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EXPERIMENTAL MODEL OF THE MURINE BREAST CARCINOMA (4T1 LUC) AND EVALUATION OF THE EFFECT OF ACETONE CYANIDINE IN THE REMISSION OF TUMOR

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ABSTRACT

Studies involving the fight against cancer have provided several advances that provide the understanding of the development of tumors, mainly regarding the molecular processes that constitute the structure that allows or enables the progressive transformation of healthy cells into malignant ones.Examinations at the molecular level of tumor development in patients is not very common, since there is a need for biopsies at various stages of transformation. This manuscript is a review that presents and discusses some aspects of the experimental approaches, techniques and strategies used in the study of tumors in murine models with emphasis on the immune response against cancer, specifically the effects of acetone cyanide on tumor remission.

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INTRODUCTION

Cancer can be roughly understood as the uncontrolled growth of cells in both human and animal bodies. Its action is to invade tissues and organs altering its functions, which can lead to death in cases whose diagnosis and the consequent onset of treatment were late. This disordered growth becomes more aggressive when allied to it we have the migration of these cells to other regions of the body (metastases). Cancer in Brazil is the second cause of death due to illness (Denorais et. al., 2002., Oms, 2015). The development of cancer depends not only on transformations within the modified cell itself, but also on the interrelations between these cells and their microenvironment. The cancer proceeds from genetic mutations and some physiological variations in the cell are essential for the disease to manifest itself.

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There are six alterations that can be considered fundamental in the cellular physiology and that allow tumor growth, that is, we have self-sufficiency in signs of growth, insensitivity to signs of growth inhibition, evasion of apoptosis, unlimited replicative potential, maintenance of angiogenesis and, finally, tissue invasion and metastasis (Pantaleão, 2010). According to Pantaleão (2010), cancers can be categorized into carcinomas - neoplasms originating from epithelial tissues (epidermoid, when originating from epithelium, and adenocarcinomas from ducts and/or glands), sarcomas (neoplasms originating from connective tissues, (neoplasms originating from the marrow) and lymphomas (neoplasms originating from lymphoid tissues). According to information obtained by Cabral (2016), the American Cancer Society categorizes cancer as a chronic disease, belonging to the group of cardiovascular diseases, diseases that affect the respiratory system and diabetes. Of the diseases responsible for approximately 70% of the world's deaths, only 15% of deaths are caused by cancers, however it would amount to 8.2 million fatalities per year.

World Health Organization (WHO) data released in 2017 report that every year 8.8 million people die from cancer, mostly in low- and middle-income countries - which has shown a resurgence in fatal cases since 2012.The prevalence of cancer cases in countries where income is medium and low is due to the scarce supply of diagnostic and treatment services. This is due to frequent structural health problems in these countries. One of the sectors that suffer the most is the one destined to the services of pathology. By 2015, according to WHO information, approximately 35% of low-income countries reported that pathology services were generally available in the public sector, compared to more than 95% of high-income countries. Breast cancer has the highest prevalence and affects women in many parts of the world, both in developed and emerging countries. This is due to the increasing longevity of the world population, which would increase the incidence of this neoplasm (Torre, SiegeL *et al.,* 2016In the year 2015 in Brazil, breast cancer was responsible for 18 thousand deaths (WHO, 2015). In addition, according to information from the World Health Organization (WHO, 2015), the spread of deaths is a global phenomenon, since 15 years ago the number of deaths did not exceed 6.9 million individuals, increasing to 8, 1 million in 2010 and 8.8 million in 2015.According to the WHO, the 22.2% increase in the number of fatalities due to cancer worldwide since the beginning of the century is one of the largest ever recorded by modern medicine. Currently, one in six deaths in the world is caused by cancer (WHO, 2017).

Experimental model of the murary mummy carcinoma (4t1 luc)

In recent years, a considerable number of researches have been devoted to the analysis of processes that seek to understand the dynamics of tumors. In this sense, efforts have been developed to try to find a cure for conditions that have cancer as the main etiological agent. As far as knowledge about carcinogenesis is concerned, most come from the analysis of tumor tissues in vitro, specifically those in very advanced stages of development, withdrawn from patients (PANTALEÃO, 2010).
To understand the dynamics of the processes associated with neoplastic progression, several experimental models have been proposed. Studies with mice stand out as the main experimental model used to represent the wide variety of events that occur in the development of human breast cancer.The advantages of its use in experiments, is the small size, which facilitates its lodging and manipulation*.,* fast reproduction and long service life*.,* complete sequencing of its already available genome*.,* manipulation with great ease and finally, share many physiological similarities with humans (Manning, Buck et. al., 2016). There are currently 32,000 murine lines, including the isogenic, non-isogenic, mutant, recombinant, among others. Each lineage is suitable for a specific study type (Eppig, Motenko *et al.,* 2015, Cabral, 2016*.,* Pantaleão, 2010). With respect to breast cancer, different murine lines and experimental models have been used in research. The most commonly manipulated models are spontaneous ones*.,* spontaneous mutations*.,* induced by chemical, physical or biological carcinogenesis*.,* transplantable xenografts and isogenic transplantable (Alvarado *et al.,* 2017).
Spontaneous models are easily recognized by the natural emergence of breast tumors - a process totally exempt from human interference. In this context there are some strains of mice such as C3H (Mao, QU *et al.,* 2014), and Kunming females (Zheng, Zhou *et al.,* 2014).
However, spontaneous models are not only limited to mice, but are also used in bitches (LIU, XIONG et. al. 2014) and felines WIESE, Thaiwong *et al.,* 2013*.,* Kojima, *et al.,* 1996*.,* Semi *et al.,* 2007). In spontaneous models induced mutations, their origin is due to the advance in the area of genetic engineering. Such mutations are caused in embryos for the formation of genetically modified species, which are subsequently cross-linked to constitute animals in homozygosis or heterozygosis of the induced gene transformation. This genetic mutation is able to generate tumors spontaneously during the growth process of the animal, since the mutations are directed to genes known to be associated with mammary carcinogenesis (Pantaleão, 2010).
Of the previously cited models that are intended to resemble what occurs in humans, there are 634Mul (Wilson, Bachawal *et al.,* 2014) and C3 (1) / SV40Tag (Steiner, Davis et al. 2014). Other genetically modified models have also been used. Many of them are knockout for the genes of interest and thus allow the functional study of this specific gene product against a transplanted tumor (Dranoff, 2011). According to Pantaleão (2010), among the experimental models of cancer, murine mammary carcinoma 4T1 is one of the most used models in the examination and a better understanding of the biological typology of the tumors. This model is recognized by the aggressiveness of its action, being a cell line deeply tumorigenic and hostile, in which metastases occur in several organs of the affected organism. In relation to the morphological aspect of the tumor, murine mammary carcinoma 4T1 is characterized by malignant epithelial propagation in solid fit, with propagation of pleomorphic cells and mitotic pattern.

Effect of cyanoid acetone in tomorrow's remission

Ramalho *et al*. (2010) analyzed the antitumor effect of acetone cyanohydrin on Ehrlich ascites tumor cells and human lymphocytes in vitro. It was possible to verify a cytotoxic effect dependent on these tumor cells, where, in the concentrations of 20 and 30 Ug.ml-1, 100% of cell death occurred in only 1 and 2 hours. At lower doses of 0.5-1.0 and 2.0 Ug.ml-1, the cytotoxic effect was less intense, increasing gradually with time. At low concentrations of 0.5-1.0 and 2.0 Ug.ml-1, more than 90% of cell death was observed only after 24 hours of incubation. Such results evidenced the ability of the tumor cell to be intoxicated cumulatively with acetone cyanohydrin. These results were compared with the results presented by human lymphocytes that, at the same doses and in the same incubation times, reached a maximum of 30% of cell death. In this study, theauthorssuggestthatthereis a differentiate drhodanaseactivitybetweenthetwocells. The action of acetone cyanidrin is based on its cytotoxic metabolite that acts in a lethal manner in the cancer cell, taking advantage of the failure of the cyanide metabolism in the cells, due to a reduced action of the enzyme rhodanase that makes the detoxification of the cells. The failure of rhodanase would be triggered by a lack of substrate for the reaction, known as sulfane sulfur, responsible for providing sulfur for the removal of cyanide in the form of thiocyanate. The authors Iciek and Wlodek (2001) reported that the regression of transplanted tumors in mice and tumor inhibition induced by carcinogens through the use of different precursors such as sulfane sulfur, confirm the above hypothesis. A perfectenvironment for cumulativecyanidepoisoningisprovided in cancercells, becauserhodanaseisonly residual.

Literature reviews contain important information such as characteristics of sulfane compounds and their affinity for cyanide reactivity, as well as evidence of sulfur metabolite in malignancy processes, cystathione deficiency in many malignant cells, and differences in the metabolism of hemocysteine between normal cells and malignant. In addition, they also evidence important information regarding the defect in sulfur metabolism in cancer cells, together with the in vivo antitumor effects of sources of sulfanesulfer. This information apparently demonstrates that the uncontrollable proliferation of malignant cells may be related to a deficiency of elements such as sulfur and to an uncontrollable action of an enzyme set that is normally inactivated by it. Tumors with high growth rates may have lower sulfur transferase activities (rhodanase, for example) than normal tissue or tumors with low growth rates.
In Ehrlich's Ascitic Tumor, cyanogenic chemotherapy using acetone cyanohydrin as a source of cyanide was able to promote a reduction in tumor volume and tumor cells, as well as prevent its development in 20% of treated animals. In the Ehrlich Solid Tumor, it was able to induce an inhibition in tumor growth, in addition to producing an increase in necrosis area (Ramalho, 2014). Studies using other active principles present also have been successful in remission of Ehrlich's Tumor.

Fukumasu (2008) investigated the action of guarana on the progression of the tumor in question and according to the results there is a considerable deceleration of cancer cells in mice fed guarana for seven days before having the cancer induced by the researchers., and another 14 days after establishment of the disease. Authors such as Bicalho (2010) and Mijan (2012) also identified tumor remission through the use of Aluminum-Chloro-Phthalocyanine (AlClFt) -mediated Photodynamic Therapy in liposomal formulation. The analyzes showed that in 100% of the animals treated with PDT, it was possible to detect the complete remission of the tumors initially induced. In the histopathological analyzes carried out by the aforementioned researchers, it was observed that PDT acted ostensibly in tumor elimination by the induction of cytotoxicity. In fact, such evidence leads to an intense necrosis process. The process in question was able to elute the tumor cells, however, resulted in the destruction of adjacent muscle tissue. Nevertheless, after 21 days after the end of the PDT, the actuators observed the healing process, in which the muscle tissue replaced by connective tissue.

Thus, Photodynamic Therapy was effective in complete remission of the tumors induced in the languages of Balb-c mice from cellular suspensions of Ehrlich's ascites tumor (Bicalho, 2010). Ehrlich's tumor is defined in the specialized literature as a transplantable neoplasm of malignant epithelial origin, resembling a mammary adenocarcinoma of female mice. It was introduced in 1986 by Ehrliche and described in 1906 as a mammary carcinoma of female mice. Initially, the tumor was experimentally developed in solid form and transplanted into animals of the same species (Dagli, 1989). But in 1932, Loewenthal and Jahnpresented theascitic form, that is, the form developed in the peritoneum of animals inoculated with tumor cells. Ehrlich's tumor is a model characterized by practicality, being widely used in antineoplastic analysis because of the reduced time interval for study, since the evolutionary progression of the neoplasia occurs very quickly, and also by the existence of previous knowledge of the amount of cells of the tumors inoculated in animals (Ramalho, 2014).

There are several studies in the literature concerning the use of cyanogenic substances, such as linamarin and amygdalin (Chen, *et al.,* 2013., Fukuda *et al.,* 2003., Jukes, 1976., Kousparou *et al.,* 2002., Lagman., Navarro, 1957., Stock *et al.,* 1978.,Swiniarski., Wodinsky, 1975., Syrigus *et al.,* 1998). However, an equally large number of articles with controversial issues associated with their use can be found (Greenberg, 1975., Khalil., Moss, 1981., Saber, 1977., Vickers *et al.,* 2006). After histopathological evaluation of the morphological alterations caused by acetone cyanohydrin in the heart, lung, liver and kidneys of healthy mice, Ribeiro (2012) concluded that there were morphological alterations of the type cell injury that lead to apoptosis, as well as cell adaptation, characterized by degeneration of the tubules recurrences and decreased size and number of renal glomeruli. In the lung, areas of fibrosis, presence of macrophages in the alveolar space, thickened septa and structural disorganization were found. There was widespread disorganization in liver structure, hepatocytes of varying sizes and loss of nucleus. And, in theheart, presenceoffatcells, hemorrhageandfibrosis. As shown above, researchers demonstrate the action of cyanide from acetone cyanidrin at alkaline pH, exploiting sulfanesulfur deficiency in malignant cells and promote irreversible intoxication with minimal effect in other normal cells, predicts the discovery of new substances for the treatment of cancer (Ramalho, 2014)

Conclusion

According to the survey carried out in this manuscript, it is notable that there is a possibility of remission of the Ehrlich Tumor by means of some substances, such as cyanide acetone, guarana and the technique of Photodynamic Therapy mediated by Aluminum-Chloro-Phthalocyanine (AlClFt) in liposomal formulation. The results make it clear, although obtained from animal experiments, that some types of cancer may be completely suppressed, or at least have their effects diminished considerably.

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