Chemico-Biological Interactions 274 (2017) 100–106

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Punicalagin, a polyphenol from pomegranate fruit, induces growth inhibition and apoptosis in human PC-3 and LNCaP cells

Oluwatosin Adaramoye [a, b, *], Bettina Erguen [a], Bianca Nitzsche [c], Michael Höpfner [c], Klaus Jung [a, d], Anja Rabien [a, d]

[a] Department of Urology, Charité–Universitätsmedizin Berlin, Berlin, Germany
[b] Drug Metabolism and Toxicology Section, Department of Biochemistry, University of Ibadan, Nigeria
[c] Institute of Physiology, Charité-Universitätsmedizin Berlin, Berlin, Germany
[d] Berlin Institute for Urologic Research, Berlin, Germany

ARTICLE INFO

Article history:
Received 11 May 2017
Received in revised form
14 June 2017
Accepted 3 July 2017
Available online 11 July 2017

Keywords:
Punicalagin
Prostate cancer cells
Apoptosis
Anti-angiogenic effects

ABSTRACT

Prostate cancer (PCa) is an international health problem and search for its effective treatment is in progress. Punicalagin (PN), polyphenol from pomegranate fruit, is known to exhibit potent anticancer activity in lung, breast and cervical cells. However, there is paucity of information on its effect in PCa. This study evaluated anti-proliferative effects of PN and its effects on extrinsic pathway of apoptosis in PCa cells, and angiogenesis in chicken chorioallantoic membrane (CAM). Antioxidant activities of PN were determined by 2,2–diphenyl-1-picryhydrazyl (DPPH) radical scavenging and inhibition of lipid peroxidation (LPO) methods. PCa (PC-3 and LNCaP) and normal prostate (BPH-1) cells were cultured and treated with PN (10, 50 and 100 μM). Cytotoxicity and viability effects of PN were determined by lactate dehydrogenase (LDH) and XTT assays, respectively. Antiangiogenic effects were measured using CAM assay, while apoptosis was assessed by DNA fragmentation, enrichment factor by Cell Death Detection ELISA kit and expressions of caspases-3 and -8. Results showed that PN (10–200 μM) significantly scavenged DPPH and inhibited LPO in a concentration-dependent manner. Furthermore, PN (10–100 μM) concentration-dependently inhibited viability in PC-3 and LNCaP, while viability in BPH-1 was insignificantly affected. PN had low toxicity on cells *in vitro* at concentrations tested. Also, PN (100 μM) increased enrichment factor in PC-3 (2.34 ± 0.05) and LNCaP (2.31 ± 0.26) relative to control (1.00 ± 0.00). In addition, PN (50 μM) decreased the network of vessels in CAM, suggesting its anti-angiogenic effect. Moreso, PN increased the expressions of caspases-3 and -8 in PC-3. Overall, PN exerts anti-proliferative activity in PCa cells via induction of apoptosis and anti-angiogenic effect.

1. Introduction

Prostate cancer is a major health problem in the world. It is the most frequently diagnosed cancer among men and the second leading cause of male cancer deaths [6]. Although, there are many therapeutic strategies including chemotherapy, radiation and combination therapies, however, high systemic toxicity and drug resistance limit the successful outcomes in most cases [8]. Natural products and their synthetic derivatives may be considered as a potential source of novel compounds for the treatment of cancers. Indeed, over 60% of the approved anticancer drug candidates developed between 1981 and 2002 are derived from natural products [30]. According to the World Health Organization (WHO), 80% of the population in Africa and some Asian countries still use herbal preparations to treat their illnesses, including cancer [10]. Therefore, research exploring the medicinal usefulness of indigenous plants and natural products is important and relevant.

Pomegranates (*Punica granatum* Linn.) have been used for medicinal purposes against diarrheal, gum, parasitic and inflammatory disorders [22]. Pomegranate extracts have been shown to exhibit anticancer effects such as anti-proliferative, pro-apoptotic, anti-invasive, and/or anti-inflammatory properties in cancer cell lines [1,2,4,23,34,37]. Also, pomegranate extract has been reported to reduce the growth of human prostate and lung cancer xenografts in immune-deficient mice and suppressed prostate tumorigenesis in

* Corresponding author. Department of Biochemistry, University of Ibadan, Ibadan, Nigeria.
E-mail addresses: aoadaramoye@yahoo.com, ao.adaramoye@ui.edu.ng (O. Adaramoye).

http://dx.doi.org/10.1016/j.cbi.2017.07.009
0009-2797/

the TRAMP mouse model [3,24]. Pomegranates contain polyphenolic compounds with high antioxidant and free-radical-scavenging activity, including flavonoids, condensed tannins and hydrolyzable tannins (ellagitannins and gallotannins) [20] and [19]. Ellagitannins are the most bioactive polyphenols of pomegranates and the most abundant ellagitannin in pomegranates is punicalagin (PN) [19]. PN is responsible for more than half of the total antioxidant capacity of the pomegranates juice [14]. Anti-atherosclerotic and antioxidant properties of pomegranate have been linked to its high polyphenol content especially of PN [1,35]. PN has been shown to elicit remarkable biological activities including anti-inflammatory [5,38], hepatoprotective [26] and [27] and anti-genotoxic activities [40]. Despite the anti-cancer effect of PN reported in literature, there is still dearth of information on its effect in androgen-dependent and −independent PCa cells. Hence, this study was designed to evaluate the anti-proliferative effects of PN and its effects on extrinsic pathway of apoptosis in human PCa cells and, angiogenesis in chicken chorioallantoic membrane.

2. Materials and methods

2.1. Chemicals and reagents

Ethylenediamine tetra-acetic acid (EDTA), 2,2−diphenyl-1-picryhydrazyl (DPPH), 2- deoxyribose, Folin-Ciocalteu reagent, catechin, 2-thiobarbituric acid (TBA), trichloroacetic acid (TCA), ascorbic acid and punicalagin were purchased from Sigma Chemical Co., Saint Louis, MO, USA. Ferrous ammonium sulphate, hydrochloric acid, naphthylenediamine dihydrochloride and sodium hydroxide were procured from British Drug House (BDH) Chemical Ltd., Poole, UK. Other chemicals were of analytical grade and purest quality available.

2.2. Cells

PC-3, LNCaP and BPH-1 cell lines were acquired from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany). For PC-3 and LNCaP, the culture medium consisted of RPMI 1640 medium (Gibco/Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal bovine serum gold (PAA Laboratories, Pasching, Austria), penicillin (100 units/ml), and streptomycin (100 μg/ml) which was used for cell growth in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. For BPH-1, the culture medium consisted of RPMI 1640 medium supplemented with 20% fetal bovine serum gold, dihydrotestosterone (20 ng/ml), transferrin (5 μg/ml), sodium selenite (5 ng/ml), insulin (5 μg/ml), penicillin (100 units/ml) and streptomycin (100 μg/ml).

2.3. Preparation of punicalagin

Prior to the experiments, 10 mg of PN (Sigma) was dissolved in 80 μl of DMSO and diluted to the desired concentrations with culture media to give a water-soluble fraction in which DMSO concentration did not exceed 0.02% in the highest of the concentrations applied.

2.4. Animals

Male Wistar rats weighing between 200-220 g were purchased from the central animal house, Department of Physiology, University of Ibadan, Nigeria. The animals were kept in well-ventilated cages at room temperature (28−30 °C) and maintained on laboratory chow (Ladokun Feeds, Ibadan, Nigeria) and water *ad libitum*. The rats were allowed a period of two weeks for acclimatization after delivery, and then used for lipid peroxidation assay. Experimental protocols, handling and treatment of rats were approved by the Animal Ethics Committee of Faculty of Basic Medical Sciences, University of Ibadan, Nigeria.

2.5. Preparation of rat liver homogenate

Rats were sacrificed under light ether anaesthesia and liver excised after dissection. Liver was removed and washed in ice-cold 1.15% KCl solution, dried and weighed. It was homogenized in 4 vol of 50 mM phosphate buffer, pH 7.4 and centrifuged at 10,000 × *g* for 15 min to obtain the post-mitochondrial fraction (PMF), which was used for lipid peroxidation assay.

2.6. Antioxidant assays

2.6.1. Determination of DPPH radical scavenging activity of PN

The stable DPPH radical was used for the determination of free radical scavenging activities of PN as described by Ref. [28]. A portion (1 ml) each of the different concentrations (10−200 μM) of PN was added to 1 ml of 1 mM DPPH in methanol. The mixtures were vortexed and incubated in a dark chamber for 30 min after which the absorbance was measured at 517 nm against a DPPH control containing only 1 ml of methanol in place of PN. The procedure above was repeated but PN was replaced with a standard antioxidant (Catechin).

All procedures were carried out in triplicates.

The inhibition of DPPH was calculated as a percentage using the expression:

$$\%I = \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \times 100$$

Where % I is the percentage inhibition of the DPPH radical, Acontrol is the absorbance of the control and Asample is the absorbance of the test compound.

2.6.2. Inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation by PN

Inhibition of Fe^{2+}/ascorbate-induced lipid peroxidation was carried out by the method of [31]. The reaction mixtures contained 0.2 ml of rat liver homogenate in varying concentrations of 30 mM tris-buffer, 0.38 ml of 0.16 mM ferrous ammonium sulphate, 0.06 ml ascorbic acid and different concentrations of PN (10−200 μM) and, were incubated for 1 h at 37 °C. The resulting thiobarbituric acid reacting substances (TBARS) formed were measured as followed; briefly, an aliquot (0.4 ml) of the reaction mixture was mixed with 1.6 ml of 0.15 M Tris-KCl buffer and 0.5 ml of 30% TCA (to stop the reaction), and placed in a water bath for 45 min at 80 °C. After which it was cooled in ice and centrifuged at room temperature for 15 min at 3000 *g* to remove precipitates. The absorbance of the clear pink coloured supernatant was measured against blank at 532 nm. Catechin was used as standard and the experiment performed in triplicate.

2.7. Assays for cell viability, cytotoxicity and DNA fragmentation

2.7.1. Measurements of cell viability

PC-3, LNCaP and BPH-1 (1×10^4) cells were cultured in a 96-well microtiter plate containing 0.1 ml of RPMI growth media/well for 24 h. Cells were incubated with PN dissolved in dimethyl sulfoxide (DMSO)/media (DMSO less than 0.02%), and PN concentrations of 10, 50 and 100 μM were used with incubation period of 24−96 h. Cells were evaluated for their viability using the XTT assay (Roche Applied Science, Mannheim, Germany) as described by Refs. [13] and [33]. After incubation period, 50 μl of the XTT solution was added to the treated cells and incubated in dark for 4 h at 37 °C