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Reactivity of a Monoclonal Antibody to Manganese Superoxide Dismutase with Human Ovarian Carcinoma[1]

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ABSTRACT

A monoclonal antibody against manganese superoxide dismutase was assessed for its use in detecting a marker for epithelial ovarian carcinoma. An enzyme-linked immunosorbent assay indicated that less than 1% of normal individuals had serum levels over 150 ng per ml of serum, whereas over 50% of such patients showed elevated amounts. The serum levels of manganese superoxide dismutase correlated with the clinical stage of the disease and with the effects of therapy. The antibody used reacts with cryopreserved epithelial ovarian carcinomas but not with normal adult ovary or other normal tissues. Determination of the levels of this enzyme should provide a useful method for detection and monitoring of responses to treatment of epithelial ovarian carcinomas.

INTRODUCTION

Gynecologists are often confronted with the difficult problem of differentiating malignant tumors from benign ones in patients with pelvic masses. Since Bast *et al.* (1) prepared a monoclonal antibody (OC 125) reactive against an ovarian carcinoma antigen (CA-125), use of the antibody to estimate serum CA-125 levels has become a relatively effective immunodiagnostic method for evaluation of such malignancies. A number of studies have shown that approximately two-thirds of patients with adenocarcinoma of the ovary have elevated serum levels of this antigen (2, 3).

The insidious onset and progression of ovarian cancer make early diagnosis very difficult. Accurate monitoring of tumor status is also difficult because patients are often in clinical remission when subclinical disease is present. Unusual antigens have been detected in the serum of humans with malignant diseases, and attempts have been made to produce antigen assays for detection of early recurrent cancer.

Recently, evidence has been presented on altered superoxide dismutase activities in neoplastic tissues (4, 5). In mammals, three types of superoxide dismutase (copper-zinc superoxide dismutase, manganese superoxide dismutase, and extracellular superoxide dismutase) occur, the three enzymes being encoded by three separate genes (6–8). These enzymes catalyze the dismutation of the superoxide radical O_2^- to O_2 and H_2O_2 and play a key role in protecting cells against direct and indirect oxidative damage (9, 10). Previous studies in our group have indicated that immunoreactive manganese superoxide dismutase increases in lung adenocarcinoma tissues (5).

An ELISA[3] specific for human manganese SOD was developed using a monoclonal antibody (11) and used to assess the clinical significance of manganese SOD in patients with ovarian carcinoma. The localization of manganese SOD in ovarian carcinoma tissue specimens was also determined histochemically.

MATERIALS AND METHODS

Subjects. Serum samples from 195 male and 207 female healthy blood donors were obtained. Serum was also taken within 1 wk before surgery or radiation therapy from 119 patients with pelvic masses and gynecological malignancies, which included 21 benign masses, 2 borderline epithelial ovarian tumors, 33 ovarian carcinomas, and 63 patients with other gynecological tumors. One ml of fresh blood was collected by venipuncture, randomly numbered, and immediately sent to the laboratory for these studies. Clinical diagnoses were not known prior to assay, and patient's diagnoses were not matched with test code numbers until the assay was complete. Diagnoses were confirmed by review of operative and pathology reports. Judgments of disease progression or regression were based on objective intraoperative observation of tumor nodules, dimension of metastases on chest roentgenography, or abdominal computed tomography. Acceptance of regression of disease required a greater than 50% reduction in the size of detectable lesions. Disease progression required the appearance of new lesions or a 25% increase in the largest dimension of previously detected tumor nodules.

All blood samples were taken in the morning before physical examination or surgery. Serum was separated and stored at −20°C within 3 h. Ovarian tumor tissues were obtained at surgery, and uninvolved ovarian tissues were obtained separately after surgical resection. The preparation of cryostat sections utilized small blocks of malignant or nonmalignant tissue which were frozen immediately at −80°C until use.

ELISA for Manganese SOD. ELISA determinations for this enzyme were carried out using a commercial kit originally developed by our group (Ube Research Laboratory, Ube Industries, Ltd.).

The sensitivity of this assay permitted detection of 2 to 200 ng of enzyme per ml. Repeat assays of control samples over a 6-day period showed a reproducibility and coefficient of variation within 3%.

Assay for CA-125. The serum levels of this antigen were measured by radioimmunoassay using a standard radioimmunoassay kit (CA-125 radioimmunoassay kit; Centcor).

Immunohistochemistry. Four-μm frozen sections of 4% paraformaldehyde-fixed tissue were washed with sucrose and mounted on glass slides. Primary incubation was carried out with a dilution of monoclonal antibody to manganese SOD in PBS for 30 min at room temperature. As negative controls, tissues were also incubated with anti-Leu-2a (IgG; Becton Dickinson immunocytometry system). The sections were then washed in PBS and incubated with biotinylated horse anti-mouse IgG (Vector Laboratories, Inc.) for 15 min. After washing in PBS, the slides were treated with avidin dehydrogenase and biotinylated horseradish peroxidase complex for 30 min at room temperature. The sections were then counterstained with hematoxylin to reveal nuclei.

RESULTS

Manganese SOD Levels in Serum. Since the enzyme level (mean ± 2 SD) for male donors was 99.8 ± 49.6 and that for female donors was 88.8 ± 41.6 ng/ml, an upper limit for normal individuals of 150 ng/ml was selected (Table 1). In our series of 119 patients, 96 proved to have invasive pelvic neoplasms.

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[3] The abbreviations used are: ELISA, enzyme-linked immunosorbent assay; SOD, superoxide dismutase; PBS, phosphate-buffered saline; IgG, immunoglobulin g.

Table 1 *Manganese SOD values in healthy controls*

Groups	Total no. tested	Mean ± SD (ng/ml)	Range (ng/ml)
Male	195	99.8 ± 24.8	47.2–141.0
Female	207	88.8 ± 20.8	50.1–149.4
Total	402	94.1 ± 23.5	47.2–141.0

Table 2 *Positivity rate of manganese SOD in the serum of patients with benign ovarian tumors and other gynecological malignancies*

Types	Total no. tested	No. of positive cases above 150 ng/ml
Ovarian tumors	19	0
Serous cystoadenoma	6	0
Mucinous cystoadenoma	5	0
Endometriosis	8	0
Tumors of the uterus		
Hydatidiform mole	2	0
Other malignant tumors	63	3
Uterine cervical cancer	33	1 (3.0)[a]
Endometrial cancer	24	2 (8.3)
Choriocarcinoma	3	0
Vulvar cancer	2	0
Vaginal cancer	1	0

[a] Numbers in parentheses, percentage.

Table 3 *Positivity rate of serum manganese SOD in patients with borderline and ovarian carcinomas*

Types	Total no. tested	No. of positive cases above 150 ng/ml
Epithelial carcinomas	23	12 (52.2)[a]
Serous cystadenocarcinoma	12	7 (58.3)
Clear cell adenocarcinoma	4	2 (50.0)
Endometrioid adenocarcinoma	2	1 (50.0)
Mucinous cystadenocarcinoma	3	0 (0)
Undifferentiated carcinoma	2	2 (100)
Germ cell tumors	4	0 (0)
Sex cord stromal tumors	2	0 (0)
Metastatic tumors	4	0 (0)
Mucinous tumor of borderline malignancy	2	0 (0)

[a] Numbers in parentheses, percentage.

Table 2 presents data for the benign masses and nonovarian gynecological malignancies along with their manganese SOD levels.

None of the 19 benign ovarian tumor patients had manganese SOD levels above 150 ng/ml. In the 63 nonovarian gynecological malignancies group, only one case of 33 uterine cervical cancer patients and 2 of 24 cases of endometrial cancer had manganese SOD levels above 150 ng/ml.

Twenty-three of 33 patients with malignant ovarian tumors had epithelial and 10 had nonepithelial carcinomas. When a serum manganese SOD value greater than 150 ng/ml was utilized as the diagnostic criterion, the positive rate was 52.2% for patients with epithelial ovarian carcinomas and 0% for patients with nonepithelial carcinomas (Table 3; Fig. 1).

The mean value of serum manganese SOD in patients with epithelial carcinomas was 194.8 ng/ml, compared with 92.4 ng/ml in patients with nonepithelial carcinomas. Statistical analysis showed a significant difference ($P < 0.01$) between these two groups (Table 4).

Among the 23 patients with epithelial ovarian carcinomas, the sensitivity of the manganese SOD assay showed a significant difference ($P < 0.05$) between those with Stage 1 *versus* other stages of disease (Table 5).

Correlation between Serum Manganese SOD and CA-125 in Epithelial Ovarian Carcinomas. Serum levels of the enzyme and CA-125 were assayed simultaneously for 23 epithelial ovarian cancer patients (Fig. 2). The positive rate of manganese SOD was 52.2% (12 of 23 patients) and that of CA-125 was 65.2% (15 of 23 patients) in epithelial ovarian carcinoma. Positive manganese SOD values were found in three of eight CA-125-negative epithelial ovarian carcinomas. No significant correlation was noted between serum manganese SOD and CA-125 levels in epithelial ovarian cancer patients ($r = 0.39$).

Correlation of Manganese SOD with Clinical Aspects. Serum specimens from 21 patients with epithelial ovarian carcinomas taken from 2 to 18 times over 2 to 36 months showed manganese SOD levels from 49 to 1925 ng/ml. Changes in enzyme levels were compared to variations in tumor mass (Fig. 3). During tumor regression, manganese SOD values exceeded 150 ng/ml in one patient and fell below 150 ng/ml in 8 patients. A rising manganese SOD level or maintenance of a level over 150

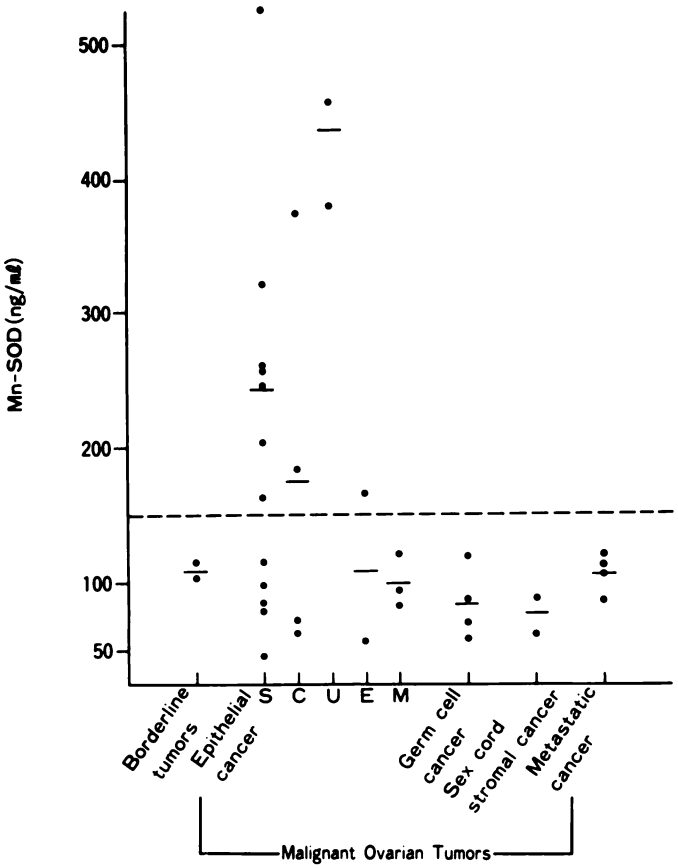

Fig. 1. Preoperative serum manganese SOD (*Mn-SOD*) levels in patients with borderline and malignant tumors of the ovary. *S*, serous cystadenocarcinoma; *C*, clear cell adenocarcinoma; *U*, undifferentiated carcinoma; *E*, endometrioid adenocarcinoma; *M*, mucinous cystadenocarcinoma.

Table 4 *Correlation between serum manganese SOD and tumor types*

Types	Total no. tested	Mean value (ng/ml)	P[a]
Epithelial	23	194.8	$P < 0.01$
Nonepithelial	10	92.4	

[a] Calculated using Wilcoxon's rank sum test.

Table 5 *Correlation between serum manganese SOD values and clinical stages of epithelial ovarian carcinomas*

Stages	Total no. tested	No. above 150 ng/ml	P[a]
I	10	2 (20.0)[b]	$P < 0.05$
II + III + IV	13	10 (76.9)	

[a] χ^2 test.
[b] Numbers in parentheses, percentage.

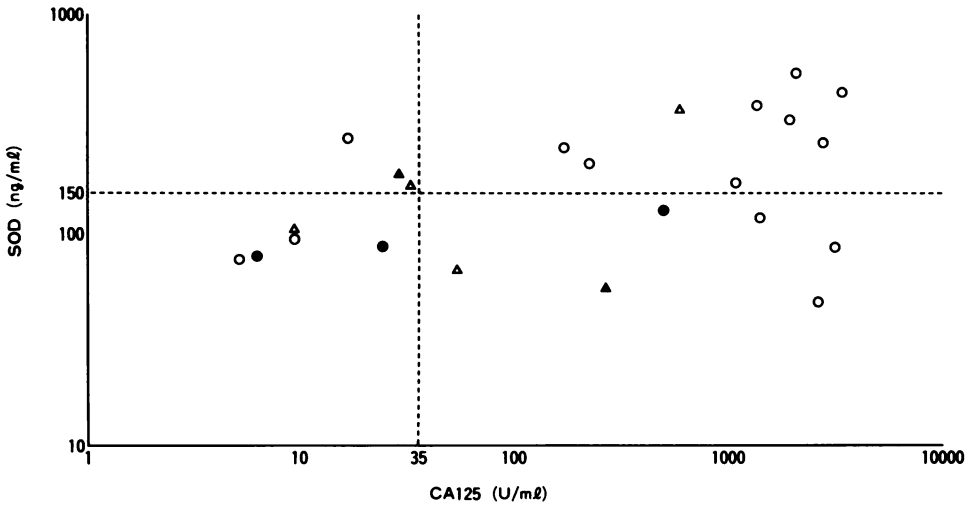

Fig. 2. Relation of serum manganese SOD to CA-125 levels in epithelial and nonepithelial ovarian carcinomas. The *dotted lines* indicate values for these tumor markers considered to be outside normal ranges. ○, serous cystadenocarcinoma; ●, mucinous cystadenocarcinoma; △, clear cell adenocarcinoma; ▲, endometrioid carcinoma.

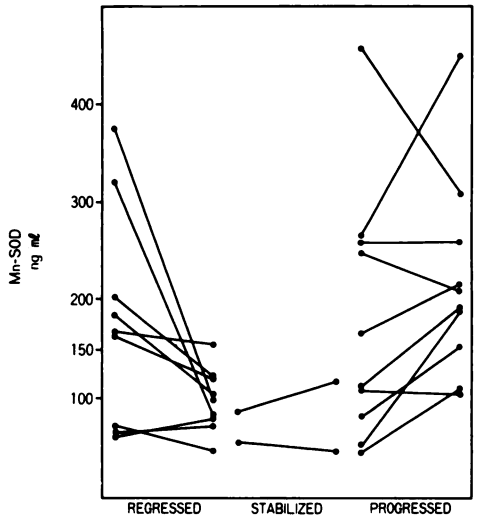

Fig. 3. Manganese SOD (*Mn-SOD*) levels before and after treatment in patients whose disease regressed or progressed. Regression or progression of disease was judged according to standard criteria outlined in "Materials and Methods."

ng/ml was associated with disease progression in 8 of 10 cases.

In one patient with Stage IIb serous cystadenocarcinoma, it was possible to monitor manganese SOD levels on 9 occasions over 30 mo (Fig. 4). After surgical cytoreduction and following chemotherapy with a combination of cyclophosphamide, Adriamycin, and cisplatin, manganese SOD levels decreased from 266 to 66 ng/ml. Laparotomy in March 1986 failed to reveal residual tumor, and further treatment was then continued with cisplatin. In March 1988, the manganese SOD level rose to 166.5 ng/ml with CA-125 rising to 150 units/ml. At this time, abdominal computed tomography revealed a small pelvic mass and ascites. Thus, an increase of manganese SOD and CA-125 was observed upon recurrence of disease.

Immunohistochemical Studies. Two of 4 ovarian serous cystadenocarcinoma tissues tested stained positively. Antibody localization of manganese SOD in the tissues is illustrated in Fig. 5. Control sections incubated with a monoclonal anti-human IgG and then stained with fluorescein-conjugated horse anti-mouse immunoglobulin failed to show uptake of antibody. No reactivity for manganese SOD could be detected with any of the normal tissues tested including ovary and uterus, nor did any of 12 other gynecological carcinomas react.

DISCUSSION

It has been reported that the superoxide dismutase activity in tumors as well as in aged tissues is decreased as compared with that of normal or younger tissues (11–17). However, the clinical significance of serum manganese SOD as a tumor marker in patients with gynecological tumors has not been reported. We have found that assay of this enzyme in the serum of such patients and study of its histological distribution in ovarian carcinoma tissue provide useful data in prognosis and in following the clinical course of this disease. A high level of manganese SOD in epithelial ovarian carcinoma cases was observed as compared with normal individuals and patients with nonepithelial ovarian carcinomas and other gynecological malignancies.

Bast *et al.* (1, 2) first suggested that serum CA-125 values greater than 35 units/ml should be defined as positive criteria for epithelial ovarian cancer. Based on this criterion, CA-125 positivity rates in this malignancy ranged from 73 to 92.5%, while in the present study the rate was 65%. However, false positive findings for serum CA-125 in patients with various benign pelvic masses have also been reported (18, 19). These rates were 10% in patients with benign epithelial ovarian tumor, 44% for leiomyomas, 9.5% in cases with pelvic inflammatory masses, and 37.5% for ovarian endometriosis (18, 19). In this respect, measurement of serum manganese SOD will be of clinical use because of its low levels in gynecological malignancies other than epithelial ovarian cancer and since all benign ovarian tumors do not show significant amounts.

In patients with epithelial ovarian carcinomas, serum manganese SOD levels increased in accordance with the progression of clinical disease. However, such measurements may not always be useful for the early diagnosis of epithelial ovarian carcinomas, since at this stage the incidence of cases with elevated serum levels was low. The decline in serum manganese SOD levels following effective therapy seems to reflect the disappearance of lesions. Decreases occurred after therapy and increases with recurrences. Thus, it appears that the measurement of this enzyme in serum can provide useful data in monitoring ovarian cancer following therapy and for the early diagnosis of recurrences.

Although the rate of detection of ovarian carcinoma is lower with manganese SOD than CA-125, the specificity to epithelial ovarian cancer seems to be relatively higher with manganese SOD than CA-125. Manganese SOD in tissue specimens was

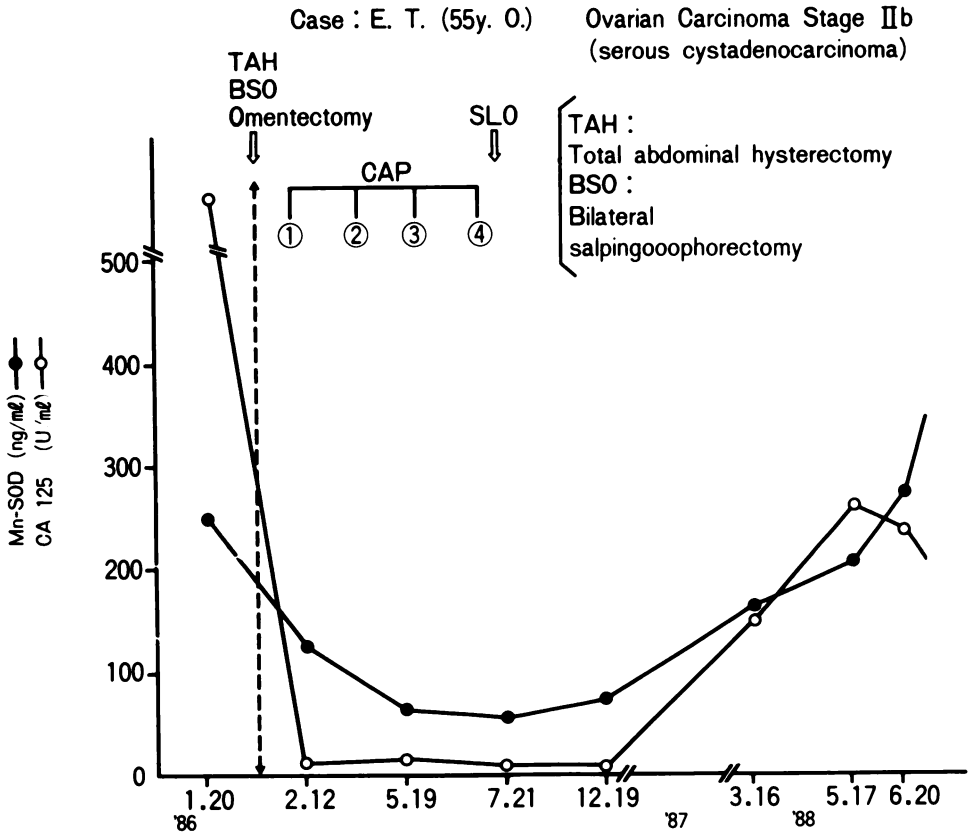

Fig. 4. Serum manganese SOD (*Mn-SOD*) levels during the course of treatment of one patient with Stage IIb serous cystadenocarcinoma. *SLO*, second look operation; *CAP*, cyclophosphamide:Adriamycin:cisplatin.

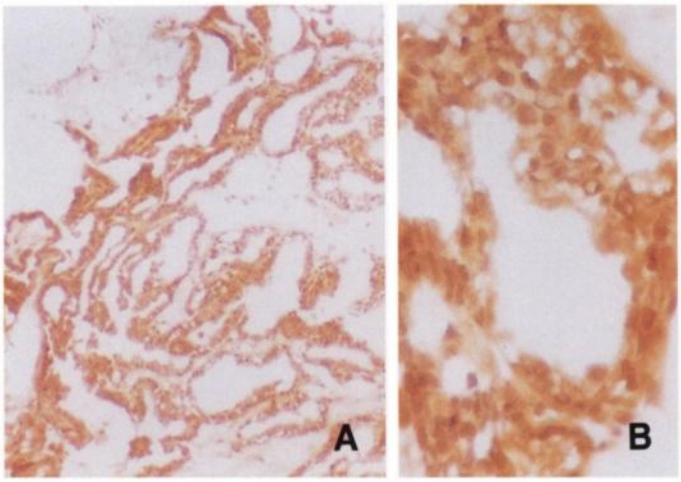

Fig. 5. A section of serous cystadenocarcinoma, stained with monoclonal antibody of manganese SOD. Bright staining is seen in cytoplasm of carcinoma cells. *A*, × 100; *B*, × 1000.

clearly detected in several histological types of epithelial ovarian carcinomas.

In any event, the results of ELISA using monoclonal antibody in the present study suggest that the measurement of manganese SOD in serum may provide one of the clinically useful markers for the presence of epithelial ovarian carcinomas and for monitoring the response to treatment and early detection of recurrences.

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