Ulcercative colitis (UC) is an immune-mediated intestinal disease characterized by periods of remission and relapse and is usually treated with aminosalicylate, sulfasalazine, or 5-aminosalicylic acid (5-ASA) followed by corticosteroids or azathioprine (AZA). However, these medications cannot improve UC in some cases. Qing-Dai (QD), a Chinese herbal medicine, despite its unapproved status as a medicine for UC in Japan, has been used to treat patients who do not respond to traditional regimens for UC. QD, or *Indigo naturalis*, is extracted from plants such as *Strobilanthes cusia* and *Isatis tinctoria*. Traditionally, QD has been used as an antiinflammatory, antipyretic, antiviral, and antimicrobial medicine to treat patients with UC in China. Some previous studies have suggested that QD treatment resulted in clinical and endoscopic improvements in patients with UC. Given that approximately 20% of patients with UC in China are treated with only Chinese herbs, Chinese herbal medicine has been used as an intriguing drug treatment for patients with UC in Japan. Recently, a prospective pilot study reported that QD was effective in inducing remission in patients with UC. However, it has been reported that patients with UC taking oral QD sometimes develop liver disorder, intussusception, and ischemic colitis. Furthermore, the first case of pulmonary arterial hypertension (PAH) as a fatal side effect caused by oral QD treatment was reported in 2016. After the publication of that report, the Japanese Ministry of Health and Welfare issued a warning on oral QD medication. When patients with UC developed PAH due to oral QD treatment, they were treated as having drug-induced PAH. Therefore, they discontinued oral QD medication and PAH was treated with pulmonary arterial-specific medications such as phosphodiesterase type 5 inhibitor.

**Background:** Qing-Dai (QD) treatment of patients with ulcerative colitis (UC) sometimes causes pulmonary arterial hypertension (PAH). However, the relationship of QD treatment to pulmonary arterial systolic pressure (PASP) in patients with UC has not been clarified.

**Methods and Results:** The 27 patients with UC who were screened for PAH by transthoracic echocardiography (TTE) and underwent repeat TTE at 1 year were analyzed in this prospective observational study. Mean age was 44.0 years old, and median follow-up duration was 392. During the follow-up, 21 patients continued QD treatment (continuous group) and 6 patients discontinued the treatment (discontinuous group). In all patients, no significant difference in PASP levels between baseline and at follow-up was observed (21.4 vs. 21.3 mmHg, P=0.802). Furthermore, the mean PASP of patients in the continuous group did not differ from baseline to follow-up (21.4 mmHg to 22.6 mmHg, P=0.212); however, in the discontinuous group mean PASP was significantly decreased (21.5 mmHg to 16.8 mmHg, P=0.005). Moreover, changes in PASP from baseline to follow-up differed between the continuous and discontinuous groups (+1.1 mmHg vs. −4.7 mmHg, P=0.004). In addition, multivariable analyses revealed that only the duration of oral QD at baseline affected the increase of PASP.

**Conclusions:** In patients with UC, QD treatment may have an undesirable association with an increase in PASP.

**Key Words:** Pulmonary arterial hypertension; Pulmonary arterial systolic pressure; Ulcerative colitis
and endothelin receptor antagonists.

However, there is no evidence that QD medications affected the pulmonary arterial systolic pressure (PASP) in those patients. Although a lot of patients with UC receive QD orally, their PASP levels were not evaluated because of the absence of symptoms suggestive of PAH. Therefore, there have been no reports of detailed studies of the association of oral QD use with PASP. Previous reports have suggested that PASP is affected by several factors, including left ventricular function and pulmonary hypertension. However, the exact mechanism by which QD affects PASP remains unclear.

Methods

Study Design and Population

This study was conducted as a single-center, prospective observational study. It study was approved by the institutional ethical committee (approval no. 2639). The patients provided written informed consent before participating in the study. A total of 40 patients with UC who were orally taking QD were prospectively and consecutively enrolled. They underwent several examinations, including blood examinations, ECG, transthoracic echocardiography (TTE), and respiratory function tests. All patients were scheduled to undergo repeat TTE at 1 year from their initial visit. UC was diagnosed by several gastroenterologists. We excluded 13 patients: 11 patients who could not undergo follow-up TTE, 1 patient who rejected follow-up TTE, and 1 patient who had un evaluable tricuspid regurgitation jet peak velocity (TRV). Finally, we analyzed 27 patients with UC who had their TRVs measured by TTE at both an initial visit and at 1-year follow-up.

Regarding administration of QD, it was not necessary to continue oral QD treatment until follow-up TTE, and it was possible to discontinue the treatment according to the severity of UC. The gastroenterologists or the patients themselves, but not cardiologists, made the decision on whether to continue QD administration. Depending on continuation of QD treatment, we divided the patients into 2 groups: continuous and discontinuous.

Study Outcomes

Primary outcome was the difference in PASP levels between baseline and at 1-year follow-up in all patients with UC.

Secondary outcomes were differences in echocardiographic parameters between baseline and at follow-up in all patients, including left ventricular ejection fraction (LVEF), LV end-diastolic diameter (LVDd), peak velocity flow in early diastole (E wave), left atrial dimension (LAD), tricuspid annular plane systolic excursion (TAPSE), and right atrial area (RA area). For the post-hoc analyses, we evaluated the differences in the same echocardiographic parameters, including inferior vena cava (IVC) dimension, between baseline and at 1-year follow-up in both the continuous and discontinuous groups.

Echocardiographic Analysis

We analyzed both the initial and follow-up TTE examinations, which were performed using a phased-array system with a 2.5-MHz transducer according to a standardized protocol by experienced echocardiographers. TRV was measured by 2 independent blinded sonographers. We measured LVEF, LVDd, E wave, TRV, IVc, TAPSE, and RA area. The tricuspid regurgitation jet pressure gradient (TRPG) was calculated using the simplified Bernoulli equation: TRPG=4×TRV²; IVC dimension was measured at end-expiration. TAPSE was acquired by placing an M-mode cursor through the tricuspid annulus and measuring the amount of longitudinal motion of the annulus at peak systole. Furthermore, PASP was calculated by adding 5 mmHg as the estimated RA pressure to the TRPG. All measures of cardiac performance were averaged over 3 cardiac cycles.

Statistical Analysis

Patient data are expressed as the mean±SD or median with interquartile range (IQR). The comparison of the data between baseline and follow-up was performed using paired t-test. We used the Mann-Whitney U test for parameters with asymmetric or distorted distribution, and ANOVA (2-sample t-test) for the other parameters. Analyses of the area under the receiver-operating-characteristic curve (ROC) were performed. The independent association with increased PASP after 1 year was evaluated using univariate and multivariate analyses. Correlations with clinical data were assessed by Spearman’s rank correlation coefficient. Statistical significance was defined as P<0.05.

Statistical analysis was performed using EZR software (Saitama Medical Center, Jichi Medical University, Japan, version 1.6–3).

Results

Baseline Characteristics

Patients’ characteristics are shown in Table 1. Mean age
was 44.0 years and 13 patients were female. Median follow-up was 392 days (IQR: 370–420). Median duration of UC was 9.0 years, and median duration of oral QD use was 36.0 months. The mean number of drugs patients received before starting QD treatment, such as 5-aminosalicylic acid and corticosteroids, was 1.8. No patients had dyspnea or any symptoms suspicious for PAH. Echocardiographic examinations revealed that mean LVEF, LVDd and PASP were 69.3%, 47.1 mm, and 21.4 mmHg, respectively (Table 2). In addition, none of the patients had structural abnormalities with suspicion of PAH, such as flattening of the interventricular septum or RV dilation. 

**Assessment of All Patients With Follow-up TTE**

To investigate the effect of oral QD on pulmonary artery pressures, we assessed 27 patients who underwent TTE at follow-up. None had any symptoms suspicious of PAH at follow-up TTE. Mean PASP at baseline and at follow-up were 21.4 mmHg and 21.3 mmHg, respectively. There was no significant difference in mean PASP between baseline and follow-up (P=0.802). The highest PASP levels were 30 mmHg at baseline and 30 mmHg at follow-up. No patient who enrolled in this study showed PAH on either initial or follow-up echocardiography. Moreover, mean LVEF, LVDd, and TAPSE at follow-up were 68.7%, 47.4 mm, and 21.3 mm, respectively. These parameters showed no significant differences between baseline and follow-up (LVEF, P=0.517; LVDd, P=0.447; TAPSE, P=0.054) (Table 2).

**Table 2. Echocardiographic Parameters at Baseline and at Follow-up in All Patients With Ulcerative Colitis**

| Parameter       | Baseline     | Follow-up    | P value |
|-----------------|--------------|--------------|---------|
| LVEF (%)        | 69.3±4.6     | 68.7±3.2     | 0.517   |
| LVDd (mm)       | 47.1±4.5     | 47.4±4.7     | 0.447   |
| E wave (cm/s)   | 76.1±17.4    | 74.6±16.2    | 0.621   |
| LAD (mm)        | 32.7±4.9     | 33.1±4.3     | 0.316   |
| PASP (mmHg)     | 21.4±3.6     | 21.3±5.2     | 0.802   |
| IVC (mm)        | 16.9±2.9     | 17.2±4.1     | 0.694   |
| TAPSE (mm)      | 22.7±4.6     | 21.3±3.9     | 0.054   |
| RA area (cm²)   | 14.3±4.6     | 14.0±3.3     | 0.597   |

Data are presented as mean±SD. The comparison between baseline and follow-up was performed using paired t-test. LAD, left atrial dimension; LVDd, left ventricular end-diastolic diameter; LVEF, left ventricular ejection fraction; IVC, inferior vena cava; PASP, pulmonary arterial systolic pressure; RA area, right atrial area; TAPSE, tricuspid annular plane systolic excursion; TRPG, tricuspid regurgitation pressure gradient.

**Table 3. Differences Between Baseline and Follow-up in the Continuous and Discontinuous Treatment Groups**

| Parameter       | Baseline     | Follow-up    | P value |
|-----------------|--------------|--------------|---------|
| Subjects (n)    | 21           | 6            | –       |
| Age (years)     | 45.7±15.3    | 37.8±16.8    | 0.366   |
| Female n (%)    | 9 (43)       | 4 (67)       | 0.700   |
| Duration of UC (years) | 10.0 (6.0–15.0) | 6.0 (4.0–10.3) | 0.198   |
| Duration of oral QD use (months) | 36.0 (19.0–84.0) | 42.0 (11.3–81.0) | 0.483   |
| Systolic BP (mmHg) | 123.1±16.9 | 123.3±21.9 | 0.977   |
| %VC (%)         | 115.9±9.8    | 115.4±8.3    | 0.935   |
| FEV1.0 (%)      | 110.4±12.4   | 108.7±13.9   | 0.953   |
| Hb (g/dL)       | 14.0±1.2     | 12.8±1.1     | 0.054   |
| BNP (pg/mL)     | 13.1 (8.1–31.8) | 15.1 (8.5–30.8) | 0.977   |

Data are presented as mean±SD or median with interquartile range. A two-sample t-test between continuous and discontinuous groups was performed on Systolic BP, Duration follow-up TTEs, %VC, and E wave at baseline, as well as in LVDd, LAD, and TAPSE at follow-up. The other indicators were analyzed using Mann-Whitney U test. 5ASA, 5-aminosalicylic acid; PSL, prednisolone; AZA, azathioprine. Other abbreviations as in Tables 1 and 2.
differences in the duration of UC, the duration of oral QD use, or PASP at baseline between the 2 groups (Table 3). Furthermore, the other parameters, including respiratory function tests and echocardiographic parameters, did not differ at baseline between the 2 groups. Similarly, we found no significant difference in various parameters at follow-up between the 2 groups except for PASP (P=0.003).

We analyzed the changes in PASP between baseline and follow-up in the respective groups (Figure 1). In the continuous group, mean PASP was 21.4 mmHg at baseline and 22.6 mmHg at follow-up. There was no statistical difference between baseline and follow-up (P=0.212). In contrast, in the discontinuous group we observed a significant difference in mean PASP between baseline and follow-up (21.5 mmHg at baseline and 16.8 mmHg at follow-up, P=0.005).

Furthermore, we compared the changes in mean PASP between the continuous and discontinuous groups. The change in mean PASP was +1.1 mmHg in the continuous group and −4.7 mmHg in the discontinuous group. We observed a significant difference in mean PASP change between groups (P=0.004, Figure 2).

Factors Contributing to the Increase in PASP
In order to investigate the effect of QD medication on PASP, we compared the features of patients in the continuous group between those with and without an increase in PASP during follow-up period.

Univariable analysis revealed that duration of QD treatment was significantly associated with increasing PASP during follow-up (Table 4). Similarly, multivariable analysis using 2 indicators with P-value <0.1 in the univariable analysis revealed that duration of QD treatment showed an independent association with enhancement of PASP after 1 year (Table 4).

In addition, ROC analysis was performed to determine the values for duration of QD treatment that defined an increase in PASP. ROC analysis revealed that the best cutoff value was 20 months and area under the curve was 0.884 (sensitivity 1.000, specificity 0.677). Using this cutoff value, we divided 21 patients in the continuous group into 2
Table 4. Predictive Factors at Baseline for Increased Pulmonary Arterial Systolic Pressure

| Indicators                             | Univariable analysis | Multivariable analysis |
|----------------------------------------|----------------------|------------------------|
|                                        | OR  | 95% CI | P value | OR   | 95% CI | P value |
| Age (years)                            | 1.01| 0.95–1.07 | 0.850 |     |       |          |
| Duration of UC (years)                 | 1.12| 0.96–1.30 | 0.165 |     |       |          |
| Duration of QD treatment (months)      | 1.05| 1.01–1.09 | 0.021 | 1.03| 1.00–1.06 | 0.032 |
| Systolic BP (mmHg)                     | 1.02| 0.96–1.07 | 0.578 |     |       |          |
| Hb (g/dL)                              | 0.74| 0.34–1.65 | 0.467 |     |       |          |
| BNP (pg/mL)                            | 1.00| 0.97–1.04 | 0.848 |     |       |          |
| LVEF (%)                               | 1.02| 0.83–1.26 | 0.829 |     |       |          |
| LVDd (mm)                              | 1.25| 0.98–1.61 | 0.076 | 1.06| 0.82–1.37 | 0.161 |
| TAPSE (mm)                             | 1.13| 0.90–1.43 | 0.296 |     |       |          |

CI, confidence interval; OR, odds ratio. Other abbreviations as in Tables 1 and 2.

Figure 3. Correlation between duration of Qing-Dai (QD) treatment and pulmonary arterial systolic pressure (PASP) at baseline. PASP significantly correlated with duration of oral QD treatment in patients with longer duration of treatment in the continuous group (A, Right). However, there is no significant association between PASP and duration of QD use in patients with shorter treatment duration in the same group (A, Left). In all groups, there is good correlation between longer treatment duration with QD and PASP at baseline (B, Right), but no significant correlation in all groups between duration of QD treatment and PASP at baseline with shorter treatment (B, Left).
groups: 15 patients with longer treatment of QD and 6 patients with shorter treatment. There was a strong correlation between PASP level and the duration of oral QD use in the longer treatment group (r=0.600, P=0.018) (Figure 3A). However, no significant association was observed between PASP and duration of QD use in patients with shorter treatment (r=-0.580, P=0.288) (Figure 3A). Moreover, in the total patients with longer treatment, we also observed a good correlation between duration of QD and PASP at baseline (r=0.504, P=0.032) (Figure 3B).

**Discussion**

In this prospective study, we clarified that oral QD use gradually and steadily increases PASP in patients with UC. Importantly, none of them were suspected of having PAH at baseline or follow-up in this study, although some patients taking QD may have developed PAH even without symptoms.

We did not observe a significant change in mean PASP between baseline and follow-up in all patients who received QD treatment at baseline; however, we detected a significant decrease in mean PASP in the discontinuous group. Moreover, we found gradual elevation of PASP in patients with QD treatment duration ≥20 months. These results suggested the following 2 important issues: (1) discontinuation of oral QD treatment could reduce PASP and (2) longer administration of QD leads to higher PASP according to the duration of oral QD administration, although within a normal range.

**Previous Reports of QD-Induced PAH**

To the best of our knowledge, there are 3 case reports of QD-induced PAH. In the first report about PAH in a 45-year-old woman with UC who received QD (2 g/day), the patient showed severe PAH with a mean pulmonary arterial pressure (mPAP) of 36 mmHg and pulmonary vascular resistance (PVR) of 8.6 Wood units. She stopped oral QD medication because she was diagnosed with drug-induced PAH and received bosentan instead. At 4 months after ceasing the QD administration, her haemodynamic parameters had improved to mPAP of 12 mmHg and PVR of 2 Wood units.

In the second report of a 59-year-old woman with UC, the patient had mPAP of 32 mmHg and 7.1 Wood units of PVR. She discontinued QD medication and was treated with 3 pulmonary arterial-specific vasodilators. At 7 months after the diagnosis of PAH, her haemodynamic parameters had improved to mPAP of 12 mmHg and PVR of 2 Wood units. In the third case, a 32-year-old man had mPAP of 48 mmHg and PVR of 7.5 Wood units. A reduction in mPAP from 48 to 12 mmHg was observed after 2 years' treatment with beraprost. Interestingly, he continued oral QD treatment while receiving beraprost. These reports suggest that oral QD treatment might decrease after discontinuation of oral QD or by administration of PAH-specific medications.

**Mechanisms of Oral QD for PASP**

In previous studies, genetic and environmental factors may have played an important role in the development of drug-induced PAH in susceptible individuals; however, the precise mechanism of the induction of PAH by QD remains to be elucidated.

We speculate that indigo, the main component of QD, is itself a candidate involved in the development of PAH. Indigo belongs to a type of indole derivative and has molecular similarity to serotonin. Serotonin has vasoconstrictive actions caused by direct α-adrenergic activations or by release of catecholamines. In particular, serotonin signaling can induce the proliferation and migration of smooth muscle cells in pulmonary arteries, which could cause PAH in susceptible individuals. The anorexigenic, including amiloride, fenfluramine, and dexfenfluramine, responsible for drug-induced PAH cause PAH by evoking the release of serotonin through their action on serotonin-transporter substrates. QD may also directly act in pulmonary arteries as an exogenous serotonin-transporter substrate, resulting in vascular constriction and remodeling.

From the previous reports and our study results, we hypothesize 2 possible mechanisms of QD on PAH. One mechanism is that QD might cause pulmonary hypertension by constricting pulmonary vessels as a serotonin analog in the acute phase. The other mechanism is that QD might damage vascular endothelial cells in the pulmonary arteries in the chronic phase. The longer duration of oral QD, the greater the endothelial damage in pulmonary arteries, leading to an increase in PAP. The phenotype of PAH in each patient with UC might be formed through a combination of these 2 different mechanisms of QD.

The reduction in PASP with oral QD discontinuation may be due to an improvement in the increase in PAP associated with vasospasm in the early phase. On the other hand, in patients taking oral QD for a long time, the poor improvement of PASP may be due to remodeling of the pulmonary artery itself.

These results suggest that when UC patients are administered oral QD, even if they do not develop PAH, the clinician managing such patients should regularly monitor their cardiac function.

Finally, it has been reported that patients with UC who had a relapse of the digestive symptoms of UC after stopping QD medication. This suggests the effectiveness of QD medication for remission of UC. When patients with UC receiving oral administration of QD develop PAH, in almost all cases they had to discontinue QD medication in order to treat PAH. By stopping QD medication, we need to pay keen attention not only to treatment of PAH but also to recurrence of UC. Therefore, to avoid unnecessary cessation of QD administration, further study is required to investigate the prevalence and predictors of PAH induced by QD in patients with UC.

**Study Limitations**

Firstly, this was a single-center study with a small sample size. Secondly, this study included patients who were referred to the cardiovascular division, so we cannot exclude the possibility of selection bias. Thirdly, we could not compare patients between with and without oral administration of QD. Fourthly, we could not collect actual dosage data of the oral QD treatment, because all patients had personally purchased many different types of QD from various companies including overseas owing to the drug being unapproved for use in Japan. Fifthly, we could not completely follow all patients undergoing follow-up echocardiography in this observational study either because of stable condition of UC or the distance of their residence from the hospital. Finally, we could not exclude facility-specific biases in the echocardiographic measurements because of being a single-center study.
**Conclusions**

Our report demonstrated a potential association between oral QD medication and an increase in PASP. PAPs in patients with UC with longer administration of QD potentially may be elevated. When treating patients with UC with QD medication, it is necessary to regularly monitor their PAPs even if they are asymptomatic.

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