Review

Breast Cancer and Oxidative Stress in Chemotherapy

Tatiane De Rossi, Specialist; Carolina Panis, MSc.; Vanessa Jacob Victorino, Graduated student; Lucas Freitas de Freitas, Graduated student; Ana Cristina da Silva do Amaral Herrera, Specialist; Alessandra Lourenço Cecchini, PhD; Rubens Cecchini, PhD

Pathophysiology Laboratory of Free Radicals - Universidade Estadual de Londrina, Londrina, Brazil

Abstract

Objective: This revision characterizes the biomarkers used for analysis of the development of oxidative stress produced during breast cancer chemotherapy. **Materials and methods**: A search of articles indexed in digital databases (Lilacs, Bireme, PubMed, Scielo and digital libraries), along with publications printed as books, periodicals and articles not available online, in the period from 1979 to 2009. **Conclusion:** Reactive oxygen and nitrogen species are produced, principally, during aerobic metabolism; however, its synthesis can be exacerbated or antioxidant defense reduced or more usually, both conditions can occurr in many pathophysiologic situations, leading to a net reactive species yelded. This unbalance is defined as oxidative stress. Stress biomarkers can be defined as predictive indicators able to detect in vivo oxidative damage and can be subdivided into antioxidant and pro-oxidants. To verify the antioxidant system, it is possible to analyze the superoxide dismutase enzymes, catalase and glutathione, along with vitamins A, E, C and glutathione among others. The analysis of pro-oxidants can be made through the verification of protein nitration and oxidation, heat shock proteins, lipoperoxidation, formation of aldehydes for malondialdehyde tests, 4-hydroxynonenal, oxidized LDL and isoprostanes or for chemiluminescent techniques. Advances in cancer detection through the identification of potential biomarkers consist of a promising strategy for the prevention and early identification of this pathology.

Keywords: Biomarkers, Free radicals, Antioxidants, Cancer, Chemotherapy, Oxidative stress

Introduction

According to data of the Instituto Nacional de Câncer - INCA, in 2005, nearly 7.6 million persons worldwide died of cancer, resulting in 13% of total deaths. Cancer estimates for the year 2009 in Brazil indicate that 466,730 new cases of cancer will be diagnosed in the country. The cancer types of greater incidence in males will be cancers of the prostate and lung, and in females, cancers of the breast and uterine cervix.[1]

Chemotherapy is one of the principal methods employed for the treatment of several types of cancer[2] being able to be used as a combination of antineoplastic drugs to increase its efficiency.[3] The principal objective of chemotherapy is to eliminate only the tumorous cells; however, most of the antineoplastic agents act in a non-specific way, harming as many malignant cells as normal.[2-3]

In several cell types, the death of a tumor cell by apoptosis is due to the use of antineoplastic drugs that act, in part, inducing oxidative stress through the formation of reactive oxygen species (ROS) and nitrogen species (RNS),[4-6] with consequent reduction of the antioxidant

Correspondence:
Rubens Cecchini
Rodovia Celso Garcia Cid Pr 445 - KM 380
86051990 - Londrina - Brazil
Fax: 554333714267
E-mail: cecchini@uel.br

defense system of organism.[7]

The direct attack of ROS and RNS produced during chemotherapy treatment causes oxidative damage in cellular structures. As a consequence, the cells can suffer adaptations through the increase in the antioxidant system or suffer oxidative damage and evolve into cell death with the persistence of this event.[8] Thus, the damage caused by reactive species in lipids, proteins and DNA, as well as antioxidant levels, can be used as markers of pro-oxidant and antioxidant activity resulting from the neoplasm in the chemotherapy process.

Materials and Methods

For revision of the chosen subject, information was obtained in the available indexed literature, in the period from 1979 to 2009, through a data search in digital databases Lilacs, Bireme, PubMed, Scielo and the digital libraries of the University of São Paulo (USP, São Paulo, Brazil) and the University of Campinas (UNICAMP, Campinas, Brazil). Keywords used in the search were "cancer", "chemotherapy", "oxidative stress", "antioxidant", "free radicals" and their respective roots in the English language. Also consulted were printed publications specifically on the subject, such as books, periodicals and articles not available online.

Results

Breast Cancer and Chemotherapy

In western countries, breast cancer represents one of the principal causes of death in women. Statistics indicate an increase of its frequency in countries both developed and developing. According to the World Health Organization (WHO), in the decades of the 60s and 70s, a 10-fold increase was registered in the incidence rates adjusted by age in the population-based cancer registers in several continents. This neoplasm represents the second most frequent type of cancer in the world; the first among women.[1]

In 1942, the TNM (Tumor-Node-Metastasis) classification system was created, which was based in attributing a cancer prognosis, in accordance with the size of the primary tumor, presence and extent of the disease in regional lymph nodes and the presence of distant metastases. The last update took place in 2002 in order to consider relevant advancements in the detection and diagnosis of metastases, such as mammographic screening of increasingly smaller tumors, sentinel lymph node biopsy technique and enhancement of immunohistochemical and molecular biology techniques.[9-10]

Chemotherapy treatment can be classified as: neoadjuvant, or in other words, to reduce the tumor dimensions to realize a more conservative surgery; adjuvant, with the purpose of eradicating micrometastases after surgical treatment; and palliative for symptom relief in the advanced disease. Adjuvant chemotherapy in breast cancer includes all the forms of endocrine therapy (hormoniotherapy), used with or without cytotoxic therapy, together with surgical resection of the tumor.[11]

Used in chemotherapy treatment, paclitaxel (PTX, Taxol®) is an isolated taxane of the stem bark of the Western yew tree (*Taxus brevifolia*), efficient against a wide variety of solid tumors, including breast cancer.[12] Because of being a hydrophobic compound, PTX needs a solvent for its administration, Cremophor EL® (CrEL), which is responsible for promoting adverse effects, such as alteration in pharmacokinetic behavior of drugs and hypersensitivity reactions is able to act as a cofactor in development of peripheral neuropathy.[12-13]

Taxol and related taxanes have a unique mechanism of action where they bind to the tubulin protein, thus inhibiting cellular division.[14] PTX interferes in the formation of the bundles of microtubules in interphase and aster cells during mitoses, where micromolar concentrations of the drug are frequently used to accent the aggregation of microtubules in the cells, inhibiting the progression of mitoses.[15-17]

Some of the direct effects of PTX and its vehicle CrEL can be observed through the alteration in erythrocyte viability due to conformational changes that result in stomatocytosis,[18] which seems to be associated to damage in the cellular membrane that leads to the formation of pre-hemolytic lesions.[19] New formulations of PTX without CrEL are already the objects of several studies, such as Genexol-PM,[20] LDE-oleate ,[21] ABI-007 and PTX loaded with nanoparticles.[22] The results have shown to be satisfactory; however, more studies should be carried out using different doses and in combinations with other drugs.[20]

Important pathways that induce cell death for PTX include the phosphorylation and activation of Raf-1, c-Jun N-terminal kinase (JNK), cdc2, phosphorylation of Bcl-2 and Bcl-xL (anti-apoptotic super expressed proteins in the development of cancer), and the p53-mediated transcriptional control for p21 expression, which inhibits cdc2 during the G_2/M phase of the cellular cycle. The

phosphorylation of Bcl-2 in the presence of PTX in this phase can result in the activation of Raf-1 or cdc2.[23]

Another common antineoplastic in breast cancer chemotherapy is doxorubicin (DOX) produced by the fungus *Streptomyces peucetius* var. *caesius* or synthesized chemically from daunorubicin. This drug has effective antitumor action and is used in chemotherapy treatment against a variety of malignant tumors.[24-25]

DOX acts in the tumor cells through DNA intercalation and inhibition of topoisomerase II, [2] as it interferes in the synthesis of DNA and RNA, inhibiting the phase of the cell cycle, inducing apoptosis of the tumor cells in the G_2 phase by block of the cell cycle and inhibition of the DNA polymerase enzyme. The break of DNA can be mediated by the formation of free radicals, contributing to an increase of oxidative stress[27-28] provoked by the reduction of the availability of other antioxidant endogens.[25]

Additionally, DOX inhibits the production of actin, troponin, myosin light chain and the isomer of creatine kinase.[25] The activation redox of the intermediary semiquinone ($DOX^{-\cdot}$) with reduction of oxygen (O_2) that produces radical superoxide ($O_2^{-\cdot}$) as a mechanism attributed to the cardiotoxicity in the treatment with DOX.[29] The cardiotoxic potential observed in chemotherapy treatment with DOX[30] is dose-dependent, where high concentrations induce necrosis, and low and submicromolar concentrations induce apoptosis.[29] The acquired cardiomyopathy results in functional alterations as congestive heart failure, serious hypotension, tachycardia, cardiac dilation and ventricular insufficiency associated to contractile depression.[25]

Other side effects, as persistent alterations in cognitive function, have been observed after treatment with DOX in patients with breast cancer, showing the sensitivity and alterations in the structure of cerebral tissue.[26] Cardoso and collaborators[31] report significant reduction of aconitase enzyme activity and an increase of calcium susceptibility by the opening of the transitory pores of permeability mitochondrial in the brain cells, which can lead to the development of neurodegenerative conditions in patients who have undergone chemotherapy.

Participation of Oxidative Stress in Carcinogenesis

ROS and RNS are produced, principally, during aerobic metabolism; however, their synthesis can be exacerbated in physiopathologic situations. In the organism, oxygen can be reduced to water, and the ROS derived mainly from the intermediate of this reaction: superoxide radical (molecular oxygen reduced by one electron), hydrogen peroxide (molecular oxygen reduced by two electrons) and hydroxyl radical (oxygen molecular reduced by three electrons). The radical superoxide can react with nitric oxide, producing a radical peroxynitrite, an RNS.[32]

Antioxidants are responsible for the neutralizing action of these reactive species. As definition, an antioxidant is any substance that, present in low concentrations in relation to the oxidizable substrate, retards or inhibits the oxidation of such a substrate, including enzymatic and non-enzymatic compounds. The neutralization of oxidant substances and reactive species can occur at several points, such as in the formation stage, during its action mechanism (intercepting and impede from acting) or repairing the biomolecules already harmed by its oxidant action. In physiologic conditions, ROS have their action neutralized by the antioxidant defenses present in tissues.[7]

Oxidative stress takes place when the generation of reactive species exceeds the capacity of neutralization of such compounds on the part of antioxidants; making the incident of oxidative damage in diverse biomolecules possible.[33] The generation of ROS can occur endogenously or exogenously. Endogenous oxidative stress comes from the normal metabolism of the cell and oxidative phosphorylation.[34] Exogenous agents, such as drugs, hormones and other xenobiotic agents, have the capacity of producing excessive quantities of ROS.[35]

The principal targets of ROS in cells are the polyunsaturated fatty acids present in cell membranes, leading to chain lipoperoxidation, represented in the stages of initiation, propagation and termination that begins with scavenging of the hydrogen of the polyunsaturated fatty acid of the cell membrane realized by a free radical or alcoxyl radical derived from prior lipoperoxidation, with consequent formation of a lipid radical. In the first propagation equation, the lipid radical reacts quickly forming peroxyl radical, which, in turn, scavenges new hydrogen of the polyunsaturated fatty acid, forming again the lipid radical in the second propagation equation. The termination of lipoperoxidation takes place when the radicals produced in the previous stages are propagated until they destroy themselves.[37]

Besides the membrane lipids, the proteins also suffer an oxidative action from ROS and RNS, very often losing their cell function. Nucleic acids are also targets of reactive species, which lead to chain breaks or base modification.

Chain breaks occur principally by hydroxyl radical action in the carbohydrate portion of the nucleotide chain, probably in carbons 3' and 4'. This type of injury to the nucleic acids has particular importance on account that the chain breaks are necessarily repaired so that the cells maintain their normal function. However, the enzymes responsible for this type of repair have reduced fidelity, which contributes to the incorporation of errors in the genetic material. The break of the nucleotide chain and hydroxylation of bases are considered important events in carcinogenesis.[38] Studies indicate that there is an increase in oxidative stress in patients with cancer, which can be observed by the levels of malondialdehyde (MDA).[39]

Anti-neoplastic chemotherapy exercises its tumoricidal effect through the generation of oxidative stress and induction of apoptosis, as much in tumor cells as in healthy cells[40-41] and as a consequence can be the generation of secondary tumors, which mechanism is related to the action of hydroxyl radicals of purines, pyrimidines and deoxyribose.[42] In DNA, the reactive oxygen species are involved as much in the phases of initiation and promotion as in the progression of tumor cells. The initiation phase is aware of the phase in which the cell suffers DNA damage and turns heritable, since this damage many times is mediated by ROS, in great part for oxidative reactions mediated by peroxyl radicals [36] and by the final products of lipoperoxidation, as malondialdehyde.[43]

The promotion phase consists of the stage in which the initiated cell suffers the action of a carcinogenic agent (promoter) for a continuous period turning the cell malignant.[2] Studies indicate that in populations of initiated cells, as well as in small pre-neoplastic foci, ROS are synthesized in greater quantity than in comparison with neighboring cells,[44] indicating that the reactive species can be related with the increase of the cell proliferation rate.

In the progression phase, uncontrolled cell multiplication takes place[2] with these cells continuing to present greater levels of oxidative stress in comparison with neighboring cells.[45] Such levels are insufficient to induce the death of tumor cells because it presents a greater resistance to oxidative stress [45-46] On account of increased oxidative stress, an increase in the expression of antioxidant defenses can take place, which gives greater resistance to the tumors to chemotherapy. Also contributing to this are greater rates of oxidative damage and some protease inhibitors.[45]

Generally, the activation of the nuclear transcription factors of NF-κB and AP-1 are modulated by oxidative stress38. The factor AP-1 controls genes involved in cell growth, and its activity is increased by compounds that induce cell proliferation. ROS can activate AP-1, as well as stimulate its synthesis.[47] Oxidative stress still can activate proto-oncogenes and increase the AP-1transcription factor.

In vitro studies show that ROS regulate the gene activity through the activation of protein kinase C , by oxidative damage or even for its direct action in the activation of transcription factors[48] besides inhibiting the apoptosis through the modulation of Myc, Bcl-2 and p53 expression, which results in an increase in the number of cells.[38]

Evaluation of Oxidative Stress in Antineoplastic Chemotherapy

According to Halliwell and Whiteman,[8] stress biomarkers can be defined as predictive indicators of the development of a pathology able to detect in vivo oxidative damage. Such markers can be subdivided into pro-oxidant and antioxidant, in accordance with the affected system.

There are a great number of defense mechanisms present in the organism that act in preventing the damage caused by free radicals.[49] Under normal conditions, the antioxidants are the only compounds able to scavenge the free radicals present in the organism, protecting it from oxidative damage and minimizing the toxicity caused by those radicals.[50]

Cell antioxidant capacity can be reduced through modification in gene expression or the reduction of the ingestion of antioxidants.[36] Nevertheless, in cases of elevated oxidative, for example, the administration of certain drugs, antioxidants become ineffectual to prevent the damage caused by the excess of free radicals in the organism.[6]

Antioxidants can be divided into two systems: enzymatic and non-enzymatic. The enzymatic system involves enzymes produced by the organism itself, as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx). The enzyme SOD acts as a defense against superoxides, while the enzymes catalase and glutathione peroxidase act on H^2O^2 .[51]

The non-enzymatic system is composed by vitamins A (retinol), E (β-tocopherol), C (ascorbic acid), and glutathione (oxidized-GSSG and reduced-GSH) among other compounds as bilirubin and uric acid, β–carotene, metallothionein, zinc, and selenium.[52-53] Vitamin A is responsible for oxidation inhibition of

compounds for the peroxides. Vitamin E participates in the protection of adipose tissue against the formation of radical peroxides. Vitamin C is able to react with simple oxygen, radical hydroxyl and radical superoxide, besides participating in the regeneration of vitamin E .[50] Thus, the antioxidant levels and enzymatic activity of these markers can be used in the evaluation of the impact of oxidative stress in several models.

The increase in free radical production during chemotherapy promotes a consequent reduction in the content of antioxidants in cells, leading the organism to a state of oxidative stress.[26] This stress state can be evidence by an increase in lipoperoxidation, reduction of total antioxidant capacity (TRAP) in the blood and plasma, reduction in the plasmatic levels of vitamins E, C, and β–carotene, as well as reduction in the tissue levels of glutathione which occurs during chemotherapy.[6]

According to Zwart et al.,[52] patients with cancer treated with DOX presented elevated levels of oxidative stress evidenced by increased plasma levels to thiobarbituric acid reactive substances (TBARS), with consequent reduction in the levels of α–tocopherol and retinol in the plasma at the end of the chemotherapy scheme.

In another study, Crohns and collaborators[54] demonstrated that some antioxidant markers have a tendency to reduce in patients with small-cell lung cancer during chemotherapy treatment with DOX, vincristine and cyclophosfamide. In at least one cycle of chemotherapy, it is possible to observe a reduction in serum urate levels, α–tocopherol, plasma ascorbic acid, serum proteins and TRAP, with these alterations observed principally during the first chemotherapy scheme, indicating that this decline in the levels of various antioxidants (principally during the first chemotherapy scheme) can be a reflection of the failure of the antioxidant defense mechanisms against the oxidative damage induced by chemotherapy and from the combat of radicals produced by this treatment.

In rats treated with DOX, Dalloz and collaborators[55] demonstrated that the plasma levels of vitamin C (non-enzymatic antioxidant of low molecular weight) presented reduced, as well as the drastic reduction of the cardiac levels of vitamin E. Additionally, an increase of cardiac catalase activity was reported, which evidenced a massive production of hydrogen peroxide in the myocardium of the rats treated with DOX.

An increase in the cell concentration of glutathione has been associated with resistance, as much with anthracyclines like DOX, as cisplatin.[56] The evidence suggests that chemotherapy treatment destroys the balance between the production of free radicals and the neutralization of the same through an antioxidant defense system, leading to oxidative stress during chemotherapy with DOX.[49]

In the treatment with DOX, different induction mechanisms of oxidative stress are manifested in the heart and brain. Interferences in normal metabolic reactions involving iron and producing ROS are observed in the heart. DOX, because of having a high affinity for cardiolipin, can accumulate in the internal mitochondrial membrane of cardiomyocytes, where in high concentrations makes the heart a place of redox reactions. In the brain, the increase of circulating TNF-α caused by the treatment with DOX, stimulates nitric oxide enzyme synthesis, becoming a ROS generation mediator.[26]

The effect of the reactive species on the proteins results in structural and functional compromise, with consequent extension of these effects through secondary damage caused by amino acid formed radicals, as faults in the DNA repair systems.[57] Nitration and protein oxidation can be detected through immunohistochemical (IHC) tests[58] and HPLC[59] by 3-nitrotyrosine or protein carbonilation.[60]

Heat shock proteins (Hsp) are described as diagnostic, prognostic and resistance markers to the treatment in human cancers, particularly Hsp-2761, for being directly related to the pathological degree of the tumor consisting in therapeutic targets important in the cancers resistant to chemotherapy.[62-63]

Lipoperoxidation is one of the principal consequences of the effects of oxidative stress on biological membranes, a phenomenon described in breast cancer. Studies point to lipoperoxidation as a future prognostic and predictive marker of human breast cancer.[64] This oxidation of lipids can occur through the action of various reactive species, which the effects can be evaluated through aldehyde formation by the tests of MDA, 4-hydroxynonenal, by the presence of lipid hydroperoxides, oxidized LDL[65] and isoprostanes[7] or by chemiluminescent techniques.[66] Studies with patients who had undergone bone marrow transplants show that there is increase of systemic lipoperoxidation due to the prior treatment with chemotherapy.[67]

Yamaguchi and collaborators[68] observed experimentally that the perfusion of liver and heart of mice with the diluent of PTX, a CrEL, led to an increase in the lipoperoxidation of these tissues,

causing cell death and early induction of apoptosis. The monitoring of oxidative damage caused in the DNA produces detectable markers by HPLC and IHC, as 8OHdG (8-hydroxy-20-deoxyguanosine).[69]

Conclusion

The oxidative damage in cell structures result in a direct attack of ROS and RNS. As a consequence, the cells can suffer adaptations by an increase in the antioxidant system or suffer oxidative damage and, in the persistence of these events, evolve into cell death. Thus, damage occurs in lipids, proteins and DNA can be used as pro-oxidant activity markers in pathological processes.

The pathogenesis of several diseases, including cancer, is related to the development of oxidative stress, increasing the migration of tumor cells, and consequently, the risk of invasion and metastasis. The free radicals produced during chemotherapy treatment cause oxidative damage in cell structures, and for this reason, the cells can suffer adaptations through an increase in the antioxidant system or suffer oxidative damage and evolve into cell death, when there is persistence of these events.

Due to the great incidence of cancer among the present population, more refined studies for the detection of potential biomarkers should be carried out for the possible advancement in the diagnosis of this pathology, consisting of promising strategies for the early prevention and identification of the cancer.

References

1 Ministério da Saúde. Estimativa 2008: Incidência de câncer no Brasil. Rio de Janeiro: INCA; 2007.

2 Almeida VL, Leitão A, Reina LCB, Montanari CA, Donnici CL. Câncer e agentes antineoplásicos ciclo-celular específicos e ciclo-celular não específicos que interagem com o DNA: uma introdução. Química Nova 2005; 28:118-29.

3 Moura JA. 2007. Uso de uma nanoemulsão rica em colesterol (LDE) como veículo para o di-dodecil metotrexato. São Paulo; 2007. [Dissertação-Mestrado-Faculdade de Medicina da Universidade São Paulo-USP].

4 Brown NS, Bicknell R. Hypoxia and oxidative stress in breast cancer - Oxidative stress: its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Research 2001; 3:323–7.

5 Sentürker S, Tschirret-Guth R, Morrow J, Levine R, Shacter E. Induction of apoptosis by chemotherapeutic drugs without generation of reactive oxygen species. Arch Biochem Biophys 2002;397:262-72.

6 Conklin KA. Chemotherapy-associated oxidative stress: impact on chemotherapeutic effectiveness. Integrat Cancer Ther 2004; 3:294-300.

7 Halliwell B, Gutteridge JMC. Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death. In: Halliwell B, Gutteridge JMC, editors. Free radicals in biology and medicine. 4th . New York: Oxford; 2007. p.187-267.

8 Halliwell B, Whiteman M. Measuring reactive species and oxidative damage *in vivo* and in cell culture: how should you do it and what do the results mean? Br J Pharmacol 2004; 142:213-55.

9 Van Zee KJ, Manasseh DM, Bevilacqua JL, Boolbol SK, Fey JV, Tan LK, et al. A nomogram for predicting the likelihood of additional nodal metastases in breast cancer patients with a positive sentinel node biopsy. Ann Surg Oncol 2003; 10:1140-51.

10 Singletary SE, Connolly JL. Breast cancer staging: working with the sixth edition of the AJCC Cancer Staging Manual. CA Cancer J Clin 2006; 56:37-47.

11 Hickey M, Saunders CM, Friedlander MM. Breast cancer in young women and its impact on reproductive function. Human Reprod Update 2009; 11-17.

12 Van Zuylen L, Verweij J, Sparreboom A. Role of formulation vehicles in taxane pharmacology. Investigational New Drugs 2001; 19:125-41.

13 Scripture CD, Frigg WD, Sparreboom A. Peripheral neuropathy induced by Paclitaxel: Recent Insights and Future Perspectives. Curr Neuropharmacol 2006; 4:165-72.

14 Adjei AA, Buolamwini JK. Novel Anticancer Agents: strategies for Discovery and clinical testing. California: Elsevier; 2006. A survey of novel molecular targets for anticancer drug Discovery; Applications of nuclear magnetic resonance and mass spectrometry; p.1-20; 107-173.

15 Jordann MA, Toso RJ, Thrower D, Wilson L. Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations. Proc Natl Acad Sci 1993; 90:9552-6.

16 Blagosklonny MV, Fojo T. Molecular effects of paclitaxel: myths and reality (A critical review). Int J Cancer 1999; 86:151-6.

17 Wang TH, Wang HS, Soong YK. Paclitaxel-induced cell death, where the cell cycle and apoptosis come together. Am Cancer Soc 2000; 88:2619-28.

18 Mark M, Walter R, Meredith DO, Reinhart WH. Commercial taxane formulations induce stomatocytosis and increase blood viscosity. Br J Pharmacol 2001;134:1207-14.

19 Oliveira RD. Estudo das lesões hemolíticas e pré-hemolíticas induzida pelo paclitaxel em eritrócitos Londrina; 2004. [Dissertação-Mestrado-Universidade Estadual do Paraná-UEL].

20 Lee KS, Chung HC, Im SA, Park YH, Kim CS, Kim SB, et al. Multicenter phase II trial of Genexol-PM, a Cremophor-free, polymeric micelle formulation of paclitaxel, in patients with metastatic breast cancer. Breast Cancer Res Treat 2008; 108:241-50.

21 Pires LA. Uso de emulsão lipídica como veículo do paclitaxel na terapia sistêmica do carcinoma de mama. São Paulo; 2006. [Dissertação-Mestrado-Faculdade de Medicina da Universidade de São Paulo-USP].

22 Danhier F, Lecouturier N, Vroman B, Jérôme C, Marchand-Brynaert J, Feron O, et al. Paclitaxel-loaded PEGylated PLGA-based nanoparticles: *In vit*ro and *in vivo* evaluation. Journal of Controlled Release 2008; 133:11-7.

23 Symmans WF. Breast cancer response to paclitaxel in vivo. Drug Resistence Updates 2001;4:297-302.

24 Chlebowski RT. Adriamycin (Doxorrubicin) Cardiopatoxicity: A Review. West J Med 1979; 131:364-8.

25 Nascimento MCMO, Martins AS. Cardiomiopatia induzida pela adriamicina: uma revisão. Arq Ciência Saúde 2005; 12:111-5.

26 Chen Y, Jungsuwadee P, Vore M, Butterfield DA, Clair DKS. Collateral damage in cancer chemotherapy: oxidative stress in nontargeted tissues. Mol Intervent 2007;7:147-56.

27 Sarvazyan N. Visualization of doxorubicin-induced oxidative stress in isolated cardiac myocytes. Am J Physiol Heart Circ Physiol 1996; 271:2079-85.

28 Zhou S, Palmeira, CM, Wallace KB. Doxorubicin-induced persistent oxidative stress to cardiac myocytes. Toxicol Letters 2001; 121:151-7.

29 Kotamraju S, Konorev EA, Joseph J, Kalyanaraman B. Doxorrubicon-induced apoptosis in endothelial cells and cardiomyocytes is ameliorated by Nitrone Spin Trap and Ebselen. J Biol Chem 2000; 275:33585-92.

30 Swain SM, Whaley FS, Ewer MS. Congestive heart failure in patients

treated with Doxorubicin. A retrospective analysis of three trials. Am Cancer Soc 2003; 97:2869-79.
31 Cardoso S, Santos RX, Carvalho C, Correia S, Pereira GC, Pereira SS, et al. Doxorrubicin increases the susceptibility of brain mitochondria to Ca2+ -induced permeability transition and oxidative damage. Free Rad Biol Med 2008; 45:1395-402.
32 Sies H. Oxidative stress: oxidants and antioxidants. Exper Physiol 1997; 82:291-5.
33 Sies H. Oxidative stress: introduction. In: H. Sies, editorOxidative stress: oxidants and antioxidants. San Diego: Academic Press; 1991. p.15-22.
34 Parke DV, Ioannides C. Role of cytochrome p-450 in mouse liver tumor production. Progress Clin Biol Res1991; 331:215-30.
35 Halliwell B. Mechanisms involved in the generation of free radicals. Pathol Biol 1996; 44:6-13.
36 Trush MA, Kensler TW. An overview of the relationship between oxidative stress and chemical carcinogenesis. Free Rad Biol Med 1991; 10:201-9.
37 Floyd RA. Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J 1990; 4:2587-97.
38 Klaunig JE, Xu Y, Isenberg JS, Bachowski S, Kolaja KL, Jiang J, et al. The role of oxidative stress in chemical carcinogenesis. Environm Health Perspect 1998; 106:289-95.
39 Huang Y-L, Sheu J-Y, Lin T-H. Association between oxidative stress and changes in trace elements in patients with breast cancer. Clinical Biochemistry 1999; 32:131-6.
40 Mignotte B, Vayssiere J. Mitochondria and apoptosis. Eur J Biochem 1998; 252:1–15.
41 Mclean L, Soto U, Agama K, Francis J, Jimenez R, Pommier Y. Aminoflavone induces oxidative DNA damage and reactive oxidative species-mediated apoptosis in breast cancer cells. Int J Cancer 2008; 122:1665–74.
42 Halliwell B. Effect of diet on cancer development: is oxidative DNA damage a biomarker? Free Radic Biol Med 2002; 32:968-74.
43 Udhary AK, Nokubo M, Marnett LJ, Blair IA. Analysis of the malondialdehyde-2'-deoxyguanosine adduct in rat liver DNA by gas chromatography/electron capture negative chemical ionization mass spectrometry. Biol Mass Spectr 1994; 23:457-64.
44 Scholz W, Schutze K, Kunz W, Schwarz M. Phenobarbital enhances the formation of reactive oxygen in neoplastic rat liver nodules. Cancer Res 1990; 50:7015-22.
45 Toyokuni S, Okamoto K, Yodoi J, Hiai H. Persistent oxidative stress in cancer. FEBS Letters 1995; 358:1-3.
46 Palozza P, Agostara G, Piccioni E, Bartoli GM. Different role of lipid peroxidation in oxidative stress-induced lethal injury in normal and tumor thymocytes. Arch Biochem Biophys 1994; 312:88-94.
47 Kerr LD, Inoue J, Verm IM. Signal transduction: the nuclear target. Curr Opinion Cell Biol 1992; 4:496-501.
48 Feig DI, Reid TM, Loeb LA. Reactive oxygen species in tumorigenesis. Cancer Research 1994; 54:1890-4.
49 Kaya E, Keskin L, Aydogdu I, Kuku I, Bayraktar N, Erkut MA. Oxidant/antioxidant parameters and their relationship with chemotherapy in Hodgkin's lymphoma. The Journal of International Medical Research 2005; 33:687-92.
50 Santos HS, Cruz WMS. A terapia nutricional com vitaminas antioxidantes e o tratamento chemotherapy oncológico. Rev Bras Cancerol 2001; 47:303-8.
51 Rover Júnior L, Höehr NF, Vellasco AP, Kubota LT. Sistema antioxidante envolvendo o ciclo metabólico da glutathione associado a métodos eletroanalíticos na avaliação do estresse oxidative. Química Nova 2001; 24:112-9.
52 Zwart LL, Meerman JHN, Commandeur JNM, Vermeulen NPE. Biomarkers of free radical damage applications in experimental animals and in humans. Free Radicals Biol Med 1999; 26:202-26.
53 Núñez-Sellés AJ. Antioxidant therapy: myth or reality? J Braz Chem Soc 2005; 16:699-710.
54 Crohns M, Liippo K, Erhola M, Kankaanranta H, Moilanen E, Alho H, et al. Concurrent decline of several antioxidants and markers of oxidative stress during combination chemotherapy for small cell lung cancer. Clin Biochem 2009; 42:1236-45.
55 Dalloz F, Maingon P, Cottin Y, Briot F, Horiot J-C, Rochette L. Effects of combined irradiation and doxorubicin treatment on cardiac function and antioxidant defenses in the rat. Free Radical Biol Med 1999; 26:785-800.
56 Lamson DW, Brignall MS. Antioxidants in cancer therapy; Their actions and interactions with oncologic therapies. Alternative Medicine Review 1999; 4:304-29.
57 Wiseman H, Halliwell B. Paclitaxel-induced cell death, where the cell cycle and apoptosis come together. Biochem J 1996 ; 313:17-29.
58 Ichimori K, Fukuyama N, Nakazawa H, Aratani Y, Koyama H, Takizawa S, et al. Myeloperoxidase has directly-opposed effects on nitration reaction – study on myeloperoxidase-deficient patient and myeloperoxidase-knockout mice. Free Radic Res 2003; 37:481–9.
59 Kaur H, Lyras L, Jenner P, Halliwell B. Artefacts in HPLC detection of 3-nitrotyrosine in human brain tissue. J Neurochem 1998; 70:2220–3.
60 Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A. Protein carbonylation in human diseases. Trends Mol Med 2003; 9:169–76.
61 Kabbage M, Chahed K, Hamrita B, Guillier CL, Trimeche M, Remadi S, et al. Protein alterations in infiltrating ductal carcinomas of the breast as detected by nonequilibrium pH gradient electrophoresis and mass spectrometry. J Biomed Biotechnol 2008; 2008: 5641-27.
62 So A, Hadaschik B, Sowery R, Gleave M. The role of stress proteins in prostate cancer. Current Genomics 2007; 8:252-61.
63 Shi P, Wang MM, Jiang L, Liu H, Sun J. Paclitaxel-doxorubicin sequence is more effective in breast cancer cells with heat shock protein 27 overexpression. Chinese Medical Journal 2008; 121:1975-79.
64 Gago-Dominguez M, Castelao JE, Pike MC, Sevanian A, Haile RW. Role of lipid peroxidation in the epidemiology and prevention of breast cancer. Cancer Epidemiol Biomarkers Prev 2005; 14:2829-39.
65 Devaraj S, Mathur S, Basu A, Aung HH, Vasu VT, Meyers S, et al. A dose-response study on the effects of purified lycopene supplementation on biomarkers of oxidative stress. J Am Coll Nutr 2008; 27:267–73.
66 Simão ANC, Suzukawa AA, Casado MF, Oliveira RD, Guarnier FA, Cecchini R. Genistein abrogates pre-hemolytic and oxidative stress damage induced by 2,2a-Azobis (Amidinopropane). Life Sciences 2006; 78:1202-10.
67 Gonçalves T, Benvegnu DM, Bonfanti G, Frediani AV, Rocha JBT. δ–ALA-D activity is a reliable marker for oxidative stress in bone marrow transplant patients. BMC Cancer 2009; 9:138.
68 Yamaguchi J-Y, Nishimura Y, Kanada A, Kobayashi M, Mishima K, Tatsuishi T, et al. Cremophor EL, a non-ionic surfactant, promotes Ca^{2+} -dependent process of cell death in rat thymocytes. Toxicology 2005; 211:179-86.
69 Collins AR, Cadet J, Moller L, Poulsen HE, Vina J. Are we sure we know how to measure 8-oxo-7,8- dihydroguanine in DNA from human cells? Arch Biochem Biophys 2004; 423:57-65.