Treatment with indigo naturalis for inflammatory bowel disease and other immune diseases

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ABSTRACT
Indigo naturalis (IN) is a herbal medicine extracted from leaves and stems of plants and is a component of crude drugs used in China. Recently, IN was reported to be effective for treating UC and psoriasis. The mechanisms of IN for UC is not clear, but aryl hydrocarbon receptor ligand, the active components of IN, can promote mucosal healing by inducing the production of interleukin-22 from type-3 innate lymphocytes cells. Although IN is effective even for refractory cases, critical adverse effects including IN-induced colitis and pulmonary arterial hypertension should be concerned. Due to adverse effects of IN, topical treatment of IN is useful for distal UC as well as psoriasis to secure patients’ safeties. Many refractory patients may be helped by IN if it becomes available in appropriate forms for clinical practice. In the near future, the mechanism that underlies the adverse effects of IN needs to be determined, and extraction of active ingredients with fewer side effects, investigated.

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1. Introduction
Inflammatory bowel diseases (IBD), such as ulcerative colitis (UC) and Crohn’s disease (CD), are chronic inflammatory conditions. The pathophysiology of IBD has been extensively studied and genetic and environmental factors and dysregulation of the immune system have been found to be involved. The fundamental treatment for UC is administration of 5-aminosalicylic acid and a corticosteroid. However, some patients do not respond to this regimen, and approximately 30% of patients who receive corticosteroids become steroid-dependent [1]. For patients who are refractory to or dependent on steroids, cyclophosphamide treatment, thiopurine, immunosuppressants, and anti-TNFα treatments are used [2–9]. In 2018, two medications became available in Japan for treating UC patients. One is an integrin antagonist that shows remarkable efficacy for induction and maintenance of remission in UC patients [10]. Another is tofacitinib, which inhibits Janus kinase (JAK) 1, 2 and 3, resulting in suppression of several cytokines [11]. Tofacitinib is orally administered and is effective even in patients who were previously unresponsive to anti-TNFα treatment.

Even with the availability of several medical treatments developed within recent the last decade, some patients do not achieve clinical remission. The rates of clinical remission are not satisfactory for UC patients in clinical trials (Table 1). Recently, Qing-Dai (Indigo naturalis, IN), which is a component of crude drugs used in China, was reported to be effective for treating UC. It is particularly efficacious for otherwise refractory cases [12–14]. In this article, the clinical efficacy and safety of IN are reviewed, and the current consideration of IN in the medical field is described.

2. General information for indigo naturalis
IN is a herbal medicine extracted from leaves and stems of plants such as Indigofera tinctoria, Strobilanthes cusia O Kuntze, and Polygonum tinctorum Lour. In China, IN is quality controlled as an herbal medicine containing more than 2.0% indigo and more than 0.13% indirubin. IN made from Fujian is regarded as being of the highest quality [15]. IN is usually used as a raw material in Japan, and is classified as a dye, rather than a medicine. IN is considered an anti-inflammatory agent in Chinese textbooks from the tenth century [15].

In Japan, IN is not categorized as an herbal medicine. Patients with IBD are not able to obtain IN from general hospitals. Recently, patients have obtained and used IN on the Internet or have received IN at private clinics. These patients do not always receive sufficient information regarding...
adverse effects caused by use of IN. Thus, the safety and efficacy of IN require adequate scientific assessment before it can be used appropriately in clinical settings.

2.1. Anti-inflammatory mechanisms of indigo naturalis for colitis

A recent study indicates that IN significantly reduces the disease activity index and colonic myeloperoxidase activity of in a DSS colitis model [16]. IN also suppresses the production of proinflammatory cytokines, such as TNF-α and IL-6 and reduces serum MCP-1 levels. It is not clear whether IN suppresses proinflammatory cytokines release directly or whether suppression is merely a result reduced inflammation. IN also suppresses LPS-induced production of TNF-α and IL-6 in vitro.

Indigo and indirubin, the active components of IN, have an indole ring in their chemical structure. A previous study indicates that Indirubin suppresses interferon-α, IL-6 [17], and nuclear factor κB (NF-κB) production [18]. Many indole compounds, such as Indole-3-carbinol (I3C) exist in nature. I3C is abundant in green-yellow vegetables [15]. When ingested, I3C reacts with gastric acid to form 6-formylindolo[3,2-b] carbazole (FICZ). In addition, indole-3-carboxaldehyde (IAld) is produced from tryptophan by intestinal bacteria. Indigo, indirubin, FICZ, and IAld are Aryl hydrocarbon receptor (AhR) ligands and AhR ligands can promote mucosal healing by inducing the production of interleukin 22 (IL-22) from type 3 innate lymphocytes cells (ILC 3) that express AhR [19].

Few direct studies in human subjects have addressed IN inhibition of colitis via promoting IL-22. However, several supportive results are available regarding therapeutic effects of IN via induction of IL-22. CARD 9 is a susceptibility gene for IBD. A recent study indicates that CARD 9 contributes to promotion IL-22 release and thus promotes recovery from colitis. CARD 9 −/− mice are more susceptible to enteritis [20]. Interestingly, microbiota produce less AhR ligands in patients with CARD9 risk alleles associated with IBD. These results suggest a role of IN that includes AhR ligands produced by intestinal bacteria. In mouse model, Monteleone, et al. demonstrated that AhR signaling, via IL-22, inhibits inflammation and colitis in the gastrointestinal tract of mice [21]. A recent study also indicates that IN shows a therapeutic effect on intestinal inflammation in a DSS colitis mouse model, and this effect was not observed in AhR-deficient mice. These results indicate that attenuation of colitis by inducing IL10 and IL22 is AhR-dependent [22]. Further, IL-22-binding protein suppressed goblet cell restitution during the recovery phase in the DSS model [23] thus promoting mucosal healing. A recent study also indicates that IL-22 activates STAT3 which enhances the transcription of anti-apoptotic and pro-proliferative genes [24]. STAT3-induced mucosal healing occurred in an IL-22-dependent manner since both IL-22 and epithelial STAT3 are found to be important in wound-healing experiments [25].

2.2. Evidence of efficacy of indigo naturalis for IBD

In general, IN was administered rectally and it was not previously used as a single agent in traditional Chinese medicine. Therefore, in most studies, the efficacy of IN was examined as part of a mixed formulation, such as Xilei-San. Fukunaga et al. investigated the efficacy of rectal Xilei-San for active UC [26]. In this study, the rate of clinical remission at Day 14 was significantly higher in patients receiving rectal Xilei-San than for patients receiving a placebo. The duration of active treatment was 4 weeks in this trial, yet remission was maintained at Day 180 for 81.8% of patients receiving active treatment while only 16.7% of patients who received the placebo remained in remission. Zhang et al. also demonstrated that the efficacy of rectal Xilei-San enema is comparable to that of dexamethasone enema in mild-to moderate UC patients [27]. Rectal Xilei-San did not cause severe adverse effects indicating that this medicine is safe and may be an effective alternative treatment for patients with mild-to-moderate UC [27].

IN and Xilei-San are used topically in China, because oral treatment may cause liver dysfunction,
headache, and nausea [13,14]. The first study to demonstrate the efficacy of oral IN was published by a Japanese group. Suzuki et al. showed that CAI therapy with IN caused mild gastrointestinal symptoms (nausea, epigastralgia), headache, and liver dysfunction in the placebo group, whereas in the IN-treated group, 40% of patients had mild liver dysfunction. The incidence of liver dysfunction was lower in the IN-treated group compared to the placebo group (Table 2). The rate of clinical response in the IN group was significantly higher than in the placebo group, and the levels recovered to those seen in healthy controls [14].

Although the efficacy of IN in UC patients was confirmed, the trial was terminated because a case of pulmonary arterial hypertension (PAH) was reported in a patient who had used self-purchased IN outside of the study protocol. Adverse effects, including PAH, are described at the next section. In the near future, the mechanism that underlies the side effects of IN needs to be determined, and extraction of active ingredients with fewer side effects, investigated.

### 3. Adverse effects of indigo naturalis

IN caused mild gastrointestinal symptoms (nausea, epigastralgia), headache, and liver dysfunction in the prospective single-center study [12] and the INDIGO study [14]. The incidence of liver dysfunction due to oral IN was 13–19%, and most cases of liver dysfunction were mild and reversible, as the increased serum transaminase levels recovered to normal levels spontaneously or after the completion of study [14]. In the recent nationwide multicenter study, no fulminant hepatitis was observed in subjects treated with IN. The mechanism of liver dysfunction is not clear; it may be related to induction of CYP1A1 in the liver [12].

Recently, IN-induced colitis has been reported. Kondo et al reported that two cases who received oral IN powdered form developed colitis with wall thickening and edema in the right colon [30]. Yanai et al. also reported reddish and edematous lesions in the ascending colon during treatment with IN [31]. Colitis disappeared after discontinuation of IN. Fhang et al reported a case of ischemic colitis induced by IN; the patient was successfully treated using laparoscopic sigmoid colectomy, and pathological examination revealed ischemic or toxic injury of the sigmoid colon [32]. More recently, Matsuno et al also reported on two cases of IN-induced colitis [33]. One was a case of colitis with wall thickness of the ascending colon and an ileocecal intussusception and the other was a case of colitis with marked wall thickening of the right colon. These cases of IN-induced colitis developed relatively early after IN administration and mainly occurred in the right colon. IN-induced colitis may be more likely to occur in right colon because IN tends to be stored in this region.

| Placebo | Clinical response rate | 13.6% | 4.5% | 13.6% |
|---------|------------------------|-------|------|-------|
| 0.5 g/day | 69.6%                  | 26.1% | 56.5% |
| 1.0 g/day | 75.0%                  | 55.0% | 60.0% |
| 2.0 g/day | 81.0%                  | 38.1% | 47.6% |

**Table 2. Main endpoints of the INDIGO study.**
PAH is another critical adverse effect induced by IN. Nishio et al. reported a case of PAH in a patient who received 2 g of IN daily for 6 months [34]. After the patient was diagnosed with PAH, IN was discontinued. Clinical symptoms and examination data for PAH had completely resolved at 4 months after discontinuation of treatment (Tamura et al. in submission). Patients did not show recurrence of PAH over the subsequent 2 years without medication for PAH. UC did become worse after discontinuation of IN therapy [35]. National surveillance data for PAH induced by IN is available and analysis of these data is ongoing.

3.1. The effectiveness of indigo naturalis for psoriasis

Traditional Chinese medicine, including IN, is widely used for UC and psoriasis, but little evidence is available regarding the efficacy of IN for other immune diseases. Although the efficacy of IN was confirmed for UC, whether IN is effective for CD is unclear. CD is characterized by transmural inflammation in the gut. IN may be less effective for this condition because IN mainly acts on the intestinal epithelium.

Table 3 summarizes the clinical use of IN for psoriasis. Mainly, IN is used as a topical therapy for psoriasis to reduce the production of toxic metabolites [36]. Lin et al. reported that IN ointment prepared by mixing IN powder with olive oil, filtering, and then mixing with petroleum jelly and wax was effective for patients with psoriasis in clinical trials. In a small randomized control study, clinical score and the percentage of plaque area was significantly improved in patients who used IN ointment than in patients who used placebo [37]. Another study indicated that the expressions of Ki-67 and CD3, markers of proliferation, were also decreased [38]. This study also confirmed that the efficacies of refined and crude IN ointments were comparable [39]. Using a refined formulation (Lindioil), a recent report indicated that Lindioil ointment is more effective than vehicle for the treatment of nail psoriasis [10]. Because indirubin, the active component of indigo naturalis, is an effective component of IN, it may be a safe alternative for topical treatment of psoriasis, which is quite different from topical corticosteroids [41]. A double-blind, dosage-controlled trial of indirubin in IN reported that Lindioil ointment with 200 μg/g of indirubin is most effective [42]. Because, according to an RCT, the therapeutic effects of topical treatment vary for psoriatic plaques, topical IN may be an alternative treatment for refractory psoriasis, which functions through a different mechanism of action [41]. Most studies have been performed by Li et al.; a multicenter RCT is needed in the near future.

3.2. Future perception and conclusion

At present, IN is used only for outpatients with mild-to-moderate UC and psoriasis. It is not clear whether the efficacy of IN is comparable to traditional medicine for patients with IBD and other inflammatory disorders. However, intractable cases, in which mucosal regeneration is disturbed, are not effectively treated even with recent biologics.
Treatments with medications with novel mechanisms of action are necessary to address the needs of patients who do not respond to existing therapy. Existing medications mainly act on the immune system and do not target epithelial regeneration. IN displays important activity by repairing colonic mucosa in aryl hydrocarbon receptor signaling involved in maintenance of homeostasis and mucosal immunity. Therefore, the mechanism of action of IN differs from that of anti-inflammatory treatments, and, as a result, IN may be effective for refractory cases. In the near future, the efficacy of IN for refractory cases should be confirmed in a large control study.

Recent reports have indicated that IN induces critical adverse effects, such as liver dysfunction, severe colitis, and PAH. To address these concerns, new medication needs to be developed by devising drug delivery system. Because topical IN is safe and effective for psoriasis, rectal IN may also be useful for UC patients. More recently, a suppository form of IN has been used in a clinical trial to confirm the safety and efficacy of rectal IN for active UC. It may also be necessary to develop medications that are not absorbed in the small intestine to alleviate adverse effects. Many refractory patients may be helped by IN if it becomes available in appropriate forms for clinical practice.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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