Serum diamine oxidase activity as a predictor of gastrointestinal toxicity and malnutrition due to anticancer drugs

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Article type: Original Article

Short title: DAO activity as predictor of GI toxicity

Abstract
Background and Aim: Objective evaluation of intestinal mucosal damage due to anticancer drugs is generally difficult. Serum diamine oxidase (DAO) activity is reported to reflect the integrity and maturity of the small intestinal mucosa. Therefore, we investigated whether serum DAO activity is an indicator of gastrointestinal toxicity or nutritional status in patients receiving chemotherapy.

Methods: We prospectively enrolled 20 patients with unresectable metastatic gastric cancer who received oral S-1 (80 mg/m²) on days 1–14, and intravenous cisplatin (60 mg/m²) and docetaxel (50 mg/m²) on day 8 every 3 weeks. Serum DAO activity was measured by colorimetry. Gastrointestinal toxicity was evaluated by CTCAE v4.0. Endoscopic examination and biopsy of duodenal mucosa assessed mucosal damage. Malnutrition was evaluated by measuring serum total protein and albumin levels.

Results: Serum DAO activity decreased step-by-step significantly during anticancer drug treatment and recovered after drug holidays. In all 14 patients who experienced diarrhea, serum DAO activity significantly decreased prior to diarrhea onset. Percent decrease in DAO activity was significantly correlated with severity of diarrhea. Significant correlation was observed between percent decrease in DAO activity and percent decrease in duodenal villus height or surface area from baseline. There were also significant correlations between percent decrease in serum DAO activity at day 14 and percent decrease in serum total protein or albumin levels at day 14.
Conclusions: Serum DAO activity sensitively indicates gastrointestinal damage prior to symptom onset and can be a useful predictor of intestinal mucosal damage and nutritional status in patients receiving chemotherapy.

Keywords: diamine oxidase, diarrhea, gastrointestinal toxicity, anticancer drugs, malnutrition
Introduction

With the development of new anticancer drugs and molecular targeted drugs, the efficacy of chemotherapy on unresectable advanced cancer has been increasing. However, gastrointestinal mucosal damage is induced by administration of anticancer drugs, resulting in gastrointestinal toxicity experienced by patients as anorexia, nausea, and/or diarrhea. Gastrointestinal toxicity causes a decrease in the quality of life and a worsening of the nutritional status of the patient, and can lead to cessation of anticancer drug therapy.\(^1\)-\(^3\) Additionally, in anticancer drug therapy, gastrointestinal mucosal damage and malnutrition can cause bacterial translocation, which can lead to fatal complications.\(^4\),\(^5\) Anticancer drug-induced gastrointestinal mucosal damage can be evaluated by gastrointestinal endoscopy. However, endoscopy is an invasive test which is often painful. In particular, evaluation of the small intestinal mucosa is difficult.\(^6\) Thus, a sensitive serum biomarker for the evaluation of gastrointestinal toxicity is needed.

Diamine oxidase (DAO) is the main enzyme for the metabolism of ingested histamine.\(^7\),\(^8\) DAO is localized mainly in the small intestinal mucosa, predominantly in the tips of the villi.\(^9\) DAO activity is known to be an indicator of the integrity and maturity of the small intestinal mucosa.\(^10\) Luk et al. reported that DAO activity in the blood significantly correlates with DAO activity in the tissues of the small intestine in animals.\(^11\) Several studies have been reported in which DAO activity was measured and small
intestinal lesions were assessed in patients with inflammatory bowel disease, acute mesenteric ischemia and other diseases.\textsuperscript{12-16} However, there have been only a few reports on the relationship between DAO activity and anticancer-drug-induced mucosal damage.\textsuperscript{17-19} Moriyama et al. measured serum DAO activity in rats, comparing gastrointestinal damage between the group receiving S-1 containing potassium oxonate (Oxo) (which reduces gastrointestinal damage) and the group receiving S-1 without Oxo.\textsuperscript{17} DAO activity in the group receiving S-1 without Oxo was significantly lower than in the group receiving S-1 containing Oxo. Histological examination revealed an apparent atrophy of the jejunal mucosa in the rats that received S-1 without Oxo. Tsujikawa et al. measured serum DAO activity during chemotherapy in 10 patients with hematological malignancies, and found that DAO activity decreased as a result of anticancer drug therapy.\textsuperscript{18} However, these investigators did not evaluate gastrointestinal symptoms and toxicities demonstrated by such symptoms as diarrhea, anorexia, and vomiting. Therefore, the relationship between DAO activity and gastrointestinal toxicity has remained unclear. Namikawa et al. reported that plasma DAO activity decreased as a result of administration of S-1 in patients receiving adjuvant chemotherapy.\textsuperscript{19} However, all the patients had received gastrectomy just before the measurement of DAO activity; therefore, the evaluation of gastrointestinal toxicity, including anorexia and DAO activity, was seemingly inappropriate because the patients’ symptoms were affected by their recent gastrectomy. Thus, the relationship between serum DAO activity and gastrointestinal toxicity and symptoms has not been
investigated, nor have histological investigations of the small intestinal mucosa been carried out. The relationship between malnutrition and DAO activity during chemotherapy is also unclear. Therefore, in the present study, we chronologically measured serum DAO activity in patients with metastatic gastric cancer who were receiving combination chemotherapy. We also investigated the relationship between change in serum DAO activity and diarrhea, a symptom of gastrointestinal toxicity, and histological changes of the small intestine. Additionally, we investigated the relationship between serum DAO activity and nutritional status during chemotherapy.

Methods

Patients and study design

This study was approved by the institutional review board of Tokushima University Hospital. We prospectively enrolled 20 patients with unresectable metastatic gastric cancer according to the classification of the International Union Against Cancer (UICC)-TNM, 7th edition, who received docetaxel (DTX), cisplatin (CDDP) and S-1 combination chemotherapy (DCS chemotherapy) as first-line chemotherapy.20,21 The patients had sufficient liver function (serum transaminase level less than 100 U/L and total bilirubin level less than 1.5 mg/dL) and renal function (normal serum creatinine level and urinary findings) for receiving DCS chemotherapy. Gastrointestinal toxicity was evaluated by the National Cancer Institute’s
Common Toxicity Criteria for Adverse Events version 4.0 (CTCAE v4.0).

Blood was drawn from each patient for measurement of serum DAO activity and nutritional parameters. Biopsy was obtained from the duodenal mucosa in 6 out of the 20 study patients on day 14 for analysis of the correlation between DAO activity and histological findings. Written consent was obtained from all study subjects.

**Treatment schedule**

DCS chemotherapy is a modified regimen of DTX, CDDP and fluorouracil (5-FU) combination chemotherapy, in which 5-FU is replaced by S-1.20-23 S-1 was given orally at a dose of 80 mg/m$^2$ daily on days 1-14, followed by a 7-day recovery period. Both DTX at a dose of 50 mg/m$^2$ and CDDP at a dose of 60 mg/m$^2$ were administered by intravenous infusion on day 8 (Fig. 1).20 For purposes of preventing nausea and vomiting, palonosetron hydrochloride 0.75 mg, a 5-HT3 receptor antagonist, dexamethasone sodium phosphate 9.9 mg, and aprepitant 125 mg, a selective NK1 receptor antagonist, were administered on day 8.24 Dexamethasone sodium phosphate 6.6 mg and aprepitant 80 mg continued to be administered for 2 more days (day 9, 10).

**Measurement of serum DAO activity**

Serum DAO activity was measured before chemotherapy on day 1, a week after the start of chemotherapy on day 8 (before administration of DTX and CDDP), at the end of chemotherapy on day 14, and after a 1-week drug holiday on day 21 (Fig. 1) using a highly sensitive colorimetric method.25
Assessment of intestinal mucosal damage

Duodenal mucosal biopsy was performed in 6 patients under upper gastrointestinal endoscopy performed before the initiation of chemotherapy and after the 1st cycle of chemotherapy (on day 14). In consideration of the invasiveness of duodenal biopsy during chemotherapy, we confined duodenal biopsy to these six patients. Three biopsy specimens were taken from the second portion of the duodenum for each patient per each endoscopy. The specimens were fixed in 10% formalin, paraffin-embedded, sectioned in the vertical direction, and stained by hematoxylin and eosin (H&E). The five largest villi were selected from each section, and villus height and width were measured under light microscopy at a magnification power of 40x. A total of 15 villi were analyzed and the average calculated. The surface area of the sample villi was calculated by the following equation according to the method by Zhang et al.\textsuperscript{26}

\[
\text{Surface area of a villus} = 3.14 \times \text{villus width} \times \text{villus height}
\]

Assessment of nutritional status

Serum total protein and albumin levels were measured by the Biuret method\textsuperscript{27} and the modified bromocresol purple (BCP) method,\textsuperscript{28} respectively, for the evaluation of nutritional status.

Statistical analysis

Differences in DAO activity between the groups at days 1, 8, 14 and 21 were
analyzed by the Kruskal–Wallis test. Then, DAO activity in each group was compared with the Wilcoxon signed-rank test. The Bonferroni correction was applied for multiple comparisons. DAO activities between the diarrhea-positive group and the diarrhea-negative group were compared by the Mann-Whitney U test. The lengths of villi or surface areas of villi before and after chemotherapy were compared by the paired t test. Correlations between DAO activity and villus height, villus surface area, and total protein or albumin levels were evaluated by Spearman’s test. Statistical analyses, including ROC analysis, were conducted using SPSS software version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Patient characteristics

Twenty patients with unresectable metastatic gastric cancer stage IV were enrolled at Tokushima University Hospital, Tokushima, Japan. Their characteristics are summarized in Table 1. There were 16 men and four women, with a median age of 61.7 years (range 36–78 years). Nineteen patients were PS 0, and one patient was PS 1. Histologically, 12 (60.0%) patients had intestinal type adenocarcinomas, and 8 (40.0%) had diffuse type. Lymph node metastases were seen in all 20 patients, and nine of the 20 patients had distant lymph node metastases to the para-aortic lymph nodes. Ten patients had distant metastases to the peritoneum, five to the liver, two
to the lungs, two to the portal vein, and one to the adrenal gland. All the patients had completed the 1st cycle of DCS when DAO activity was measured.

**Decrease in serum DAO activity during chemotherapy**

We measured serum DAO activity in the 20 patients to investigate the chronological changes during chemotherapy. The median serum DAO activities (25th and 75th percentiles) on day 8 and day 14 were 3.5 (2.8, 5.2) U/L and 2.4 (1.9, 4.7) U/L, respectively. These values were significantly lower than the values before treatment (median 4.2 (3.2, 6.5) U/L) ($P=0.03$ and $P<0.01$, respectively). The median DAO level increased to 3.5 (2.6, 4.1) U/L after the drug holiday (Fig. 2A). Thus, serum DAO activity decreased during administration of anticancer drugs and recovered after the drug holiday.

**Decrease in serum DAO activity preceded onset of diarrhea**

We next examined when diarrhea, a representative of gastrointestinal toxicity, appeared in relationship with the decrease in DAO activity. Diarrhea (grade 1 or higher) occurred in 14 of the 20 patients (70%). The onset was between 10 and 18 days after the start of anticancer drugs. In all 14 of the patients who experienced diarrhea, DAO activity significantly decreased on day 8 (and on day 14) before diarrhea appeared, and recovered after the drug holiday (Fig. 2B). The severity of the diarrhea was grade 1 in eight patients, grade 2 in three patients, and grade 3 in three patients (Table 2). In the 6 patients without diarrhea, there was no significant difference in DAO
activity between each day (Fig. 2C). Significant differences in DAO activity were observed between the diarrhea-positive group and the diarrhea-negative group on day 8, day 14, and day 21 ($P<0.01$, $P=0.011$ and $P=0.013$, respectively) (Fig. 2D). There was a significant correlation between percent decrease in DAO activity at day 8 ($\Delta$ day 1–8) and severity of diarrhea (Supplemental Fig. 1A). In addition, ROC curves were drawn to assess percent decrease in DAO activity ($\Delta$1–8) for predicting diarrhea. For occurrence of diarrhea (grade 1–3), the optimal cut-off value of percent decrease from the maximum AUC was 9.7%, and the odds ratio was 117 (Supplemental Fig. 1B).

**Correlation between DAO activity and villus atrophy**

To explore the relationship between DAO activity and histological findings of small intestinal villi, we assessed villus height and surface area in the duodenal biopsy specimens from the 6 patients (Fig. 3). No clear erosion or ulceration was found under endoscopic observation in the duodenal mucosa before or after chemotherapy. A representative H&E staining pattern is shown in Fig. 3A. The villus size in the duodenal mucosa after chemotherapy (on day 14) was apparently decreased compared with the size before chemotherapy. The number of goblet cells after chemotherapy (on day 14) was significantly reduced compared with that before chemotherapy. Moreover, the median villus height ($25^{\text{th}}$, $75^{\text{th}}$ percentiles) on day 14 was significantly shorter than that before chemotherapy (302 (225, 311) vs 457 (410, 518), $P<0.01$) (Fig. 3B). Likewise, the villus surface area on day 14 was
significantly lower than that before chemotherapy (median 0.078 (0.069, 0.099) mm$^2$ vs 0.17 (0.16, 0.19) mm$^2$, $P<0.01$) (Fig. 3C). Thus, villus height and villus surface area decreased significantly after the administration of anticancer drugs. DAO activity on day 14 in the 6 patients was significantly lower than on day 1 (median 1.7 (1.1, 2.1) U/L vs 5.5 (3.7, 7.1) U/L, $P<0.01$) (Fig. 3D). The percent decrease in DAO activity from day 1 to day 14 correlated significantly with the percent decrease in villus height from baseline (before chemotherapy) to day 14 (correlation coefficient: 0.94, $P<0.01$) (Fig. 3E). Likewise, there was a significant correlation between the percent decrease in DAO activity from day 1 to day 14 and the percent decrease in villus surface area from baseline (before chemotherapy) to day 14 (correlation coefficient: 0.92, $P<0.01$) (Fig. 3F). We also analyzed the association between severity of diarrhea and duodenal villus height or surface area (Supplemental Fig. 2). The patients with a more severe grade of diarrhea showed a greater decrease in villus height or a greater decrease in surface area.

Eventually, the patients who received duodenal biopsy consisted of 1 without diarrhea (grade 0), 3 with grade 1 diarrhea and 2 with grade 3 diarrhea, representing a relatively balanced distribution.

**Correlation between serum DAO activity and serum total protein or albumin levels**

We then measured serum total protein and albumin levels in the 20 patients to examine the relationship between serum DAO activity and
nutritional status. The median serum total protein level decreased after initiation of chemotherapy from 6.9 (25th and 75th percentiles 6.2, 7.0) g/dL at day 1 to 6.3 (6.2, 6.8) g/dL at day 8; 5.9 (5.3, 6.2) g/dL at day 14; and then to 5.6 (5.1, 5.8) g/dL at day 21 (Fig. 4A). Similarly, the median albumin level decreased significantly from 3.5 (3.0, 3.9) g/dL at day 1 to 3.3 (3.1, 3.8) g/dL at day 8; to 3.2 (2.8, 3.6) g/dL at day 14; and then 3.1 (2.4, 3.4) g/dL at day 21 (Fig. 4B). Thus, the serum protein and albumin levels decreased after chemotherapy up to day 21, and recovered later. The percent decrease in serum DAO activity at day 14, when the decrease was maximum (based on baseline levels before chemotherapy), correlated significantly with the percent decrease in serum total protein at day 21, when the decrease was maximum (based on baseline levels before chemotherapy) (correlation coefficient: 0.77, \( P<0.01 \)) (Fig. 4C). Similarly, the percent decrease in serum DAO activity at day 14 correlated significantly with the percent decrease in serum albumin at day 21, when the decrease was maximum (based on baseline levels before chemotherapy) (correlation coefficient: 0.702, \( P<0.01 \)) (Fig. 4D).

Subsequently, we elucidated the relationship between clinical symptoms and nutritional parameters. In the analysis of the associations between diarrhea and serum total protein (TP) levels, the patients with more severe diarrhea tended to show a greater decrease in serum TP levels (\( P=0.10 \)), although there was no statistically evident correlation between severity of diarrhea and serum albumin levels (\( \Delta \text{Alb}_{1-21} \)) (Supplemental Fig. 3). In addition, there were no statistically significant correlations between nausea
and/or anorexia and serum TP or albumin levels (Supplemental Figs. 4 and 5).

**Discussion**

The present study showed that serum DAO activity significantly decreases with the administration of anticancer drugs in humans and recovers during drug holidays. Moreover, histological examinations revealed that the decrease in serum DAO activity was significantly correlated with decreases in villus height and villus surface area. This is the first report showing that serum DAO activity decreases as a result of villus damage of the small intestine due to administration of anticancer drugs in humans. The fact that there were significant correlations between percent decrease in DAO activity (day 1–14) and percent decrease in length and surface area of villi (day 1–14) indicates that serum DAO activity sensitively reflects mucosal damage during chemotherapy. Importantly, serum DAO activity decreased (day 8) before the onset of diarrhea in all patients who developed diarrhea. The decrease in DAO activity in the diarrhea-positive group was significantly higher than in the diarrhea-negative group. Moreover, the DAO activity decreased in correlation with the severity of diarrhea, and a more than 9.7% decrease in DAO activity suggested the occurrence of diarrhea ($P<0.01$, areas under the ROC curve: 0.93; sensitivity: 92.9% and specificity: 100% for an optimal cut-off value of 9.7%) (Supplemental Fig. 1), indicating that DAO
activity can serve as a useful predictor of gastrointestinal toxicity due to anticancer drugs. In other words, measurement of DAO activity, via a simple blood test, enables prediction of mucosal damage and gastrointestinal toxicity from anticancer drug therapy, which in the past has been difficult.

In our analysis of the relationship between DAO activity and nutritional parameters, we showed a significant correlation between percent decrease (Δday1–14) of DAO activity and later decrease (Δday1–21) in total protein or albumin. This suggests that serum DAO activity may also serve as a sensitive predictor of malnutrition resulting from the administration of anticancer drugs. Tsujikawa et al. reported that there were no positive correlations between changes in DAO activity and changes in total protein, albumin, or cholinesterase. However, there was no description of the timing of when serum DAO activity, total protein, albumin, and cholinesterase were assessed. In the present study, the percent decrease in serum DAO activity at day 14, when the decrease was maximum, correlated significantly with the percent decrease in serum total protein or albumin at day 21, when the decrease was maximum. In short, comparing the levels of DAO activity with total protein and albumin levels at the times of maximum change in these levels showed significant correlations. Based on our results, we consider the following hypothesis as a mechanism of association between DAO activity and nutrition parameters: anticancer drugs damage intestinal mucosal epithelia due to their high proliferation activity, leading to mucosal (villus) atrophy, thereby decreasing the level of DAO activity (Figs. 2 and 3). This mucosal damage induces malabsorption, and also increases intestinal
permeability, which may cause diarrheal symptoms, leading to low serum TP and albumin levels.

The limitations of this study were the small sample size and the fact that the subjects had only one type of cancer. Therefore, future large-scale investigations on serum DAO activity are needed in connection with other types of cancer besides gastric cancer, as well as other therapeutic regimens. It may also be necessary to investigate the link between serum DAO activity and non-duodenal small intestinal lesions using capsule endoscopy, a relatively low-invasive examination. Other nutritional parameters than total protein and albumin should also be investigated.

In conclusion, serum DAO activity is a useful predictive marker for gastrointestinal damage and nutritional status in patients receiving chemotherapy. DAO activity sensitively indicates gastrointestinal damage prior to symptom onset of gastrointestinal toxicity.

Acknowledgements

The authors are grateful to Dr. Hiroaki Mikasa (Department of Clinical Epidemiology, University of Tokushima), M.S. Eriko Aoyagi, and Mayumi Kajimoto (Department of Gastroenterology and Oncology, University of Tokushima) for their expert technical assistance.
Conflict of interest

The authors have declared no conflicts of interest in connection with this study.
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### Table 1. Patient characteristics

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<th>Number of patients (n = 20)</th>
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Table 2. Onset and grade of diarrhea due to anticancer drugs

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