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Influence of galangin on HL-60 cell proliferation and survival

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Abstract

The effect of galangin, a flavonol component of India root spice and the 'herbal' medicine propolis, on HL-60 human leukaemia cell survival is characterised. Galangin (1–100 μM) exerted an antiproliferative effect that, with dose and exposure longevity, was progressively associated with an elevated hypodiploid DNA content and expression of the active form of caspase-3, principally prior to membrane damage. At ≥50 μM, plasmamembrane phosphatidylserine exposure was observed. There was no evidence for intracellular oxidative stress as an orchestrator of cytotoxicity and significant phagocyte-like differentiation was not detected. We discuss whether such cytotoxicity will be therapeutically exploitable or contribute to cancer prevention within a pharmacological or dietary context.

Keywords: Apoptosis; Cytotoxicity; Flavonoids; Galangin; HL-60

1. Introduction

Diet is intimately linked to both the incidence and avoidance of many types of cancer. In particular, fruit and vegetable-rich diets are correlated with a lower incidence of disease [1,2]. Partly from such observations there is interest in exploiting non-nutritive dietary phytochemicals as pharmacological agents for both cancer prevention and treatment [3–5]. In addition, driven by a multitude of lifestyle and philosophical factors which are occasionally in opposition to scientific rationale, there is a burgeoning market for the use of non-nutritive phytochemicals and plant extracts as dietary supplements or as herbal medicines. Despite this, whether individual non-nutritive phytochemicals or groups of such compounds specifically confer protection is unresolved [3,6–8].

The flavonoids represent a group of highly dietary abundant non-nutritive phyto-polyphenols, categorised into flavones, flavonols, flavanones, flavanols, isoflavones and anthocyanidins [9]. Estimates of flavonoid intake vary widely with population, region and culture but are generally in the range of 1–160 mg per day [9–11]. Intakes may also be enhanced through supplements and herbal mixtures [11]. Flavonoids exert an extensive array of activities that may contribute to anti-carcinogenic effects including the mitigation of oxidative damage, antiproliferative effects, promotion of differentiation, induction of apoptosis and inhibition of malignant transformation [12,13].

Understandably, the majority of research has concentrated on the more generally abundant flavonoids such as the flavonol, quercetin (3,5,7,3, 4-pentaydroxyflavone) which, comprises 40–70% of an average 23–33 mg flavone/flavonol intake within The Netherlands [14,15]. Many other flavonoids, perhaps with less general abundance, are nevertheless present in particular regional or ethnic diets and enriched within herbal

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medicinal preparations. For example, galangin (3,5,7-trihydroxyflavone) is present at high concentrations in propolis, a resinous material produced by the activities of honeybees, and in India root, *Alpinia officinarum*, which is common spice and herbal medicine in Asia, comprising ca.10% of the ethanolic extract of the root [16].

As the disruption, but rarely the complete loss of the ability to execute apoptosis is a critical feature for neoplastic progression, flavonoid induced apoptosis has attracted considerable interest as a mechanism by which these non-nutritive phytochemicals may exert cancer preventative or therapeutic effects [12,13,17]. Thus, here we describe the effect of galangin on the survival of HL-60 'leukaemia cells'. HL-60 cells represent an excellent model for studying drug or natural product-cell interactions, possessing a well defined apoptotic response to chemotherapeutic agents and other stressors [18–20].

2. Materials and methods

2.1. Reagents

HL-60 cells were purchased from the European Collection of Cell Cultures (98070106). RPMI medium and foetal calf serum (Cyclone) was purchased from Gibco-BRL (Paisley, UK). Tissue culture flasks were supplied by Greiner Labortecknik (Gloucesterhire, UK). Dihydrorhodamine-123 (DHR) and propidium iodide (PI) were purchased from Molecular Probes (Leiden, The Netherlands). Active Caspase-3 detection kit, Cell Cycle Plus DNA Reagent Kit and QC DNA particles were purchased from Becton Dickinson (Oxford, UK). The Annexin V-FITC kit was purchased from Bender Medsystems (Vienna, Austria). Galangin (>95% purity), etoposide, camptothecin, retinoic acid, phorbol 12-myristate 13-acetate (PMA), dimethylsulfoxide (DMSO) and all other reagents were purchased from Sigma Chemical Co. (Poole, Dorset, UK) unless stated otherwise.

2.2. Cell culture and treatment

HL-60 cells were cultured in RPMI medium supplemented with 5 mL L^{-1} 100× non-essential amino acids, 2 mM glutamine, 50 μg mL^{-1} streptomycin, 50 units mL^{-1} penicillin and 10% (v/v) foetal calf serum. Cells were seeded at a density of 5×10^5 cells mL^{-1} in 75 cm^2 flasks in a humidified atmosphere of 5% CO_2 in air at 37 °C. For treatment, cells (5×10^5 mL^{-1}) were incubated with water, galangin (1–100 μM final concentration, DMSO final concentration 0.02%, v/v) or 0.02% (v/v) DMSO supplemented culture media for up to 96 h. Cells were also incubated with 1 μM *all trans* retinoic acid (RA)/1.25% (v/v) DMSO to provide a positive control for differentiation [19] and 1 μM etoposide or 4 μM camptothecin, both prepared in DMSO, to provide a positive control for apoptosis [20].

2.3. Cell proliferation and viability

Cells were collected by centrifugation (300×*g* for 5 min); culture media removed and the pellet suspended in PBS comprising trypan blue (0.2%, w/v final concentration). Cells were counted using a haemocytometer (Neubauer).

2.4. Phosphatidylserine exposure and membrane integrity

The exposure of phosphatidylserine at the extracellular surface of the plasmamembrane was determined by FITC-Annexin-V binding using a commercial assay kit. Annexin V binding and the relationship to membrane damage was assessed by co-incubation with propidium iodide (PI) as per the manufacturer's protocol (BenderMedsystems, Vienna, Austria).

2.5. Caspase-3 detection

The presence of the active form of caspase-3 was analysed by flow cytometry following cell fixation, permeabilization and labelling with PE-conjugated polyclonal rabbit anti active caspase-3 according to manufacturers recommendations. Camptothecin treatment and mock (water) treated cells, positive and negative controls, respectively, were used to define positive caspase-3 expression using Cell Quest software (Becton Dickinson, Oxford, UK). 10,000 events were recorded.

2.6. Internucleosomal DNA fragmentation

The presence of internucleosomal DNA fragmentation giving rise to the characteristic apoptotic 'DNA ladder' was identified following DNA extraction and separation by agarose gel electrophoresis. Briefly, 1×10^6 cells were pelleted by centrifugation at 1000×*g* for 5 min at room temperature. The supernatant was discarded and the pellet lysed by incubation on ice with 500 μL of 5 mM tris (pH 8.0) containing 20 mM EDTA and 0.5% (w/v) triton X-100. The suspension was then centrifuged at 16,000×*g* for 5 min at RT and the supernatant treated with 10 μL RNAase A (10 mg mL^{-1}) for 60 min at 37 °C. Next, 10 μL of proteinase K (20 mg/mL) was added and incubation continued at 50 °C for 60 min, followed by addition of 2 μL pellet paint co-precipitant (Calbiochem, Nottingham, UK), 60 μL of sodium acetate (pH 5.2), mixed and 662 μL of 2-propanol added. Following mixing by inversion, the sample was incubated for 2 min at RT, centrifuged at 16,000×*g* for 5 min at RT and the supernatant carefully removed. The pellet was then rinsed with 70 and 100% ethanol, interspersed by centrifugation and supernatant removal. The resulting pellet was air dried and resuspended in 10 mM Tris containing 1 mM EDTA. Aliquots were diluted in 50% (v/v) glycerol