Phytochemicals in diets for breast cancer prevention: The importance of resveratrol and ursolic acid

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Abstract

Breast cancer is the second leading cause of death from cancer in women in the United States. A growing emphasis is being placed on alternative medicine and dietary approaches toward prevention of potential diseases. Phytochemicals are bioactive compounds that are naturally present in foods that, when acting in synergy, bestow potential anti-cancer properties. Resveratrol, a phytoalexin, and ursolic acid, a pentacyclic triterpenoid, are two bioactive compounds that are at the forefront in scientific research. Previous animal studies have documented the anti-cancer properties of resveratrol on breast cancer cells and research groups have recently been able to identify the anti-cancer, anti-inflammatory and induction of apoptosis properties of resveratrol along with the signal transduction pathways that the compound affects. Ursolic acid has been cast into the limelight with the recent discovery documenting its anti-inflammation and anti-cancer activities by targeting signal pathways, especially in the prevention of breast cancer.

Keywords: Phytochemicals; Cancer; Resveratrol; Ursolic acid; Diet and cancer

1. Introduction

Cancer along with cardiovascular disease (CVD) are two of the leading causes of death in the United States; the prevalence of which is also seen at higher rates in developed and industrialized countries. According to the 2010 Cancer Statistics [1], an estimated 1.5 million individuals will be diagnosed with different forms of cancer. The most prevalent form of cancer in men is that of the prostate, accounting for 28% of the total cases diagnosed, with lung cancer being the second most probably at 15%. In the cases of women, breast cancer is the leading cause accounting for 28% of the total individuals diagnosed, with lung cancer being the second most probably at 14%.

Traditional cancer treatments including chemotherapy, radiation therapy, surgery, immunotherapy and biologic therapy are regularly used in treating cancer at various stages. Though they offer an effective way to reduce cancer, the patient often suffers from numerous side-effects. This has led to the emergence of alternate forms of cancer treatment such as nutrition therapy, which serves to fight cancer through a healthy diet while presenting none of the side-effects often encountered by patients undergoing treatment.

Strong associations between high dietary intakes of fruits, vegetables and whole grains, with a reduced risk in chronic disease development have consistently been reported in numerous epidemiological studies [2–4]. It has been estimated that 1/3 of all cancer related deaths in the United States can be avoided through appropriate dietary modification; this correlation between diet and health suggests a change in dietary behavior, through an increase in consumption of fruits, vegetables and whole grains, along with related lifestyle, as a potential strategy for significantly reducing the incidence of cancer.

The cells present in human body as well as in other organisms are often exposed to a varying variety of oxidizing agents, a few of which are necessary for life. These oxidizing agents can be present in the air, food, as well as water, or they can even be produced as a result of metabolic activities within the cells. The importance lies in the careful balance between oxidants and...
anti-oxidants ensuring the sustenance of an optimal physiological condition. In situations where oxidants are overproduced, the imbalance induced as a result of oxidative stress, especially in chronic bacterial, viral as well as parasitic infections [5]. The onset of oxidative stress results in oxidative damage to large biomolecules such as lipids, proteins and DNA leading to an increase in the risk of cancer [5–7].

The formation of cancer is a multi-stage process with the damage induced by oxidation linked to the formation of tumors through different mechanisms [5,6]. Free radical induced oxidative stress can result in significant DNA damage. Unless proper repair mechanisms are initiated, this damage can cause base mutation, single as well as double strand breakages, DNA cross linking as well as chromosomal breakage and rearrangement [6].

1.1. Dietary approach to cancer treatment

This potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants in fruits and vegetables. The anti-oxidant activities of fruits and vegetables are a consequence of the present phytochemicals.

Phytochemicals are defined as bioactive non-nutrient compounds in fruits, vegetables, whole grains and other plant foods that have been linked to reducing the risk of major chronic diseases [8]. More than 5000 phytochemicals have been identified in fruits, vegetables and grains. They are generally classified into carotenoids, alkaloids, phenolics, nitrogen containing compounds and organosulfur compounds. Phytochemicals obtained through the diets have the capability to prevent cancer or interfere with cancer progression at various stages of its development. Present studies also indicate the ability of phytochemicals to express anti-oxidant activity as a result of their free radical scavenging property; to induce cell cycle arrest and apoptosis; to modulate enzyme activities in detoxification, oxidation and reduction; to stimulate the immune system; and to regulate hormone-dependent carcinogenesis and have anti-bacterial and anti-viral effects [9–15].

Amongst the many phytochemicals present, the compounds with greatest importance include resveratrol and ursolic acid. Ever since the inverse relationship between the consumption of wine and cardiovascular disease was established [16–18], the underlying phytochemicals that may be responsible have received a lot of focus in the scientific domain attributing to its numerous health benefits. With the discovery of resveratrol in red wine [19] numerous researches have been conducted for documenting its anti-cancer, anti-aging, anti-inflammatory and a plethora of other beneficial properties. The compound has been used to conduct a lot of potential research to further gauge its capabilities. Ursolic acid is a phytochemical present in numerous herbs, fruits and vegetables, and has often been associated with herbal medicine. Though research data on the potential benefits of the compound was initially scarce owing to the lack of oral bioavailability data, with detection of ursolic acid in cranberries, apples and other fruits along with anti-cancer properties, the compound has generated renewed interest in scientific research exploring its potentials. Recently with the discovery of ursolic acid to reduce obesity and burn calories, the compound is currently in the spotlight in terms of its potential health benefits.

This review will focus on the anti-cancer properties of the two phytochemicals through an analysis of various epidemiological, animal model and clinical trial studies with a focus on breast cancer.

2. Resveratrol

Resveratrol was first isolated in 1940 from hellebore roots [20,21]. Research into the potential health benefits of the compound was carried out shortly after the discovery of resveratrol production in grape vines when they were infected by fungus [21]. Following the discovery of resveratrol in wine [19] the compound was hypothesized as the primary underlying factor responsible for the “French Paradox”, a term coined by Dr. Serge Renaud [22]. The paradox serves to describe the decrease in incidences of cardiovascular disease amongst the French population that consume a diet which is much higher in saturated fat compared to an American diet. One of the hypotheses was proposed due to the regular consumption of wine along with the traditional diet [18,19,22]. On the basis of this link to a potential health benefit, scientists then began to fully explore the potential cardiovascular benefits associated with resveratrol. Shortly after, resveratrol was revealed to inhibit tumor initiation, promotion as well as progression for cancer prevention [23].

A number of scientific studies report a plethora of applications of resveratrol pertaining to anti-aging activity [24,25], anti-cancer properties [26,27], cardio-protective effects [28,29], anti-diabetic properties [30,31], as well as anti-inflammatory [32,33], anti-viral [34,35] and neuro-protective effects [36,37].

2.1. Structure and chemistry

Resveratrol is a phytoalexin produced by plants as a result of bacterial and fungal attack. It is a defense mechanism adopted by plants suited for their survival against pathogen attack. Phytoalexins are toxic compounds synthesized de-novo by plants designed to delay cell maturation, prevent reproduction of the pathogen and puncture pathogen cell walls.

Resveratrol is a stilbenoid, a derivative of stilbene and is produced with the assistance of the enzyme stilbene synthase. The chemical name for resveratrol is usually given as 3,5,4’-trihydroxystilbene. Stilbenes are a form of phyto-estrogens consisting of trans-ethane double bond substituted with a phenyl group on both the double the carbon atoms of the double bonds. They are mainly constituents of the heartwood of the widely known eucalyptus genera, along with Pinu and Malcura. Stilbenes consist of two aromatic rings joined by a C2 bridge and generally exist in two forms: the trans-stilbene and the cis-stilbene. As with all stilbenes, resveratrol exists in a cis- and trans- form with the trans-resveratrol known to be ideally more stable [38]. The inter-conversion of trans-resveratrol to its cis-form can be seen upon exposure to ultraviolet radiation [39]. Resveratrol also has three different analogs: (E)-5-(2-(Quinolin-4-yl) vinyl) benzene-1,3-diol,
(E)-4-(3,5-dimethoxystyrlyl) quinoline and (E)-4-(3,5-dimethoxystyrlyl) phenol [40], which are similar in structures to resveratrol and possess anti-oxidant activity [41].

2.2. Sources

Resveratrol has been identified in more than 72 plant species including red grapes, peanuts and mulberries, which are common in the human diet [23,42,43]. The resveratrol content varies from different food sources with peanuts being found to have approximately half the amount of resveratrol as compared to the amount in wines. While considering blueberries, they were found to have twice the amount of resveratrol compared to bilberries with a pattern of regional variation being observed. Since resveratrol is not heat stable, cooking or any form of thermal processing involving these fruits will subsequently degrade the resveratrol present in them. It should be noted that in the case of grapes, the amount of resveratrol varies with respect to the grape cultivar, geographic origin and probable exposure to fungal infection [39,44].

2.3. Bioavailability and intra-cellular absorption and metabolism

One of the main reasons for a low cellular concentration of resveratrol, even though approximately 70% of it administered orally are absorbed, is due to its rapid breakdown into the conjugated metabolites: glucuronate and sulfonate [45], amongst which the pre-dominant forms are trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate. The glycosylated form of resveratrol, being more stable and resistant to oxidative degradation, is absorbed in the human gastro-intestinal tract [46]. Unchanged or free resveratrol in negligible concentrations below 5 ng/mL after an oral dose of 25 mg [45], is lower than the concentration of 5 μmol which is required to elicit a pharmacologic activity [47–49]. Needless to say, it has been seen that a high dosage of resveratrol has cytotoxic properties toward breast cancer cells owing to structural similarity to phytoestrogen [50,51] as well as resveratrol’s ability to slow down the potential development of blood vessels, which may result in a delay of healing [52]. They have been shown to display antioxidant, anti-mutagenic as well as anti-initiation, promotion and progression activities [23].

Taking the proposed beneficial health properties displayed by the bioactive compound in conjunction with it being a regular constituent in the human diet, resveratrol is an important phytochemical in its contribution toward breast cancer prevention.

2.4. Inhibition of cell proliferation and apoptosis

Resveratrol has been shown to inhibit cancer cell proliferation through the subsequent down-regulation of numerous proliferative and anti-apoptosis gene products [53]. The anti-proliferative activity of resveratrol has recently been documented in MCF-7 breast cancer cells, where the treatment caused a dose-dependent inhibition of cell growth along with an accumulation of cells in the S phase of the cell cycle at lower resveratrol concentrations [54]. The inhibition of cell growth is attributed to the ability of resveratrol to initiate apoptosis as evidenced by the presence of condensed chromatin as well as the detection of a sub-G1 fraction. Treating synthetic resveratrol on ER+ and ER- breast cancer cell lines show a similar anti-proliferative and apoptotic activity [55]. Three cell lines were used in this study, KPL-1 and MCF-7, both of which were ER+ and MKL-F which is ER−. Low concentrations of resveratrol induce cell proliferation in the ER+ cell lines at concentrations of <4 μmol/L in the case of MCF-7 and <22 μmol/L for KPL-1. At higher concentrations (>44 μmol/L), all three cell lines displayed a reduction in cancer cell growth, which is attributed to the induction of apoptosis as evidenced by the presence of sub-G1 fractions.

Despite the potential of resveratrol as an anti-oxidant and an anti-cancer agent, it is present at a small concentration in red wine compared to the other phenolic compounds present such as catechin, epicatechin and quercitin, which together account for more than 70% of the total phenolic content [56], and micromolar level concentrations are required to produce any significant benefit [56]. When the anti-proliferative activity of red wine was tested on hormone resistant and hormone sensitive breast cancer cell lines, phenolic concentrations at the picomolar and the nanomolar range were found to decrease cancer cell proliferation at a time- and dose-dependent manner [57]. They were able to observe a specific interaction between individual phenolic compounds and their respective steroid receptors in the case of hormone sensitive breast cancer cell lines, MCF-7 and T47D. Distinct anti-oxidant activities were also seen in the tested cell lines at similar concentrations. Bowers [51] reported that resveratrol exhibited agonist/antagonist activity toward alpha- and beta-estrogen receptors. In light of that observation, the effect of resveratrol treatment, in combination with estradiol, on the cell growth of ER positive breast cancer cell line, MCF-7, and ER negative cell line, MDA-MB-468, was studied [58]. Resveratrol at a concentration of 10 μmol/L, inhibited 1 nmol/L estradiol mediated cell growth along with an alteration in the expression of autocrine growth modulators and receptors in the MCF-7 breast cancer cell line. Resveratrol also inhibited cell proliferation in the ER negative breast cancer cell line, MDA-MB-468. These findings substantiated the estrogen receptor antagonist activity of resveratrol and proposed an alternative mechanism for inhibiting cell proliferation through the alteration of autocrine growth modulators and receptors.

The efficacy of resveratrol as a potential anti-proliferative agent for both hormone resistant and hormone sensitive, breast cancer cell lines was substantiated in a study where synthetic resveratrol at concentrations of 5, 10, 20 or 40 μg/mL were treated with MCF-7, MCF-10F and MDA-MB-231 breast cancer cells [59]. The treatment period ranged from 24 h to 144 h and a time- and dose-dependent inhibition of proliferation for all three cell lines were observed along with a decrease in the number of viable cells, which suggested resveratrol inhibited cell proliferation thorough the estrogen receptor independent mechanism.

Depending on the concentration, resveratrol exhibited estrogenic/anti-estrogenic effect on the ER positive and ER negative breast cancer cells and was associated with growth
inhibition through different targets in the proliferative and apoptotic pathways [60]. A recent study aimed at investigating the biological properties of a boronic acid derivative of resveratrol on ER positive MCF-7 breast cancer cells [61]. The trans-4 analog was seen to induce the cell cycle G1 arrest and as such displayed no obvious effect on the estrogen stimulated MCF-7 cells.

2.5. In vivo studies

The properties of resveratrol in the prevention of breast cancer was observed in a N-methyl-N-nitrosourea (NMU) induced mammary tumors in Sprague–Dawley rats [60] since the underlying cause or origination of cancer in this model is relevant to human breast cancer [62]. Resveratrol treatment reduced the onset of tumor multiplicity as well as increasing the latency period by 27 days. The tumor formation in the treated rats reduced by 50% over a period of 69 days, following which the incidence continued to increase to reach that of the control.

A recent animal study showed resveratrol’s ability to prevent the shedding of tumor cells from mouse mammary cancer spheroids and to inhibit cancer cell invasion [63]. The tumor growth was found to be inhibited through a phenol mediated expression of the proliferation marker Ki-67 and the phenol mediated down-regulation of MMP-9, and ROS generation was responsible for the prevention of tumor cell shedding. In contrast, when pre-pubertal female rats were treated with resveratrol, it resulted in endocrine system disruption along with an accelerated development of MNU-induced mammary carcinoma in Sprague–Dawley rats [64]. Another study showed that high doses of trans-resveratrol administered to Sprague–Dawley rats for a period of 28 days at a dose of 20 mg/kg/day was found to be harmless [65]. The post treatment analysis indicated no negative effect on growth as well as normalcy to most vital organs. The mean growth rate, final body weight and the lipoprotein profiles were all seen to be affected by the high dose treatment with resveratrol. When pharmacologic levels of resveratrol were used to treat ER- and ERβ+ MDA-MB-231 tumor cells in nude mice, a decrease in angiogenesis, tumor growth and apoptotic index was observed when compared to the control [66]. A significantly reduced extracellular vascular endothelial growth factor (VEGF), a protein that stimulates vasculogenesis and angiogenesis, was also seen. Resveratrol is known to inhibit angiogenesis by the suppression of VEGF action through a reduction in the MAP kinase phosphorylation thereby leading to a block in the VEGF receptor mediated response [52]. This suggests a possible mechanism exhibited by resveratrol in affecting angiogenesis through VEGF secretion as well as influencing the downstream cell signaling pathways of the VEGF receptor [66].

2.6. Molecular targets and signal pathway studies

Resveratrol has been shown to inhibit the invasive and migration properties of MDA-MB 435 breast cancer cells through the inhibition of insulin-like growth factor-1 (IGF-1), a stimulant for breast cancer cell migration [67]. IGF-1 induces cancer cell migration in the estrogen receptor negative (ER-negative) breast cancer cells through the activation of the PI-3K cellular signaling pathway. Resveratrol was found to suppress cell migration through the inhibition of the PI-3K/AKT pathway in a dose dependent manner. IGF-1 also up-regulated the Matrix metalloproteinase (MMP) activity in cancer cells, which was responsible for their invasive and metastasis properties [68]. Based on the research findings, resveratrol was revealed to modulate the MMP-2 levels in a dose-dependent manner through the post-translational regulation of protein synthesis. HRG-β1 (growth factor) mediated MMP-9 expression was also seen to be inhibited by resveratrol along with the suppression of ERK 1/2 phosphorylation [69]. This indicates the inhibitory effect of resveratrol on breast cancer cell invasion and MMP-9 expression being attributed to the down-regulation of the MAPK/ERK signaling pathway. Following the findings of a dose-dependent inhibition of the PI3K pathway, a study was conducted to observe the downstream signaling of PI3K in order to understand the mechanism of resveratrol-induced apoptosis. Bcl-2 was found to be down-regulated along with the inhibition of necrosis factor kappa-beta (NF-κβ), a regulator of Bcl-2 expression, and calpain protease activity. These findings were in conjunction to a decrease in mitochondrial membrane potential and an increase in nitric oxide production and reactive oxygen species (ROS). Based on these findings, Bcl-2 and NF-κβ were considered to be potential targets for the anti-cancer activity of resveratrol [70].

Resveratrol has been found to bind with Integrin αVβ3, a hetero-dimer, at the receptor which is close to the Arg-Gly-Asp (RGD) recognition site on the Integrin which is required for the transduction of the stilbene signal to a p53 dependent apoptosis of breast cancer cells [71]. Taking it into consideration in accordance with the limited bioavailability of resveratrol, a comparative study on signaling pathways elicited by resveratrol and its derivatives was carried out. The findings suggested that on binding of resveratrol and trans-resveratrol to integrin αVβ3 the p38 kinase pathways were activated, subsequently followed by the activation of p53; but in the case of trimethoxy-resveratrol, binding to integrin αVβ3 activated a different MAPK pathway leading to the activation of p53 and the induction of apoptosis [72]. This serves to suggest the different convergent and divergent signaling pathway as well as numerous mechanisms prior to the binding with integrin αVβ3 and the subsequent activation of p53. Cyclooxygenase-2 (COX-2) is an enzyme that is measured at high levels during inflammation; it is an anti-apoptotic. Treatment of breast cancer cells with resveratrol has been found to increase the nuclear accumulation of COX-2 where they interact with p53 and p300, a co-activator of p53 gene expression, and form a complex resulting in the facilitation of apoptosis in resveratrol treated MCF-7 and MDA-MB-231 breast cancer cells [73]. This finding served to distinguish between the cancer promoting properties of constitutive COX-2 expression and the proapoptotic properties of inducible COX-2 expression that supports resveratrol induced p53 expression. Breast cancer 1 and breast cancer 2 (BRCA 1 and BRCA 2) are genes that produce proteins responsible for DNA repair. The mRNA expression of breast cancer susceptibility genes, BRCA 1 and BRCA 2, was found to be increased following resveratrol treatment. Following
the treatment of MCF7, MDA-MB 231 and HBL 100 breast cancer cell lines with 10, 30 and 50 μM trans-resveratrol at varying exposure times, a blockade of cancer cells in the S phase in the three cancer cell lines was observed along with an increase in S phase cells and a decrease in cells in the G1 phase. The mRNA expression of the BRCA 1 was found to be increased with no change being detected in the case of the BRCA 2 genes [74].

A limitation encountered while weighing the benefits associated with administering resveratrol is the limited bioavailability. Trans-Resveratrol is metabolized in the body, particularly in the intestine and the liver, and is stored as glucuronide and sulfate conjugates. The uptake and metabolism of resveratrol was recently studied in ZR-75-1 and MDA-MB-231 breast cancer cell lines where they were able to observe the extensive formation of resveratrol-3-O-sulfate in the ZR-75-1 cells compared to the MDA-MB-231 cell line [75]. Contrary to traditional metabolism, resveratrol glucuronide was not detected in either cell lines. They were able to identify a significant co-relation between SULT1A1 (sulfotransfase) expression and resveratrol-3-O-sulfate formation in ZR-75-1 cells while a low co-relation was seen in the case of MBA-MB-231 cell line. The increase in the resveratrol-sulfate metabolite resulted in the excretion of the product from the cells which subsequently caused the lowering of the intra-cellular resveratrol concentrations as an offset [75]. This explains the lower anti-cancer activity of resveratrol on the ZR-75-1 breast cancer cell line. Resveratrol is passively absorbed into the cells where they are metabolized into respective glucuronide and sulfate metabolites. The efflux of the resveratrol metabolism products occurred through the ABC transporters, multi-drug resistance associated protein (MRP2) and breast cancer resistance protein (BCRP) [76]. The functional importance of BCRP was further established in a study where wild type and Brcep−/− mice where orally administered 60 mg/kg of resveratrol followed by a trans-resveratrol and metabolite concentration analysis in the intestine, plasma and tissues. A considerable reduction in metabolite efflux was observed in the knockout variant with an inhibition of 70% and 95% for glucuronide conjugate and sulfate conjugate respectively, which indicated at a substrate specificity of the sulfate conjugate to brcp1 [77]. The formation of resveratrol-3-O-sulfate was considered to be dose-dependent on resveratrol concentration. At higher concentrations of resveratrol, the formation of sulfate decreased as a result of non-competitive substrate inhibition [78]. When resveratrol-3-O-sulfate metabolite was leveled where compared between malignant and non-malignant breast cells, the sulfate conjugate was found at a greater extent in the tumor tissue owing to the over-expression of steroid sulfatase (STS) in the control [78].

An in vitro and in vivo study on the metabolism of resveratrol in human, rat and mouse models showed trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate as the most abundant metabolites of resveratrol [79]. The in vitro experiments involved the incubation of resveratrol with human liver chromosomes, human hepatocytes and rat hepatocytes while the in vivo studies involved oral administration of resveratrol to rats and mice. From the studies of human microsomes and hepatocytes, intact resveratrol but no metabolites were detected in human liver microsomes. Abundant resveratrol metabolites like trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate were observed in rat urine, mouse serum and the rat and human hepatocytes incubations. In order to confirm the structures of the conjugates, incubation with glucuronidase and sulfatase was performed to release free resveratrol. They were only able to observe trace amounts of cis-resveratrol indicating that isomerization did not play a significant role in the metabolism of resveratrol.

In order to address the limitations associated with bioavailability, a solid nanoparticle (SLN) mediated uptake of resveratrol (RSV) was studied by Teskac and Kristl [80]. This study was done to explore the potential for colloidal carriers in delivering chemo preventive drug resveratrol to specific, desired locations. The effects of the SLN-RSV complex on factors such as growth, morphology, metabolic activity and genetic material of keratinocytes were observed compared to that of RSV alone in solution. The results showed that approximately 15% of RSV was not bound to SLN, with a slow and sustained release of RSV after 15 min indicating a peripheral location of RSV. Additional colloidal structures in SLN such as micelles, mixed micelles and liposome provided additional spaces for RSV. The cells were found to rapidly cross the membrane and a significant amount of the administered dose was taken up by the cells. The internalized SLV did not stay in one place and was in constant motion in the cytosol with the cells remaining completely viable. SLN-RSV at a concentration of 10 μM led to no change in the cell morphology but at a concentration of 100 μM, the cells were slightly enlarged and their number reduced when compared to RSV alone in solution. An increase in metabolic activity by 20% relative to the control was observed on treating cells with SLN-RSV and SLN alone did not influence the metabolic activity. On incubation of cells with SLN-RSV, cells moved from the G1 phase to the S phase, followed by a significant decrease in G2/M phase. An increased metabolic activity was seen in the case of SLN-RSV indicating intracellular transport of SLN increases RSV efficacy. A sparse confluence was observed after incubation of cells with RSV or SLN-RSV alone. The overall cytotoxicity associated with RSV was avoided by its delivery with SLN. The research data indicated the cytostatic effectiveness of the SLN-RSV complex compared to RSV alone in solution.

Treating cancer cells with resveratrol has been shown to result in a plethora of metabolic changes. In a recent analytical study on the metabolic effect induced by resveratrol on MCF-7 and MDA-MB-231 cancer cells, treatment was found to increase the synthesis of 21 amino acids 100 fold at a concentration of 100 μmol, to modulate the bio-synthesis of polyamine, and to stimulate the putrescine and spermidine synthesis, to increase extracellular arachidonic acid, to reduce prostaglandin E2 levels (PGE2), as well as to increase in expression of tryptophan, serotonin and kynurenine [81].

An analysis on the pharmacokinetics of resveratrol showed that (BCRP and MRP 3) played a vital role [82]. Both BCRP as well as MRP 3 play an important role in the transport of resveratrol and its metabolites: resveratrol sulfate and resveratrol glucuronide. BCRP has a lower affinity to resveratrol.
glucuronide compared to MRP 3 and as such the two proteins are responsible for the disposition of resveratrol in the cells [82]. A lack of MRP proteins reduces the amount of resveratrol that is secreted through the urine whereas a lack of BCRP protein results in the formation of resveratrol di-sulfate along with an increase in resveratrol secretion through the urine.

Resveratrol suppresses the synthesis of PGE2 through the inhibition of COX-2 enzyme activity [83]. Upon phorbol ester mediated (PMA) induction of COX-2, an increase in PGE2 production was seen. They were able to observed that resveratrol caused a dose-dependent suppression of PGE2 synthesis in human mammary cells and when compared to a selective inhibitor of COX-2, the synthesis decreased by 10% to control level indicating that more than 90% of the remaining activity in the mammary epithelial cells was due to the COX-2 isoforms. Resveratrol suppressed the higher rates of synthesis along with a marked decrease in c-jun expression; PMA-mediated induction of c-myc transcription was not inhibited. A 6 fold increase in COX-2 activity following treatment with PMA was seen with resveratrol inhibiting this induction. PMA and resveratrol were mediated via a cyclic AMP response. Resveratrol was also found to inhibit PMA-mediated activation of protein kinase C along with the over expression of Cjun, ERK1 and protein kinase c-alpha, all of which increased the COX-2 promoter activity [83]. Resveratrol also blocked the AP-1 mediated gene expression activation through the PKC signaling cascade [83].

Recently an analog of resveratrol, cis-3,4′,5-trimethoxy-3′-hydroxystilbene, has been shown to display anti-cancer properties at concentrations lower than that of resveratrol [84]. The proposed compound functions by disrupting the polymerization of microtubules. The reported potency of the hydroxyl-stilbene analog over resveratrol was due to the ability of the analog’s hydroxyl group to form hydrogen bonds with Val/α181 of tubulin. The cis-3,4′,5-trimethoxy-3′-hydroxystilbene analog was shown to accumulate the MDA-MB-231 breast cancer cells in the G2/M phase, which was in conjunction with reported data showing long term accumulation of cells in the M phase results in apoptotic cell death known to be mediated by caspase-3, PARP and Bax.

Resveratrol arrested cell proliferation, induced cell death, and decreased the number of cell colonies that were sensitive to caspase-3-dependent apoptosis [85]. The activation of Beclin-1 dependent autophagy in the two cell lines by resveratrol was responsible for triggering cell death but the functionality was blocked by the expression of caspase-3. This serves to indicate the potential process through which resveratrol initiates caspase-independent cell death.

Resveratrol is known to act as an ER agonist and an aryl hydrocarbon receptor (AHR) antagonist with previous studies indicating that the expression of ERα limits the compounds ability to inhibit the AHR dependent transcription [86]. Resveratrol has been shown to be a competitive antagonist of dioxin (TCDD) along with numerous other ligands of AHR and has been demonstrated to be involved with the extracellular translocation of the receptor through subsequent binding with the dioxin-response element. The research group was able to observe that resveratrol was a competitive antagonist of AHR and in this particular study competed with TCDD for the AHR binding and was also responsible for blocking the CYP1A1 and Il-1β expression at micromolar levels of concentration [86]. Recent studies indicated that the expression of ERs was independent to the ability of resveratrol to inhibit AHR dependent transcription and that metabolites of phytochemicals might contribute toward resveratrol induced inhibition of AHR mediated activities along with the mediation of certain biological and anti-neoplastic properties of the phytochemical compounds [87].

2.7. Human clinical trials

Based on the well documented beneficial properties attributed to resveratrol treatment, human clinical trials are the logical steps to further explore the benefits and limitations associated with administering resveratrol at controlled dosages.

A phase-1 dose escalation resveratrol pharmacokinetic study was conducted in healthy volunteers [49]. The study of oral resveratrol included single doses of 0.5, 1, 2.5 or 5 g and was conducted on 10 healthy individuals. Resveratrol was administered in the form of immediate-release caplets containing a dosage of 500 mg. The peak levels of resveratrol at the highest dose were 559 ± 384 ng/mL which occurred 1.5 h after dosage. It was found that the peak levels of two mono-glucuronides and resveratrol-3-sulfate were 3 to 8 fold higher. Resveratrol-3-sulfate and resveratrol monoglucuronides were up to 23 times greater as compared to resveratrol. The plasma half-lives of the three resveratrol metabolite conjugates were 3.2–11.5 h for the sulfate metabolites and 2.9–10.6 h for the glucuronides and were similar to the half-life of parent resveratrol which was 2.9–8.9 h. A rapid urinary excretion of resveratrol was also observed, with 77 percent of all the urinary agent derived species excreted within 4 h after the lowest dose was administered. Based on the pharmokinetics study, even on ingesting resveratrol equivalent to the amount found in several hundred red wine bottles, the concentrations will still fall between 0.3 and 2.4 μmol/L, well below the 5 μmol/L required to show anti-cancer properties [49].

A similar study in a Spanish population was conducted by the European Prospective Investigation into Cancer and Nutrition (EPIC) [88] in 2007 to assess the concentrations of resveratrol and its derivatives in foods and to estimate the principal dietary sources of these compounds in the Spanish adult population. A food composition database (FCDB) of resveratrol and piceids in Spanish food was compiled. 40,685 subjects aged between 35 and 64 years old were included in the study from both the northern and southern regions of Spain with their food intake assessed through a series of personal interviews employing a computerized version of a validated diet history method. Following the interview process, a FCD with 160 items were compiled. In order to assess the resveratrol and piceid intake with respect to various attributes such as age, region, education level, etc., estimations of the portions of consumers and median resveratrol and piceid intake was calculated using linear regression analysis. The average intake of resveratrol and piceid was found to be 933 μg/days with a median of 100 μg/days. A total of 13,175 participants, which included 39% men and 20% women, had a total resveratrol intake of 0 μg/days. Trans-Piceid contributed 53.7% of total
resveratrol intake, trans-resveratrol 20.8%, cis-piceid 19.3% and cis-resveratrol 6.2%. The resveratrol and piceid consumption was lower in quantity and percentage amongst women than in men. It was found that the most important source of resveratrol and piceid was wines with 98.4% followed by grape and grape juices with 1.6%. Peanuts, pistachios and berries contributed to less than 0.01%. They were able to conclude that trans-piceid is a common component of the Mediterranean diet.

From a double blind, placebo controlled study investigating four increasing doses of oral trans-resveratrol, bioavailability of resveratrol is found to be greatest when administered in the morning [89]. The groups were studied in ascending order of doses with the trans-resveratrol being administered orally in the form of capsules in varying concentrations of 25 mg/placebo in group 1, 50 mg/placebo in group 2, 100 mg/placebo in group 3 and 150 mg/placebo in group 4 and were given at 4 h intervals, 6 times a day for 13 doses. The peak plasma concentrations of trans-resveratrol was reached at 0.8–1.5 h post dose and the mean apparent terminal half-life ranged from 1 to 3 h following trans-resveratrol single-dose and from 2 to 5 h following repeated dosing. After the 13th dose of trans-resveratrol 25, 50, 100 and 150 mg, the mean peak concentration of plasma (Cmax) was 3.89, 7.39, 23.1 and 63.8 ng/mL and the mean AUC (area under the plasma concentration curve) was 3.1, 11.2, 33.0 and 78.9 ng/(h mL). The trans-resveratrol pharmacokinetics showed circadian variation. A total of 18 treatment-emergent adverse effects (AE) were reported. No case of related AE with placebo was reported. The adverse effects were mild in severity and similar amongst the groups. They concluded that even though the repeated dose administration was well tolerated only a relatively low plasma concentration of trans-resveratrol was produced despite of the high doses and short dosing interval.

2.8. Summary

Vast research has been conducted analyzing the anti-cancer potential of resveratrol toward the treatment of breast cancer. Even with a limitation in terms of a lower bioavailability, studies are exploring different avenues to bypass this. Clear evidence exists documenting the anti-proliferative and apoptosis activities associated with resveratrol treatment as well as research data on cell signaling pathways and metabolite that are specifically affected.

3. Ursolic acid

Ursolic acid was first reported to be present in cranberries [90,91] as well as in cranberry press cakes and was not given much importance due to the assumption that it was a waste product associated with cranberry juice production. Relatively little information regarding the phytochemical is currently known due to a lack of scientific research data on the compound’s oral bioavailability. However, over the years numerous in vitro and in vivo researches have been conducted with regards to the potential health benefits of this particular phytochemical and among the reported findings are ursolic acid’s anti-cancer and anti-inflammatory activities [92–94].

The compound has recently been identified to be present in apple peels [95] as well as high blue blueberries [96] and is typically present in the fruit’s wax layer. Hence, processing methods used and the relative presence of fruit peel, stands to have an effect on the amount of ursolic acid present [91]. The importance is further substantiated in a recent research report which links the ability of apple peels to lower obesity and burn calories [97]. In light of these research findings, the health benefits associated with the consumption of apples and other fruits that are similar, which can be attributed to the presence of ursolic acid.

3.1. Structure and chemistry

Ursolic acid is a pentacyclic triterpenoid compound that exists either in its free acidic form or as aglycones for the saponins. Terpenoid compounds are present in most plant species and serves as a primary metabolite taking part in processes such as respiration, development and growth and photosynthesis [98]. Certain terpenoid compounds also function as secondary metabolites to protect the plants against pathogens and herbivores [99,100]. The compound has a very low water solubility which in turn affects their bioavailability [101].

Terpenoid compounds in plants are synthesized through two different pathways, the mevalonic (MVA) acid and the mevalonolate 4-phosphate (MEP). The biosynthesis of a majority of the terpenoid compounds displays a common route in the synthesis pathway from isopentenyl diphosphate (IPP) to dimethyl-allyl-diphosphate (DMAPP), the essential precursors for the formation of ursolic acid [102–105].

3.2. Sources

Ursolic acids are tri-terpenoid compounds in medicinal herbs, food as well as certain plants such as apples, cranberries, prunes, lavender and oregano. They are mainly found along with its isomer oleanolic acid [106]. Apple peels have been shown to contain high amounts of ursolic acid [95,107]. They have also been identified in numerous plant species [99,100,108,109].

3.3. Uses

Ursolic acid mainly exists in the form of free acids or aglycones for certain triterpenoid saponins. Initially ursolic acid was considered to be a biologically inactive terpenoid compound [106], but due to its low toxicity and pharmacological effects [92] along with various biological functionalities [110], ursolic acid is being studied to further explore its beneficial properties [111–114].

Due to the relatively low level of toxicity [115], ursolic acid is generally used in medicine formulations that can be applied both orally and topically. In terms of acute toxicity, the LD50 value resulting from an intra-peritoneal administration in mice is 637 mg/kg and from oral administration is 8330 mg/kg [116]. Ursolic acid has recently been used as a radio-sensitizer, where an in vitro analysis showed its ability to bolster the ionization radiation induced apoptosis of DU145, CT26 and B16F10 breast cancer cell lines [117]. A similar radio-sensitizing effect was also
observed in vivo where ursolic acid administration in conjunction with ionizing radiation, inhibited the formation of tumor in mice implanted with the B16F10 melanoma cancer cells [117].

The health benefits of ursolic acid include anti-inflammatory activity [118,119], anti-cancer activity [120–125], and anti-hyper lipidemic activity [126].

3.4. In vitro proliferation and apoptosis studies

Pomegranate extract has been shown to inhibit the proliferation of various cancer cells [127] and an HPLC analysis reveals that it contains approximately 70% total phenolic compounds, a composition that is similar to the components found in pomegranate juice [128]. One of the phenolic components present in a pomegranate extract is ursolic acid. Pomegranate extract inhibited the proliferation of MMTV-Wnt-1 mouse mammary cancer stem cells in a time and concentration dependent manner by initiating a cell cycle arrest in the G0/G1 phase [129]. A similar study observing the antiproliferative activity of two herbal extract mixtures on human breast cancer cell lines, MDA-MB-453 and MDA-MB-361 [130]. The two herbal extract mixtures act as a good source of ursolic acid or rosmarinic acid. Ursolic acid has been isolated from apple peel extracts using a bioactivity-guided fractionation process [95] and shows significant anti-proliferative activity when treated with MCF-7 breast cancer cells. Similar reports have been observed on treating MDA-MB-231 cells with ursolic acid which results in the induction of apoptosis along with the suppression of cancer cell proliferation [131]. The anti-proliferative activity associated with ursolic acid treatment was dose-dependent with an IC50 value of approximately 40 μmol/L at a 24 and 48 h time period in comparison with the control. Morphological changes of the cancer cells in terms of shape, size and density were also detected following treatment. On treating the MDA-MB-231 cells with a 40 μmol/L concentration of ursolic acid for a 24 and 48 h period, a time-dependent increase in the G1 apoptotic fraction was seen with an increase from 3.58% to 10.51% in 24 h and 5.19% to 33.09% in 48 h, indicating an induction of cancer cell apoptosis [131]. A concentration-dependent inhibition of cell proliferation and the induction of apoptosis were also observed when MCF-7 breast cancer cells were treated with ursolic acid [132]. After a 24 h time period following ursolic acid treatment, the IC50 of ursolic acid was found to be approximately 22.6 ± 3.0 μmol/L, with a concentration of 50 μmol/L found to arrest the cells in the G0–G1 phase of the cell cycle.

3.5. In vivo studies

Ursolic acid is one of the active phytochemical compounds present in Rosemary extract. Rosemary extract when administered to rats with DMBA (7,1-dimethylbenz[a]anthracene) induced mammary tumorigenesis resulted in a decrease in the incidence of tumor formation in comparison to the control treatment [133]. The study observed a decrease in DMBA binding to the mammary epithelial cell DNA by approximately 42% as well as a decrease in the DMBA derived adduct binding to deoxyguanosine (dGuo). A 51% inhibition of dGuo adduct formation was also seen. A similar study to investigate the anti-tumor effects of ursolic acid on post-menopausal breast cancer resulted in a decrease in tumor proliferation being observed [134]. Ursolic acid caused a dose-dependent opposing effect as 0.10% ursolic acid was seen to significantly decrease final tumor growth compared to ursolic acid treatments at 0.25% with higher concentrations observed to induce tumor growth. Ursolic acid at 0.10% induced the maximum tumor inhibition of 40% as well as decrease cyclin D1 levels while both the concentrations, 0.10% and 0.25%, decreased the phosphorylation of Akt, MAPK as well as S6 a downstream effector of Akt/mTOR.

3.6. Molecular target and signal pathway studies

Ursolic acid treatments of MCF-7 breast cancer cells induced apoptosis through internal mitochondrial pathways [135]. This finding is further substantiated when on treating MDA-MB-231 cells with ursolic acid an induction of mitochondrial mediated cancer cell apoptosis was observed [131]. Ursolic acid was found to release cytochrome C from the mitochondria to the cytosol, to up-regulate the Bax protein as well as a down-regulate Bcl-2. This was in conjunction with the cleavage of caspase-9 along with a decrease in the mitochondrial membrane potential [131].

Treatment with ursolic acid caused an increase in nuclear translocation of GR as well as the down regulation of Bcl-2 causing a release of cytochrome C. This was subsequently followed by an activation of caspase-9 and caspase-3, proteases that are involved with apoptosis [136]. Poly ADP-ribose polymerase (PARP) is cleaved by capase-9 following its activation. Even though studies indicated that ursolic acid induced the binding of AP-1 to its target DNA elements [137,138], however, ursolic acid was seen to not induce any trans-repression or trans-activation of AP-1 activity which serves to suggest that ursolic acid responds through a mechanism that does not involve AP-1 signaling activity. A recent study indicates that MCF-7 apoptosis by ursolic acid was mediated through the inhibition of Forkhead Box protein M1 (FoxM1), a protein responsible for cell cycle progression, as well as the inactivation of CyclinD1/CDK4, the downstream targets of FoxM1 [139]. The FoxM1 transcription factor is known to regulate target genes that are involved with the initiation of mitosis and as such is over expressed in most tumors. Ursolic acid is also found to up-regulate the expression of the p53 protein expression when treated with MCF-7 cells providing a possible link to the induction of apoptosis following treatment [132].

The migration and invasive properties of the breast cancer cell line MDA-MB231 is inhibited on treatment with ursolic acid [140]. Ursolic acid suppressed the MDAMB231 cell growth at concentrations of 25 or 50 μmol/L and viability decreases in a dose- and time-dependent manner. At concentrations ranging from 0 to 50 μmol/L colony formations were suppressed and at 10 μmol/L cell motility was inhibited. Concentrations above 2.5 μmol/L decreased cell migration and invasion in a dose- and time-dependent manner. Ursolic acid treatment also reduced MMP-7 and plasminogen activator (u-PA) activities by 35% and 42%, respectively, at a 10 μmol/L concentration which corresponded to an increase in TIMP-2 (tissue inhibitor
of MMP) and PAI-1 (plasminogen activator inhibitor) protein. This reduction in MMP activity was linked to the inactivation of Akt, mTOR and JNK by ursolic acid in a time-dependent manner with the inactivation being responsible for the inhibition of cell invasion and migration. Ursolic acid treatment also reduced NF-κB, c-Jun and c-Fos protein levels in the nucleus and also inhibits the metastasis and angiogenesis related signaling cascades RhoA (Rho-like GTPases), Grb2, Ras and vascular endothelial growth factor (VEGF). NF-κB activity was suppressed through the inhibition of IκBα kinase and p65 phosphorylation [141]. Ursolic acid prevented the DNA binding of the NF-κB that consists of p50 and p65 along with the inhibition of NF-κB reporter gene expression through tumor necrosis factor receptor (TNFR), tumor necrosis factor receptor type 1-associated death domain protein (TRADD), tumor necrosis factor receptor associated factor-2 (TRAF2), necrosis factor kappa-B inducing kinase (NIK), IκB kinase (IKK) as well as p65.

COX-2 is an enzyme that is typically over-expressed in various forms of cancer. The enzyme acts on arachidonic acid and converts it into Prostaglandin H2 (PGH2). PGH2 is then acted on by Prostaglandin E2 synthase (PGE2) converting it into Prostaglandin E2 (PGE2), a compound that is known to increase the rate of cancer progression. Ursolic acid inhibited the COX-2 transcription in human mammary epithelial cells treated with phorbol 12-myristate 13-acetate (PAM), a tumor promoter [142,143]. Ursolic acid suppressed COX-2 activity by inhibiting the PKC signaling pathway through a blockage of the translocation of the PKC signaling from the cytosol to the membrane as well as inhibiting the activation of ERK1/2, JNK and p38 MAPK [144]. The TNF-related apoptosis inducing ligand (TRAIL) is a protein that induces the process of apoptosis. The protein typically binds to the TNFR, DR4 and DR5 in order to initiate the process of apoptosis. Even though a majority of the cancer cells are resistant to TRAIL induced apoptosis [145], treatment with ursolic acid was shown to sensitize the cancer cells to the TRAIL induced process [146]. Ursolic acid treatment was revealed to up-regulate the expression of death receptors (DR) through JNK mediation, activate the ROS and to down-regulate the expression of survival proteins and DCR2, a receptor that contains a truncated death domain [146].

3.7. Summary

The beneficial effects of ursolic acid have traditionally been observed through the use of herbal medicine due to its presence in many plant species. The identification of the compound in apples and fruits followed by reported data on its potential anti-cancer properties served to pave way for future scientific research exploring its health beneficial potential. Numerous researchers have documented the anti-proliferative and apoptosis activity attributed to treating breast cancer cells with ursolic acid. It has also been documented to affect cellular signaling pathways as well as individual metabolites.

4. Conclusion

There is a growing emphasis placed by consumers on eating healthy as a means toward preventing the onset of disease. In light of that very change, resveratrol and ursolic acid are at the forefront in terms of exposure and potential health benefits. The discovery of resveratrol as a component in red wine and its potential link to providing cardio-vascular protection has opened doors to explore further its health beneficial capabilities. Various researches report the anti-tumor, anti-inflammatory and apoptosis properties of resveratrol along with the signal transduction pathways that are affected by the compound. With clinical trials reporting the benefits of resveratrol in terms of anti-cancer properties, the commercial application of administering resveratrol has been suggested. With the bio-availability still being a drawback, further research needs to be made in this avenue in terms of a potential delivery system or encapsulation system which can increase the bioavailability of the compound.

Ursolic acid has been used in traditional medicine for a long time and is commonly attributed to be one of the underlying factors responsible for bestowing the health beneficial effects. Following the recent discovery of the ability of ursolic acid to reduce obesity and burn calories, the compound in currently in the lime light. The anti-cancer and anti-inflammatory activities have been documented by various research groups across different cell lines. With more research currently being done on the oral bioavailability of the compound, based on the future research findings, a potential avenue to explore will be to conduct clinical trials.

References

[54] H. Nakagawa, Y. Kiyozuka, Y. Uemura, et al., Resveratrol inhibits human breast cancer cell growth and may mitigate the effect of linoleic acid,


[95] X. He, R.H. Liu, Triterpenoids isolated from apple peels have potent antiproiferative activity and may be partially responsible for apple’s...


