ORIGINAL ARTICLE

Effect of Diarylpentanoid Analogues on Lipopolysaccharide-Induced Interleukin-6 and Interleukin-8 Gene Expression in Airway Inflammation

Kong Yen Liew[1], Nur A'dilah Rosly[1], Faridah Abas[2,3], Sze Wei Leong[3], Daud Ahmad Israf[1], Chau Ling Tham[1]

[1] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Darul Ehsan, Malaysia
[2] Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia
[3] Laboratory of Natural Products, Institute of Bioscience, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

ABSTRACT

Introduction: Airway inflammation is the pathological hallmark of chronic inflammatory airway diseases, especially asthma and chronic obstructive pulmonary disease (COPD). Airway epithelium plays an indispensable role in these diseases by secreting inflammatory mediators and cytokines in response to foreign substances, such as lipopolysaccharide (LPS). Previous studies have shown that diarylpentanoid analogues, especially 5-(3,4-dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl)penta-2,4-dien-1-one (DHHPD) and 2-benzoyl-6-(3,4-dihydroxybenzylidene)cyclohexen-1-ol (BDHBC), significantly inhibited nitric oxide (NO) production; suggesting their anti-inflammatory property. However, the therapeutic potential of DHHPD and BDHBC in airway inflammation has not been explored. Thus, this study aims to investigate their effects on interleukin (IL)-6 and IL-8 gene expression in LPS-induced Calu-3 cells, a cellular model of human airway epithelium. **Methods:** MTT cytotoxicity assay was carried out to identify non-cytotoxic concentrations of DHHPD and BDHBC on Calu-3 cells. RT-PCR was done to determine IL-6 and IL-8 gene expression levels. **Results:** DHHPD and BDHBC were not cytotoxic on Calu-3 cells up to 200μM. Four non-cytotoxic concentrations were chosen – 6.25, 12.5, 25 and 50μM to determine the effect of both compounds on gene expression. All four concentrations of DHHPD and BDHBC significantly inhibited LPS-induced mRNA expression of IL-6 while all concentrations of BDHBC, except 6.25μM, significantly reduced IL-8 mRNA expression. Similar finding was obtained for DHHPD, except that at 50μM, there was no inhibition of IL-8 mRNA expression. **Conclusion:** Diarylpentanoid analogues, DHHPD and BDHBC, are proven to be effective in suppressing LPS-induced IL-6 and IL-8 gene expression. However, further studies are required to confirm their inhibitory effects on the production of pro-inflammatory cytokines.

Keywords: Airway inflammation, Airway epithelium, Diarylpentanoid analogue, Interleukin-6, Interleukin-8

Corresponding Author:
Chau Ling Tham, PhD
Email: chauling@upm.edu.my
Tel: +603-8609 2952

INTRODUCTION

The initiation of an inflammatory response within human airways is a self-protective mechanism towards harmful environmental stimuli. These include pathogens such as bacteria, viruses and airborne irritants, such as dust, chemicals and tobacco smoke. While the response is usually protective and beneficial in its nature, it may also lead to tissue injury (1). Lipopolysaccharide (LPS), which is present as a contaminant on airborne particles, is capable of inducing cytokine production and inflammatory responses within the respiratory system, resulting in lung damage (2). Airway inflammation is the predominant feature of various chronic airway diseases, especially asthma and COPD (3). As airway epithelium acts as both physical and chemical barrier against pathogenic microorganisms or foreign substances to which human airways are constantly exposed, it is a crucial player in the development of inflammatory airway diseases. They are able to recruit immune cells and secrete cytokines, chemokines and antimicrobial peptides in response to harmful pathogens in the air (4). Thus, airway epithelial dysfunction represents one of the important events during asthma and COPD (5).

Cytokines are the crucial factors responsible for the initiation and progression of chronic inflammation

underlying various respiratory diseases (4). IL-6 is a pro-inflammatory cytokine secreted by a vast array of immune cells, such as leukocytes, mast cells and dendritic cells, as well as endothelial cells and airway epithelium (6). On the other hand, IL-8 functions as a chemoattractant that mediates neutrophils accumulation in the airways (7). Although IL-4, IL-5 and IL-13 are commonly implicated in the pathogenesis of allergic asthma, evidence has shown that IL-6 is responsible for regulating CD4 T cells responses by stimulating IL-4 secretion, Th2 differentiation or/and Th17 differentiation (8). Besides that, IL-6 is also required for IL-13 production and mucous hypersecretion by lung epithelial cells (9). Due to the important roles played by IL-6 and IL-8 as described above, it is not surprising that IL-6 and IL-8 were reported to be negatively correlated with pulmonary function of asthmatic and COPD patients (10, 11). Current treatment options for asthma and COPD include three different classes of inhaled therapies, which are inhaled corticosteroids (ICS), long-acting beta-2 (β2) agonists (LABAs) and long-acting muscarinic antagonists (LAMAs) (12). However, inflammation which often fails to resolve and resistance to corticosteroid remain the major hindrances for a successful treatment (13). Thus, efforts to look for alternative anti-inflammatory therapies for the management of both severe asthma and COPD are still underway.

Curcumin, a major constituent of turmeric (Curcuma longa), is popular among Chinese and Indian communities as a healing agent for diseases. The potency of curcumin as an anti-inflammatory and anti-cancer agent has been well-validated in various studies (14). However, the therapeutic benefits of curcumin were hindered by its low bioavailability, low water solubility and poor chemical stability (15). Due to these reasons, researchers have been prompted to overcome the problems by using different drug delivery systems and through structural modifications of curcumin (14). As a result, there have been numerous analogues of curcumin being synthesised, including cyclohexanone, cyclopentanone and diarylpentanoid derivatives, with most of them being mono-carbonyl analogues of curcumin (16-19).

Several diarylpentanoid derivatives of curcumin have been proven to be excellent anti-inflammatory agents through their inhibition on different inflammatory mediators, cytokines and enzymes, such as IL-6, IL-10, tumour necrosis factor-alpha (TNF-α), monocyte chemoattractant protein-1 (MCP-1), nitric oxide (NO), lipo-oxygenase (LOX) and cyclooxygenase (COX) (17, 20-23). Leong et al. (22) synthesised a series of 97 diarylpentanoid analogues and subsequently screened the compounds for anti-inflammatory potential using RAW264.7 macrophages challenged with both interferon gamma (IFN-γ) and LPS. More than half of the diarylpentanoids significantly inhibited NO production with 5-(3,4-dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl)penta-2,4-dien-1-one (DHHPD) (Fig. 1A) as one of the compounds with the highest inhibition (98.9 ± 1.6%), comparable to that of curcumin (99.3 ± 0.2%). The NO suppression activity was attributed to the catechol or 3,4-dihydroxyl moiety of DHHPD, which is a polyphenolic diarylpentenedione analogue (22). Furthermore, DHHPD was found to exhibit both central and peripheral anti-nociceptive activities in animal models of nociception, which might be partly due to attenuated production of inflammatory mediators (24). In another series of 24 diarylpentanoid analogues, 2-benzoyl-6-(3,4-dihydroxybenzylidene)cyclohexen-1-ol (BDHBC) (Fig. 1B) was found to be the most effective compound in suppressing NO production by RAW264.7 macrophages induced with both IFN-γ and LPS with an IC_{50} value of 4.2 ± 0.2μM, which was much lower than that of curcumin (14.7 ± 0.2μM). Most importantly, BDHBC demonstrated higher water solubility and stability compared to curcumin. Similar to DHHPD, the 3,4-dihydroxylphenyl ring was the crucial determinant of the bioactivity of BDHBC (23).

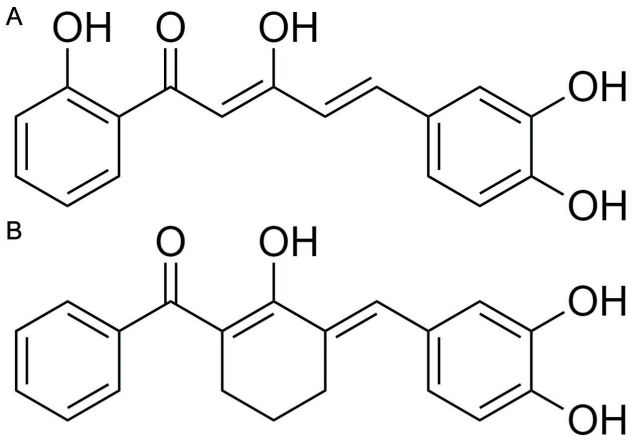

Figure 1: Chemical structure of (A) DHHPD and (B) BDHBC. (A) 5-(3,4-dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl) penta-2,4-dien-1-one (DHHPD) and (B) 2-benzoyl-6-(3,4-dihydroxybenzylidene)cyclohexen-1-ol (BDHBC).

Despite numerous findings proving prominent anti-inflammatory nature of diarylpentanoids, especially DHHPD and BDHBC, there has not been any study on these two diarylpentanoid analogues which explores their therapeutic potential in airway inflammation. Thus, this study aims to determine anti-inflammatory activities of DHHPD and BDHBC, particularly by assessing their effects on the gene expression of IL-6 and IL-8, which are key inflammatory cytokines released during airway inflammation.

MATERIALS AND METHODS

Reagents

Lipopolysaccharide (O111:B4) was purchased from Sigma Aldrich (St. Louis, MO, USA). Dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were obtained from Amresco (Solon, OH, USA). RNeasy Plus Mini Kit

and OneStep RT-PCR Kit were obtained from Qiagen (Valencia, CA, USA).

Synthesis of DHHPD and BDHBC

Both diarylpentanoid analogues, DHHPD (Fig. 1A) and BDHBC (Fig. 1B), used in this study were synthesised at Institute of Bioscience, University Putra Malaysia. DHHPD and BDHBC were synthesised as previously described (22, 23).

Cell Culture

Calu-3, a human airway epithelial cell line derived from lung adenocarcinoma, was purchased from American Type Culture Collection (ATCC). Calu-3 was propagated in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS), 1% sodium pyruvate, 100U/mL penicillin and 100µg/mL streptomycin at 37°C in humidified incubator with 5% carbon dioxide. Cell culture medium was replaced every two days to ensure optimal growth condition for the cells. In this study, only Calu-3 cells between passage 15 to passage 25 were used. The selected range was within the passage range routinely used for this cell line, which was between 15 to 42 (25-27).

Cytotoxicity Assay

The cytotoxicity of both DHHPD and BDHBC on Calu-3 cells was determined using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) as described previously with slight modifications (20). Firstly, Calu-3 cells were seeded onto a 96-well plate (2.0×10^4 cells/well) in 100µL of complete EMEM medium. On the next day, the cells were treated with DHHPD or BDHBC, ranging from 10µM to 200µM, for 24 hours. After that, the medium with/without treatment was replaced with fresh EMEM medium and 20µL MTT solution (5mg/mL) was added into each well. After incubation in the dark for another 3 hours, the MTT solution was removed and the formazan crystals formed in each well were dissolved with 50µL DMSO. The amount of formazan crystals was quantified by determining the absorbance at 570nm with Tecan microplate reader (Mannedorf, Zurich, Switzerland).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

IL-6 and IL-8 gene expression levels of Calu-3 cells were determined as described previously with slight modifications (20). Calu-3 cells were seeded onto a 6-well plate (8.0×10^5 cells/well) in 2mL of complete EMEM medium. Upon reaching 100% confluence, the cells were concurrently treated with DHHPD or BDHBC (6.25, 12.5, 25 and 50µM) and induced with LPS (10µg/mL) for 24 hours. Dexamethasone (10µM) served as a positive drug control in this study. After 24 hours, total RNA was extracted from the cells using RNeasy Plus Mini Kit according to the manufacturer's instructions. The purity and concentration of RNA were assessed using Implen NanoPhotometer (Schatzbogen, Munich, Germany) and 100ng total RNA were used as the initial template for reverse transcription. RT-PCR was done using Qiagen OneStep RT-PCR Kit under the following conditions: reverse transcription at 50°C for 30 min, initial PCR activation at 95°C for 15 min, 30 cycles of denaturation (94°C for 1 min), annealing (60°C, 45 sec for IL-6 or 55°C, 45 sec for IL-8) and elongation (72°C for 1 min), followed by final extension at 72°C for 10 min. The primers used in this study were purchased from Integrated DNA Technologies (Coralville, IA, USA) and the sequences were as follows: IL-6, forward 5′-TGTAGCCGCCCCACACAGACAGCC-3′ and reverse 5′-GGCAAGTCTCCTCATTGAATCCAGAGATTG-3′; IL-8, forward 5′-ATGACTTCCAAGCTGGCCGTGGCT-3′ and reverse 5′-TCTCAGCCCTCTTCAAAAACTTCTC-3′; and GAPDH, forward 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ and reverse 5′-CATGTGGGCCATGAGGTCCACCAC-3′. Upon completion, separation of the RT-PCR products was carried out using a 2% agarose gel containing ethidium bromide (0.5µg/mL). After that, the gel was visualized with Fusion Fx imaging system (Vilber Lourmat) under UV light. The intensities of the immunoblots were determined using ImageJ software and normalized to that of glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical Analysis

All values were presented as mean ± standard error of mean (SEM) of three separate experiments. GraphPad Prism 7.0 was used as the tool to perform statistical analyses. Data from different experimental groups were compared using one-way analysis of variance (ANOVA), followed by post hoc Dunnett's test. Statistical significance was set at $p < 0.05$.

RESULTS

Effect of DHHPD and BDHBC on Calu-3 cell viability

The cytotoxicity of both diarylpentanoid analogues, DHHPD and BDHBC, on Calu-3 cells was determined using MTT cytotoxicity assay. As shown in Fig. 2, both DHHPD and BDHBC have no significant effect on Calu-3 cell viability for all tested concentrations. Four non-cytotoxic concentrations of DHHPD and BDHBC – 6.25, 12.5, 25 and 50µM were chosen to be used in the subsequent assay.

Effect of DHHPD and BDHBC on IL-6 mRNA expression in LPS-induced Calu-3 cells

The effect of both diarylpentanoid analogues, DHHPD and BDHBC, on IL-6 and IL-8 gene expression was examined using RT-PCR. In comparison to Calu-3 cells without LPS induction, non-treated LPS-induced Calu-3 cells showed more than two-fold increase of IL-6 mRNA expression (Fig. 3A and 3B). However, Calu-3 cells concurrently treated with DHHPD and induced with LPS showed significant reduction in IL-6 mRNA expression levels at all selected concentrations of

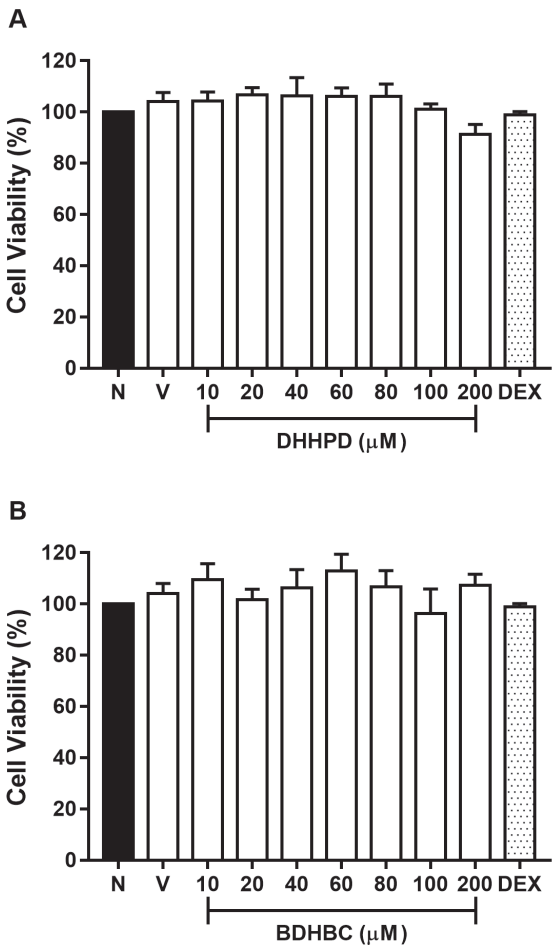

Figure 2: Effect of (A) DHHPD and (B) BDHBC on Calu-3 cell viability. Both (A) DHHPD and (B) BDHBC were not cytotoxic to Calu-3 cells at the range of concentrations used (10μM to 200μM). Data are expressed as mean ± standard error of mean (SEM) (n = 3). Cell viabilities are presented as percentage of normal control (N). N: Calu-3 cells without any treatment; V: Vehicle control with 0.1% DMSO; DEX: Dexamethasone (10μM) as a positive drug control.

DHHPD. At 6.25μM, DHHPD attenuated LPS-induced IL-6 mRNA expression by almost 50%, whereas at 12.5, 25 and 50μM approximately 70 – 80% inhibition was observed (Fig. 3A). Similar finding was observed in Calu-3 cells concurrently treated with BDHBC and induced with LPS. BDHBC at 6.25μM significantly reduced IL-6 mRNA expression level by more than 50% compared to LPS-stimulated but non-treated Calu-3 cells. On the other hand, the other three concentrations of BDHBC (12.5, 25 and 50μM) significantly reduced LPS-induced IL-6 mRNA expression by approximately 70% (Fig. 3B). Dexamethasone, a corticosteroid commonly prescribed for inflammatory airway diseases, also significantly suppressed IL-6 mRNA expression by about 75 – 85% (Fig. 3A and Fig. 3B). Thus, these indicated that both DHHPD and BDHBC were able to counteract the effect of LPS in airway inflammation by inhibiting IL-6 gene expression in Calu-3 airway epithelial cells.

Effect of DHHPD and BDHBC on IL-8 mRNA expression in LPS-induced Calu-3 cells

Similar to IL-6, mRNA expression levels of IL-8 were significantly increased in LPS-induced Calu-3 cells (Fig.

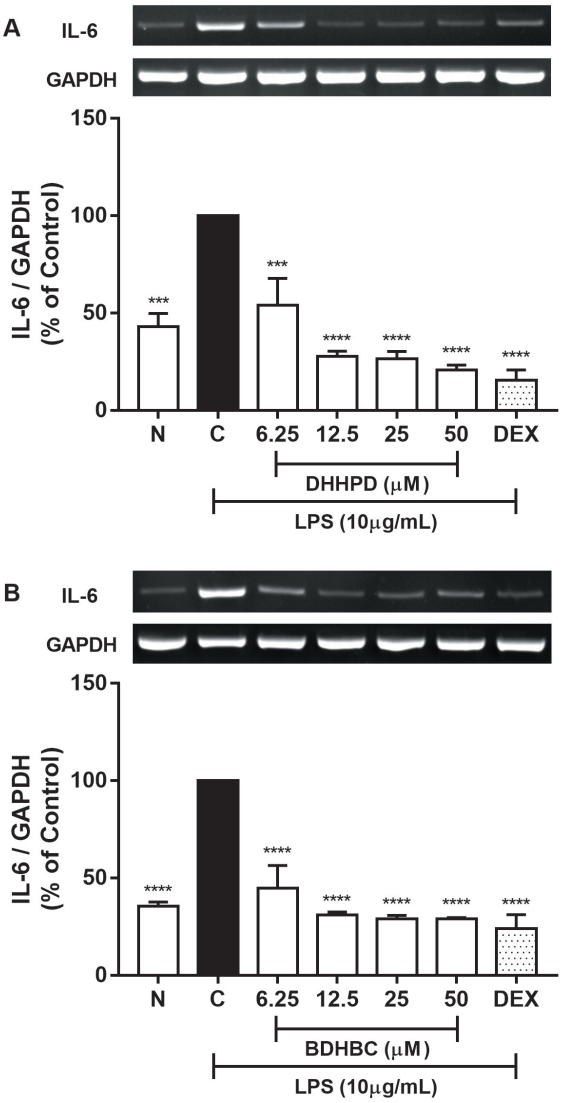

Figure 3: Effect of (A) DHHPD and (B) BDHBC on IL-6 mRNA expression in LPS-induced Calu-3 cells. Both (A) DHHPD and (B) BDHBC significantly inhibited IL-6 mRNA expression in LPS-induced Calu-3 cells at 6.25, 12.5, 25 and 50μM. Data are presented as mean ± standard error of mean (SEM) (n = 3). ***p < 0.001 and ****p < 0.0001 represent significant difference compared to LPS-induced Calu-3 cells without any treatment (C: black bar). N: Calu-3 cells without LPS induction and treatment; DEX: Dexamethasone (10μM) as a positive drug control.

4A and 4B). DHHPD was able to significantly decrease LPS-induced IL-8 mRNA expression at 12.5 and 25μM, both with approximately 40% inhibition. However, both the lowest and the highest concentration of DHHPD used, which were 6.25μM and 50μM respectively, were not effective in inhibiting the increase in IL-8 mRNA expression levels (Fig. 4A). On the other hand, BDHBC significantly suppressed IL-8 mRNA expression in LPS-induced Calu-3 cells at 12.5, 25 and 50μM, with about 28, 40 and 45% inhibition, respectively. The inhibitory effect on IL-8 mRNA expression increased when the concentration of BDHBC was higher. However, BDHBC at the lowest concentration (6.25μM) gave an insignificant inhibition of less than 10% (Fig. 4B). The inhibition on LPS-induced IL-8 mRNA expression due to dexamethasone was approximately 35% (Fig. 4A and 4B).

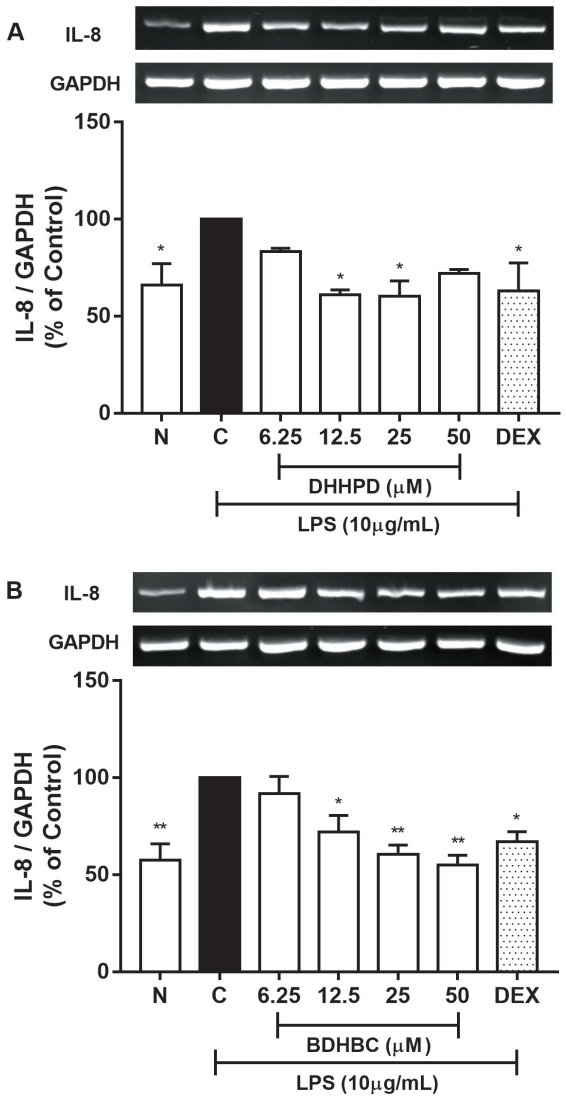

Figure 4: Effect of (A) DHHPD and (B) BDHBC on IL-8 mRNA expression in LPS-induced Calu-3 cells. (A) DHHPD significantly inhibited IL-8 mRNA expression in LPS-induced Calu-3 cells at 12.5 and 25μM. (B) BDHBC significantly inhibited IL-8 mRNA expression in LPS-induced Calu-3 cells at 12.5, 25 and 50μM. Data are presented as mean ± standard error of mean (SEM) (n = 3). *p < 0.05 and **p < 0.01 represent significant difference compared to LPS-induced Calu-3 cells without any treatment (C: black bar). N: Calu-3 cells without LPS induction and treatment; DEX: Dexamethasone (10μM) as a positive drug control

DISCUSSION

Cytokines are the key players that orchestrate diverse inflammatory responses. During chronic airway inflammation, various inflammatory cytokines/chemokines are highly secreted by immune cells and bronchial epithelium, contributing to the disease severity of asthma and COPD. Thus, targeting pro-inflammatory cytokines, such as IL-6 and IL-8, may represent a potential therapeutic avenue for the treatment of these chronic inflammatory airway diseases (4).

IL-6 is one of the cytokines which is of clinical importance in chronic inflammatory airway diseases, especially asthma and COPD. Neveu et al. (28) suggested that IL-6 is an indicator of disease severity and progression of asthma, rather than just a pro-inflammatory marker, as the levels of IL-6 were increased in the sputum of patients with mild to moderate allergic asthma without active inflammation. In the same study, IL-6 levels were found to be positively correlated with that of IL-13, a cytokine responsible for stimulating mucous production by airway epithelial cells, subepithelial fibrosis and other pathological changes of asthma (28-30). Moreover, IL-6, together with IL-8, were shown to be determinants of the severity of COPD as increased levels of IL-6 and IL-8 were identified in COPD patients with marked obstruction of airways (31). Recently, Liu et al. (32) also revealed that higher IL-8 levels were seen in patients with COPD exacerbations compared with asthma attacks, which was in line with the previous finding by Nocker et al. (33) that the increase in IL-8 was more pronounced in COPD patients in comparison to patients with asthma. Based on the results of our current study, it has been shown that BDHBC is effective in inhibiting the expression of IL-6 and IL-8 at mRNA level in human airway epithelium, Calu-3 cells. With the aforementioned clinical relevance of both cytokines in asthma and COPD, inhibitory effects of BDHBC on IL-6 and IL-8 gene expression justified the need for further study on BDHBC in airway inflammation.

On the other hand, DHHPD significantly inhibited IL-6 mRNA expression at all concentrations. However, only 12.5 and 25μM of DHHPD were able to significantly decrease LPS-induced IL-8 mRNA expression levels. It was surprising to note that DHHPD, at the highest concentration – 50μM, was not able to significantly suppress IL-8 mRNA expression. This unusual trend is similar to a biphasic dose response. This phenomenon, also known as hormesis, occurs when a drug or compound does not exhibit a classical linear or threshold dose-response relationship. In simple words, the drug elicits desirable effects at a low dose, but harmful effects at a high dose, even it is not toxic, resulting in a U-shape or an inverted U-shape dose-response curve (34). At 50μM, DHHPD was proven to be non-cytotoxic on Calu-3 cells in MTT assay (Fig. 2A), thus, suggesting that the effect of DHHPD on IL-8 mRNA expression may follow a biphasic response.

Interestingly, curcumin has been suggested as one of the dietary phytochemicals that exhibit hormetic or biphasic mode of action. It is well-known that curcumin has health-promoting benefits, which were later found to be partly attributed to the activation of adaptive cellular stress response pathways. Mild stress activates different signalling pathways and triggers intrinsic changes within the cells, conferring resistance to more severe and detrimental stress responses. However, the protection is lost when the dosage is beyond tolerable level (32). Since DHHPD was derived from the structure of curcumin, there is possibility that DHHPD may share similarity with curcumin, to a certain extent, in terms of its mechanism of action. But it should also be noted

that the claim on the biphasic response of curcumin was made on the basis of its neuroprotective effects (35) and may not apply to other conditions. Nonetheless, further testing of the effect of DHHPD on IL-8 mRNA expression at higher concentrations may give a clearer picture on its pharmacological activity.

Previously, there have been quite a number of studies testing the effectiveness of curcumin against airway inflammation (36-40). In particular, an in vitro study by Lin et al. (36) elucidated that curcumin inhibited LPS-induced inflammatory responses and mucous secretion in human bronchial epithelial (NCI-H292) cells through activation of nuclear factor erythroid 2-related factor 2 (Nrf-2). The inhibitory effect of curcumin on LPS-induced IL-6 and IL-8 expression has also been reported in a few in vitro studies using different cell lines. Jin et al. (41) revealed that curcumin was able to suppress IL-6 expression at mRNA levels in LPS-induced BV2 microgial cells through inhibition of nuclear factor-κB (NF-κB) pathway. Jeong and Yun (42) also found that curcumin was capable of inhibiting LPS-induced neutrophils activation, including IL-6 and IL-8 protein levels, by attenuating the activation of p38 mitogen activated protein kinases (p38), c-Jun amino-terminal kinases (JNK) and NF-κB. In another more recent study by Ma et al. (43), it has been shown that curcumin suppressed LPS-induced IL-6 mRNA expression in RAW264.7 macrophages by decreasing microRNA-155 (miR-155) expression through phosphatidylinositol 3-kinase (PI3K)/ Protein kinase B (Akt) pathway. These findings may give some hints on how the curcumin analogues could also possibly work to reverse the effect of LPS. Curcumin is not a clinically approved drug for any inflammatory airway diseases. Furthermore, although it has shown potential effect in reversing airway inflammation, its effect on LPS-induced inflammatory responses in Calu-3 human airway epithelial cells, particularly on IL-6 and IL-8 expression, has not been previously investigated. Thus, a currently prescribed medication for airway inflammation – dexamethasone, was used as a positive drug control.

Notably, the inhibition on LPS-induced IL-6 mRNA levels was stronger compared to that of IL-8 for both DHHPD and BDHBC. As indicated by the findings of Liu et al. (44), LPS-induced expression of pro-inflammatory cytokines, including IL-6 and IL-8, in human airway epithelial cells is mediated by nuclear factor-κB (NF-κB) and activator protein-1 (AP-1). Even so, either pathway may be more dominant in regulating LPS-induced IL-6 or IL-8 gene expression. Sawa et al. (45) reported that inhibition of NF-κB resulted in greater reduction in IL-6 levels compared to that of IL-8 in LPS-induced human lymphatic endothelial cells, indicating that NF-κB pathway plays a more critical role in IL-6 expression. This may be the same in LPS-induced human airway epithelial cells. Thus, the target signalling molecule of the diarylpentanoid analogues, once identified, will give better explanation on why they exerted stronger inhibition on IL-6 mRNA expression. This is beyond the scope of our current study but should be the subject of further investigation.

This study serves to provide preliminary findings on the therapeutic potential of these two diarylpentanoid analogues in airway inflammation. At this point, both DHHPD and BDHBC seem to target pro-inflammatory cytokines, which are IL-6 and IL-8. There are other cytokines which are also implicated in airway inflammation, such as IL-1β and tumour necrosis factor (TNF)-α. The effect of the two diarylpentanoid analogues on the expression of these cytokines, at both mRNA and protein levels, should be assessed as well. This is because mRNA levels do not always correlate well to protein levels due to variations in cell cycle and delay in the translation process (46). As such, measurement of protein abundances of the cytokines could be done using Western blot or enzyme-linked immunosorbant assay (ELISA) to confirm their effect on cytokine production. Nevertheless, BDHBC and DHHPD deserve seats as candidate compounds worth to be further investigated to validate their therapeutic benefits in airway inflammation.

CONCLUSION

In conclusion, both diarylpentanoid analogues, DHHPD and BDHBC, are proven to be effective in inhibiting the gene expression of IL-6 and IL-8 in LPS-induced airway inflammation. Since this is a preliminary study to give insights on the therapeutic potential of diarylpentanoid analogues in airway inflammation, further investigations are needed to elucidate their mechanisms of action in regulating pro-inflammatory cytokines, such as IL-6 and IL-8, or other key cytokines - IL-1β and TNF-α, in order for them to be developed as potential therapeutic drug for asthma and COPD.

ACKNOWLEDGEMENTS

The authors would like to thank the staffs of Cell Signalling Lab, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for providing all the required technical support for this study. This work was supported by Fundamental Research Grant Scheme (FRGS/1/2018/SKK10/UPM/02/2) under Ministry of Education Malaysia. The authors also want to acknowledge Miss Audrey Kow Siew Foong for her kind contribution in proofreading the manuscript.

REFERENCES

1. Larsen GL, Holt PG. The concept of airway inflammation. American journal of respiratory and critical care medicine. 2000 Aug 1;162(supplement_1):S2-6.
2. Eutamene H, Theodorou V, Schmidlin F, Tondereau V, Garcia-Villar R, Salvador-Cartier C, et al. LPS-

induced lung inflammation is linked to increased epithelial permeability: role of MLCK. European Respiratory Journal. 2005 May 1;25(5):789-96.
3. O'byrne PM, Postma DS. The many faces of airway inflammation: asthma and chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1999 May 1;159(supplement_2):S1-63.
4. Garth J, Barnes J, Krick S. Targeting Cytokines as Evolving Treatment Strategies in Chronic Inflammatory Airway Diseases. International journal of molecular sciences. 2018 Oct 30;19(11):3402.
5. Gohy ST, Hupin C, Pilette C, Ladjemi MZ. Chronic inflammatory airway diseases: the central role of the epithelium revisited. Clinical & Experimental Allergy. 2016 Apr;46(4):529-42.
6. Rincon M, Irvin CG. Role of IL-6 in asthma and other inflammatory pulmonary diseases. International journal of biological sciences. 2012;8(9):1281.
7. Oishi K, Sonoda F, Kobayashi S, Iwagaki A, Nagatake T, Matsushima K, et al. Role of interleukin-8 (IL-8) and an inhibitory effect of erythromycin on IL-8 release in the airways of patients with chronic airway diseases. Infection and immunity. 1994 Oct 1;62(10):4145-52.
8. Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. Clinical immunology. 2009 Jan 1;130(1):27-33.
9. Neveu WA, Allard JB, Dienz O, Wargo MJ, Ciliberto G, Whittaker LA, et al. IL-6 is required for airway mucus production induced by inhaled fungal allergens. The Journal of Immunology. 2009 Jan 1:jimmunol-0802923.
10. He JQ, Foreman MG, Shumansky K, Zhang X, Akhabir L, Sin DD, et al. Associations of IL6 polymorphisms with lung function decline and COPD. Thorax. 2009 Apr 8.
11. Huang AX, Lu LW, Liu WJ, Huang M. Plasma inflammatory cytokine IL-4, IL-8, IL-10, and TNF-α levels correlate with pulmonary function in patients with asthma-chronic obstructive pulmonary disease (COPD) overlap syndrome. Medical science monitor: international medical journal of experimental and clinical research. 2016;22:2800.
12. Horita N, Goto A, Shibata Y, Ota E, Nakashima K, Nagai K, et al. Long-acting muscarinic antagonist (LAMA) plus long-acting beta-agonist (LABA) versus LABA plus inhaled corticosteroid (ICS) for stable chronic obstructive pulmonary disease (COPD). Cochrane Database of Systematic Reviews. 2017(2).
13. Gross NJ, Barnes PJ. New therapies for asthma and chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2017 Jan 15;195(2):159-66.
14. Vyas A, Dandawate P, Padhye S, Ahmad A, Sarkar F. Perspectives on new synthetic curcumin analogs and their potential anticancer properties. Current pharmaceutical design. 2013 Apr 1;19(11):2047-69.
15. Schneider C, Gordon ON, Edwards RL, Luis PB. Degradation of curcumin: from mechanism to biological implications. Journal of agricultural and food chemistry. 2015 Apr 2;63(35):7606-14.
16. Liang G, Li X, Chen L, Yang S, Wu X, Studer E, et al. Synthesis and anti-inflammatory activities of mono-carbonyl analogues of curcumin. Bioorganic & medicinal chemistry letters. 2008 Feb 15;18(4):1525-9.
17. Lee KH, Aziz FH, Syahida A, Abas F, Shaari K, Israf DA, et al. Synthesis and biological evaluation of curcumin-like diarylpentanoid analogues for anti-inflammatory, antioxidant and anti-tyrosinase activities. European journal of medicinal chemistry. 2009 Aug 31;44(8):3195-200.
18. Bukhari SN, Jantan IB, Jasamai M, Ahmad W, Amjad MW. Synthesis and biological evaluation of curcumin analogues. J. Med. Sci. 2013 Oct 1;13(7):501-13.
19. Zhang Y, Zhao L, Wu J, Jiang X, Dong L, Xu F, et al. Synthesis and evaluation of a series of novel asymmetrical curcumin analogs for the treatment of inflammation. Molecules. 2014 Jun 4;19(6):7287-307.
20. Tham CL, Liew CY, Lam KW, Mohamad AS, Kim MK, Cheah YK, et al. A synthetic curcuminoid derivative inhibits nitric oxide and proinflammatory cytokine synthesis. European journal of pharmacology. 2010 Feb 25;628(1-3):247-54.
21. Ahmad W, Kumolosasi E, Jantan I, Bukhari SN, Jasamai M. Effects of novel diarylpentanoid analogues of curcumin on secretory phospholipase A2, cyclooxygenases, lipo-oxygenase, and microsomal prostaglandin E synthase-1. Chemical biology & drug design. 2014 Jun;83(6):670-81.
22. Leong SW, Faudzi SM, Abas F, Aluwi MF, Rullah K, Wai LK, et al. Synthesis and sar study of diarylpentanoid analogues as new anti-inflammatory agents. Molecules. 2014 Oct 9;19(10):16058-81.
23. Leong SW, Faudzi SM, Abas F, Aluwi MF, Rullah K, Lam KW, et al. Nitric oxide inhibitory activity and antioxidant evaluations of 2-benzoyl-6-benzylidenecyclohexanone analogs, a novel series of curcuminoid and diarylpentanoid derivatives. Bioorganic & medicinal chemistry letters. 2015 Aug 15;25(16):3330-7.
24. Kamarudin N, Hisamuddin N, Ong H, Ahmad Azmi A, Leong S, Abas F, et al. Analgesic Effect of 5-(3, 4-Dihydroxyphenyl)-3-hydroxy-1-(2-hydroxyphenyl) penta-2, 4-dien-1-one in Experimental Animal Models of Nociception. Molecules. 2018 Aug 21;23(9):2099.
25. Haghi M, Young PM, Traini D, Jaiswal R, Gong J, Bebawy M. Time-and passage-dependent characteristics of a Calu-3 respiratory epithelial cell model. Drug development and industrial

pharmacy. 2010 Oct 1;36(10):1207-14.
26. Kreft ME, Jerman UD, Lasič E, Hevir-Kene N, Rižner TL, Peternel L, Kristan K. The characterization of the human cell line Calu-3 under different culture conditions and its use as an optimized in vitro model to investigate bronchial epithelial function. European Journal of Pharmaceutical Sciences. 2015 Mar 10;69:1-9.
27. Zemans RL, Briones N, Campbell M, McClendon J, Young SK, Suzuki T, Yang IV, De Langhe S, Reynolds SD, Mason RJ, Kahn M. Neutrophil transmigration triggers repair of the lung epithelium via β-catenin signaling. Proceedings of the National Academy of Sciences. 2011 Sep 20;108(38):15990-5.
28. Neveu WA, Allard JL, Raymond DM, Bourassa LM, Burns SM, Bunn JY, et al. Elevation of IL-6 in the allergic asthmatic airway is independent of inflammation but associates with loss of central airway function. Respiratory research. 2010 Dec;11(1):28.
29. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. The Journal of clinical investigation. 1999 Mar 15;103(6):779-88.
30. Whittaker L, Niu N, Temann UA, Stoddard A, Flavell RA, Ray A, et al. Interleukin-13 mediates a fundamental pathway for airway epithelial mucus induced by CD4 T cells and interleukin-9. American journal of respiratory cell and molecular biology. 2002 Nov;27(5):593-602.
31. Hacievliyagil SS, Gunen H, Mutlu LC, Karabulut AB, Temel İ. Association between cytokines in induced sputum and severity of chronic obstructive pulmonary disease. Respiratory medicine. 2006 May 1;100(5):846-54.
32. Liu HC, Lu MC, Lin YC, Wu TC, Hsu JY, Jan MS, et al. Differences in IL-8 in serum and exhaled breath condensate from patients with exacerbated COPD or asthma attacks. Journal of the Formosan Medical Association. 2014 Dec 1;113(12):908-14.
33. Nocker RE, Schoonbrood DF, van de Graaf EA, Hack E, Lutter R, Jansen HM, et al. Interleukin-8 in airway inflammation in patients with asthma and chronic obstructive pulmonary disease. International archives of allergy and immunology. 1996;109(2):183-91.
34. Bhakta-Guha D, Efferth T. Hormesis: decoding two sides of the same coin. Pharmaceuticals. 2015;8(4):865-83.
35. Son TG, Camandola S, Mattson MP. Hormetic dietary phytochemicals. Neuromolecular medicine. 2008 Dec 1;10(4):236.
36. Lin XP, Xue C, Zhang JM, Wu WJ, Chen XY, Zeng YM. Curcumin Inhibits Lipopolysaccharide-Induced Mucin 5AC Hypersecretion and Airway Inflammation via Nuclear Factor Erythroid 2-Related Factor 2. Chinese medical journal. 2018 Jul 20;131(14):1686.
37. Moghaddam SJ, Barta P, Mirabolfathinejad SG, Ammar-Aouchiche Z, Garza NT, Vo TT, et al. Curcumin inhibits COPD-like airway inflammation and lung cancer progression in mice. Carcinogenesis. 2009 Sep 30;30(11):1949-56.
38. Chong L, Zhang W, Nie Y, Yu G, Liu L, Lin L, et al. Protective effect of curcumin on acute airway inflammation of allergic asthma in mice through Notch1–GATA3 signaling pathway. Inflammation. 2014 Oct 1;37(5):1476-85.
39. Yuan J, Liu R, Ma Y, Zhang Z, Xie Z. Curcumin Attenuates Airway Inflammation and Airway Remolding by Inhibiting NF-κB Signaling and COX-2 in Cigarette Smoke-Induced COPD Mice. Inflammation. 2018 Oct 1;41(5):1804-14.
40. Abidi A, Gupta S, Agarwal M, Bhalla HL, Saluja M. Evaluation of efficacy of curcumin as an add-on therapy in patients of bronchial asthma. Journal of clinical and diagnostic research: JCDR. 2014 Aug;8(8):HC19.
41. Jin CY, Lee JD, Park C, Choi YH, Kim GY. Curcumin attenuates the release of pro-inflammatory cytokines in lipopolysaccharide-stimulated BV2 microglia 1. Acta Pharmacologica Sinica. 2007 Oct;28(10):1645-51.
42. Jeong H, Yun C. Effect of curcumin on LPS-induced neutrophil activation and acute lung injury. European Respiratory Society Annual Congress 2012. Available from: https://erj.ersjournals.com/content/erj/40/Suppl_56/P4635.full.pdf
43. Ma F, Liu F, Ding L, You M, Yue H, Zhou Y, et al. Anti-inflammatory effects of curcumin are associated with down regulating microRNA-155 in LPS-treated macrophages and mice. Pharmaceutical biology. 2017 Jan 1;55(1):1263-73.
44. Liu X, Yin S, Chen Y, Wu Y, Zheng W, Dong H, et al. LPS induced proinflammatory cytokine expression in human airway epithelial cells and macrophages via NF κB, STAT3 or AP 1 activation. Molecular medicine reports. 2018 Apr 1;17(4):5484-91.
45. Sawa Y, Ueki T, Hata M, Iwasawa K, Tsuruga E, Kojima H, et al. LPS-induced IL-6, IL-8, VCAM-1, and ICAM-1 expression in human lymphatic endothelium. Journal of Histochemistry & Cytochemistry. 2008 Feb;56(2):97-109.
46. Liu Y, Beyer A, Aebersold R. On the dependency of cellular protein levels on mRNA abundance. Cell. 2016 Apr 21;165(3):535-50.