Andrographolide and its Attributes Against Triple-Negative Breast Cancer: A Review of the Literature

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Abstract

Breast Cancer is associated with high incidents and mortality rates and considered to be the second most frequent type of cancer among women worldwide. One of its invasive modalities is the Triple-Negative subtype. Lacking the expression of ER/PR and HER2 contributes to its severity. Current treatment strategies for TNBC diagnosed patients are believed to be hazardous and do not improve the disease-free survival (DFS) neither the overall survival (OS). Thus, scientist and researchers are seeking alternative remedies that have little or no consequences on the patients. *Andrographis paniculate* with its remarkable active constituent Andrographolide are attracting the world's attention due to its pharmacological activities against metabolic maladies especially hyperlipidemia, obesity, and diabetes as well as its attribute as an anti-cancer compound. Here, we review the effect of Andrographolide on Triple-Negative Breast Cancer.

Keywords: *Andrographolide; Breast Cancer; Triple-Negative Breast Cancer; Alternative and Complementary Treatment; Andrographis paniculate.*

Introduction

Estrogen, progesterone, and HER2 are the hallmark for the presence of a Breast Tumor, it serves by mimicking the receptors found in the normal breast tissues and sends signals to cells permitting its growth. Nearly 50% of Breast tumors express ER, PR, and HER2 but not in case of the TNBC, in which the expression of the biomarkers is idle [26]. TNBC severity exceeds the positive BC. It has a high risk of re-occurrence and death [20] and can metastasize viscerally to other organs (lymph nodes, bilateral lungs, and the liver) [26]. Metastatic TNBC has a low life expectancy rate and it does not exceed 12 months in spite of the response to chemotherapy [8]. Annually, from 1.3 million Breast Cancer patients 15-20% will be identified as Triple-Negative Breast Cancer subtype [2]. The survival rate of TNBC varies depending on the stage of the disease. Stage 0 and 1 have a 100% chance of survival, stage 2 and 3 have 93%, 72% survival rate respectively. Metastatic TNBC patients have 22% [9].

A. paniculata is part of *Acanthaceae* family, a herbal plant natively found in Sri Lanka and India and it is also found in the southeast of Asian, America, and China [16]. *A. paniculata* has been used traditionally in treating various types of diseases like fever, dysentery, diabetes, and malaria [12]. Andrographolide is the major active constituent of in *A. paniculata*, it belongs to the labdane diterpenoid chemical class. Andrographolide possess many pharmacological properties, it has anti-inflammatory, antihyperglycemic, antimicrobial, antiparasitic and anticancer effects [12].

Current treatment strategies for Triple-Negative Breast Cancer (TNBC)

Chemotherapy (CT) remains the standard strategy for treating TNBC [13]. Despite the fact that TNBC is aggressive in nature, yet, it shows a higher vulnerability to Chemotherapy than any other types of Breast Cancers [25]. Neoadjuvant systemic treatment (NST) chemotherapy is used for those who are at the early stages of TNBC and willing to undergo BCS [17]. frequently used neoadjuvant agents are anthracycline alone or in combination with taxane (anthracycline-taxane CT) [23]. In adjuvant settings, anthracycline-taxane regiments show small but important improvement in disease-free survival (DFS) [6].

Breast Conservative surgery is advised for patients with early stage of TNBC alongside adjuvant RT [10]. One of its advantages is the low rate of local recurrence, but, it does not imply on all types of Breast Cancer. [25]. Mastectomy is wanting to BC because of the better outcomes of BCS, in terms of overall and disease-free

survival rates [7].

Radiotherapy, as reported by Langlands *et al,* has little influence on TNBC and can withstand it. Although most of TNBC tumors are associated with BRCA1/2 mutations which means it carries DNA repair defects, however, it showed no significant sensitivity to RT[15]. A contrasting study suggested otherwise, in which RT can enhance survival outcomes if implemented with surgery [28] and it could provide ideal tumor management with trivial side effects [14].

Targeted therapies have shown its efficacy in targeting the tumor's cell cycle, gene defects, and growth factors. Affecting cell cycle regulators (CDK4/6) can be tricky in the case of TNBCs since not all express cell cycle regulators [19]. But, a different study tested palbociclib (CDK inhibitor) on Luminal Androgen Receptor (LAR) a subtype of TNBC and exhibited high sensitivity towards it (*in vitro* and *in vivo*) [3].

Another potential targeted therapy candidate is the tumor suppresser gene (p53) through checkpoint kinase 1(CHK1). Since the majority of TNBCs are Basal-Like tumors that carry gene defects in P53 [24]. Bryant *et al,* reported that TNBC cell line can be inhibited through arresting cell cycle at the S and G2/M phases mainly by inhibiting CHK1 through the activation of P53 [11].

Targeting growth factors such as FGFR, EFGFR, and VEGFR can beneficial in eliminating TNBC since they are the causative agent for the overgrowth of the tumor [4]. Drugs such as cetuximab (anti-EGFR antibody), PD173074 (anti-FGFR inhibitor) and bevacizumab as a VEGFR inhibitor currently being tested on TNBC [21].

Andrographis paniculate

A. paniculata is part of *Acanthaceae* family, a herbal plant natively found in Sri Lanka and India and it is also found in the southeast of Asian, America, and China [16]. *A. paniculata* is a herbal grows annually and extend about half to a meter above the soil, it is known for its bitter taste hence the name King of Bitter [16]. *A. paniculata* has been used traditionally to cure diseases like fever, dysentery, diabetes, and malaria [12]. In Asia, the roots and the aerial parts have been used to treat inflammation, pyrexia, intermittent fevers and stomach-aches [16]. The essence of leaves is used to treat fever, infectious diseases, colic pain and diarrhea [16]. the aerial part of the plant is used in Malaysia to treat snakebites and malaria [16].

The active compounds are distributed throughout the parts of the plant for instance terpenoids. The most isolated terpenoid from the plant is Andrographolide in terms of quantity and occurrence [16]. Andrographolide after it is isolated, it appears crystalline and colorless and has a bitter taste [16]. Apart from Andrographolide, neoAndrographolide and deoxyAndrographolide are also common terpenoids isolated from the aerial parts and the roots [16].

Flavonoids such as Flavone-1, 2′methylether, 7-O-methylwogonin and flavone-1, 2′-O-glucoside are found in the whole body, roots and the aerials of *A. paniculata* [16]

Andrographolide

Andrographolide is the major active constituent found in *A. paniculata*, it belongs to the labdane diterpenoid chemical class [12]. Andrographolide is the causative agents for the extremely bitter taste of *A. paniculata* [16]. Andrographolide must be harvested after cultivating the plant for 110 days or just before the flowering of the plant [12]. It can be found in several parts of the plant including the roots, leaves, stem, the aerial parts or in the whole plant [16]. As for the process of isolation, it can be achieved through extraction by solvents like ethanol (EtOH), methanol (MeOH), acetone, dichloromethane and chloroform (CHCl3) [12]. *A. paniculata* synthesizes Andrographolide through two pathways, the mevalonic acid pathway or through deoxyxylulose pathway (DXP) [22]. Andrographolide possess many pharmacological properties, it has anti-inflammatory, antihyperglycemic, antimicrobial, antiparasitic and anticancer effects [12].

Anti-Cancer Effect of Andrographolide on TNBC cell lines

Anti-proliferative and viability effect

Several studies suggested that among all TNBC cell lines, MDA-MB-231 was the most susceptible type of TNBC cell line towards Andrographolide [27, 5, 18]. Weber *et al, proposed that* 39.6μg/mL *of Andrographolide was cap*able of eliminating 50% of cells, meanwhile, a higher dose of Andrographolide (77.6μg/mL) was able to inhibit MDA-MB-468 population [27]. A different report was done by M Banerjee *et al, where they* implemented Andrographolide on multiple BC cell lines (MCF-7, MDA-MB-231, and T-47D), and used MCF-10A (normal breast tissue cell line) to examine its effect on the normal breast epithelia. The pattern of exposure

to Andrographolide was in both time and concentration-dependent manners. MDA-MB-231 cell line was found to be the most vulnerable amongst the other cell lines, and the exposure to the treatment introduced inhibitory effect on both viability and propagation of cells. The IC_{50} was estimated to be 51.98μM at 24 hrs. and 30.28μM at 48 hrs. Worth mentioning that these values did not cause any effect on the MCF-10A, however, to introduce an effect the needed concentrations must be 3-5 folds higher [5]. Similar outcomes were reported by Peng *et al*, in which approximately 50μM of Andrographolide eliminated MDA-MB-231 population [18].

The induction of cell cycle arrest

Andrographolide has the ability to induce cell cycle arrest as reported by several studies. *Increase in cellular population was reported by* M Banerjee *et al, at S phase and a decrease occurred at G1/G0 accompanied by* the formation of Reactive Oxygen Species (ROS), the increase in ROS was proportional to a longer time of exposure and might indicate that the inhibition was due to ROS accumulation. *Different results were suggested by another study, the increase in population happened at the G1 and the decrease* was noticed at the S phase. Downregulation in the levels of P27 (G1 phase inhibitor) and CDK6 were detected, and no accumulation in ROS was observed. [27], [5]. *While in a study done by* Alqouqa *et al, the arrest occurred at G_2/M phase, followed by downregulation of Cyclin D1, CDK4, Cyclin E and P-cdc2, and upregulation of P21, Cyclin B1 and Waf1/Cip (cell cycle regulators proteins)* [1].

The introduction of cellular and nuclear morphological modifications

Nuclear morphological modifications were described as well, nuclear pyknosis, chromatin condensation, irregular edges and body fragments formation occurred during the time of exposure [5].

Mitochondrial and apoptotic effects

Release of cytosolic cytochrome c from the mitochondria of MDA-MB-231 was recorded. The release might be caused by the changes in the ratio of apoptotic and anti-apoptotic proteins (Bax/Bcl-2), whereas Andrographolide elevated their expression [5]. In the same fashion, Peng *et al.* detected the translocation of cytochrome c from the intermembrane to the cytosol. Bcl-2, Bax and cleaved caspase-3/9 were spotted after 24 hrs. dosage of Andrographolide [18]. Caspase-3/9 activation correlates with the finding of M Banerjee *et al*, [5].

Conclusion and Future Directions

Breast Cancer still remains a threat to women around the world. Despite technological advancement and the increase in awareness, many patients are not transitioning from ill to healthy.

The undenied fact that the current treatments (Radiation Therapy (RT), Chemotherapy (CT) and Adjuvant Endocrine) have proved its effectiveness. But, its complications on the patients are treacherous and the medical community is due to overcome it.

Andrographolide affects TNBC cells through multiple pathways such as the arresting of the cell cycle, inducement of cellular and nuclear morphological changes, upregulation apoptotic and anti-apoptotic proteins (Bax/Bcl-2). Involving Andrographolide in animal or human trials could elucidate its potential as an anti-cancer agent and provide knowledge and assessment for the future references.

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List of Symbols and Abbreviations

Abbreviation

BAX: BCL2-Associated X protein

Bcl: B-Cell Lymphoma

BCS: Breast-Conserving Surgery

BL1: Basal-Like 1

BL2: Basal-Like 2

BRCA: BReast CAncer gene

CDC: Cell Division Control protein.

CDK: Cyclin-Dependent Kinase

CHK: Checkpoint Kinase

CT: Chemotherapy

DFS: Disease-Free Survival

DNA: DeoxyriboNucleic Acid

EGFR: The Epidermal Growth Factor Receptor

ER: Estrogen Receptor

ET: Endocrine Therapy

HER2: Human Epidermal Growth Factor Receptor

HR: Hormone- Receptor

Hr: Hour

LAR: Luminal Androgen Receptor

M: Molar

MCF7: Michigan cancer foundation-7

ml: Milliliter

mm: Millimeter

OS: Overall Survival

PR: Progesterone Receptor

RT: Radiotherapy

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