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The attenuating effects of 1,2,3,4,6 penta-O-galloyl- β -D-glucose on inflammatory cytokines release from activated BV-2 microglial cells

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Abstract

Alzheimer's disease (AD) is the most common cause of neurodegeneration and dementia in the elderly. Dysregulated, chronic activation of microglia, the brain's resident macrophages, induces the release of excessive amounts of pro-inflammatory cytokines which has been implicated in the early stages of AD pathology. Therefore, suppressing the expression of these inflammatory mediators may decrease or delay the progression of AD. Many natural compounds derived from plants have shown anti-inflammatory activity. The naturally occurring 1,2,3,4,6 Penta-O-Galloyl- β -D-Glucose (PGG), is a polyphenolic compound highly enriched in *Rhus chinensis* Mill plant. It is a potent anti-inflammatory agent that act through the inhibition of many cytokines in different experimental models. In the present study, we investigated the role of PGG as an anti-inflammatory agent in LPS/IFN γ activated BV-2 microglia cells. Mouse cytokine antibody arrays were used to assess the effect of PGG on the release of pro-inflammatory cytokines, and ELISA experiments were performed to validate the results from the arrays. The results obtained from the cytokine arrays, and ELISA assays showed that PGG decreased the expression of monocyte chemotactic protein-5 (MCP-5) 8-fold, and pro-matrix metalloproteinase 9 (Pro MMP-9) 10-fold. Both of these cytokines are upregulated during the inflammatory process and have been shown to be involved in brain injury, inflammation, and neurodegeneration. Therefore, these findings suggest that the anti-inflammatory effect of PGG on activated microglia involving the attenuation of MCP-5 and ProMMP-9 cytokines.

Keywords

Alzheimer's disease; Monocyte chemotactic protein-5; Matrix metalloproteinase-9

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Conflict of interest
None declared.

1. Introduction

It has been several decades since the proposal that neuroinflammation might promote the progression of neurodegenerative diseases such as Alzheimer's (AD) and Parkinson's diseases (PD) (Glass et al., 2010; Ransohoff, 2016). These diseases are characterized by slow progressive loss of neurons in the central nervous system (CNS), which leads to deficits in specific brain functions, such as memory, movement, and cognition (Gao and Hong, 2008). One of the hallmarks of AD is the abnormal extracellular deposition of neurotoxic amyloid- β (A β) peptides that accumulate in senile plaques which are toxic to neurons and contribute to an inflammatory process and activation of glial cells. These activated cells produce inflammatory mediators such as macrophage inflammatory proteins, monocyte chemoattractant proteins, and other proinflammatory cytokines (Rubio-Perez and Morillas-Ruiz, 2012). Therefore, suppressing the expression of these inflammatory mediators may decrease the incidence of inflammatory diseases (Oh et al., 2004).

It is well-known that the chronic use of non-steroidal anti-inflammatory drugs (NSAID) are associated with the decreased risk of AD (McGeer et al., 1996). It has been proposed that NSAID exerts their beneficial effects by reducing the anti-inflammatory responses in the brain, suggesting that anti-inflammatory agents may offer some protection against cognitive function decline, and appear to be a promising option for AD patients (McGeer et al., 1996). However, clinical use of NSAID drugs has the disadvantage of side effects and/or high cost. An alternative to these drugs are natural products with anti-inflammatory effects (Gautam and Jachak, 2009).

On the other hand, 1,2,3,4,6-Penta-O-galloyl-Beta-D-glucose (PGG), is a naturally occurring polyphenolic compound highly enriched in some medicinal herbs such as *Rhus chinensis Mill* (Oh et al., 2004; Cao et al., 2014). This plant has been utilized as a traditional Chinese medicine in the treatment of diarrhea, dysentery, rectal and intestinal cancer, diabetes mellitus, sepsis, oral diseases and inflammation (Djakpo and Yao, 2010). *In vivo* and *in-vitro* studies have shown that PGG exhibits multiple biological activities that implicate a great potential for PGG in the therapy and prevention of several diseases, including inflammatory diseases. PGG has been shown to exhibit anti-cancer effects, anti-angiogenesis and anti-diabetic activities (Yu et al., 2011; Bruno et al., 2013; Hu et al., 2008; Zhang et al., 2009). Also, in our laboratory we were able to establish that PGG is a potent inhibitor for hLDH-A, which is an anti-cancer therapeutic target with an established role in tumor initiation, progression, and metastasis (Deiab et al., 2015).

PGG has also exhibited an anti-inflammatory effect through the inhibition of the expression of TNF- α in LPS-stimulated human peripheral blood mononuclear cells (Feldman et al., 2011), and the secretion of TNF- α and IL-6 induced by IL-1- β in rat synoviocytes (Wu and Gu, 2009). It also inhibited IL-8 mRNA expression and secretion in human monocytic U937 cells stimulated with PMA or TNF- α (Oh et al., 2004). Studies in peritoneal and colonic macrophages showed that PGG reduced activation of NF- κ B and MAPK signaling pathways by directly interacting with the MyD88 adaptor protein, having a potential to ameliorate inflammatory diseases such as colitis (Jang et al., 2013). Also, PGG also suppressed gene expression and secretion of pro-inflammatory cytokines in a dose-dependent manner and

blocked the activation of NF-kappaB, indicating a potential for the treatment of inflammatory diseases through the down-regulation of NF-kappaB-mediated activation of human mast cells (Lee et al., 2007).

Although many studies indicated PGG ability to reduce inflammation, the effect of PGG on microglia cells has not been evaluated yet. Microglia are the resident macrophage-like cell with a broad role in the brain's innate immunity and inflammatory neuropathologies and have been examined in many studies in primary cultures (Henn et al., 2009). In the present study, we investigated the effect of PGG on LPS/IFN γ activated BV-2 microglia cells and examine its potential for the treatment of neuroinflammatory/neurodegenerative diseases.

2. Material and methods

2.1. Chemicals and reagents

Dulbecco's Modified Eagle Medium-High Glucose Medium (DMEM) and Heat Inactivated Fetal Bovine Serum (FBS-HI) were obtained from Atlanta Biologicals (Flowery Branch, GA). Penicillin/streptomycin and Hank's Balanced Salt Solution (HBSS) were obtained from GIBCO (Waltham, MA). Dimethyl sulfoxide (DMSO), PGG, lipopolysaccharide from *Salmonella enterica* (LPS), interferon γ (IFN γ), and other general reagents and supplies were all purchased from Sigma-Aldrich Co. (St. Louis, MO) and VWR International (Radnor, PA).

2.2. Cell culture

BV-2 microglia cells were generously provided by Dr. Elizabetta Blasi (Blasi et al., 1990). Cells were cultured in DMEM media supplemented with 10% Heat Inactivated Fetal Bovine Serum (FBS-HI) and 1% penicillin/streptomycin (100 U/ml penicillin and 0.1mg/ml streptomycin) in a humidified atmosphere of 5% CO₂ at 37 °C in an incubator. Plating media for each experiment consisted of DMEM, 2.5% of FBS-HI, and no penicillin/streptomycin-penicillin. LPS was prepared in HBSS at 1 mg/ml and IFN γ in PBS at 200 ng/ml; both of them stored at -20 °C. For experiments, LPS and IFN γ were added to the culture media at a working concentration of 0.2 μ g/ml and 0.2 ng/ml, respectively.

2.3. Cell viability

Alamar Blue (Resazurin) assay was used to assess BV-2 cell viability. Approximately 5.0×10^5 cells/ml (100 μ l/well) were plated in 96 well plates and incubated overnight to attach. The next day cells were replaced with experimental media. Our samples were prepared as follows: control (cells + media), control-DMSO (cells + DMSO), and cells treated with different concentrations of PGG (6.25 – 200 μ M) and incubated at 37 °C. After 1 h cells were stimulated with a combination of 0.2 μ g/ml LPS and 0.2 ng/ml IFN γ . PGG was dissolved in DMSO before dilution in the media and the final concentration of DMSO did not exceed 0.1% (Elsisi et al., 2005). After 24 h 20 μ l of Alamar Blue solution (0.5 mg/ml) was added and the plate was incubated again for 4 h. The fluorescent signal was monitored using 550 nm excitation and 580 nm emission wavelengths using a microplate reader Infinite M200 (Tecan Trading AG). The fluorescent signal generated from the assay is proportional

to the number of living cells in the sample. The data were expressed as a percentage of live untreated controls.

2.4. Measurement of nitric oxide (NO) production

The effect of PGG on NO production was studied in LPS/IFN γ -stimulated BV-2 cells. The released amount of nitrite (NO_2^-), a soluble oxidation product of NO, was measured with a colorimetric assay using Griess reagent (1% sulfanilamide and 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride in 5% phosphoric acid (H_3PO_4)) (Subedi et al., 2016). BV-2 cells (5×10^4 cells/well, in a 96-well plate) were seeded overnight to attach the cells, then stimulated with either LPS/IFN γ (0.2 $\mu\text{g/ml}$ and 0.2 ng/ml , respectively) in the presence or absence of PGG (25 to 200 μM) for 24 h. Control cells were treated with DMSO. Fifty microliters of supernatant were mixed with an equal volume of the Griess reagent and OD was measured at 550 nm. Sodiumnitrite was used as a standard to calculate NO_2^- concentrations.

2.5. iNOS assay

To examine the effect of PGG in iNOS protein expression, MyBioSource Mouse Inducible Nitric Oxide Synthase ELISA Kit (Cat#MBS701257) was used. The samples consisted of Control (cells + DMSO), and LPS/IFN γ (0.2 $\mu\text{g/ml}$ and 0.2 ng/ml , respectively), in the presence or absence of different concentrations of PGG (25 to 200 μM). Each experiment was performed in triplicate according to the manufacturer's instructions. Briefly, 100 μl of standard and sample were added per well and incubated for 2 h at 37 °C and any iNOS present in the sample was bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for iNOS was added to the wells, washed after 1 h., and then avidin conjugated Horseradish peroxidase (HRP) was added. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color developed in proportion to the amount of iNOS bound in the initial step. The intensity of the color was measured at 450 nm.

2.6. Mouse cytokine antibody arrays

RayBiotech Mouse Cytokine Antibody Arrays (Cat#AAM-CYT-1000) were used to study the effect of PGG on 96 cytokine proteins released by LPS/IFN γ activated BV-2 microglia cells. Each experiment was performed in triplicate and according to the manufacturer's instructions. Briefly, antibody-coated array membranes were first incubated for 30 min with 1 ml of blocking buffer. After 30 min, blocking buffer was decanted and replaced with 1 ml supernatant from control (cells + DMSO) samples, cells treated with PGG (25 μM) only, LPS/IFN γ only, and PGG (25 μM) + LPS/IFN γ (0.2 $\mu\text{g/ml}$ and 0.2 ng/ml , respectively). Membranes were incubated overnight at 4 °C with mild shaking. The next day, the media were decanted; membranes were washed, and subsequently incubated with 1 ml biotin-conjugated anti-bodies for 2 h. Lastly, biotin-conjugated antibodies were removed, and membranes were incubated with HRP-conjugated streptavidin for 2 h. Detection of spots using chemiluminescence was acquired with semi-quantitative analysis of signal intensities from Quantity One software (Bio-Rad). Intensities were normalized as a percentage of positive controls on each membrane.

2.7. Cytokine ELISAs

Supernatants from the control (cells + DMSO), stimulated, and treated (24 h) BV-2 microglia cells were collected and centrifuged at 1000g for 5 min at 4°C. Specific ELISAs were performed using Murine Monocyte chemoattractant protein-5 (MCP-5) (Cat# ELM-MCP-5) and Matrix metalloproteinase-9 (MMP-9) (Cat# ELM proMMP-9) ELISA kits (RayBiotech, Norcross, GA, USA) following manufacturer's instructions. Briefly, 100 µl of supernatants from samples and standards were added to 96 well plates pre-coated with capture antibody. After incubation, 100 µl of prepared biotinylated antibody mixture was added to each well. After 1 h, the mixture was decanted, and 100 µl Streptavidin solution was placed in each well and incubated. Substrate reagent (100 µl) was then added to each well for 30 min followed by the addition of 50 µl of stop solution. Data was quantified by optical density at 450 nm.

2.8. Data analysis

Statistical analysis was performed using Graph Pad Prism (version 6.07). All data was expressed as mean ± standard error from at least 3 independent experiments and the significance of the difference between the groups was assessed using a one-way ANOVA, followed by Tukey *post hoc* means comparison test or Student's *t*-test.

3. Results

The cell viability of BV-2 cells treated with PGG and LPS/IFN γ was assessed by Alamar Blue assay after 24 h. Both, PGG and LPS/IFN γ combination, initiated a mild loss of cell viability. PGG caused a concentration-dependent decrease in cell viability in concentrations over 25uM (Fig. 1A). The tested concentrations of LPS/IFN γ to establish an optimum dose ranged from 0.05 to 0.40 µg/ml of LPS and 0.05 to 0.40 ng/ml of IFN γ (Fig. 1B). Based on the data from the cell viability assays and supported by the literature (Sheng et al., 2011) we selected to use 25uM of PGG and 0.2 µg/ml of LPS together with 0.2 ng/ml of IFN γ as our working concentrations for subsequent evaluation (Fig. 1C).

Nitric Oxide production in the LPS/IFN γ -stimulated BV-2 microglia cells was increased by 17-fold as compared to control (Fig. 2A). However, 25uM of PGG caused no inhibition of NO in the stimulated cells. By increasing the concentration of PGG, a decrease of NO production was observed, which can be explained by a decrease of cell viability as shown in the cell viability experiments (Fig. 2B). In the iNOS expression assay, LPS/IFN γ combination caused a significant increase in iNOS expression in the activated BV-2 cells, but 25uM of PGG didn't inhibit it significantly. In higher concentrations it followed the same pattern of NO assay, causing inhibition of iNOS expression but probably compromising cell viability (Fig. 2C).

To profile the effect of PGG, LPS/IFN γ or a co-treatment of PGG and LPS/IFN γ vs. control on pro-inflammatory cytokines, Mouse Cytokine Antibody Arrays with 96 cytokine proteins were used (Figs. 3A and 4A). The dot blot intensity analysis of the supernatants derived from activated BV-2 microglia cells and the co-treatment of PGG with LPS/IFN γ showed that PGG attenuated LPS/IFN γ -induced MCP-5 (Monocyte Chemotactic Protein-5) and Pro

MMP-9 (Pro Matrix Metalloproteinase-9) release (Figs. 3B and 4B). Protein expression as normalized intensity showed that PGG inhibited significantly the release of MCP-5 (8-fold) and Pro MMP-9 (10-fold) pro-inflammatory cytokines in activated BV-2 microglia cells (Figs. 3C and 4C). MCP-5 and Pro MMP-9 individual ELISAs were used to corroborate the cytokine array findings. The results confirmed that LPS/IFN γ induces up-regulation of MCP-5 and ProMMP-9 in BV-2 microglia cells, which was attenuated by PGG co-treatment (Fig. 5A and B).

4. Discussion

AD as the most common neurodegenerative disorder is characterized by the neuropathological hallmarks of A β plaques deposits and neurofibrillary tangles. AD is also accompanied by inflammation which contributes significantly to the pathogenesis of the disease. During the pathogenesis of the disease, activated microglia cells induce the overexpression of pro-inflammatory cytokines (Rubio-Perez and Morillas-Ruiz, 2012). Studies have shown that PGG exhibits an anti-inflammatory effect through the inhibition of the expression of many cytokines, such as TNF- α , IL-6, and IL-8 in different cell models (Oh et al., 2004; Feldman et al., 2011; Wu and Gu, 2009). PGG presented an anti-cancer potential by inhibiting the enzyme hLDH-A (Deiab et al., 2015), and inhibiting epidermal growth factor (EGF)-induced cell invasiveness and MMP-9 expression in a dose- and time-dependent manner by reducing the MMP-9 transcriptional activity, suggesting that PGG may be a therapeutic drug candidate for the treatment of advanced metastatic prostate cancer (Zhang et al., 2009).

The data obtained from this study indicate that the administration of 25uM PGG caused no changes of NO in LPS/IFN γ -stimulated BV-2 microglia cells. By increasing the concentration of PGG, a decrease of NO production was observed, which can be explained by a decrease of cell viability shown in our cell viability experiments. In the iNOS expression assay, LPS/IFN γ combination caused a significant increase in iNOS expression in the activated BV-2 cells, but 25uM of PGG didn't inhibit it significantly. In higher concentrations it followed the same pattern of NO assay, causing inhibition of iNOS expression but probably compromising cell viability. These results clearly indicate that the lack of PGG effect on nitric oxide might indicate that the action of PGG on inflammation does not involve the inhibition of nitric oxide system.

The data also show that PGG can downregulate the expression of MCP-5 by 8-fold and Pro-MMP-9 by 10-fold; two pro-inflammatory cytokines induced by LPS/IFN γ activated BV-2 microglia cells. MCP-5 belongs to a large family of chemokines that controls the recruitment of leukocytes in immune and inflammatory responses. It was shown that brain cortical amount of MCP-5 correlates strongly with the amount of tau protein phosphorylated at Ser396/404 in a tau transgenic mice model (Garwood et al., 2010). The biological activity of tau, primarily a neuronal protein, in promoting assembly and stability of microtubules is regulated by its degree of phosphorylation. Normal tau contains 2–3 mol phosphate/mol of the protein (Köpke et al., 1993), which represents the level of phosphorylation of its optimal activity. Hyperphosphorylation of tau depresses its microtubule assembly activity, and its binding to microtubules. Tau in AD brain is abnormally hyperphosphorylated and in this

state is the major protein subunit of paired helical filaments/straight filaments (PHF/SF) which form neurofibrillary tangles, a hallmark lesion of this disease (Iqbal et al., 2005). These studies indicate that MCP-5 could be associated with the development of tau pathology, and therefore the inhibition of MCP-5 expression would be beneficial to AD patients.

Meanwhile, Pro MMP-9 belongs to a large family of zinc-mediated metallo-proteinases and exerts activity in the turnover of extracellular matrix (ECM) and physiological and pathological processes (Yong et al., 1998). In general, MMP-9 s are produced as inactive pre-forms and activated through the proteolytic splitting of the N-terminal domain. They are also known as 92-kDa gelatinase B/type IV collagenase secreted from neutrophils, and macrophages. Its concentration is low and usually can be induced by various pro-inflammatory factors such as cytokines, peptides, pathogenic, and other stress in neuronal or neuroglial cells (Hsi-Lung and Chuen-Mao, 2014; Rosenberg, 2002). In CNS, MMP-9 is essential in various physiological function, such as morphogenesis, neurite outgrowth, and injured repairing. Moreover, up-regulation of MMP-9 may be involved in the pathogenesis of many CNS disorders including stroke, AD, multiple sclerosis, and malignant glioma (Yong et al., 2001). Previous studies have revealed that MMP-9 is induced by the presence of A β and highly expressed and secreted by astrocytes (Fujimoto et al., 2008). Its expression was observed in the cytoplasm of neurons, neurofibrillary tangles, senile plaques, and vascular walls of the hippocampus and cerebral cortex of AD patients (Xiang-Xiang et al., 2014; Mizoguchi et al., 2009). Its accumulation in the CNS is highly neurotoxic and deteriorates synaptic functions (Fujiwara et al., 2009; Selkoe, 2002; Wirths et al., 2004). MMP-9 can induce and degrade A β and compact plaques, and it is released, along with nerve growth factor precursor (pro-NGF) in an activity-dependent manner (Yan et al., 2006). Therefore, the inhibition of MMP-9-mediated inflammatory pathways may provide a therapeutic target for brain inflammation and neurodegenerative diseases.

Currently, drugs available for AD such as acetylcholinesterase inhibitors exert only a temporary benefit on cognitive dysfunction, and they do not prevent or reverse the formation of A β deposits (Darreh-Shori et al., 2000; Park et al., 2000). A potentially promising strategy for developing more effective anti-dementia drugs is the inhibition of A β fibril formation or destabilization of aggregated A β or a combination of both. *In vivo* studies using transgenic mice expressing the human A β precursor protein showed that PGG reduced the accumulation of A β in brain tissue and also disassembled pre-formed A β fibrils, decreasing the number of A β positive spots in the cortex and hippocampus in the treated animals (Fujiwara et al., 2009).

In summary, our findings show that PGG inhibits the expression of MCP-5 and ProMMP-9 cytokines, which may be associated with the formation of senile plaques and neurofibrillary tangles in AD disease. Therefore, PGG inhibitory effect on the release of these cytokines may provide a therapeutic target to treat brain inflammation. Future studies using Alzheimer's disease animal model are required to investigate further the potential of PGG in AD therapy.

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References

- Blasi E, Barluzzi R, Bocchini V, Mazzolla R, Bistoni F. Immortalization of murine microglial cells by a v-raf/v-myc carrying retrovirus. *J Neuroimmunol.* 1990; 27(2–3):229–237. [PubMed: 2110186]
- Bruno E, Pereira C, Roman KP, Takiguchi M, Kao P, Nogaj LA, Moffet DA. IAPP aggregation and cellular toxicity are inhibited by 1,2,3,4,6-penta-O-galloyl- β -D-glucose. *Amyloid.* 2013 Mar; 20(1): 34–38. [PubMed: 23339420]
- Cao Y, Himmeldirk KB, Qian Y, Ren Y, Malki A, Chen X. Biological and biomedical functions of penta-O-galloyl-D-glucose and its derivatives. *J Nat Med.* 2014; 68(3):465–472. [PubMed: 24532420]
- Darreh-Shori T, Hellström-Lindahl E, Flores-Flores C, Guan ZZ, Soreq H, Nordberg A. Long-lasting acetylcholinesterase splice variations in anticholinesterase-treated Alzheimer's disease patients. *J Neurochem.* 2000; 88:1102–1113.
- Deiab S, Mazzi E, Eyunni S, McTier O, Mateeva N, Elshami F, Soliman KFA. 1,2,3,4,6-Penta-O-galloylglucose within *Galla Chinensis* inhibits human LDH-A and attenuates cell proliferation in MDA-MB-231 breast cancer cells. *Evid Based Complement Alternat Med.* 2015; 2015:276946. [PubMed: 25918543]
- Djakpo O, Yao W. *Rhus chinensis* and *Galla Chinensis* - folklore to modern evidence: review. *Phytother Res.* 2010; 24:1739–1747. [PubMed: 20564459]
- Elsisi N, Darling-Reed S, Lee EY, Oriaku ET, Soliman KF. Ibuprofen and apigenin induce apoptosis and cell cycle arrest in activated microglia. *Neurosci Lett.* 2005; 375:91–96. [PubMed: 15670648]
- Feldman KS, Sahasrabudhe K, Lawlor MD, Wilson SL, Lang CH, Scheuchenzuber WJ. *In vitro* and *In vivo* inhibition of LPS-stimulated tumor necrosis factor- α secretion by the gallotannin beta-D-pentagalloylglucose. *Bioorg Med Chem Lett.* 2011; 11(14):1813–1815.
- Fujimoto M, Takagi Y, Aoki T, Hayase M, Marumo T, Gomi M, Nishimura M, Kataoka H, Hashimoto N, Nozaki K. Tissue inhibitor of metalloproteinases protect blood-brain barrier disruption in focal cerebral ischemia. *J Cereb Blood Flow Metab.* 2008; 28(10):1674–1685. [PubMed: 18560439]
- Fujiwara H, Tabuchi M, Yamaguchi T, Iwasaki K, Furukawa K, Sekiguchi K, Ikarashi Y, Kudo Y, Higuchi M, Saido TC, Maeda S, Takashima A, Hara M, Yaegashi N, Kase Y, Arai HA. Traditional medicinal herb *Paeonia suffruticosa* and its active constituent 1,2,3,4,6-penta-O-galloyl-beta-D-glucopyranose have potent anti-aggregation effects on Alzheimer's amyloid beta proteins *in vitro* and *in vivo*. *J Neurochem.* 2009; 109(6):1648–1657. [PubMed: 19457098]
- Gao H-M, Hong J-S. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol.* 2008; 29:8.
- Garwood CJ, Cooper JD, Hanger DP, Noble W. Anti-inflammatory impact of minocycline in a mouse model of Tauopathy. *Front Psych.* 2010; 1:136.
- Gautam R, Jachak SM. Recent developments in anti-inflammatory natural products. *Med Res Rev.* 2009; 29:767–820. [PubMed: 19378317]
- Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell.* 2010; 140:918–934. [PubMed: 20303880]
- Henn A, Lund S, Hedtjörn M, Schrattenholz A, Pörzgen P, Leist M. The suitability of BV-2 cells as alternative model system for primary microglia cultures for animal experiments examining brain inflammation. *ALTEX.* 2009; 26(2):83–94. [PubMed: 19565166]
- Hsi-Lung H, Chuen-Mao Y. The role of matrix metalloproteinase-9 in pro-inflammatory factors-induced brain inflammation and neurodegenerative diseases. *Inflammation and Cell Signaling.* 2014; 1(e 124)
- Hu H, Lee HJ, Jiang C, Zhang J, Wang L, Zhao Y, Xiang Q, Lee EO, Kim SH, Lu J. Penta-1,2,3,4,6-O-galloyl-beta-D-glucose induces p53 and inhibits STAT3 in prostate cancer cells *in vitro* and

suppresses prostate xenograft tumor growth *in vivo*. Mol Cancer Ther. 2008; 7:2681–2691. [PubMed: 18790750]

Iqbal K, Alonso AC, Chen S, Chohan MO, El-Akkad E, Gong CX, Khatoon S, Li B, Liu F, Rahman A, Tanimukai H, Grundke-Iqbal I. Tau pathology in Alzheimer disease and other Tauopathies. Biochim Biophys Acta. 2005; 1739(2–3):198–210. [PubMed: 15615638]

Jang SE, Hyam SR, Jeong JJ, Han MJ, Kim DH. Penta-O-galloyl- β -D-glucose ameliorates inflammation by inhibiting Myd88/NF- κ B and Myd88/MAPK signaling pathways. Br J Pharmacol. 2013; 170(5):1078–1091. [PubMed: 23941302]

Köpke E, Tung YC, Shaikh S, Alonso AC, Iqbal K, Grundke-Iqbal I. Microtubule associated protein tau: abnormal phosphorylation of a non-paired helical filament pool in Alzheimer disease. J Biol Chem. 1993; 268:24374–24384. [PubMed: 8226987]

Lee SH, Park HH, Kim JE, Kim JA, Kim YH, Jun CD, Kim SH. Alloose gallates suppress expression of pro-inflammatory cytokines through attenuation of NF-kappa B in human mast cells. Planta Med. 2007; 73(8):769–773. [PubMed: 17599281]

McGeer PL, Schulzer M, McGeer EG. Arthritis and anti-inflammatory agents as possible protective factors for Alzheimer's disease: a review of 17 epidemiologic studies. Neurology. 1996; 47(2): 425–432. [PubMed: 8757015]

Mizoguchi H, Takuma K, Fukuzaki E, Ibi D, Someya E, Akazawa KH, Alkam T, Tsunekawa H, Mouri A, Noda Y, Nabeshima T, Yamada K. Matrix metalloproteinase-9 inhibition improves amyloid β -mediated cognitive impairment and neurotoxicity in mice. J Pharmacol Exp Ther. 2009; 331:14–22. [PubMed: 19587312]

Oh GS, Pae HO, Choi BM, Lee HS, Kim IK, Yun YG, Kim JD, Chung HT. Penta-O-galloyl-beta-D-glucose inhibits phorbol myristate acetate-induced interleukin-8 [correction of interleukin-8] gene expression in human monocytic U937 cells through its inactivation of nuclear factor-kappa B. Int Immunopharmacol. 2004; 4(3):377–386. [PubMed: 15037215]

Park CH, Lee YJ, Lee SH, Choi SH, Kim HS, Jeong SJ, Kim SS, Suh YH. Dehydroevodiamine HCl prevents impairment of learning and memory and neuronal loss in rat models of cognitive disturbance. J Neurochem. 2000; 74:244–253. [PubMed: 10617126]

Ransohoff RM. How neuroinflammation contributes to neurodegeneration. Science. 2016; 353(6301): 777–783. [PubMed: 27540165]

Rosenberg GA. Matrix Metalloproteinases in Neuroinflammation. Glia. 2002; 39:279–291. [PubMed: 12203394]

Rubio-Perez JM, Morillas-Ruiz JM. A review: inflammatory process in Alzheimer's disease, role of cytokines. Sci World J. 2012; 2012:756357.

Selkoe DJ. Alzheimer's disease is a synaptic failure. Science. 2002; 298:789–791. [PubMed: 12399581]

Sheng W, Zong Y, Mohammad A, Ajit D, Cui J, Han D, Hamilton JL, Simonyi A, Sun AY, Gu Z, Hong J, Weisman GA, Sun GY. Pro-inflammatory cytokines and lipopolysaccharide induce changes in cell morphology, and upregulation of ERK1/2, Inos and Spla2-IIA expression in astrocytes and microglia. J Neuroinflammation. 2011; 8:121. [PubMed: 21943492]

Subedi L, Gaire BP, Do MH, Lee TH, Kim SY. Anti-neuroinflammatory and neuroprotective effects of the Linderia Neesiana fruit *in vitro*. Phytomedicine. 2016; 23:872–881. [PubMed: 27288923]

Wirths O, Multhaup G, Bayer TA. A modified B-amyloid hypothesis: intraneuronal accumulation of the B-amyloid peptide – the first step of a fatal cascade. J Neurochem. 2004; 91:513–520. [PubMed: 15485483]

Wu M, Gu Z. Screening of Bioactive compounds from moutan cortex and their anti-inflammatory activities in rat synoviocytes. Evid Based Complement Alternat Med. 2009; 6(1):57–63. [PubMed: 18955220]

Xiang-Xiang W, Meng-Shan T, Jin-Tai Y, Lan T. Matrix metalloproteinases and their multiple roles in Alzheimer's disease. Biomed Res Int. 2014; 2014 Article ID 908636.

Yan P, Hu X, Song H, Yin K, Bateman RJ, Cirrito JR, Xiao Q, Hsu FF, Turk JW, Xu J, Hsu CY, Holtzman DM, Lee JM. Matrix metalloproteinase-9 degrades amyloid- β fibrils *in vitro* and compact plaques *in situ*. J Biol Chem. 2006; 281:24566–24574. [PubMed: 16787929]

- Yong VW, Krekoski CA, Forsyth PA, Bell R, Edwards DR. Matrix metalloproteinases and diseases of the CNS. *Trends Neurosci.* 1998; 21:75–80. [PubMed: 9498303]
- Yong VW, Power C, Forsyth P, Edwards DR. Metalloproteinases in biology and pathology of the nervous system. *Nat Rev Neurosci.* 2001; 2:502–511. [PubMed: 11433375]
- Yu WS, Jeong SJ, Kim JH, Lee HJ, Song HS, Kim MS, Ko E, Lee HJ, Khil JH, Jang HJ, Kim YC, Bae H, Chen CY, Kim SH. The genome-wide expression profile of 1,2,3,4,6-penta-O-galloyl- β -D-glucose-treated MDA-MB-231 breast cancer cells: Molecular target on cancer metabolism. *Mol Cells.* 2011; 32(2):123–132. [PubMed: 21614488]
- Zhang J, Li L, Kim SH, Hagerman AE, Lu J. Anti-cancer, anti-diabetic and other pharmacologic and biological activities of penta-galloyl-glucose. *Pharm Res.* 2009; 26(9):2066–2080. [PubMed: 19575286]

Fig. 1.

A- The effect of PGG on cell viability of BV-2 microglia cells ($n=5$); **B-** The effect of combined LPS and IFN γ (0.05 to 0.40 $\mu\text{g/ml}$ and 0.05 to 0.40 ng/ml , respectively) on cell viability of BV-2 microglia cells ($n=3$); **C-** The effect of PGG on cell viability of BV-2 microglia cells stimulated with LPS/IFN γ ($\mu\text{g/ml}$ and ng/ml , respectively) ($n=5$). All the experiments were performed at 5% CO_2/Atm for 24 h. The data are presented as mean $\pm$ S.E.M, and the significance of differences from the control were determined by a one-way ANOVA, with a Tukey *post hoc* test * p 0.05, ** p 0.01, *** p 0.001 and **** p 0.0001.

Fig. 2.

A- The effect of PGG on the Nitric Oxide production (μ M) in BV-2 microglia cells stimulated with LPS/IFN γ (μ g/ml and ng/ml, respectively) ($n=5$); **B-** The effect of PGG on the Nitric Oxide production and Cell viability as % of control (cells + DMSO) in BV-2 microglia cells stimulated with LPS/IFN γ (μ g/ml and ng/ml, respectively) ($n=5$). **C-** The effect of PGG on iNOS expression in activated BV-2 microglia cells ($n=3$). All the experiments were performed at 5% CO₂/Atm for 24 h. The data are presented as mean $\pm$ S.E.M., and the significance of differences from the control (**A** and **B**) and LPS/ IFN γ treatment (**C**) was determined by a one-way ANOVA, with a Tukey *post hoc* test * p 0.05, ** p 0.01, *** p 0.001 and **** p 0.0001.

Fig. 3.
A- Microarray layout. MCP-5, monocyte chemotactic protein 5. Positive controls are located in the upper left ($n=2$) and lower right ($n=1$) corners to ensure equal distribution of supernatant. **B-** Microarray chemiluminescent spot intensity analysis of supernatant derived from BV-2 microglia cells. Treatments consisted of control (cells + DMSO), PGG only, cells activated with LPS/IFN γ and BV-2 microglia cells treated with PGG and LPS/IFN γ . **C-** Protein expression of MCP-5 as normalized intensity, expressed as % of control arrays (mean $\pm$ S.E.M. $n = 3$). Statistical significance from LPS/IFN γ vs PGG + LPS/IFN γ was evaluated by a Student's t -test, $p^* = 0.05$.

Fig. 4.
A-Microarray layout. ProMMP-9, pro-matrix metalloproteinase 9. Positive controls are located in the upper left ($n=2$) and lower right ($n=1$) corners to insure equal distribution of supernatant. **B**- Microarray chemiluminescent spot intensity analysis of supernatant derived from BV-2 microglia cells. Treatments consisted of control (cells+DMSO), PGG only, cells activated with LPS/IFN γ and BV-2 microglia cells treated with PGG and LPS/IFN γ . **C**- Protein expression of MMP-9 as normalized intensity, expressed as % of control arrays (mean $\pm$ S.E.M. $n = 3$). Statistical significance of LPS/IFN γ vs PGG + LPS/IFN γ was evaluated by a Student's t -test, $p^* = 0.05$.

Fig. 5. ELISA assay. **A-** The effect of PGG (25 μ M) on MCP-5 expression in resting and LPS/IFN γ (0.2 μ g/ml and 0.2 ng/ml, respectively) activated BV-2 microglia cells. **B-** The effect of PGG (25 μ M) on ProMMP-9 expression in resting and LPS/IFN γ (0.2 μ g/ml and 0.2 ng/ml, respectively) activated BV-2 microglia cells. Control cells were treated with DMSO. The data are presented as the mean $\pm$ S.E.M. ($n = 3$), and significance was determined by a one-way ANOVA, with a Tukey *post hoc* test $*p < 0.05$ compared to the LPS/IFN γ treatment.