Plasma Diamine Oxidase Activity Is a Useful Biomarker for Evaluating Gastrointestinal Tract Toxicities during Chemotherapy with Oral Fluorouracil Anti-Cancer Drugs in Patients with Gastric Cancer

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Key Words
Diamine oxidase activity • Gastric cancer • Chemotherapy • Quality of life • Gastrointestinal tract toxicity

Abstract
Objectives: Diamine oxidase (DAO) is an enzyme that catalyzes oxidation and is highly active in the mature upper villus cells of the intestinal mucosa. This study sought to evaluate plasma DAO activities during adjuvant chemotherapy in patients with gastric cancer. Methods: We investigated 20 patients with gastric cancer who were treated with oral fluorouracil anti-cancer drugs as adjuvant chemotherapy. Plasma DAO activity was measured in all patients before chemotherapy and at 2, 4 and 6 weeks after the start of chemotherapy, and quality of life was evaluated simultaneously. Results: The median DAO activity after 4 weeks of chemotherapy was significantly decreased compared to the pre-chemotherapy levels (6.6 vs. 7.5 U/l; \( p = 0.038 \)). The changes in the rate of DAO activity at 2 and 6 weeks following the start of chemotherapy in patients with gastrointestinal tract toxicity were significantly lower than in those without toxicity (\( p = 0.021 \) and 0.047, respectively). The patient cohort showed a slightly positive correlation between DAO activity and global health status and a negative correlation between DAO activity and appetite loss. Conclusions: Plasma DAO activities may be useful for monitoring and evaluating gastrointestinal tract toxicities induced by adjuvant chemotherapy with oral fluorouracil in patients with gastric cancer.

Introduction

Gastric cancer is the fourth most common cancer worldwide and is a leading cause of cancer-related death [1]. Results from a recent large-scale randomized controlled trial indicated that S-1, an oral fluoropyrimidine, is an effective adjuvant treatment for patients who have undergone a D2 dissection for locally advanced gastric cancer [2]. Consequently, using S-1 in adjuvant chemotherapy is now standard clinical practice for stage II/III gastric cancer patients in Japan. Adverse events caused by S-1 include gastrointestinal tract toxicities.
such as anorexia, nausea, diarrhea and stomatitis, which can negatively affect the nutritional status by decreasing food intake and result in disturbance or even discontinuation of chemotherapy. Therefore, preventing such gastrointestinal toxicities during chemotherapy is extremely important for improving the prognosis of cancer patients.

Diamine oxidase (DAO) is an enzyme that catalyzes oxidation, including the oxidative deamination of several polyamines, which are essential factors in cell proliferation. Therefore, DAO is an important regulator in rapidly proliferation tissues such as bone marrow and intestinal mucosa [3, 4]. In humans and rodents, DAO is found in various tissues, with small intestinal mucosa showing the highest enzymatic activity [5]. Furthermore, plasma DAO activity increases in parallel with DAO activity in the villi of the small intestinal mucosa in maturing rats [6, 7] and correlates with the severity of small intestinal mucosal lesions induced by anti-cancer drugs [8]. In addition, serum levels of DAO activities seem to be a reliable indicator of intestinal mucosal integrity and reflect quantitative changes in the small bowel mucosal mass [6, 9, 10].

Despite the common use of adjuvant chemotherapy after curative surgery for gastric cancer, the association between predictive indicators and gastrointestinal toxicities or quality of life (QOL) scores in patients undergoing chemotherapy has not been investigated. The present study measured DAO activity to evaluate mucosal injury in patients with gastric cancer undergoing postsurgical adjuvant chemotherapy. To the best of our knowledge, this is the first examination of DAO activities associated with gastrointestinal toxicities caused by chemotherapy and patient QOL.

Patients and Methods

Twenty patients with stage II or III gastric cancer, treated with oral fluorouracil anti-cancer drugs as adjuvant chemotherapy after curative operation at the Department of Surgery, Kochi Medical School (Nankoku, Japan) in 2010, were enrolled in this study. The hospital ethics committee approved the protocol and written informed consent was obtained from each patient. All patients received 80 mg of S-1 per square meter of body surface area per day, for 4 weeks, followed by 2 weeks of no chemotherapy. The plasma DAO activity was measured before chemotherapy and at 2, 4 and 6 weeks after the beginning of treatment. Simultaneously, QOL was evaluated using the European Organization of Research and Treatment of Cancer QOL Questionnaire (EORTC QLQ-C30) [11].

Measurement of Plasma DAO Activity

Blood samples were anticoagulated with heparin and centrifuged to obtain plasma for the determination of DAO activities, which was carried out according to the method of Takagi et al. [12]. Briefly, plasma was added to a cadaverine solution and incubated. The incubation mixture was then mixed with a color reagent containing DA-67 and peroxidase. After a given period, the absorption of the reaction product was measured colorimetrically at 668 nm against the blank solution using a spectrophotometer. The plasma DAO activity was expressed as units per liter.

Assessment of QOL

We assessed the QOL during chemotherapy by administering the EORTC QLQ-C30 [11]. The EORTC QLQ is an integrated system for assessing health-related QOL in cancer patients participating in international clinical trials. The QLQ-C30 contains scales and items addressing functional aspects of QOL and symptoms that commonly occur in patients with cancer. These include five functional scales, three symptom scales, a global health status scale, and six single items. All of the scales and single-item measures range in score from 0 to 100, with a high–scale score representing a higher response level. Thus, a high score for a functional scale represents a high or healthy level of functioning, and a high global health status scale represents a high QOL, but a high score for a symptom scale or item represents a high level of symptomatology or problems. Kobayashi et al. [13] confirmed the validity and reliability of the Japanese version of the EORTC QLQ-C30 in Japanese cancer patients. The EORTC QLQ-C30 questionnaire was delivered to the patients during the plasma DAO activity measurements.

Statistical Analysis

Significances of difference between mean values were assessed by the two-tailed Mann-Whitney U test or the two-tailed Welch t test. The χ² test was used to evaluate differences between qualitative variables. Correlation between DAO activity and QOL scores using EORTC QLQ-C30 was evaluated by calculating Pearson’s product moment correlation coefficient. All data are presented as the mean ± standard deviation. p values <0.05 were considered to indicate statistical significance. Statistical analysis was performed with SPSS® for Windows version 13.0 (SPSS, Chicago, Ill., USA).

Results

Patient Characteristics

Table 1 summarizes the clinical characteristics of all patients (n = 20) in this study. Our cohort comprised 16 men and 4 women with a median age of 67 years (range 44–78). The patients underwent distal gastrectomy in 13 and total gastrectomy in 7 cases and included 4 cases in stage IIA, 3 cases in stage IIB, 5 cases in stage IIIA, 4 cases in stage IIIB, and 4 cases in stage IIE classified according to the International Union against Cancer TNM classification [14] and the TNM Supplement [15]. Six patients...
had adverse events related to gastrointestinal toxicities of grade 1 or 2 (defined according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0) including stomatitis, appetite loss, nausea and diarrhea.

**DAO Activity during Chemotherapy**

The median DAO activity before chemotherapy and at 2, 4 and 6 weeks after chemotherapy was 7.5 (range 2.5–23.9), 6.9 (range 2.5–30.8), 6.6 (range 1.5–18.0) and 5.7 U/l (range 1.5–24.6), respectively. DAO activity at 4 weeks after the start of chemotherapy was significantly decreased compared to the levels measured before chemotherapy (p = 0.038; fig. 1). DAO activity at 6 weeks after the beginning of chemotherapy was decreased compared to the pre-chemotherapy level, while there was no significant difference.

Changes in DAO activities during chemotherapy relative to gastrointestinal toxicities are shown in figure 2. Changing rates of DAO activity at 2 and 6 weeks after the beginning of the chemotherapy in patients with gastrointestinal tract toxicity were significantly lower than in those patients without toxicity.

**Table 1.** Patient characteristics

<table>
<thead>
<tr>
<th>Median age (range), years</th>
<th>67 (44–78)</th>
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<tbody>
<tr>
<td>Gender</td>
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<td>IIIC</td>
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<tr>
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<td>Appetite loss</td>
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<td>Nausea</td>
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<td>Diarrhea</td>
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**Fig. 1.** Changes in DAO activities during chemotherapy. DAO activity at 4 weeks after the beginning of the chemotherapy was significantly decreased compared to the measured activity before chemotherapy.

**Fig. 2.** Changes in DAO activities during chemotherapy relative to gastrointestinal toxicities. Changing rates of DAO activity at 2 and 6 weeks after the beginning of the chemotherapy in patients with gastrointestinal tract toxicity were significantly lower than in those patients without toxicity.
Correlation between DAO Activities and QOL

Figure 3 indicates the correlation between DAO activities and QOL based on the EORTC QLQ-C30 scores. DAO activities showed a significant positive correlation with global health status ($\gamma = 0.246$). On the other hand, there was a slight, but significant, negative correlation between DAO activities and appetite loss ($\gamma = -0.351$). There were no significant correlations between DAO activities and other domains of the QOL scores including functional scales and symptom scales.

Discussion

Our study demonstrated that plasma DAO activities were significantly decreased by adjuvant chemotherapy using S-1 in patients with gastric cancer who had undergone curative surgery. Furthermore, the findings indicated lower rates of change in DAO activity in those patients also showing gastrointestinal tract toxicity induced by chemotherapy.

A study in rats showed decreased plasma DAO activity in animals administered an oral fluorouracil anti-cancer drug and a correlation between the changes in plasma DAO activity and the severity of histopathological findings in the jejunal mucosa and mucosal area induced by the anti-cancer agent [16]. These results suggested that mucosal injury could be caused by a decreased release of intestinal mucosal DAO into the systemic circulation. Our report of plasma DAO activities in human patients given oral fluorouracil anti-cancer drugs for gastric cancer is the first to mirror the findings in animals.

In the present study, plasma DAO activities correlated slightly with some QOL scores, namely global health status and appetite loss. Abdominal symptoms including diarrhea and appetite loss are common gastrointestinal tract toxicities induced by cancer treatment and are caused by intestinal mucosal dysfunction due to the chemotherapeutic drugs. Diarrhea and appetite loss are the most frequent adverse events in adjuvant chemotherapy for gastric cancer, with an incidence of 59.8–61.1% [2]. Our study revealed a correlation between the severity of gastrointestinal tract toxicity due to anti-cancer drugs and plasma DAO activity in patients on chemotherapy. In patients with hematological malignancies, plasma DAO activity was significantly correlated with the severity of small intestinal mucosal lesions induced by anti-cancer drugs [8]. From the standpoint of both gastrointestinal tract toxicities and QOL, plasma DAO activity may be a useful indicator of mucosal injury following chemotherapy.

It has been reported that treatment with irinotecan hydrochloride (CPT-11), a topoisomerase I inhibitor highly effective for various cancers, caused severe diarrhea and simultaneously decreased mucosal DAO activity [17]. However, to the best of our knowledge, there is no study demonstrating a relationship between plasma DAO ac-
tivity and diarrhea, including our study. When intestinal mucosa cells became necrotic and are shed into the intestinal lumen under conditions of diarrhea, the intestinal mucosal villi decrease. This could be the case because injury of the small intestine leads to reduced DAO activity in the mucosal villi, increasing the likelihood that plasma DAO activity will decrease. Further studies are needed to elucidate whether plasma DAO activity reflects diarrhea caused by anti-cancer drugs directly.

Gastrointestinal tract symptoms induced by anti-cancer drugs are difficult to evaluate quantitatively. Previous studies have shown that glutamine, a semi-essential amino acid used as a special nutrient by intestinal mucosal cells, may protect against intestinal barrier dysfunction [18, 19]. Glutamine is also known to reduce intestinal permeability increased by stress, such as surgery and severe trauma [20]. In addition, medium-chain triglycerides enhance cell proliferation in the intestinal epithelium and mucous secretion from goblet cells in the small intestine of rat, both of which may improve mucosal injury [21]. Gastrointestinal tract toxicity was clarified by measuring plasma DAO activity, which may be one of the important quantitative biomarkers to evaluate preventive effectiveness of glutamine or medium-chain triglycerides against the gastrointestinal mucosal disorder caused by anti-cancer drugs. Furthermore, since DAO activity decreases before the manifestation of gastrointestinal tract toxicities, measuring such activity could serve as a predictive indicator of adverse events due to anti-cancer drugs and of chemotherapy tolerability.

We recognize the following limitations of the present study. First, the sample size was insufficient to clarify definitive and long-term changes in DAO activities with chemotherapy. Another limitation is that the correlation between DAO activities and QOL scores was only slight. Further studies are needed to examine the reliability and accuracy regarding the usefulness of plasma DAO activities during chemotherapy. In the future, biomarkers must be developed for estimating manifestations of adverse events or chemosensitivity with the view to selecting patient-appropriate anti-cancer drugs and improving therapeutic outcomes for all patients.

In conclusion, our study confirmed that DAO activities decrease during chemotherapy in patients being administered S-1 as adjuvant treatment after curative resection for gastric cancer. It also showed that these enzyme activity rates were lower by S-1 chemotherapy in patients with gastrointestinal toxicity than in those without toxicity. Furthermore, the level of DAO activities was slightly correlated with QOL scores including global health status and appetite loss. These findings suggested that measuring DAO activity in gastric cancer patients could be useful not only as an indicator of mucosal injury but also for the evaluation of coinciding gastrointestinal tract toxicities induced by the anti-cancer drug.

Disclosure Statement

The authors declare no conflict of interest.

References


