Impact of Itraconazole After First-line Chemotherapy on Survival of Patients with Metastatic Biliary Tract Cancer

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Abstract. *Aim: We evaluated the efficacy and safety of itraconazole after first-line chemotherapy in patients with metastatic biliary tract cancer (BTC). Patients and Methods: We retrospectively reviewed data from patients with histologically-diagnosed BTC with distant metastases who had received one or more lines of chemotherapy and subsequent itraconazole chemotherapy. Results: Among 28 enrolled patients, 26 (93%) received docetaxel (35 mg/m^2), gemcitabine (1,000 mg/m^2), and carboplatin (AUC4) on day 1 and oral itraconazole solution (400 mg) on days −2 to 2, repeated every 2 weeks. Two patients received docetaxel plus itraconazole with irinotecan. Two complete responses and 14 partial responses were observed, with a response rate of 57%. The median overall survival was 12.0 months. During 160 cycles, 21 (75%) and 17 (61%) patients had grade 3/4 neutropenia and thrombocytopenia, respectively. Two patients (7%) experienced febrile neutropenia. Conclusion: Combination chemotherapy with itraconazole after first-line chemotherapy is promising for patients with metastatic BTC.*

Biliary tract cancer (BTC) includes cancer arising from the gall bladder, bile ducts, and ampulla of Vater. The incidence of BTC is relatively rare, and varies by geographic region and racial ethnic group. In Japan, 11,345 patients were newly-diagnosed in 2010 (1), and the incidence is approximately 10-fold that in the UK. The prognosis of non-resectable, recurrent, or metastatic disease is poor. A combination of gemcitabine and either cisplatin or oxaliplatin is regarded as the standard first-line regimen (2, 3). There is insufficient evidence to recommend second-line chemotherapy, although some benefits of second-line chemotherapy have been reported. There have been no randomized trials investigating second-line chemotherapy for BTC. However, 63 out of 410 (15%) patients in the UK's ABC-02 trial, and 63 out of 84 (75%) patients in Japan's BT22 trial received second-line chemotherapy, and the median overall survival (OS) was similar in the two trials (11.7 and 11.2 months, respectively) (2, 4). A systematic review of phase II trials and retrospective analyses revealed a mean OS of 7.2 months after second-line chemotherapy (5).

Itraconazole, a common anti-fungal agent, is a potent inhibitor of P-glycoprotein (P-gp; ATP-binding cassette sub-family B member 1, multiple drug resistance 1), which plays a critical role in chemoresistance (6, 7). Itraconazole also inhibits Hedgehog (Hh) signals in cancer stem cells (CSCs) (8). Since 2008, we have treated patients with BTC using combination chemotherapy with itraconazole, and herein we report our retrospective exploratory study that aimed to assess the efficacy and toxicity of itraconazole-containing regimens for the treatment of refractory BTC.

Patients and Methods

We retrospectively reviewed the medical records of patients with histologically confirmed BTC who had a history of progression during or after prior chemotherapy and who subsequently received chemotherapy in conjunction with itraconazole. All patients provided written informed consent, and the treatment protocol was approved by the Institutional Review Board (IRB no.: 2007-0302).

Treatment protocol. Docetaxel-containing cytotoxic regimens were administered in combination with itraconazole. An oral itraconazole solution was administered at 400 mg/day on days −2 to 2, every 2 weeks. Dose modifications were made to maintain the nadir of white blood cell (WBC) and platelet counts within 1,000-1,500/mm^3 and 30,000-50,000/mm^3, respectively. Granulocyte colony-stimulating factor (G-CSF) was administered according to the manufacturer's recommendations until the WBC and absolute neutrophil counts recovered. The regimen was continued until disease progression or

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until other cytotoxic regimens with itraconazole were used to prevent chemotherapy-induced peripheral neuropathy.

Efficacy was evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1. OS was defined as the time from the first itraconazole administration to death. Adverse events were graded according to the National Cancer Institute Common Toxicity Criteria, version 4.0. Statistical analyses were performed on the observed distributions of OS using the Kaplan–Meier method. All the analyses were performed using XLSTAT 2014 (Addinsoft, Paris, France).

Results

Patients' characteristics. All patients were referred to our Institution from tertiary Hospitals, and 20 (71%) were referred after progression during prior chemotherapy. Between January 2008 and August 2013, 28 patients with metastatic BTC were concurrently treated with chemotherapy and itraconazole. According to the RECIST 1.1 criteria, all patients had distant metastases and 19 (68%) had multiple organ metastases. Eighteen (64%) patients had previously undergone two or more lines of chemotherapy. Twenty-three (82%) patients had progressive disease during prior chemotherapy, and 18 (64%) started itraconazole-containing regimens within 30 days of the last date of prior chemotherapy. Bile duct stents were placed in six (21%) patients before treatment of chemotherapy with itraconazole. The patients' characteristics are summarized in Table I.

The regimens contained docetaxel, gemcitabine, and carboplatin plus itraconazole (DGC plus itraconazole) in 25 patients and docetaxel and irinotecan plus itraconazole in two patients. The median number of cycles was 6 (range=2-17). Twenty-five (89%) patients discontinued the initial cytotoxic regimen with itraconazole within six cycles. Five (18%) patients experienced disease progression during the initial regimen. Details of the DGC plus itraconazole regimen were previously reported (9). Briefly, the starting doses of intravenous docetaxel, carboplatin, and gemcitabine were 35 mg/m^2 (day 1), area under the curve of 4 mg min^{-1} ml^{-1} (day 1), and 1000 mg/m^2 (day 1), respectively.

Table I. *Patients' characteristics (n=28).*

	No.
Median age (range), years	62 (34-72)
Gender	17 (61%)
Male	
Female	11 (39%)
Primary tumor location	
Gall bladder	12 (43%)
Intrahepatic bile duct	6 (21%)
Extrahepatic bile duct	10 (36%)
Stage	
I-III	5 (18%)
IV A	11 (39%)
IV B	12 (43%)
Prior treatment	
Chemotherapy	28 (100%)
Surgery	17 (61%)
Radiotherapy	2 (7%)
Lines of prior chemotherapy	
1	10 (36%)
≥2	18 (64%)
Prior chemotherapy drugs	
Gemcitabine	17 (61%)
S-1	14 (50%)
Cisplatin	4 (14%)
Paclitaxel	1 (14%)
Treatment-free interval*	
<30 days	18 (64%)
30-90 days	8 (29%)
>90 days	2 (7%)
ECOG performance status	
0	25 (89%)
1	3 (11%)
Site of metastases	
Liver	14 (50%)
Peritoneum	9 (32%)
Lung	6 (21%)
Number of metastatic sites	
1	9 (32%)
2	14 (50%)
>3	5 (18%)

*Treatment-free interval, time between the end of prior chemotherapy and the day of initiation of combination chemotherapy with itraconazole; n, Number; ECOG, Eastern Cooperative Oncology Group.

Efficacy of combination chemotherapy with itraconazole. During a median of six cycles of chemotherapy with itraconazole, two complete responses and 14 partial responses were observed (Table II), yielding a chemotherapy response rate of 57% [95% confidence interval (CI)=39-75%). After the induction of combination chemotherapy with itraconazole, four patients underwent primary surgery and three underwent radiotherapy. The median OS was 12.0 months (95% CI=9.1-24.6 months), with data from five patients censored (Figure 1).

Toxicities. During the administration of 160 cycles, all patients received G-CSF (Table III). Nineteen (68%), 21 (75%), and 17 (61%) patients had grade 3/4 anemia, neutropenia and thrombocytopenia, respectively. Nineteen (68%) required packed red blood cell transfusion when their hemoglobin levels decreased to <8.0 g/dl. No patients required a platelet transfusion. Two (7%) patients experienced febrile neutropenia. No patients had grade 2 or more sensory peripheral neuropathy. There were no treatment-related deaths.

Discussion

Herein, we demonstrated that administration of a combination of chemotherapy with itraconazole was safe and

Table II. *Tumor response to combination chemotherapy with itraconazole (n=28).*

Response	No.
Complete response	2 (7%)
Partial response	14 (50%)
Stable disease	8 (29%)
Progressive disease	4 (14%)
Response rate	57 (95% CI=39-75%)

CI, Confidence interval.

Table III. *Toxicities during 160 cycles of combination chemotherapy with itraconazole (n=28).*

Adverse events	Grade 1	Grade 2	Grade 3	Grade 4	Grade 3/4
Anemia	1	8	17	2	19 (68%)
Leukopenia	2	4	17	5	22 (79%)
Neutropenia	3	4	10	11	21 (75%)
Thrombocytopenia	3	8	12	5	17 (61%)
Febrile neutropenia					2 (7%)
Transfusion					
Packed RBCs*					19 (68%)
G-CSF					28 (100%)
Increased T. Bil	6	1	0	0	0
Increased ALT	9	5	3	0	3 (11%)
Increased Cr	3	0	0	0	0

*Packed red blood cells were transfused when hemoglobin levels fell below 8.0 g/dl. T. Bil, Blood total bilirubin; AST, serum aspartate aminotransferase; Cr, serum creatinine; G-CSF: granulocyte-colony stimulating factor.

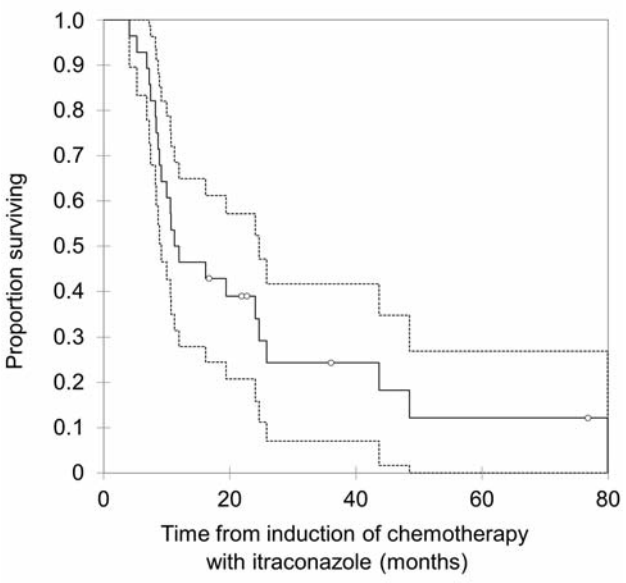

Figure 1. *Kaplan–Meier analysis of overall survival (OS) after chemotherapy with itraconazole (n=28). OS is shown by the solid line while the 95% confidence lines are indicated by dashed lines The median OS was 12.0 months (95% confidence interval=9.1-24.6 months).*

efficacious for the treatment of patients with refractory metastatic BTC, with more than half of patients demonstrating a response. The median OS was 12.0 months (95% CI=9.1-24.6 months), which was favorable compared to that of historical controls. A systematic review revealed a mean OS after second-line chemotherapy was 7.2 months (95% CI=6.2-8.2 months) (5).

The current protocol of adding itraconazole to cytotoxic chemotherapy was primarily based on previous reports, indicating that itraconazole enhanced the effect of taxanes by inhibiting P-gp (6, 7). After exposure to cytotoxic agents, residual tumors typically harbor CSCs (10). The concept of CSCs, or tumor-initiating cells, was proposed to explain metastasis and recurrence after exposure to chemotherapy. CSCs are characterized by self-renewal, multi-differentiation, and chemoresistance, and they have been isolated from various types of cancer including cholangiocarcinoma (11). CSCs have also been associated with the epithelial–mesenchymal transition, which plays a pivotal role in tumor invasion (12). Pre-treated CSC-rich residual tumors overexpress P-gp (13, 14). Itraconazole has the highest affinity among anti-fungal agents, which serve as substrates for P-gp (15), and the resistance of pretreated cancer cells or CSCs to docetaxel can be reversed with itraconazole treatment (7, 16).

In 2010, Kim *et al*. reported that itraconazole inhibited the Hh signaling pathway in CSCs and in cancer cells (89. The Hh signaling pathway plays a key role in embryogenesis, and is re-activated in various cancer types (17). The expression of Smoothend (Smo) was detected in approximately 70% of pancreatic cancer specimens (18), and the Hh signaling pathway has been extensively studied in pancreatic cancer. Inhibition of the Hh signaling by cyclopamine reduced the self-renewal of pancreatic CSCs, and reversed chemoresistance (19). In a murine model of pancreatic cancer, the Hh inhibitor AZD8542 prevented tumor growth and metastasis by affecting the surrounding tumor microenvironment (20). A recent interim analysis of a single-arm phase II study on patients with untreated pancreatic cancer showed that the Smo antagonist vismodegib in combination with gemcitabine plus nab-paclitaxel had favorable efficacy with acceptable toxicities (21). Itraconazole inhibits the Hh signaling pathway (22). We administered cytotoxic agents with itraconazole to 38 patients with refractory pancreatic cancer and found favorable efficacy compared to historical controls (23).

Li *et al*. confirmed the activation of the Hh signaling pathway in gallbladder cancer; 76 (82%) and 66 (70%) specimens expressed sonic Hh and Gli1, respectively, and the expression status correlated with known prognostic factors and OS (24). Matsushita *et al*. reported that cyclopamine and Smo siRNA inhibited proliferation and Smo siRNA inhibited epithelial–mesenchymal transition and invasion *in vitro* (25). Kisslich *et al*. demonstrated inhibition of BTC cell growth in vitro using cyclopamine and the Hh transcription inhibitor Gant-61, and a synergistic cytotoxic effect was apparent in combination with cisplatin (26).

Since 2013, several studies have reported the clinical benefit of itraconazole for various cancer types including prostate, lung, skin, ovarian, breast, and pancreatic cancer (9, 23, 27-31). The addition of itraconazole to cytotoxic agents was reported to have improved OS in a prospective, randomized, phase II study on the second-line treatment of non-small cell lung cancer and in a retrospective multivariate analysis of refractory ovarian cancer (28, 30). Cytotoxic regimens in combination with itraconazole should be further investigated considering the efficacy, toxicities, and compliance in daily practice.

The dose modification in the present study was complicated and demanded for close monitoring. Based on the promising results of DGC plus itraconazole for patients with refractory breast or ovarian cancer (9, 30, 31), we launched a phase II study of combined docetaxel, gemcitabine, and itraconazole for gynecological malignancy using a simplified dose modification and continuation until disease progression protocol (UMIN000013951).

Limitations of the present study include the small sample size, the observational and retrospective nature, and the complicated dose modification protocol. Nevertheless, the findings herein are encouraging for patients with refractory BTC, because combination chemotherapy with itraconazole demonstrated favorable efficacy with acceptable toxicity after first-line chemotherapy for treatment-refractory metastatic BTC. Furthermore, itraconazole is not an expensive drug and would be affordable to patients in less developed countries, and may reduce medical costs in developed countries.

Conflicts of Interest

The Authors declare no financial conflicts of interest with regard to this study.

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