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Anti-inflammatory properties of potato glycoalkaloids in stimulated Jurkat and Raw 264.7 mouse macrophages

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ABSTRACT

Aims: The potato glycoalkaloids, α-chaconine, α-solanine and solanidine, along with potato peel extracts were investigated for potential anti-inflammatory effects in vitro. Their potential to reduce two biomarkers of inflammation, cytokine and nitric oxide (NO) productions, were assessed in the stimulated Jurkat and macrophage models, respectively.
Main methods: Cytokine and nitric oxide productions were stimulated in Jurkat and Raw 264.7 macrophages with Concanavalin A (Con A; 25 μg/ml) and lipopolysaccaride (LPS; 1 μg/ml), respectively. Selective concentrations of glycoalkaloids and potato peel extracts were added simultaneously with Con A or LPS for 24 h to investigate their potential to reduce inflammatory activity.
Key findings: α-Chaconine and solanidine significantly reduced interleukin-2 (IL-2) and interleukin-8 (IL-8) productions in Con A-induced Jurkat cells. The potato peel extracts did not influence cytokine production. In LPS-stimulated Raw macrophages, α-solanine, solanidine and two potato peel extracts significantly reduced induced NO production.
Significance: Our findings suggest that sub-cytotoxic concentrations of potato glycoalkaloids and potato peel extracts possess anti-inflammatory effects in vitro and with further investigation may be useful in the prevention of anti-inflammatory diseases.

Introduction

Inflammation has been implicated in the development of acute and chronic diseases such as pancreatitis, cancer, rheumatoid arthritis and irritable bowel disease (IBD) (Bhatia et al., 2012; Kunda and Surh, 2012; Marrelli et al., 2011; Neuman, 2007). Acute inflammation is characterised by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes and inflammatory mediators such as cytokines (Feghali and Wright, 1997). During chronic inflammation, development of specific humoral and cellular immune responses occurs. Within both inflammatory responses, soluble factors play a pivotal role in leukocyte recruitment by elevating expression of cellular adhesion molecules and chemoattraction. These soluble factors include (1) inflammatory lipid metabolites such as platelet activating factor (PAF) and derivatives of arachidonic acid, (2) three cascades of soluble proteases/substrates (clotting, complement and kinins), (3) nitric oxide (NO) and (4) cytokines (Feghali and Wright, 1997).

Cytokines are the major regulators of inflammatory response and are classified as cytokines involved in acute inflammation and those associated with chronic inflammation. Tumour necrosis factor-α (TNF-α) and interleukins (ILs) such as IL-1, IL-6 and IL-8 are the predominant cytokines associated with acute inflammation. Chronic inflammation is subdivided into cytokines mediating cellular responses such as IL-1, IL-2, IL-10, interferon (IFN) and TNF-α and cytokines mediating humoral responses such as IL-4 and IL-6. NO is another signalling molecule that displays anti-inflammatory characteristics under normal physiological conditions but when produced at excessive levels it possesses pro-inflammatory properties (Sharma et al., 2007). NO is also involved in immune responses where it is released at high concentrations by cytokine-activated macrophages (Choi et al., 2008). NO is synthesized from the amino acid L-arginine by isoforms of nitric oxide synthase enzymes (NOS). Cells, especially macrophages, can be induced to produce NO upon exposure to stimulants such as bacterial lipopolysaccharide (LPS) and/or cytokines (Choi et al., 2008; Konkimalla et al., 2010; Zha et al., 2011).

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The potato aglycone, solanidine shares structural similarities to diosgenin, a precursor of steroidal hormones and anti-inflammatory steroids (Fig. 1); solanidine is a nitrogen containing equivalent. To date, the potential effects of glycoalkaloids on inflammation are linked to those originating from tomatoes and potatoes (Chiu and Lin, 2008; Choi and Koo, 2005; Filderman and Kovacs, 1969; Iablokov et al., 2010; Milner et al., 2011; Patel et al., 2002). Tomato glycoalkaloids have shown remarkable potential as a vaccine adjuvant (Heal et al., 2001; Morrow et al., 2004). The aim of this study was to investigate whether potato glycoalkaloids and potato peel extracts have the ability to reduce biomarkers of inflammation including pro-inflammatory cytokines (IL-2 and 8) and NO in vitro. The cytokines IL-8 and IL-2 were selected for investigation in this study as IL-8 plays a key role in the early stages of inflammation and suppression of this chemokine could prevent amplification of inflammatory signals and further progression of inflammatory activity. IL-2 production was evaluated as it is widely utilised in the investigation of inflammatory activity in the stimulated-Jurkat cell model (Aherne and O'Brien, 2008; McCarthy et al., 2013; Verlengia et al., 2004).

Materials and methods

Materials

All chemicals and cell culture reagents were purchased from Sigma-Aldrich Chemical Co. (Dublin, Ireland) unless otherwise stated. α-Chaconine was purchased from Apin Chemicals (Abingdon, Oxon, UK). Solanidine was purchased from Extrasynthese (Lyon, France). The potato peel extracts (one crude and three semi-purified) were generated by co-researchers within this research project (Fig. 2).

Preparation of potato peel extracts

Dried potato peel powder (550 g) was mixed with 11 l methanol and shaken for 16 h at 175 rpm at room temperature (Fig. 2). The extract was filtered through a Buchner funnel (~1 μm pore size) and dried using a rotary evaporator at 60 °C under low pressure. The dried crude extract (CE; 15 g) was redissolved in chloroform to separate the non-polar fraction (NPF) from the polar fraction (PF). The PF (11 g) was floating on the surface of the NP chloroform fraction (4 g). The PF was then isolated, dried and redissolved in 1 l water. This extract was kept at 4 °C overnight. Upon centrifugation, a precipitate was obtained. This precipitate (1.2 g) was dried and dissolved in 30 ml methanol. Silica (3 g) was added to the methanol extract and the solvent was dried using a rotary evaporator. The dried extract containing glycoalkaloids dispersed by silica was loaded onto the normal phase DASi™ cartridges of flash chromatography system. Partial purification was achieved by using Varian flash chromatography system (Intelliflash 310, Varian Inc., USA) equipped with SuperFlash silica column (SF25-120 g, 39.2×2.82 cm, 50 μm particle size). The extract was fractionated using the following mobile phases: ethylacetate (A) and methanol (B). The flow rate was kept constant at 25 ml/min for a total run time of 90 min. A stepwise gradient from 10 to 100% methanol was applied with an increment of 10% every 10 min. A total of 9 fractions were collected (Fig. 2). Similar to the PF, the NPF was also fractionated by flash chromatography system using the procedure mentioned above generating 9 fractions (Fig. 2).

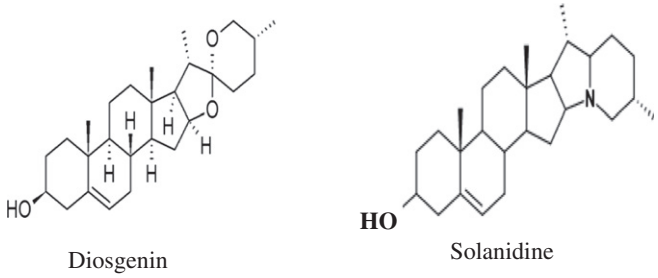

Fig. 1. Structural similarities of the potato aglycone solanidine to the saponin diosgenin.

Identification and quantification of glycoalkaloids in potato peel

Glycoalkaloids were identified and quantified using Waters Acquity (Waters Corporation, MA, USA) ultra-performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS). The compounds were separated on a Waters Acquity BEH C18 column (50×2.1 mm, particle size 1.7 μm) using 0.5% formic acid in water (solvent A) and 0.5% formic acid in acetonitrile (solvent B). The following gradient programme was carried out: 10% B to 21% B in 0.5 min, 21% B until 4.5 min, 40% B in 6 min, 40% B until 7 min, 90% B in 7.5 min, 90% B until 8.5 min, 10% B in 9 min and 10% B until 10 min at a flow rate of 0.5 ml/min. The injection volume for all samples was 5 μl. All quantification standards, ranging from 0.1 to 1 μg/ml were dissolved in methanol. MS detection of ions was performed in the positive mode using multiple reaction monitoring (MRM). The parameters for the MRM transitions are shown in Table 1. The ionization source conditions were as follows: capillary voltage 3 kV, cone voltage 30 V, extractor voltage 3 V, source temperature 120 °C, desolvation temperature 350 °C, desolvation gas flow 800 l/h, cone gas flow 50 l/h, collision gas flow 0.10 ml/min. The MRM traces were acquired using the Waters MassLynx V4.1 software while the quantifications of the data were carried out using the Waters TargetLynx software. Glycoalkaloid composition of each potato peel extract is outlined in Table 2.

Maintenance of cells in culture

Human Jurkat T cells and RAW 264.7 mouse macrophages were purchased from the European Collection of Animal Cell Cultures (Salisbury, UK). Jurkat cells were grown and maintained in RPMI media supplemented with 10% (v/v) foetal bovine serum (FBS). RAW mouse macrophages were grown and maintained in DMEM media supplemented with 10% FBS. Cells were incubated in an atmosphere of CO_2/air (5:95 v/v) at 37 °C and were maintained in the absence of antibiotics. Both cell lines were sub-cultured every two to three days. Exponentially growing cells were used throughout the experiments.

Cytotoxicity

The cytotoxic effects of glycoalkaloids and potato peel extracts were examined in both cell lines using the microculture tetrazolium (MTT) assay. This assay is routinely used for the assessment of cell proliferation and viability. Both cell lines were seeded at a density of 2×10^5/ml into 96-well plates. RAW mouse macrophages were allowed to adhere for 24 h. To determine the IC50 values for each of the test compounds, cells were exposed to increasing concentrations of glycoalkaloids and potato peel extracts. After 24 h incubation, 10 μl of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT solution) was added to each of the samples. The samples were further incubated for 4 h, prior to the addition of the solubilisation solution (10% SDS in 0.01 M HCl) and incubated overnight. The absorbance of each sample was read on a Tecan Spectrafluor Plus plate reader at a wavelength of 570 nm with a reference wavelength of 690 nm. Viability was also assessed after dose response trials involving the mitogens Concanavalin A (Con A) and Phorbol-12-Myristate-13-Acetate plus Ionomycin (PMA/IoM) in Jurkat T cells. Similarly, cell viability was assessed after dose response trials using LPS in RAW mouse macrophages.