Cancer Chemoprevention by Pomegranate: Laboratory and Clinical Evidence

Vagar Mustafa Adhami, Nagmina Khan, and Hasan Mukhtar

INTRODUCTION

The fruit of the tree Punica granatum, grown mainly in the Mediterranean region, has been shown to possess many medicinal properties such as being antioxidant and anti-inflammatory (1). The antioxidant activity of flavonoids obtained from pomegranate juice (PJ) was observed to be close to that of butylated hydroxyanisole, green tea, and significantly greater than red wine (2,3). Commerically available pomegranate juices tested for their antioxidant activity by the Trolox Equivalent Antioxidant Capacity (TEAC) assay showed antioxidant activity of \(-18\) to \(20\) TEAC that was three times higher than those of red wine and green tea (4–6 TEAC). Interestingly, the antioxidant activity was higher in commercial juices that were extracted from whole pomegranates than in experimental juices that were obtained from the arils only (7). Antioxidant activities of freeze-dried preparations of pomegranate and its 3 major anthocyanidins (delphinidin, cyanidin, and pelargonidin) were evaluated by Noda et al. (8) by the method of electron spin resonance technique and spin trapping. Pomegranate extract exhibited scavenging activity against \(\cdot\text{OH}\) and \(\cdot\text{O}_2^-\). The anthocyanidins were found to inhibit a Fenton reagent \(\cdot\text{OH}\) generating system possibly by chelating with ferrous ion. Also, anthocyanidins scavenged \(\cdot\text{OH}\) in a dose-dependent manner, and ID_{50} values of delphinidin, cyanidin, and pelargonidin were 2.4, 22, and 456 \(\mu\text{M}\), respectively. Anthocyanidins inhibited \(\text{H}_2\text{O}_2\)-induced lipid peroxidation in the rat brain homogenates and ID_{50} values of delphinidin, cyanidin, and pelargonidin were 0.7, 3.5, and 85 \(\mu\text{M}\), respectively (9). Pomegranates have only recently been studied for their anticancer effects (10). The following sections will summarize the studies on the effects of pomegranate against various cancers.

POMEGRANATE AND BREAST CANCER

Polyphenolic fractions from pomegranate fruit were assessed in vitro for their possible chemopreventive activity or as an adjuvant in a therapeutic setting against human breast cancer cells (11). Polyphenols obtained from fermented juice at concentrations ranging from 100 to 1,000 \(\mu\text{g/ml}\) inhibited aromatase and \(17\beta\)-hydroxysteroid dehydrogenase type 1 activity by 60–80%. Human breast cancer cell lines MCF-7 and MB-MDA-231 cells were treated with fermented pomegranate juice and fresh pomegranate juice. Polyphenols from fermented juice showed about twice the antiproliferative effect as compared to polyphenols from fresh pomegranate juice. Pomegranate seed oil (PGO; 100 \(\mu\text{g/ml}\) of medium) resulted in an antiproliferative effect as compared to polyphenols from fresh pomegranate juice. Pomegranate fermented juice polyphenols.

POMEGRANATE AND PROSTATE CANCER

The effects of pomegranate on prostate cancer (PCa) have been investigated in the cell culture system, animal models, and in a phase II clinical trial in humans. Various preparations of pomegranate, in the form of oils, fermented juice polyphenols, and pericarp polyphenols, were tested on human PCa cell growth both in vitro and in vivo (12–14). Each preparation inhibited growth of human PCa LNCaP, PC-3, and DU 145 cells, whereas normal prostate epithelial cells were significantly less affected (15). These effects were observed to be mediated by changes in cell cycle distribution and induction of apoptosis. Androgen-independent DU 145 cells were treated with pomegranate cold pressed oil (35 \(\mu\text{g/ml}\) and found to...
accumulate in the G2/M phase of the cell cycle that was associated with a significant upregulation of the cyclin-dependent kinase inhibitor (cdk) p21 and downregulation of e-cadherin (e-cad). In contrast, cell proliferation was inhibited predominantly by induction of apoptosis in PC-3 cells through a caspase-3-mediated pathway. All forms of pomegranate preparations were found to inhibit PC-3 cell invasion through Matrigel and also inhibited the growth of PC-3 xenograft in athymic nude mice (9,13). These findings have suggested an overall significant antiproliferative and antimigratory action of the polyphenol fraction of pomegranate fruit extract against human Pca cells. In vivo studies have demonstrated that pomegranate fruit extract effectively inhibited cell growth and invasion of prostate cancer xenografts in mice.

Antiproliferative and proapoptotic properties of pomegranate fruit extract (PFE) against human Pca cells were demonstrated by the authors (15-18) both in the cell culture system and in a xenograft mouse model. Human Pca PC-3 cells treated with PFE (10-100 µg/ml) for 48 h resulted in a dose-dependent inhibition of cell growth and induction of apoptosis (15). The induction of apoptosis and cell cycle arrest was associated with upregulation of proapoptotic genes (such as Bak, downregulation of anti-apoptotic Bel-2 and Bel-1, induction of WAF1/p21 and KIP/flip, a decrease in the protein expression of cyclin-dependent kinase (cdk)2, -4 and -6). To demonstrate the efficacy of PFE in an in vivo setting, athymic nude mice were implanted with androgen-responsive CWR22Rv1 cells and given 0.1% and 0.2% (wt/vol) PFE in drinking water starting simultaneously after cell implantation (16). The selection of doses, 0.1% and 0.2%, was based on the assumption that a typical healthy individual (~70 kg) may be persuaded to drink 250 or 500 ml of pomegranate juice extracted from one or two fruits, respectively. Oral infusion of PFE to mice resulted in a significant inhibition in tumor growth as observed by prolongation of tumor appearance. Tumor volumes were consistently lower in mice that received PFE, with effects being dose-dependent and the maximum inhibitory effect observed in the 0.2% PFE-fed group. Tumor growth inhibition was accompanied with a concomitant decrease in serum prostate-specific antigen and serum PSA levels were 70–85% lower in PFE-fed mice as compared to water-fed mice (16). The reduction in prostate tumor growth with concomitant reduction in PSA levels observed in the xenograft model suggested that PFE may have clinical relevance.

PCAs initiate as an androgen regulated disease; however, advanced disease acquires androgen-independence. Overexpression of the androgen receptor promoter drives the development of androgen independence. Hong et al. (17) investigated the effects of pomegranate polyphenols, ellagitannin-rich extract, and whole juice extract on the expression of genes for key androgen-synthesizing enzymes and the androgen receptor. Genes HSD3B2 (3β-hydroxysteroid dehydrogenase type 2), AKR1C3 (aldo-keto reductase family 1 member C3), and SRD5A1 (steroid 5α-reductase 1) in LNCaP, LNCaP-AR, and DU-145 human Pca cells. Pomegranate polyphenols inhibited gene expression and AR most consistently in the LNCaP-AR cell line where androgen receptor was overexpressed (17). These studies have suggested that pomegranate polyphenols may be of particular importance in androgen-independent Pca cells and the subset of human prostate cancers where the androgen receptor is upregulated.

In a phase II clinical trial, Pantuck et al. (18) recruited patients with rising PSA and gave them 8 ounces of pomegranate juice daily until disease progression. PSA doubling time significantly increased with treatment from a mean of 15 mo at baseline to 54 mo posttreatment (P < 0.001). A major drawback of this study was the absence of a proper placebo control; however, statistically significant prolongation of PSA doubling time suggested a potential of pomegranate for prevention of human Pcas (18). This initial clinical trial bears evidence in support of PFE because it suggests that pomegranate consumption may retard Pca progression, which may prolong not only the survival but also improve the quality of life of patients.

**POMEGRANATE AND LUNG CANCER**

The effects of PFE on lung tumorigenesis were examined by authors both in vitro and in vivo (19-23). Normal human bronchial epithelial cells (NHBE) and human lung carcinoma A549 cells were treated with PFE (0.05–500 µg/ml) for 72 h. Whereas PFE resulted in a significant decrease in the viability of A549 cells, only minimal effects were observed on NHBE cells (19). PFE treatment of A549 cells resulted in dose-dependent arrest of cells in Go/G1 phase of the cell cycle, which was associated with induction of WAF1/p21 and KIP/flip and accompanied by decrease in the expression of downstream cell cycle regulatory proteins. PFE treatment also resulted in inhibition of several signaling pathways, including MAPK/Erk, PI3K/Akt, and NF-κB. The effect of PFE was tested in mice implanted with A549 cells (19). The appearance of tumors was observed in animals receiving water as early as 15 days post cell inoculation. This latency period was prolonged to 19 days in animals receiving PFE in drinking fluid. In mice that received water, the average tumor volume of 1,200 mm3 was reached in 53 ± 2 days after tumor cell inoculation. At this time point, the average tumor volumes in the 0.1 and 0.2% PFE-treated groups were 621 and 540 mm3, respectively (19). The average tumor volume of 1,200 mm3 was achieved in 67 ± 4 days after tumor cell inoculation in the 0.1% PFE group, and a 28-fold increase in tumor growth inhibitory response in which the targeted average tumor volume of 1,200 mm3 was reached at 79 ± 3 days after tumor cell inoculation. These observations indicated that PFE could be a useful chemopreventive/chemotherapeutic agent against human lung cancer.

To further explore the benefits of PFE against lung tumorigenesis, authors (20) examined the effect of oral consumption of a human achievable dose of PFE in two mouse lung tumor protocols. Benzo(a)pyrene [BaP] and N-nitro-tris-chloroethyurea (NTC) were used to induce lung tumors, and PFE was given in drinking water to A/J mice. Lung tumor yield was examined on the 84th day and 140 days after BaP dosing and 240 days after NTCU treatment. Mice treated with PFE and exposed to BaP and NTCU had statistically significant lower lung tumor multiplicity than mice treated with carcinogens only (20). Tumor reduction was 54% and 66% in the Balp and NTCU group at 84 and 140 days, respectively, compared with the Balp group. The NTCU + PFE group had 65% tumor reduction compared with the NTCU group at 240 days (20). Tumors from these animals were examined for effects on cell proliferation and various signaling pathways. Tumors with low proliferative indexes as examined by ki-67 and PCNA staining. PFE treatment also resulted in inhibition of NfKb, MANP, and p16/Kip signaling. Since the mammalian target of rapamycin (mTOR) is downstream of both p16/Kip and Akt, it was determined whether phosphorylation of mTOR was a result of p16/Kip activation (20). Treatment with B(a)P and NTCU caused increased phosphorylation of mTOR at Ser2448, whereas PFE administration resulted in inhibition of phosphorylation of mTOR. This observation was significant since the mTOR inhibitor temsirolimus activates AKT and p70 S6 kinase that control protein translation and cell cycle progression. Phosphorylation of AMPK, an upstream downregulator of mTOR, that was decreased in B(a)P and NTCU treated mice was restored in mice that received oral infusion of PFE (20).

**POMEGRANATE AND COLON CANCER**

The effect of PGO was studied in mice on the occurrence of colon aberrant crypt foci induced by azoxymethane (AOM) (22-24). Colonic tumors were induced in 6-week-old male F344 rats by subcutaneous injections of AOM (20 mg/kg body weight) once, a week for 2 wk (22). At 1 wk before the first AOM injection, the rats were divided into three groups, one of which received a daily dose of 0.1% PGO for 240 days after AOM treatment. The average tumor volume of 1,200 mm3 was achieved in 67 ± 4 days after tumor cell inoculation in the 0.1% PFG group. In AOM-treated animals, the incidence of colon tumors was 81% with a tumor multiplicity of 1.88/mice. Administration of PGO in the diet significantly inhibited the incidence and multiplicity of colon adenomas/carcinomas; however, a dose-response relationship was not observed (22). The inhibition of tumor incidence was associated with increased expression of peroxisome proliferator-activated receptor (PPAR) gamma protein in the nontumor mucosa (22). These findings suggest beneficial effects of pomegranate against the development of colon tumors in mice.

Inflammation plays a key role in the development of colon cancer, and many anti-inflammatory agents have shown promise for prevention of colon cancer. Adams et al. (23) examined the effects of pomegranate juice (PJ) on inflammatory cell signaling proteins in HT-29 human colon cancer cell line. At a concentration of 50 mg/L PJ significantly suppressed TNFα-induced (COX)-2 protein expression by 79% and also
reduced phosphorylation of the NF-κB/p65 subunit and its binding to the NF-κB response element. PJ also abolished TNFα-induced AKT activation, needed for NF-κB activity (24). These data suggest that polyphenolic constituents in the pomegranate can play an important role in the modulation of inflammatory signals in colon cancer cells.

POMEGRANATE AND SKIN CANCER

PGO has been investigated for possible skin cancer chemopreventive efficacy in mice (25–29). Skin tumors were initiated in 3-week-old, female, CD-1 mice with an initial topical application of DMBA followed by biweekly promotion using 12-O-tetradecanoylphorbol 13-acetate (TPA). Tumor incidence was 100% in control mice compared to 93% in mice pretreated with 5% PGO prior to each TPA application (26). The effect of PGO on TPA-stimulated ornithine decarboxylase (ODC) activity, an important event in skin cancer promotion, showed a 15% reduction in ODC activity. These initial observations suggested that PGO is a safe and effective chemopreventive agent against skin cancer (25). We evaluated antitumor-promoting effects of PFE in a similar animal model of skin cancer development (26). Topical application of PFE (2 mg/mouse) 30 min prior to TPA (3.2 nmole/mouse) application on mouse skin afforded significant inhibition, in a time-dependent manner, against TPA-mediated increase in skin edema and hyperplasia, epidermal ODC activity, and protein expression of ODC and COX-2 (26). PFE treatment also resulted in inhibition of UV-B-mediated phosphorylation of ERK1/2, p38, and JNK1/2, as well as activation of NF-κB (27). The effect of skin application of PFE on TPA-induced skin tumor promotion in DMBA-initiated CD-1 mouse was also investigated. In TPA-treated group, 100% of the mice developed tumors at 16 wk on test; whereas at this time in the PFE-treated group, only 30% of mice exhibited tumors. Skin application of PFE prior to TPA application also resulted in a significant delay in latency period from 9 to 14 wk and afforded protection when tumor data were considered in terms of incidence and tumor multiplicity. These observations provide clear evidence that PFE possesses anti-skin-tumor-promoting effects in CD-1 mouse by inhibiting conventional as well as novel biomarkers of TPA-induced tumor promotion.

Excessive exposure of solar ultraviolet (UV) radiation, particularly its UV-B component, to humans causes many adverse effects that include erythema, hyperplasia, hyperpigmentation, immunosuppression, photoaging, and skin cancer. To investigate the effect of PFE for humans, authors (27) determined its effect in normal human epidermal keratinocytes (NHEK) exposed UV-B, PFE (10–40 μg/ml) for 24 h before UV-B (40 μJ/cm²) exposure. PFE dose dependently inhibited UV-B-mediated phosphorylation of ERK1/2, JNK1/2, and p38 protein (27). PFE treatment of NHEK also resulted in a dose- and time-dependent inhibition of UV-B-activation of NF-κB (27). These data demonstrated protective effects of PFE against UV-B radiation and provided a molecular basis for the observed effects. In a recent study, protective effects of pomegranate fruit extract against UV-A and UV-B-induced damage were studied in SKU-1604 human skin fibroblast cells (28). Pomegranate extract (PE), in a range from 5 to 60 μg/ml, was effective at protecting human skin fibroblasts from cell death following UV exposure, which were attributed to a reduced activation of the proinflammatory transcription factor NF-κB, downregulation of proapoptotic caspase-3, and an increased Go/G1 phase associated with DNA repair (28). However, higher polyphenolic concentrations (500–1,000 μg/ml) were needed to achieve a significant reduction in UV-induced reactive oxygen species levels and increased intracellular antioxidant capacity (from 1.9 to 8.6 μM Trolox equivalents/ml) (28). UV-A is the major portion of solar radiation reaching the earth’s surface and has been shown to lead to formation of benign and malignant tumors. UVA exposure to NHEK led to an increase in phosphorylation of STAT3, AKT, and ERK1/2, which were inhibited when cells were pretreated with PFE (60–100 μg/ml) for 24 h (29). PFE pretreatment also resulted in a dose-dependent inhibition in the phosphorylation of mTOR and p70S6K (29). These observations suggest that PFE is an effective agent for ameliorating UVA-mediated damages by modulating cellular pathways. Overall results suggest protective effects of pomegranate against UVA- and UVB-induced cell damage and the potential use of pomegranate polyphenolics in topical applications.

CONCLUSIONS

Interest in the biological activity of pomegranate-derived products, especially their anticancer properties, is being investigated in earnest. This interest is largely attributed to initial experiments that have reported activity of PJ that was found to be greater than that of red wine or even green tea. Various dietary agents are being investigated for their potential beneficial effects against PCs. PJ has shown an initial promise in a phase II clinical trial against PCs. There is a need to undertake similar clinical studies in other cancers such as colon and breast. Although identifying individual active ingredients in the PJ would be ideal, it is interesting to note that many studies have observed the extract or the juice to be more beneficial compared to the individual or purified compound. This suggests the existence of a synergistic effect of the compound. The use of a chemical synthesis of a molecule rather than a purified compound could explain the inhibition of multiple targets observed in many studies and thus greater likelihood for producing cancer chemopreventive effects in humans. This may account for the synergistic preventive and/or anti-cancer effects, and the approach can be explored in laboratory, animal, clinical, and epidemiological studies in the future. It is anticipated that in-depth research into the anticancer activities of naturally occurring compounds would enable one day to develop a cocktail of such molecules for effective prevention cancers.

ACKNOWLEDGMENTS

The original work from the author’s (H. Mukhtar) laboratory outlined in this review was supported by United States Public Health Service Grants ROI CA 78890, ROI CA10139, P30 DK056330-01, and RO1 CA 120431.

REFERENCES


