Original article

Prognostic significance of serum superoxide dismutase activity in patients with gastric cancer

MITSUHIRO YASUDA, FUMIO TAKESUE, SADA AKI INUTSUKA, MASAYUKI HONDA, TADAHIRO NOZOE,
and DAISUKE KORENAGA

Department of General Surgery, Fukuoka Dental College Hospital, 2-15-1 Tamura, Sawara-ku, Fukuoka 814-0193, Japan

Abstract

Background. Although superoxide dismutase (SOD) may play an important role in helping to protect against carcinogenesis or tumor progression, little information is available regarding the clinical evaluation of antioxidant defense in patients with gastric cancer.

Methods. Serum SOD activity in 34 patients with gastric cancer was estimated and the data compared with clinicopathological parameters.

Results. The mean serum SOD activity in the patients was $15.9\% \pm 2.6\%$, which was higher than the value obtained in healthy donors. The serum SOD activity in patients over 70 years of age was $14.5\% \pm 3.0\%$, which was significantly lower than the value of $16.6\% \pm 7.3\%$ in those under 70 years of age ($P < 0.01$). According to the stage of disease, the reduction in SOD activity in the patients aged over 70 years was significant in those with far advanced tumor, classified as stage IV ($P < 0.01$), but not in those with stage I–III disease. When the cutoff value for high- and low-SOD groups was determined as 18.0%, based on the median value for serum SOD activity in 15 patients with stage IV gastric cancer, the survival rate of patients with stage IV tumor was significantly higher in the high-SOD group than in the low-SOD group ($P < 0.05$).

Conclusion. These findings suggested that the reduction in serum SOD activity in elderly patients may be due to weakness of the antioxidant defense of the host, thus resulting in a poor prognosis in those with far-advanced (stage IV) gastric cancer.

Key words Gastric cancer · Superoxide dismutase · Serum activity · Prognosis

Introduction

Reactive oxygen metabolites, such as hydrogen peroxide, superoxide anion, and hydroxyl radical, are implicated in the initiation and promotion of cancer [1–3]. This reactive oxygen can mediate DNA damage induced by various carcinogens during multistep carcinogenesis [4]. Normal cells are protected by antioxidant enzymes from the toxic effects of high concentrations of reactive oxygen species generated during cellular metabolism. Superoxide dismutase (SOD), is one of the most important antioxidant enzymes involved in the primary cellular defense, and may thus help to protect against carcinogenesis or tumor progression.

There have been many reports concerning SOD in human cancerous tissues. Several studies suggested that the SOD activity of tumor tissue was decreased in colon cancer [5,6], oral squamous cell carcinoma [7], renal carcinoma [8], cervical carcinoma [9], and hepatocellular carcinoma [10]. Conversely, in other reports, SOD activity was reported to be increased in tumor tissue, including carcinomas of esophagus, stomach, and colon [11–13]. The results on the SOD status of various organs thus remain controversial, possibly depending on differences in the cancerous tissues studied. Some investigators reported a significant association of SOD with clinicopathological findings [7,11]; however, these studies investigated SOD in tumor tissues. Until now, few studies have been performed to clarify the role of SOD in serum and its possible relation to antioxidant defense against cancer.

The purpose of this study was, therefore, to determine the potential benefits of evaluating serum SOD activity in patients with gastric cancer. Moreover, in order to clarify the biological significance of serum SOD activity, the data were also compared with the tissue SOD results obtained in patients with gastric cancer.

Patients and methods

Patients

Thirty-four Japanese patients with gastric cancer treated in our department from March 1998 to August 1999 were selected for this study. Among these 34 patients, total gastrectomy was performed in 8, partial gastrectomy in 13, and gastrojejunostomy in 4 patients. The remaining 9 patients received chemotherapy alone. In order to rule out the influence of invasive surgical operation or chemotherapy upon the data, all patients entered the study at the first date of attendance our outpatients clinic, and blood samples were taken before the individual treatments. Data on 47 healthy donors, whose mean age was 48.8 ± 11.6 years (range, 27–69 years), were collected and used for comparison purposes as a control group. The general rules for gastric cancer study in surgery and pathology in Japan were used for clinical and pathological descriptions [14]. The cutoff age for elderly and younger patients was chosen to be 70 years, according to previous clinical reports on gastric cancer patients [15,16].

Assay for serum SOD activity

Serum SOD activity was estimated using the nitroblue tetrazolium (NBT) dye reduction test (SOD Test Wako, Wako Pure Chemical Industries, Osaka, Japan). A serum sample (0.1 ml) was added to the reaction mixture (2 ml), which contained 0.20 mM xanthine, 0.12 mM nitroblue tetrazolium, 0.049 IU xanthine oxidase, and 0.1 M phosphate buffer (pH 8.0). The mixture was incubated for 20 min at 37°C. In the reaction mixture, the superoxide radical is produced, which reduces NBT to NBTH₂. The rate of reduction of NBT was measured at 560 nm. The results for SOD activity were expressed as the percent inhibition of these reactions by SOD. The serum SOD activity was compared with clinicopathological factors and the prognosis of patients with gastric cancer.

Assay for tissue SOD activity

Carcinoma and paired normal mucosal tissue specimens were resected from ten patients with gastric cancer to compare the SOD activities in these tissues with those in the serum. The tissue samples were obtained from surgical specimens and were frozen immediately in liquid nitrogen and kept at -80°C until use. Before analysis, the tissue specimens were washed adequately with phosphate-buffered saline to eliminate any blood cells containing SOD. The tissues were then homogenized in nine volumes of 1.15% KCl, and their supernatants were used for assays. The NBT dye reduction test

was performed as described in the above-mentioned procedure.

The SOD activity in normal mucosa (N) was compared with that in the corresponding carcinoma tissue (T). The normal/tumor ratio of tissue SOD activity (N/T ratio) in each case was then calculated and the data were compared with the serum SOD activities in the corresponding patients.

Statistical analyses

The values for SOD activity and the clinicopathological parameters were expressed as means $\pm$ SD, and the Mann-Whitney *t*-test was used to determine statistical significance.

Survival curves were calculated by the Kaplan and Meier method, and survival rates were analyzed using the log-rank test. The SOD activity in normal mucosa and carcinoma tissue specimens was compared using the paired Student's *t*-test. Any correlations between the N/T ratio and serum SOD activity were compared using a regression analysis.

Results

Serum SOD activity and clinicopathological factors

The mean serum SOD activity in gastric cancer patients was $15.9\% \pm 2.6\%$, which was significantly higher than the $10.3\% \pm 3.3\%$ obtained in the 47 healthy donors ($P < 0.0001$; Fig. 1). For a more detailed analysis, 11 patients and healthy donors matched for age were selected. The SOD activity in these 11 patients with gastric cancer was $15.9\% \pm 7.8\%$, which was significantly higher than the value of $11.3\% \pm 3.3\%$ in the healthy donors ($P = 0.002$).

The relationship between serum SOD activity and clinicopathological factors is shown in Table 1. No significant correlation was found, except for patient age. The serum SOD activity in elderly patients (those over 70 years of age) was $14.5\% \pm 3.0\%$, which was significantly lower than the value of $16.6\% \pm 7.3\%$ observed in younger patients (under 70 years; $P < 0.01$). When the enzyme activity in elderly and younger patients was compared according to the stage of disease, patients over 70 years of age had significantly lower SOD activity than did the younger patients (under 70 years) in those with stage IV tumors ($P < 0.01$), but not in those with stage I–III tumors (Fig. 2).

Serum SOD activity and survival in stage IV patients

To assess the possible relationship between changes in SOD levels in serum and the systemic antioxidant de-

Fig. 1. Distribution of serum superoxide dismutase (SOD) activity in patients with gastric cancer

Fig. 2. Comparison of serum SOD activity in elderly patients (≥ 70 years) and younger patients (< 70 years)

fense system against cancer, special attention was given to patients with far advanced cancer with diffuse metastases, classified as stage IV disease. Survival curves of the stage IV patients classified according to serum SOD

Fig. 3. Survival curves of patients with stage IV gastric cancer classified according to serum SOD activity. $P < 0.05$ (log-rank test)

activity are shown in Fig. 3. The cutoff value of the high- and low-SOD groups was determined to be 18.0%, based on the median value for serum SOD activity obtained in 15 patients with stage IV gastric cancer. The high-SOD patients survived for significantly longer than the low-SOD patients ($P < 0.05$). There were no significant differences with regard to clinical background factors, including age, sex, blood counts, and blood chemistry findings between the high-SOD and low-SOD groups (Table 2). Similarly, no significant differences regarding such pathological factors as gross type or histological differentiation of the tumor were observed. Neither the type of surgery for the gastrectomy nor the use of chemotherapy demonstrated any significant differences between the patients in these two groups.

Comparison of tissue SOD activity with serum SOD activity

The results of comparisons between tissue SOD activity and serum SOD activity in ten patients with gastric cancer are shown in Table 3. There was no significant correlation between the tissue SOD activity of the normal mucosa and the corresponding carcinoma tissues. Moreover, there was no significant correlation between the N/T ratio and serum SOD activity in the corresponding patients ($R^2 = 0.010$).

Discussion

Some previous reports have demonstrated the clinico-pathological significance of the serum elevation of SOD

Table 1. Serum superoxide dismutase (SOD) activity in gastric cancer patients according to various clinicopathological parameters

Parameters	Percent inhibition of SOD (<i>n</i>)	<i>P</i>
Age (years)		
<70	16.6 ± 7.3 (23)	<i>P</i> = 0.005
≥70	14.5 ± 3.0 (11)	
Sex		
Male	16.3 ± 2.6 (24)	<i>P</i> = 0.08
Female	14.9 ± 2.6 (10)	
Gross type		
Borrmann I, II	15.0 ± 0.1 (13)	<i>P</i> = 0.1
Borrmann III, IV	16.3 ± 0.0 (14)	
Differentiation		
Well and moderately	15.6 ± 3.1 (15)	<i>P</i> = 0.4
Poorly	15.8 ± 2.0 (17)	
Depth of invasion		
T1, 2	15.7 ± 1.8 (12)	<i>P</i> = 0.26
T3, 4	16.3 ± 3.0 (18)	
Lymph node metastasis		
N0–2	15.6 ± 2.3 (18)	<i>P</i> = 0.09
N3, 4	17.1 ± 2.6 (12)	
Liver metastasis		
Absent	15.5 ± 2.3 (27)	<i>P</i> = 0.14
Present	17.1 ± 3.5 (7)	
Peritoneal dissemination		
Absent	15.7 ± 2.4 (22)	<i>P</i> = 0.46
Present	15.6 ± 3.0 (11)	
Ascites		
Absent	15.5 ± 2.3 (27)	<i>P</i> = 0.47
Present	15.6 ± 3.3 (6)	
Stage		
I–III	15.2 ± 1.9 (17)	<i>P</i> = 0.06
IV	16.6 ± 3.1 (17)	

In some cases with stage IV gastric cancer, data were unknown. These cases were excluded from statistical analysis

Table 2. Background of stage IV gastric cancer patients according to low and high serum SOD activity

Parameters	High SOD (<i>n</i> = 7)	Low SOD (<i>n</i> = 8)	<i>P</i>
Age (years)	65.7 ± 3.3	69.4 ± 7.7	<i>P</i> = 0.27
Leukocytes (/μl)	6900 ± 1579	7463 ± 3024	<i>P</i> = 0.67
Lymphocytes (/μl)	1973 ± 987	2049 ± 799	<i>P</i> = 0.88
Hemoglobin (g/dl)	9.87 ± 3.52	10.60 ± 2.47	<i>P</i> = 0.65
Total protein (g/dl)	6.71 ± 0.64	6.53 ± 0.73	<i>P</i> = 0.61
Albumin (g/dl)	4.01 ± 0.52	3.81 ± 0.53	<i>P</i> = 0.47
Cholesterol (mg/dl)	177.8 ± 44.0	212.0 ± 45.0	<i>P</i> = 0.18
GOT (U/l)	32.1 ± 12.7	41.6 ± 33.4	<i>P</i> = 0.49
GPT (U/l)	22.0 ± 13.0	27.3 ± 26.8	<i>P</i> = 0.65
LDH (U/l)	496.5 ± 126.2	1115.8 ± 1045.5	<i>P</i> = 0.18
CRP (mg/dl)	1.00 ± 1.16	1.33 ± 1.74	<i>P</i> = 0.70

as a tumor marker in Hodgkin's disease [17], ovarian cancer [18], and malignant melanoma [19]. On the other hand, there has also been an investigation that emphasized the decrease of serum SOD in patients with leukemia [17]. The findings of these studies may suggest that the clinical significance of the serum elevation of SOD in human tumors remains controversial.

In this study, we found the mean SOD activity in the serum of gastric cancer patients to be higher than that in the normal serum. Because the serum samples were collected before the therapy, the cytotoxic effect of chemotherapeutic drugs and surgical treatment was thought to be negligible. Some studies have suggested the SOD activity in tumor tissue to increase in carcino-

Table 3. Comparison of tissue SOD activity with serum SOD activity in gastric cancer patients

Patient no.	Age (years)	Sex	Tissue SOD activity (% inhibition)		N/T ratio	Serum SOD activity (% inhibition)
			Normal mucosa	Carcinoma		
1	77	Male	12.3	30.3	0.41	10.9
2	63	Female	14.4	31.8	0.45	13.8
3	93	Female	21.3	38.1	0.56	10.1
4	63	Male	12.9	21.3	0.61	13.5
5	64	Female	24.0	34.2	0.70	14.1
6	79	Female	23.1	31.5	0.73	13.5
7	50	Male	41.4	30.3	1.38	13.5
8	65	Male	33.3	23.7	1.41	12.6
9	39	Female	38.4	18.9	2.03	14.7
10	60	Female	45.7	16.5	2.76	10.3

N/T, Normal mucosa/corresponding carcinoma tissue

mas of the gastrointestinal tract [11–13]. Therefore, one possible explanation for the elevated SOD in the serum of gastric cancer patients could be the seepage of SOD enzymes from the carcinoma tissue into the blood. However, other reports have given conflicting explanations for the origin of serum SOD. Canbolat et al. [20] showed the absence of differences between pre- and postoperative serum SOD activities in patients with laryngeal cancer. Furthermore, we found no significant differences in tissue SOD activity between normal mucosa and carcinoma, and no significant correlation between tissue SOD activity and serum SOD activity was indicated. Considering these observations, serum SOD activity is thus not considered to necessarily reflect tissue SOD activity.

The mechanism of elevated SOD activity in the serum of patients with gastric cancer remains to be elucidated. The activity of this enzyme is frequently elevated in patients with various diseases, including ischemic or inflammatory diseases [21]. In normal human tissue specimens, the content of SOD is much higher in liver, lung, and renal cortex than in gastrointestinal tissue [22]. It is well known that tumor necrosis factor α and interleukin 1 α induce Mn-SOD expression in vascular endothelial cells [23], and they are also thought to contribute to increased serum levels of Mn-SOD [24]. It is therefore feasible to speculate that SOD upregulation in the serum of gastric cancer patients may be a host response to the increased production of reactive oxygen species derived from either the gastric tissue or the other organs.

Some investigators have reported that a high SOD content in tumor tissues was closely associated with poor prognosis in patients with colorectal cancer [11] and in patients with oral squamous cell carcinoma [7]. In the present study, we obtained evidence that the serum SOD activity might represent the antioxidant defense of

the host, because the serum SOD activity in elderly patients (over 70 years of age) was significantly decreased, and this reduction of SOD was seen particularly in those with stage IV (advanced) tumor. Our previous experimental study showed that an impaired antioxidant defense system in mucosal tissue might be critical for cancer development associated with chronic colitis in mice [25]. Although it is clinically difficult to establish the possible role of serum SOD in relation to the systemic antioxidant defense against tumor, the results in this study suggested that an impaired enzymatic antioxidant defense system in elderly patients with far-advanced gastric cancer might be closely associated with the development of diffuse metastasis and invasion, thus resulting in a poor prognosis for these patients.

The relationship between SOD and cancer treatments such as radiotherapy and chemotherapy is of great interest, because reactive oxygen metabolites are involved in the mechanisms by which several anticancer treatments exert their therapeutic effects [26,27]. Regarding the cytoprotective function of antioxidant enzymes, SOD has been reported to contribute to resistance to the harmful side effects of radiation [26,28,29] and several anticancer drugs [30,31]. Therefore, caution should be exercised when treating elderly patients with stage IV gastric cancer, especially patients with lower levels of serum SOD. An analysis of serum SOD is likely to provide pertinent information regarding the antioxidant defense of the host. In this respect, serum SOD activity may be useful for determining the indications for aggressive treatment, including surgery and chemotherapy, in elderly patients with advanced gastric cancer.

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