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ORIGINAL ARTICLE

Oxidative and antioxidative status after anthracycline-based chemotherapy in breast cancer patients

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Summary

Purpose: *The present study was undertaken to evaluate the effects of adjuvant anthracycline-based chemotherapy on thiobarbituric acid reactive substances (TBARS) and superoxide dismutase (SOD) levels in patients with breast cancer who had undergone surgery.*

Methods: *Body mass index (BMI), serum lipids (total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides), serum TBARS and SOD values were assessed in 30 patients with stage III breast cancer receiving adjuvant anthracycline-based chemotherapy.*

Results: *Anthracycline-based chemotherapy had no effect on BMI, blood pressure and lipid profile. A significant elevation was noted in TBARS (5.5±0.6 vs 5.9±0.9 μmol/L; p=0.038) and a significant reduction to baseline values in SOD levels (226.5±61.0 vs 203.1±48.3 U/mL; p=0.037) in patients following 6 cycles of adjuvant chemotherapy.*

Conclusion: *The TBARS levels increased, whereas the SOD levels descreased after anthracycline-based chemotherapy. We suggest that oxidative stress is not always detrimental, as it can be beneficial in cancer treatment.*

Key words: *anthracycline, breast cancer, SOD, TBARS*

Introduction

Oxidative stress has been implicated as mediator of apoptosis [1]. Increased oxidative stress has been detected emanating from apoptotic cells, and antioxidants can block apoptosis in a variety of systems [2,3].

Thus, accumulating evidence strongly suggests that oxidative stress might play a role in the antineoplastic action on the anticancer drugs. Glutathione S transferase (GST) and superoxide dismutase (SOD) are major antioxidant enzymes and are involved in detoxification processes. Anthracyclines are believed to be related to generation of oxidative stress [4,5] and their oxidative effects are modulated by the antioxidative system. Although these agents can induce oxidative stess, little is known about the effects of anthracyclines on the antioxidant system.

Since biochemical data concerning the antioxidative status in anthracycline-induced oxidative stress are not well documented, we investigated and assessed serum TBARS and SOD values in 30 patients with stage III breast cancer receiving adjuvant anthracycline-based chemotherapy.

Methods

Inclusion/exclusion criteria

Thirty women with breast cancer who applied to our clinic between January 2008 - January 2011 were included in this study. Written informed consent was obtained from each patient and the study was approved by the Ethics Commitee of our Institution (Izmir Bozyaka Research and Training Hospital). Patients were included if they had: 1) histologically confirmed operable stage III breast adenocarcinoma; 2) had primary surgical resection within one month from the beginning of adjuvant chemotherapy; 3) were older than 18 years; and 4) were scheduled to receive postoperative

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adjuvant chemotherapy.

Exclusion criteria included: 1) patients with breast cancer who were already receiving neoadjuvant chemotherapy and were talking antihyperlipidemic and antihypertensive drugs; and 2) patients with a history of diabetes mellitus, coronary artery disease or hypo- or hyperthyroidism and smoking.

Study design

The adjuvant chemotherapy regimen used in this study was docetaxel (75 mg/m^2), epirubicin (100 mg/m^2) and cyclophosphamide (500 mg/m^2) (TEC), all given intravenously (i.v.) on day 1. TEC was administered every 3 weeks for 6 cycles. Methylprednisolone 16 mg/day i.v. for 3 days was given during treatment to prevent docetaxel reactions. The patients were also administered 5 mg/kg filgrastim for prophylaxis of neutropenia on days 2-7 after the end of each treatment cycle.

Patients with breast cancer were evaluated on two occasions: at baseline (on day -1 of the first chemotherapy cycle), and 1 week after the completion of the 6th cycle.

Other measurements

BMI was calculated according to the Quetelet's index as the ratio of weight (kg) to height (m) squared (kg/m^2).

Laboratory evaluation

After overnight fasting, venous blood samples were taken on 8-9 a.m. for the lab tests. Serum lipids, total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were assayed on the same day according to standard procedures. Serum samples for the measurement of TBARS and SOD were preserved at -80°C until analysis.

TBARS serum concentration was determined by using the method described by Ohkawa [6] based on TBA reactivity. Briefly, 0.2 mL of sample was combined with 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid, 1.5 mL of 0.8% TBA solution, and 0.6 mL of distilled water, and incubated at 95 °C for 1 h. After n-butanol/pyridine (15:1, v/v) extraction, absorbance of the pink chromophore was read at 532 nm. Tetramethoxypropane (5 nM) was used as the standard solution, and TBARS levels were expressed as µmol/L [6].

Superoxide dismutase (SOD)

Serum SOD activity was measured using the SOD Assay Kit (Cayman Chemical, Ann Arbor, MI, USA), according to the manufacturer's instructions. The assay uses a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine, which yields a chromophore with a maximal absorbance at 525 nm. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. SOD activity was expressed as U/mL.

Table 1. Clinical and laboratory features at baseline and after 6 cycles of adjuvant chemotherapy in breast cancer patients (N=30). Values are means±standard deviation

Features	*Pre-treatment*	*Post-treatment*	*p-value*
BMI (kg/m^2)	32.4±4.1	32.8±3.0	0.4
FPG (mg/dL)	90.3±9.0	87.1±7.0	0.1
Systolic blood pressure (mmHg)	117.6±16.1	117.1±12	0.2
Diastolic blood pressure (mmHg)	78.7±6.0	77.6±6.9	0.7
Tc (mg/dL)	199.5±30.4	190.2±12.7	0.1
Tg (mg/dL)	188.7±88.6	187.1±25.8	0.9
HDL-C (mg/dL)	42.6±10.0	40.6±4.6	0.2
LDL-C (mg/dL)	119.5±23.5	112.3±11.5	0.1
SOD (U/L)	226.5±61.0	203.1±48.3	0.037
TBARS (mmol/L)	5.5±0.6	5.9±0.9	0.038

BMI: body mass index, FPG: fasting plasma glucose, Tc: total cholesterol, Tg: triglyceride, SOD: superoxide dismutase, TBARS: thiobarbituric acid reactive substances

Statistics

Results were expressed as mean±standard deviation. Pre- and post-treatment values of the parameters were compared with a paired t-test sample. The relationships between different variables were analysed with Pearson's correlation test. Statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS), version 11.0 (SPSS, Chicago, IL). A p-value of <0.05 was considered to be statistically significant.

Results

The patient mean age was 50.7±10.7 years (range 32-73). Thirteen patients were postmenopausal. No patient had metastatic, node positive breast cancer.

Patient pre- and postchemotherapy demographic features and labaratory findings are depicted in Table 1. No statistically significant differences were noted in patients' BMI and blood lipid measurements taken at the baseline and after 6 cycles of chemotherapy (p>0.05). However, significant elevation was noticed in TBARS (5.5±0.6 vs 5.9±0.9 µmol/L;p=0.038) and significant reduction was seen in SOD levels (226.5±61.0 vs 203.1±48.3 U/mL;p=0.037) in patients following 6 cycles of adjuvant chemotherapy compared to baseline values. Pearson's correlation analysis revealed that pretreatment serum SOD levels were positively

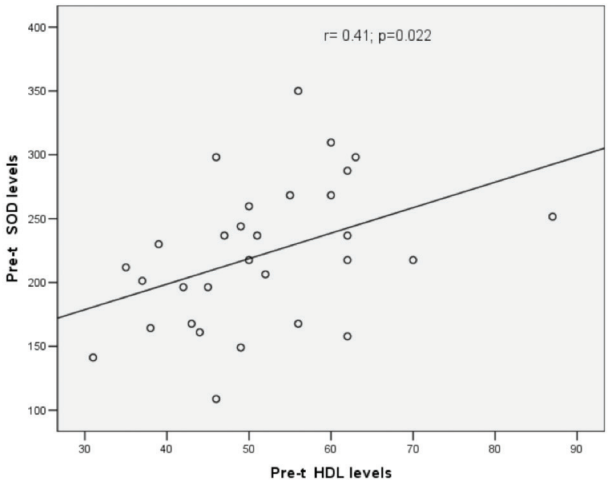

Figure 1. Pretreatment serum SOD levels were positively correlated with pre-HDL cholesterol levels.

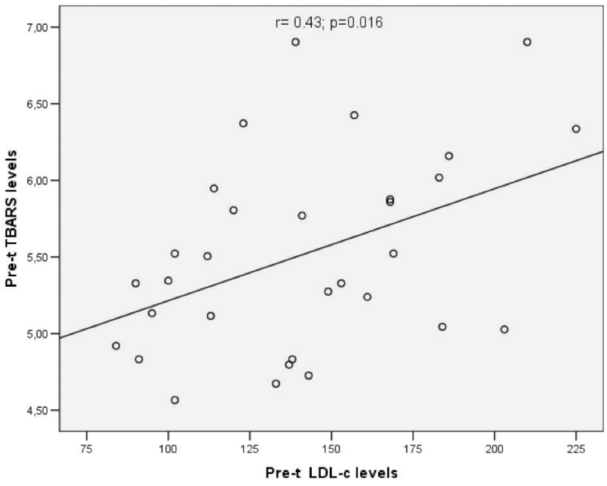

Figure 2. Pretreatment serum TBARS levels were positively correlated with pre-treatment LDL cholesterol levels.

correlated with HDL cholesterol (r= 0.41; p=0.022; Figure 1) and negatively with triglycerides levels (r= -0.40; p=0.026). Pearson's correlation analysis also revealed that pretreatment serum TBARS levels were positively correlated with LDL cholesterol levels (r= 0.43; p=0.016; Figure 2) . Increased TBARS levels and decreased SOD levels did not correlate significantly with lipid profile or BMI after 6 cycles of chemotherapy.

Discussion

In this study, we demonstrated that adjuvant chemotherapy significantly decreased the baseline SOD activity and increased TBARS levels in patients with breast cancer.

There is an equilibrium between a free radical/reactive oxygen species (ROS) formation and endogenous antioxidant defense mechanisms, but if this balance is disturbed, it can produce oxidative stress. TBARS are commonly used to measure systemic oxidative stress [7]. In the pretreatment period, LDL level was positively correlated with TBARS levels. Most patients have not only increased levels of LDL, cholesterol and triglycerides [8] but also icreased oxidative stres [3,4] leading to LDL conversion into oxidized low-density lipoprotein (oxLDL). Lipid peroxidation has been implicated in neoplastic transformation [8]. In our study, pretreatment serum TBARS levels were positively correlated with LDL cholesterol levels. Excessive accumulation of lipids derived from oxLDL induces oxidative stress in the body and causes a compensatory increase in the synthesis of the endogenous antioxidant SOD acticity. Superoxide is generated within the mitochondria and is sequentially reduced to hydrogen peroxide and hydroxyl radicals [9]. Human tumor cell lines in vitro produce ROS at a far greater rate than do nontransformed cell lines [10] and markers of constitutive oxidative stress have been detected in samples from *in vivo* breast carcinomas [11].

In the present study, fasting glucose and blood pressure did not differ significantly between the pre and posttreatment period. Therefore, these factors may not affect LDL particle diameter in breast cancer subjects receiving anthracycline-based chemotherapy. Interestingly, after 6 cycles of chemotherapy reduction of SOD and elevated TBARS levels were noticed, without changes in lipid profile, glucose profile and blood pressure. However, changes in SOD and TBARS were not significantly correlated with the observed decrease in other parametres which influence the oxidative system.

Some chemotherapies are thought to work in part by increasing the oxidative stress on cancer cells. Oxidative stress, however, is not always detrimental. Selective oxidative stress sometimes is desirable and can be utilized therapeutically also. Severe oxidative stress leads to apoptosis. Anthracyclines have been described to be able to induce significant oxidative and nitrosative stress to different kinds of cells [12].

Under normal conditions, antioxidant mechanisms, including small-molecular-weight antioxidants and antioxidant enzyme systems, scavenge ROS and protect the organism from the damaging effects of oxidative stress. However, under conditions of excessive oxidative stress, these occur with the administration of certain drugs, and cellular antioxidant mechanisms may be unable to prevent the adverse impact of ROS on critical cellular processes [13]. High oxidative stress level

kills cells either by necrosis or by apoptosis [14,15].

This is evident by the elevation of lipid peroxidation products, the reduction of total radical-trapping capacity of blood plasma, the reduction in plasma levels of antioxidants such as vitamin E, vitamin C, and β-carotene, and the marked reduction of tissue glutathione levels that occurs during chemotherapy. This happens because one of the pathways of drug-induced apoptosis involves the release of cytochrome-C from the mitochondria [16]. When this occurs, electrons are diverted from the electron transport system to oxygen by NADH dehydrogenase and reduced coenzyme Q10, resulting in the formation of superoxide radicals. Antioxidants may decrease the anticancer activity of cancer chemotherapy by reducing the oxidative stress during chemotherapy-induced oxidative stress [17].

This observation suggests that anthracycline-based chemotherapy may lower the risk of disease progression through mechanisms other than biochemical parametres like lipid profile and glucose levels. Prolonged accumulation of high levels of free radicals in cells may cause irreversible cellular injury and ultimately result in cell death. Since SOD is the key enzyme in the first metabolic step of superoxide elimination, deficiency in SOD or inhibition of the enzyme activity may cause severe accumulation of superoxide radicals in cells and lead to cell death. Compared to normal proliferating cells, cancer cells are active in energy metabolism and have developed the following metabolic characteristics: enhanced glucose uptake, aerobic glycolysis, glutaminolysis, nucleic acid synthesis and lipid synthesis, reduced mitochondrial respiration, pyruvate oxidation and acetyl-CoA oxidation [18]. This may render maligant cells more depended on SOD to eliminate the toxic superoxide radicals. It is a plausible hypothesis that inhibition of SOD may preferentially kill malignant cells through a free radical-mediated mechanism [19].

We suggest that oxidative stress is not always detrimental, and could be beneficial in cancer treatment. Obviously, there is a need of conducting larger clinical trials to prove the therapeutic efficacy and selectivity of these potent anticancer agents (such anthracyclines and taxanes) acting through antioxidative stress mechanisms.

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