INFLUENCE OF SELECTED TRITERPENOIDS ON CHEMOPREVENTION AND THERAPY OF BREAST CANCER

Minireview

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Abstract

Breast cancer is hormone signaling failures-related disease and the second leading cause of female cancer mortality. Among plant-based agents, triterpenoids have emerged as a promising group of phytochemicals with proposed anti-cancer activity. They are the major components of some oriental and traditional medicine herbs wildly distributed all over the world. Triterpenoids are relatively non-toxic and could be used as chemopreventive / chemoprotective agens in clinical praxis. Numerous studies using cell culture assays and animal models of cancer have revealed, that triterpenoids hold great potential not only in the therapy of breast cancer but also in preventing this disease. However, further studies are needed to explore the full potential of these agents.

Keywords: triterpenoids, breast cancer, chemoprevention

1 Introduction

Breast cancer is the leading worldwide cause of death among women between the ages of 40 and 55 [1]. Screening is not offered to younger or older participants, because the benefits in these age groups do not outweigh the harms. One could argue that it is not so much age that determines the benefits but the risk of developing preclinical and treatable cancer. Cancer risk varies with age but is also affected by other factors [2].

Breast cancer is known to be influenced by several hormones, specifically by estrogen and progesterone which are known to be capable of increasing breast cancer risk. Approximately two-thirds of breast cancers are positive for estrogen and/or progesterone receptors. In premenopausal women, the primary source of estrogen is the ovaries and in postmenopausal women, estrogen is produced in adipose tissue and the adrenal glands. The enzyme aromatase plays a critical role in the production of estrogen in postmenopausal women [1].

At present, tamoxifen is widely used for estrogen receptor-positive breast cancers [3]. Trustazumab is used in HER2/neu-positive breast cancers and have proven benefits [4]. Nevertheless, these drugs carry significant adverse effects along with their known benefits. Tamoxifen shows a positive effect on bone decreasing the osteoporosis and on the other hand it increases the risk of endometrial cancer and venous thromboembolism [3]. Trustazumab, a monoclonal antibody, has potential concern of severe cardiac dysfunction [1, 5]. Therefore, there is increasing need for searching for novel preventive and therapeutic approaches for this disease, especially for agents which will be effective in decreasing the incidence of breast cancer in high-risk women.

One such strategy to consider is chemoprevention, an approach by which the occurrence of the disease can be prevented, slowed, or reversed by the administration of one or more naturally occurring and/or synthetic compounds [1, 6-9]. Due to generally being an

estrogen-dependent cancer, breast carcinomas are ideal candidates for hormonal and other types of chemoprevention [1, 10]. During the last decade several bioactive agents have been identified in plants and in human diets and are being developed as chemopreventive and therapeutic agents for various cancers including breast cancer [1].

2 Relationship of natural compounds supplementation and breast cancer

Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of developing specific cancers [11, 12]. Furthermore, nutrition is an important part of cancer treatment. Eating the right kinds of foods before, during, and after treatment can help the patient feel better and stay stronger. A large number of epidemiologic data supports the fact that diet and nutrition play a vital role in carcinogenesis [11, 13, 14].

In the past years, both encouraging and discouraging results about the associations between natural compounds supplementation and cancer have been reported to public and scientific community. Their safe and favorable toxicity profile makes them suitable to be investigated in a preventive setting [15].

Evidence for an association between fruit and vegetable intake and breast cancer risk is inconclusive. In meta-analysis of the evidence from prospective studies of fruit and vegetable intake and breast cancer risk until April 30, 2011, high intake of fruits, and fruits and vegetables combined, but not vegetables, is associated with a weak reduction in risk of breast cancer [16]. From the opposite point of view, measurement errors in the dietary assessment of fruit and vegetable intake may attenuate associations with breast cancer risk and might explain the weak associations observed in epidemiologic studies. Aune D. et al. [17] compared dietary intake with blood concentrations of carotenoids and breast cancer risk. They tried to use concentrations of these natural compounds in blood as biomarkers of fruit and vegetable intake. According to their results, blood concentrations of carotenoids are more strongly associated with reduced breast cancer risk than are carotenoids assessed by dietary questionnaires.

The most intensively studied and discussed group of natural compounds connected with chemoprevention of breast cancer is isoflavones. Isoflavones are substances produced by plants. They possess weak estrogenic properties. Their molecular structures are similar with 17-β-estradiol (Fig.1). Hence, the molecular binding mechanisms of estradiol and isoflavones are similar, but their transcriptional potencies are weaker. There are many apparently contradictory results published on the effects of isoflavones on a variety of estrogen-regulated organs point to both beneficial as well as adverse effects on human health [18, 19]. There is dispute as to whether isoflavones derived from soy or red clover have negative, positive or any effect at all on the mammary gland or endometrium. It is beyond any doubt that soy products may have cancer preventing properties in a variety of organs including the mammary gland. However, these properties may only be exerted if the developing organ was under the influence of isoflavones during childhood and puberty. This may also explain the often quoted "Japanese Phenomenon", the fact that breast cancer occurs to a lesser extent in Japanese women. When administered to isoflavone "inexperienced" women at the time of menopause, the phytoestrogens appear to share the same effects as estrogen used in classical preparations for hormone replacement therapy, i.e. they may stimulate the proliferation of endometrial and mammary gland tissue with at present unknown and unpredictable risk to these organs [18, 20].

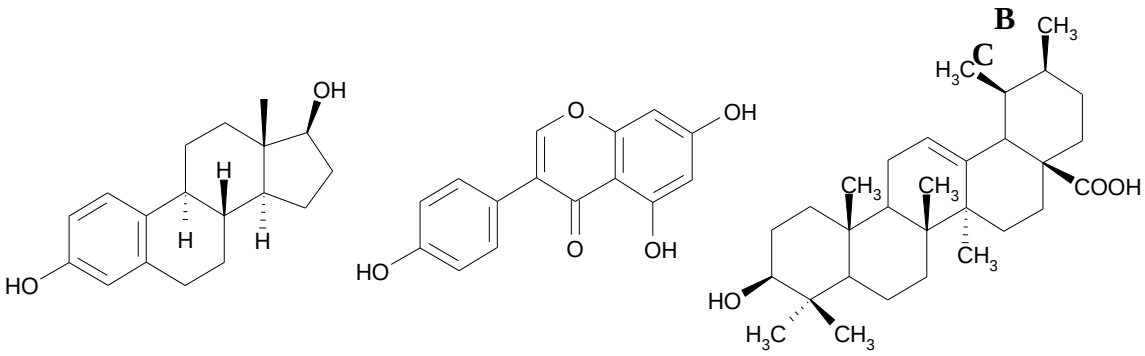

Fig.1 *Chemical structures of A: 17-β-estradiol; B: genistein (isoflavone); C: ursolic acid (triterpene)*

3 Triterpenoids

Triterpenoids are compounds naturally found in various plants such as sea-weeds, the wax-like coatings of fruits and many medicinal herbs, e.g. rosemary, thyme, oregano and lavender. They were long considered to be biologically inactive but in recent years, they have attracted the interest of medical scientists because of their pharmacological effects, combined with a low toxicity [21, 22]. The traditional usages of plants containing triterpenoids in folk medicine are multiple, in terms of anti-inflammatory, hepatoprotection, analgesia, cardiotonic, sedative and tonic effects, etc. Many of these therapeutic effects have been confirmed by contemporary scientific research [21, 23].

Recent evidences support the beneficial effects of naturally occurring triterpenoids against several types of human diseases, including various types of cancers. Anticancer potential of triterpenoids and their anti-inflammatory, anti-proliferative, and pro-apoptotic effects have been investigated both in *in vitro* and *in vivo* models. Importantly, a large number of preclinical efficacy studies using chemically-induced, as well as tumor xenograft models provided evidence that both naturally occurring and synthetic derivatives had chemopreventive and therapeutic effects [24].

As a large number of *in vitro* studies have successfully shown that triterpenoids possess potent cytotoxic effects against several breast cancer cell lines, *in vivo* studies by different laboratories have investigated whether some of these triterpenoids and their semi-synthetic analogs lead to positive outcomes in preclinical animal models of breast cancer. Most of these studies have utilized tumor growth in immunocompromised mouse model whereas a few investigators have used the chemically-induced mammary tumor development protocol [1].

Triterpenoids exert a plethora of biological activities including suppression of inflammation, reduction of oxidative stress, regulation of cell cycle, inhibition of cell proliferation, induction of apoptosis, and interaction with tumor microenvironment through modulation of multiple signal transduction pathways. In essence, this could explain, at least in part, their antineoplastic properties in breast cancer. According to a recent landmark study, mutations in nearly 200 genes have been detected in human breast and colon cancers showing the genetic complexity of these specific neoplastic diseases [25]. With this background, agents like triterpenoids that can alter multiple dysregulated cellular pathways may have a significant potential for breast cancer prevention and control [1].

Scanning of pertinent literature reveals that although there are a large number of *in vitro* studies demonstrating the cytotoxicity of triterpenoids against various breast cancer

cells, only a few compounds have yet been evaluated in preclinical animal models of breast cancer. One of the reasons for the limited number of preclinical breast cancer studies on triterpenoids, including lack of *in vivo* studies on agents which have already showed efficacy in cell culture systems, could be due to the fact that most of the triterpenoids are insoluble in aqueous media limiting their bioavailability in the body which is very important for *in vivo* efficacy. One approach to enhance the water solubility of triterpenoids could be the structural modification of naturally occurring compounds to generate more polar analogs. Several other possibilities of improving the hydrophilicity of triterpenoids include design and generation of formulations containing cyclodextrin complexes, liposomes, colloids, micelles as well as nanoparticles [1, 26-28].

4 Supposed molecular mechanism of action of selected triterpenoids

Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid; UA) and its isomer oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid; OA) are common phytochemicals, which are naturally found in various plants and many medicinal herbs, and thus their distribution in human diet is extensive [11]. Several studies suggest that UA has potent cancer-preventive activity and great therapeutic potential [11, 23]. After UA treatment, breast cancer cells MCF-7 exhibited typical apoptotic features, including chromatin clumps and aggregation and DNA fragmentation, which was in correlation with the down-regulation of Bcl-2 and up-regulation of caspase-3 [29]. In human breast cancer cell line MDA-MB-231, UA decreased cell proliferation rate and induced apoptosis by inducing various apoptotic molecules related to either extrinsic or intrinsic apoptosis signal pathway. In MDA-MB-231 cells UA induced the appearance of Fas receptor and cleavage of caspase-8, -3 and PARP. Moreover, UA induced Bax up-regulation and Bcl-2 down-regulation and release of cytochrome C to the cytosol from mitochondria [30]. Bcl-2 down-regulation may be therefore one of the molecular mechanisms by which UA induces apoptosis [31]. OA and UA isolated from the leaves of *W. tomentosa* inhibited cell proliferation of MCF-7 and MDA-MB-231 cells, whereas there was devoid of significant cell inhibiting activity in non-cancer originated cells, HEK-293. In both MCF-7 and MDA-MB-231, OA and UA induced cell cycle arrest and apoptosis [32]. In another study, OA showed significant cytotoxic effect on human MCF-7 breast cancer cell line. OA inhibited proliferation probably by cell cycle arrest [33]. Moreover, both UA and OA showed significant inhibition on parent and multi-drug resistance breast cell lines MCF-7 and MCF-7/ADR possibly by down-regulation of expressions of apoptosis antagonistic proteins, Bcl-2 and Bcl-XL [34].

Celastrol (3-hydroxy-9β,13α-dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oic acid) is a triterpene isolated for example from the traditional Chinese medicine "God of Thunder Vine" or *Tripterygium wilfordii Hook F*. used for the treatment of cancer and other inflammatory diseases [35]. Celastrol inhibited approximately 60% of tumor growth in breast cancer xenograft through NF-κB inactivation including inhibition of its DNA-binding activity and inhibition of IκB-α degradation which was induced by TNF-α or phorbol myristyl acetate. Further investigation showed that celastrol suppressed the NF-κB activation by targeting cysteine 179 in the IκB-α kinase [31, 36]. Celastrol was able to potentiate apoptotic effects induced by TNF and chemotherapeutic agents. It was also able to inhibit the TNF-induced activation of IκB-α kinase, IκB-α phosphorylation, IκB-α degradation, p65 nuclear translocation and phosphorylation, and NF-κB-mediated reporter gene expression [31, 37]. Finally, celastrol treatment suppressed expression of several TNF-induced genes that are involved in anti-apoptosis, proliferation, invasion, and angiogenesis [31]. Celastrol is also a potential agent for clinical use in preventing the invasion and metastasis of human malignant breast tumors. It inhibits for example NF-κB-mediated MMP-9 expression, resulting in

suppression of MCF-7 breast cancer cell invasion and migration that is induced by 12-myristate 13-acetate [38].

Pristimerin (3-hydroxy-9β,13α-dimethyl-2-oxo-24,25,26-trinoroleana-1(10),3,5,7-tetraen-29-oic acid, methyl ester) is a natural analog of celastrol which was isolated from Celastrus and Maytenus spp. Previous studies have shown that pristimerin has anti-inflammatory activity and can inhibit tumor cell proliferation by inhibiting the NF-κB pathway and cell cycling. In addition, prestimerin has been reported to induce caspase-dependent apoptosis of breast cancer cells [39]. Pristimerin-triggered caspase activation was observed in human breast cancer cells. MDA-MB-231 cells treated with pristimerin showed rapid induction of apoptosis through caspase activation. Treatment of breast tumor cells with pristimerin resulted in a rapid release of cytochrome c from mitochondria, which preceded caspase activation and the decrease of mitochondrial membrane potential. This process did not depend on Bcl-2 family (Bcl-2, Bcl-XL and Bax) protein levels and does not require translocation of Bax to the mitochondria [31, 40].

Withaferin A (4β,5β,6β,22R)-5,6-Epoxy-4,22,27-trihydroxy-1-oxoergosta-2,24-dien-26-oic acid; WA) is derived from the medicinal plant *Withania somnifera*, which has been used for over centuries in Indian Ayurvedic medicine and as a dietary supplement in the United States recently [31]. WA inhibits growth of MDA-MB-231 and MCF-7 human breast cancer cells in culture and MDA-MB-231 xenografts *in vivo* in association with apoptosis induction, but the mechanism of cell death is not fully understood [41]. Suppressed human breast cancer growth by WA, is correlated with apoptosis induction characterized by DNA condensation, cytoplasmic histone-associated DNA fragmentation, and cleavage of PARP. WA-mediated DNA fragmentation was significantly attenuated by knockdown of protein levels of Bim and its transcriptional regulator forkhead box O3 in breast cancer MCF7 and MDA-MB-231 cells [31].

5 Conclusion

This minireview highlights the role of natural compounds and especially triterpenoids in chemoprevention of breast cancer. Among these plant-based agents, ursolic acid, oleanolic acid, celastrol, pristimerin and withaferin A are promising phytochemicals that selectively kill breast cancer cells with a pleiotropic mode of action while sparing normal cells. From numerous studies using cell culture assays and animal models of cancer, it is clear that triterpenoids hold great potential not only in preventing of a wide variety of breast cancers but also in the therapy these diseases. Further investigation to explore their full biological potential may prove to be worthwhile.

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