Better efficacy of S-1 treatment for thymic carcinoma: case report and review of the literature

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Abstract Primary thymic carcinoma is a rare tumor of the thymus gland. The role of chemotherapy in treating advanced thymic carcinoma is unclear. It has recently been reported that thymidylate synthase (TS), dihydropyrimidine dehydrogenase (DPD), and orotate phosphoribosyltransferase (OPRT) expression may be important factors when predicting the effectiveness of 5-fluorouracil-based chemotherapy (Kaira et al., 74:419–425, 2011). In this report, we describe three cases in which S-1 or S-1 combination treatment was given as second-line therapy for thymic carcinoma, and examine the relationships between the immunohistochemical expression of TS, DPD, and OPRT and treatment effectiveness. There were no deaths due to toxicity. Two patients achieved partial remission (PR), and one patient achieved stable disease (SD). These results suggest that S-1 is a safe and promising regimen for thymic carcinoma. Immunohistochemical staining for OPRT expression tended to be stronger in the two PR cases than in the SD case. Based on the present results, it appears that S-1 may be useful for treating unresectable thymic carcinoma, and that OPRT expression may predict the response to S-1 treatment. Further clinical studies are needed to confirm these findings.

Keywords Thymic carcinoma · S-1 · Thymidylate synthase (TS) · Dihydropyrimidine dehydrogenase (DPD) · Orotate phosphoribosyltransferase (OPRT)

Introduction

Thymic epithelial tumors include thymoma, thymic carcinoma, and thymic carcinoid. Thymic carcinoma is a very rare malignancy of the anterior mediastinum, accounting for less than 0.06 % of thymic neoplasms [2] and invasive mediastinal neoplasms, and it often metastasizes [3]. Therefore, the optimal chemotherapeutic regimen for inoperable thymic carcinoma remains uncertain [4].

5-Fluorouracil (5-FU), a pyrimidine analog metabolized by pyrimidine metabolic pathways, has been used worldwide for chemotherapy in individuals with various solid organ malignancies. S-1 (Taiho Pharmaceutical Co., Ltd., Tokyo, Japan) is an oral anticancer agent composed of tegafur (TF), 5-chloro-2,4-dihydroxypyridine (CDHP), and potassium oxonate (Oxo), in the molar ratio 1:0.4:1 [5].

From 2008 to 2011, eight cases of thymic carcinomas treated by S-1-based chemotherapy have been reported, and all showed good responses. Recently, Kaira et al. [1] summarized 56 cases of thymic tumors, of which 17 were thymic carcinomas; they found a significant correlation between immunohistochemical expressions of TS, DPD, and OPRT, and the World Health Organization (WHO) International Histological Classification of Tumors grade of malignancy. Other than this report, there have been very few reports of thymic carcinoma patients who were treated with S-1 therapy, and no reports have described the details of immunohistochemical staining for TS, DPD, and OPRT and the effects of S-1 treatment.

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In this paper, three cases of unresectable thymic carcinoma that were treated with S-1 or S-1 combination chemotherapy and in which TS, DPD, and OPRT protein expression were evaluated by immunohistochemical staining are described. The relationships between the TS, DPD, and OPRT protein expression levels and the efficacy of S-1 were evaluated.

Methods

Three patients diagnosed with thymic carcinoma were included in this study. Three fine needle biopsy samples were collected from consecutive patients with unresectable thymic carcinoma who were treated at the National Hospital Organization Disaster Medical Center (Tokyo, Japan) from 2008 to 2011. The study was approved by the hospital's institutional review board. Written, informed consent was obtained from all living patients.

The responses were defined according to the Response Evaluation Criteria in Solid Tumors [6].

Immunohistochemical staining for TS, DPD, and OPRT

Formalin-fixed and paraffin-embedded 3- μ m-thick tissue sections were deparaffinized and rehydrated. For antigen retrieval steps, the sections were treated with microwaves for 10 min in a 10 μ M hot citrate buffer solution at pH 6.0. After quenching the endogenous peroxidase activity with 0.3 % H_2O_2 for 20 min, the sections were pre-incubated with peroxidase blocking reagent for 15 min. The sections were then incubated overnight at 4 °C with anti-TS [7], anti-DPD [8], and anti-OPRT polyclonal antibodies provided by Taiho Pharmaceutical Co. Ltd. (Saitama, Japan) (diluted to 1:500). Biotinylated goat anti-rabbit IgG (Dako, Kyoto, Japan) was applied as a secondary antibody for 20 min at room temperature, followed by streptavidin-biotinylated peroxidase complex for 20 min at room temperature. Peroxidase activity was visualized with a diaminobenzidine tetrahydrochloride solution for 5 min at room temperature. The sections were then counterstained with hematoxylin.

The slides were examined by a pathologist who was blinded to the clinical characteristics of the patients (Hist Science Laboratory Co., Ltd., Oume, Japan). Intensity was classified as 0 (no staining), +1 (weak staining), +2 (distinct staining), +3 (very strong staining), +4 (very, very strong staining).

Cases

Case 1

A 61-year-old female smoker presented with a history of eyelid drooping lasting several months. The clinical diagnosis was myasthenia gravis. Mediastinal imaging by computed tomography (CT) revealed a mass extending to the pleura that was initially thought to be malignant mesothelioma. Thoracoscopy and biopsy led to a diagnosis of thymic carcinoma (squamous cell carcinoma). Treatment with a regimen of cisplatin, doxorubicin, vincristine, and cyclophosphamide (ADOC) was started in January 2011. This treatment regimen was not effective, because the primary site showed growth within only two courses. Accordingly, she was switched to a second-line regimen of carboplatin (AUC, 5) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on days 1–14. Grade 1 nausea and grade 2 thrombocytopenia were recorded in the treatment cycles. Chemotherapy was repeated every three weeks for four cycles. The best objective tumor response was stable disease (SD) (Fig. 1).

Case 2

A 37-year-old man was found to have an abnormal chest shadow at an annual physical examination. Examination of an ultrasound-guided fine needle biopsy specimen led to a diagnosis of thymic carcinoma (squamous cell carcinoma). The tumor, which invaded the main pulmonary artery, could not be surgically resected (Masaoka–Koga classification IVb). The patient was treated with cisplatin, doxorubicin, vincristine, and cyclophosphamide (ADOC) as the first-line regimen. However, the tumor grew after treatment, necessitating additional palliative treatment. Carboplatin (AUC, 5) on day 1 plus oral S-1 (40 mg/m^2 twice per day) on days 1–14 were administered as a second-line therapy from March 2011 for the maximum of six cycles. Grade 2 neutropenia and grade 2 thrombocytopenia were recorded during treatment cycles. The mediastinal tumor shrank markedly, and a left pleural effusion disappeared. The best objective tumor response was partial response (PR) (Fig. 1).

Case 3

A 75-year-old man was found to have an abnormal chest shadow at an annual physical examination. Examination of a CT-guided fine needle biopsy specimen led to a diagnosis of thymic carcinoma (squamous cell carcinoma) (Masaoka–Koga classification IVb). The patient was treated with multiple chemotherapy and radiation therapy. The

	TS expression	DPD expression	OPRT expression	Treatment course	
Case 1
SCORE	1	0	2	Initial	2 Months
Regimen				carboplatin+S-1	
Case 2
SCORE	2	0	4	Initial	2 Months
Regimen				carboplatin+S-1	
Case 3
SCORE	3	0	4	Initial	8 Months
Regimen				S-1	

Fig. 1 Immunostaining results and tumor reduction with S-1 treatment. In the first case, no tumor reduction is seen (SD). Apparent tumor shrinkage is seen in cases 2 and 3, who achieved PR. TS protein is significantly expressed in cases 2 and 3, which show tumor shrinkage. Similarly, OPRT is prominently expressed in cases 2 and

3. High TS expression may serve to dampen the antitumor effect. Therefore, OPRT expression may have a strong relationship with the success of S-1 therapy. *SD* stable disease, *PR* partial response, *TS* thymidylate synthase, *DPD* dihydropyrimidine dehydrogenase, *OPRT* orotate phosphoribosyltransferase

tumor regrew after two regimens of chemotherapy (carboplatin + paclitaxel, carboplatin + CPT-11). The best objective tumor response was progressive disease (PD). Then, S-1 (40 mg/m² twice per day) single-agent chemotherapy was given on days 1–28 as a third-line regimen and continued for eight cycles. Grade 2 thrombocytopenia was recorded during treatment cycles. The best objective tumor response was partial response (PR) (Fig. 1).

Results

Case 1 showed no tumor shrinkage in response to carboplatin + S-1 therapy and was thus evaluated as SD. Immunohistochemical staining showed expression of the TS and OPRT proteins, but not of the DPD protein. However, the expression of both the TS protein and the OPRT protein was weak (i.e., 1+ and 2+, respectively; Fig. 1).

Case 2 responded to CBDCA + S-1 therapy, showing marked shrinkage of the tumor, and was evaluated as having achieved PR. Similar to case 1, the TS and OPRT proteins were expressed, but the DPD protein was not. Unlike case 1, TS expression was strong, and OPRT

expression was even stronger (i.e., 2+ and 4+, respectively; Fig. 1).

Case 3 was evaluated as achieving PR with S-1 monotherapy. Protein expression was similar to that in case 2; TS and OPRT proteins were expressed (i.e., 3+ and 4+, respectively; Fig. 1), but DPD protein was not. Also similar to case 2, OPRT protein expression was especially marked (Fig. 1).

Three cases of DPD protein expression stains look like positive results. The pathologist concluded that these were not positive results, since the nucleus was stained without the cytoplasm.

Discussion

Three patients with unresectable thymic carcinoma treated with S-1 and S-1 combination agents achieved good results, and the expression of various marker proteins in their tumors was evaluated. In particular, in two patients in whom tumor shrinkage was seen, although TS protein expression was strong, OPRT protein expression was striking.

Kaira et al. analyzed protein expression in 57 patients with thymic tumors, and 17 patients had thymic carcinoma. Three of the thymic carcinoma patients were treated with S-1, resulting in PR, SD, and progressive disease (PD) in one case each. The PR case showed 3+ expression of OPRT protein, which was stronger than the 2+ expression seen in the PD case. The present results were similar: in the patient whose thymic carcinoma responded to S-1 therapy, it was possible to expect a tumor shrinkage effect if a high level of OPRT protein expression was present, even if TS protein expression was high.

5-FU expresses its antitumor effect primarily through the inhibition of DNA synthesis and RNA function. The mechanism underlying its inhibition of DNA synthesis is thought to be as follows. In vivo, 5-FU is converted to its active form, 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP). Then, with reduced folic acid as a coenzyme, it strongly binds with TS, which catalyzes the integrity of thymidine necessary for DNA synthesis, forming a tripartite complex. This reduces the activity of TS, thereby inhibiting DNA synthesis. However, when TS is highly expressed in tumor tissues, DNA synthesis occurs due to the residual excess TS, and the antitumor effect of 5-FU is attenuated. TS expression in the tumor tissues of various solid cancers was investigated, and it was found to be lower in lung cancer than in other cancers, including colon cancer [9]. In view of the above, though 5-FU should be effective against most lung cancers based on the expression patterns, its efficacy is actually poor. A proposed reason for this is that, although 5-FU is activated in vivo, it is conversely rapidly broken down by DPD, a 5-FU-degrading enzyme. Furthermore, in carcinoma types with high intratumoral levels of DPD activity, 5-FU is rapidly degraded and its efficacy is decreased. DPD activity was reported to be at least twofold higher in lung carcinoma than in gastric and colon cancers [9]. Therefore, when 5-FU is used to treat carcinoma types with high DPD activity levels, inhibition of DPD is considered to be essential, and this realization led to the development of TS-1, which contains gimeracil.

Several investigators have also reported that squamous cell carcinoma of the lung exhibits a lower level of DPD than adenocarcinoma evaluated by immunohistochemistry or radioenzyme assay [9–11]. On the other hand, squamous cell carcinoma of the thymic carcinoma as the histopathologic type is seen in 62 % of thymic carcinomas, suggesting that the DPD expression in the three reported cases was low, possibly due to the histopathology [3]. In all three of our cases of squamous cell-type thymic carcinoma, DPD protein expression was absent. This suggests that S-1 might be effective as well as 5-FU in thymic carcinoma.

In non-small-cell lung cancer, TS expression is nearly twofold higher in squamous cell carcinoma than in adenocarcinoma; therefore, it can be surmised that the

antitumor effect of S-1 is attenuated. In fact, pemetrexed, which inhibits TS, showed inferior efficacy for squamous cell carcinoma than for adenocarcinoma [12].

As noted above, the second mechanism of action of 5-FU is inhibition of RNA function, which is mediated by the conversion of OPRT to fluorouridine monophosphate (FUMP) by a phosphorylating enzyme. In this case, it is known that higher OPRT expression results in higher antitumor efficacy of 5-FU. Investigation of OPRT expression in lung cancer showed that it was at least twofold higher in squamous cell carcinoma than in adenocarcinoma [9]. It can be surmised that S-1 exerts an antitumor effect on squamous cell carcinoma, mainly by inhibiting RNA function.

With regard to the thymus, Kaira et al. reported that, in an in vitro study with cell lines, TS and OPRT expression was markedly increased in thymic carcinoma cells compared with thymic tumor cells and thymic fibroblast cells. They reported three patients who received S-1 therapy: one of the tumors with high TS expression showed a good response, whereas that with hypo TS expression showed stable disease and progressive disease. Two cases with high OPRT expression showed good responses (PR and SD) [1]. We have described three cases of patients who received S-1 therapy; the tumor with high TS and OPRT expression, especially OPRT expression, showed a remarkable response. The present results suggest that OPRT expression might be more important than TS expression for treating S-1 therapy.

It is generally believed that potassium oxonate accumulates selectively in the digestive tract after oral administration of S-1 and then reduces gastrointestinal toxicity by blocking the OPRT pathway without affecting the antitumor activity of S-1. Tanaka et al. [12] reported that potassium oxonate prevents gastrointestinal toxicity without loss of the antitumor effect, as oxonate inhibits the phosphorylation of 5-FU in the gastrointestinal tract but not in tumor tissue.

A limitation of this study is that our sample size was small. Another limitation is that the tissue specimens available from small needle biopsy for the diagnosis of thymic carcinoma were small, as pathological examination of the entire gland was not possible. Saito et al. [13] reported two cases that were diagnosed as thymoma by needle biopsy before surgery, but were then found to have thymic cancer after surgery.

In thymic carcinoma patients with squamous cell carcinoma showing strong OPRT expression, the same mechanisms of response to S-1 therapy as those described for lung cancer and other types of cancer may be present. It is also possible that 5-FU-type anticancer drugs, especially S-1, might express their efficacy preferentially on thymic carcinoma.

TS, DPD, and OPRT protein expression and the related tissue systems have been documented for lung cancer and other types of cancer, but there are few detailed reports on differences in their characteristics for thymic tumors, especially thymic carcinomas.

In conclusion, therapy including S-1 should be considered a promising treatment option for unresectable thymic carcinoma in the future. Better efficacy of S-1 can be expected in patients who show high OPRT protein expression levels. Larger-scale clinical studies to evaluate the changes in TS, OPRT, and DPD expression after treatment with the S-1 regimen are warranted.

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Conflict of interest The author has any no conflicts of interest to declare.

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