RESEARCH COMMUNICATION

Superoxide Dismutase Isoenzyme Activities in Plasma and Tissues of Iraqi Patients with Breast Cancer

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Abstract

Breast cancer is the first of the most common ten cancers in Iraq. Its etiology is multifactorial, oxidative stress and lipid peroxidation being suggested to play important roles in carcinogenesis. The purpose of this study was to investigate the oxidant-antioxidant status in breast cancer patients, by measuring SOD isoenzyme activities (total SOD, CuZn-SOD, Mn-SOD and EC-SOD) in plasma and breast tumors, and by estimating thiobarbituric reactive substances (TBRS) in tissue homogenates. General increase in total SOD activity was observed in plasma and tissue samples of breast tumors, greater in the malignant when compared to benign group ($p<0.05$). Mn-SOD showed a significant decrease in tissue malignant samples ($p<0.05$), and insignificant decrease in plasma malignant samples compared with control and benign samples. Plasma EC-SOD activity in both patient benign and malignant breast tumors demonstrated 3.5% and 22.8% increase, respectively. However, there was a decrease in tissue EC-SOD activity in malignant breast tumors when compared with benign. A similar tendency was noted for TBRS. We suggest that elevated total SOD might reflect a response to oxidative stress, and then may predict a state of excess reactive oxygen species in the carcinogenesis process. If there is proteolytic removal of the heparin binding domain, EC-SOD will lose its affinity for the extracellular matrix and diffuse out of the tissue. This will result in a decreased EC-SOD activity, thus leading to an increase in the steady-state concentration of O^{2-} in this domain, and increase in EC-SOD activity in the extracellular fluid. This might explain the results recorded here concerning the decrease in tissue EC-SOD activity and increase in plasma of breast cancer patients.

Keywords: Breast cancer - lipid peroxidation - total SOD - CuZn-SOD - Mn-SOD - EC-SOD

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Introduction

Breast cancer is the first of the commonest ten cancers in Iraq, according to the latest of Iraq Cancer Registry (Alwan, 2010). The development of breast cancer is a multifactorial process whose mechanism is still largely unknown. It has been hypothesized that reactive oxygen species (ROS) and oxidative stress may play a critical role in breast cancer etiology and progression and be associated with the proliferation potencies of breast cancer cells (Brown & Bicknell, 2001; Mobley & Brueggemeier, 2004; Xing et al., 2008).

Reactive oxygen is related to both the arrest of the growth and the start of cell differentiation. Low concentrations of reactive oxygen intermediates may be beneficial, or even indispensable in processes such as intracellular messaging and defense against microorganisms, but higher amount of active oxygen may be harmful to cells and organisms (Dean et al., 1997; Buettner, 2011). If oxidative stress persists, oxidative damage to critical biomolecules (i.e. ,DNA ,RNA ,proteins and lipids) accumulates and eventually disrupts normal metabolism, resulting in a wide variety of biological effects ranging from alterations in signal transduction and gene expression (Demple, 1991; Guyton et al.,1996; Monteiro & Stem, 1996; Sun & Oberley, 1996) to mitogenesis (Budroe et al., 1992), transformation (Ames, 1983; Cerutti, 1985; Cerutti et al., 1989), mutagenesis (Shay & Werbin, 1989; Moraes et al., 1990) and cell death (Spitz et al., 1990; Spitz et al., 1992; Sullivan et al.,1992).

A wide variety of non enzymatic and enzymatic antioxidant defense exists. One of the most important enzymatic antioxidants are superoxide dismutase (SOD, EC 1.15.1.1). SOD(s) are a family of enzymes important in biology and pathology of reactive oxygen species since they catalyze the conversion of superoxide to hydrogen peroxide and molecular oxygen (Halliwell & Gutteridge, 1999). There are three known isoform of SOD(s) in mammals: the intracellular CuZn-containing SOD which is located primarily in the cytoplasm and the nucleus of cells (Saez et al., 1982), Mn- superoxide dismutase is a nuclear-encoded antioxidant enzyme that localizes to the mitochondria (Halliwell & Gutteridge, 1985; Holley et al., 2012). The third isoenzyme of SOD is the extracellular SOD (EC-SOD), which also contains Cu & Zn in its active site (Hassan & Fridovich, 1981). EC-SOD is localized

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predominantly in the extracellular matrix of the tissues as well as in the extracellular. EC-SOD in extracellular fluid is heterogeneous in its affinity for heparin, and three subtypes exist: type C with high affinity; type B with intermediate affinity; and type A without affinity for heparin (Karlsson & Marklund, 1987;: 1988).

Biochemical free radical reactions have been inferred by identifying the products of lipid peroxidation, in particular, malondialdehyde (MDA) (Gutteridge, 1995), a by-products that has been speculated to have a critical role in the early phases of tumor growth if they are excessively generated (Akbulut et al., 2003). The ability of lipid peroxidation by-products in generating mutagenesis and DNA adducts formation suggest their possible role in carcinogenesis (Srivastava et al., 2009).

It was reported that the antioxidant defense system altered in various human tumors, and a reversed relationship was found between antioxidant enzyme activities and lipid peroxidation in patient with some of these tumors (Alagol et al., 1999; Rizwan et al., 2008).

Yamanaka and Deamer (1974) were the first to show that the SOD activity in transformed cells is abnormal. Changes in serum and tissue SOD activity, and other scavengers of free radicals, have been studied in various pathological conditions (Nakada et al., 1987; Nakamura et al., 1988). However, the obtained data remain unclear because SOD activity varies greatly, according to the different conditions in each study (Iwase et al., 1997). Since the causes and biochemical profile of breast cancer are not yet fully known and there is no a clear mechanism by which oxygen radicals may affect the outcome of breast cancer, the purpose of this study was to investigate the oxidant-antioxidant statue in breast cancer patients, by measuring SOD isoenzymes activities (total SOD, CuZn-SOD, Mn-SOD and EC-SOD) in plasma and tissues of breast tumors, and by estimating thiobarbituric reactive substance (TBRS) in tissues homogenate of breast tumor patients.

Materials and Methods

Chemicals

Common laboratories chemicals and reagents (annular grade) were used without further purification.

Patients

Breast tissue samples were obtained from women with benign and malignant breast tumors .Blood samples were collected from healthy women to be used as control group and from hospitalized females with benign and malignant breast diseases .All patients were admitted for diagnostic or surgical operations to either Medical City, Al-Yarmok hospital, Al-Jadria private hospital and Al-Karada private hospital .The diagnosis is confirmed by cytological and histopathological examination , which were carried in the laboratories of the above mentioned hospitals . Any women with a significant coexisting disease are excluded.

Preparation of samples

Plasma, five milliliters of venous blood was taken for each sample and collected in heparinized tube, then centrifuged at 3000 x g for 10 min.

Tissue, all the homogenization steps were performed on ice, the tumor tissue was taken out of saline and prepared for SOD activities measurement using Marklund's method (Marklund, 1984).

Protein determination

The protein content of samples was determined by modified Lowry's method (Lowry et al., 1951), using bovine serum albumin as a standard protein.

Determination of the lipid peroxidation

The level of the lipid peroxidation was estimated by the measuring the amount of lipid peroxidation in tissue samples. The extent of lipid peroxidation was assessed by measuring thiobarbtiuric acid reactive substance (TBARS), using OKKawa (OKKawa et al., 1979) method with minor modifications (Hirayama et al., 2000).

Determination of the SOD activities

Total SOD, CuZn-SOD and Mn-SOD activities were measured using the modified nitrite method of Oyanagui (1984), employing xanthine/xanthine oxidase as an enzyme generator of($.O-_2$). One unit of the enzyme activity was expressed as the amount of the sample that cause 50% decrease in the enzymatic nitrite formation.

Unlike intracellular SOD (CuZn-SOD and Mn-SOD), EC-SOD is a glycoprotein, and binds to Con A Sepharose (Conrade, 1998). EC-SOD was separated from intracellular superoxide dismutase by passing the sample over a concanavalin A Sepharose column as described by Marklund et al. (1982).

Results

Superoxide dismutase activity was measured by nitrate method, that based on xanthine/xanthine oxidase as $.O_2$-generator system and hydroxylamine as detector system as described by Oyanagui (1984).

Figure 1 (a and b), show that when percent inhibition is plotted against amount of protein in tissue extract (μg/ml), maximum inhibition was 82% & 70% for malignant and benign tissue samples respectively. While, the maximum inhibition was correspond to 92% for plasma of the control group as shown in Figure 3.

General increase in total SOD activity was observed in plasma & tissue samples of malignant breast tumors. A significant increase in tissue total SOD activity was recorded in malignant group when compared to benign group ($p<0.05$), Table 1.

Mn-SOD shows a significant decrease in tissue malignant samples, and insignificant decrease in plasma malignant samples compared with that of control & benign samples, as presented in Table 1. However, there was a significant increase in tissue CuZn-SOD in cancerous tissue. In contrast, a significant decrease was observed in plasma CuZn-SOD (as illustrated in Figures 2 & 3).

Plasma EC-SOD activity in both patient with benign & malignant breast tumors show 3.5% & 22.8% increase respectively. However, a decrease in tissue EC-SOD activity was observed in malignant breast tumors when compared with benign, as presented in Table 1.

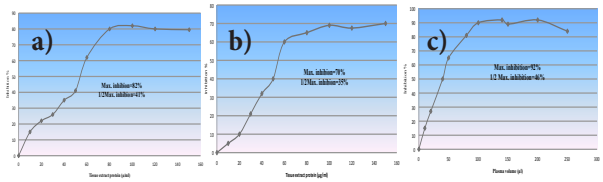

Figure 1. Inhibition Curve of Nitrite Formation as a Function. a) Tissue Extract Protein Concentration of Malignant Samples, b) Tissue Extract Protein Concentration of Benign Samples, c) Plasma Volume

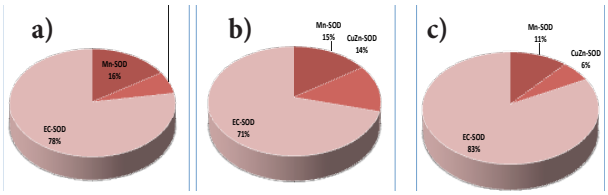

Figure 2. The Percentage of SOD Isoenzymes Activities in Plasma of a)Control, b)Benign and c)Malignant Groups

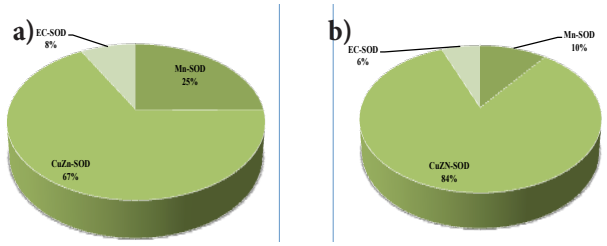

Figure 3. The Percentage of SOD Isoenzymes Activities in Tissue of Human Breast Tumors a) Benign & b) Malignant

Table 1. Superoxide Dismutase Isoenzymes Activities in Plasma and Tissues Samples of Women with Breast Cancer.

Patients	No. of samples	SOD† Total	Mn-SOD Mitochondrial	CuZn-SOD Cytosolic	EC-SOD Extracellular
Control					
plasma	34	21.4±2.08	3.40±0.35	1.40±0.12	16.6
Benign					
plasma	15	[a]23.3±1.28	[a]3.75±0.27	[a]3.32±0.71	[a]17.2
tissue	9	36.4±6.19	9.90±1.87	26.6±4.85	3.2
Malignant					
plasma	38	[a]24.9±1.64	[b]2.83±0.53	[b]* 1.47±0.25	[a]20.4
tissue	13	[a]*42±9.6	[b]*4.6±3.7	[a]*37±6.7	[b]2.7

* '†Activities express as U/mL in plasma samples and as U/mg of protein in tissue samples, [a]Increase or [b]Decrease in SOD activity compared with control & (benign, malignant) in plasma, benign & malignant in tissue.*Significant when p<0.05.

The products of lipid peroxidation reactions were analyzed in benign & cancer human breast tissue. Observed pink color is due to the formation of an adduct between thiobarbituric acid and malondialdehyde under acidic conditions, TBRS levels showed insignificant decrease in cancer group compared with benign group (p>0.05).

Discussion

Due to its key position in the antioxidative network, SOD is of marked pathophysiological importance (Nakada et al., 1987). It was studied primarily as a defense mechanism against the consequences of free radical production (Nakamura et al., 1988). A recent study demonstrate a novel therapeutic strategy to inhibit cell death and apoptosis caused by ROS, via increasing antioxidant potential, which can be overcome by treatment with SOD mimic (Arid et al., 2012).

During the past few years, SOD activity in tumor cells has received increasing attention. Numerous studies have reported a wide variation in the SOD activity in different cancerous tissue: - skin (Perchellet & Perchllet, 1989), colon (Ozturk et al., 1998), bladder (Durak et al., 1994) and laryngeal tissue (Durak et al., 1993). The interpretation of the result is difficult, as they seem to vary from one study to another. The inconsistency in the result is likely due to the heterogeneity of the tumor tissue (Punnonen et al., 1994), and the method which was used to measure this enzyme activity.

Generally elevated total SOD might reflect a response to oxidative stress, and then may predict a state of excess reactive oxygen species in the carcinogenesis process. In our study, total SOD activity in breast cancer patient's plasma & tissues was found to be higher than that of the control group, although the net differences were statistically insignificant. Our results agree with Japanese team's results which showed a slightly positive association between serum SOD level and cancer mortality (Pham et al., 2009).

Lin et al. reported that serum levels of CuZn-SOD were significantly elevated in gastric cancer patient, and they suggested that the high CuZn-SOD levels may be associated with an increased risk of gastric cancer (Lin et al., 2002). Such increase in CuZn-SOD activity was reported by others in many cancerous cells and tissues (Wang et al., 1996; Gönenç et al., 2006).

Throughout the present study's results, Mn-SOD activity showed a significant decrease in breast malignant tissues (p<0.05), Table 1 and Figure 2. And insignificant decrease in the plasma samples of these patients. It is known that within mitochondria manganese superoxide dismutase (Mn-SOD) provides a major defence against oxidative damage by reactive oxygen species (Mitrunen et al., 2001; Robbins & Zhao, 2011). MnSOD appears to be a central player in the redox biology of cells and tissues (Buettner, 2011). Mitochondria are often said to be the most important intracellular source of ROS (Wiseman & Halliwell, 1996) where that mitochondrial DNA is damaged by them. Since oxygen free-radicals are produced by mitochondrial membrane bound electron transport chains and mitochondria have been shown to be structurally abnormal in almost all malignant tumor cells, it can be concluded that these biologically damaging intermediates are responsible in part for the abnormalities that observed in cancer cells (Guo et al., 2003). Mn-SOD was reported to be reduced in tumors (Dionisi et al., 1975; Oberley et al., 1978; Margaret et al., 2011). It has been suggested that not only Mn-SOD reduced in all tumors, but the degree of reduction in this enzyme activity seems to be correlated with the level of differentiation, speed of growth, and the degree of malignancy of these tumors (Guo et al., 2003). Many relatively recent studies have

focused on the tumor-suppressive effects of Mn-SOD (Ridnour et al., 2004; Oberley, 2005; Zhang et al., 2006). Similar results among these studies have indicated that increased levels of Mn-SOD suppressed the malignant phenotype as evidenced by slower cell growth rate and lower colony formation. All these findings may contribute in explicate the presence of the low level of Mn-SOD in cancerous samples.

In extracellular space, there are many potential sources of O_2.-. All leukocytes, except lymphocytes, produce O_2.- on activation (Halliwell, 1982). EC-SOD is the main enzymatic scavenger of O_2.- in the extracellular matrix of tissue. Thus, alteration in EC-SOD activity will have important consequences on the steady-state concentration of O_2.- in the extracellular spaces of tissue (Enghild et al., 1999). EC-SOD has a high affinity for heparin sulfate proteoglycan, which appears to be the important physiological ligand of EC-SOD (Sandstrom et al., 1993). All three types of EC-SOD exist in plasma, virtually all of the EC-SOD in the extracellular matrix of tissue is type C (strongly bound to heparin). If there is proteolytic removal of the heparin binding domain, EC-SOD will lose its affinity for the extracellular matrix and diffuse out of the tissue. This will result in a decreased EC-SOD activity in tissue, thus leading to an increase in the steady-state concentration of O_2.- in this domain, and increase in EC-SOD activity in extarcellular fluids (Enghild et al., 1999). All this can explain the result recorded here concerning the decrease in tissue EC-SOD activity and increase in plasma of breast cancer patients. Our results agree with previous study carried out in our laboratory, which reported an increase in serum EC-SOD activity in patient with brain tumors (Hasan & Numan, 2009).

Our results showed that, insignificant decrease in level of TBRS in cancer tissues, when compared to benign tissues. Wang et al. (1996) study reported that, tumor tissues displayed significantly lower levels of MDA adducts, than their corresponding normal adjacent tissues. A study by Gönenç et.al (2006) reported that, serum & tissue MAD levels were found to be decreased in breast cancer patient compared to the benign group. These findings support the hypothesis that lipid peroxidation in serum and tissue of benign breast disease patients is greater than in corresponding breast cancer patients.

Previous studies have suggested that MDA is a lipid peroxidation marker, and low plasma levels of MDA are associated with advanced stages of breast cancer (Wang et.al, 1996; Saintot et al., 1996). These findings suggest that a change in oxidant-antiaxidant status might accompany the proliferative capacities of tumor cells (Saintot et al., 1996). Zaridze et. al. were reported that, the risk of breast cancer is decreased in association with an increased level of polyunsaturated fatty acids in the erythrocyte membranes (Zaridze et al., 1990). They also suggested that increased antioxidant capacities and decreased peroxidable substrates gave transformed cells a selective growth advantage. Also, in several experimental models, anti-oxidants have been found at increased levels in tumor tissues where there existed a lower lipid peroxidation (Hietanen et al, 1989; Gutteridge, 1995). It has been proposed that initiated cancer cells develop protective mechanism(s) which facilitate their proliferation for an effective promotion. However, studies with more patients and oxidative stress-related parameters are required, to explore the association between free radicals and antioxidants, in relation to benign and malignant breast disease.

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