Neuroendocrinology Letters Volume 35 No. 7 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

ORIGINAL ARTICLE

Increased oxidative damage to membrane lipids following surgery for breast cancer

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Submitted: 2014-10-05 Accepted: 2014-11-05 Published online: 2014-12-27

Key words: **oxidative stress; lipid peroxidation; breast cancer; breast tumor; surgery**

Neuroendocrinol Lett 2014; **35**(7):602–607 **PMID:** 25617883 NEL350714A05

Abstract

OBJECTIVES: To evaluate the level of oxidative damage to membrane lipids due to the breast cancer surgery in the early postoperative period.
PATIENTS AND METHODS: Blood samples were collected on the preoperative day and 24 hours postoperatively in 71 women operated for breast cancer, and preoperatively in 38 female patients with benign breast tumour. Lipid peroxidation (LPO) in the blood samples was estimated by measuring the concentrations of malondialdehyde+4-hydroxyalkenals (MDA+4-HDA) with spectrophotometry.
CLINICAL DATA INCLUDED: tumour site, tumour histological findings, cancer stage, grade, tumour volume, state of lymph nodes, type of surgery for breast, type of surgery for axilla.
RESULTS: Blood LPO level was similar in breast cancer patients and benign tumour patients (2.01±0.46 nmol/ml vs. 1.92±0.39 nmol/ml, respectively; $p>0.05$). In cancer patients, MDA+4-HDA increased on the first postoperative day, i.e. from 2.01±0.46 nmol/ml to 2.58±0.98 nmol/ml ($p=0.0001$). In women with benign breast tumour, LPO did not relate to the histological finding ($p=0.8915$). In the breast cancer group, preoperative LPO did not correlate with age, tumour volume and number of metastatic lymph nodes. Level of MDA+4-HDA was similar in stages I/II (2.03±0.46 nmol/ml) compared to stages III/IV (1.69±0.26 nmol/ml, $p=0.1521$). Consequently, levels of MDA+4-HDA did not relate to disease stage ($p=0.1364$).
CONCLUSIONS: Surgery for breast cancer causes peripheral increase in oxidative damage to macromolecules in the early postoperative period. Therefore, perioperative antioxidant supplementation should be considered.

INTRODUCTION

Each surgical procedure is a physical intervention on tissues which requires subsequent healing of the surgical wound. Early healing and recovery enables patients to return to their normal life and results in the aesthetically pleasing scar. Such scenario is particularly desired in the breast cancer patients, where the postoperative local and systemic adjuvant therapy – radio- and chemotherapy, respectively – can be initiated promptly only in cases of the uneventful healing.

Trauma caused by surgery is associated with excessive release of reactive oxygen species (ROS) and with the impaired antioxidant defense (Hiki *et al.* 2006). The increased ROS production during surgery results from mechanical damage, bacterial invasion or hypoxia (Arsalani-Zadeh *et al.* 2011). Impaired antioxidant protection is due to redistribution and increased consumption of ROS scavengers (Rizzo *et al.* 2010). In the clinical setting, the adverse effect of oxidative stress was documented in the range of general, trauma, orthopedic cardiac and plastic surgery (Rosenfeldt *et al.* 2013). Significance of the oxidative stress in the elective breast cancer surgery has not been well documented yet.

Oxidative damage to macromolecules was extensively studied in the several experimental and clinical scenarios by author of the present study (Karbownik *et al.* 2000; 2001; Karbownik-Lewinska *et al.* 2012a; 2012b; 2012c; Milczarek *et al.* 2013; Stepniak *et al.* 2013; Kokoszko-Bilska *et al.* 2014). Usually, the indirect assessment of oxidative stress is performed with detection of stable markers of lipid, protein or DNA oxidation (Pande *et al.* 2012). They include, among others, malondialdehyde (MDA), F2-isoprostanes and 8-oxo-7,8-dihydroguanine (8-oxo-G) (Brucknerova *et al.* 2013; Rosenfeldt *et al.* 2013). Products of oxidation of polyunsaturated fatty acids (lipid peroxidation, LPO) can correspond to the early tissue oxidative damage, because the most outer components of cells exposed to oxidative injury are membranes containing lipids.

The aim of the study was to evaluate the level of oxidative damage to membrane lipids due to the breast cancer surgery in the early postoperative period.

MATERIALS AND METHODS

The Ethics Committee of the Mother's Poland Memorial Hospital & Research Institute approved the case control study. For the present work we enrolled 71 women with newly diagnosed breast cancer, who constituted the breast cancer group. The control group included the age-matched 38 female patients with benign breast tumour. Table 1 shows the general characteristics of patients included in the study. Exclusion criteria were clinical or pathologic evidence of cancer at any other site, state after neo-adjuvant therapy, liver dysfunction, diabetes mellitus, heart failure, renal failure, oral contraceptives or antioxidant supplementation.

Patients with breast cancer were classified using the TNM-UICC staging system. They were operated under general anaesthesia with endotracheal intubation. Premedication included intravenous midazolam (7.5–15 mg). Anesthetic induction was done with midazolam (2 mg), propofol (1.5–2 mg/kg), fentanyl (0.1 mg), and rocuronium (0.5–0.8 mg/kg). After endotracheal intubation, all patients were provided with mechanical ventilation using sevoflurane (2–3%) and an air/O_2 mixture (30% O_2). The surgical procedures for breast included breast conserving therapy or mastectomy, whereas surgeries for lymph nodes comprised sentinel lymph node biopsy or axillary lymph node dissection. The benign breast lesions were expounded or excised with margins under intravenous general anaesthesia. Two surgeons performed operations. Clinical data of patients were collected from the case notes and included: tumour site, tumour histological findings, cancer stage, grade, tumour volume, state of lymph nodes, type of surgery for breast, type of surgery for axilla.

Fasting blood samples were collected the day before surgery and 24 hours postoperatively in the cancer group. In the control group, routine blood samples were taken only preoperatively and, thus, we measured LPO level only before surgery in women with benign breast tumour. Blood samples were obtained by venous arm punctures into EDTA tubes. Immediately after collection, the plasma was separated by centrifugation at 4,000 rpm for 5 min and stored in cryovials at –80 °C.

In order to estimate oxidative damage to membrane lipids in the blood plasma samples we measured the concentrations of malondialdehyde+4-hydroxyalkenals (MDA+4-HDA), as an index of LPO using the ALDetect Lipid Peroxidation Assay Kit obtained from Enzo Life Sciences, Inc. (Zandhoven, Belgium). The chemicals and reagents used in the study were of analytical grade. The blood plasma (200 µl) was mixed with 650 µl of a methanol:acetonitrile (1:3, v/v) solution with a chromogenic reagent N-methyl-2-phenylindole and then vortexed. After addition of 150 µl of methanesulphonic acid (15.4 M), the incubation was carried out at 45 °C for 40 min. The reaction between MDA+4-HDA and N-methyl-2-phenylindole yields a chromophore, which is spectrophotometrically measured at the absorbance of 586 nm, using a solution of 4-hydroxynonenal (10 mM) as the standard. The level of LPO in tissue homogenates was expressed as the amount of MDA+4-HDA nmol/ml of plasma.

Data were analyzed statistically. The continuous parameters in the breast cancer group were compared with their corresponding variables in the control group with t-test for independent samples. Changes in LPO level as a result of surgery was assessed with t-test for dependent samples. Pearson correlation coefficient was calculated for relationships between the measured continuous parameters. Univariate logistic regression analysis was used to determine whether continuous variable, such as pre- or postoperative level of lipid

peroxidation, might have predicted the dichotomized variables. The results were presented as mean±standard deviation (SD). Statistical significance was determined at the level of $p<0.05$.

RESULTS

The breast cancer patients were aged 57.05±12.59 years and the benign tumour patients were aged 52.78±8.36 years (Table 1). The correlation between LPO and patients' age has already been well established (Karbownik-Lewinska *et al.* 2012b). Therefore, the breast cancer group and the benign tumour group have been age-matched ($p=0.0633$). The tumour volume in the breast cancer group (10.26±20.23 cc) was similar to the benign tumour volume (29.12±77.74 cc) ($p=0.0611$).

The level of lipid peroxidation in blood plasma was similar in breast cancer patients 2.01±0.46 nmol/ml, in comparison to benign tumour patients 1.92±0.39 nmol/ml ($p>0.05$) (Figure 1). In the benign breast tumour group, LPO level did not determine the histological finding (OR=0.89, 95%CI=0.16–4.93, $p=0.8915$) and the tumour site (OR=0.62, 95%CI=0.10–3.79, $p=0.5998$).

In the breast cancer group, the preoperative levels of LPO did not correlate with age, tumour volume and number of metastatic lymph nodes (Table 2). We did not see correlation between oxidative stress and patients' age and we suggest this finding can reflect the relatively small age range of participants. Levels of MDA+4-HDA were similar in stages I/II (2.03±0.46 nmol/ml) compared to stages III/IV (1.69±0.26 nmol/ml, $p=0.1521$). Consequently, the levels of MDA+4-HDA did not determine the disease stage (OR=0.09, 95%CI=0.00–2.26, $p=0.1364$). High quality of the samples storage regimen and the analytical technique was proven by the low background preoperative levels of MDA+4-HDA (Kilic *et al.* 2014).

The degree of oxidative stress, as measured with MDA+4-HDA, increased significantly 24 hours after the surgery from 2.01±0.46 nmol/ml to 2.58±0.98 nmol/ml ($p=0.0001$) (Figure 1). Postoperatively, level of LPO did not determine the operated site, the type of breast surgery related to the disease stage, type of axilla surgery or the surgeon-related technique (Table 3).

DISCUSSION

The previous studies on breast malignancies reported imbalance between production of ROS and antioxidant status, however the results were controversial and contrary (Ray *et al.* 2000; Yeh *et al.* 2005). Overproduction of ROS with the enhanced lipid peroxidation was reported in the breast cancer patients, both in malignant tissue and blood specimens (Pande *et al.* 2012). Other authors hypothesized that elevated levels of antioxidants in malignant tumours made cancer cells more resistant to oxidative damage (Iscan *et al.* 2002). Consequently, patients operated for breast cancer would be at predefined higher risk of impaired healing due to the additional, predetermined oxidative imbalance caused by the oncological disease. However, breast cancer patients were also reported to have reduced lipid peroxidation (Gonenc *et al.* 2006). In fact, we did not observe any relationship in our series between degree

Tab. 1. General characteristics of the breast cancer patients and those with benign breast tumour

Breast cancer group			**Benign tumour group**		
Number of patients		71	**Number of patients**		38
Age (years, mean ± SD)		57.05±12.5	**Age (years, mean ± SD)**		52.78±8.36
Cancer site	Left breast	60.6%	**Tumour site**	Left breast	48.6%
	Right breast	39.4%		Right breast	51.4%
Tumour histological type	Ductal carcinoma	85.9%	**Tumour histological type**	Fibroadenoma	51.4%
	Lobular carcinoma	5.6%		Dysplasia	48.6%
	Other	8.5%			
Stage	I	47.9%			
	II	46.5%			
	III	4.2%			
	IV	1.4%			
Grade	I	24.6%			
	II	55.7%			
	III	19.7%			

SD – standard deviation

Tab. 2. Correlation between the pre- and postoperative LPO with the clinical variables in the breast cancer patients.

LPO in blood plasma	Clinical variable	Correlation	
		r	*p*
Preoperative MDA+4-HDA	Age	–0.009	0.934
	Tumour volume	0.160	0.191
	Number of metastatic lymph nodes	0.021	0.859
Postoperative MDA+4-HDA	Age	–0.167	0.236
	Tumor volume	–0.045	0.757
	Specimen volume	–0.026	0.853
	Number of metastatic lymph nodes	0.091	0.529

r – correlation coefficient; *p* – level of significance

Tab. 3. Univariate logistic regression analysis of one univariate determinant, i.e. postoperative LPO, for 5 clinical variables in the breast cancer patients.

LPO in blood plasma	Clinical variable	OR	–95%CI	+95%CI	*p*
Postoperative MDA+4-HDA	Cancer site	1.27	0.66	2.45	0.458
	Stage (I/II or III/IV)	0.69	0.10	4.75	0.702
	Type of breast surgery	1.09	0.60	1.97	0.769
	Type of axilla surgery	1.20	0.66	2.19	0.535
	Surgeon	0.76	0.40	1.43	0.381

OR – odds ratio; CI – confidence interval; *p* – level of significance

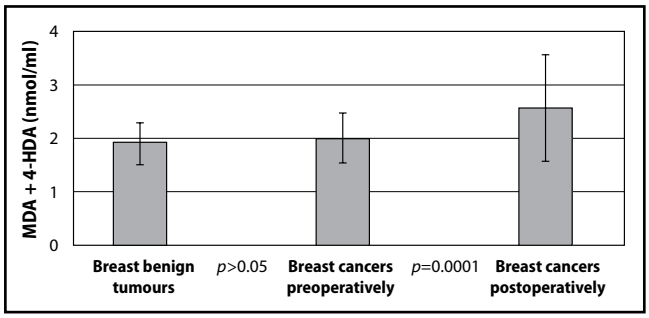

Fig. 1. Blood LPO level in breast cancer patients before and after oncological surgery, and in breast benign tumour patients preoperatively; MDA+4-HDA – malondialdehyde+4-hydroxyalkenals; *p* – level of significance

of oxidative stress and breast cancer, independently from tumour volume, disease stage or involvement of regional lymph nodes.

Oxidative damage due to surgery has been previously extensively assessed in different clinical scenarios (Uzunkoy *et al.* 2000; Zengin *et al.* 2002; Bentes de Souza *et al.* 2003; Brucknerova *et al.* 2013). Patients undergoing intraabdominal operations, gynaecological procedures or even open hernia repair demonstrated systemic stress response in the early postoperative period, detected as the increased level of plasma MDA (Uzunkoy *et al.* 2000; Zengin *et al.* 2002; Bentes de Souza *et al.* 2003). In our study on the breast cancer patients, LPO levels in blood plasma were significantly elevated 24 hours after the operation, regardless the patients' age, tumour volume, excised specimen volume, number of metastatic lymph nodes, cancer site, disease stage, type of breast surgery, type of axilla surgery or the operating surgeon.

In the clinical practice, harmful effects of oxidative stress due to surgical treatment in the breast cancer patients could potentially be diminished with the antioxidant therapy. The previously proposed perioperative antioxidant regimens reduced complications rate and mortality, which was particularly significant in the elderly patients (Rosenfeldt *et al.* 2013). Oral supplementation with vitamins A, C, and E attenuated ischaemia-reperfusion injury and necrosis in skin flaps (Bilgin-Karabulu *et al.* 2001; Yoshida & Campos 2005; Fukushima & Yamazaki 2010). In the mastectomy patients, skin flap necrosis was reduced with topical antioxidant, dimethylsulfoxide (DMSO), however no mechanism of action or need for systemic therapy were analyzed (Celen *et al.* 2005). The previously reported antioxidant treatment in the surgical patients included also other scavengers, such as coenzyme Q10, magnesium orotate, selenium, lipoic acid and omega-3 fatty acids (Calo *et al.* 2005; Linnane *et al.* 2007; Lymbury *et al.* 2008; Shay *et al.* 2009). Melatonin was reported to attenuate oxidative stress and thus its antioxidant properties could be potentially curative in case of different types of surgery (Gitto *et al.* 2001; Di Bella & Colori 2012; Di Bella *et al.* 2012; 2013). With such a wide range of treatment alternatives, clinical guidelines on the perioperative antioxidant therapy in the breast cancer patients would be beneficial.

Postoperative inflammation and the corresponding level of oxidative stress are affected not only by the degree of surgical trauma but also by the anaesthetic drugs (Arsalani-Zadeh *et al.* 2011). Sevoflurane is the golden standard for inhalation of intubated patients and it was used during ventilation of our patients. In our series we used propofol only in premedication. However, sevoflurane was reported to decrease level of antioxidants, contrary to propofol, which in turn decreased the peripheral oxidative stress (Tsuchiya *et al.* 2008). Consequently, use of sevoflurane in combination with antioxidants should be verified in the further studies.

Our study supports surgeons with validated, clinically useful data suggesting the need for the preoperative antioxidant therapy; however the interpretation of our results has some limitations. Oxidative stress measured in blood is a result of systemic inflammation and injury, and does not usually correlate with a local tissue trauma (Kerkweg *et al.* 2010). We examined the

blood plasma specimens, and not the breast cancer tissue itself, because we aimed to assess the detectable systemic changes caused clearly by surgery, which was undertaken for the localized malignant tumour with no diagnosed distant metastases. Method for measuring biomarkers in peripheral blood is clinically applicable and less invasive than measuring tissue oxidative stress. Additionally, LPO levels were measured in a short time frame in only two time points and thus we could not analyze the trends of oxidative stress and the resulting antioxidant requirements. However, the highest oxidative stress caused by surgery was previously reported in the first 24 hours and therefore we suggest that majority of the associated complications result from the damage caused in the above postoperative time (Bentes de Souza *et al.* 2003). Finally, influence of oxidative stress and antioxidant therapy on the subsequent recovery and postoperative outcomes also requires further research.

In conclusion, surgery for breast cancer causes peripheral increase in oxidative damage to macromolecules in the early postoperative period. Therefore, perioperative antioxidant supplementation should be considered.

Disclosure

Disclosure of any commercial interest that they may have in the subject of study and the source of any financial or material support: none

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