SHORT COMMUNICATION

THE ROLE OF POMEGRANATE (*PUNICA GRANATUM L.)* IN COLON CANCER

SEHER A. KHAN*

Department of Pharmaceutical Sciences, Lake Erie College of Osteopathic Medicine (LECOM) School of Pharmacy, 1858 West Grandview Blvd., Erie, PA 16509, USA

ABSTRACT

Colon cancer is one of the major causes of cancer-related death in the Western world. Although cytotoxic chemotherapeutic agents are available to treat the disease, these agents become ineffective as the disease advances to an invasive state. An alternative but viable approach to reduce the incidence of this deadly disease is then, to increase the dietary intake of relatively non-toxic fruits and vegetables. An example of a fruit with antioxidant, antidiabetic and anti-atherosclerotic properties is pomegranate. Pomegranate produces anticancer effects in experimental models of lung, prostate and skin cancer. More recently, pomegranate has been found to be anti-carcinogenic in the colon. This communication discusses pomegranate's effect in colon cancer.

Keywords: Colon; cancer; pomegranate; prevention; apoptosis.

INTRODUCTION

Colon cancer is one of the most frequent cancers of the Western world. In the United States alone, this disease constitutes the second leading cause of cancer death. Approximately, forty thousand people may die of colon cancer in USA this year. Current treatment options such as surgical intervention and adjuvant chemotherapy have several limitations in counteracting the disease. Furthermore, patients with advanced stage colon cancer are usually unresponsive to any form of treatment. In this regard, increasing efforts are focused towards formulating effective preventive strategies to reduce the number of incidence of the disease. One of the primary modes of preventing colon cancer therefore, will be, increasing the consumption of food containing anticancer compounds.

Pomegranate (*Punica granatum* L.*;* Punicaceae) is a small plant, widely cultivated in Afghanistan, India, Iran, Japan, China, Russia and some parts of USA. Pomegranate is consumed either as an edible fruit or in the form of a beverage such as fruit juice. Pomegranate's role in health is well documented. In experimental studies, pomegranate fruit and other plant parts produced beneficial effects against atherosclerosis (de Nigris *et al.,* 2005), hypertension (Aviram and Dornfeld, 2001), diabetes (Das *et al.*, 2001; Huang *et al.*, 2005a), cardiac fibrosis (Huang *et al.*, 2005b) and cancer of prostate (Malik *et al.*, 2005) skin (Afaq *et al.,* 2005) and lung (Khan *et al.,* 2007). Promising results from studies on pomegranate's role in colon cancer are presented in this article.

Inhibition of aberrant crypt formation and adeno-carcinoma

Pomegranate fruit juice reduced the number of aberrant cryptic foci (ACF) of the colon by 91 % in male F-344 rats (Boateng *et al*., 2007). In this study, animals were given access to 20% pomegranate fruit juice, before and after treatment with colon-specific chemical carcinogen, azoxymethane. Histopathology of rat colon after 17^{th} week of treatment revealed a marked decrease in the number of large crypts in pomegranate juice-fed rats. The number of crypts/ACF was also less in these animals. When compared to other fruit juices, pomegranate juice was found to be superior to other fruit juices (e.g. water melon and cranberry) as an inhibitor of ACF in rat colon. Increase in weight gain and feed intake ($P<0.05$) was observed in pomegranate fruit juice-fed rats, suggesting a possible protective effect against cancer cachexia.

Hepatic glutathione S transferase (GST) activity was approximately 3 fold higher in rats receiving pomegranate juice. The enzyme is well known for scavenging free radicals resulting from oxidative stress. This induction of enzyme activity also supports the mechanism of pomegranate's antioxidative actions in other experimental models (Rosenblat *et al.,* 2007).

In a separate study (Kohno *et al*., 2004a), pomegranate seed oil incorporated in the diet, markedly reduced the incidence and multiplicity of colonic carcinoma (measured as no of tumors/rat) induced by azoxymethane. In this experiment, pomegranate seed oil was added to AIN-76A diet at an increased concentration of 0.01%, 0.1% and 1% (w/w), respectively. Although, a dose response effect was not clearly established, anti-carcinogenic effects were observed in all the doses used in the study. Duration of this study was obviously longer (32 weeks) than the study discussed above.

Pomegranate seed oil enriched (75%) with 9 *cis*, 11 *trans*, 13 *trans* conjugated linolenic acid (CLN), shown in a

**Corresponding author:* e-mail: seherkhan@lecom.edu

separate study, to possess anti-carcinogenic effect in the colon (Kohno *et al*., 2004b). CLN is a C-18 fatty acid with conjugated double bonds in carbon 9, 11, and 13 with geometric (*cis* and *trans*) isomers. In this study, CLN is metabolized to conjugated linoleic acid (CLA) in liver and colonic mucosa of the pomegrante juice-treated animals. Lipid analysis failed to detect any CLN, but interestingly a specific CLA, 9 *cis* 11 *trans* CLA was increased in a dose-dependent manner in these tissues. Most likely, an enzyme catalyzed saturation step occurred *in vivo.* CLA collectively refers to a group of linoleic acid (18:2, c9, c12) derivatives with several positional (double bonds in carbon 9 and 11 or 10 and 12) and geometric (*cis*, Z and *trans*, E) isomers. CLAs are relatively abundant in ruminant meat and heat-processed dairy products and elicit anti-cancer effects in rodents. Both CLA and CLN can activate peroxisome proliferator-activated receptor gamma (PPARγ), a nuclear receptor associated with cellular differentiation including those of colon and adipose tissues. In the non-tumor colonic mucosa PPARγ protein levels were increased as compared to control rats. Although not shown in this study, conjugated fatty acids (CLN and CLA) might have activated PPARγ and provided protection in colon neoplasia by promoting differentiation and growth arrest. It will be interesting to determine which conjugated fatty acid (CLA or CLN) binds to PPARγ more avidly in an *in vitro* receptor-binding assay.

Cell culture studies

While studying antiproliferative activities in cell culture systems, different constituents of the fruit were used. These include pomegranate juice, two important antioxidant polyphenols namely, punicalagin and ellagic acid, and total pomegranate tannin (TPT). All the constituents significantly inhibited the proliferation of different colon cancer cells (SW480, HT29, HCT116, SW620) with the most prominent effect observed with pomegranate juice (Seeram *et al.,* 2005). Cell cycle analysis revealed that punicalagin and ellagic acid caused cell cycle arrest in Caco-2 cells (Larossa et al., 2006). These ingredients down regulated cyclin A and cyclin B1, members of cyclin class of regulatory proteins. Cyclin A and cyclin B are responsible for passage thorough S-phase and regulation of mitosis, respectively. Cyclin E, responsible for activation of cyclin-dependent kinases near S-phase was markedly up regulated.

Ellagic acid, punicalagin and TPT failed to induce apoptosis in HT-29 and HCT-116 cells when treated at doses equivalent to found in pomegranate juice (Seeram *et al.,* 2005). They were only effective when treated at equivalent doses of 100μg/ml.

As an antioxidant, pomegranate juice exhibited a better response than individually isolated constituents, possibly because of synergistic effects of multiple constituents present in the juice.

Mechanistic studies

Punicalagin and ellagic acid induced apoptosis of colon cancer cells (Larossa *et al.,* 2006). Leakage of mitochondrial cytochrome c in the cytosol by punicalagin and ellagic acid suggested an intrinsic pathway of apoptosis. Antiapoptotic bcl-XL protein was down regulated with 30 μM ellagic acid and 100 μM punicalagin, respectively. Similarly, both compounds induced caspase 9 and procaspase 3, member of caspase family of proteases. Neither ellagic acid nor punicalalgin activated caspase 8, another member of caspase family associated with extrinsic pathways (e.g.-induced by cytokines) of apoptosis. When an anti-Fas ZB4 antibody was incubated with either ellagic acid or punicalagin, no inhibitory effect on apoptosis was observed. This antibody was used to block any interaction of ellagic acid or punicalagin with Fas receptor. Altogether, these findings support the role of intrinsic mechanism in pomegranate's apoptotic effect in colon carcinogenesis.

In a separate study, pomegranate juice, punicalagin, and TPT markedly suppressed tumor necrosis factor-alpha (TNFα) mediated expression of COX-2, an inducible member of COX family of regulatory proteins in HT-29 cells (Adams *et al.,* 2006). Increased expression of COX-2 has been implicated in inflammation of the colon and COX-2 suppression by chemical antagonists reduces the occurrence of colon cancer in animal and *in vitro* models (Marnett and Dubois, 2002). Western Blot data indicated that pomegranate juice elicited maximum inhibition of COX-2 protein followed by TPT and punicalagin, respectively.

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT)/nuclear factor kappa-B (NFκB) pathway is essential for COX-2 activation. TNFα induced AKT activity, necessary for activation of NFκB, was suppressed by pomegranate juice in a dose-dependent manner (Adams *et al.,* 2006). NFκB acts as an upstream regulator of COX-2 gene. NFκB phosphorylation (phosphorylation of p65 subunit) was also inhibited by pomegranate juice. DNA binding of this transcription factor was effectively suppressed by punicalagin, although ellagic acid was totally ineffective.

Since pomegranate's apoptotic effect is *via* intrinsic pathway only (Larossa *et al.,* 2006), the role of COX-2 in apoptotic process is not clearly understood.

CONCLUSION

Pomegranate's anticancer effects in the colon involve various cell signaling pathways. These include:

modulation of cyclins, induction of caspases, inhibition of COX-2 expression, induction of glutathione S transferase (GST) and induction of PPARγ. Although these cellular events are fascinating, at this time they are not well connected to derive a total picture of pomegranate's role in colon cancer. Further studies will provide greater insights on these signaling events.

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