Pomegranate and breast cancer: possible mechanisms of prevention

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Several mechanistic studies in cell culture and mouse models suggest possible estrogen receptor-mediated and non-estrogen receptor-mediated benefits of pomegranate juice with respect to breast cancer risk. These studies demonstrate that various constituents of pomegranates can inhibit aromatase and 17β-hydroxysteroid dehydrogenase enzymes or have antiestrogenic activity. Additional large, well-controlled human studies are warranted to elucidate the effects of pomegranate juice intake on serum hormone levels. Clarifying the effects of pomegranate constituents on key hormones known to be involved in breast cancer could result in important information for consumers and shed further light on the impact of diet on breast cancer risk.

INTRODUCTION

In 2008, an estimated 182,460 new cases of breast cancer were diagnosed in the United States, and an estimated 40,480 deaths due to breast cancer occurred among US women.[1] Generally accepted risk factors for breast cancer include older age, family history of breast cancer, early age at menarche, late age of menopause, long-term use of estrogen-replacement therapy, and later age at birth of first-born child. As many of these established risk factors suggest, steroid hormones, particularly estrogens, are centrally involved in the development of breast cancer.[2] The dietary-related factors most convincingly associated with breast cancer risk, i.e., postmenopausal obesity and alcohol intake, are also likely to be linked, at least in part, to higher cumulative estrogen exposure.

An active area of research concerning the dietary effects on breast cancer has been the connection between soy intake and breast cancer risk. Isoflavonoid compounds in soybeans, such as genistein and daidzein, act as phytoestrogens that bind to estrogen receptors, exerting weak estrogenic effects that may lower breast cancer risk.[3] A more recent development in the area of diet and breast cancer is the potential beneficial effect of phytochemical components in pomegranates. Pomegranate fruits, beverages, and related products are frequently highlighted for their putative beneficial health effects on arthritis, diabetes, cardiovascular disease, prostate cancer, and other medical conditions. Reviews of the potential health effects of pomegranate fruit are available, but these are generally focused on other disease outcomes or broader aspects of health.[4–8] The purpose of this review is to bring together some of the available in vitro and in vivo evidence relevant to the possible mechanistic effects of pomegranates and its constituents with respect to breast cancer risk and to identify areas that need additional study. Consideration of the available evidence on this topic is of interest because of the pomegranate fruit's unique phytochemical properties and its potential effects on estrogenic hormonal activity and health. In vitro cell culture studies, animal studies, and available human data are described.

THE POMEGRANATE: AN OVERVIEW

The pomegranate fruit (*Punica granatum* L.) is revered in the traditions and mythology of ancient civilizations.[9] In modern times, pomegranates are frequently used in the

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cuisine of Eastern and Middle Eastern countries. In the United States, fresh pomegranate fruit has become increasingly popular over the past decade.[6] Sales of refrigerated pomegranate juice soared from zero in 2001 to more than $63 million in 2005, according to A.C. Nielsen, a market research firm.[10] According to industry reports,[11] 190 new pomegranate-flavored foods and beverages were introduced in the United States in the first 10 months of 2005, up from 33 products in 2003. Other countries, including Japan[12] and England[13] have also observed a surge in pomegranate popularity.

The pomegranate fruit has three main components: the seeds, the juice, and the peel (i.e., pericarp or husk), which also includes the white interior membranes. Typically, the numerous seeds and the fleshy sacs containing them are eaten whole, with the pomegranate peel and its associated white membranes discarded. Each ruby-colored juicy sac covers a small, hard, white seed and is known as an aril. Commercial juice is typically obtained by pressing whole fruits.

The largest class of pomegranate phytochemicals includes the polyphenolic compounds, which are characterized by multiple phenol rings bearing several hydroxyl groups.[7] Research interest related to the putative health benefits of this fruit has primarily been generated because of two major classes of polyphenols: flavonoids, particularly anthocyanidins (delphinidin, cyanidin, and pelargonidin), which are present in the peel or juice,[14,15] and hydrolyzable tannins, including the ellagitannin, punicalagin, which is unique to pomegranates. Other flavonoids of possible interest include flavonols (kaempferol, quercetin),[16,17] and flavones (luteolin),[16] also found in pomegranate peels[18,19] and commercially available juices.[20]

The exceptionally high antioxidant activity of pomegranate juice is primarily attributable to the ellagitannins.[18,21] Hydrolysis of ellagitannins releases ellagic acid, some of which is absorbed, while a portion is further metabolized to urolithins A and B by human intestinal microflora, which are subsequently excreted in both urine and feces.[20,22] Although ellagic acid disappears from plasma 6 hours after administration of pomegranate juice, the urolithin derivatives persist in plasma for somewhat longer and may be excreted in human urine for up to 2 days after juice consumption.[20] Based on their putative antioxidant potency, ellagitannin-enriched pomegranate extracts, typically made from whole pomegranate fruits, are also widely available in dry and liquid supplements.[23] In addition to polyphenolic compounds, other pomegranate phytochemicals include punicic acid, a unique conjugated form of linolenic acid found in pomegranate seed oil,[24] and coumestrol, an isoflavone present in pomegranate seeds.[25]

POTENTIAL EFFECTS OF POMEGRANATES ON ESTROGEN SYNTHESIS

Estrogen synthesis

In premenopausal women, synthesis of the estrogens estrone (E1) and estradiol (E2) and other sex steroids takes place in the theca cells of the ovary, where cholesterol undergoes a series of irreversible reactions that ultimately produce androstenedione. In ovarian granulosa cells, a P450 enzyme known as aromatase, or CYP19, can convert androstenedione to estrone, which has relatively weak estrogenic activity (Figure 1). Estrone can then be reduced to estradiol, the most biologically active form of estrogen, by the enzyme 17β-hydroxysteorid dehydrogenase. In an alternative pathway, androstenedione is first reduced to testosterone, which is subsequently converted to E2 by the same aromatase enzyme. Both E1 and E2 are secreted by the ovaries, although estrone can also be synthesized in peripheral tissues, including adipose tissue. Estriol (E3) is a much less bioactive form of estrogen that is produced peripherally from estrone primarily during pregnancy, at which time E3 is produced by the placenta in relatively high quantities. After menopause, most circulating estrogen is derived from the conversion of adrenal androgens to E1 by aromatase in adipose tissue; some E1 is further metabolized to E2, which is the most biologically active estrogen in breast tissue.[26]

Although most circulating estrogen is bound to sex-hormone binding globulin (SHBG), the free, unbound E2 is thought to be the biologically active fraction.[27] Estrogens are involved in cell proliferation in the mammary gland and are believed to play a pivotal role in breast cancer initiation and progression. Epidemiologic studies indicate that elevated serum levels of E1 and E2 and lower levels of SHBG after menopause substantially increase the risk of breast cancer.[28] Thus, dietary interventions that reduce estrogen synthesis in postmenopausal women have the potential to lead to effective prevention strategies for breast cancer.

Aromatase inhibition

Kim et al.[29] reported that both fermented pomegranate juice and unfermented polyphenols extracted from pomegranate peels have substantial aromatase inhibitory activity. In a placental microsome system, aminoglutethimide (a known aromatase inhibitor) at a dose of 100 μmol/L was associated with approximately 65% inhibition of aromatase activity. Comparable figures for fermented pomegranate juice and pericarp polyphenols were 51% and 24% inhibition. Thus, fermented pomegranate juice and pericarp polyphenols had nearly 80%

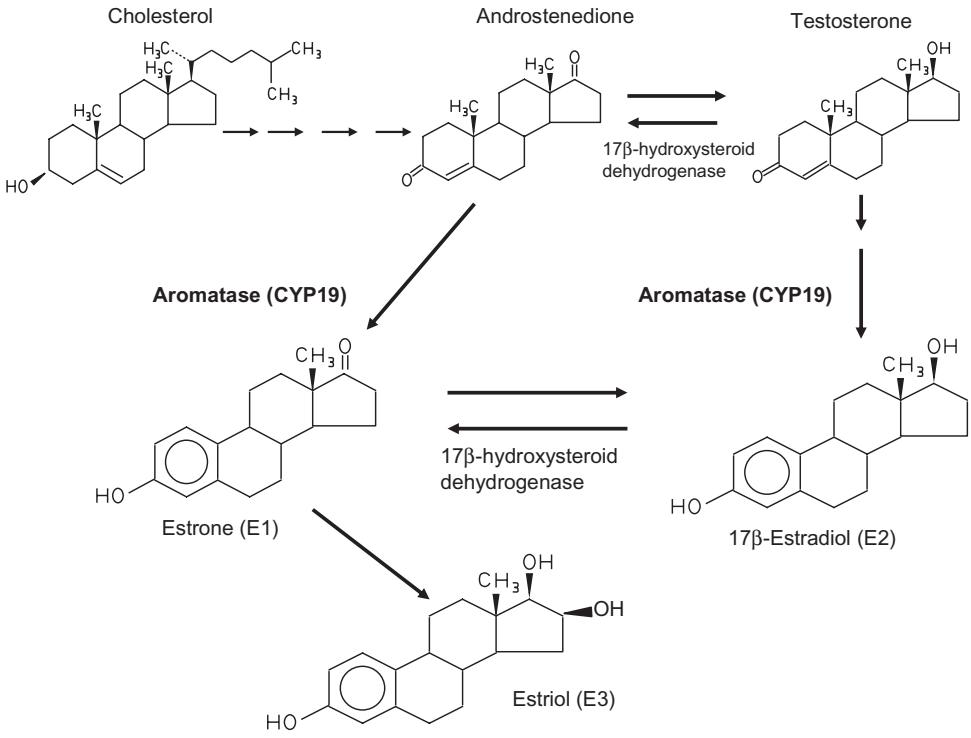

Figure 1 **Summary of estrogen synthesis.**

and 40%, respectively, of the aromatase inhibitory activity of a known clinical aromatase inhibitor. The observed stronger inhibition of the fermented juice compared to extracted pericarp polyphenols was attributed to breakage of flavonoid-sugar complexes during fermentation, a process that was compared to acid hydrolysis in the stomach.[29] The specific compounds or combination of compounds present in pomegranate juice contributing to its putative estrogen inhibition are unknown, but its effects are consistent with competitive inhibition of aromatase. Several flavonoids found in pomegranates (e.g., luteolin, coumestrol, kaempferol) have been shown to competitively inhibit aromatase in a human preadipocyte cell culture system.[30]

Cyclooxygenase inhibition

Constituents of the pomegranate fruit could also inhibit aromatase expression via effects on cyclooxygenase-mediated pathways. Cyclooxygenase (COX) catalyses the rate-limiting step in the synthesis of prostaglandins, including prostaglandin E2 (PGE2), primarily from arachidonic acid. The COX enzyme exists in two isoforms: COX1, which is constitutively expressed in most cells and COX2, an inducible form associated with inflammation. Decreased production of PGE2 has been shown to downregulate aromatase expression,[31] and inhibition of COX-2 reduces breast cell proliferation.[32,33] Alterations in COX-mediated pathways may explain the observed inverse associations between use of non-steroidal anti-inflammatory medications (NSAIDS), which inhibit both COX isoforms, and breast cancer risk.[34,35] In a study by Schubert et al.[24] cold-pressed pomegranate seed oil (although not fermented juice with seeds included) inhibited sheep COX by 31% to 41%.

Punicic acid. Over 65% of pomegranate seed oil is punicic acid, a polyunsaturated fatty acid found almost exclusively in pomegranates. Punicic acid has previously been reported to substantially inhibit prostaglandin production and it may be involved in the COX inhibition associated with pomegranate seed oil. Recently, Shukla et al.[36] reported that plasma obtained from rabbits after ingesting a pomegranate fruit extract inhibited activities of both COX-1 and COX-2 enzymes ex vivo, a finding which indicates that pomegranate metabolites present in blood after pomegranate juice consumption have measurable physiologic effects. In addition, fermented pomegranate polyphenols and whole pomegranate polyphenols and whole pomegranate oil were shown to substantially reduce the number of lesions from a known carcinogen in a mouse mammary model.[29,37] While fermented polyphenols produced a 42% reduction in the number of lesions compared to controls, a purified peak of pomegranate juice phenols and pome-

granate seed oil each effected an 87% reduction in lesions. Mehta et al.[37] speculate that the effect of pomegranate seed oil was due to punicic acid and its ability to inhibit prostaglandin biosynthesis in humans. Additional research is needed to determine whether COX inhibition by these pomegranate-derived compounds ultimately influences breast cell proliferation in humans.

Ellagic acid and anthocyanins. Constituents of pomegranates other than punicic acid may also modulate COX activity and inflammatory responses. For instance, ellagic acid reportedly modifies subcellular signaling pathways by decreasing COX2 activity.[38] Ellagic acid has been shown to exhibit apoptosis, inhibit activation of inflammatory pathways, and inhibit angiogenesis. In addition, a prodelphinidian isolate from the blackcurrent berry (*Ribes nigrum*) has been shown to inhibit COX and prostaglandin production, suggesting that delphnidins (anthocyanins also present in the arils of pomegranate fruit) may inhibit inflammatory responses.[39]

17β- hydroxysteroid dehydrogenase inhibition

Pomegranate juice has also been shown to inhibit 17β-hydroxysteroid dehydrogenase (HSD),[29] the enzyme that catalyzes conversion of E1 to the more biologically active E2 (Figure 1). Using an established laboratory method involving transfected embryonic kidney cells to evaluate the activity of different fractions of pomegranate juice (fermented juice, polyphenols from pericarp, and pure pomegranate seed oil), Kim et al.[29] found that all three fractions substantially inhibited 17β-HSD. These data are supported by additional evidence indicating that flavonoids, which are present in the juice and pericarp, are inhibitors of 17β-HSD activity.[29] However, pomegranate oil does not contain these flavonoids, which suggests that other components in the oil may also be inhibitory.

Antiestrogenic and estrogenic activity

Flavonoids. In addition to influencing enzymes involved in estrogen synthesis, pomegranate constituents may also act as phytoestrogens – nonsteroidal plant compounds that possess estrogenic or antiestrogenic activity. Phytoestrogens have been implicated in breast cancer etiology. In a high-estrogen milieu, phytoestrogens are thought to compete with natural estrogens, binding to the estrogen receptor and thereby reducing breast cancer risk. In a low-estrogen milieu, phytoestrogens may exert estrogenic effects and increase breast cancer risk.[40] Most epidemiologic studies to date have focused on the effects of isoflavones, phytoestrogens found predominately in soy products, and breast cancer risk and have reported protective effects with higher soy intakes.[3] There is still concern among some that soy intake may be an estrogenic stimulus, thereby potentially increasing breast cancer risk; soy intake has been associated with an increase in nipple aspirate volume secretion and an increase in cathepsin D and pS2, estrogen-inducible proteins, in nipple aspirate fluid.[41,42]

Fewer epidemiologic data are available on the possible roles of phytoestrogens from non-soy sources on breast cancer risk, primarily due to historically limited information on flavonoid concentrations in food. Several recent epidemiologic studies, although not all,[43] have observed reductions in postmenopausal breast cancer risk with increasing intakes of flavones[44–46] and flavonols.[44,46] These studies have generated interest in the possibility that phytoestrogens from other foods, such as pomegranates, may be protective against breast cancer.

Evidence from in vitro studies suggests a possible antiestrogenic effect of pomegranate constituents. In a yeast estrogenic screen, lyophilized pomegranate reduced estrogenic activity from 100 units to 40 units (56% inhibition).[29] According to Kim et al.[29] these findings were most likely attributable to competitive binding to estrogen receptors by the non-steroidal estrogenic flavonoids in pomegranates, including luteolin, kaempferol, quercetin, naringenin, and coumestrol and the weakly estrogenic 17-alpha-estradiol. Pomegranate juice has also been shown to have strong or moderate antiproliferative activity on estrogen-receptor (ER)-positive and ER-negative breast cancer cell lines, respectively.[29] The appearance of effects in both ER-positive and ER-negative cell lines was taken to indicate that pomegranates can inhibit cell proliferation through both ER- and non-ER-mediated mechanisms.

Despite evidence of antiestrogenic effects, limited animal data suggest a possible estrogenic action of pomegranates. Sharaf et al.[47] reported that pomegranate seed oil injected into ovariectomized mice increased cornification of vaginal cells. Similarly, uterine weight was higher in immature rabbits injected with pomegranate seed oil than in the control rabbits.[47] In another study,[12] administration of pomegranate juice and seed extract to ovariectomized mice prevented uterine weight loss and increased bone volume in the pomegranate-treated group compared to control animals.

Sex steroids. Although several historical reports in the literature[48,49] suggest that pomegranate seeds contain estrone that is identical to that found in humans, a more recent report using high performance liquid chromatography mass spectrometry methods found no evidence of

steroidal hormones, including estrone, estradiol, and testosterone, in pomegranate seeds, juice, or concentrated extract.[50]

Ellagitannins. As previously mentioned, ellagitannins are found in the outer part of the pomegranate fruit. Ellagitannins, including punicalagin, the most abundant ellagitannin in pomegranates, are metabolized to ellagic acid. Recent evidence indicates that ellagic acid may possess either estrogenic or antiestrogenic activity, depending on its concentration and the estrogen receptor to which it binds. In a study using HeLa cells, Papoutsi et al.[51] reported that, in low concentrations, binding of ellagic acid to the estrogen-alpha receptor produced estrogenic activity, whereas binding to the estrogen-beta receptor led to antiestrogenic activity. Moreover, in an osteoblastic cell line, ellagic acid produced estrogenic effects (nodule mineralization), which were reversed by exposure of the cells to an estrogen antagonist, whereas in MCF-7 breast cancer-derived cells, ellagic acid demonstrated potent antiestrogenic activity. Further evidence of antiestrogenic activity was shown in a study using ACI rats (a rodent model of breast cancer) that received implants of 17β-estradiol. Aiyer et al.[52] found that treatment with ellagic acid (400 mg per kg of diet) decreased estrogen-induced mammary tumor volume by 75% and reduced the number of tumors by 44% compared to the control rats.

In addition to these compounds, the ellagic acid metabolites urolithin A and B, which are produced by colonic microflora, also appear in plasma and may exert physiologic effects. Larossa et al.[22] reported that both urolithin A and B exhibited dose-dependent estrogenic activity that was somewhat weaker than that of other phytoestrogens, such as genistein. However, the urolithins also showed antiestrogenic activity that was more pronounced than that of the other phytoestrogens evaluated. These findings suggest that these gut-derived metabolites may function as dietary "prophytoestrogens."[22]

Human evidence

To our knowledge, no studies of the effect of pomegranate juice intake on serum hormones among postmenopausal women have been published in peer-reviewed journals. One small study of postmenopausal women ($n = 11$) involved a 7-day intervention period consisting of eight ounces daily of commercial pomegranate juice.[6] Although the author reported a statistically significant ($P < 0.001$) increase in serum estrone levels (40.8 pg/mL to 64.6 pg/mL), no change in serum estradiol levels was noted. Whether these observed changes in serum estrone levels are real or represent an important biologic effect is unclear. Increased circulating levels of estrone have been associated with a 40% higher risk of postmenopausal breast cancer.[53]

CONCLUSION

In summary, several mechanistic studies in cell culture and mouse models suggest possible estrogen receptor-mediated and non-estrogen receptor-mediated benefits of pomegranate juice with respect to breast cancer risk. Large, well-controlled studies are warranted to elucidate the effects of pomegranate juice intake on serum hormone levels. Clarifying the effects of pomegranate constituents on key hormones known to be involved in breast cancer could result in important information for consumers and shed further light on the impact of diet on breast cancer risk.

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