RESEARCH PAPER

Correlation of Dickkopf-1 with Inflammation in Crohn Disease

Mi Jin Kim[1,2] and Yon Ho Choe[1]

From [1]Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul; and [2]Chungnam National University Hospital, College of Medicine, Chungnam National University, Daejeon; Korea.

Correspondence to: Mi Jin Kim, Department of Pediatrics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul, 06351, Korea. mijin1217.kim@samsung.com
Received: March 14, 2019;
Initial review: April 16, 2019;
Accepted: September 03, 2019.

Objective: To explore the potential roles of Dickkopf-1 (DKK-1) and β-catenin in Crohn disease, and to evaluate the effects of a tumor necrosis factor (TNF)-α inhibitor on Wnt signaling in patients with the disease. **Methods:** We enrolled 21 patients who received infliximab treatment for one year and achieved clinical remission during the treatment period. Disease activity was graded according to the Pediatric Crohn's Disease Activity Index (PCDAI). Peripheral blood and colonic mucosal specimens were collected from all patients with Crohn disease and from 14 healthy controls. DKK-1 levels in serum were detected by enzyme-linked immunosorbent assay (ELISA). Total RNA for DKK-1 and β-catenin from the frozen colonic tissue were obtained *via* real-time quantitative reverse transcription-polymerase chain reaction (RT-PCR). Serum C-reactive protein (CRP) levels, erythrocyte sedimentation rates (ESR), and albumin were also measured in patients with Crohn disease before and after infliximab therapy. **Results:** The serum levels of DKK-1 were significantly higher in patients with Crohn disease than in healthy controls (P=0.003) and were decreased in those treated with infliximab (P=0.026). Serum DKK-1 level was correlated with levels of ESR (r=0.527, P=0.025), CRP (r=0.502, P=0.034), albumin (r=0.363, P=0.021) and PCDAI (r =0.462, P=0.054) in Crohn disease. DKK-1 mRNA expression in the colonic mucosa was higher in patients than in controls and decreased after infliximab treatment. β-catenin expression in the colonic mucosa was lower in patients than in controls and increased after infliximab treatment. However, the differences were not significant (P>0.05). **Conclusions:** DKK-1 might be an important mediator of the pathogenesis of Crohn disease, and changes in DKK-1 levels may serve as biomarkers of inflammation in these patients.

Keywords: β-*catenin, Infliximab*, *Pathogenesis*, *Wnt signaling.*

Crohn disease (CD) is a multifactorial disease of unknown etiology characterized by a chronic inflammation of the entire gastrointestinal tract, most commonly occurring in the distal ileum and colon [1]. Immune, genetic, and environmental factors are thought to contribute to CD [2]. Cytokine tumor necrosis factor (TNF)-α contributes substantially to the pathology of Crohn disease, a role that is highlighted by the responsiveness of the disease to TNF-α blocking agents [3].

Signaling by the Wnt family of secreted lipoproteins plays a central role in embryogenesis and tissue homeostasis [4,5]. Abnormal Wnt/β-catenin signaling is associated with many diseases in humans, including cancer and osteoporosis [5,6]. β-catenin is a fundamental component of the Wnt signaling pathway, which acts as a coactivator through its ability to recruit components that promote chromatin remodeling and the transcriptional process [7]. Dickkopf-1 (DKK-1) is a secreted glycoprotein that has been shown to act as a potent inhibitor of the canonical Wnt/β-catenin signaling pathway [8,9]. DKK-1 plays essential roles in many biological processes, ranging from the induction of anterior mesoderm formation and head development during embryogenesis to bone formation and bone mass regulation in adult organisms [10]. However, relatively little is known about the localization of Wnt signaling components and the importance of DKK-1 within the intestine.

The Wnt signaling pathway is associated with regulation of homeostasis within the colonic stem cell compartment, controlling the balance between proliferation and differentiation [11,12]. Questions regarding whether DKK-1 is associated with inflammation in Crohn disease, or whether there are correlations between DKK-1 and clinical and laboratory characteristics, remain unanswered (***Web Fig.* 1**).

In this study, we explored the potential roles of DKK-1 and β-catenin in CD and evaluated the effects of a TNF-α inhibitor (infliximab) on Wnt signaling in patients with CD.

METHODS

From among pediatric patients who were diagnosed with Crohn disease in accordance with the European Society for Pediatric Gastroenterology, Hepatology and Nutrition - Porto criteria [13] at the Samsung Medical Center between January 2010 and August 2013, we enrolled 21 patients who received infliximab treatment for one year and who achieved clinical remission during the treatment period. Disease activity was graded according to the Pediatric Crohn's Disease Activity Index (PCDAI) [14]. We recruited 14 children with macroscopically and histologically normal mucosa and no evidence of any underlying gastrointestinal conditions as healthy controls.

A monoclonal immunoglobulin G1 chimeric antibody directed against TNF-α (infliximab) was administered to CD patients by intravenous infusion at a dose of 5 mg/kg at weeks 0, 2, and 6, and this course was repeated every 8 weeks for 10 months thereafter.

This study was approved by the Institutional Review Board of the Samsung Medical Center. All participants provided written informed consent before participation in this study.

Peripheral blood samples were collected to assess DKK-1 levels by enzyme-linked immunosorbent assay (ELISA) from all Crohn disease patients and healthy control individuals. In patients with Crohn disease, blood collection was performed before infliximab therapy and after the eighth course of infliximab therapy. Serum C-reactive protein (CRP) levels, erythrocyte sedimentation rates (ESR), and albumin were also measured in patients with Crohn disease before and after infliximab therapy.

Colonic mucosal specimens from all 21 CD patients and 14 healthy controls were assessed for mRNA expressions of DKK-1 and β-catenin. Colonoscopy was performed for all patients by a single pediatric gastroenterologist, who collected one or two additional biopsies within close proximity of the area in which biopsies were taken for routine histology. In patients with Crohn disease, mucosal biopsies were performed before infliximab therapy and after eight courses of infliximab therapy. The specimens were frozen and stored at -80°C for RNA isolation.

DKK-1 levels were determined in serum samples from Crohn disease patients and healthy controls using a commercially available LINCOplex kit (Millipore, Billerica, MA, USA) and a Luminex analyzer, according to the manufacturer's instructions. The result was calculated through the Bio-Plex Manager Software (Bio-Rad Laboratories, Hercules, CA, USA) and the cytokine concentration in plasma was expressed as pg/mL.

Total RNA for DKK-1 and β-catenin from the frozen colonic tissue was obtained using Trizol reagent (Invitrogen) according to the manufacturer's instructions. The amount and purity of the obtained RNA was determined using a ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA). Reverse transcription was performed using the SuperScript III First-Strand Synthesis System for RT-PCR (Invitrogen). cDNA was prepared from 1 μg of mRNA with oligo/dT according to the manufacturer's instructions. Real-time PCR was constructed using commercially available assays for the *DKK-1* gene (assay ID Hs00183740_mL, Genbank accession number NM_012242.2; Applied Biosystems, Foster City, CA, USA), β-catenin (assay ID Hs01076483_mL, Genbank accession number NM_001904.3), and human endogenous control GAPDH (assay ID Hs99999905_mL, Genbank accession number NM_002046.3) in combination with the TaqMan Universal PCR Master Mix (Applied Biosystems). Then, PCR was performed in a 7900HT real-time PCR system (Applied Biosystems). Comparative analyses of each gene were performed using computer programs SDS 2.3 and RQ 2.1 (Applied Biosystems). The relative gene expressions (RQ) were calculated using the 2_∇∇CT method.

Statistical analyses: Statistical analyses were performed using the Mann-Whitney U-test for unpaired samples and the Wilcoxon signed-rank test for paired samples using SPSS 24.0 (SPSS, Chicago, IL, USA). Correlations between DKK-1 or β-catenin expression from CD patients and disease activity were determined by simple linear regression. Values of $P<0.05$ were considered statistically significant.

RESULTS

The enrolled children included 21 with Crohn disease (16 males) and 14 healthy controls (12 males). The mean age of the healthy controls was patients with Crohn disease did not differ significantly [15.9 (1.4) *vs* 14.4 (2.1) years]. After one year of infliximab treatment, disease activity according to PCDAI score, and from ESR and C-reactive protein declined significantly (***Table* I**).

The plasma level of DKK-1 in CD patients before infliximab therapy was significantly higher than that of healthy controls (***Fig.* 1a**, $P=0.003$). After infliximab therapy, the level of DKK-1 in CD patients was decreased compared to that before therapy (***Fig.* 1a**, $P=0.03$). Correlations were found between DKK-1 in plasma from

TABLE I BASELINE CHARACTERISTICS AND CLINICAL OUTCOMES

	Controls (n=14)	*Patients (n=21)*	
		Before IFX Tx	*After IFX Tx*
Albumin	4.7 (0.2)	3.9 (0.4)	4.5 (0.3)
ESR (mm/hr)	11.1 (11.8)	61.9 (31.0)	16.1 (14.2)
CRP (mg/dL)	0.6 (1.8)	2.1 (2.2)	0.2 (0.3)
PCDAI score		35.2 (10.8)	4.2 (4.9)

IFX Tx: *infliximab therapy; ESR*: *Erythrocyte sedimentation rate; CRP*: *C-reactive protein; PCDAI*: *Pediatric Crohn Disease Activity Index.*

children with Crohn disease and ESR (r=0.527, *P*=0.025), CRP (r=0.502, *P*=0.034), albumin (r=0.363, *P*=0.021) and PCDAI (r=0.462, *P*=0.054) (***Fig***. **1b**).

DKK-1 mRNA expression in the colonic mucosa was higher in patients than in controls [2.18 (1.69) *vs* 1.60 (1.48)] and decreased after infliximab treatment [2.18 (1.69) *vs* 1.83 (2.32)]. β-catenin expression in the colonic mucosa was lower in patients than in controls [0.69 (1.19) *vs* 1.02 (1.25)] and increased after infliximab treatment [0.69 (1.19) *vs* 0.92 (0.71)]. However, there were no significant differences between each groups before and after infliximab therapy or compared to normal controls. There were no correlations between DKK-1 or β-catenin in the colonic mucosa from children with Crohn disease and disease activity.

DISCUSSION

Since Wnt signaling is known to cause cancer and malformations, many animal models are now in use to assess the pathogenesis of various diseases and the toxicity of therapeutic agents. Recently, a number of diseases have been associated with abnormalities in Wnt signaling, including adipogenesis [15], schizophrenia [16], and Alzheimer disease [17], as well as cancer and developmental difficulties. Therefore, the role of Wnt signaling as a pathogenic process in various diseases has been examined in the context of efforts to identify the role of Wnt signaling in cancer. DKK-1 is a downstream target gene for Wnt/β-catenin signaling, and has been shown to regulate Wnt signaling through negative feedback [18,19]. Inflammatory stimuli such as TNF-α induce DKK-1 release in various cells [20]. Inflammatory bowel disease can lead to chronic relapsing inflammation of the gastrointestinal tract. The Wnt antagonist DKK-1 is induced by inflammatory cytokines and that exacerbates intestinal inflammation. However, little is known about the localization of Wnt signaling components and the importance of DKK-1 in inflammatory bowel disease, especially CD.

In this study, we found that DKK-1 was inhibited by TNF-α inhibition in CD patients. The plasma level of DKK-1 in CD patients before infliximab therapy was significantly higher than that of healthy controls. After infliximab therapy, the level of DKK-1 in CD patients was decreased compared to that before therapy. Correlations were also found between DKK-1 in plasma from CD patients and inflammatory marker or disease activity. Inhibition of DKK-1 may increase the transcriptional activity and survival signaling pathway of β-catenin, thereby promoting epithelial cell proliferation and wound repair. These results suggest that the Wnt signaling

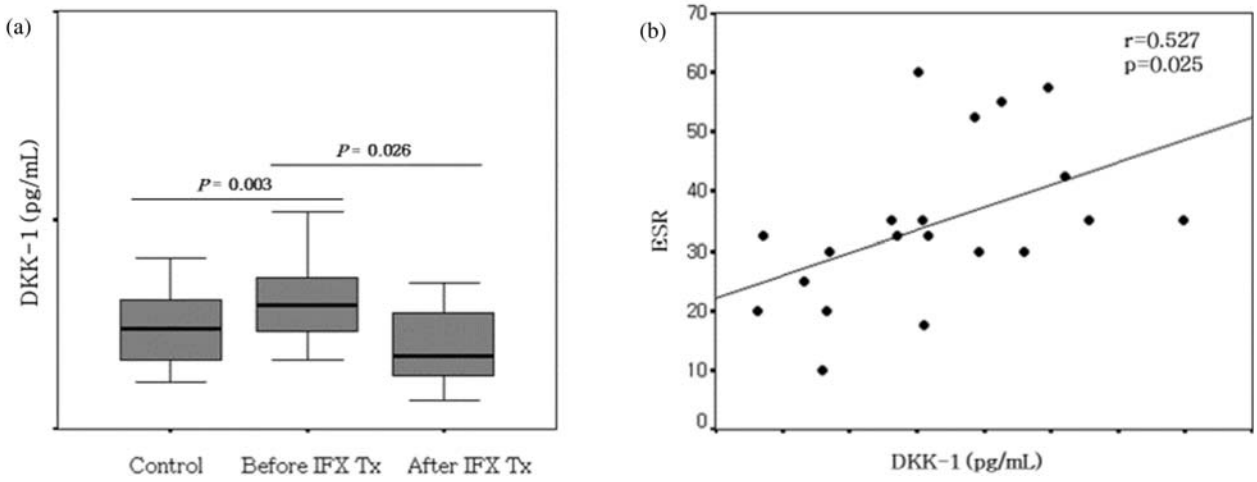


FIG. 1 *Expression of Dickkopf-1 in peripheral blood. (a) The serum levels of Dickkopf-1 were significantly higher in patients with Crohn disease than in healthy controls (P=0.003) and were decreased in Crohn disease patients treated with infliximab (P=0.026). (b) Serum Dickkopf-1 levels were correlated with levels of ESR (r=0.527, P=0.025) in patients with Crohn disease.*

WHAT THIS STUDY ADDS?

- Dickkopf-1 (DKK-1) may be a mediator in the pathophysiology of Crohn disease.
- DKK-1 level are higher with increasing inflammation and decrease with treatment in children with Crohn disease.

activator can be used to maintain the regeneration of gut epithelium, homeostasis, and to treat CD.

The number of patients was relatively low, which requires a cautious interpretation of the results. Preventive and therapeutic Wnt/β-catenin activation led to a significant improvement of CD. Future *in vitro* and *in vivo* studies will certainly provide more insights into the principles underlying decreased Wnt/β-catenin signaling in CD, and whether therapeutic Wnt/β-catenin activation will present as a future therapeutic tool in this chronic disease.

Since DKK-1, as a part of the canonical Wnt/β-Catenin pathway, has already been shown to be involved in pathological processes such as cell migration and invasion, this observation also supports the hypothesis that DKK-1 might be involved in the pathogenesis of CD patients. DKK-1 may be an important mediator in the pathophysiology of CD, and DKK-1 level may be a good marker for predicting the inflammation and prognosis of CD patients. Our findings warrant further research examining the potential of DKK-1 as a therapeutic agent of CD.

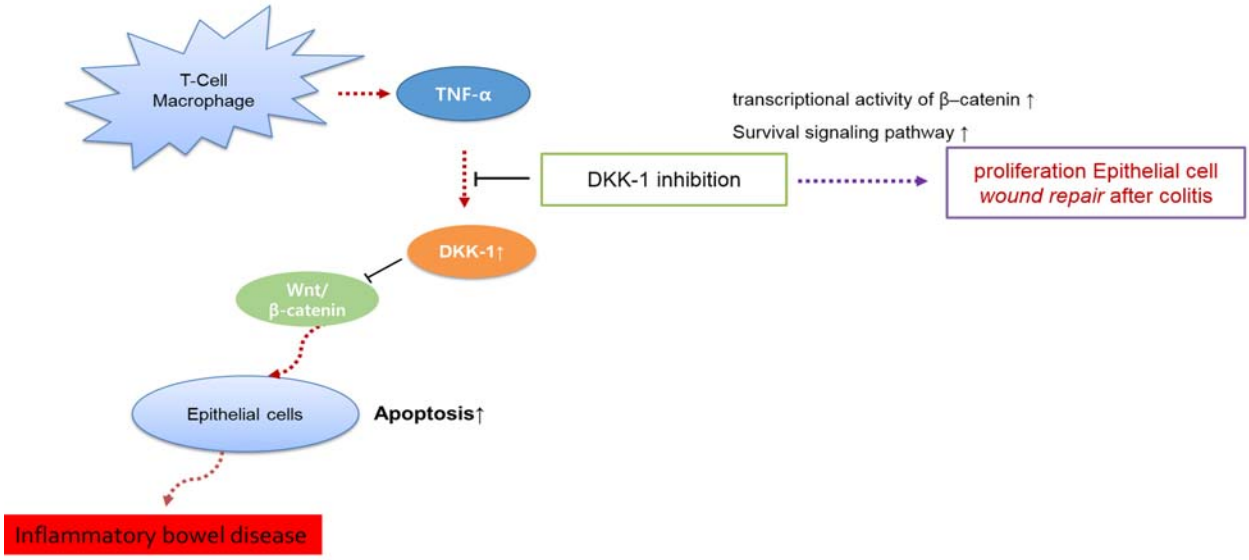
Contributors: Both authors have contributed, designed and approved the final version of manuscript, and are accountable for all aspects related to the study.

Funding: This work was supported by the research fund of Chungnam National University.

Competing interests: None stated.

REFERENCES

1. Podolsky DK. Inflammatory bowel disease. N Engl J Med. 2002;347:417-24.
2. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427-34.
3. Papadakis KA, Targan SR. Role of cytokines in the pathogenesis of inflammatory bowel disease. Annu Rev Med. 2000;51:289-98.
4. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol. 2004;20:781-810.
5. Clevers H. Wnt/beta-catenin signaling in development and disease. Cell. 2006;127:469-80.
6. Moon RT, Kohn AD, De Ferrari GV, Kaykas A. WNT and beta-catenin signalling: diseases and therapies. Nat Rev Genet. 2004;5:691-701.
7. Willert K, Jones KA. Wnt signaling: is the party in the nucleus? Genes Dev. 2006;20:1394-404.
8. Fedi P, Bafico A, Nieto Soria A, Burgess WH, Miki T, Bottaro DP, *et al*. Isolation and biochemical characterization of the human Dkk-1 homologue, a novelinhibitor of mammalian Wnt signaling. J Biol Chem. 1999;274:19465-72.
9. Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. Nature. 1998;391:357-62.
10. Niehrs C. Function and biological roles of the Dickkopf family of Wnt modulators. Oncogene. 2006;25:7469-81.
11. de Lau W, Barker N, Clevers H. WNT signaling in the normal intestine and colorectal cancer. Front Biosci. 2007;12:471-91.
12. Pinto D, Clevers H. Wnt control of stem cells and differentiation in the intestinal epithelium. Exp Cell Res. 2005;306:357-63.
13. Inflammatory bowel disease in children and adolescents: Recommendations for diagnosis-the Porto criteria. J Pediatr Gastroenterol Nutr. 2005;41:1-7.
14. Hyams JS, Ferry GD, Mandel FS, Gryboski JD, Kibort PM, Kirschner BS, *et al*. Development and validation of a pediatric Crohn's disease activity index. J Pediatr Gastroenterol Nutr. 1991;12:439-47.
15. Bennett CN, Ross SE, Longo KA, Bajnok L, Hemati N, Johnson KW, *et al*. Regulation of Wnt signaling during adipogenesis. J Biol Chem. 2002;277:30998-1004.
16. Miyaoka T, Seno H, Ishino H. Increased expression of Wnt-1 in schizophrenic brains. Schizophr Res. 1999;38:1-6.
17. Jaworski T, Dewachter I, Lechat B, Gees M, Kremer A, Demedts D, *et al*. GSK-3alpha/beta kinases and amyloid production in vivo. Nature. 2011;480:E4-5; discussion E6.
18. Niida A, Hiroko T, Kasai M, Furukawa Y, Nakamura Y, Suzuki Y, *et al*. DKK1,anegative regulator of Wnt signaling, is a target of the beta-catenin/TCF pathway. Oncogene. 2004;23:8520-6.
19. Gonzalez-Sancho JM, Aguilera O, Garcia JM, Pendas-Franco N, Pena C, Cal S, *et al*. The Wnt antagonist DICKKOPF-1 gene is a downstream target of beta-catenin/TCF and is downregulated in human colon cancer. Oncogene. 2005;24:1098-103.
20. Diarra D, Stolina M, Polzer K, Zwerina J, Ominsky MS, Dwyer D, *et al*. Dickkopf-1 is a master regulator of joint remodeling. Nat Med. 2007;13:156-63.



WEB FIG. 1 *Diagrammatic cascade of Dickkopf-1 in inflammatory bowel disease.*