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Circulating oxidative stress parameters in pre- and post-menopausal healthy women and in women suffering from breast cancer treated or not with neoadjuvant chemotherapy

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ABSTRACT

We evaluate here the redox status in pre- and post-menopausal healthy women and in women with breast cancer in order to understand the consequences of the hormonal alterations of menopause for the oxidative stress status, its modifications with breast cancer and the influence of neoadjuvant chemotherapy (NC). To that, serum oxidative stress parameters (total antioxidant capacity, lipid peroxidation and protein oxidation), non-enzyme antioxidant defenses (total glutathione, uric acid and bilirubin) and enzyme antioxidant defenses (superoxide dismutase, catalase and glutathione peroxidase activities) were measured in healthy women and in women with breast cancer divided according to their menopausal status and that received or not NC. Circulating estradiol, progesterone, FSH and LH were also analyzed. We found that menopause itself modifies the redox status of healthy women, being most of these differences also reflected in women with breast cancer. However, several changes occur as a consequence of the disease. Furthermore, NC increases oxidative damage, decreases antioxidant defenses and eliminates the differences found in menopause. We conclude that the normal redox balance is disrupted by breast cancer but is also affected by the hormonal status promoted by menopause. In fact, NC nullifies the differences found between pre- and postmenopausal women in several antioxidant defense systems.

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1. Introduction

Reactive species are abundantly formed during both physiological and pathological processes, primarily through oxygen reduction in the mitochondrial respiratory system. The damage that can cause to the cell does depend not only on their intracellular concentration but also on the equilibrium between them and the endogenous antioxidant defenses. Thus, when the pro-oxidant/anti-oxidant equilibrium is lost, oxidative stress is generated, altering and damaging many intracellular molecules, including lipids, proteins and nucleic acids [1,2]. In this sense, the cell membrane is rich in polyunsaturated lipids that are susceptible to oxidation by reactive species which increase the permeability of the cell membrane and could lead to cell death [3]. Proteins are the molecules most affected by a cellular environment with a high concentration of reactive species. Proteins suffer from the generation and accumulation of carbonyl groups (i.e., aldehydes and ketones) and thiol groups (–SH) that may be converted into sulfur reactive radicals [4]. Due to this oxidation-induced modification, there is an alteration in the protein structure and, consequently, changes or loss of protein function. Finally, reactive species also cause nicks in the DNA and malfunctions in the DNA repair mechanism. On the contrary,

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endogenous antioxidants constitute the defense mechanisms that scavenge reactive species in cells, which include non-enzyme and enzyme antioxidant defense systems such as glutathione (GSH), alpha-lipoic acid, coenzyme Q, ferritin, uric acid, bilirubin, metallothionein, L-carnitine, melatonin, and enzymatic superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) respectively [2]. Therefore, antioxidants coexist in a delicate balance with oxidative inputs.

Several studies support findings that reactive species are involved in the etiology and progression of breast cancer because certain markers of oxidative stress, including lipid peroxidation products, such as malondialdehyde (MDA) [5] and 8-isoprostanes [6], protein oxidation products such as carbonyls and diene-conjugates [7,8] and DNA adducts [9] are frequently identified in breast cancer patients. Furthermore, it has been very recently described that circulating redox status is closely correlated to estrogen levels, which may control, almost in part, antioxidant gene expression [10]. Oxidative stress has been also shown to participate in the structural modification of the estrogen and progesterone receptors, altering the clinical evolution of patients with endocrine-responsive breast cancer [11]. In this sense, natural menopause or chemotherapy induced-menopause supposes the reduction of ovarian hormone activities. Therefore, in the present report we evaluate the redox status in healthy pre- and post-menopausal control women and women with breast cancer in order to understand the consequences of the hormonal alterations of menopause for the oxidative stress status, its modifications with breast cancer and the influence of neoadjuvant chemotherapy (NC).

2. Subjects and methods

2.1. Subjects and study design

A total of 198 women were recruited at the Unit of Breast Pathology at the University Hospital of Jaén, and 78 healthy women volunteers composed the control group. This study was approved by the Ethical Committee of the University Hospital of Jaén and all patients signed a term of free, informed consent. Patient characterization included age at diagnosis, tumor size, tumor histology, pathologic T classification, Scarff–Bloom–Richardson grade, hormonal and HER-2/neu status, molecular subtype and circulating LH, FSH, estradiol and progesterone hormone levels. Patients were divided in premenopausal or postmenopausal who received or not NC (premenopausal women without chemotherapy n = 39; with chemotherapy n = 63; postmenopausal women without chemotherapy n = 44; with chemotherapy n = 52). The control group consisted of healthy women, aged 28 to 69 years old (premenopausal women with regular menstrual periods n = 38; postmenopausal women with spontaneous menopause for at least one year n = 40), with no previous history of any type of cancer, chemotherapy, hormonal or antioxidant therapy, or chronic diseases. Women were excluded if they were current smokers, regular alcohol consumers, antioxidant supplement users, pregnant or lactating, presented hepatic, cardiac or renal dysfunction, obesity, use of drugs, hypertension, diabetes, and other eventual chronic conditions.

Patients treated with NC received an anthracycline/taxane-based regimen including 4 courses of EC (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2, every 21 days), followed by 8 courses of 100 mg/m^2 paclitaxel once a week or 4 courses of 75 mg/m^2 docetaxel every 21 days. Patients with a HER2/neu-overexpressing tumor also received trastuzumab (14 courses at 6 mg/kg every 21 days). Women with triple-negative breast cancer received 6 cycles of 75 mg/m^2 docetaxel plus carboplatin (AUC 6).

2.2. Sample acquisition

Blood samples were obtained after an overnight fast by venous arm puncture in tubes without anticoagulants. Blood specimens were centrifuged at 2500 *g*, for 5 min, at 4 °C. Serum samples were collected, kept on −80 °C, and after defrosting centrifuged at 11,000 *g*, for 1 min, at 4 °C. Clarified serum preparations were used for assays.

2.3. Oxidative stress parameter assays

2.3.1. Total antioxidant capacity (TAC) assay

TAC was measured using copper(II)-neocuproine as chromogenic oxidant, as previously described by Apak et al. [12] as the CUPRAC method. Results were compared with a standard curve obtained with trolox and are expressed in μmol trolox equivalents/mg of protein.

2.4. Lipid peroxidation and protein oxidation assays

Lipid peroxidation was measured by analyzing the amount of thiobarbituric acid reactive substances (TBARS) as previously described [13]. Results were expressed as mg/mg of protein against a malondialdehyde (MDA) standard curve. Protein oxidation was measured by analyzing the carbonyl group content of proteins also as described [13]. Results were expressed as nmol per mg of protein using an extinction coefficient of $2.1 \times 10^4\ M^{-1}\ cm^{-1}$.

2.5. Determination of non-enzyme antioxidants

GSH levels were measured as previously described [13]. Data are presented as nmol of total GSH per mg of protein. Uric acid and direct bilirubin levels were assessed using commercial kits (Boehringer Mannheim) with the automated Roche-Hitachi 917 system. Results are expressed in mg/dL for both compounds.

2.6. Determination of antioxidant enzymes

SOD, CAT and GPx activities were measured as previously described [13]. Results were expressed in U/mL. One unit of SOD activity is defined as the amount of enzyme necessary to produce a 50% inhibition of the NADH oxidation rate under the assay conditions. One unit of CAT activity is defined as 1 μmol of H_2O_2 decomposed per minute under the assay conditions. One unit of GPx activity is defined as 1 μmol of NADH oxidized per minute under the assay conditions.

2.7. Circulating hormone assays

Serum samples were measured by dissociation enhanced lanthanide fluorescence immunoassay (DELFIA) for estradiol and progesterone using a PerkinElmer autoanalyzer. Circulating LH and FSH hormones were analyzed using the Unicel DxI 800 autoanalyzer from Beckman Coulter.

2.8. Statistical analysis

All values represent the mean ± standard error of the mean (SEM). Data were analyzed by multiple analysis of variance (MANOVA) plus Newman–Keul's post-hoc test, using IBM SPSS V.19 software. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Subject population

This study involves a population sample characterized by the clinicopathological parameters presented in Table 1. The entire population studied was diagnosed according to the histological type of breast cancer disease (100%) infiltrative ductal carcinoma. Table 2 shows circulating levels of estradiol, progesterone and the LH/FSH ratio in pre- and postmenopausal healthy control women and pre- and postmenopausal women with breast cancer treated or not with NC. An induced menopause as a consequence of chemotherapy is clearly shown in treated

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