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Bioactive Actions of Pomegranate Fruit Extracts on Leukemia Cell Lines In Vitro Hold Promise for New Therapeutic Agents for Leukemia

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Studies suggest that pomegranates contain bioactive chemicals with potential for treatment and prevention of cancer. Pomegranate juice extracts (PJE) have been shown to inhibit cellular proliferation and tumour growth and induce cell death via apoptosis in a number of cancer cell lines. However, to date, few studies have investigated the potential of PJE in the treatment of leukemia. We investigated the potential effect of PJE on induction of apoptosis and inhibition of cellular proliferation in 8 leukemia cell lines (4 lymphoid and 4 myeloid) and non-cancer human periperipheral blood mononuclear cells (control cells). Apoptosis was assessed by 2 methods: Annexin V-FITC/propidium iodide staining with flow cytometric analysis and 4′-6-diamidino-2-phenylindole (DAPI) morphological assessment. Cell cycle stage was investigated using propidium iodide staining of DNA content and flow cytometric analysis. Live cell counts were also performed using a trypan exclusion assay. PJE significantly induced apoptosis in all cell lines, including non-cancer control cells, although lymphoid cells and 2 of the myeloid cell lines were more sensitive. Furthermore, PJE induced cell cycle arrest. These results were confirmed by DAPI analysis and viable cell counts using trypan blue exclusion assay. Our results provide evidence that PJE contains bioactive components that could be used in the treatment of leukemia.

INTRODUCTION

Leukemia is defined as a clonal malignant disorder of white cells in the blood and blood-forming organs. Leukemia is characterized by uncontrolled proliferation of white cells, resulting in bone marrow failure and decreased production and function of normal hematopoietic cells. From the time of Virchow's initial discovery, the overall classification of leukemia has become more complex (1). Currently, leukemia is classified based on the predominant cell of origin (myeloid or lymphoid) and the rate of progression (acute or chronic), together with numerous sub-classifications (2).

Leukemia is a major problem worldwide, affecting millions of people each year (3). In the UK, leukemia is the most common childhood cancer, and, overall, leukemia shows a slightly higher incidence in males than females (3). In 2008, it was estimated that 4,362 deaths were caused from leukemia in the UK, and it was ranked as the 11th most common cause of death from cancer (3).

Treatment of leukemia depends on many factors, such as histologic type of leukemia, its stage, and prognostic features (patient's age and overall health) (4). However, current treatment options such as chemotherapy, bone marrow transplantation, and radiotherapy still have several limitations—not least, the cytotoxicity of these therapies to normal cells and the fact that certain chemotherapy agents may cause bone marrow toxicity (5,6) and organ damage (7). In addition, the major causes of treatment failure in leukemia are drug resistance and metastasis to other tissues (8). A number of groups worldwide are investigating new cancer treatment strategies using natural plant products. Evidence is developing on the basic mechanisms of action using in vitro culture and animal models as well as human trials in a number of solid tumors which have shown responses on important markers of disease progression (9,10).

Pomegranate (Punica Granatum) is an ancient fruit that has been used in alternative medicine in many cultures (11). Polyphenolic compounds make up the highest proportion of phytochemicals in pomegranate, which are characterized by multiple phenol rings that bear a number of hydroxyl groups (12). The 2 major types of polyphenolic components found in pomegranates are hydrolysable tannins, which account for the majority of antioxidant activity of the fruit, and anthocyanins (13). The cellular antioxidant activity and total phenolic contents of pomegranate have been shown to be higher than commonly

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ORIGINAL RESEARCH

Polyphenols are responsible for the proapoptotic properties of pomegranate juice on leukemia cell lines

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Abstract

Pomegranates have shown great promise as anti-cancer agents in a number of cancers including clinical trials in prostate cancer. We have previously shown pomegranate juice (PGJ) induced apoptosis and preferentially alters the cell cycle in leukemia cell lines compared with nontumor control cells. However, the agents responsible have not yet been fully elucidated. Treatment of four leukemia cell lines with five fractions obtained from PGJ by solid phase extraction demonstrated that only the acetonitrile fractions decreased adenosine triphosphate (ATP) levels in all leukemia cell lines. Acetonitrile fractions also significantly activated caspase-3 and induced nuclear morphology characteristic of apoptosis. S phase arrest was induced by acetonitrile fractions which matched S phase arrest seen previously following whole PGJ treatments. The acetonitrile fractions contained higher phenol content than whole PGJ whereas only low levels of phenols were seen in any other fraction. Liquid chromatography mass spectrometry (LC–MS) analysis demonstrated that acetonitrile fractions were enriched in ellagitannins, ellagic acid, and hydroxycinnamic acid derivatives but depleted in anthocyanins. Individual treatments with identified compounds demonstrated that the ellagitannin: punicalagin was the most active and mimicked the responses seen following acetonitrile fraction treatment. Bioactive components within pomegranate were confined to the acetonitrile fraction of PGJ. The enrichment in ellagitannins and hydroxycinnamic acids suggest these may provide the majority of the bioactivities of PGJ. Individual treatments with compounds identified demonstrated that the ellagitannin: punicalagin was the most active agent, highlighting this compound as a key bioactive agent in PGJ.

Introduction

Despite improvement in early detection and advances in treatments, leukemia continues to be a major cause of morbidity and mortality worldwide (Cancer Research U.K. 2011). Treatment options remain limited and are fraught with adverse side effects (Fielding et al. 2012; Richardson et al. 2012; Shamdal and Haddad 2012; Suttorp et al. 2012). Thus, studies into the use of nontoxic dietary agents are rapidly gaining ground and many natural agents are currently under investigation as potential chemo-preventive as well as chemotherapeutic agents including investigations in a number of clinical trials (Khan et al. 2008; Syed et al. 2008; Paller et al. 2012).

The fruit of the pomegranate tree (*Punica granatum*) has shown great promise as an anti-cancer agent in lung (Khan et al. 2007), prostate (Paller et al. 2012), skin (Afaq et al. 2009), colon (Adams et al. 2006), and breast cancer (Kim et al. 2002), which has been taken into phase II clinical trials in prostate cancer (Adhami et al. 2009; Paller et al. 2012). We have previously shown that crude extracts of pomegranate juice (PGJ) induce apoptosis and inhibit cell cycle in a number of leukemia cell lines, which demonstrated greater sensitivity than nontumor control cells (Dahlawi et al. 2012).

However, to date, the compounds responsible for the anti-leukemic properties of pomegranate remain unknown. PGJ contains a number of potential active