Biological Activity of Phytochemical Compounds in Pomegranate - A Review

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Abstract

Plant phytochemicals in pomegranate including flavonoids, punicalagin, ellagic acid and punicic acid have a wide range of potential applications in human pathology. Classically they act as anti-oxidants through several mechanisms including auto-oxidation and inhibition of lipid peroxidation however their role has expanded into other areas. By interacting with important cellular signal cascades including NFκB, MAPK and PI3K/Akt they can attenuate deleterious processes in inflammation and atherogenesis. Mediation of these signal cascades as well as regulation of other mechanisms like nuclear micro-RNA expression and the ubiquitin-proteosome pathway allows these compounds to decrease aberrant cell cycle progression and inhibit abnormal anti-apoptotic cell survival mechanisms resulting in antagonism of oncogenic and tumorigenic processes. Other possible applications include management of diabetes through PPAR agonism and anti-microbial capabilities. These remarkable properties present promising potential in critical areas of human disease and highlight the value of further investigation into the therapeutic applications of pomegranate compounds and other phytochemicals.

Keywords: Pomegranate; Punicic acid; Flavonoids; Biological activity

Introduction

In recent years there has been increasing interest in the medical applications and nutritional benefits of natural compounds found in a wide variety of foods such as green tea, red wine and many fruits and vegetables. In particular there is considerable interest in the phytochemical constituents of pomegranate (Punica granatum) including specific flavonoids, tannins and fatty acids [1]. While there is a substantial amount of literature focusing on the anti-oxidant properties of these phytochemicals the proposed applications have expanded dramatically over the last decade including critical areas such as inflammatory disease, cardiovascular disease, metabolic disease, infectious disease and cancer [2]. This review aims to present some of the most recent literature concerning the biological activity and potential applications of the phytochemical compounds in pomegranate.

Composition

While there is some inconsistency concerning the exact composition of biologically active phytochemicals in pomegranate most beneficial effects are attributed to polyphenolic flavonoids, tannins and specific fatty acid compounds present to varying degrees in different parts of the fruit. In addition to these compounds pomegranate is high in vitamins C, E, K as well as other important nutrients and minerals [2,3-7].

Flavonoids

The flavonoids are a group of polyphenol plant metabolites involved in plant protection against damaging processes such as UV radiation [8]. Particularly important flavonoid compounds in pomegranate include the anthocyanidins, the flavonol quercetin, the flavanol
Anthocyanidins (Figure 1) are aglycones of anthocyanins, a subset of flavonoid present in the peel, juice and seeds of pomegranate that are responsible for their pigmentation and other properties. These compounds have been shown to mediate important cellular signaling pathways. For example delphinidin, the major anthocyanidin in pomegranate, inhibits several pathways including the mitogen activated protein kinase (MAPK) pathway in certain in vitro breast cancer cell lines at concentrations as little as 50 mcg/ml [11]. Despite these effects the anthocyanidins are largely degraded by gastric pH and there is debate concerning the potential in vivo efficacy of these compounds [3,10].

Quercetin, luteolin and catechin (Figure 2) are members of flavonoid subclasses with a similar phenolic structure and are present in the juice and peel of pomegranate. These compounds have recently been shown to have a remarkably broad range of effects not only as anti-oxidants but as mediators of numerous cellular pathways including but not limited to inflammatory, proteosomal, apoptotic, survival and proliferative pathways [7-9,12-17]. It is important to note that these studies have largely been limited to in vitro and in vivo animal models thus questions regarding their bioavailability and metabolism in humans continue to be investigated.

Table 1: Proposed benefits of Flavonoid compounds.

| 1.     | Antioxidants [3] |
| 2.     | Anti-inflammatory action via TNFα & NfκB pathway antagonism [16] |
| 3.     | Decreased tissue injury during ischemia-reperfusion [17] |
| 4.     | Inhibition of oncogenic cell migration [14] |
| 5.     | Decreased abnormal cell survival related to aberrant anti-apoptotic factors [15] |
| 6.     | Inhibition of abnormal proteosome activity with a reduction of deleterious intracellular events [8] |
| 7.     | Mediation of microRNA expression affecting a broad array of cellular events [7] |

**Tannins**

The tannins are large hydrolysable polyphenolic acid structures that play important roles in regulating plant growth and protection. An important characteristic of tannins is their ability to form insoluble complexes with amino acids, carbohydrates, nucleic acids and alkaloids [18, 19]. The important tannins in pomegranate are the ellagitannins and the gallocateins along with their metabolic acid derivatives such as ellagic acid (Figure 3). The exact metabolism of the tannin compounds is complex and still under investigation but studies indicate that some acid derivatives may enter the systemic circulation and persist for a few days after gastric hydrolysis. However, intestinal hydrolysis by local flora, absorption and subsequent metabolic conjugation predominates [3, 5, 7, 8].

Of the tannins punicalagin is potentially the most important. It is the major antioxidant in pomegranate juice [3] and is also present in the peel and the seeds. Like the flavonoids studies have shown punicalagin to have a broad range of beneficial effects related to combating atherogenesis, inflammation and cancer as well as viral, bacterial and fungal infection [3, 6, 18-20, 22]. The question of bioavailability and metabolism is again a primary concern with some studies indicating that in vivo benefits may be primarily related to the ellagic acid derivative [7]. Several recent studies have even shown that ellagic acid itself was responsible for up regulating apoptotic pathways in hepatocellular carcinoma cells in vitro [22] and inhibiting proliferation of breast cancer cells through tumour growth factor Beta (TGFB) pathways [23]. A general summary of the effects of tannins and their derivatives is given in table 2.

**Punicic Acid**

In addition to the polyphenolic compounds in pomegranate there are other compounds of importance. The seeds of the fruit contain a high concentration of fatty acids such as Palmitic acid and stearic acid however punicic acid (Figure 4) comprises up to 80% of the fatty acid component of the seed oil [4]. Punicic acid is an eighteen carbon polyunsaturated fatty acid and is virtually unique to pomegranate [24]. Research has shown that punicic acid may play a critical role in the beneficial effects of pomegranate compounds in inflammatory and metabolic disease through agonism of Peroxisome proliferator activated receptors (PPAR) as well as mediation of tumor necrosis factor alpha (TNFα) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) pathways [3, 24]. Reported benefits of punicic acid are summarized in table 3.

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Figure 3: Examples of tannin compounds. These compounds are large hydrolysable polyphenolic molecules with complex ring structures, their activity is broad but includes antioxidant and anti-oncogenic function. In addition to ability to interact with glycosaminoglycans and other cell surface molecules in an anti-microbial fashion. Larger molecules are broken down into metabolic derivatives such ad Ellagic Acid which are reputed to be responsible for some of the effects seen with these compounds.

1. Antioxidants [3]
2. Inhibition of viral entry into host cells by blocking cell surface glycosaminoglycans [19]
3. Inhibition of bacterial motility and biofilm formation [21]
4. Decreased fungal cell wall stability [18]
5. Inhibition of abnormal cell cycle progression [22]
6. Decreased abnormal cell survival related to aberrant anti-apoptotic factors [22]
7. Inhibition of abnormal proteosome activity with a reduction of deleterious intracellular events [8]

Table 2: Proposed benefits of Tannin compounds.

1. Control of blood glucose through PPAR agonism [24]
2. Anti-inflammatory action via TNFα antagonism & decreased neutrophil activation [4]

Table 3: Proposed benefits of Punicic acid.

Antioxidant Properties

Many of the constituents of pomegranate have antioxidant properties. It is important to note that these constituents contain a high concentration of antioxidants even compared to other foods known for their antioxidant properties. For example pomegranate juice contains roughly 5 mmol/liter of polyphenolic compounds while other juices and drinks like green tea range from 1.3-4 mmol/liter [3]. Several studies indicate the primary antioxidant properties of pomegranate lies in the tannin compounds, particularly punicalagin [3,6]. It is interesting to note that the tannin punicalin does not express the same protein binding capacity as other tannin compounds and may act solely as an antioxidant [25]. The exact mechanisms of these antioxidant properties have not been fully elucidated however several mechanisms have been demonstrated.

The simplest explanation for these properties may be the ability of these compounds to scavenge damaging reactive oxygen species (ROS) via auto-oxidation. The molecular composition of the polyphenolic compounds presents reactive sites for this process such as abundant hydroxyl groups [9]. These compounds can also mediate enzymes related to oxidative homeostasis including augmentation of catalase, superoxide dismutase, glutathione peroxidase and glutathione reductase [9,10]. Several studies have also critically shown that these compounds can inhibit lipoxgenase in addition to acting as copper, iron and mercury metal chelation agents leading to a decrease in membrane lipid peroxidation, one of the most critical aspects of oxidative stress [3,9,26]. Additionally the antioxidant compounds have been shown to protect other biologically active molecules and processes from oxidation with one example being the protection of endothelial nitric oxide [27].

These protective abilities have been demonstrated both in vitro as well as in vivo. In one study it was shown that placental syncytiotrophoblast ischemic oxidative stress was attenuated by daily ingestion of 8 oz pomegranate juice in pregnant women at 35-38 weeks gestation. On analysis of placental tissue obtained < 30 minutes post-labor the researchers found reduced trophoblastic markers of oxidative stress in the study group receiving pomegranate juice in addition to increased stability of E-cadherin cellular adhesion and decreased markers of apoptotic cysteine-dependent aspartate specific protease (caspase) activation [5]. Other examples have shown mediation of ROS in UVB mediated DNA damage, gonadal oxidative damage and ischemia reperfusion injury [12,17,28]. While these specific examples are notable the role of oxidative stress in pathological processes is vast and the potential use of these compounds merits further investigation.

Role in Inflammation

Mediation of inflammatory processes may underlie many of the proposed benefits of the compounds present in pomegranate. Perhaps the most compelling findings are related to the ability of these compounds to interact with and mediate critical signal transduction pathways within cells. One of these pathways is the NFκB pathway. NFκB is a DNA transcription mediator present initially as an inactive cytosolic complex in the majority of cells. After being activated by ubiquination and proteosomal degradation of the primary NFκB complex the active subunit enters the nucleus and regulates genes involved in response to numerous stress factors such as infection.

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inflammation, osmotic stress and free radical damage. It accomplishes this through regulation of cytokines, including those critical in inflammatory processes such as TNFα, IL-1, IL-6, IL-8 as well as important chemokines and cellular adhesion molecules [4,10,16, 24].

Several studies have shown pomegranate constituents to regulate factors in the NFκB pathway. One study showed that punicic acid was able to suppress TNFα expression in an animal model after oral dosing [24]. A later study examined the effects of the flavonoid luteolin in human keratinocytes and found that in certain cell lines 10-100 µM of luteolin was able to reduce TNFα induced nuclear binding & mRNA expression of NFκB subunits in a concentration dependent fashion. In turn this reduced the expression of IL-6, IL-8 and downstream effects on Th17 and TNFα expression itself, interrupting the positive feedback cycle between NFκB and TNFα, a mechanism proposed as an important process in the pathogenesis of chronic inflammation [16]. One explanation for the action of luteolin and other flavonoids is related to their structural similarity to endogenous steroid hormones, allowing access to cellular and nuclear sites where mediation of these pathways at multiple steps may occur [8,9].

Another critical regulatory signal transduction pathway shown to be affected by pomegranate compounds is the MAPK pathway. The MAPK signal cascade is one of the most critical in the cell. It is responsible in part for mediation of cytokines and the cellular response to epidermal growth factor (EGF) and other extracellular growth factor signals via membrane receptor tyrosine kinases (RTK). In the cytosol the pathway involves important signal transducers including the RAF family of cytosolic serine threonine kinases. Additionally the pathway is subject to further mediation through cytosolic G-protein related messengers which are themselves mediated by cytokines, chemokines and other signaling molecules at membrane g-protein coupled receptors (GPCRs) [4,10].

In the inflammatory process the MAPK pathway has been shown to regulate subunits of NADPH oxidase through mediation of p47phox as well as numerous other enzymes and proteins related to the activation of neutrophils in preparation for their function in inflammation. The priming of neutrophils by cytokines like TNFα and IL-8 has also been shown to be mediated in part by MAPK signaling. Data has shown that administration of 0.5 ml pomegranate seed oil composed primarily of punicic acid over a course of 10 days was able to down regulate in vivo neutrophil activation signals in pathologically inflamed rat colon by inhibition of MAPK with a subsequent reduction in inflammatory damage [4]. Another animal study showed acute inflammatory damage measured by bi-fold skin thickness was reduced in UVB exposed mouse epidermis after pretreatment with 14 days oral 0.2% w/v pomegranate extract in drinking water. It was noted that lipid peroxidation was also affected shown by generation of oxidative byproducts of approximately 200% in the UVB exposure only control versus 135% in the group receiving extract [12]. This finding may reflect the theory that some beneficial effects of the compounds in pomegranate display synergistic properties and may in fact rely on them [7,8,10,18].

Role in Cardiovascular Disease

The role of pomegranate constituents in cardiovascular disease is mechanistically related to the antioxidant and anti-inflammatory properties placed in the context of atherosclerosis and other cardiovascular pathology. Whereas the previously mentioned studies discussed neutrophil mediated damage the anti-atherogenic properties of these compounds are largely related to these mechanisms in regard to how they affect macrophages and the vascular endothelium.

Pomegranate compound inhibition of NADPH oxidase by down regulation of p47phox and other related molecules decreases the macrophage generation of superoxide and its respiratory burst. This reduces the ability of macrophages to take up plaque LDL and subsequently form foam cells, a constituent of the early fatty streaks seen in atherogenesis. An example of this concept was presented by the in vitro analysis of tissue from patients with carotid artery stenosis who had consumed approximately 240 ml of pomegranate juice/day over a period of 3 months. Results showed not only a significantly reduced concentration of lipid peroxides but a 43% reduced capacity of the fatty lesion to oxidize LDL. Additionally pomegranate compounds in concentrations of 1-50 µM have been shown to dose-dependently decrease triglyceride (TG) synthesis by up to 30%. This effect is thought to be related to up regulation of paraoxonase 2 (PON2) as shown by a 1.7 fold increase in its activity and an approximately 50% down regulation of diacylglycerol

Numerous studies propose that whole pomegranate and its constituents have potential benefit in metabolic disease [3,24,32,33]. While there has been some research on the general dietary health effects there is noted effect of pomegranate compounds in the mediation of HDL, LDL and TG through the mechanisms previously discussed [3]. However, the vast majority of research into the effects of these compounds in metabolic disease examines their role in diabetes mellitus.

Perhaps the most compelling effect of any pomegranate component in this regard is the action of punicic acid as a PPAR agonist. PPAR are a family of nuclear receptors that act as regulators of gene transcription related to cellular regulation, immunity and metabolism. PPAR ligands are endogenous lipid molecules such as prostaglandins or polyunsaturated fatty acids. Of the sub families β/δ are notably increased in the CNS and skin while α and γ are present in skeletal muscle, cardiac muscle and visceral organs. PPARγ is of particular importance and has been reported to play a role in not only type 2 diabetes but also inflammatory bowel disease, colorectal cancer and infection. Research has shown that oral administration of punicic acid moderates abnormal fasting plasma glucose in a mouse model through PPAR γ and α agonism as well as suppressing activation of NFκB and expression of TNFα [24]. The use of naturally occurring compounds such as punicic acid as PPAR agonists is a potential alternative to current glitazone diabetic therapy and continues to represent an attractive target for research.

Role in Infectious Diseases

While the majority of current research into the uses of phytochemicals like those in pomegranate focuses on intrinsic cellular processes there is a growing body of evidence supporting promising effect in combating viral, bacterial and fungal infection. In this regard it is important to recall the properties of the tannin compounds such as punicalagin.

Evidence has shown that punicalagin is able to block access to host cell glycosaminoglycans (GAGs) and subsequently inhibit attachment of viruses that use glycoproteins for entry into host cells including CMV, HCV, RSV and HIV. While remarkable this effect requires direct interaction of the tannin compound and exploration into methods of application is required [19]. Tannin compounds have...
also been shown to inhibit the motility of bacteria and decrease their ability to form biofilms potentially through similar mechanisms. This effect has been demonstrated against Pseudomonas Aerugenosa, E. Coli, Salmonella spp, Proteus Mirabilis and several other bacterial species [21]. Lastly it has been shown that Candida spp displayed abnormal cell wall morphology and variable degrees of lysis after exposure to punicalagin [29]. While achieving therapeutically feasible in vivo applications of these compounds presents a challenge the importance of combating these species, particularly in nosocomial infection merits continued effort. One interesting study was presented where polyphenolic compounds were shown to maintain their activity bound to nanoparticle polymers [8]. In this example the polyphenols were used to increase chemotherapeutic drug sensitization, however if compounds such as the tannin polyphenols maintained their properties bound to such polymers applications in other areas such as medical and prosthetic devices may present a topic for future investigation.

**Anti-Oncogenic and Anti-Tumorigenic Properties**

The anti-oncogenic and anti-tumorigenic effect of phytochemicals like pomegranate flavonoids represents one of the most critical areas of current research. The process through which these compounds exert their effect is complex and multifaceted but can fundamentally be categorized into action between cells, extracellular signal mediation, membrane processes and the modulation of intracellular or intranuclear signals and regulators. The NFκB and MAPK pathways are important examples of these signaling processes.

A primary role of NFκB is regulation of cytokine signaling in response to stress, however its role can be expanded when discussing oncogenic and tumorigenic processes. For example NFκB is activated in response to UVB induced DNA damage in mammalian epidermal cells [12]. This stress response plays a role in cellular damage signaling and cell survival, a process displayed by the subsequent up regulation of anti-apoptotic factor Bcl-2 expression as a result of nuclear NFκB activation. At the same time this UVB damage activates the tumor suppressor p53/p21 signal cascade resulting in cell cycle arrest permitting damage repair or allowing facilitation of apoptosis [8]. Numerous studies have shown the ability of pomegranate compounds to inhibit NFκB while augmenting p53 and other tumor suppressor pathways to prevent pro-tumorigenic or oncogenic events in cells experiencing stress while sparing healthy cells [6,8,11-13,16,17,34,35].

The role of MAPK signal transduction cascade is a pivotal player in oncogenesis and tumorigenesis, regulating processes related to cellular proliferation, differentiation, cell cycle progression, inflammation and apoptosis. There are many examples of pomegranate compound mediation of specific MAPK involvement in human cancer [6,9,11,15,23]. For example in vitro analysis of chronic myelogenous leukemia myeloid precursor cells pretreated with 20-100 µg/ml of pomegranate extract (19% punicalagin/punicalin, 4% free ellagic acid, 77% oligomeric ellagic/gallic acid) resulted in selective attenuation of ERK, JNK (MAPK subclasses) and reduced binding of NFκB to DNA transcription sites leading specific reduction in gene expression of IL-6 and IL-8 resulting in an anti-oncogenic effect. In this study it was also noted that the potency of this effect was increased when quercetin was added, supporting the theoretical synergistic capacity of these compounds [10].

Another important signaling pathway shown to be affected by pomegranate constituents is the phosphatidylinositol-4,5-bisphosphate 3-kinase/protein kinase B (PI3K/Akt) pathway. This pathway is mediated by IGF signaling at membrane RTKs and plays an important role cell growth and survival through the mediation of NFκB, G-protein second messengers, apoptosis and other signal cascades like mammalian target of rapamycin/phosphate and tensin homolog (mTOR/PTEN). Like the MAPK pathways PI3K/Akt dysfunction has been shown to play a role in several human cancers including breast cancer, lung carcinoma and colon cancer [6,13,15]. An example of pomegranate compound mediation of these pathways showed that direct in vitro administration of 30 µM luteolin for 24 hours was able to inhibit migration of human glioblastoma cells by down regulating PI3K/Akt activation after proteosomal lysis of cell division control protein 42 (cdc42), a small GTPase involved in many cell functions [14].
The interaction between PI3K/Akt, MAPK, NFκB and other signal transduction cascades represents one of the most complex yet compelling areas of investigation for pomegranate compounds and other phytochemicals. However this is not the limit of their application and in fact the downstream processes these pathways effect may play a more critical role in combating oncogenic and tumorigenic processes. In this regard it is important to address two cellular processes classically involved in oncogenesis, namely abnormal cell cycle progression and dysregulation of apoptosis.

Many of the previously discussed pathways normally operate to regulate the cell cycle and under pathological conditions can promote tumorigensis and oncogenesis. An important example of cell cycle regulation is the cyclin/cyclin dependent kinase (CDK) system. Cyclins are a protein family that control specific cell cycle progression by complexing with CDKs, allowing them to exert their function as serine/threonine kinases. In general, cyclin A, D and E along with CDK 2, 4 and 6 induce G1 to S cycle progression and cyclin B/CDK1 induces G2 to M. In addition several classic tumor suppressors exert their function on cyclin/CDKs notably p15 and p16 inhibition of cyclinD/CDK4 and p21/p27 inhibition of cyclinE/CDK2, with p21 itself being induced by seminal tumor suppressor p53 [6,15,22,23,34]. Pomegranate compounds have been shown to mediate the function of these pathways in pathologic cells. An in vitro analysis of 50-150 mcg/ml whole pomegranate extract application on A459 lung carcinoma cells demonstrated dose-dependent 65-72% G1 cell cycle arrest related Induction of p21/27 and concomitant reduction in cyclin D,E and CDKs 2, 4 and 6 [6]. Other evidence shows the effects of ellagic acid derivatives on an in vitro human hepatocellular carcinoma cell line and found that application of 45-90 µmol/L ellagic acid derivative concentrations down regulated CCND1 (cyclin D1 gene) expression leading to inhibition of unregulated G1 to S cell cycle transition [22]. This effect may have been produced by increasing expression of or protecting tumor suppressors such as retinoblastoma (RB) from inactivation related to CCND1 expression, however the exact mechanism is unknown [22,23].

Apoptosis is an important function in humans, responsible for programmed cell death and in part for determination of cell survival. Dysregulation of apoptosis is the basis for many pathological conditions and plays a large role when discussing oncogenesis and tumorigenesis. The process of apoptosis can be divided into extrinsic and intrinsic mediation. Extrinsic mediation induces apoptosis in several important ways including TNFα binding to TNFα receptor associated death domains (TRADD), Fas/FasL interaction and killer T-cell perforin/granzyme induction. Intrinsic apoptosis is intimately related to the mitochondrial membrane permeability reflecting cellular damage states and the release of cytochrome C. Both apoptotic pathways result in facilitation of caspase cascades ultimately causing cell death. Regulation and dysregulation of apoptosis is reliant upon a balance between pro-apoptotic and anti-apoptotic factors with their respective signal associations. An important example is the Bcl-2 family including anti-apoptotic factors such as Bcl-2 and Bcl-XL and pro-apoptotic factors Bax and Bad [8,12,13,15,22]. Disruption of the balance between anti-apoptotic and pro-apoptotic factors is one basis of abnormal cell survival in oncogenic processes and represents a crucial area where phytochemicals may play a role. Luteolin affects colon cancer cell lines expressing abnormal ratios of Bcl-XL related to IGF-II and janus tyrosine kinase/signal transducer and activator of transcription (JAK/STAT) signaling. In vitro dosing of 0-60 µmol/L luteolin is able to inhibit Bcl-XL and disinhibit p53 induction of pro-apoptotic Bax by antagonizing the expression of p53 inhibitor mdm2 leading to decreased abnormal cell survival [15]. Other evidence shows that ellagic acid in similar concentrations was able to decrease anti-apoptotic Bcl-2 while increasing pro-apoptotic Bax and caspase activation in human hepatocellular carcinoma cell lines leading to selective apoptosis of the cancer cells [22].

Exactly how phytochemicals like those in pomegranate accomplish these anti-oncogenic and anti-tumorigenic effects is an active area of investigation. While it is likely related to the summation of their activity as a whole on multiple pathological regulatory levels several specifics have been presented. Two of the most compelling examples of pomegranate compound activity are in the regulation of the ubiquitin-proteosome pathway (UPP) and micro-RNA (miRNA) expression.

The UPP is responsible for proteolysis of intracellular proteins marked by ubiquination. This allows for the removal of misfolded or damaged proteins and the regulation of many cellular functions with notable roles in the cell cycle, stress response and immunity. Evidence suggests that the UPP may accomplish this through regulation of cyclin/CDK effectors, apoptotic factors like Bcl-2 and Bax, tumor suppressors and NFκB activation. Flavonoids may exert some of their effect by inhibiting the UPP. For example epigallocatechin (EGCG), a compound of the flavonoid catechin & gallic acid was found to bind a specific β subunit of the proteosome inhibiting its proteolytic activity. A specific region of EGCG was found to mimic tyrosine and bind a separate site on the proteosome mediating its function. Other tannin compounds were found to contain ester groups whose reaction resulted in inhibition of proteosomal function and increase in Bax and p27. Another relevant finding is the UPPs ability to increase ATP binding cassette (ABC) and multidrug resistance (MDR) efflux pumps through enhancement of p-glycoprotein glycosylation, an important mechanism of drug resistance. Catechins were found to inhibit this function and thereby reduce chemotherapeutic drug resistance to agents such as tamoxifen, doxorubicin and cytarabine. Lastly, the UPP may mediate DNA repair mechanisms and homologous recombination events [8].

The function of miRNA in healthy and pathologic cells is still not completely understood however its primary function is likely the regulation of genetic expression at several important levels. While miRNA may exert some effect through DNA and histone modification its primary regulatory function is accomplished by binding to the 3’ UTR of messenger RNA and subsequently decreasing the expression of protein. Evidence has shown that variation of miRNA expression may be related to homologous recombination events. Mediation of these events may lead to Down regulation of aberrant miRNA and up regulation of tumor suppressor miRNA potentially explaining the effect of some phytochemicals like those found in pomegranate [5,7-9,11,13,34]. One recent study demonstrated that treatment of breast cancer cell lines with 2.5-10 μg/ml whole pomegranate polyphenols mediated the expression of miRNA-27a and miRNA-155 leading to induction of tumor suppressor src homology-2 domain inositol phosphatase (SHIP1), decreased activation of PI3K/Akt and NFκB with subsequent reduction in angiogenic and inflammatory mediators [13]. A separate study found that homologous recombination variation of miRNA may have mediated aberrant increases in dsDNA break repair in breast cancer cells. Administration of pomegranate extract reversed this effect and led to increased dsDNA strand breaks in these cancer cells [35]. Lastly a compelling study found that in prostate cancer cell lines a combination of Luteolin, ellagic acid and punicic acid upregulated tumor suppressing miRNA and down regulated oncogenic miRNAs. Notable examples include increase in miRNA-34c/127 and miRNA-124 with reduced expression of oncogenic Bcl-2 and CDK 6 respectively and reduced miRNA-29b/181b and miRNA-27a increasing tumor suppressors PTEN and p21 respectively. While these examples are important the list of relevant miRNA is expansive and should not be understated. Other critical areas such as angiogenesis, E-cadherin stability and regulation of tissue migration factors are affected by miRNA expression suggesting a role in oncogenic invasion and metastasis making further investigation into the area vitally important [7].

Discussion

The broad range of effects represented by the compounds within whole pomegranate is daunting and represents a challenge in discerning effect of specific molecules. Of the main groups of compounds presented the most striking action appears to be from the flavonoid compounds and the tannins. As antioxidants their action in reducing free radical damage and lipid peroxidation is at least in part explained by auto-oxidation. However it is their apparent ability to interact with cellular processes that remains to be fully elucidated.

Punicalagin is often cited as one of the primary substances responsible for the effects of the tannins. It is known that the molecule is able to form insoluble complexes with amino acids, carbohydrates and nucleic acid. This ability may offer some explanation for the anti-microbial effect of punicalagin by blocking cell surface glycosaminoglycan binding sites preventing viral host cell entry and inhibiting bacterial biofilm formation. The compelling findings in regard to aberrant cell cycle, apoptotic, proteosomal and miRNA are less apparent. Given the observations presented it is likely that these effects are related to the ellagic & gallic acid derivatives of the tannin compounds. While the exact mechanisms are unknown the capacity to form ester bonds with other compounds and present a biologically active site for nucleophilic attack is an appealing avenue of investigation [36].
The flavonoids are an equally interesting enigma. While the effects proposed for these compounds are comparable to the tannins there are some key differences and similarities in molecular action. One of the most striking differences is the fact that some flavonoids are structurally similar to steroid hormones such as estrogen. This could explain not only the capacity to gain entry into the cell itself but also nuclear sites where interaction with transcription factors and other nucleic acid elements may result in some of the more compelling effects observed in regards to NFκB activation or miRNA expression. In addition these molecules are not subject to the same amount of concern regarding metabolic hydrolysis and thus they may be more promising in clinical application. The capacity of flavonoids to form a biologically active ester bonds may present a similarity in function to tannins. The fact that both flavonoids and tannins hold this ability presents an interesting observation of possible synergy with an excellent example being epigallocatechin, an ester of catechin and gallic acid. This compound is reputed to be responsible for the beneficial effects of green tea with specific action on the proteasome complex among others. This specific interaction of ester bond functionality, the flavonoids and the tannins could potentially hold the key to their beneficial effects [37].

While the observed benefits of the compounds are notable the disparity between in vitro observations versus the reality of clinical application in real patients cannot be ignored. One of the critical downfalls with polyphenolic compounds is the extensive metabolism which they undergo, most notably vulnerability to gastric acid and intestinal hydrolysis. The majority of human observations relies upon long term supplementation of whole pomegranate juice or extracts potentially allowing beneficial compounds to reach appreciable systemic levels. While beneficial the effects are debatable in their clinical utility and their efficacy as an actual treatment demands further refinement. Several options have been discussed including potential acetylated pro-drug formulations but to the best of our knowledge nothing has been investigated in depth. If any of the dramatic effects observed in the literature are to ever be realized further investigation is required.

Conclusion

Compounds in pomegranate display an incredibly broad range of activity in critical areas of human disease and present promising avenues of investigation into new treatment modality. The potential applications in cardiovascular, inflammatory and infectious diseases as well as diabetes and cancer underlie the crucial importance of developing effective ways in which to apply these compounds. While some effect has been observed in human studies with simple ingestion of juice or extract questions concerning the bioavailability, metabolism and actions of specific constituents’ remains and much of the future challenge for these compounds may be in developing effective methods of specific therapeutic application. Despite these issues the human and economic cost of cardiovascular disease, diabetes and cancer alone demands continual effort into new therapeutic options and in this capacity the pursuit of potential benefits of phytochemicals like those in pomegranate is a vital direction of continued research.

Bibliography


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