

# Melatonin bioengineered: A New Possible Strategy for Treatment of Breast Cancer

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**Abstract**— Breast cancer is an important public health problem, with an estimated 3.2 million new cases by the year 2050. Diet plays a key role in the etiology of breast cancer and breastfeeding is associated with a lower incidence of breast cancer. On the other hand, the improvement of the therapeutic properties of bioactive compounds through their incorporation into microcarriers is an important strategy in obtaining new therapies, since cyclical changes in concentration are eliminated; there is biological availability of the compound as well as the reduction in toxicity, number dose and suppression of adverse reactions. Studies using hormones such as melatonin extracted from human milk adsorbed onto polyethylene glycol (PEG) microspheres showed that the controlled release of this compound was able to reduce viability and induce apoptosis in MCF-7 cell lines. Colostrum differs from most of the secretions because it contains viable leukocytes during the first days of lactation with a quantity and activity comparable to blood leukocytes, and has several defense components such as antibodies and hormones, such as melatonin (MLT). This review details the influence of the soluble and cellular components present in human colostrum, such as the MLT hormone, as the modified release systems influence the action of MLT and the possible

mechanisms involved that contribute to the hypothesis of reduction of breast cancer in women who breastfed.

**Keywords**— colostrum, melatonin, polyethylene glycol, breast cancer, bioengineering.

## I. INTRODUCTION

Breast cancer cases have increased worldwide, and are directly related with more life expectancy, exposure to risk factors, and habits. Breast cancer is a multifactorial disease with a higher incidence among women, leading to death [1]. Cancer and chronic inflammation are closely linked and the imbalance between reactive oxygen species and antioxidant enzymes favors the emergence of these diseases. Thus the control of the production of reactive species as well as the maintenance of oxidative balance are primordial for the control of tumor progression [2, 3].

Some factors such as healthy lifestyle and breastfeeding are related to the prevention of breast cancer [4]. Human colostrum differs from other secretions by containing large quantities of viable leukocytes comparable to those found in blood acting as anti-inflammatory mediators. It is believed that both soluble and cellular components interact with each other and may be important for antitumor immunity [5,6]. Breast milk is rich in soluble and cellular components, such as

phagocytes, secretory IgA immunoglobulin (SIgA), and hormones, especially melatonin (MLT) [5,7,8].

Melatonin, a hormone produced by the pineal gland, is involved in several physiological processes, including the functional regulation of breast milk. In milk it is related to the anti-inflammatory effects [9] and pro-oxidant and antioxidant effects of paramount importance in the oxidative stress balance as a protection mechanism [10].

Some studies have reported that the bioavailability and biofunctional function of melatonin may be potentiated when associated in modified release systems [11,12]. Among these systems, polyethylene glycol (PEG) microspheres have been considered an important vehicle for the administration of various drugs, natural products and hormones [13,14,15,16,17].

The administration of drugs adsorbed to carrier systems such as PEG microspheres has been an alternative treatment for various diseases [18], including breast cancer. These release systems are promising for the release of the hormone melatonin [11], preventing it from the degradation promoted by the metabolic enzymes increasing their bioavailability in the organism [19]. How much combined with the MLT has been demonstrated its ability to reduce cell viability and induce apoptosis in tumor cell lines from breast cancer [12,11].

The control of the process of carcinogenesis is closely related to the control of apoptosis, since the tumor cell is able to alter this system, favoring its proliferation and promotion [20]. Thus, cancer treatments are directed at inducing increased apoptosis of tumor cells [21, 22], which may be favored by the use of immunomodulatory agents such as MLT, for acting on immunocompetent cells and assisting in tumor eradication.

This review details the influence of the soluble and cellular components present in human colostrum, such as the MLT hormone, and how they may be responsible for mechanisms that reinforce the hypothesis that breastfeeding reduces the risk of breast cancer.

## II. BREAST CANCER

Breast cancer is a public health problem, with around 59,700 new cases in Brazil in the year 2018 [23]. The worldwide incidence and mortality of this disease are highly related and it is estimated that by the year 2050 will appear about 3.2 million new cases of breast cancer in the world. Despite technological advances, there are still several mechanisms that must be elucidated in the eradication of this disease that affects the world population [24].

Breast cancer is considered a heterogeneous disease, both morphologically and clinically, and is due to a disordered proliferation of breast tissue cells. About

80% of the types of breast tumors originate in the ductal epithelium, known as invasive ductal carcinoma [25]. Invasive carcinomas are so called because they have high metastatic potential, since carcinomas in situ have low metastatic potential and may arise in both lobes and mammary ducts [26]. During carcinogenesis, genetic mutations are accumulating and the cell phenotype is changing through malignant lesions, evolving into invasive cancer [27].

The structure of the breast is composed of glandular tissues composed of the milk producing glands and the ducts through which milk produced and stromal tissues pass, which are fibrous and fibrous connective tissues. In addition to these tissues the breast is also composed of the tissue of the immune system and lymphatic system [28]. For normal development of breast tissue to occur, there is a need to balance cell proliferation and apoptosis. In tumor growth, there is a reduction in apoptosis and an increase in cell proliferation [27].

The balance between a protective cytotoxic response and a non-protective response can be regulated by the individual's overall immune status [29]. A major challenge for tumor research has been the identification of molecular and immunological changes associated with the different stages of tumor progression, and advances in these studies have been hampered by technical limitations to the pre-invasive stages of tumors [30].

The study with in vitro breast cancer cells began in 1973 from isolated cells from pleural effusion of a 69-year-old woman with metastatic disease [31]. MCF-7 cells are useful for in vitro breast cancer studies by having several particular ideal characteristics of the mammary epithelium, such as the ability to process estrogen in the form of estradiol via estrogen receptors in the cell cytoplasm. This cell line is positive for the estrogen receptor (ER) and for the progesterone receptor and negative for HER2. These cells are very well studied with the immense number of protocols defined which allows researchers to use this cell line for study pathogenesis and in the search for treatment of breast cancer through reliable means in vitro assays [32]. During the last decade, several work on the mechanisms related to the interaction between the cells of the immune system and tumor progression. The results indicate that an immune response to a tumor is determined by the different cell types, such as lymphocytes, NK cells, neutrophils and others, as well as by the interactions between hormones, proteins and receptors present on the cell surface [33]. On the other hand, tumor cells arise from a mutation in DNA (deoxyribonucleic acid) that can be caused by radiation, bacteria, fungi, viruses, chemicals, etc. Although the components of the immune system are present and active, cancer cells can progressively grow

and spread, thus, the body weakened by poor diet, genetic predisposition, advanced age and exposure is the perfect environment for the development of cancer. In addition the cancer cells are very similar to the own cells of the organism, which hampers even more the response of the immune system [28].

Among the mutations, the most important alterations that occur are self-sufficiency in signs of proliferation, insensitivity to growth inhibitory signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis and tissue invasion and metastasis [20].

The carcinogenesis process is directly involved with the generation of reactive oxygen species. Oxidative stress participates in all stages of cancer development. At initiation, oxidative species damage DNA by introducing genetic mutations and structural alterations; in the promotion, there is an increase of the initiated cell population, which is proliferation with consequent decrease of apoptosis; already in progression participate in the development of irreversible cancer growth [34].

### III. BREASTFEEDING AND THE IMPORTANCE OF BREAST CANCER PREVENTION

Breast cancer is the neoplasm most feared by women, since its occurrence causes great psychological, functional and social impact, acting negatively on issues related to self-image, social interaction and the perception of sexuality. It is considered of great importance in the health care of women, due to the high prevalence, morbidity and mortality [35].

The role of the immune system in cancer prevention is complex and partially understood. It is widely known that diet plays a fundamental role in the etiology of cancer [33] and that breastfeeding is associated with lower incidence of breast cancer. In this sense, studies have tried to elucidate the effects of lactation on breast cancer [36, 37]. There is evidence that human milk may confer long-term benefits and an increasing number of studies have indicated that breastfeeding provides protection against ovarian and breast cancer [38,39].

However, the effects of breastfeeding on the risk of breast cancer have been difficult to study because of the high correlation with parity [40, 41]. Reproductive factors may induce permanent changes in the epithelium of the mammary gland or in the surrounding stromal tissue [42,43]. Although the mechanisms have not been fully elucidated, the hypothesis of reducing the risk of breast cancer through breastfeeding seems to occur because of breast tissue differentiation or reduction in the number of ovulatory cycles [44].

Studies have revealed that the relative risk of having breast cancer reduces about 7,0% for each child

born and about 4,3% for every 12 months of breastfeeding. This suggests that breastfeeding duration mothers is crucial to ensure the immunity components acts against the breast cancer [37,45] There is still evidence that breastfeeding protects women who have had their children under 50 years of age [46]. Another study reported that women who breastfed several children had the lowest risk of developing breast cancer, and mothers who breastfed four or more children had a 60 percent reduction in breast cancer risk. The magnitude of the protective effect is directly related to the time of breastfeeding [47].

Lactating mammary glands are an integral part of the mucosal immune system, and the antibodies and cells present in the milk reflect the antigenic stimulation of the Mucosa-Associated Lymphoid Tissue (MALT) in both the intestine and the respiratory tract. The literature reports that antibodies and human milk cells have specificity for a variety of antigens from intestinal and respiratory pathogens [48].

Colostrum differs from most of the secretions by containing viable leukocytes ( $10^9$  cells / ml) during the first days of lactation [49], with amount and activity comparable to blood leukocytes [5]. On the other hand, other defense components present in the secretion that may be associated with protective activity, such as lactoferrin, analogous complexes (receptors), fatty acids (lipids), mucins [50], cytokines and chemokines [51,52,53,54], antibodies such as IgA [55,56], lysozymes [52], probiotics [57], antioxidant factors [58], among other components produced by the maternal immune system, as well as several hormones such as to melatonin [59, 60, 15].

The concentrations of melatonin in human colostrum and mature milk are similar the concentration of this hormone in the bloodstream. Also, immunocompetent colostrum cells can start to produce melatonin after stimuli from injuries, such as those caused by bacteria or fungal metabolites. Studies have shown that lymphocytes and peritoneal macrophages from rats and human colostrum phagocytes produce melatonin in response to activation, and this production of melatonin in the site activates lymphocytes and macrophages to produce IL-12, IL-6, IFN- $\gamma$  which increases the production of T lymphocytes, the presentation of antigens and the phagocytic activity of macrophages, thus increasing the local inflammatory aspect [61,62,63] and this synthesis of melatonin occurs by the same enzymatic pathway that occurs in human pinealocytes [64].

Immunocompetent breast cells remain highly permeable after childbirth, which makes this type of cell suitable for signaling pathways when collected in a non-invasive manner, suggesting that these cells play an

important role in the protection of the newborn in pathological conditions these cells will be the defense of the newborn, thus consolidating the importance of breastfeeding [62].

#### IV. MELATONIN

Melatonin is synthesized by the pineal gland [65]. It plays an important role in circadian rhythm control, reproduction, sleep-wake, is directly linked to the regulation of several neuroendocrine axes, protection against cancer and action against free radicals, acting on cell protection [66, 67]. Studies have shown that melatonin may increase the action of innate and acquired immunity and stimulate mainly leukocytes, an immunomodulatory property, which represents an important mechanism of protection for several diseases [9, 68, 8], as well as showing remarkable functional versatility oncotic properties, antioxidants and antiaging [69].

The action of direct melatonin against free radicals has been increasingly studied and its indirect role as an antioxidant has been tested and the effect has been highly effective in reducing oxidative stress in the body when compared to the antioxidants better known as vitamins C and E. Melatonin and its metabolites have positive aspects that make them effective in fighting free radicals. They easily cross the blood-brain and placental barrier, in addition to all maternal organs which leads to greater protection of the placenta and the fetus. Another positive aspect of melatonin is that it can be produced in other compartments, external to the pineal gland [66], and it has been speculated that all cells can synthesize melatonin, mainly in their mitochondria and this local production has the function of protection against radicals free [70].

The production of melatonin by other kinds of cells and organs has been reported, such as the retina, thymus, brain, intestine, bone marrow, ovary, testis, placenta, skin and lymphocytes [71]. High concentrations of melatonin have been found in skin keratinocytes, suggesting that the production of melatonin outside the pineal gland is not only related to the light / dark circadian rhythm, but rather as an antioxidant and anti-inflammatory agent and as a mechanism of stress protection oxidative. This production of melatonin in response to oxidative stress occurs in all living beings, such as plants, unicellular beings, animals and man, and must have been the main function of melatonin in the primitive beings, since they did not have resources in the fight against free radicals.

The production of melatonin by cells of the immune system occurs by activation of pro-inflammatory agents such as cytokines, increases the phagocytic

capacity of macrophages and lymphocytes and induces the synthesis of interleukin-2 (IL-2), which has autocrine action and paracrine [69].

Melatonin acts on inflammatory processes and allergic diseases by attenuating the activation of NF- $\kappa$ B, reducing the production of TNF- $\alpha$  and IL-6 and promoting the survival of mast cells via a series of enzyme kinase activation and inhibition processes [72].

Melatonin exerts antioxidant action, which decreases the formation of free radicals, reducing the number of lesions that may affect cellular DNA [73]. It exerts an antiproliferative effect on physiological dose dependent breast cancer MCF-7 (human breast adenocarcinoma cell line) cells, in addition to reducing the rates of invasive and metastatic properties of this cell type [74]. Studies have shown that melatonin decreased cell proliferation and increased expression of p53 and p21 proteins in MCF-7 cells, inhibiting proliferation and inducing apoptosis. The p53 protein is an important tumor suppressor gene and is involved in the regulation of the cell cycle [75]. Melatonin, via activation of the melatonin 1 receptor (MT1) [76], is associated with suppression of growth and development of breast cancer through regulation of growth factors, regulation of gene expression, inhibition of tumor cell invasion and metastasis and by regulation of mammary gland development [77].

#### V. POLYETHYLENE GLYCOL (PEG) AND THERAPEUTIC APPLICATION IN BIOENGINEERING

Studies aimed at reducing adverse drug effects have been developed as novel therapeutic systems, known as modified release systems [78, 79, 80, 81, 82, 83]. The improvement of the therapeutic properties of bioactive compounds through their incorporation into microcarriers is an important strategy in obtaining new therapies, since cyclical changes in concentration are eliminated; there is biological availability of the compound as well as the reduction in toxicity, number of administered doses and suppression of adverse reactions [84].

PEG-drug conjugates and microemulsions-drug, are being studied as possible modified release systems for a variety of molecules and drugs [85, 86, 87, 88, 89, 90, 91, 92, 83]. This combination has many advantages such as prolonged residence in the organism, decreased degradation by metabolic enzymes and reduction or elimination of the immunogenicity of proteins [87].

Several studies have shown that the association of PEG microspheres with molecules, hormones or proteins increases the immunomodulatory capacity of both blood and colostrum phagocytes and suggests that the adsorption of these compounds to PEG microspheres has

immunostimulatory effects and can be considered a important material/vehicle, with potential for future therapeutic applications in infectious diseases or tumors [13, 15, 14, 17, 16, 93].

Studies using hormones such as melatonin and secretory IgA antibodies extracted from human milk adsorbed onto PEG microspheres showed that the controlled release of this compound was able to reduce viability and induce apoptosis in MCF-7 cell lines [11,12]. Other herbicidal and barium chloride studies, adsorbed to PEG microspheres on human blood mononuclear cells co-cultured with breast cancer cell lines (MCF-7), showed a pro-apoptotic effect in breast cancer cells MCF-7 human [94, 95]. Immunotherapy for tumor treatments based on cytotoxic properties of immunocompetent cells has also been the focus of many studies. Both T cells and phagocytic cells are considered effectors with antitumor activity [96]. The melatonin adsorbed to the PEG microsphere was able to increase the functional activity of colostrum phagocytes and that this modified hormone release system may represent an alternative in the treatment of diseases [17].

Here we hypothesize that melatonin adsorbed on PEG microsphere may be effective in the treatment of breast cancer. The possible interactions between components present in human milk and therapy of bioengineered melatonin as a strategy for the prevention and treatment of breast cancer are shown in figure 1.

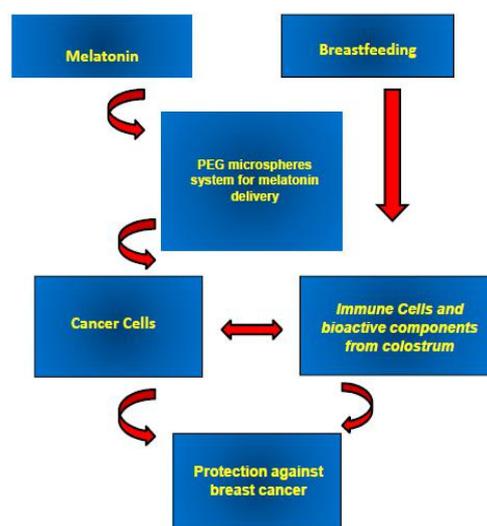


Fig.1: Hypothetical model of therapy with Melatonin bioengineered

Additionally the chronotherapy, the practice of drug administration according to the circadian rhythm, is intended to maximize efficacy and minimize toxicity in the body. This approach has proven advantageous in several diseases, such as cancer and asthma [97] and has been an alternative of treatments based on melatonin.

On the other hand, the expected response by chemotherapy, radiotherapy, hormone treatments, is the induction of apoptosis, since the balance between cell proliferation and apoptosis is determinant for its growth [98]. After initiation of chemotherapy, within 24 h there is a significant increase in apoptosis in breast tumors, associated with decreased proliferation. This response may occur differently in the various tumor types, and at the end of the chemotherapies there is an increase in the levels of the anti-apoptotic Bcl-2 protein, which favors the occurrence of chemoresistant residual cells, which may be important for tumor recurrences [99].

The anti-apoptotic role in normal and pro-apoptotic cells in cancer cells has been reported in studies with melatonin, giving prominence to this hormone, since conventional cancer treatments can not do this discrimination between healthy cells and cancer cells, placing it in a prominent position in the search for effective treatment against various types of cancer, and especially against breast cancer [100].

## VI CONCLUSION

Major advances in cancer therapy have been occurring, and the study of the use of melatonin in cell culture or in vivo oncology has shown promise. The mechanisms of action of melatonin in reducing oxidative stress and the activation of apoptosis in cancer cells has put this hormone as a highlight in the adjuvant use of cancer treatment.

And considering that the breast tissue is in constant and direct contact with the soluble and cellular immune components in the milk, and the numerous immunological constituents of the breast milk, among these high concentrations of melatonin, macrophages, it is possible that interactions of these components, directly or modified release systems with factors present in tumor cells may be an alternative for tumor immunotherapy.

There is still much to study and develop to further increase the cure rates of cancer patients, as well as eradicate the occurrence of adverse reactions that both discomfort and incapacitate the patient, often leading to withdrawal of treatment. There is a need to improve the studies around melatonin as an immunomodulatory agent of colostrum phagocytes in the action against breast cancer cells, since these cells are present in large quantities, mainly in women who have breastfed, which can increase even more the chances of prevention against breast cancer.

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