Evaluation of SOD Activity in Gastric Cancer

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ABSTRACT : For the purpose of clarifying the defensive capacity of the tumor-bearing host against the tumor was evaluated from the standpoint of superoxide dismutase (SOD) activity in the peripheral blood of untreated gastric cancer patients as compared with clinicopathologic factors. There was not significant difference in SOD activity between the entire gastric cancer patients and normal subjects. In contrast, a significant difference in SOD in monocytes (MNC) and polynuclear leucocytes (PMN) was found between early and advanced gastric cancer patients. SOD activities in advanced gastric cancer patients were obviously depressed. According to the disease stage, those in Stage IV patients were also apparently reduced when compared to reduction of superoxide dismutase (SOD) activity in MNC was observed in patients with hepatic metastasis (p<0.05) and that in MNC and PMN was seen in patients with peritoneal dissemination (p<0.01). As for gross findings by Borrmann' classification, Borrmann IV gastric cancer revealed significantly reduced SOD activity in MNC and PMN (p<0.01).

It is concluded that SOD activity in the peripheral blood was depressed in accordance with advanding cancer stage. On the in vitro basic study to clarify the mechanism on SOD activity in gastric cancer patients it was certified that there was no influence of extracellular factors such as serum factor obtained from cancer-bearing patients, cultured cells, immunodepressant factor and nutrients on SOD activity. In contrast, intracellular factor of inhibitors of RNA and protein synthesis induced by adriamycin, actinomycin puromycin was greatly responsible for SOD activity in cultured cells.

However, the inhibition of DNA synthesis could be not entirely neglected. It is dubious in association with inhibition of DNA synthesis.

It was warned that the use of chemotherrapy of adriamycin and actinomycin D contributed to reduced SOD activity for the treatment of advanced cancers such as stage IV and Borrmann IV gastric cancers.

INTRODUCTION

Since superoxide dismutase was discoverd in 1969 by McCord and Friduvitch[1] as an enzym to uniformly dissolve superoxide to oxygen and superoxide oxidor, it has become well known in living cells. In mammalian, two kinds of SOD, Cu, Zn-sensitive SOD in cytoplasma and Mn-sensitive SOD in mitochondria, were identified[2,3]. Recently a presence of very small amount of EC-SOD also was recognized by Marklund[4].

To date, clinical application of SOD has become prevalent in the field of inflammation, carcinogenesis degenerative process, ARDS, irradiation injury, chromosome abnormality, ischemia, reperfusion syndrome[7] and so on.

With respect to SOD in tumor cells, since Yamanaka[8] reported disappearance and/or

absence of Mn-SOD in carcinogenetic process in VI-38 cells by SV40, the study on SOD has been performed in cultured cells and experimentally implanted cells.

It is clarified that the majority of cancer cells in Morris hepatoma 39244 (Dionisi9), Ehrlich ascitic liver cancer cells, H_6 hepatoma (Oberley), CH_3 breast cancer cells[11] represent a decrease in total SOD, marked reduction of disapperance of Mn-SOD activity. In the human, a decrease in SOD activity in non-cancer tissues was observed in cases of renal cell carcinoma by Westmann[14] and lung cancer by Hartz[15]. A few reports concerning sequential study on SOD activity with advancing cancer are now available. It corresponds only to an experimental study on Yoshida' sarcoma by Sakuma[7], It is clarified that reduction of Cu, Zn-SOD activity is more remarkable in late stage rather than in early stage in the liver, heart, brain and lung.

On the basis of the fact[17] that the levels of SOD activity in tissues proportionate to those in the blood, this study dealt with changes in total SOD and Mn-SOD activities in RBC, PMN and MNC in combination with the effect of anticancer agents on blood corpscules.

MATERIAL AND METHOD

1. Clinical evaluation

Fourty-four preoperative gastric cancer patients were eligible for this study. Sex distribution of men to women was 29 : 15. The ages ranged from 38 to 79 with an average of 62.5 years old. As a control, 39 healthy subjects were selected.

SOD activities in MNC, PMN and RBC obtained from untreated patients and healthy candidates were measured and compared to the clinicopathologic factors.

2. Basic evaluation

In vivo study was made on cultured lymphocytes in 10% FBS to make clear of changes in SOD activity of tumor-bearing host.

1. Evaluation of extracellular factor

Experiment

1) mixed culture with human cultured cancer cells and lymphocytes

HeLa cells were used for the evaluation. Changes in SOD activity were observed in admixture of HeLa cell to 10 times leucocytes for 72 hours.

2) lymphocytes culture with the serum from cancer patients

SOD activity in leucocytes was compared with before and after culture with the use of the serum from colon cancer patients for 72 hours.

3) lymphocyte culture in addition to acid soluble glycoprotein (ASP)

The effects of ASP on SOD activity in cultered lymphocytes were evaluated at the concentrations of 125, 250 and 500mg/dl. ASP was supplied from Otsuka Assay Co, which was one of immunosuppressive proteins.

4) The effects of different concentrations of glucose on cultured lymphocytes.

The lymphocytes from healthy subjects humans were cultured in RPMI without glucose, and adding 100, 200, 400mg/dl glucose for 72 hours.

5) The effects of different concentrations of FBS on cultured lymphocytes

SOD activity in cultured lymphocyte was measured at 1, 5, 10% concentrations of FBS in RPMI.

2. Evaluation of intracellular factors

1) Blastogenesis of lymphocytes stimulated by PHA. SOD activity was observed at the time of lymphocyte blastogenesis stimulated by PHA-P. And also nuclear DNA and RNA contents were measured by using flow cytometry according to Darzynkiewicz and Tagawa' method.[18,19]

2) The effect of inhibitors of protein synthesis on SOD activity

The lymphocytes were cultured with DNA synthesis inhibitors (cisplatinum 10μg/ml), (mitomycin C 1.0μg/ml), adriamycin 0.5μg/ml, RNA synthesis inhibitor (actinamycin D 0.1μg/ml), protein synthesis inhibitor (puromycin 1.0μg/ml), which concentrations were set up at the cytostatic concentration against the tumor cells.

Method

SOD activity was measured according to the method reported by McCord and Fridovich[1]. Inhibition of cytochrom deoxidized reaction were estimated by using spectrometry (Hidachi Model 557) at 25°C in reflection of changes in cytochrom reduction in accordance with

superoxide production. Inhibitory rates of cyto-chrom reduction were calculated as reported by Kobayashi[22)] as one unit of 50% SOD inhibition. SOD activity in PMN, MNC and RBC were expressed as per blood corpuscles.

SOD activity in mitochondria also was measured by the addition of 1mM KCN. The values averaged and t-test was used for estimation of statistic difference of the average values.

RESULTS

Clinical evaluation

SOD activity in gastric cancer patients averaged $48.0 \pm 10.1 \mu/10^8$ in RBC, $8.8 \pm 2.4 \mu/10^7$ in PMN and MnSOD activities were $1.4 \pm 0.6 \mu/10^7$ as a whole, $25.6 \pm 9.9 \mu$ in MNC $2.8 \pm 1.4 \mu/10^7$ in MnSOD. There was not significant difference between healthy and gastric cancer patients (**Table 1**) and between both sexes.
2. SOD activity in blood corpuscles between early and advanced gastric cancer patients.

Although there was not significantly different in RBC, significant difference was seen in PMN. In advanced cases, it was depressed (p<0.01) SOD activity in MNC in advanced patients was

23.1 ± 6.7 on the average in contrast with $28.5 \pm 9.4 \mu/10^7$ which was significantly depressed as compared to $28.3 \pm 11.5 \mu/10^7$ early gastric cancer (**Fig. 1**).
3. SOD activity and gross finding of gastric cancer.

Total SOD activities in PMN, MNC, and whole blood were significantly suppressed in Borrmann IV gastric cancer patients (p<0.01) (**Fig. 2**). Only SOD in MNC in Borrmann II and III gastric cancer was reduced.
4. Disease stage of gastric cancer

SOD activity in whole blood corpuscles was depressed with advances in the disease stage although that in Stage II somewhat increased. In ly (+) gastric cancer, SOD activities in PMN were appearently reduced as compared to those in ly (−). This tendency in v (+) gastric cancer was similar to ly (+).
Basic evaluation
(1) Mixed culture with human tumor cells and lymphocytes

In mixed culture with HeLa cells and 10 human lymphocytes, proliferation of HeLa cells was not inhibited and no alternation of lymphocytes in MLTR noted (**Fig. 4**). There was not remarkable changes in SOD in lymphocytes

Table 1. SOD activity of patients with gastric cancer and of normal controls

|	RBC[1)]		PMN[2)] $\mu/1 \times 10^7$		MNC[3)] $\mu/1 \times 10^7$	
	μ/mgHg	$\mu/1 \times 10^8$	Total-	Manganase	Total-	Manganase
Gastric Cancer (n=44)	17.9±4.8	48.0±10.1	8.8±2.4	1.4±0.6	25.6± 9.9	2.8±1.4
Normal Controls (n=39)	19.1±4.3	49.3±11.1	8.9±1.9	1.3±0.3	28.1± 9.5	3.1±1.0
Sex Male (n=24)	18.3±4.0	47.4±12.6	8.2±1.6	1.2±0.3	28.0± 9.7	3.2±1.1
Female (n=15)	19.1±6.0	49.3±12.5	9.6±2.3	1.4±0.3	28.6± 9.8	3.1±0.7
Age 20~ (yr) (n= 5)	23.0±4.2	64.4± 5.0	9.1±1.3	1.2±0.2	27.6±13.3	3.3±1.1
30~ (n=17)	18.7±4.2	45.3±10.9	8.9±2.2	1.4±0.3	29.4± 7.9	3.4±1.0
50~ (n=10)	18.6±4.0	50.3±11.1	8.1±2.2	1.2±0.4	24.9± 7.8	2.6±0.9
70~ (n= 7)	18.3±4.9	48.8± 7.5	9.1±1.7	1.1±0.2	30.0±14.1	3.2±0.9

1) RBC : erythrocytes
2) PMN : polymorphonuclear leukocytes
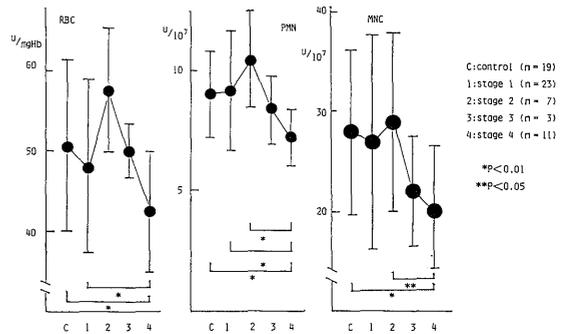3) MNC : mononuclear leukocytes

Table 2. SOD activity of peripheral blood cells in gastric cancer patients and P, N, H, PS factors

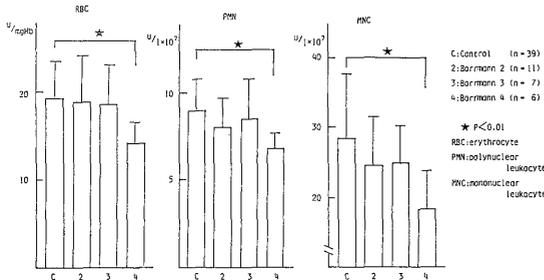|		RBC (μ/mgHb)	PMN (total) $\mu/10^7$ cells	MNC (total) $\mu/10^7$ cells
P (−)	(34)	18.7 ± 4.8]*	9.2 ± 2.3]*	26.4 ± 9.7
P (+)	(6)	13.8 ± 2.0	6.5 ± 0.9	18.4 ± 6.4
N (−)	(24)	17.2 ± 3.6	9.3 ± 2.6	27.1 ± 10.5
N (+)	(19)	18.9 ± 6.0	8.4 ± 2.0	23.3 ± 8.3
H (−)	(40)	18.0 ± 5.0	8.9 ± 2.4	26.0 ± 9.8]**
H (+)	(2)	18.0 ± 5.2	7.6 ± 1.0	17.5 ± 2.6
PS (−)	(31)	18.3 ± 5.0	9.3 ± 2.4	27.2 ± 10.1
PS (+)	(12)	17.2 ± 4.6	7.9 ± 2.0	20.2 ± 6.6

$*\,p<0.01$
$**\,p<0.05$
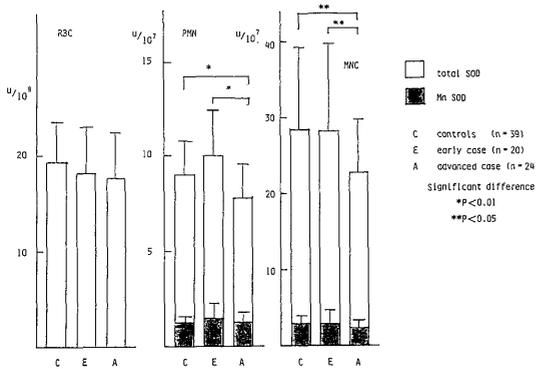
Fig. 1. SOD activity of peripheral blood cells between early and advanced cases in gastric cancer patients

Fig. 2. SOD activity of peripheral blood cells classfied by Borrmann types

Fig. 3. SOD activity of peripheral blood cells in each stage in gastric cancer patients
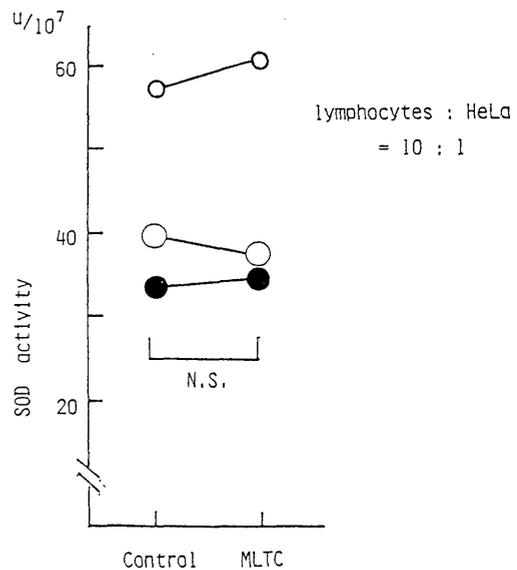
Fig. 4. Mixed Lymphocytes Tumor Reaction for 72 hours

between MLTR and control groups. **Fig. 5** showed changes in SOD activity in the serum taken from the patients with colon cancer, which contained 714μ/ml IAP. SOD activities were depressed as being $14.3\mu/10^7$ in total-SOD and $0.8\mu/10^7$ in Mn-SOD. However, there was not
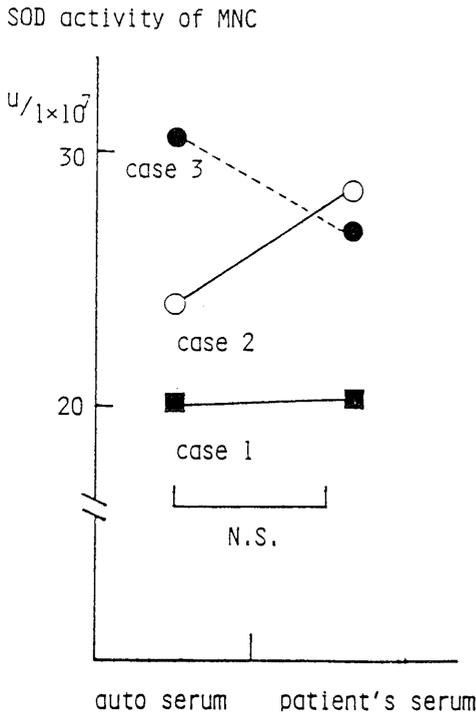
SOD activity of MNC

Fig. 5. SOD activity of healthy volunteers' lymphocytes after 72 hour incubation with serum of advanced cancer patient
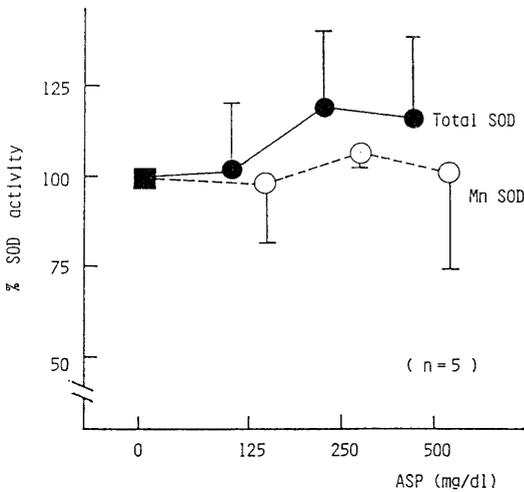

Fig. 6. SOD activity of healthy volunteers' lymphoctes after 72 hour incubation with various ASP concentration

significant difference in SOD activity between healthy and patient's sera.

3. The effect of the addition of ASP on SOD

activity

There was not remarkable change in SOD activity between with and without the addition of each concentration of ASP to culture medium as shown in **Fig. 6** in comparison with the control, which did not contain ASP and was regarded as being 100%.

4. The effect of different concentrations of glucose in culture medium on SOD activity.

It aimed at the effect of different blood sugar levels in patients on SOD activity. **Fig. 7** showed that the higher the concentration of glucose, the greater Cu-Zn SOD activity and the lesser, Mn-SOD had become. There was not satistically significant difference in a total of SOD activity between with and without the addition of glucose.

5. The effect of lymphocyte culture at different concentrations of FBS

With advancing the disease stage, the nutritional condition of a tumor-bearing host has become aggravated. In carcinomas of the digestive tract, poorly nutrionatal condition is in proportion to progression of the tumor. The effect of poor nutrition on SOD activity was tested at different FBS concentrations. There was not statistically significant difference in total and Cu Au SOD activities between different concentration of 5% and 1% as compared with the control of a value of SOD activity in 10% FBS, which was regarded as 100% (**Fig. 8**).
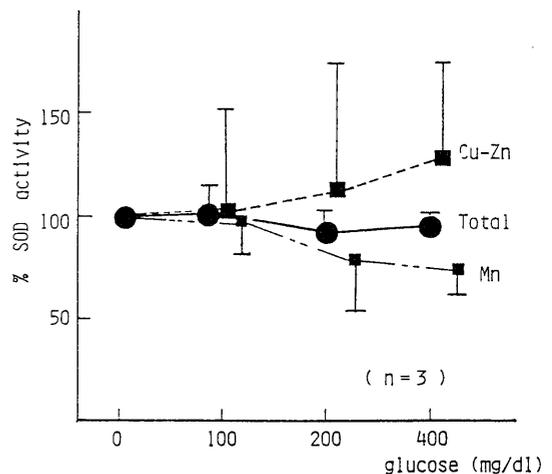

Fig. 7. SOD activity of healthy volunteers' lymphoctes after 72 hour incubation with various glucose concentration
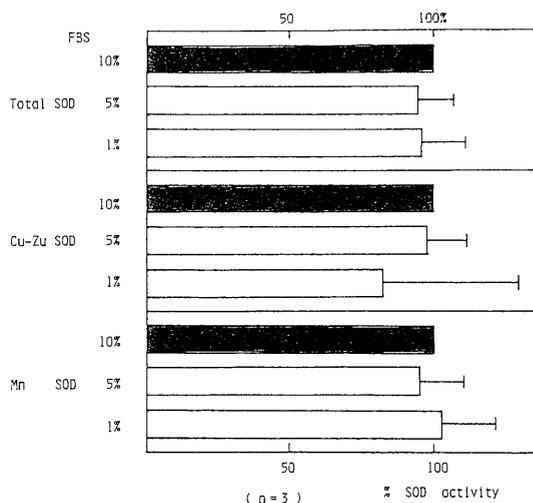
Fig. 8. SOD activity of healthy volunteers' lympho-
cytes after 72 hour incubation with various
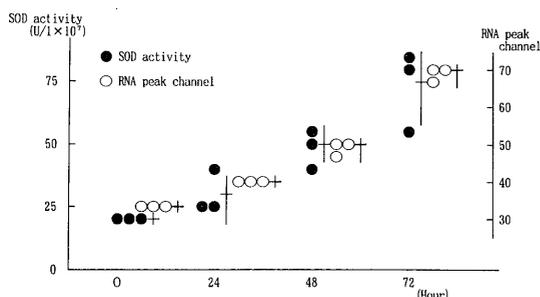FBS concentration

Fig. 9. Relationship between SOD activity and RNA
peak channel (RNA contents) in PHA-P
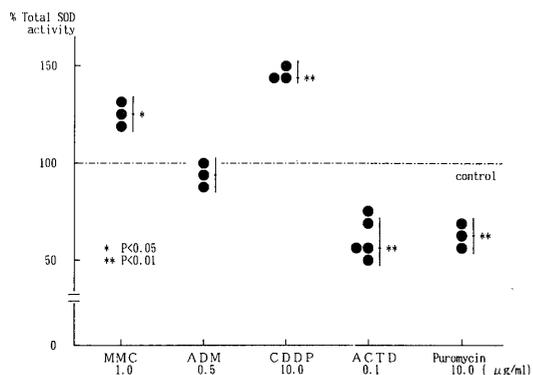stimulated lymphocytes (n=3)

Fig. 10. SOD activity of lymphoctes for 2 hour
exposure to agents following 72 hour
incubation

2. Intracellular factor

1) lymphoblastogenesis by stimulation with
PHA-P

SOD activity stimulated by PHA-A in lym-
phocytes increased with elapse of time and it
reached to 3.7 times as high as prior values after
72 hours (**Fig. 9**). RNA contents also rose 1.4
times after 48 hours and 1.97 times after 72
hours.

2) The effect of anticancer agents and protein
synthesis inhibitor on lymphocyte culture.

SOD activity in lymphocytes in contact with
CDDP and MMC of protein synthesis inhibitor
increased 1.43 and 1.25 times as compared with
the control. On the other hand, in case of ADM
it dereased 1.92 times. in contact with
actinomycin-D, it reduced to 62%, Meanwhile, in
case of puromycin, inhibitor of portein
synthesis, it was decreased to 60%.

DISCUSSION

It is assumed that SOD activity is indispen-
sable for action as scavenger of free radical
produced in body as already reported in gastric
cancer by Nakagoe[23]. In this series, SOD
activity in RBC, MN and MNC had become
reduced with advances in disease stage.
Furthermore, in advanced cancer patients with
peritoneal dissemination, hepatic metastasis as
well as ly (+) v (+), it was apparently inhibited.

In malignant diseases, many researchers
reported reduced SOD activities, for example,
SOD in MN in malignant lymphoma, Mn-SOD
in lymphocytes in breast cancer as reported by
Michelson[24]. SOD in RBC in 11 malignant
lymphomas in spite of no change in SOD of RBC
of lung cancers by Saito[25]. Sato[26] also in-
vestigated Cu-Zn SOD and Mn-SOD activities
in sera and lymphocytes obtained from patients
with malignant diseases by using polyclonal
antibodies. Consequently he reported high
Cu-Zn SOD and Mn SOD activities in sera and
lymphocytes irrespective of the kinds of malig-
nant diseases. It seemed that this discrepancy
would be based on the differences in measure-
ment method which might be associated with
the differences between enzym-action and
antigen-presenting sites.

From above results, it is suggestive that SOD

molecules be not reduced in accordance with progression of the tumors but the molecules have become inactive because of most of inactive molecules and/or because of the surrounding circumstances to make them inactivated.

It is well known that diethyl dithiocarbamate (DDC)[27] inhibits SOD activity and also thiocurbanyl acid makes Cu-Zn-SOD activities inactive in spite of no evidence of their presence in humans. It is also accepted that SOD activity in RBC is inhibited in cupper dificiency.[28] However, recovery to 60% Cu level in RBC made it possible to revert to the normal SOD activity.

In this series, it was certified that reduced SOD activity in advanced cancer patients was not in association with Cu deficiency because of normal levels of Cu in sera.

Okahatake[29] reported that T lymphocytes had higher SOD activity rather than B lymphocytes. In this series, lymphocyte fractions did not show remarkable variations between early and advanced cancer patients. In this study, there was not significant difference in leucocyte fraction except that OKIa1 fraction somewhat increased in advanced cancer patients. In this series, SOD activity in lymphocytes was not altered in culture medium which added sera from healthy human and cancer patient's and/ of the addition of ASP, immunosuppressive protein.

Kasemset and Oberly[30] clarified that SOD activity in the myocardium was reduced by adding glucose into the peritoneal cavity in mice, in particular Mn-SOD activity was much more inhibited in reflection of inhibition of superoxide production, followed by inhibited Mn-SOD activity. There was not influenced on SOD activity in lymphocytes by differenct concentrations of FBS in culture medium.

In this series, it was certified that activated SOD activity was contributary for an increase of RNA content. In contrast, DNA inhibition by MMC, ADM, CDDP led to increased SOD activity. It is accepted the reasons are that MMC causes damage to nucleoic acid by production of OH^- with help of a small amount of metal. On the other hand, it converts to semiquinone type which produes O_2^- by action of NADPH-P450 reductase[31]. And also CDDP

contributes to production of O_2^- H_2O_2 from macrophages[32]. High SOD activities imply induction of SOD by O^-. In contrast, it is different in case of ADM with which SOD is not easy to be induced[33]. RNA synthesis inhibitor reduced SOD activity to 60% of the previous level. It is substantiated that SOD activity is closely associated with RNA synthesis. Niwa[17] clarified that in comparative study on SOD in RBC, WBC platelets in patients with various cancers, SOD and OI (Oi/SOD oxygen intermediate) did not remarkably vary and he contemplated that in early stage of carcinomas, excess OI might be generated by phagocytic cells as a result of enhanced defensive ability, thereafter, with progression of the tumor, OI production would be inhibited because of depressed viability of phagocytes. His suggestion is consistent with the result in this study. As reported by Nakagoe[23], it is reliable to consider that depressed SOD activity is due to nothing to induce SOD or difficulty in induction of SOD. It is known that dose effects of Cu-Zn SOD exist in chromosome 21. On the other hand, Feaster[34] explained that Cu-Zn SOD activity in Down' disease (21 trisomy) was 1.5 times as high as the normal in RBC, and fibroblasts in reflection of gene dose effect. Joenje[35] reported that repair process from damage to chromosome was impaired by high concentration of O_2^- which was caused by depressed SOD activity in patients with Fanconi anemia. On the contrary, Yoshimitsu[36] insisted that abnormality of chromosome might result in depressed SOD activity.

On the basis of a result of this study on SOD activity in advanced cancer patients, it is interest to emphasize that anticancer agents produce inhibited SOD activity in addition to a low SOD activity seen in tumor-bearing hosts. Recent studies focus on the use of L-SOD which makes up for too short half-life time of SOD and makes it possible to penetrate into the inside of cells, and also to explore drugs of free radical scavenger.

In conclusion, this study on SOD activity in advanced cancer patients warned of the treatment of anticancer drugs and radiation.

ACKNOWLEDGEMENT

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