Crohn's disease: a clinical update. Therap Adv Gastroenterol 2015; 8: 352-359.

90) Dasari B, McKay D, Gardiner K. Laparoscopic versus surgery for small bowel Crohn's disease. Cochrane Database Syst Rev 2011; (1): CD006956.