Influences Exercised By The Intensity Of The Oxidative Stress Over Wounds Healing In Surgical Therapy Of Breast Cancer

TUDORASCU BRUJAN IULIA(1), C. MARGARITESCU(2), M. NOVAC(3), CRISTINA SIMIONESCU(2), CAMELIA FOARFĂ(2), MARIA VRABETE(1)

(1) Dept. of Physiopathology, (2) Dept. of Pathology, (3) Dept. of Anesthesia-Intensive Care, University of Medicine and Pharmacy, Craiova

ABSTRACT

The objectives of our study were: Determination of the plasmatic oxidative status over the patients with operated breast cancer (CS). Assessment of the ratio between the intensity of the induced S.Ox (tumour + surgical act) and the plasma antioxidant potential. Evidencing of parameters, allowing elaboration of a prediction with regard the quality of the post-operation wound cure. Methods: We investigated 32 patients with breast cancer (CS) and 37 healthy patients (witness lot), 69 cases, in total. We have calculated the total anti/oxidant potential of plasma (TAOP), measured the concentration of total peroxides from plasma and calculated the ratio between the two values, which is expressed as an index of oxidative stress (ISO). These values are used in order to appreciate the oxidative status of plasma. We achieved four groups of study, considering the morphopathological aspect of tumours and the prognosis over the quality of wound healing, post-surgery. In order to explain the psychopathological mechanisms involved in healing the surgery wounds done on the neoplastic field, we checked the evolution of clinical parameters: clinical: wounds appearance, umorals interactions between the residing cells and those migrating from the blood vessels into the wound tissues. We associated to the conventional therapy, post-surgery a diet with exogenous antioxidants (C vitamin, selenium, and beta carotene) and we noted down the features of wound healing, after 3/6 weeks post/operation, up to 2-4 years of life, as appropriate. Outcomes: TAOP has been reduced, the index of the oxidative stress significantly increased at the patients with operated CS, who had a faulty wound healing. The additional exogenous anti/oxidants have different effects, from none effect, to the patients with a lipids and glucides rich diet up to defaultless healing and improvement of the general clinical condition. Conclusions: Reduction of the oxidative stress intensity has a positive role in wound healing and for this reason the administration of some exogenous anti/oxidants could influence the evolution of the general condition of the patients operated for breast cancer (CS), with a favourable sense, for life.

KEY WORDS oxidative stress, cathepsin D, TAOP, IOS, peroxides, breast tumor, antioxidants

Introduction

The cells of breast malign tumours are submitted to a persistent antioxidant stress (S.Ox.p), as a result of maintaining, within a vicious circle of the hypoxia through the following physiopathological mechanisms: increase of tumour, determine the compression of blood vessels and production of some inadequate vascular nets, that cannot provide the contribution of O2, requested to cover the accelerated metabolic needs of tumoral tissues. In such conditions the inducible factor of hypoxia (HIF-1) is released, whose presence determine the amplification, progressive of Sox, activating a series of adaptive mechanisms, required for protection and providing the survival of tumoral cells. Of those mechanisms are explained: 1) Activation of proangiogenic factors (angiogenesis), that associatively sustain tumourgenesis but also the increase of vascular permeability (increase the tumour metastasis potential); 2) Recruiting of cell phenotype resistant to hypoxia, apoptosis and conventional therapy (radio, chemotherapy); 3) Transposition of glucides metabolism to anaerobic glycolisis; 4) protein glication. [1,2]

Reaction of tissues beside the tumour is also protective for the healthy cells and consists in blocking or reduction of S.Ox through mechanisms stimulating the development of enzymatic antioxidant systems [Superoxid dismutasis (SOD), Catalysis (CAT), Glutation peroxidasis (GI.Px)] and non-enzymatic. [3,4] Biochemically, the Sox production, starts from the mitochondrial respiration, where from the O2 free radicals accumulate, manifesting as reactive species of O2 (SRO) that may be measured, by special lab tests, after prelevation of tissues at the solid tumour level. The mechanisms of Sox intracellular production are: 1) Superexpression of timidinphosphorilasis termines the change of the timidine into timine+2 deoxi-D ribozis-1 phosphate, reduction of glucoses, protein glication and generation of reactive species of O2 (ROS).

2). Degradation of estrogens in the presence of lactoperoxidasis, in the galactophore channel area is associated with acceleration of the oxidation
reaction of an electron from the estradiol radical that passes into phenoxil and intensifies S.Ox in vivo; 3) The inadequate vascular nets generate a large level of fluctuations of the blood flow contribution, stimulating the repetition of hypoxia cycles (ischemic) – reperfusion. At the same time with occurrence of the reperfusion phenomena, recruiting and tumoral infiltration with macrophages takes place, with consequences over SRO production; 4) radiotherapy, general photodynamic therapy, administration of tamoxiphen (antiestrogenic) are some means or techniques used for cancer treatment, but all of them generating SRO.

The S.Ox effects are known namely: production of genetic mutations, fragmentation of the DNA helix, alteration of purinic and pirimidinic , chromatidic exchanges, inhibition of the suppressor genes from tumoral cells, increase of protooncogenes super expression, a high genetic instability meaning development of the malign tumour potential. At cell level, the S.Ox effects are interpreted by activation of many types of cell signalling cascade, having as effect the MAPK activation, triggering the cell proliferation. Besides proliferation, stimulated by MAKP, the fibroblast proliferation produces also, this being triggered by transformation of oncogene RAS into rac and NADPH oxidasis, in the SRO presence. Through this biochemical mechanism, the progression of cells within the cell cycle is provided, without the presence of MAKP is requested.

Activation of iNOS and hemoxygenasis, with production of NO and CO, as supporting elements of vessel dilatation and vascular permeability, increases the tumour metastasis due to intensification of cell detachment from the basal membrane and their obstruction to outrun the lamina. At the same time, take place the reorganization of the active cytoskeleton and MDA-MB-231 migration as a result of HSP 27 phosphorilation and rac-1 activation and NADPH oxidasis [6,7,8,9]. Blocking and local re-equilibration of the oxidative excess by the tumoral microenvironment is often overrun, and for this reason many researchers recommend the antioxidative therapy in order to maintain the oxidative/antioxidative balance at plasmatic level, providing in this way minimizing the extension of tumoral process and maintaining the mechanisms involved in wound healing. Acknowledgement of mechanisms and times required to maintain the oxidant/antioxidant balance supposes knowing the methods through which the permanent control of the relationship among the contradictory elements, placed both intracellular and extra cell, is done. The results of genomic, proteomic researches transposed in physiopathological mechanisms, shown, in detail, the interrelations among the intracellular signalling ways, membrane receptors, growing factors etc, suggesting the need of finding some biochemical, umoral and cellular markers, through which structural and functional status of cell (tissues), forced to function in some hypostasis, is expressed. Existence of markers shall be used to elaborate the prediction over the evolution of some cell population and shall enable the intervention with the view of therapeutically redirection of this evolution. In this sense, the prediction over the effects induced by the surgical therapy of breast cancer, using umoral markers, became a reality where the herein study affirms the value of potential antioxidant plasma as indicator of Sox intensity.[10]

Study objectives:

Determination of the value plasma oxidative status, at the breast cancer patients (BC) after surgical intervention (tumoral excision) in order to evaluate the relationship between the intensity of the tumoral induced S.Ox and healing of the operated wound, after the antioxidant effect, triggered by tumour excising and activation of antioxidative mechanisms at the healthy cell level, surrounding.

Material and methods

The study was carried out with the approval of the Ethic Commission of the Craiova UMF, approval of the Head of Department and the acceptance of the patients operated in the Surgery department II of the Craiova Clinical Hospital no.1. The lab determinations were achieved with the support of specialized labs of Craiova UMF (Immunohystochemistry) and Physiopathology Department of Cluj UMF.

Criteria of selection for the study group, comparable to the witness had in view the following demographic characteristics: age, index of corporeal mass, life style (diet) and compulsorially, the absence of co-morbidities, shown in the table below.

We followed up clinically, functionally and umoral intra- and postoperation evolution, recording for every case, the parameters followed up: at 3-7-10 days, up to hospital leaving, for the witness group and for study group we kept on with the control of all parameters during the same periods, to which examinations at every 6 months have been added, of the effects of cytostatic cures,
radiotherapy in correlation with the antioxidant status of plasma and quality of wound healing.

Table no.1 Demographic characteristics and diagnosis of the studies group

<table>
<thead>
<tr>
<th>Witness group-Healthy women - Diagnosis (37 Cases)</th>
<th>Group with cancer - Diagnosis (32 Cases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scler atrophic adenoma</td>
<td>Invasive ductal adenoma (G3)</td>
</tr>
<tr>
<td>Ductal hyperplasia</td>
<td>Invasive lobular carcinoma(G1)</td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>Invasive mucinous carcinoma</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>30-40 years</th>
<th>Age</th>
<th>30-40 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corporeal mass index (IMC)</td>
<td>21-23</td>
<td>Corporeal mass index (IMC)</td>
<td>22-24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Fats</th>
<th>25%</th>
<th>diet</th>
<th>Fats</th>
<th>40%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coffee</td>
<td>25%</td>
<td></td>
<td>Coffee</td>
<td>35%</td>
</tr>
<tr>
<td></td>
<td>Alcohol</td>
<td>15%</td>
<td></td>
<td>Alcohol</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>Fruits-vegetables</td>
<td>25%</td>
<td></td>
<td>Fruits-vegetables</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>Smoking</td>
<td>10%</td>
<td></td>
<td>Smoking</td>
<td>10%</td>
</tr>
</tbody>
</table>

| Absence of co-morbidity |

Clinical parameters: Early discovery of breast cancer allowed the surgical act, as primary treatment, as conservatory surgery (lumpectomy with auxiliary dissection) or (mastectomy with auxiliary dissection). We followed up the number of drain retention days, as indicator of serous collection presence, on wound place (reduction of wound healing capacity). The criterion for drain removal, was achieving of a flow of < 50 ml in 48 hours.

Duration of hospitalization was correlated to the occurrence or not of some post operative complications (blooding, infections, etc). The post operative duration was considered the duration when another adjuvant therapy was not administrated. The interval required to complete wound healing (removal of threads at the suture level, after closing of the external edges of wound). The general postoperational complications, found to any type of surgical intervention, are the same for the operated breast cancer as well. They are: infections, secondary suture, serums, and uncontrollable pain.

Umoral parameters: Plasma antioxidant potential (TAOP)

Plasma antioxidant potential was determined in the Cluj UMF’s labs, in compliance with Benzie and Strain technique (1996) using the redox power of the ferric powder (FRAP). The test is done at 37 degree Celsius. It is obtained a solution whose colour may be read at the absorption of 593 nm at a pre-determined time (0-4 mn) after mixing of the reagent with the plasma sample. The arbitrary unit of wavelength expression is the concentration of the Trolox equivalent, as measure of TAOP, according to Gao G., Prior RL (1998). Outcomes are expressed in micromole Trolox equivalent/l.

Concentration of total peroxides:

It was determined using the FOX 2 method, changed, that is based on oxidation of ferrous ion to ferric ion in the presence of different peroxides of plasma and that produces an orange colour whose absorption length may be measured at 560nm. The variation ratio for every individualized plasma sample was lower than 5%.

Index of oxidative stress (IOS):

Represents the value of the ratio between the total peroxide concentration and the total plasma antioxidant potential and has values between: 3, 20-6, and 10.

Plasmatic level of TNF-alpha cytokines, interferon /gamma:

Dosing of cytokines IL1, TNF alpha, IL8, IFN gamma. After prelevation of 2ml of blood on sodium citrate in vacuum containers, the plasma is insulated so that the cytokines may be measured, using the ELISA technique. Reading at 450nm allows the achievement of standard curves expressing the log. of cytokines concentration on y-axis and the log. Of optical density on x-axis; the values were determined through the analysis by regression. Test sensitivity was between the limits 15.7-998 15.8-950 and 31.5-2000 pg/ml for TNF-alpha, IFN-gamma.

Immunohystochemical determination of oxidant/antioxidant profile at tissular level:

From the tumoral tissue we determined: catepsine D, SOD, Gl.Px., reduced glutatiol / oxidated glutatiol bcl- 2 and p 53 with the purpose of appreciation the tumour malignity level so that we can correlate it with the serum antioxidant potential and postoperation evolution of wound.

Working technique:

We sampled blood into heparinized tubes, from the tumours vessels that was to be extirpated and from peripheral blood of patients. The blood was centrifuged at 1500 tours for 10 min. and the plasma was immediately stored at -80 degrees C, in order to determine the values of total antioxidant capacity of plasma, total concentration of plasma peroxides. Other blood samples were: determination of leukocytes number and smear of peripheral blood, Giemsa coloured, for leukocytes formula.

AFM technique (atomic force microscopy):

Assessment of interrelat ion between the resident and migraine elements of blood.

Outcomes
Table no. 2 Plasmatic indicators of the oxidative stress during the first post operation three days

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Witness group</th>
<th>Group in study progress</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAOP (Micromole Trolox equiv/l)</td>
<td>620± 98,3</td>
<td>314 ± 57,1.</td>
<td>p= 0.027</td>
</tr>
<tr>
<td>Total level of peroxides Micromole H2O2/l</td>
<td>20,2± 2,6</td>
<td>35,4± 6,4</td>
<td>p = 0.001</td>
</tr>
<tr>
<td>IOS</td>
<td>3,93± 5,4</td>
<td>8,12± 4,1</td>
<td>p = 0.001</td>
</tr>
</tbody>
</table>

Table no. 3 Plasmatic indicators of Sox on the study group, on group s of cancer severity In evolution, dynamic during three weeks

<table>
<thead>
<tr>
<th>Parameters</th>
<th>A (n=12)</th>
<th>B (n=14)</th>
<th>C (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAOP (Micromol Trolox equiv/l)</td>
<td>435± 22</td>
<td>334± 76</td>
<td>253± 78</td>
</tr>
<tr>
<td>Peroxides Micromol H2O2/l</td>
<td>26± 4,3</td>
<td>32,2± 2,4</td>
<td>38,6± 3,4</td>
</tr>
<tr>
<td>IOS</td>
<td>14,2± 1,2</td>
<td>10,4± 4,3</td>
<td>7,52± 1,22</td>
</tr>
</tbody>
</table>

Discussions

We observed a normal hospitalization period or even reduced, for patients whose the TAOP value was unchanged to the witness (p = 0.003). Reduction of number of days for drain retention (p = 0.001) and for applied suture, after the drain removal (p = 0.03) was manifested to witness and patients as well whose TAOP and IOS valued have been maintained within the normal limits. It is important the decrease of TNF alpha level, after seven postoperative days, at the groups with normal TAOP and IOS (p < 0.001).

Immediately postoperation, an increase of leucocytes number was found, physiologic phenomenon with the process of unspecific defence, triggered by the programmed traumatic injury (surgical act). Decrease of number of white figurate elements after 1-2 weeks (3700 ± 700 and 6000 ± 600) is explained by their participation in the process of unspecific defence, when increase of S.Ox is beneficial for postoperation point cleaning. After 4,6 and 8 weeks, the number of leucocytes revert to the physiological values (7900 ± 338 and 9400 ± 658), as a result of simulation of the feed-back type mechanisms (central production / peripheral destruction of figurate elements through stimulation of red cell formatting marrow).

The AFM appearance distinguishes the agglomeration of white and red figurate elements. After there days it can be observed the occurrence of figurate elements, large whites, of monocyes - macrophage type, with fringed membranes and even with cytoplasmatic hollows or destructions. These aspects have been coordinated with intensification of postoperation Sox, earlier (24 – 72 hours), at the same time with decrease of TAOP and IOS.

Tissular antioxidant profile rendered evident a deprived activity of reductase glutation, immediately after surgical intervention (35.6 ± 2.8 to 115.2 ± 11.8 mU/mg protein) but that reverted to normal after 72 postoperation hours The peroxidase glutation activity was very lithe changed, phenomenon that could be explained due to the fact that this enzyme comes from erythrocytes that are present and approximately normal, structurally and functionally, due to blood transfusions. Glutation S/transpherasis and superoxide dismutasis were not significantly changed after 48 hours nor later, this suggesting that blocking of S.Ox intensity is achieved especially through the elements produced by the microenvironment that transfers then into plasma where an increase of TAOP is determined and a decrease of IOS. Lipid peroxidation measured through production of malondialdehyde (MDA) is not an excessive one and does not contribute to increase of plasmatic peroxide concentration.

Fig. 1 Immunohystochemical expression of D catepsine in invasive ductal carcinoma
Outcomes achieved suggested the marked expression of D catepsine in malign tumors to benign ones, positiveness being present both in neoplastic epithelial cells but also in stromal cells.

Post operation wound healing as dynamic physiological process, associates the activity of fixed population tissular cells residing with the activity of movable cells, of blood migrating in compliance with the chemotaxia mechanisms. Migrating cells interact with the extra cell matrix of tissular areas, surrounding the area where the tumours were excised from. The physiologic tissular behaviour is construed through healing per primam. This kind of healing expresses the collaboration and interaction among the cell, biochemical and umoral elements, that within the haemostasis limits are grouped in contradictory elements, adjusted so that to balance each other. So it is cited the equilibrium or balance among oxidant and antioxidant, extra/intracellular, tissular/plasmatic factors, the acide/base balance, balance among pro-anti angiogenesis and pro-anti-inflammatory etc. [11,12]. All these balances are provided at cell level by the mitochondria activity, at its turn in correlation with proteome expressivity, manifested in metabolic activity and cell functionality. Maintaining of these activities is reflected, also, in maintaining the cell haemostasis, being the guarantor for cell progression in cell cycle and transition of its evolution steps: cell development, proliferation.
and apoptosis. At cell level the biochemical mechanisms are described maintaining the glutaniol in a reduced form. Its oxidation is effective only when it is triggered by the intracellular reductasis glutathione. This aspect have been cited by a series of authors, as a phenomenon produced secondary after administration of chemotherapy and it is considered as triggering element for unbalancing the balance between the oxidative stress and antioxidant reserves [13].

The purpose of our study was to observe the effects of an antioxidant diet administration where we included C, E vitamins, selenium found in fruits and vegetables, during pre/and post operation period. At social, cooperative patients, we associated gym and breathing exercises, specific to lung affections, secondary of breast neoplasm. [14] Decrease of postoperation complications during the immediate period and also decrease of mortality, after some years after surgical intervention, concurring with decrease of S.O.x.p intensity, promote the possibility to activate some protective physiopathological mechanisms. Our research acknowledges the hypothesis that the cancer survivors need to change their life style, starting with reduction of oxidative stress and increase of TAOP level.

Even that the diet with C vitamin did not convinced us of its capacity to change the TAOP value, nevertheless it has a remarkable role of vascular tonic. Selenium and E vitamin reduce the major effects of S.o.x.p. [15] Recent data, in oncology literature, suggests that the alteration of DNA< proteins and cell membranes and mitochondria by Sox is one of the main causes of development, production and evolution of breast cancer, more Sox has a permanent action (Sox.p).

The researchers from the “National Center for Toxicological Research” certified that SOD, the intramitochondrial enzyme (MnSOD) is a complex protein, existing in two different forms, with two different expressions that are coded at the level of two different alleles. The Mn dependent superoxide dismutasis (MnSOD) seems to be an enzymes required for protection of mitochondria (“power plant”).

The MnSOD alleles are not equivalent and for this reason the two forms of MnSOD are not equivalent in relation with mitochondria protection. It was found that the women genetically predisposed to have MnSOD, the effective form, in lower quantity have a higher risk to develop the breast cancer. This finding, acknowledges the decrease of endogenous antioxidant capacity, at the same time with increase of total peroxide concentration, determining Sox.p installation and intensification. This description provides the argument for supplementation of conventional therapy with endogenous antioxidant therapy. [16,17,18]

**Statistic processing**

In order to process the statistic data achieved, we referred to an analysis method through hierarchic regression that enabled us to maintain that the values TAOP, ISO, TNF alpha and some increase factors (unpublished data) may be considered as predictive markers of general patient status evolution with breast cancer and healing of wounds produced as a result of excision of this kind of cancer. Statistic analysis, achieved in order to underline the variance, dependent on proposed variables within the study, evidenced the value of every type of variables: dependent and independent. The dependent variables were: number of days required to maintain the drain, period required for recovery of the area where the drain has been removed, duration of hospitalization, postoperation duration equivalent to the interval between the surgical act and starting of a new therapy, adjuvating. The independent therapies were: age, type of surgical intervention, tumour size, occurrence of postoperation complications, surgical re-interventions, need of transplantation or plastic surgery with splitted free skin (PPLD) due to the lack of substance, non-healing due to the incapacity of wound edge closing.

In the pattern approaches, took into consideration, the evolution of independent variables, during the postoperation period enabled to elaborate the prognosis over the healing type, to which the association of TAOP, ISO and TNF-alpha transformed these variables into predictive character variables. The outcomes, statistically processed suggested the possible benefits of antioxidant therpay, which applied, dependent on the unbalance oxidants/antioxidants value, at the right time, could reduce the postoperatory complications to patient breast cancer operated.

**Conclusions**

- Age, as independent variable, points out that the premenopausis stage is a risk factor in breast cancer development, because hypothetically during this period of hormonal unbalance, the intracell Sox, becomes very intensive and cannot be decreased because of decrease of protective enzyme effectiveness, of MnSOD type.
- Endogenous antioxidant enzymes as: glutation peroxidaza, catalaza and
superoxid dismutazis (enzymatic antioxidant system, intracell) are used within the serious malign and aggravating tumor process due to progressive intensification of SOx.

- Presence of endogenous antioxidants in the extracellular space is very low, nearly absent, and for this reason the tumoral microenvironment is forced to work in metaboloi conditions, identical to those of the tumor, meaning it is submitted to changes of oxidative type.

- Food rich in sweets and fats increase the malignizart capacity due to decrease of tissular and plasmatic endogenous antioxidant effects.

- The breast cancer to which the content of pro-inflammatory cytokines and development factors is increased, secondarily, to oxidative stress, suggests that the presence of a redox unbalance, stimulates the superexpression of genes that encode the proteins involved in adaptive and protective activities, during the postaggressive period.

- Default of healing of remaining tissues, after excision of cancerous tumor or accelerated evolution of cancer are aspects, that can be correctly prognosticated as a result of association of some umoral parameters that may be considered as markers of the postoperation evolution: TAOP, ISO, VEGF, TNF alpha.

- Food with apport of fruits and vegetables, developed as common hygienic/dietetic mesure, starting from childhood, seems to be a method of prevention of breast cancer development due to provision of constant concentration of exogenous antioxidants. They may be used under the conditions of rough and sudden occurrence of oxidants/antioxidants unbalances, due to installation of some neuro-hormonal unbalances:pubescence, pre- and postmenopause.

References


Adress: Dr. Ilulia Tudorascu, Dept. of Physiopathology, University of Medicine and Pharmacy, Craiova, ibrjurjan@yahoo.com