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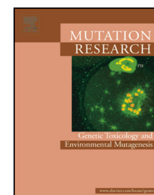

Genotoxic and cytostatic effects of 6-pentadecyl salicylic anacardic acid in transformed cell lines and peripheral blood mononuclear cells

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ABSTRACT

In Mexico, as in many other countries, traditional medicine is used for the treatment of several diseases. In particular, *Amphipterygium adstringens* infusion is used for gastritis, gastric ulcers, and gastric cancer. Extracts from this tree have microbicidal effects against *Helicobacter pylori*, an important risk factor for gastric cancer development. Anacardic acids are constituents of *A. adstringens*, and 6-pentadecyl salicylic acid (6-PSA) is the most abundant. However, there is a lack of information regarding the effects of 6-PSA on cancer cells. Therefore, we investigated whether 6-PSA has differential effects on the induction of genotoxicity, cytostaticity, and apoptosis in normal human peripheral blood mononucleated cells (PBMCs), bone marrow polychromatic erythrocytes of Balb/c mice, and human transformed cell lines derived from both gastric cancer (AGS cells) and leukaemia (K562 cells).

Treatment with 6-PSA (30–150 μM) reduced the viability of AGS and K562 cells together with a moderate, but significant, increase in the frequency of micronucleated cells and the induction of DNA breakage (Comet Assay). Moreover, 6-PSA increased the apoptosis rate in both the AGS and K562 cell lines in a caspase 8-dependent manner. In contrast, neither cytotoxicity nor genotoxicity were observed in PBMCs or bone marrow polychromatic erythrocytes of Balb/c mice after treatment with low doses of 6-PSA (0.2–2.0 mg/Kg). Instead, 6-PSA treatment resulted in the inhibition of PBMC proliferation, which was reversible after the compound was removed. Additionally, 6-PSA treatments (2–20 mg/Kg) increased the frequency of mature polychromatic erythrocytes in the bone marrow, suggesting a possible effect on the differentiation process of immune cells.

The present results indicate that 6-PSA induces cytotoxicity and moderate genotoxicity, together with an increase in the apoptosis rate, in a caspase 8-dependent manner in gastric cancer cells. In contrast, a low toxicity was observed when PBMCs were exposed to 6-PSA.

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1. Introduction

Gastric cancer is a major health concern in several countries. In Mexico, gastric diseases such as gastritis, gastric ulcers, and gastrointestinal cancers are some of the most common health concerns. As an alternative to chemotherapy for gastric cancer, patients often turn to empirical "ethnopharmacology" for treatment. The traditional use of extracts of *Amphipterygium adstringens* (*A. adstringens*) to control some gastric diseases, including gastric cancer, has shown some anti-inflammatory and antitumour activity *in vivo* [1].

A. adstringens is a rich source of anacardic acids, anacardic aldehydes, alkyl naphthalenes, triterpenoids, and sterols [2]. The chemical compounds that *A. adstringens* produces in the largest quantities are the anacardic acids, among which 6-pentadecyl salicylic acid (C15:0, 6-PSA) is the most abundant [3] (Table 1).

Abbreviations: 5F, 5-fluorouracil; 6-PSA, 6-pentadecyl salicylic anacardic acid; AGS, human gastric adenocarcinoma cell line; CFSE, carboxyfluorescein succinimidyl ester; ER, endoplasmic reticulum; K562, human myelogenous leukaemia cells; MN, micronuclei; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-biphenyltetrazolium; NCE, normochomatic erythrocytes; PBMCs, peripheral blood mononuclear cells; PCE, polychomatic erythrocytes; PHA, phytohemagglutinin; RT, room temperature; T, taxol; Z-DEVD-FMK, benzyloxycarbonyl (Asp-Glu-Val-Asp) fluoromethyl keton; Z-IETD-FMK, benzyloxycarbonyl (Ile-Glu-Thr) aspfluoro methylketon; Z-LEHD-FMK, benzyloxycarbonyl (Leu-Glu-His-Asp) fluoromethylketon.

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Some extracts from *A. adstringens*, which contain a mixture of anacardic acids, can inhibit prostaglandin synthase, tyrosinase, lipoxygenase, histone acetyltransferase, p300, and PCAF (p300/CBP-associated factor) [4]. Other reports indicate that a mixture of anacardic acids induces apoptosis in tumour cells, and several mechanisms have been proposed [5].

One common mechanism for the induction of tumour cell death by several chemotherapeutic agents is the induction of severe genotoxic damage to the cells, as such, a characteristic of most antineoplastic drugs is their ability to induce genetic damage. Despite the information in the literature regarding the possible molecular targets for anacardic acid mixtures in tumour cells, there is a lack of information on the genotoxic activity of these compounds, both in normal or tumour cells that could indicate some of the safety features of the treatment with these compounds. Therefore, we sought to investigate whether 6-PSA (C15:0), a purified anacardic acid found in large amounts in anacardic acids mixtures from *A. adstringens*, mediates the cytotoxic effects reported for other mixtures of anacardic acids and whether it has differential effects on the induction of genotoxic damage and apoptosis in normal human cells (peripheral blood mononucleated cells, PBMCs) and in human transformed cells (K562 - lymphoblastic cells). In addition to K562 cells, because *A. adstringens* is traditionally used for gastric diseases, we evaluated the effects of purified 6-PSA (C15:0) on human gastric cancer cells (AGS – human gastric adenocarcinoma cells).

2. Materials and methods

2.1. Materials

6-pentadecyl salicylic anacardic acid (C15:0) (6-PSA, 348.5 MW, 98% purity) was obtained from Calbiochem (San Diego, CA, USA); dimethyl sulfoxide (DMSO), methanol and acetic acid were purchased from J.T. Baker (Phillipsburg, NJ, USA); Ficoll-hypaque, cytochalasin B (CytB), phytohemagglutinin (PHA), Taxol (T), and 5-fluorouracil (5F) were obtained from Sigma Chemicals (St. Louis, MO, USA); Dulbecco's modified eagles medium (DMEM), RPMI-1640, foetal bovine serum (FBS), L-glutamine, non-essential amino acids, streptomycin, and penicillin were purchased from Invitrogen Life Technologies (Carlsbad, CA, USA).

2.2. PBMCs isolation

Plasma was separated by centrifugation at 250 × *g* for 15 min from 10 ml of heparinised peripheral blood from four healthy male volunteers. Peripheral blood mononuclear cells (PBMCs) were then isolated by density centrifugation using Ficoll-hypaque (2:1) at 180 × *g* for 25 min at room temperature (RT). Isolated PBMCs were washed with phosphate-buffered saline (PBS: 2 g KCl, 80 g NaCl, 2 g KH_2PO_4, and 11.5 g Na_2HPO_4, J.T. Baker) and resuspended at 1×10^6 cell/ml in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% L-glutamine (200 mM), and 1% nonessential amino acids (100 mM).

2.3. Cell cultures

Human gastric adenocarcinoma cells (AGS) were cultured in DMEM medium supplemented with 10% heat-inactivated FBS, 1% L-glutamine, 1% nonessential amino acids, and 1% streptomycin and penicillin (5000 IU). Human myelogenous leukaemia cells (K562) were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% L-glutamine, 1% nonessential amino acids, and 1% streptomycin and penicillin. PBMCs were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated FBS, 1% L-glutamine, and 1% nonessential amino acids. Cells were stimulated with phytohemagglutinin (PHA, 5 μg/ml); all cell types were incubated at 37 °C in a humidified atmosphere with 5% CO_2. Because AGS cells are adherent, they required a 2 min incubation with trypsin (0.25 mg/ml) before the manual removal of the cells.

2.4. Treatments

6-PSA was dissolved in DMSO, stored at −20 °C, and diluted as needed in cell culture medium to obtain final concentrations of 0.15, 0.3, 1.5, 3, 15, 30, and 150 μM, with a maximum concentration of 0.5% of DMSO in the culture medium. Taxol (T, 2.5 μM) and 5-fluorouracil (5F, 2.5 μM) were used as positive controls. All solutions were prepared fresh for each experiment.

2.5. Viability

2.5.1. Trypan blue exclusion test

All cell types were seeded in 96-well tissue culture plates at a density of 1×10^5 cells/ml and treated with 6-PSA for 24, 48, or 72 h.

Table 1
Anacardic acid constituents from different species.

Species	Extract[a]	AA (Carbon side chain:double bonds)	% AA[b]	Reference
A. adstringens	MeOH	6-[16′Z-nonadeceny)] salicylic acid (C19:1)	19.4	[3]
		6-nonadecenyl salicylic acid (C19:0)	9.2	
		6-[8′Z-pentadecenyl] salicylic acid (C15:1)	13.0	
		6-pentadecyl salicylic acid (C15:0)	58.5	
Anacardium occidentale	MeOH–water	6-(8(Z),11(Z),14-pentadecatrienyl) salicylic acid (C15:3)	50.0	[20,21]
		6-(8(Z),11(Z)-pentadecadienyl) salicylic acid (C15:12)	17.0	
		6-(8(Z)-pentadecenyl) salicylic acid (C15:1)	33.0	
Ginkgo biloba	Petroleum ether	6-[10(Z)-heptadecenyl] salicylic acid (C17:1)	49.1	[22]
		6-(8(Z),11(Z)-heptadecadienyl) salicylic acid (C17:2)	3.3	
		6-(8′Z,11′Z,14′Z-heptadecatrienyl) salicylic acid (C17:3)	Trace	
		6-(8′Z-pentadecenyl) salicylic acid (C15:1)	37.0	
		6-pentadecyl salicylic acid (C15:0)	4.6	
		6-tridecyl salicylic acid (C13:0)	5.8	
Ozoroa insignis	EtOAc	6-[nonydecyl] salicylic acid (C19:0)	21.2	[23]
		6-[10(Z)-heptadecenyl] salicylic acid (C17:1)	33.3	
		6-[8(Z)-pentadecenyl] salicylic acid (C15:1)	27.3	
		6-tridecyl salicylic acid (C13:0)	18.2	
Viola websteri	Petroleum ether	6-(8′Z,11′Z,14′Z-heptadecatrienyl) salicylic acid (C17:3)	68.2	[24]
		6-(8′Z-pentadecenyl) salicylic acid (C15:1)	31.8	

AA – anacardic acid.
[a] Solvent used for AA extraction.
[b] Percentage of the total AA content.

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