J Gastroenterol (2016) 51:853–861
DOI 10.1007/s00535-016-1220-2

The Japanese Society of Gastroenterology

CrossMark

REVIEW

Indole compounds may be promising medicines for ulcerative colitis

Shinya Sugimoto[1] · Makoto Naganuma[1] · Takanori Kanai[1]

Received: 21 March 2016 / Accepted: 25 April 2016 / Published online: 9 May 2016
© Japanese Society of Gastroenterology 2016

Abstract Indole compounds are extracted from indigo plants and have been used as blue or purple dyes for hundreds of years. In traditional Chinese medicine, herbal agents in combination with Qing-Dai (also known as indigo naturalis) have been used to treat patients with ulcerative colitis (UC) and to remedy inflammatory conditions. Recent studies have noted that indole compounds can be biosynthesized from tryptophan metabolites produced by various enzymes derived from intestinal microbiota. In addition to their action on indole compounds, the intestinal microbiota produce various tryptophan metabolites that mediate critical functions through distinct pathways and enzymes. Furthermore, some indole compounds, such as indigo and indirubin, act as ligands for the aryl hydrocarbon receptor. This signaling pathway stimulates mucosal type 3 innate lymphoid cells to produce interleukin-22, which induces antimicrobial peptide and tight junction molecule production, suggesting a role for indole compounds during the mucosal healing process. Thus, indole compounds may represent a novel treatment strategy for UC patients. In this review, we describe the origin and function of this indole compound-containing Chinese herb, as well as the drug development of indole compounds.

Keywords Inflammatory bowel diseases · Ulcerative colitis · Chinese herbal medicine · Aryl hydrocarbon receptor · Indole

✉ Takanori Kanai
takagast@z2.keio.jp

[1] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Introduction

Wilks and Moxon from Guy's Hospital in England first reported on ulcerative colitis (UC) in 1875 [1]. Although UC is a relatively new disease, its incidence has increased drastically in developed Western countries over the past 50 years [2]. In developed Asian countries such as Japan, Korea, and China, the emergence of UC has coincided with the hosting of the Olympic games, and increased thereafter [3–5]. In addition to colorectal cancer, UC is now a critical illness in developed countries [6]. Crohn's disease (CD) is another form of inflammatory bowel disease (IBD), and while the incidence of CD was initially lower than that of UC, it is gradually increasing as well in developed Western countries [5]. Because both UC and CD are thought to be chronic immune-mediated diseases, various types of immunological control therapies are applicable to these patients [6–9]. However, it is obvious that immunodysregulation is not the cause of UC but rather the effect, and possible causes of UC may be rooted in genetic and environmental factors [10].

The main cause of UC is likely related to modern lifestyle practices in Western and Asian countries rather than genetic factors, given that humans emerged a half-million years ago, but the incidence of UC has increased only within the past 100 years [3]. Specifically, increasingly hygienic environments, Western-style diets (high fat and low dietary fiber), increased mental stress, and inadequate exercise may have acted in concert to alter the intestinal luminal environment and induce "dysbiosis," which includes the loss of microbiota diversity and an imbalanced microbial composition, resulting in the emergence and increased incidence of UC in developed countries [11–16]. It is notable that this dysbiosis may be a major cause not only of intestinal diseases such as IBD [17] and

irritable bowel syndrome (IBS) [18], but also of extra-intestinal diseases such as rheumatoid disease [19], obesity, diabetes mellitus [20], neuropsychiatric disorders, multiple sclerosis [21], and cancer [22].

Humans in modern times may be consuming smaller amounts of indole compound-containing green vegetables than they were in the past. Although broccoli and cabbage are known to be rich in indole compounds [23], it is likely that in the past, humans may have consumed appreciable amounts of plants, including natural herbs from the wilderness. Of note, the indole compound-containing herb Qing-Dai has been used as an anti-inflammatory and anti-fever drug in traditional Chinese medicine.

Recently, doctors practicing traditional Chinese medicine have used Qing-Dai in combination with other herbs to treat UC patients [24]. Although most gastroenterologists and gastroendoscopists are familiar with indigo carmine (5,5′-indigodisulfonic acid sodium salt) dye, many microbiologists are now focusing on indole compounds derived from herbs and tryptophan metabolites produced by microbiota for the treatment of inflammatory diseases.

How do treatment strategies differ?

Traditional Chinese medicine has been evolving for over 3000 years, and differs from conventional Western medical practices [25]. In Western medicine, doctors target inflammation when treating individuals. However, Chinese herbal medicine focuses on the whole individual and his or her interactions with the environment, rather than on the molecular targets of the disease. In other words, according to Chinese literature, maintaining balance results in maintaining health (Fig. 1). "Qi" represents the energy that is integral to thoughts, emotions, tissue, and blood, and the manipulation of "Qi" within the body affects health or illness [26]. Chinese medicine is based on the idea that the human body is a perfect reflection of nature. Chinese herbal medicine can be classified into four groups according to their thermal nature: hot, warm, cool, or cold. Hot and warm herbs are used to treat cold diseases, whereas cool and cold herbs are used to treat heat diseases [26]. In Chinese herbal medicine, some UC symptoms represent the accumulation of heat within the individual, and therefore, Qing-Dai, classified as a cold herb, is used to treat UC [26]. The differences between Western and Chinese medicines may be a considerable barrier to the acceptance of Chinese herbal medicine by Western doctors, and there is the lack of scientific explanation for the possible efficacy and safety of traditional Chinese medicines [27]. However, Chinese medicine may be integrated into Western medicine through the use of Chinese herbs as supplements to compensate for certain deficiencies. For instance, Qing-Dai may improve environmental factors such as unbalanced dietary habits and intestinal dysbiosis.

What is Qing-Dai?

Historically, Qing-Dai has been used as a dye extracted from the leaves and stems of plant species that contain high concentrations of indigo, such as *Baphicacanthus cusia* (Nees) Bremek. (Acanthaceae), *Polygonum tinctorium* Ait. (Polygonaceae), and *Isatis indigotica* Fort. (Cruciferae) (Fig. 2). The composition of indigo-containing plant species differs due to harvest location and the particular species of Qing-Dai [24]. Qing-Dai that is extracted from *Baphicacanthus cusia* Bremek. and produced in Fujian is considered the highest quality version of Qing-Dai in China. The organic components of Qing-Dai include indigo, indirubin, tryptanthrin, sterols, and amino acids [28, 29]. Qing-Dai also contains a small amount of inorganic components including $CaCO_3$ and trace elements. According to the 2015 edition of the Chinese Pharmacopoeia, an official drug compendium designed to enhance the overall quality of drugs in China, Qing-Dai requires $\geq$2.0 % indigo and $\geq$0.13 % indirubin content. To produce Qing-Dai, fresh leaves are dipped in a clear water tank for a couple of days. The leaves are then removed, and the water is mixed thoroughly with caustic lime. After the blue/purple color of the water is confirmed, the foams are harvested and dried to make the powdered form of Qing-Dai.

The history of indigo can be traced back to the west across Europe in ancient Greece and Rome. The anti-fever and anti-inflammatory effects of Qing-Dai were described in "Kai Bao Ben Cao", which was written during the Song dynasty of China (tenth century). Chinese herbal medicines partly containing Qing-Dai have been used mainly as anti-fever and anti-inflammatory drugs, e.g., in the form of enema treatments for UC patients. Furthermore, topical and oral Qing-Dai is used to treat various diseases such as psoriasis [30], oral ulcers [24], radiation proctitis [31], chronic myelocytic leukemia [32], and herpes zoster. Although Chinese herbal medicines containing Qing-Dai (e.g., Qing-Dai San, Xilei-San, etc.) are available as over-the-counter drugs in China, Qing-Dai is not approved as a drug but is categorized into other food categories in other countries, including Japan. Although Qing-Dai has been used to treat UC patients in Japan, these individuals must purchase the necessary products and administer the drugs to themselves without cautionary education regarding the possible adverse effects or visit some particular clinics at their own expense. However, most doctors have little knowledge and do not have a positive impression of Qing-Dai due to a lack of evidence and English publications regarding Qing-Dai for UC patients. In actuality, some

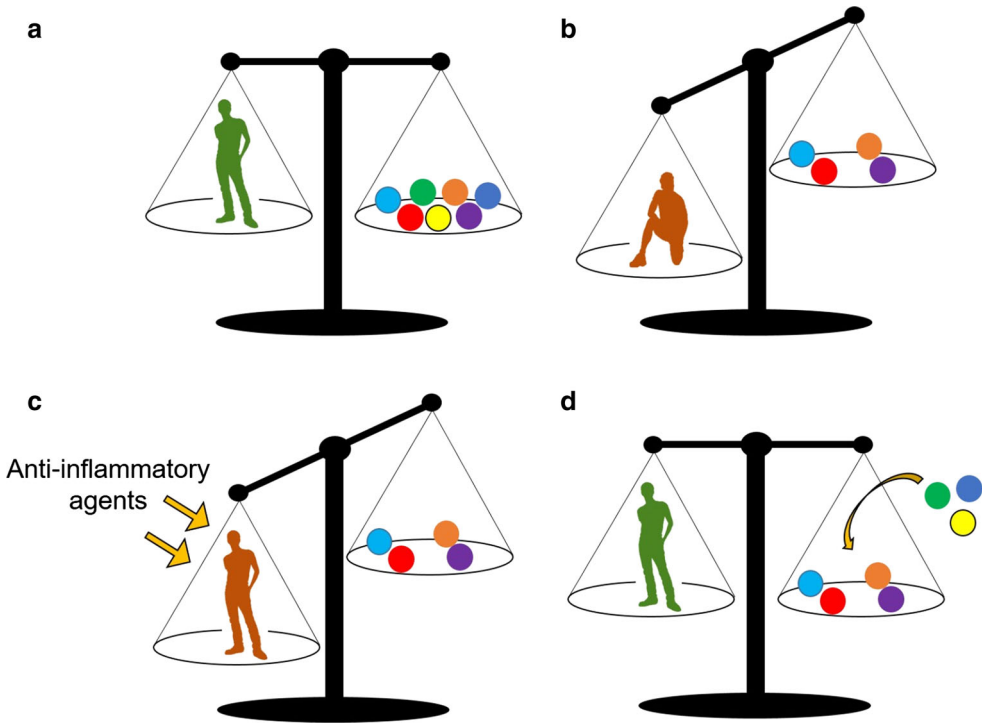

Fig. 1 Different conceptions of treatment between Western and Chinese medicines. **a** Healthy state; **b** inflammatory disease state; **c** treated state in the Western approach; **d** treated state in the Chinese approach. *Colored circles* indicate multifactorial etiology, where complex interactions exist among genetics, epigenetics, and environmental factors (including diet, infections, antibiotics, smoking, alterations of the microbiota, and sanitation). This balance should be regarded as the sole contributing factor to disease pathogenesis. In the Western approach, patients with inflammation are treated by anti-inflammatory agents or immunosuppressants, resulting in suppressed inflammation, although the environmental factors remain imbalanced. However, in the Chinese approach, the patients, not the disease, are treated by resolving the deficiency and maintaining the balance

patients use Qing-Dai without informing their doctors, and these situations are dangerous.

What are indole compounds?

Over 400 types of indole molecules that bear indole ring structures, such as indigo and indirubin, are known. For instance, food-derived indole molecules, such as indole-3-carbinol (I3C), are abundant in green vegetables, including broccoli, cabbage, Brussels sprouts, and kale. In addition, indole-3-carboxaldehyde (IAld) is produced from the essential amino acid tryptophan through currently unidentified biosynthetic enzymes derived from intestinal microbiota, such as lactobacillus species [23, 33]. The reduced intake of fresh vegetables in Western countries and the resultant dysbiosis [3] may have changed the total amount of indole compounds typically present in the body. Furthermore, indigo, indirubin, I3C, and IAld have all been suggested as endogenous aryl hydrocarbon receptor (AhR) ligands [33], whereas indigo carmine is not. Orally administered I3C is converted to the higher order AhR ligands, 6-formylindolo[3,2-b]carbazole (FICZ) and 3,3′-diindolylmethane (DIM), by gastric acids [34]. The AhR pathway has been known for a long time because it has been associated with certain classes of hazardous chemicals, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), which is an inducer of CYP1A1 via AhR [35]. In addition, pigmented virulence factors derived from various infectious bacteria, such as tuberculosis bacteria, induce CYP1A1 and CYP1B1 via AhR to suppress the expansion of harmful bacteria [36].

Why are indole compounds crucial for maintaining intestinal homeostasis?

The intestinal epithelium, lined by simple columnar cells, is the most rapidly self-renewing tissue in the mammalian body. Intestinal stem cells, which exclusively express the *Lgr5* gene, lay at the bottom of intestinal crypts and have the ability to self-renew as well as the capacity for multipotent differentiation [37]. These cells undergo stochastic symmetric cell division every 24 h, and the intestinal epithelium completely renews every 3–5 days [38]. Intestinal stem cells are crucial for epithelial regeneration

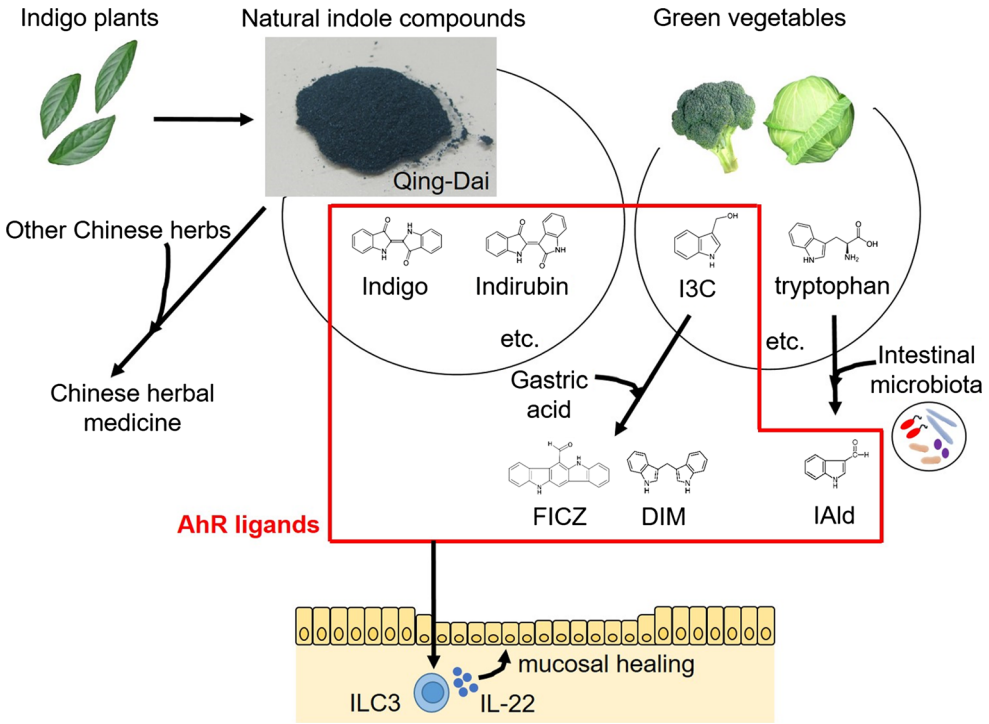

Fig. 2 The natural indole compound Qing-Dai is extracted from plants, and food- and microbiota-derived indoles act as aryl hydrocarbon receptor ligands. Qing-Dai contains indole compounds that bear indole ring structures, such as indigo, indirubin. Food-derived indole molecules, such as I3C, are abundant in green vegetables, such as broccoli and cabbage. In addition, IAld is produced from the essential amino acid tryptophan and derived from intestinal microbiota. I3C is converted to FICZ and DIM by gastric acids. These indole compounds are endogenous AhR ligands and promote mucosal healing. Although the actual mechanisms of action of Qing-Dai still remain unknown, Qing-Dai can potentially promote mucosal healing through stimulating mucosal type 3 innate lymphoid cells to produce interleukin-22. *I3C* indole-3-carbinol, *IAld* indole-3-carboxaldehyde, *FICZ* 6-formylindolo[3,2-b] carbazole, *DIM* 3,3′-diindolylmethane, *ILC3* innate lymphoid cell type 3, *IL-22* interleukin-22, *AhR* aryl hydrocarbon receptor

and for maintaining barrier and epithelial function after intestinal injury [39, 40]. Additionally, the human intestine contains approximately 100 trillion bacterial cells, mainly consisting of commensal bacteria, and these bacteria belong to as many as a thousand different species, which mainly include Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes [41, 42].

Of note, recent studies have revealed that AhR ligands are crucial molecules for stimulating AhR-expressing type 3 innate lymphoid cells (ILC3s) in the mucosa to produce IL-22 [43, 44], which is known to reinforce epithelial permeability by induction of tight junction molecules and the production of antimicrobial peptides [45–50]. Thus, it is possible to use these AhR ligands as therapies for IBD to induce mucosal healing, which is the ultimate treatment goal [51–54]. In addition, it is interesting that signaling through the TNF-α and AhR pathways activates the same transcription factor, p300/Sac-1, which suggests that a combinational therapy of anti-TNF-α biologics and AhR ligands may achieve complete remission in UC patients [55]. Furthermore, it has been demonstrated that IL-22 induces STAT3 phosphorylation by acting directly on intestinal stem cells, and STAT3 is essential for enhancing organoid growth and IL-22-dependent epithelial regeneration [56]. Thus, IL-22 regulates intestinal immune homeostasis and mucosal healing.

Do indole compounds suppress the development of colitis in animal models?

Li et al. [57] have reported that the administration of vegetable-derived I3C suppresses the development of dextran sulfate sodium (DSS)-induced acute colitis in mice, and $AhR^{-/-}$ mice developed more severe DSS-induced colitis compared to that of control C57BL/6 mice. Zelante et al. [44] have also demonstrated that microbiota-derived IAld suppresses the development of acute DSS-induced colitis. In addition to the IBD model, $AhR^{-/-}$ mice are resistant to models of psoriasis [58] and acute pancreatitis [59] that are mediated by epithelia injury. Furthermore, Xiao et al. [28] have reported that the administration of Qing-Dai suppressed the inflammatory responses of colonic macrophages during DSS-induced colitis and the production of TNF-α, IL-1β, and IL-6 in colonic tissue.

Is Qing-Dai effective for patients with ulcerative colitis?

Due to concerns of side effects, such as infection, and prior loss of response or intolerance to well-known approved medicines, many patients with UC seek complementary and alternative medicines [60, 61]. Recently, some worldwide clinical trials have examined the usefulness of herbal medicines for treating UC. *Aloe vera* gel (Xanthorrhoeaceae) [62], *Triticum aestivum* (Poaceae; wheat grass juice) [63], *Andrographis paniculata* (Acanthaceae) extract (HMPL-004) [64], and Xilei-San suppositories [65, 66] were superior to placebo at inducing remission or response. HMPL-004 [67] and gum resin from *Boswellia serrata* (Burseraceae) [68] were as effective as mesalamine, and a Xilei-San enema [69] was as effective as a dexamethasone enema. Additionally, *Vaccinium myrtillus* (Ericaceae; anthocyanin-rich bilberry preparation) [70], *Potentilla tormentilla* (Rosaceae; tormentil) extract [71], Fufangkushen colon-coated capsules [72], and Jian Pi Ling tablets [73] induced remission in some patients with UC. Curcumin [74] was superior to placebo in maintaining remission, and *Plantago ovata* (Plantaginaceae) seeds [75] and Myrrhinil intest® (herbal combination of myrrh, dry extract of chamomile flowers, and coffee charcoal) [76] were as effective as mesalamine. Additionally, *Oenothera biennis* (Onagraceae; evening primrose oil) [77] and germinated barley foodstuff [78] induced remission in some patients with UC. Nevertheless, some of these studies examining herbs for the treatment of UC showed encouraging results but remain limited, and further high-quality research is required [79].

As shown in Table 1, some of abovementioned herbal medicines contain Qing-Dai [65, 66, 69]. In traditional Chinese medicine, rectal administration of Qing-Dai has been used for patients with acute UC, although almost all the publications reporting this were written in Chinese. In China, Qing-Dai is not used as a single agent but is instead used as a mixed formulation, such as Xilei-San. However, the exact scientific reason for Qing-Dai to be used as a mixed formulation is unknown [80]. According to Chinese literature, it is known that liver dysfunction, headache, and nausea are the main adverse effects of Qing-Dai [30, 81]. In Japan, Fukunaga et al. [66] previously reported that Xilei-San suppositories were statistically effective for patients with UC who were refractory to topical mesalamine or corticosteroid treatment in a placebo-controlled study. Suzuki et al. [82] also reported that the oral administration of Qing-Dai powder was safe and seemed to be effective in a retrospective analysis.

In addition, Dr. Amano of Hiroshima, Japan, has treated patients with intractable UC using an undisclosed combination of Chinese herbs, including modified Xilei-San, based on his experience over many years. This treatment, the so-called Hiroshima kampo, was initially considered an unscientific folk remedy, but incontrovertible evidence of its efficacy has caused it to garner more recent attention. Over 3000 patients with UC in Japan and from other countries such as Korea were treated with Amano's formulation; however, the results of this treatment, regrettably, have not been published and still have not been subjected to scientific evaluation.

To scientifically examine the efficacy and safety of Qing-Dai, our group recently conducted a pilot clinical trial using capsulated Qing-Dai (daily dose, 2 g) that was administered to 20 patients with moderate UC activity who were refractory to corticosteroids, immunomodulators, and/

Table 1 Studies of herbal therapies containing Qing-Dai to induce remission patients with ulcerative colitis

Authors	Country	Year	Intervention	Duration	Number of patients	Comparator	Rates of clinical response/remission/mucosal healing	
							Test agent	Comparator
Fukunaga et al. [65]	Japan	2007	Xilei-San suppository	6 months	6	None	N/A	–
Gong et al. [72]	China	2012	Fufangkushen colon-coated capsule with Huidi	8 weeks	320	Placebo with Huidi	73/42/55 %	65/41/55 %
Fukunaga et al. [66]	Japan	2012	Xilei-San suppository	2 weeks	30	Placebo	N/A/46 %/N/A	N/A/0 %/N/A
Zhang et al. [69]	China	2013	Xilei-San enema	8 weeks	35	Dexamethasone enema	N/A	N/A
Suzuki et al. [82]	Japan	2013	Oral powdered Qing-Dai	not determined (1–4 months)	9	None (case series)	N/A	–
Sugimoto et al. [27]	Japan	2016	Oral capsule Qing-Dai	8 weeks	20	None	72/33/61 %	–

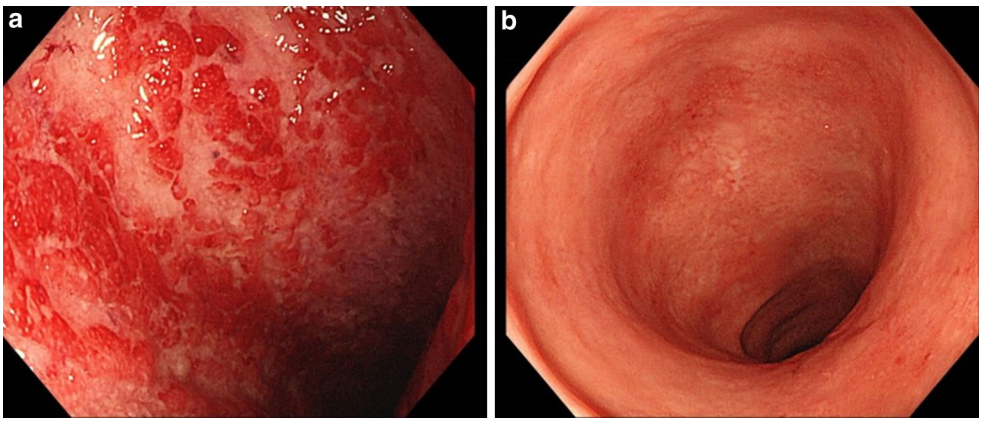

Fig. 3 Representative endoscopic findings before (**a**) and after (**b**) the administration of Qing-Dai for 8 weeks. This patient with active ulcerative colitis was a 29-year-old woman with chronic continuous disease. She had been previously treated with prednisolone, granulocytapheresis, tacrolimus, infliximab, and golimumab (clinical trial), and she had an allergy to mesalamine. Although she had been treated with 100 mg of azathioprine and adalimumab, she was currently experiencing secondary failure. Due to exacerbation, the administration of 40 mg of prednisolone was started; however, the patient did not respond, and her symptoms worsened. After 2 weeks of adalimumab discontinuation and a dose reduction in prednisolone to 30 mg, the administration of Qing-Dai (daily dose of 2 g) was started. During the prednisolone dose reduction, the patient's symptoms and endoscopic findings improved markedly (Mayo score of 9 to 3) at week 8. After finishing our trial, this patient has continued to purchase and use Qing-Dai (daily dose of 1 g) and has maintained clinical remission

or anti-TNF-α therapies [27]. This open-cohort single-arm study demonstrated that capsulated Qing-Dai was tolerable in terms of adverse events and remarkably effective in terms of clinical response (72 %), clinical remission (33 %), and endoscopic remission (61 %). The Mayo score, partial Mayo score, Mayo endoscopic score [83], and clinical activity index [84] improved significantly, from a mean of 7.9 to 2.7, 5.7 to 1.4, 2.4 to 1.2, and 9.4 to 3.5, respectively ($p < 0.001$) [27]. The partial Mayo scores were reduced even in patients who did not achieve a clinical response by definition. Notably, these patients included intractable cases (12 patients with chronic continuous disease courses), and 5 of 20 had experienced secondary failure of anti-TNF-α agents. Treatment with Qing-Dai allowed these patients to discontinue use of these drugs (Fig. 3). In this study, 10 % mild liver dysfunction was reported, and we have cautioned against this side effect. The efficacy of Qing-Dai was substantially superior even to that of other approved therapies, and thus Qing-Dai may be considered as an alternative treatment strategy to avoid the use of corticosteroids or other immunosuppressants [27]. Following our pilot study, trials were initiated at other institutions (UMIN000017359, UMIN000019103). To confirm the safety and efficacy of Qing-Dai, our group is currently conducting a large, multi-center, double-blind, randomized, dose-dependent, placebo-controlled trial (UMIN000021439).

Specific molecules and indole derivatives may also be promising candidates for the treatment of UC. A phase II trial of Natura-alpha (meisoindigo; *N*-methyl-Δ3,3′-dihydroindole-2,2′-diketone) [85] is in progress, spearheaded by Natrogen Therapeutics International, Inc. and Takeda Pharmaceutical Co., Ltd. (ClinicalTrials.gov identifier: NCT01216280). These indole compounds may change the course of medicine and provide physicians with options to treat intractable inflammatory conditions.

On the other hand, there have been no reports regarding the efficacy of Qing-Dai in patients with CD. However, it is generally known that AhR is highly expressed in cells at body surfaces such as intestinal mucosa, including the small intestine, and particularly in ILC3s [33]. While it is possible that Qing-Dai is also effective for patients with CD, the clinical condition of CD is usually complex, and CD can affect all layers of the intestinal wall, whereas UC affects only the lining of the colon. We are currently conducting a pilot study to investigate the clinical efficacy of Qing-Dai for patients with CD who have lost responsiveness to anti-TNF-α agents (UMIN000019249), although careful further investigation is needed.

What are the concerns about Qing-Dai?

Qing-Dai has potential as a new treatment strategy for mild-to-moderate UC after doctors have considered treatment with corticosteroids, granulocytapheresis, anti-TNF-α agents, or tacrolimus. Qing-Dai is an encouraging alternative, especially for those who have refractory disease that is corticosteroid-resistant or corticosteroid-dependent, who do not respond to anti-TNF agents, or who cannot tolerate mesalamine. In addition, with the exception of corticosteroids, conventional treatment to induce remission in

patients with active UC is quite expensive. Qing-Dai is extremely inexpensive (85 Chinese yuan per 500 g; converted to approximately 20 dollars per year in China, 100 dollars per year after importation to Japan); its cost is approximately one-hundredth that of mesalamine and one-thousandth that of anti-TNF-α agents on an annual basis. Qing-Dai may eventually have a large impact on patient outcomes as well as on medical economics. The quality control of Chinese herbal medicines is an important problem. The contents of Qing-Dai may differ between batches according to species, harvest locations, years, and seasons [24]. To resolve this issue, the application of chromatographic fingerprints and related chemometric analysis is essential during safety evaluations of Qing-Dai, as is maintaining a stable supply of good-quality drug [86].

Although it has been scientifically demonstrated that indole compounds promote mucosal healing, the actual mechanisms of action of Qing-Dai still remain unknown [27]. While IL-22/STAT3 signaling promotes mucosal healing and protects against tumor formation during inflammation, IL-22 can also promote intestinal tumorigenesis during the resolution of inflammation [87, 88]. From this viewpoint, the regulation of IL-22 production is important, and the timing of Qing-Dai discontinuation is of concern in light of the possible risk of tumorigenesis. However, indole compounds such as indirubin and Natura-alpha are considered potential anticancer agents [89]. In addition, the suppression of superoxide generation has been suggested as another mechanism of action for Qing-Dai [82, 90]. The safety of long-term Qing-Dai use should be investigated.

The adverse side effects of Qing-Dai include liver dysfunction, headache, and digestive problems, and patients should be cautioned regarding these symptoms even though they may tolerate Qing-Dai [27]. Further basic research and clinical investigation of indole compounds are warranted.

Conclusion

The indole compounds discussed in this paper may be promising candidates for old and new natural drugs not only for UC, but also for other intractable diseases that are mediated by epithelia injury. The AhR ligand environment in the gut comprises food-derived and microbiota-derived indole compounds, and may play a critical role in stemming the rising incidence of microbiota-mediated diseases in Western countries. In other words, different approaches, such as the intake of foods that favor indole-inducing bacteria, microbiota that produce indoles from tryptophan, and indole compounds themselves, should be attempted for the prevention of disease as well as its maintenance and remission. The combination of probiotics that are efficient producers of indole compounds may also be a promising new treatment strategy for UC. Finally, although food- or microbiota-derived indole compounds may be safe, we should not forget that dioxins (e.g., TCDD) are also high-affinity AhR ligands, and the adverse effects of these molecules should be considered during clinical development.

Acknowledgments We thank the past and present members of the Keio IBD Group for their continued support. We also thank Dr. Kuniki Amano (Sky Clinic, Hiroshima, Japan) for useful suggestions.

Compliance with ethical standards

Conflict of interest Takanori Kanai received a research grant and lecture fees from Takeda Pharmaceutical Co., Ltd. However, this funding was not related to the generation of this article.

References

1. Kirsner JB. Historical aspects of inflammatory bowel disease. J Clin Gastroenterol. 1988;10:286–97.
2. Danese S, Fiocchi C. Ulcerative colitis. N Engl J Med. 2011;365:1713–25.
3. Kanai T, Matsuoka K, Naganuma M, et al. Diet, microbiota, and inflammatory bowel disease: lessons from Japanese foods. Korean J Intern Med. 2014;29:409–15.
4. Kim HJ, Hann HJ, Hong SN, et al. Incidence and natural course of inflammatory bowel disease in Korea, 2006–2012: a nationwide population-based study. Inflamm Bowel Dis. 2015;21:623–30.
5. Ng SC, Zeng Z, Niewiadomski O, et al. Early course of inflammatory bowel disease in a population-based inception cohort study from 8 countries in Asia and Australia. Gastroenterology. 2016;150(86–95):e3.
6. Annese V, Daperno M, Rutter MD, et al. European evidence based consensus for endoscopy in inflammatory bowel disease. J Crohns Colitis. 2013;7:982–1018.
7. Naganuma M, Sakuraba A, Hibi T. Ulcerative colitis: prevention of relapse. Expert Rev Gastroenterol Hepatol. 2013;7:341–51.
8. Naganuma M, Hisamatsu T, Matsuoka K, et al. Endoscopic severity predicts long-term prognosis in Crohn's disease patients with clinical remission. Digestion. 2016;93:66–71.
9. Brown SJ, Mayer L. The immune response in inflammatory bowel disease. Am J Gastroenterol. 2007;102:2058–69.
10. Liu TC, Stappenbeck TS. Genetics and pathogenesis of inflammatory bowel disease. Annu Rev Pathol. 2016;. doi:10.1146/annurev-pathol-012615-044152 **(in press)**.
11. Lee D, Albenberg L, Compher C, et al. Diet in the pathogenesis and treatment of inflammatory bowel diseases. Gastroenterology. 2015;148:1087–106.
12. Sheehan D, Moran C, Shanahan F. The microbiota in inflammatory bowel disease. J Gastroenterol. 2015;50:495–507.
13. Yurist-Doutsch S, Arrieta MC, Vogt SL, et al. Gastrointestinal microbiota-mediated control of enteric pathogens. Annu Rev Genet. 2014;48:361–82.
14. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol. 2014;49:785–98.
15. Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. Gastroenterology. 2014;146:1489–99.

16. Clemente JC, Ursell LK, Parfrey LW, et al. The impact of the gut microbiota on human health: an integrative view. Cell. 2012;148:1258–70.
17. Matsuoka K, Kanai T. The gut microbiota and inflammatory bowel disease. Semin Immunopathol. 2015;37:47–55.
18. Lee KJ, Tack J. Altered intestinal microbiota in irritable bowel syndrome. Neurogastroenterol Motil. 2010;22:493–8.
19. Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21:895–905.
20. Tilg H, Moschen AR. Microbiota and diabetes: an evolving relationship. Gut. 2014;63:1513–21.
21. Smits LP, Bouter KE, de Vos WM, et al. Therapeutic potential of fecal microbiota transplantation. Gastroenterology. 2013;145:946–53.
22. Sears CL, Garrett WS. Microbes, microbiota, and colon cancer. Cell Host Microbe. 2014;15:317–28.
23. Gullett NP, Ruhul Amin AR, Bayraktar S, et al. Cancer prevention with natural compounds. Semin Oncol. 2010;37:258–81.
24. Stasiak N, Kukula-Koch W, Glowniak K. Modern industrial and pharmacological applications of indigo dye and its derivatives—a review. Acta Pol Pharm. 2014;71:215–21.
25. Zhang GB, Li QY, Chen QL, et al. Network pharmacology: a new approach for chinese herbal medicine research. Evid Based Complement Alternat Med. 2013;2013:621423.
26. Fu J, Pang J, Zhao X, et al. The quantitative ideas and methods in assessment of four properties of Chinese medicinal herbs. Cell Biochem Biophys. 2014;71:1307–12.
27. Sugimoto S, Naganuma M, Kiyohara H, et al. Clinical efficacy and safety of oral Qing-Dai in patients with ulcerative colitis: a single-center open-label prospective study. Digestion. 2016;93:193–201.
28. Xiao HT, Peng J, Hu DD, et al. Qing-dai powder promotes recovery of colitis by inhibiting inflammatory responses of colonic macrophages in dextran sulfate sodium-treated mice. Chin Med. 2015;10:29.
29. Lin YK, Leu YL, Huang TH, et al. Anti-inflammatory effects of the extract of indigo naturalis in human neutrophils. J Ethnopharmacol. 2009;125:51–8.
30. Zhou D, Chen W, Li X, et al. Evidence-based practice guideline of Chinese herbal medicine for psoriasis vulgaris (Bai Bi). Eur J Integr Med. 2014;6:135–46.
31. Yuan G, Ke Q, Su X, et al. Qing Dai, a traditional Chinese medicine for the treatment of chronic hemorrhagic radiation proctitis. Chinese-German J Clin Oncol. 2009;8:114–6.
32. Han R. Highlight on the studies of anticancer drugs derived from plants in China. Stem Cells. 1994;12:53–63.
33. Stockinger B, Di Meglio P, Gialitakis M, et al. The aryl hydrocarbon receptor: multitasking in the immune system. Annu Rev Immunol. 2014;32:403–32.
34. Shertzer HG, Senft AP. The micronutrient indole-3-carbinol: implications for disease and chemoprevention. Drug metabol Drug interact. 2000;17:159–88.
35. Vaas S, Kreft L, Schwarz M, et al. Cooperation of structurally different aryl hydrocarbon receptor agonists and beta-catenin in the regulation of CYP1A expression. Toxicology. 2014;325:31–41.
36. Moura-Alves P, Fae K, Houthuys E, et al. AhR sensing of bacterial pigments regulates antibacterial defence. Nature. 2014;512:387–92.
37. Barker N, van Es JH, Kuipers J, et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. Nature. 2007;449:1003–7.
38. Sato T, Clevers H. Growing self-organizing mini-guts from a single intestinal stem cell: mechanism and applications. Science. 2013;340:1190–4.
39. Karin M, Clevers H. Reparative inflammation takes charge of tissue regeneration. Nature. 2016;529:307–15.
40. Okamoto R, Watanabe M. Role of epithelial cells in the pathogenesis and treatment of inflammatory bowel disease. J Gastroenterol. 2016;51:11–21.
41. Maynard CL, Elson CO, Hatton RD, et al. Reciprocal interactions of the intestinal microbiota and immune system. Nature. 2012;489:231–41.
42. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59–65.
43. Qiu J, Guo X, Chen ZM, et al. Group 3 innate lymphoid cells inhibit T-cell-mediated intestinal inflammation through aryl hydrocarbon receptor signaling and regulation of microflora. Immunity. 2013;39:386–99.
44. Zelante T, Iannitti RG, Cunha C, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity. 2013;39:372–85.
45. Spits H, Di Santo JP. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. Nat Immunol. 2011;12:21–7.
46. Eberl G. Development and evolution of RORgammat+ cells in a microbe's world. Immunol Rev. 2012;245:177–88.
47. Goldberg R, Prescott N, Lord GM, et al. The unusual suspects–innate lymphoid cells as novel therapeutic targets in IBD. Nat Rev Gastroenterol Hepatol. 2015;12:271–83.
48. Mizuno S, Mikami Y, Kamada N, et al. Cross-talk between RORgammat+ innate lymphoid cells and intestinal macrophages induces mucosal IL-22 production in Crohn's disease. Inflamm Bowel Dis. 2014;20:1426–34.
49. Kiss EA, Vonarbourg C, Kopfmann S, et al. Natural aryl hydrocarbon receptor ligands control organogenesis of intestinal lymphoid follicles. Science. 2011;334:1561–5.
50. Monteleone I, Rizzo A, Sarra M, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. Gastroenterology. 2011;141:237–48, 248.e1.
51. Zelante T, Iannitti RG, Fallarino F, et al. Tryptophan feeding of the IDO1-AhR axis in host-microbial symbiosis. Front Immunol. 2014;5:640.
52. Veldhoen M, Ferreira C. Influence of nutrient-derived metabolites on lymphocyte immunity. Nat Med. 2015;21:709–18.
53. Froslie KF, Jahnsen J, Moum BA, et al. Mucosal healing in inflammatory bowel disease: results from a Norwegian population-based cohort. Gastroenterology. 2007;133:412–22.
54. Saigusa K, Matsuoka K, Sugimoto S, et al. Ulcerative colitis endoscopic index of severity is associated with long-term prognosis in ulcerative colitis patients treated with infliximab. Dig Endosc. 2016;. doi:10.1111/den.12655 **(in press)**.
55. Ke S, Rabson AB, Germino JF, et al. Mechanism of suppression of cytochrome P-450 1A1 expression by tumor necrosis factor-alpha and lipopolysaccharide. J Biol Chem. 2001;276:39638–44.
56. Lindemans CA, Calafiore M, Mertelsmann AM, et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. Nature. 2015;528:560–4.
57. Li Y, Innocentin S, Withers DR, et al. Exogenous stimuli maintain intraepithelial lymphocytes via aryl hydrocarbon receptor activation. Cell. 2011;147:629–40.
58. Di Meglio P, Duarte JH, Ahlfors H, et al. Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. Immunity. 2014;40:989–1001.
59. Xue J, Nguyen DT, Habtezion A. Aryl hydrocarbon receptor regulates pancreatic IL-22 production and protects mice from acute pancreatitis. Gastroenterology. 2012;143:1670–80.

60. Koning M, Ailabouni R, Gearry RB, et al. Use and predictors of oral complementary and alternative medicine by patients with inflammatory bowel disease: a population-based, case–control study. Inflamm Bowel Dis. 2013;19:767–78.
61. Weizman AV, Ahn E, Thanabalan R, et al. Characterisation of complementary and alternative medicine use and its impact on medication adherence in inflammatory bowel disease. Aliment Pharmacol Ther. 2012;35:342–9.
62. Langmead L, Feakins RM, Goldthorpe S, et al. Randomized, double-blind, placebo-controlled trial of oral aloe vera gel for active ulcerative colitis. Aliment Pharmacol Ther. 2004;19:739–47.
63. Ben-Arye E, Goldin E, Wengrower D, et al. Wheat grass juice in the treatment of active distal ulcerative colitis: a randomized double-blind placebo-controlled trial. Scand J Gastroenterol. 2002;37:444–9.
64. Sandborn WJ, Targan SR, Byers VS, et al. Andrographis paniculata extract (HMPL-004) for active ulcerative colitis. Am J Gastroenterol. 2013;108:90–8.
65. Fukunaga K, Hida N, Ohnishi K, et al. A suppository Chinese medicine (xilei-san) for refractory ulcerative proctitis: a pilot clinical trial. Digestion. 2007;75:146–7.
66. Fukunaga K, Ohda Y, Hida N, et al. Placebo controlled evaluation of Xilei San, a herbal preparation in patients with intractable ulcerative proctitis. J Gastroenterol Hepatol. 2012;27:1808–15.
67. Tang T, Targan SR, Li ZS, et al. Randomised clinical trial: herbal extract HMPL-004 in active ulcerative colitis—a double-blind comparison with sustained release mesalazine. Aliment Pharmacol Ther. 2011;33:194–202.
68. Gupta I, Parihar A, Malhotra P, et al. Effects of gum resin of Boswellia serrata in patients with chronic colitis. Planta Med. 2001;67:391–5.
69. Zhang F, Li Y, Xu F, et al. Comparison of Xilei-san, a Chinese herbal medicine, and dexamethasone in mild/moderate ulcerative proctitis: a double-blind randomized clinical trial. J Altern Complement Med. 2013;19:838–42.
70. Biedermann L, Mwinyi J, Scharl M, et al. Bilberry ingestion improves disease activity in mild to moderate ulcerative colitis—an open pilot study. J Crohns Colitis. 2013;7:271–9.
71. Huber R, Ditfurth AV, Amann F, et al. Tormentil for active ulcerative colitis: an open-label, dose-escalating study. J Clin Gastroenterol. 2007;41:834–8.
72. Gong Y, Zha Q, Li L, et al. Efficacy and safety of Fufangkushen colon-coated capsule in the treatment of ulcerative colitis compared with mesalazine: a double-blinded and randomized study. J Ethnopharmacol. 2012;141:592–8.
73. Chen ZS, Nie ZW, Sun QL. Clinical study in treating intractable ulcerative colitis with traditional Chinese medicine. Chin J Integr Med. 1994;14:400–2.
74. Hanai H, Iida T, Takeuchi K, et al. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. Clin Gastroenterol Hepatol. 2006;4:1502–6.
75. Fernandez-Banares F, Hinojosa J, Sanchez-Lombrana JL, et al. Randomized clinical trial of *Plantago ovata* seeds (dietary fiber) as compared with mesalamine in maintaining remission in ulcerative colitis. Spanish Group for the Study of Crohn's Disease and Ulcerative Colitis (GETECCU). Am J Gastroenterol. 1999;94:427–33.
76. Langhorst J, Varnhagen I, Schneider SB, et al. Randomised clinical trial: a herbal preparation of myrrh, chamomile and coffee charcoal compared with mesalazine in maintaining remission in ulcerative colitis—a double-blind, double-dummy study. Aliment Pharmacol Ther. 2013;38:490–500.
77. Greenfield SM, Green AT, Teare JP, et al. A randomized controlled study of evening primrose oil and fish oil in ulcerative colitis. Aliment Pharmacol Ther. 1993;7:159–66.
78. Kanauchi O, Mitsuyama K, Homma T, et al. Treatment of ulcerative colitis patients by long-term administration of germinated barley foodstuff: multi-center open trial. Int J Mol Med. 2003;12:701–4.
79. Langhorst J, Wulfert H, Lauche R, et al. Systematic review of complementary and alternative medicine treatments in inflammatory bowel diseases. J Crohns Colitis. 2015;9:86–106.
80. Teng L, Xin HW, Blix HS, et al. Review of the use of defined daily dose concept in drug utilisation research in China. Pharmacoepidemiol Drug Saf. 2012;21:1118–24.
81. Ferber KH. Toxicology of indigo. A review. J Environ Pathol Toxicol Oncol. 1987;7:73–83.
82. Suzuki H, Kaneko T, Mizokami Y, et al. Therapeutic efficacy of the Qing Dai in patients with intractable ulcerative colitis. World J Gastroenterol. 2013;19:2718–22.
83. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317:1625–9.
84. Lichtiger S, Present DH, Kornbluth A, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med. 1994;330:1841–5.
85. Glatigny S, Blaton MA, Mencher SK, et al. Treatment of collagen-induced arthritis by Natura-alpha via regulation of Th-1/Th-17 responses. Eur J Immunol. 2010;40:460–9.
86. Liang Y, Xie P, Chau F. Chromatographic fingerprinting and related chemometric techniques for quality control of traditional Chinese medicines. J Sep Sci. 2010;33:410–21.
87. Huber S, Gagliani N, Zenewicz LA, et al. IL-22BP is regulated by the inflammasome and modulates tumorigenesis in the intestine. Nature. 2012;491:259–63.
88. Sonnenberg GF, Artis D. Innate lymphoid cells in the initiation, regulation and resolution of inflammation. Nat Med. 2015;21:698–708.
89. Huang M, Lin HS, Lee YS, et al. Evaluation of meisoindigo, an indirubin derivative: in vitro antileukemic activity and in vivo pharmacokinetics. Int J Oncol. 2014;45:1724–34.
90. Saito R, Tamura M, Matsui H, et al. Qing Dai attenuates nonsteroidal anti-inflammatory drug-induced mitochondrial reactive oxygen species in gastrointestinal epithelial cells. J Clin Biochem Nutr. 2015;56:8–14.