

Breast cancer: Metronomic therapy focused on muscarinic acetylcholine receptors

María Elena Sales*

Centro de Estudios Farmacológicos y Botánicos (CEFYBO)-CONICET, 2da Cátedra de Farmacología, Facultad de Medicina, Argentina

Muscarinic receptors in breast cancer

Muscarinic acetylcholine receptors (mAChRs) belong to the G protein coupled receptor group (GPCRs) and together with nicotinic receptors formed the cholinergic receptor family [1]. Both types of receptors respond to acetylcholine but trigger different signaling pathways since the first one exerts metabotropic actions while the latter is ionotropic. Several authors reported the expression of mAChRs in tumor cells and tissues [2]. Five subtypes of mAChRs (M_1 - M_5) have been cloned. Their activation by acetylcholine triggers not only the activation of classical signal transduction pathways like phospholipase C/inositol triphosphate/calcium for M_1 , M_3 and M_5 subtypes and the inhibition of adenylyl cyclase for M_2 and M_4 subtypes; but may activate also non-canonical signals like Ras-Raf-1-Erk-AKT for M_3 receptor subtype [1,3].

We described, for the first time the presence of different subtypes of mAChRs in murine and human breast tumors. The five subtypes of mAChRs are expressed in three different mammary tumors (LM2, LM3 and LMM3) that spontaneously aroused in BALB/c mice, but they are absent in normal murine mammary epithelial cells (NMuMG) [4-6]. Meanwhile, the pattern of expression of mAChRs in human mammary breast tumors differs depending on the cell line analyzed. MCF-7 cells express M_3 and M_4 receptor subtypes while MDA-MB231 cells exhibit M_1 , M_2 , M_4 and M_5 receptors expression [7,8]. Similarly to that observed in mice, mAChRs were not detected in the human non-tumorigenic mammary cell line, MCF-10A. Preliminary results from our laboratory demonstrate that the transfection of MCF-10A cell with mAChRs induces the malignant transformation promoting three dimensional growth in vitro and angiogenesis and tumor generation in nude mice [9].

We also reported that the activation of mAChRs with the synthetic non-degradable agonist carbachol, for short periods of time stimulates tumor cell proliferation via different metabolic pathways [10]. In MCF-7 cells, the stimulatory action of carbachol on cell proliferation was mediated mainly by M_3 receptor inactivation that triggers phospholipase C/nitric oxide synthase (NOS) pathway [7].

On the contrary, the addition of the agonist for longer periods of time reduced cell viability either in mice or in human tumor breast cell lines [11,12]. The latter was also reported for the treatment of brain tumor cells with the selective M_2 receptor agonist arecaidine propargyl ester (APE) [13].

Muscarinics as repurposing drugs in metronomic therapy

The results mentioned above indicate that muscarinic agonists may act as anti-tumor drugs, and should be considered as repositioning drugs. The latter refers to the assignation of new uses for existing drugs

and represents an alternative drug development strategy. In oncology, there is an increasing interest in the prescription of non-cancer drugs for cancer treatments due to the knowledge of pharmacokinetics/dynamics, side effects, and because most of them are available at low cost [14]. Many repurposing drugs have been studied to be used in breast cancer treatment: β_2 adrenergic receptor blockers [15], the anti-diabetic drug metformin [16] or the PPAR γ -ligand, pioglitazone [17]. Another important aspect of chemotherapy in cancer treatment is its schedule of administration. Traditional chemotherapy is based on the usage of cytotoxic/cytostatic drugs administered at maximal tolerable dose in order to kill as much tumor cells as possible, although as it is well known this treatment causes severe side effects. Based on this background Hanahan et al. in 2000 devised a different way of administering chemotherapy named metronomic therapy (MT) [18]. The term MT was used by Hanahan referring to the “close, regular administration of a chemotherapeutic drug for a long time with no extended drug-free breaks” [18]. It should be useful as a strategy to break resistance to drug treatment and also to target the tumor vasculature besides the tumor cells. Phase II/III studies for the treatment of triple negative breast cancer patients are currently being developed with encouraging results [19,20]. MT is not only applied with traditional drugs alone but also combined with repurposing drugs. Roy et al., [21] have recently demonstrated that the addition of suboptimal concentrations of the glycolytic metabolite, methylglyoxal in a combination regimen with traditional chemotherapeutic drugs against breast cancer like doxorubicin or cisplatin at metronomic concentrations produces a synergistic increment in doxorubicin and cisplatin mediated cytotoxicity in human breast cancer cell lines MDA-MB231 or MCF-7. This treatment promoted apoptosis or necroptosis in tumor cells respectively and also reduced breast cancer stem cells in both cell lines [21].

In our laboratory we, designed a combination of the muscarinic agonist carbachol plus the chemotherapeutic agent paclitaxel, usually administered for breast cancer treatment scheduled as MT. The administration of carbachol plus paclitaxel at sub threshold concentrations induced MCF-7 cell death by up-regulating NOS3 expression and reducing arginase II activity [12]. This kind of treatment also reduced de percentage of cancer stem cells (CD44+/CD24-) in MCF-7 cells [22]. This result could be advantageous to prevent tumor

*Correspondence to: María Elena Sales, Centro de Estudios Farmacológicos y Botánicos (CEFYBO)-CONICET, 2da Cátedra de Farmacología, Facultad de Medicina, UBA, Argentina, E-mail: malegazpio@yahoo.com.ar

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metastasis and recidivas. In addition, we observed that this metronomic combination was also effective on MDA-MB231 cells and on murine breast cancer cells promoting more apoptosis than necrosis [11]. Moreover, we observed that if paclitaxel is replaced by doxorubicin in the combination, a significant cytotoxic effect is also obtained on both MCF-7 and MDA-MB231 cells (unpublished results). Further in vitro and in vivo studies should be done to demonstrate beneficial effects of MT focused on mAChRs in pre-clinical studies for further insurance positive effects on breast cancer patients.

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