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Natural product HTP screening for attenuation of cytokine-induced neutrophil chemo attractants (CINCs) and NO2 — in LPS/IFNγ activated glioma cells

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ABSTRACT

Chronic and acute central nervous system (CNS) inflammation are contributors toward neurological injury associated with head trauma, stroke, infection, Parkinsons or Alzheimers disease. CNS inflammatory illnesses can also contribute toward risk of developing glioblastoma multiforme (GBM). With growing public interest in complementary and alternative medicines (CAMs), we conduct a high throughput (HTP) screening of >1400 natural herbs, plants and over the counter (OTC) products for anti-inflammatory effects on lipopolysaccharide (LPS)/interferon gamma (IFNγ) activated C6 glioma cells. Validation studies were performed showing a pro-inflammatory profile of [LPS 3 μg/ml/ IFNγ 3 ng/ml] consistent with greater release [>8.5 fold] of MCP-1, NO2—, cytokine-induced neutrophil chemo-attractants (CINC) 1, CINC 2a and CINC3. The data show no changes to the following, IL-13, TNF-a, fracktaline, leptin, LIX, GM-CSF, ICAM1, L-Selectin, activin A, agrin, IL-1α, MIP-3a, B72/CD86, NGF, IL-1b, MMP-8, IL-1 R6, PDGF-AA, IL-2, IL-4, prolactin R, RAGE, IL-6, Thymus Chemokine-1, CNTF,IL-10 or TIMP-1. A HTP screening was conducted, where we employ an *in vitro* efficacy index (*i*EI) defined as the ratio of toxicity (LC_{50})/anti-inflammatory potency (IC_{50}). The *i*EI was precautionary to ensure biological effects were occurring in fully viable cells (ratio > 3.8) independent of toxicity. Using NO2 — as a guideline molecule, the data show that 1.77% (25 of 1410 tested) had anti-inflammatory effects with *i*EI ratios >3.8 and IC_{50}s <250 μg/ml. These include reference drugs (hydrocortisone, dexamethasone N6-(1-iminoethyl)-L-lysine and NSAIDS: diclofenac, tolfenamic acid), a histone deacetylase inhibitor (apicidin) and the following natural products; Ashwaganda (*Withania somnifera*), Elecampagne Root (*Inula helenium*), Feverfew (*Tanacetum parthenium*), Green Tea (*Camellia sinensis*), Turmeric Root (*Curcuma longa*) Ganthoda (*Valeriana wallichii*), Tansy (*Tanacetum vulgare*), Maddar Root (*Rubia tinctoria*), Red Sandle wood (*Pterocarpus santalinus*), Bay Leaf (*Laurus nobilis*, Lauraceae), quercetin, cardamonin, fisetin, EGCG, biochanin A, galangin, apigenin and curcumin. The herb with the largest *i*EI was Ashwaganda where the IC_{50}/LC_{50} was 11.1/>1750.0 μg/ml, and the compound with the greatest *i*EI was quercetin where the IC_{50}/LC_{50} was 10.0/>363.6 μg/ml. These substances also downregulate the production of iNOS expression and attenuate CINC-3 release. In summary, this HTP screening provides guideline information about the efficacy of natural products that could prevent inflammatory processes associated with neurodegenerative disease and aggressive glioma tumor growth.

1. Introduction

1.1. CNS inflammation, glial cells and neurodegeneration

Age related central nervous system (CNS) degenerative diseases such as Parkinson's (PD) and Alzheimer's disease (AD) are becoming significant global health concerns. Inflammatory processes occurring within CNS (by glial cells) can contribute toward vascular damage (Doyle et al., 2015; Weaver et al., 2010), neurological insult (Bodnar et al., 2015; Garcia et al., 2016) and destruction to the blood brain barrier (BBB). (Adelson et al., 1998) While CNS glial cells exert a positive influential role in neurodevelopment, neuronal homeostasis and detoxification, on the flip side, age related pro-inflammatory neurodegeneration can be evoked by head trauma (Lopez-Rodriguez et al., 2015), ischemia (Li et al., 2015c) infection (Ben Haim et al., 2015a) lysosomal storage disease (Rama Rao and Kielian, 2015) and protein aggregates of amyloid β (Aβ) (Ben Haim et al., 2015a) and α-synuclein A53T (Ben Haim et al., 2015a, Yang et al., 2015) common to AD and PD, respectively. Once neuronal insult has taken place, reactive gliosis (Mohn and Koob, 2015) can

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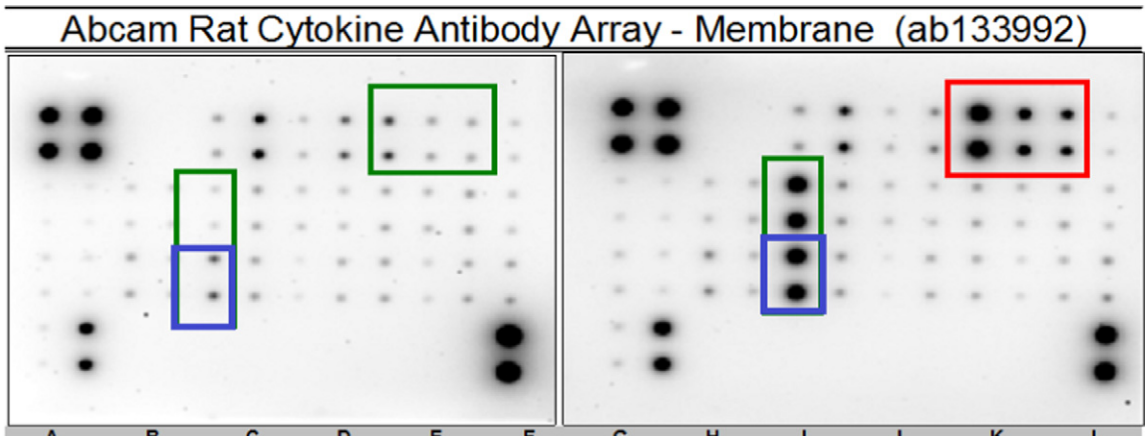
A



	A	B	C	D	E	F	G	H	I	J	K	L
1	Pos	Pos	Neg	Neg	Activin A	Agrin	B72/CD86	B-NGF	CINC-1	CINC-2α	CINC-3	CNTF
2	Pos	Pos	Neg	Neg	Activin A	Agrin	B72/CD86	B-NGF	CINC-1	CINC-2α	CINC-3	CNTF
3	Fas Ligand	Fracktaline	GM-CSF	ICAM-1	IFN-g	IL-1α	IL-1b	IL-1 R6	IL-2	IL-4	IL-6	IL-10
4	Fas Ligand	Fracktaline	GM-CSF	ICAM-1	IFN-g	IL-1α	IL-1b	IL-1 R6	IL-2	IL-4	IL-6	IL-10
5	IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3a	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
6	IL-13	Leptin	LIX	L-Selectin	MCP-1	MIP-3a	MMP-8	PDGF-AA	Prolactin R	RAGE	Thymus Chemokine-1	TIMP-1
7	TNF-α	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos
8	TNF-α	VEGF	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Pos

B

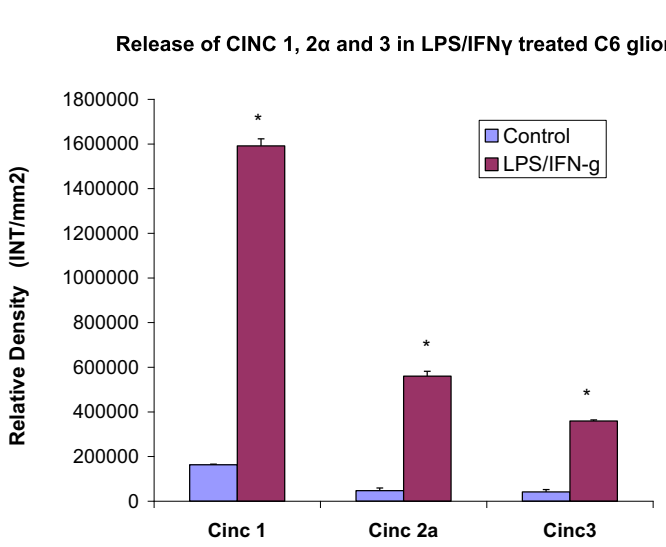


Fig. 1. [A] Cytokine release in LPS/IFNγ treated C6 glioma cells at 24 h. The blot image (Top) and corresponding array grid layout (Bottom) are presented. [B] CINC 1,2a and 3 release were significantly upregulated in LPS/IFNγ treated C6 glioma. The data represents relative density and are expressed as the Mean ± S. E. M., n = 4. Differences between resting and activated cells were determined using a Student's *t*-test, (*) $P < 0.001$.

circumscribe degenerating neurons and perpetuate the release of pro-inflammatory danger-associated molecular patterns (DAMPs) (Frank et al., 2016, Kigerl et al., 2014) leading to perpetuated release of neurotoxic cytokines (Hammond et al., 2015, Mohn and Koob, 2015). CNS inflammation can also manifest itself systemically, where high elevated cytokines are often reported in the cerebral spinal, synovial or serum in patients with PD (Bessler et al., 1999) or AD (Blum-Degen et al., 1995, Brodacki et al., 2008).

1.2. CNS inflammation, glial cells and malignancy

While aging and chronic inflammation are associated with degenerative disease, both events are risk factors for developing malignant glioblastoma multiforme (GBM), and its radiotherapeutic resistance.(Li and Liu, 2015) Glial tumors can arise from chronic irritation/inflammation and once initiated perpetuate release of pro-tumor cytokines which further augment tumor proliferation, resistance, and immune escape (Salazar-Ramiro et al., 2016). Glioma cells have enormous capacity to release tumor promoting chemokines such as MCP-1 which can recruit tumor associated monocytes (TAMS) (Leung et al., 1997) to infiltrate the glioma tumor bed (Lindemann et al., 2015, Polyzoidis et al., 2015). These immune driven processes are responsible for heightening malignant aggressivity of glioblastomas and astrocytomas (Liang et al., 2008, Lin et al., 2013).

With a greater public awareness about complementary and alternative medicine (CAMS), here we conduct a HTP screening of commonly used natural products (herbs, seeds, roots, leaves, stems) to reduce inflammation in glioma cells, relative to known drugs. As a chosen model, C6 glioma cells were used because they are of malignant origin, contain an immune-competent phenotype and are widely used to