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Original Article

Oxidative Stress in Breast Cancer

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Abstract

The present study was undertaken to evaluate the place of oxidative stress on breast cancer. Lipid peroxidation as evidenced by malondialdehyde (MDA) and the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were estimated in tissues of 10 fibroadenoma and 40 breast cancer patients. Lipid peroxidation in breast cancer tissues was enhanced compared to nonmalignant tissues ($p < 0.001$). Similarly, antioxidants SOD ($p < 0.001$) and GPx ($p = 0.007$) in tumor tissues significantly were increased. On the contrary, CAT activity was found significantly decreased ($p < 0.001$). We found that oxidant/antioxidant status was independent from any prognostic factors concerning breast cancer. The results of our study have shown higher oxygen-free-radical production and decreased CAT activity support the oxidative stress hypothesis in breast carcinogenesis.

Key Words: Breast cancer; catalase; GPx; MDA; oxidative stress; SOD.

Introduction

In normal aerobic cells there exists a balance between oxidative damage and antioxidant protection. Inadequate antioxidant scavenging or excess oxygen-free-radical formation creates a condition known as oxidative stress. Excess generation of oxygen free radicals can cause oxidative damage to biomolecules resulting in lipid peroxidation, mutagenesis, and carcinogenesis. Oxygen-free-radical–induced lipid peroxidation has been implicated in malignant transformation *(1–3).*

Breast cancer is a leading cause of morbidity and mortality in women's lives. In recent years, there have been enormous advances and developments in our knowledge of the mechanism and factors involved in breast carcinogenesis. The precise mechanisms of oxidative stress being induced in breast cancer cells are still not exactly understood and documented. There are only few reports on the oxidant–antioxidant profile in breast cancer patients *(3–9).*

We therefore examined the extent of lipid peroxidation as evidenced by the formation of malondialdehyde (MDA) and the status of the antioxidants superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in tumor tissues of patients with carcinoma of the breast.

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Material and Methods

Patients

Forty newly pathologically diagnosed women with breast cancer, median age of 56 (31–84) yr and 10 women with fibroadenoma, median age of 38 (17–52)

yr were chosen for the study. The patients were categorized as stage; 10 patients were accepted per stage.

Determination of Oxidative/Antioxidative Status

Fresh tumor and fibroadenoma tissues obtained from patients immediately after surgery were placed in cold 0.9% NaCl solution. The tissues were blotted on filter paper, weighed and homogenized under standardized conditions, and 20% tissue homogenate was performed. The supernatant was kept in an ice-cold condition until assayed. For histopathological analysis, portions of the tissues were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylen and eosin.

Lipid peroxidation, as evidenced by the formation of malondialdehyde (MDA), was assayed by the method described by Angel et al. *(10).* The reaction mixture containing 0.5 mL of 20% tissue homogenate, 1.5 mL of 0.75% thiobarbituric acid, 1 mL of 30% tricloracetic acid, and 0.1 mL 5 *M* hydrochloric acid was incubated in boiling water for 15 min. Products of lipid peroxidation was read at 535 nm. It was assessed by μ*M* MDA/per mg protein.

Superoxide dismutase (SOD) activity was assayed using the spectrophotometric method of Sun *(11).* The assay mixture contained 0.1 mL 20% tissue homogenate. It was read at 560 nm and assessed by U/mg protein.

Catalase (CAT) activity was determined by the method of Beers–Sizer *(12).* One milliliter of 0.025 *M* hydrogen peroxide was added to 0.1 mL of 20% tissue homogenate. The utilization of hydrogen peroxide was read at 240 nm. It was assessed by U/mg protein.

Glutathione peroxidase (GPx) was measured spectrophotometrically using a technique based on the method of Paglia–Valentine *(13).* Using 20 mL of 20% tissue homogenate, the value of GPx was determined with semimicro method by Randox Ransel 504 kit. It was assessed by U/mg protein.

Determination of Other Prognostic Markers Related to Breast Cancer

p53

Zymed 08–170 Mouse Anti p53 (Pab 1801) as primary antibody and Histostain SP kit as staining process were used. Positive immunoreactivity was accepted only when cell nucleus was stained in more than 5% of the stained cells.

c-erb B2

As primary antibody Zymed 08–107 Monoclonal Mouse Anti c-erb B2 was used. The conditions of only stained cell cytoplasm membrane were accepted as immunoreactive. Weak staining was negative.

DNA Flow Cytometric Determination

Tissue specimen was assessed by Coulter Epics Profile II Flow Cytometry. Values of DNA ploidy, DNA index, and %S phase fraction were determined by statistical analyses using Phoenix Multicycle Program.

Receptor Analyses

Determinations of estrogen and progesterone receptor analyses were done immunohistochemically using DAKO ER-ICA and PR-ICA monoclonal kits.

CEA and CA 15.3 Analyses

In serum specimen before surgery, serum CEA levels (ng/mL) were determined as enzyme immunoassay method using MAGIA ASSAY CEA kit. Analysis of serum CA 15.3 was done by immunoradiometric method using IRMA-Count BR-MA kit.

Statistical Analysis

Frequency tables and statistical analyses were calculated with SPSS for Windows 7.5 (SPSS, Chicago, IL, USA). The data for analyses are expressed as mean±SD. Statistical comparisons were performed by Student's *t*-test. Pearson correlation test was used in correlation between all parameters. A value of $p < 0.05$ was considered statistically significant.

Results

Table 1 shows the extent of lipid peroxidation and the status of antioxidants in patients with fibroadenoma and breast cancer. Lipid peroxidation by measuring MDA was found to be significantly increased in tumor tissue ($p < 0.001$). Likewise, in these cases the activity of antioxidants such as SOD ($p < 0.001$) and GPO ($p = 0.007$) were significantly elevated while the activity of catalase ($p < 0.001$) was significantly decreased in cancer tissue compared with the nonmalignant breast tissue.

The relationships between oxidants and antioxidants in breast cancer tissues are shown in Table 2.

The correlations between oxidant/antioxidant profile and known prognostic factors in breast cancer

Table 1
Tissue Lipid Peroxidation and Antioxidant Status in Patients with Fibroadenoma and Breast Cancer

	Breast cancer patients			Benign breast patients			
Parameter	mean ± SD	median	range	mean ± SD	median	range	*p*
MDA (μM/mg)	27.4 ± 4.9	25.4	20.3–42.6	20.0 ± 2.1	20.2	15.6–22.4	< 0.001
CAT (U/mg)	45.7 ± 6.5	45.9	31.2–60.0	66.7 ± 12.7	69.1	51.4–86.0	< 0.001
SOD (U/mg)	110.7 ± 28.7	104.3	71.6–187.1	80.1 ± 13.3	80.5	62.7–106.0	< 0.001
GPx (U/mg)	1.4 ± 0.4	1.4	0.8–2.9	1.2 ± 0.1	1.2	1.0–1.4	0.007

Table 2
Correlations Between Oxidant and Antioxidants in Breast Cancer Tissues

		CAT	SOD	GPx
Pearson correlation	MDA	–0.257	0.056	0.014
	CAT		–0.313*	–0.314*
	SOD			0.523**
Sig. (two-tailed)	MDA	0.07	0.7	0.9
	CAT		0.027	0.027
	SOD			< 0.001

* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

are presented in Table 3. We found that generally oxidant/antioxidant status was independent of any prognostic factors concerning breast cancer. Only relationships between SOD and serum CA 15.3 (p = 0.001) and GPx and c-erbB2 (p = 0.04) were determined.

Except oxidant/antioxidant status the interrelationships with known prognostic factors each other are shown in Table 4. We determined similar data existed in literature.

Discussion

The evidence for increased action of oxidants in breast cancer is the increase in the level of oxidative damage. In our study, it was found that there was a higher production of oxygen free radicals in breast cancer tissues. Similarly, MDA, the lipid peroxidation product, a well-characterized mutagen, is also significantly increased in breast cancer tissue in other studies *(3,7).* But, in 23 patients with breast cancer tissues, Punnonen et al. *(6)* found that the content of thiobarbituric-acid-reactive material was slightly lower in the cancerous tissue.

The increase in tissue lipid peroxidation in breast cancer seen in the present study was associated with enhanced antioxidant capacities. Increased generation of oxygen free radicals can induce SOD and GPx, not CAT. An increase in SOD and GPx activities due to overexpression has been reported *(3,6–8).* In our study, SOD and GPx activities were found significantly higher in breast cancer tissues. The activity of SOD is significantly increased, which may catalyze the conversion of O_2 into H_2O_2 and cause an increase in the level of hydrogen peroxide. The latter is usually detoxified with the help of GPx and CAT. In the cancerous tissue, CAT activity was lower than in the reference tissue, similar to the findings of Punnonen et al. *(6).* However, higher tissue levels of CAT were also observed *(3,7).* As CAT activity was significantly lower, GPx alone was probably unable to detoxify H_2O_2 into H_2O completely. As a result there might an accumulation of H_2O_2 resulting to higher production of the OH^- radical, which was supported by the higher production of MDA contents. Higher oxygen-free-radical production, MDA concentration, and lower CAT activity indicate an oxidative stress in breast cancer patients. Administration of antioxidant enzymes, particularly CAT, may be helpful in the management of breast cancer.

Moreover, in our study the decrease in the activity of CAT, which degrades hydrogen peroxide, suggests an increase in this oxygen species in cancer tissue. It showed that breast cancer tissues produced

Table 3
Correlations between Oxidant/Antioxidant Profile and Known Prognostic Factors in Breast Cancer

Parameters	MDA (μM/mg) (mean ± SD)	CAT (U/mg) (mean ± SD)	SOD (U/mg) (mean ± SD)	GPx (U/mg) (mean ± SD)
Tumor size				
T1-2	26.2 ± 4.7	45.4 ± 7.5	114.2 ± 30.0	1.3 ± 0.3
T3-4	29.0 ± 4.8	46.1 ± 5.1	105.9 ± 27.0	1.4 ± 0.5
Axillary node				
(–)	27.7 ± 5.5	46.2 ± 5.8	116.1 ± 24.6	1.3 ± 0.2
(+)	27.5 ± 4.2	46.6 ± 6.7	97.2 ± 31.7	1.4 ± 0.5
Stage				
I + II	26.9 ± 4.7	45.7 ± 6.0	113.7 ± 23.4	1.3 ± 0.2
III + IV	27.9 ± 5.1	45.7 ± 7.2	107.7 ± 33.6	1.4 ± 0.5
Grade				
I + II	26.9 ± 5.0	46.2 ± 6.0	112.8 ± 25.9	1.4 ± 0.5
III	28.7 ± 5.7	43.1 ± 7.6	109.6 ± 28.3	1.5 ± 0.3
ER				
(–)	29.3 ± 6.3	45.1 ± 6.2	117.1 ± 35.2	1.4 ± 0.4
(+)	26.3 ± 3.7	46.0 ± 6.8	107.3 ± 24.6	1.4 ± 0.4
PR				
(–)	27.8 ± 4.4	46.8 ± 6.8	110.6 ± 31.7	1.4 ± 0.3
(+)	26.7 ± 5.8	43.5 ± 5.6	110.8 ± 23.3	1.4 ± 0.5
CEA				
Normal	27.6 ± 5.0	45.8 ± 6.7	108.4 ± 29.5	1.3 ± 0.3
Elevated	25.6 ± 4.3	44.9 ± 5.8	126.9 ± 15.8	1.6 ± 0.8
CA 15.3				
Normal	27.3 ± 4.8	46.2 ± 7.0	**104.6 ± 25.2**[a]	1.3 ± 0.3
Elevated	27.8 ± 5.5	43.7 ± 4.3	**131.8 ± 31.6**	1.7 ± 0.5
cerbB2				
(–)	27.6 ± 5.1	46.8 ± 6.7	107.9 ± 25.9	**1.3 ± 0.3**[b]
(+)	26.7 ± 4.2	42.4 ± 5.1	119.1 ± 36.2	**1.6 ± 0.6**
p53				
(–)	26.7 ± 4.5	44.4 ± 6.7	109.6 ± 26.4	1.4 ± 0.4
(+)	29.1 ± 5.4	48.6 ± 5.3	113.4 ± 34.6	1.4 ± 0.3
DNA ploidy				
Aneuploid	27.8 ± 5.0	45.6 ± 6.1	112.7 ± 30.1	1.4 ± 0.4
Diploid	25.8 ± 4.5	46.0 ± 8.6	102.9 ± 22.2	1.2 ± 0.3
DNA index				
<median	27.1 ± 4.9	45.4 ± 6.8	113.0 ± 29.2	1.4 ± 0.5
>median	27.7 ± 4.9	46.0 ± 6.4	108.4 ± 28.8	1.3 ± 0.3
%S phase				
<median	26.8 ± 3.7	46.0 ± 7.3	110.2 ± 31.5	1.3 ± 0.5
>median	28.0 ± 5.9	45.4 ± 5.8	111.2 ± 26.5	1.4 ± 0.3

[a] $p = 0.001$. [b] $p = 0.04$

large amounts of hydrogen peroxide. However, in our study the increase in activity of GPx, an enzyme that may also destroy the hydrogen peroxide, was also observed.

In conclusion, the breast adenocarcinoma cells are more exposed to oxidative stress with higher free radical production and decreased CAT activity. GPx and SOD levels are increased mainly due to the response

Table 4
Interrelationships with Known Prognostic Factors Except Oxidant/Antioxidant Status[a]

	Axillary node	Stage	Grade	ER	PR	CEA	CA 15.3	cerb B2	P53	DNA ploidy	DNA index	%S phase
Tumor size							**0.001**		**0.044**			
Axillary node		**<0.001**						**0.046**	0.052			
Stage			**0.046**				0.061		**0.005**			
Grade				**<0.001**	**0.022**		0.067		**0.02**			**0.025**
ER					**0.006**		**0.002**	0.058	**0.005**	**0.020**		**0.048**
PR								0.058	**0.02**			
CEA							**0.032**					
CA 15.3									0.06	**0.012**		0.074
cerbB2										0.071		
p53												
DNA ploidy											<0.00	**0.001**
DNA index												**0.007**

[a]Boldface entries are significant (p<0.005) parameters.

of increased free radical production. Increased levels of GPx and SOD may be failed to detoxify high levels of H_2O_2 into H_2O. Therefore, exogenous CAT enzyme administration may be a reasonable tool for treating breast cancer patients. Further understanding of tumor biology from the standpoint of reactive oxygen species may be helpful for establishing a new strategy for cancer therapy. For example, tamoxifen, a potent suppressor of lipid peroxide formation in both human and animal systems, and tamoxifen therapy may exert significant positive effects on lipid peroxidation and protective systems *(9).*

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