Free Radical Biology and Medicine 131 (2019) 309–317

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Original article

Impact of dibenzocyclooctadiene lignans from *Schisandra chinensis* on the redox status and activation of human innate immune system cells

Maarit Kortesoja[a,1], Elina Karhu[a,1], Elin Soffia Olafsdottir[b], Jona Freysdottir[c], Leena Hanski[a,*]

[a] Drug Research Program, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, P.O. Box 56, FI-00014 University of Helsinki, Finland
[b] Faculty of Pharmaceutical Sciences, University of Iceland, Reykjavik, Hofsvallagata 53, 107 Reykjavik, Iceland
[c] Department of Immunology and Center for Rheumatology Research, Landspitali-The National University Hospital of Iceland and Faculty of Medicine, University of Iceland, Eiriksgata, 101 Reykjavik, Iceland

A B S T R A C T

Redox signaling has been established as an essential component of inflammatory responses, and redox active compounds are of interest as potential immunomodulatory agents. Dibenzocyclooctadiene lignans isolated from *Schisandra chinensis*, a medicinal plant with widespread use in oriental medicine, have been implicated to possess immunomodulatory properties but their effects on the human innate immune system cells have not been described. In this contribution, data are presented on the impact of schisandrin, schisandrin B and schisandrin C on human monocytic cell redox status, as well as their impact on dendritic cell maturation and T cell activation capacity and cytokine production. In THP-1 cells, levels of intracellular reactive oxygen species (ROS) were elevated after 1 h exposure to schisandrin. Schisandrin B and schisandrin C decreased cellular glutathione pools, which is a phenotype previously reported to promote anti-inflammatory functions. Treatment of human primary monocytes with the lignans during their maturation to dendritic cells did not have any effect on the appearance of surface markers HLA-DR and CD86 but schisandrin B and schisandrin C suppressed the secretion of cytokines interleukin (IL)-6, IL-10 and IL-12 by the mature dendritic cells. Dendritic cells maturated in presence of schisandrin C were further cocultured with naïve CD4 + T cells, resulting in reduced IL-12 production. In THP-1 cells, schisandrin B and schisandrin C reduced the IL-6 and IL-12 production triggered by *E. coli* lipopolysaccharide and IL-12 production induced by an infection with *Chlamydia pneumoniae*. In conclusion, the studied lignans act as immunomodulatory agents by altering the cytokine secretion, but do not interfere with dendritic cell maturation. And the observed effects may be associated with the ability of the lignans to alter cellular redox status.

1. Introduction

Schisandra chinensis represents one of the oldest medicinal plants used in traditional Chinese and other East-Asian medicine [1]. During the past few decades, the berry extract of this herb has become more popular also in the western world. Traditionally the berry extracts of *S. chinensis* are considered as tonic and liver protective agents and they are currently often referred as adaptogens [1–3]. Dibenzocyclooctadiene lignans are the dominant secondary metabolite components in the berries and various biological activities have been associated with these compounds [3,4]. Schisandrin and schisandrin B are the most abundant lignan constituents in the berries and, therefore, the most studied ones as well.

The biological and pharmacological activities associated with the *S. chinensis* lignans include neuro-, cardio- and hepatoprotective effects, as well as anti-cancer activities [5–8]. As illustrative examples, Lee and colleagues demonstrated that schisandrin B inhibits cortical neuronal damage in a rat stroke model [6]. Schisandrin B has also been reported to protect murine brain against beta amyloid toxicity [9] and to protect liver against chemically induced toxicity [8,10,11]. Regarding anti-cancer properties, schisandrin B has been shown to inhibit the lung and bone metastases in BALB/c mice inoculated with breast cancer 4T1 cells, by inhibiting the epithelial-mesenchymal transition (EMT) [7]. Another S. *chinensis* metabolite, schisandrin C, induces cell cycle arrest and apoptosis in human leukemia U937 cells [12]. Furthermore, schisandrin lignans may reverse P-glycoprotein mediated drug resistance of several multidrug resistant cell lines [13].

To date, the anti-inflammatory properties of the *S. chinensis* lignans have mostly been studied in murine cell lines and animal models stimulated by bacterial lipopolysaccharides (LPS) [14–17]. In these

Abbreviations: DC, dendritic cells; DMSO, dimethyl sulfoxide; GSH, glutathione; iDC, immature dendritic cell; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; mDC, mature dendritic cell; NF-κB, nuclear factor κB; NO, nitric oxide; Nrf2, nuclear erythroud-derived factor 2; ROS, reactive oxygen species; TNF, tumor necrosis factor

* Corresponding author.
E-mail address: leena.hanski@helsinki.fi (L. Hanski).
[1] MK and EK contributed equally to the manuscript.

https://doi.org/10.1016/j.freeradbiomed.2018.12.019
Received 9 October 2018; Received in revised form 5 December 2018; Accepted 17 December 2018
Available online 19 December 2018

studies, the impact of different lignans on reactive nitrogen species (RNS) and reactive oxygen species (ROS) have been addressed. ROS and RNS are essential mediators and messenger molecules of immune system and gene expression [18,19]. Owing to its direct antimicrobial actions and ability to induce cytokine production, nitric oxide (NO) plays an important role in immunity [17,20]. Despite the physiological role of NO, other RNS and ROS in inflammatory responses, their overproduction may result in tissue injury [18]. *S. chinensis* berry extract has been shown to inhibit LPS-induced NO production and suppress NO synthetase levels in murine macrophages [20]. Schisandrin, schisandrin B and other lignin constituents of the extract have been reported with similar effects both in vitro and in vivo [14,17]. However, while NO production is a prominent and well-established property of monocytic and macrophage cell of rodent origin, NO production by human monocytic cells has been a matter of debate [21–25].

Another previously studied aspect of immunomodulatory effects exhibited by schisandrin lignans is their impact on cytokine secretion [16,17]. Cytokines are small protein molecules that have various functions which regulate the immune reactions of the cells [26]. They are produced by various cell types as a response to endogenous or exogenous stimuli and are able to activate and inhibit cell functions, regulate cell differentiation and act chemotactically in case of tissue injury [27]. In systemic level, the interplay of cytokines acting pro-inflammatory to destroy pathogens by inflammation and anti-inflammatory to prevent unnecessary tissue damage is essential to maintain body homeostasis [28].

Previous studies have implicated that schisandrin B suppresses interleukin (IL)-6 and IL-1β secretion in LPS-treated microglia [29] and IL-2, IL-4 and IL-6 production in murine splenic lymphocytes [16]. Schisandrin B and schisandrin C have also been reported to diminish IL-1β, IL-6 and tumor necrosis factor (TNF)-α production in LPS-stimulated murine macrophage cell line, RAW264,7 [17]. All these studies involve the use of rodent models, and only a few studies have been carried out with human cell models. Lin et al. [30] reported that schisandrin B inhibited the LPS-induced production of IL-8 and TNF-α in human umbilical vein endothelial cells (HUVEC) and increased the expression of transcription factor Nrf2 and blocked the activation of NF -κB. In human monocytic THP-1 cells, suppression of acne-related inflammation by the lignans has recently been reported [31] but otherwise, the impact of *S. chinensis* -derived compounds on human innate immune system cells remain unstudied.

The antioxidant properties of schisandrin lignans have been suggested to mediate their anti-inflammatory responses [14]. In mouse splenic T lymphocytes, schisandrin B has been shown to increase the production of ROS and alter the redox balance in the cells, leading to increase in the redox sensitive transcription factor Nrf2 levels and decrease in activation of pro-inflammatory transcription factor NF -κB [16]. Also, altering cellular glutathione (GSH) homeostasis has been linked to its anti-inflammatory activities. Schisandrin B increased the oxidation level of glutathione (GSH:GSSG ratio) in T lymphocytes [17]. The increase in hepatic GSH levels by schisandrin B has been suggested to mediate its hepatoprotective effect [5] and increase in reduced GSH levels in solar irradiated human fibroblasts has been reported after schisandrin B and schisandrin C treatment [32].

In the present study we examined the effects of schisandin, schisandrin B and schisandrin C (Fig. 1) on human innate immune system cells. Two widely used and well established models were used: monocyte derived dendritic cells (DCs) obtained from healthy donors and THP-1 cell line [33,34]. The effect of the schisandrin, schisandrin B and schisandrin C on monocytic glutathione pools was evaluated. Based on these findings DC maturation was studied by evaluating their expression of surface marker proteins. In addition, the impact of schisandrin C treated DCs on T cell activation was addressed. Cytokine secretion by DCs, T-cells and monocytes was evaluated after stimulation with *E. coli* LPS. As an alternative trigger for the inflammatory state the infection of monocytes by *Chlamydia pneumoniae* was used to gain more comprehensive picture of the anti-inflammatory properties of the lignans.

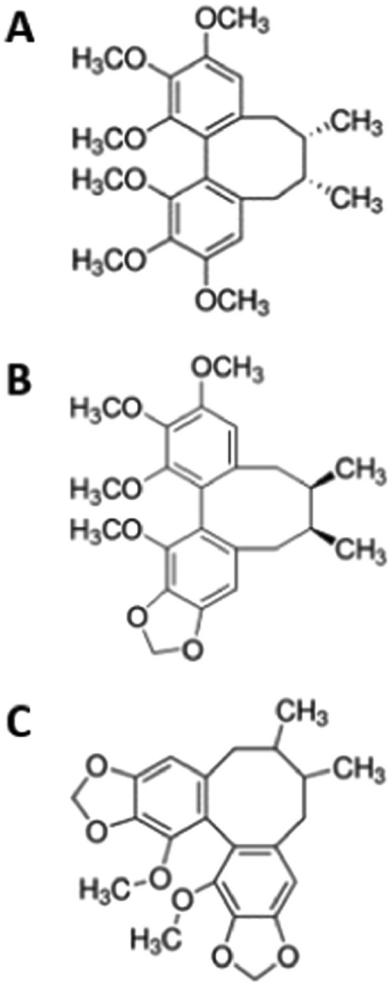

Fig. 1. Chemical structures of dibenzocyclooctadiene lignans a.) schisandrin, b.) schisandrin B and c.) schisandrin C.

2. Materials and methods

2.1. Compounds

Schisandrin was purchased from Sigma-Aldrich (St. Louis, MO, USA). Schisandrin B and schisandrin C were obtained from Fine Tech Industries (London, UK). 20 mM stock solutions of the lignans were prepared in DMSO and diluted in cell culture media at final concentrations indicated for each experiment.

2.2. THP-1 culture

All cell cultures were maintained and incubated during the experiments at 37 °C, 5% CO^2 and 95% air humidity unless otherwise mentioned in the text.

Human monocyte cell line THP-1 (ATCC TIB202) was cultured in RPMI 1640 (Dutch edition) medium (Gibco, Invitrogen, Thermo Fisher Scientific, Paisley, UK) with 10% fetal bovine serum (FBS) (BioWhittaker, Lonza, Basel, Switzerland), 2 nM L-glutamine (BioWhittaker), 0.05 mM 2-mercapthoethanol (Gibco) and 20 μg/ml Gentamicin (Fluka, Buchs, Switzerland).

For cytokine secretion experiments THP-1 cells were seeded in 48-well plate into density at 5×10^5 cells/ml. In experiments stimulated by LPS, 50 U/ml of interferon (IFN)-γ (R&D Systems) were added and

cells were incubated for 3 h. Then, 0.75 μg/ml of *E. coli* LPS (Fluka, Sigma) and schisandrin lignans in final concentration of 25 μM and 50 μM were added. As vehicle control, 0.25% DMSO was applied. In experiments involving *C. pneumoniae* infection the bacterium inoculum (strain K7, prepared as previously described [35]) was added into the wells at multiplicity of infection (MOI) 2 and the plates were centrifuged at 550 × g for 1 h. After consequent 1 h incubation at 37 °C The lignans and vehicle control were added as described above. With either stimulus the cultures were incubated for 48 h and centrifuged at 1100 rpm for 10 min. Supernatants were collected and stored at −80 °C.

2.3. Culture of dendritic cells

DCs were obtained as previously described [36]. In short, peripheral blood mononuclear cells (PBMCs) were obtained by density gradient centrifugation of blood of healthy volunteers using Histopaque® 1077 (Sigma, Münich, Germany). Monocytes expressing CD14 were isolated from peripheral blood mononuclear cells using beads-labeled anti-CD14 antibodies (CD14 MicroBeads) and magnetic cell sorting (MACS) (both from Miltenyi Biotech, Bergisch Gladbach, Germany). The monocytes were cultured at 5×10^5 cells/well in 48-well plates (Nunc, Roskilde, Denmark) in RPMI 1640 medium (Gibco) with 10% FBS (Gibco) and differentiated to immature DCs (imDCs) with GM-CSF (25 ng/well) and IL-4 (12.5 ng/well) (both from R&D Systems). Fresh medium and cytokines were added on day three. On day 7 the cells were harvested and cultured at 1.25×10^5 cells/well in RPMI 1640 medium with 10% FBS. The cells were cultured in the presence of IL-1β (5 ng/well, TNF-α, 25 ng/well (both from R&D Systems) and LPS 250 ng/well (Sigma), which turns the imDCs into mature DCs (mDCs). The schisandrin lignans were added to the wells at 25 and 50 μM. Some wells were left without compounds and served as negative control. For solvent control, 0.25% DMSO was included in some well as vehicle control. Cells were incubated for 48 h. On day 9 the cells were harvested and the maturation evaluated by measuring expression of surface molecules by flow cytometry and secretion of cytokines by ELISA. In some experiments the DCs were used in co-culture experiments.

2.4. Co-culture of mCDs and allogenic CD4+ T cells

In order to investigate the effect of DCs matured in the presence of schisandrin lignans on the activation and differentiation of T cells, mDCs were co-cultured with allogeneic CD4+ T cells. The CD4+ T cells were obtained from the whole blood using the same method as described for CD14 monocytes except using CD4 MicroBeads (Miltenyi). The CD4+ T cells were diluted to a final concentration of 2×10^6 cells/ml. mDCs which had been maturated with 50 μM schisandrin C or vehicle control only were collected on day 9 and resuspended at 2×10^5 cells/ml. The mDCs and T cells were cultured in U-bottomed 96-well plates (Nunc) at 1:10 ratio (2×10^4 DCs/well:2×10^5 T cells/well). The cells were cultured for six days. Then the cells were harvested and the activation and differentiation of the T cells evaluated by measuring expression of surface molecules by flow cytometry and secretion of cytokines by ELISA.

2.5. Measurement of surface marker expression by flow cytometry

The purity of isolated CD4+ T cells and CD14+ monocytes, the differentiation of monocytes into imDCs, maturation of imDCs into mDCs, the effect of schisandrin lignans on DC maturation and the effect of schisandrin C-treated DCs on activation and differentiation of CD4+ T cells was analyzed by flow cytometry. The cells were stained with fluorochrome labeled monoclonal antibodies and incubated on ice for 20 min. The cells were washed with staining buffer and resuspended in 300 μl of 1% paraformaldehyde. Ten thousand cells for each staining were collected with FACScaliber flow cytometer (BD Biosciences, San Jose, CA) and analyzed with CellQuest Pro software (BD Biosciences). The results were given as percentage positive cells or mean fluorescence intensity (MFI). The antibodies used for staining of CD14+ monocytes, imDCs and mDCs were against CD86, HLA-DR and CD14 (all from BD Biosciences). The antibodies for staining of CD4+ T cells were against CD4, CTLA4 and CD69 (all from BD Biosciences). Appropriate isotype control antibodies were used to set gates.

2.6. Cell viability assay

The impact of studied lignans on THP-1 cell viability was evaluated with resazurin cytotoxicity assay. The cells were seeded into 96-well plates at density of 6×10^4 cells/well and the lignans were added at 25 μM and 50 μM. 200 μM usnic acid (Sigma) and 1% Triton X-100 (Sigma) were used as positive controls and 0.25% DMSO as a vehicle control. After 4 h, 24 h and 48 h incubation, resazurin (Sigma, 3 mM stock dissolved in H_2O) was added to yield a final concentration of 20 μM. The cultures were incubated for 2 h and fluorescence was recorded at 570/590 nm with Varioskan Lux plate reader.

2.7. Measurement of cytokines by ELISA

The effect of schisandrin lignans on cytokine secretion by mDCs, T cells and THP-1 cells was measured by sandwich ELISA by using DuoSets kits from R&D Systems, according to the manufacturer's instructions. IL-6, IL-10 and IL-12p40 levels were measured in supernatants from THP-1 cells or mDCs and IL-6, IL-10. IL-12p40, IL-13 and IFN-γ in co-culture supernatants. In brief, the 96-well plates were coated overnight in room temperature with the capture antibody diluted in PBS. Next day the capture antibody was discarded, wells were washed with wash buffer (0.05% Tween 20 in PBS) and blocking solution (1% BSA in PBS for THP-1, 1% BSA, 5% sucrose and 0.05% NaN_3 in PBS for DCs and T cells) was added. After 1 h incubation at RT the wells were washed, samples and standards were added and incubated for 2 h at RT. Then, plates were washed, detection antibody were added and incubated 2 h at RT. Then the wells were washed and HRP –conjugated Streptavidin was added and incubated for 20 min at RT. The wells were washed again and the substrate solution were added and incubated until the color developed in the highest standard. The stop solution (2 N sulphuric acid) was applied and the optical density was determined by Varioskan lux plate reader set to 450 nm. The concentration of cytokines in pg/ml were read from seven-point standard curves. The results were given as secretion index (SI) which was calculated by dividing the concentration of cytokine in supernatant from cells treated with schisandra lignans by the concentration of cytokine in supernatant from cells treated with vehicle control (DMSO).

2.8. Intracellular ROS detection

The intracellular ROS levels of THP-1 cells were studied with dichlorodihydrofluorescein diacetate (DCFH-DA) (Sigma-Aldrich). After being washed with PBS, 1.5×10^5 of THP-1 cells were incubated in microcentrifuge tubes with 20 μM DCFH-DA for 30 min. After incubation, DCFH-DA was removed, cells washed with PBS and lignan samples (25 μM and 50 μM) and 0.25% DMSO vehicle control were added to the tubes. After 1 h incubation cells suspensions were transferred to 96-well plate for the fluorescent readout. The fluorescence was recorded at 503/523 nm with Varioskan lux plate reader (Thermo Fisher Scientific). One mM hydrogen peroxide (Sigma-Aldrich) was used as a positive control.

2.9. Glutathione and glutathione disulfide quantification assay

The intracellular GSH and GSSG levels of THP-1 cells were studied using method described previously by Rahman et al. [37]. In brief, THP-1 cells were seeded into 24-well plates at density of 4×10^5 cells/ml and incubated 4 h, 24 h or 48 h with schisandrin, schisandrin B and schisandrin C at 25 μM and 50 μM. 0.25% DMSO was used as vehicle control. Cells were lysed and stored at −70 °C. To measure total GSH, 83.3 units/ml of glutathione reductase (GR) were added to the sample with 666 μg/ml of 5.5-dithio-bis(2-nitrobenzoic acid) (DTNB). After 30 s, 666 μg/ml of β-NADPH were added and the formation of TNB chromophore product was recorded by Multiskan Sky plate reader at 412 nm. To detect the GSSG, 9.5% 2-vinylpyridine was added and after 1 h incubation 16.3% triethanolamine was applied. Then GR, DTNB and β-NADPH were added and the amount TNB was recorded as described above.

2.10. Nitrite quantification assay

For analysis of NO production in THP-1 cells were seeded into 24-well plates at density of 4×10^5 cells/ml. To differentiate cells to macrophage-like phenotype, the cultures were treated with 160 nM phorbol 12-myristate 13-acetate (PMA) for 48–72 h. Cells were then treated with 1 μg/ml *E. coli* LPS (Fluka, Sigma) for 4 – 72 h. Luteolin (10 μM) was used as positive control and 0.25% DMSO as vehicle control. The total nitrate concentrations, indicative of NO, were quantified using Griess Reagent which is a mixture of two solutions: N-1-naphtylethylenediamine dihydrochloride (Sigma-Aldrich) and Milli-Q H_2O; 2% sulfanilamide (Sigma-Aldrich) and 5% phosphoric acid diluted from 85% orto-phosphoric acid. Sample of 100 μl of each culture supernatant were pipetted into wells on 96-well plates and 100 μl of Griess reagent, freshly prepared, was added. Absorbance was determined by Varioskan lux plate reader (Thermo Fisher Scientific) set to 540 nm.

2.11. Data analysis

The data were expressed as mean ± standard error of mean (SEM). Statistical analyses were performed using SPSS Statistics 24 software. The statistical significance of results of THP-1 cytokine studies were analyzed using one-way ANOVA with Games-Howell post hoc test. Kruskall-Wallis test was used for studies on DCs and Student *t*-test with Bonferroni correction for GSH and ROS assays. *P* values < 0.05 were considered as statistically significant.

3. Results

3.1. Impact of schisandrin lignans on intracellular ROS and glutathione levels

The study was initiated by examining the impact of the three schisandrin lignans on human monocytic cell redox status. THP-1 cells were incubated with schisandrin, schisandrin B and schisandrin C for 1 h and intracellular ROS levels were determined. As shown in Fig. 2A, schisandrin increased the intracellular ROS levels at both 25 μM (43%) and 50 μM (50%) concentrations. No differences in ROS levels compared to the vehicle control were observed with schisandrin B or schisandin C.

Next, GSH and GSSG levels of THP-1 cells were determined after 4 h, 24 h and 48 h incubation with the schisandrin lignans. Shcisandrin B lowered the total GSH concentrations by 35% (25 μM) and 50% (50 μM) after 4 h incubation and schisandrin C (50 μM) lowered the levels by 38% (Fig. 2B). After 24 h incubation schisandrin B at 50 μM concentration lowered the total GSH levels by 48% and after 48 h schisandrin B 25 μM and 50 μM, as well as schisandrin C 50 μM lowered the GSH levels by 53%, 73% and 50%, respectively. In the conditions used,

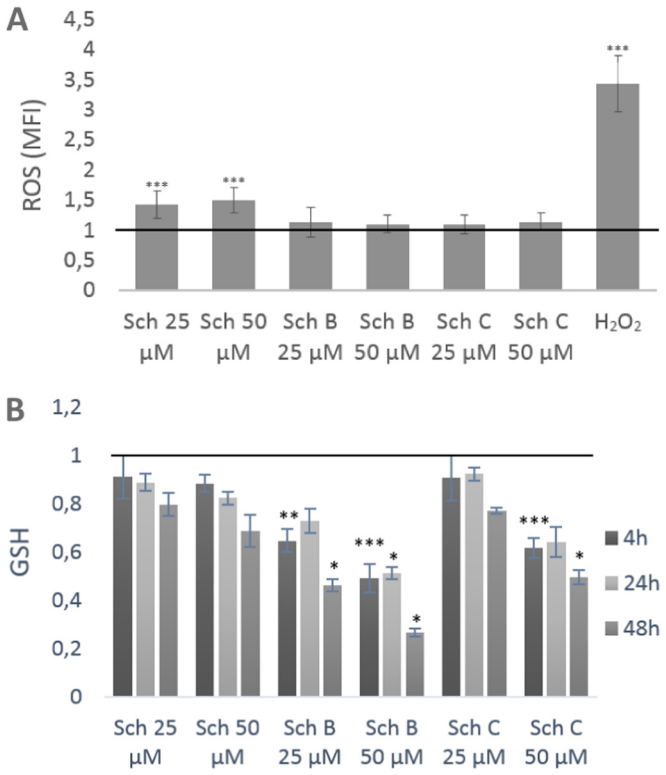

Fig. 2. Effects of schisandrin lignans on intracellular ROS and total GSH levels in THP-1 cells. The cells were treated with 25 μM and 50 μM concentration of schisandrin, schisandrin B and schisandrin C and basal intracellular ROS levels were measured after 1 h incubation with DCFH-DA (A). Total intracellular GSH concentration of THP-1 cells after 4 h, 24 h and 48 h incubation with 25 μM and 50 μM schisandrin, schisandrin B and schisandrin C (B). Data are normalized as a ration of DMSO control and shown as mean ± SEM and statistical significance is presented as marks of P values: < 0.05: *; < 0.01: **; < 0.001: ***. n ≥ 4.

GSSG concentrations remained below the detection limit of the assay and thus are not reported. To rule out direct cytotoxic effects of the lignans, resazurin cell viability assay on THP-1 cells was carried out after 4 h, 24 h and 48 h exposure, respectively. In these assays, cell viability was not affected in any of the lignin-treated samples (data not shown).

3.2. NO production in THP-1 cells

Earlier reports on the immunomodulatory properties of *S. chinensis* lignans have presented data on the impact of these compounds on murine macrophage NO production [14]. In our studies, data essentially similar to the previously published were obtained with RAW264.7 cells (supplementary Fig. S1). *E. coli* LPS induced significant NO production in these cells, and the total nitrate levels quantified with the Griess reagent were suppressed by the studied lignans schisandrin, schisandrin B and schisandrin C. However, in contrary to previous findings [15], altering the administration times did not significantly affect the cellular responses in our study.

Despite the obvious activation of NO production in RAW264.7 cells (suppl. Fig. 1), no elevation of nitrate levels was observed in the human leukemia cell line THP-1, neither in its monocytic nor macrophage form in any of the time points studied (from 4 h to 72 h; data not shown). While some controversial data have been presented in the literature, this finding was in line with earlier observations [38–40], and the impact of the *S. chinensis* lignans on this aspect of human monocyte immune responses was thus not addressed in this study

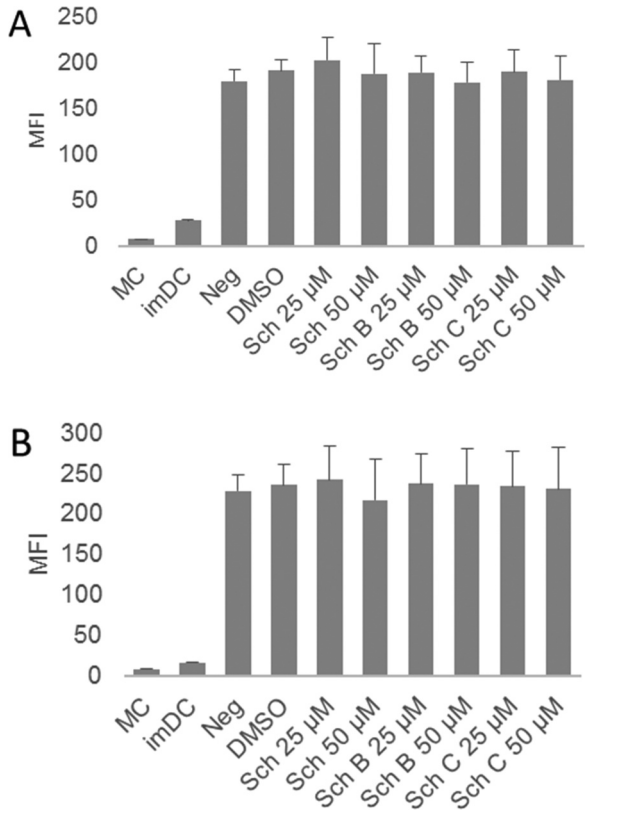

Fig. 3. Effect of the schisandrin lignans on the expression of CD86 and HLA-DR. Monocytes (MCs) were differentiated into immature dendritic cells (imDCs) and then maturated with IL-1β, TNF-α and LPS into mature dendritic cells (mDCs) with nothing added (Neg), vehicle only (DMSO) or in the presence of schisandrin, schisandrin B and schisandrin C at 25 and 50 µM. The expression of HLA-DR (A) and CD86 (B) was measured by flow cytometry and the results give as mean fluorescence intensity (MFI). Data are presented as mean MFI ± SEM, n = 8.

3.3. The effect of schisandrin lignans on surface marker expression on DCs

We then evaluated the impact of the three studied lignans on the maturation process of dendritic cells, applying CD14+ monocytes isolated from healthy human volunteers. Differentiation of CD14+ monocytes into imDCs and subsequent maturation into mDCs was confirmed by staining the cells with antibodies against CD86 and HLA-DR, which are expressed in low levels on monocytes and imDCs but at high levels on mDCs. As expected, mature dendritic cells had significantly higher levels of CD86 and HLA-DR expression as compared with imDCs and monocytes ($p < 0.05$), indicating that maturation had occurred by the treatment with maturation factors (Fig. 3). Maturing the DCs in the presence of schisandrin, schisandrin B or schisandrin C had no effect on the expression of CD86 and HLA-DR as compared with vehicle-treated DCs, indicating that the schisandrin lignans did not affect the maturation of the DCs.

3.4. Impact on cytokine secretion by mature dendritic cells

After confirming that the schisandrin lignans did not affect the maturation of the DCs their effect on cytokine secretion was evaluated. All three lignans showed some tendency towards downregulation of IL-6, IL-10 and IL-12p40 secretion by the mDCs as compared with vehicle-treated DCs. DCs matured in the presence of schisandrin B and schisandrin C at 25 and 50 µM secreted lower levels of IL-6 as compared with DCs treated with vehicle (concentration 30260 pg/ml) (Fig. 4A). DCs treated with 25 µM schisandrin B secreted lower levels of IL-10 than DCs treated with vehicle (5800 pg/ml) (Fig. 4B). DCs matured in the presence of schisandrin at 25 and 50 µM and schisandrin C at 25 and 50 µM secreted lower levels of IL-12p40 compared with DCs treated with vehicle (18368 pg/ml) (Fig. 4C).

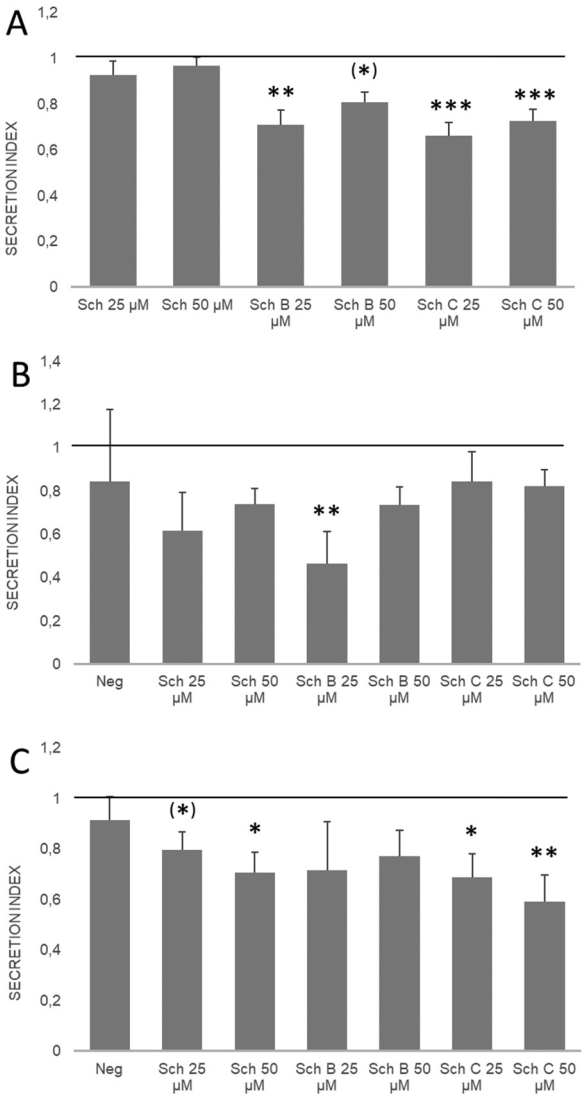

Fig. 4. IL-6, IL-10 and IL-12p40 levels. DCs were matured with IL-1β, TNF-α and LPS in the presence of presence of schisandrin, schisandrin B and schisandrin C at 25 and 50 µM or vehicle only and their secretion of IL-6 (A), IL-10 (B) and IL-12p40 (C) was measured with ELISA. The results are given as secretion index (SI) which is calculated by dividing the concentration of cytokine in supernatant of DCs maturated in the presence of schisandrin lignans by the concentration of cytokines in supernatant of DC treated with vehicle. Data are presented as mean SI ± SEM, n = 8. Statistical significance is presented as marks of P values: < 0.05: *; < 0.01: **; < 0.001: ***. Full datasets of detected cytokine concentrations are available in Mendeley data repository as a MS Excel file linked to this article.

3.5. Impact of schisandrin C on cytokine secretion of the co-cultured DCs and T cells

In order to investigate the subsequent differentiation of naïve CD4+ T cells when co-cultured in the presence of mDCs, their levels of IL-6, IL-10, IL-12p40, IL-13, and IFN-γ secretion was measured. The DCs in the co-culture were matured in the presence of 50 µM schisandrin C. Based on data presented above, schisandrin C was chosen to this analysis. The concentrations of IL-6, IL-10, IL-12p40, IL-13 and IFN-γ were all elevated implying that T cells had become activated by mDCs and produced cytokines. The co-culture of T cells and the mature DCs

Table 1
Effect of schisandrin C-treated DCs on T cells. DCs matured in the presence of 50 μM schisandrin C (DC-Sch C) or vehicle only (DC-DMSO) were co-cultured with allogeneic CD4+ T cells. The secretion of IL-6, IL-10, IL12p40, IL-13 and IFN-γ in the culture supernatant was measured by ELISA. The results are given as secretion index which is calculated by dividing the concentration of cytokine in supernatant of co-culture of DC-Sch C and T cells by the concentration of cytokines in supernatant of co-culture of DC-DMSO and T cells. Data are presented as mean SI ± SEM, n = 6. Statistical significance is presented as marks of P values: < 0.05: *; < 0.01: **; < 0.001: ***. Full datasets of detected cytokine concentrations are available in Mendeley data repository as a MS Excel file linked to this article.

Cytokine	Secretion index (DC-Sch C+T/DC-DMSO+T)
IL-6	0.899 ± 0.069
IL-10	0.861 ± 0.327
IL-12p40	0.617 ± 0.069***
IL-13	1.888 ± 0.365
INF-γ	0.778 ± 0.232

treated with schisandrin C secreted significantly lower levels of IL-12 (SI 0,62) compared with cocultures with untreated DCs (Table 1). Schisandrin C also seemed to decrease the levels of IL-6 (SeI 0.90, p = 0.17), IL-10 (SeI 0.86, p = 0.68) and IFN-γ (SeI 0.78, p = 0.36)) compared to vehicle control. The level of IL-13 (SeI 1.89, p = 0.20) seemed to be increased in presence of schisandrin C.

The impact of schisandrin C-treated DCs on T cell activation and differentiation was also evaluated by measuring the T cell surface proteins CTLA-4 and CD69. However, no differences in the expression of these two T cell activation markers were observed between T cells co-cultured with schisandrin C-treated DCs or vehicle-treated DCs (data not shown).

3.6. Impact of schisandrin lignans on IL-6 and IL-12 production in THP-1 cells

For comparison, the human monocytic cell line THP-1 was also applied for analyzing the ability of schisandrin lignans to suppress cytokine production. THP-1 cells were stimulated with LPS from *E. coli* and IFN-γ and then treated with schisandrin, schisandrin B and schisandrin C to evaluate the impact of the lignans on their cytokine secretion. All three schisandrin lignans seemed to lower the concentrations of LPS induced IL-6 levels compared with DMSO control (2778 pg/ml or 2152 pg/ml) (Fig. 5A). Both concentrations of schisandrin B and achisandrin C lowered the IL-6 levels statistically significantly. All three compounds also seemed to reduce the IL-12p40 concentrations compared to DMSO control (644 pg/ml or 1327 pg/ml) (Fig. 5B). At both, 25 μM and 50 μM, concentrations, the IL-12p40 reduction was statistically significant with schisandrin B. Schisandrin C lowered the IL-12p40 levels statistically significantly at 50 μM concentration. Schisandrin also seem to lower the IL-12p40 levels but the reduction was not statistically significant. IL-10 production of THP-1 cells was also studied, but the production was not activated significantly when treated with IFN-γ and LPS. Highest IL-10 concentrations determined were only 10 pg/ml in these conditions.

Besides the activation by *E. coli* LPS and IFN-γ, THP-1 cells were also stimulated to produce cytokines by an infection with the obligate intracellular bacterium *Chlamydia pneumoniae* and treated with schisandrin, schisandrin B and schisandrin C. Schisandrin B and schisandrin C decreased the IL-12p40 levels in statistically significant manner, compared to DMSO control (47 pg/ml at most) (Fig. 5C). The IL-12p40 concentrations of untreated samples of cells induced by LPS were much higher than *C. pneumoniae* induced cells indicating the differences in the antigenic properties of LPS of *E. coli* and *C. pneumoniae*. THP-1 cells did not produce significant amount of IL-6 when induced with *C. pneumoniae* infection.

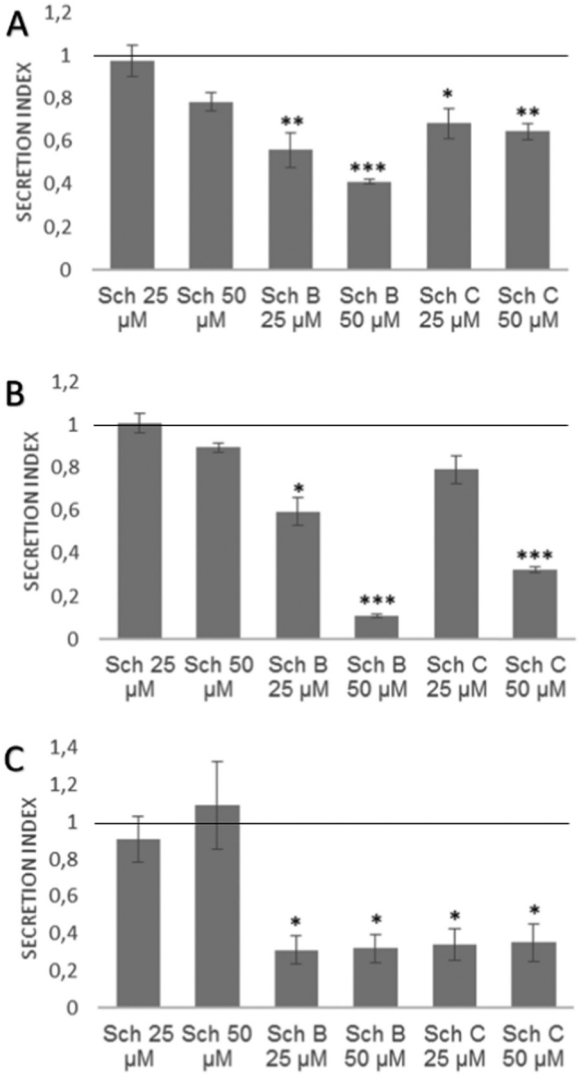

Fig. 5. Cytokine production of THP-1 cells. LPS induced IL-6 (A) and IL-12p40 (B) concentrations and *C. pneumoniae* induced IL-12p40 (C) concentrations in THP-1 cell samples in presence of INF-γ (LPS induced samples) and Scisandrin lignans. THP-1 cells were treated with 25 μM and 50 μM concentration of schisandrin, schisandrin B and schisandrin C. Data is presented as secretion index (SeI) as mean ± SEM and statistical significance is presented as marks of P values: < 0.05: *; < 0.01:* *; < 0.001:* **. n ≥ 4. Full datasets of detected cytokine concentrations are available in Mendeley data repository as a MS Excel file linked to this article.

4. Discussion

The dibenzocyclooctadiene lignans isolated from *Schisandra chinensis* have been widely studied for their biological activities, involving among others, anti-inflammatory and immunomodulatory properties. The current work contributes to the knowledge on these commonly consumed compounds regarding their impact on the redox status and activation of the cells of human innate immune system. Despite the widespread use of *S. chinensis* extract, no data on human plasma or target tissue concentrations of individual lignans after their oral administration are available. In recently published pharmacokinetic studies of schisandrin and schisandrin B in rats, micromolar plasma peak concentrations of both lignans have been reported after single dose oral administration [41,42]. Even though the influence of multiple dosing, tissue distribution and inter-species variation in the kinetics of the lignans remain unknown, the concentrations used in this study can be considered achievable in vivo.

GSH is an essential endogenous antioxidant agent present both inside cells and in extracellular space [43]. It protects cells from oxidative damage via eliminating radicals by the virtue of GSSG formation, and depletion of cellular glutathione pools may occur as a response to chemical toxicant exposure. Previously, it has been reported that schisandrin B elevates the chemically depleted GSH levels in different cells and tissues [5,32,44,45]. In the current contribution, we studied the effects of schisandrin lignans on basal GSH levels in THP-1 cells, a human monocytic cell line. According to our data (Fig. 2B), schisandrin B and schisandrin C but not schisandrin, cause a drastic decrease in cellular GSH pools already after 4 h incubation, and similar effect is seen after 48 h exposure. While Checker et al. have reported a decrease in GSH:GSSG ratio after a 4 h treatment of murine splenic lymphocytes with schisandrin B [16], no earlier reports on decrease in total cellular glutathione content upon schisandrin B or schisandrin C are available.

Owing to homeostatic mechanisms, decrease of intracellular glutathione pools may also have beneficial effects on cellular physiology [46]. Several studies have reported the suppression of NF-κB mediated transcriptional activity by chemical agents which induce cellular GSH depletion [47–49]. The suggested mechanisms underlying this phenomenon involves the oxidation of a cysteine residue in the DNA-binding domain of NF-κB, resulting in weakened ability of the activated transcription factor to bind DNA. Furthermore, the Nrf2 transcription factor is activated by oxidative stress [50] which downregulates NF-κB, shifting the cell towards an anti-inflammatory phenotype [47]. Both suppression of NF-κB activity and activation of Nrf2 have been reported to occur as a result of schisandrin B and C treatment [16,17,30], and even though not a subject of experiments in the current work, this can be speculated to result from altered GSH levels.

In earlier studies on schisandrin lignans, it has been suggested that the oxidative metabolism of these lignans results in the increase in intracellular ROS levels which may mediate the protective effects [16,32,51–53]. It seems, however, that such events are dependent on cell type and additional stimuli, as other studies have linked schisandrin lignan treatment with either no changes in ROS production [54,55] or suppression of ROS production after various stimuli [9,56–61] In human monocytic THP-1 cells, only schisandrin elevated ROS levels while schisandrin B and schisandrin C did not affect the ROS levels after 1 h (Fig. 2A). Based on our data, the possible ROS elevation by schisandrin B and schisandrin C after longer exposure cannot be ruled out but it seems obvious that the ability of the lignans to decrease cellular GSH pools is not associated with a rapid increase in cytosolic ROS. A similar phenotype involving GSH depletion with no cytosolic ROS elevation has recently been linked to mitochondrial oxidative stress [34]. Furthermore, the impact of schisandrin B and schisandrin C on GSH levels was less obvious at 24 h than at 4 h or 48 h, implying that a more detailed study with more specific probes on the kinetics of cellular redox status after exposure to different lignans would be necessary to elucidate the underlying molecular events. It can be speculated, however, that the immediate ROS production by schisandrin may contribute to the GSH level maintenance through homeostatic mechanisms and result in ineffective anti-inflammatory response in THP-1 cells. In contrast, decrease in cellular GSH upon schisandrin B and schisandrin C exposure can be hypothesized to mediate the anti-inflammatory effects discussed below.

DCs are a diverse population of immune cells with an essential role in induction and regulation of immune responses [62]. Specialized in antigen capture, processing and presenting this small fraction of hematopoietic cells is responsible for priming naïve T cells and thereby govern the immunogenic and tolerogenic features of the adaptive immune system [63]. Originating from bone marrow, the DC precursors migrate to the blood stream and non-lymphoid tissues where they develop to imDCs under the influence of various growth factors. Phenotypic and functional maturation to mDCs occurs upon recognition of foreign matter by toll-like receptors (TLRs) [64]. Interference of DC maturation may involve functions like antigen-processing capacity, cytokine secretion, expression of co-stimulatory molecules, migration to lymph nodes, and ultimately their ability to prime naïve T cells [65].

As a model for DC maturation, CD14+ monocytes from healthy human volunteers were induced to different stages of DC maturation by a combination of relevant cytokines and LPS from *E. coli*. When the *S. chinensis* lignans were present in the culture medium during the maturation, decrease in the secretion of IL-6, IL-10 and IL-12p40 was control. A statistically significant ($p < 0.05$) decrease was achieved with schisandrin B and schisandrin C for IL-6 (Fig. 4A), schisandrin B in the case of IL-10 Ievels (Fig. 4B) and schisandrin and schisandrin C in the case of IL-12p40 levels (Fig. 4C), respectively. Based on earlier literature, glutathione depletion is linked to alter dendritic cell maturation as seen by suppressed IL-6 and Il-12 secretion, yet IL-10 levels were not affected in these studies [66,67].

For comparison, the impact of the lignans on production of IL-6, IL-10 and IL-12p40 was also studied with human monocyte THP-1 cells. THP-1 cells are isolated from peripheral blood of a patient suffering from acute monocytic leukemia and it has been widely applied as a model for innate immune system cell functions [68]. Consistent with previous reports [68] the concentrations of secreted interleukines were lower in THP-1 cell cultures compared to those determined from primary DC culture supernatants. Concentration of IL-6 in vehicle-treated DCs was 30260 pg/ml and in THP-1 samples 2600 pg/ml. IL-12 concentrations were 18368 pg/ml and 1326 pg/ml in DCs and THP-1 cells, respectively, indicating over 10-fold difference in these conditions. Also, THP-1 cells did not produce IL-10 at detectable levels as did DCs (3976 pg/ml).

Schisandrin, schisandrin B and schisandrin C lowered the IL-6 and IL-12p40 levels in THP-1 culture supernatants at least in 50 μM concentration (Fig. 5A, B).

As an alternative model of monocyte activation, the THP-1 cytokine production was also studied by stimulating the cells with an infection by an intracellular bacterium *Chlamydia pneumoniae* which is known to induce DC maturation [69]. LPS from *E. coli* and various other gram-negative bacteria is recognized by monocytic cells through Toll-like receptor 4 (TLR4), triggering signal transduction cascades involving MyD88 and TRAM -mediated pathway and ultimately leading to up-regulation of proinflammatory gene expression [62]. As a gram-negative bacterium, *C. pneumoniae* also carries LPS molecules in its outer membrane, but the chlamydial LPS is considered to have only a minor role in monocyte activation [70]. Instead of LPS-mediated TLR-4 activation, stimulation of innate immune system cells by *C. pneumoniae* is primarily mediated via TLR-2 [71,72] but involves downstream signaling cascades similar to TLR-4, including NF-B and mitogen-activated protein kinases [73,74]. *C. pneumoniae* is also reported to elevate intracellular ROS levels of different cell types, at least in epithelial HEp2 cell line [75] and THP-1 cells [76]. In macrophage – T Cell cultures, reduced GSH:GSSG ratio has been reported upon *C. pneumoniae* infection [77]

Overall, the differential immunostimulatory nature of the isolated LPS from *E. coli* and whole cells of *C. pneumoniae* is well illustrated by the less intense activation of THP-1 cells by *C. pneumoniae* infection. Schisandrin B and schisandrin C yet inhibits the production of IL-12 also in *C. pneumoniae* infected samples (Fig. 5C), demonstrating that their mechanism of proinflammatory cytokine suppression is not limited to TLR-4-mediated triggers.

As mentioned, glutathione depletion has been reported to mediate altered DC maturation [66,67], and thus the impact of *S. chinensis* lignans on monocyte-derived DC maturation was further evaluated by analyzing the appearance of DC surface proteins. The major histocompatibility complex II (HLA-DR) is applied by DCs for antigen presenting, while CD86, in turn, is a costimulatory protein expressed on mature antigen-presenting cells. In contrast to earlier reports on immunosuppressants with GSH depleting activity, none of the studied

lignans affected the appearance of HLA-DR or CD86 on DC surface upon maturation (Fig. 3), indicating that despite the obvious GSH decreasing activity of schisandrin B and C, the immunomodulatory properties of these lignans are not similar to classical immunosuppressive therapeutics.

Similar to increased cytokine secretion, emergence of HLA-DR and CD86 on DC surface is driven by TLR-mediated activation of MYD88-dependent signaling cascades. However, in contrast to cytokine secretion, the surface proteins are primarily regulated by altering their stability and subcellular localization [78,79]. Absence of HLA-DR or CD86 modulation by the lignans indicates that these compounds do not interact directly with TLR4 or MYD88 and are not able to interfere with the ubiquitin-dependent trafficking of the DC surface markers. Thus, the molecular targets of the lignans are likely to lie in downstream cascades and may rather involve transcriptional regulation.

Consistent with the lack of HLA-DR and CD86 modulation, schisandrin C did not affect the ability of DCs to activate allogenic CD4 + T cells, as no changes in T cell activation markers were observed and out of the studied cytokines, only IL-12 levels were decreased in a statistically significant manner. Also in this respect, the immunomodulatory profile of schisandrin C differs from GSH-depleting immunosuppressants, vaccine adjuvants, or sensitizers which affect DC maturation by either suppressing allogenic T cell activation of altering the Th1/Th2 balance [34,66,67]. Collectively, the presented data suggests that while the impact of schisandrin B and C on cellular glutathione pools is likely to contribute to the anti-inflammatory actions of these lignans. However, their full spectrum of biological activities is likely mediated in a cell type and trigger-dependent fashion and warrants further studies to elucidate the relative significance and interplay of different signaling pathways.

5. Conclusions

Previous studies on anti-inflammatory activities of schisandrin B have indicated that this lignan triggers its biological activities by affecting cellular redox status, but no data on human peripheral blood mononuclear cells has been available in this respect. In this study we report that schisandrin B and schisandrin C are able to decrease cellular GSH pools in human monocytic cells. In our study, the immunomodulatory properties of schisandrin B and schisandrin C are demonstrated on human monocyte-derived DCs and THP-1 cell line as their ability to suppress IL-6, IL-10 and IL-12p40 secretion.

Despite acting as immunomodulatory agents by altering the cytokine secretion, the studied lignans do not interfere with dendritic cell maturation or T cell activating capacity as measured by surface marker expression. Collectively, these results increase our understanding on the potential of dibenzocyclooctadiene lignans as immunomodulatory agents.

Acknowledgments

We would like to acknowledge CIMO (The Centre for International Mobility) for a Nordplus- programme grant for EK. Nina Franko, Milka Lohtaja and Krista Virtanen are acknowledged for excellent technical assistance. We would also like to acknowledge Ilkka Miettinen and Lasse Karhu for excellent assistance in data analysis.

Conflict of interest

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.freeradbiomed.2018.12.019.

References

[1] A. Szopa, R. Ekiert, H. Ekiert, Current knowledge of *Schisandra chinensis* (turcz.) baill.(chinese magnolia vine) as a medicinal plant species: a review on the bioactive components, pharmacological properties, analytical and biotechnological studies, Phytochem. Rev. 16 (2017) 195–218.
[2] A. Panossian, G. Wikman, Pharmacology of *Schisandra chinensis* bail.: an overview of russian research and uses in medicine, J. Ethnopharmacol. 118 (2008) 183–212.
[3] K. Sowndhararajan, P. Deepa, M. Kim, S.J. Park, S. Kim, An overview of neuroprotective and cognitive enhancement properties of lignans from *Schisandra chinensis*, Biomed. Pharmacother. 97 (2018) 958–968.
[4] Y. Lu, D. Chen, Analysis of *Schisandra chinensis* and *Schisandra sphenanthera*, J. Chromatogr. A 1216 (2009) 1980–1990.
[5] P.Y. Chiu, M.H. Tang, D.H. Mak, M.K. Poon, K.M. Ko, Hepatoprotective mechanism of schisandrin B: role of mitochondrial glutathione antioxidant status and heat shock proteins, Free Radic. Biol. Med. 35 (2003) 368–380.
[6] T.H. Lee, C.H. Jung, D. Lee, Neuroprotective effects of schisandrin B against transient focal cerebral ischemia in Sprague–Dawley rats, Food Chem. Toxicol. 50 (2012) 4239–4245.
[7] Z. Liu, B. Zhang, K. Liu, Z. Ding, X. Hu, Schisandrin B attenuates cancer invasion and metastasis via inhibiting epithelial-mesenchymal transition, PLoS One 7 (2012) e40480.
[8] A. Stacchiotti, G.L. Volti, A. Lavazza, R. Rezzani, L.F. Rodella, Schisandrin B stimulates a cytoprotective response in rat liver exposed to mercuric chloride, Food Chem. Toxicol. 47 (2009) 2834–2840.
[9] J. Song, X. Lin, R.N. Wong, S.C. Sze, Y. Tong, P. Shaw, Y. Zhang, Protective effects of dibenzocyclooctadiene lignans from *Schisandra chinensis* against beta-amyloid and homocysteine neurotoxicity in PC12 cells, Phytother. Res. 25 (2011) 435–443.
[10] P.Y. Chiu, H.Y. Leung, A.H.L. Siu, M.K.T. Poon, K.M. Ko, Schisandrin B decreases the sensitivity of mitochondria to calcium ion-induced permeability transition and protects against carbon tetrachloride toxicity in mouse livers, Biol. Pharm. Bull. 30 (2007) 1108–1112.
[11] S. Ip, K. Ko, The crucial antioxidant action of schisandrin B in protecting against carbon tetrachloride hepatotoxicity in mice: a comparative study with butylated hydroxytoluene, Biochem. Pharmacol. 52 (1996) 1687–1693.
[12] C. Park, Y. Choi, S.K. Hyun, H.J. Kwon, H.J. Hwang, G. Kim, B.T. Choi, B. Kim, I. Choi, S. Moon, Induction of G1 arrest and apoptosis by schisandrin C isolated from *Schizandra chinensis* baill in human leukemia U937 cells, Int. J. Mol. Med. 24 (2009) 495–502.
[13] J. Slanina, G. Pachnikova, M. Čarnecká, L. Porubová Koubíková, L. Adámková, O. Humpa, K. Smejkal, I. Slaninová, Identification of key structural characteristics of *Schisandra chinensis* lignans involved in P-glycoprotein inhibition, J. Nat. Prod. 77 (2014) 2255–2263.
[14] L.Y. Guo, T.M. Hung, K.H. Bae, E.M. Shin, H.Y. Zhou, Y.N. Hong, S.S. Kang, H.P. Kim, Y.S. Kim, Anti-inflammatory effects of schisandrin isolated from the fruit of *Schisandra chinensis* baill, Eur. J. Pharmacol. 591 (2008) 293–299.
[15] L.Y. Guo, T.M. Hung, K. Bae, S. Jang, E.M. Shin, J.W. Chung, S.S. Kang, H.P. Kim, Y.S. Kim, Effects of schisandrin on transcriptional factors in lipopolysaccharide-pretreated macrophages, Arch. Pharm. Res. 32 (2009) 399–405.
[16] R. Checker, R.S. Patwardhan, D. Sharma, J. Menon, M. Thoh, H.N. Bhilwade, T. Konishi, S.K. Sandur, Schisandrin B exhibits anti-inflammatory activity through modulation of the redox-sensitive transcription factors Nrf2 and NF-κB, Free Radic. Biol. Med. 53 (2012) 1421–1430.
[17] S. Oh, Y.H. Kim, D.S. Bae, B.H. Um, C. Pan, C.Y. Kim, H.J. Lee, J.K. Lee, Anti-inflammatory effects of gomisin N, gomisin J, and schisandrin C isolated from the fruit of *Schisandra chinensis*, Biosci. Biotechnol. Biochem. 74 (2010) 285–291.
[18] S. Di Meo, T.T. Reed, P. Venditti, V.M. Victor, Role of ROS and RNS sources in physiological and pathological conditions, Oxid. Med. Cell. Longev. 2016 (2016).
[19] D.A. Wink, H.B. Hines, R.Y. Cheng, C.H. Switzer, W. Flores-Santana, M.P. Vitek, L.A. Ridnour, C.A. Colton, Nitric oxide and redox mechanisms in the immune response, J. Leukoc. Biol. 89 (2011) 873–891.
[20] Y.S. Kang, M.H. Han, S.H. Hong, C. Park, H.J. Hwang, B.W. Kim, K.H. Kyoung, Y.W. Choi, C.M. Kim, Y.H. Choi, Anti-inflammatory effects of *Schisandra chinensis* (turcz.) baill fruit through the inactivation of nuclear factor-kappaB and mitogen-activated protein kinases signaling pathways in lipopolysaccharide-stimulated murine macrophages, J. Cancer Prev. 19 (2014) 279–287.
[21] D.A. Wink, H.B. Hines, R. Cheng, C.H. Switzer, W. Flores-Santana, M.P. Vitek, L.A. Ridnour, C.A. Colton, Nitric oxide and redox mechanisms in the immune response, J. Leukoc. Biol. 89 (2011) 873–891.
[22] M. Zembala, M. Siedlar, J. Pryjma, J. Marcinkiewicz, Human monocytes are stimulated for nitric oxide release in vitro by some tumor cells but not by cytokines and lipopolysaccharide, Eur. J. Immunol. 24 (1994) 435–439.
[23] M. Denis, Human monocytes/macrophages: NO or no NO? J. Leukoc. Biol. 55 (1994) 682–684.
[24] M. Schneemann, G. Schoeden, Macrophage biology and immunology: man is not a mouse, J. Leukoc. Biol. 81 (2007) 579.
[25] R. Korhonen, A. Lahti, H. Kankaanranta, E. Moilanen, Nitric oxide production and signaling in inflammation, Curr. Drug Targets-Inflamm. Allergy 4 (2005) 471–479.
[26] J.W. Steinke, L. Borish, 3. Cytokines and chemokines, J. Allergy Clin. Immunol. 117 (2006) S441–S445.
[27] I. Striz, E. Brabcova, L. Kolesar, A. Sekerkova, Cytokine networking of innate immunity cells: a potential target of therapy, Clin. Sci. 126 (2014) 593–612.
[28] J.M. Zhang, J. An, Cytokines, inflammation, and pain, Int. Anesthesiol. Clin. 45 (2007) 27–37.
[29] K. Zeng, T. Zhang, H. Fu, G. Liu, X. Wang, Schisandrin B exerts anti-

neuroinflammatory activity by inhibiting the toll-like receptor 4-dependent MyD88/IKK/NF-κB signaling pathway in lipopolysaccharide-induced microglia, Eur. J. Pharmacol. 692 (2012) 29–37.
[30] Q. Lin, X. Qin, M. Shi, Z. Qin, Y. Meng, Z. Qin, S. Guo, Schisandrin B inhibits LPS-induced inflammatory response in human umbilical vein endothelial cells by activating Nrf2, Int. Immunopharmacol. 49 (2017) 142–147.
[31] M. Guo, F. An, X. Wei, M. Hong, Y. Lu, Comparative effects of schisandrin A, B, and C on acne-related inflammation, Inflammation 40 (2017) 2163–2172.
[32] P.Y. Chiu, P.Y. Lam, C.W. Yan, K.M. Ko, Schisandrin B protects against solar irradiation-induced oxidative injury in BJ human fibroblasts, Fitoterapia 82 (2011) 682–691.
[33] F. Groell, O. Jordan, G. Borchard, In vitro models for immunogenicity prediction of therapeutic proteins, Eur. J. Pharm. Biopharm. (2018).
[34] I. Ferreira, A. Silva, J.D. Martins, B.M. Neves, M.T. Cruz, Nature and kinetics of redox imbalance triggered by respiratory and skin chemical sensitizers on the human monocytic cell line THP-1, Redox Biol. 16 (2018) 75–86.
[35] L. Hanski, D. Ausbacher, T.M. Tiirola, M.B. Strøm, P.M. Vuorela, Amphipathic $\beta^{2,2}$-amino acid derivatives suppress infectivity and disrupt the intracellular replication cycle of *Chlamydia pneumoniae*, PLoS One 11 (2016) e0157306.
[36] I. Hardardottir, E.S. Olafsdottir, J. Freysdottir, Dendritic cells matured in the presence of the lycopodium alkaloid annotine direct T cell responses toward a Th2/treg phenotype, Phytomedicine 22 (2015) 277–282.
[37] I. Rahman, A. Kode, S.K. Biswas, Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method, Nat. Protoc. 1 (2006) 3159.
[38] W. Chanput, J. Mes, R.A. Vreeburg, H.F. Savelkoul, H.J. Wichers, Transcription profiles of LPS-stimulated THP-1 monocytes and macrophages: a tool to study inflammation modulating effects of food-derived compounds, Food Funct. 1 (2010) 254–261.
[39] G.I. Perez-Perez, V.L. Shepherd, J.D. Morrow, M.J. Blaser, Activation of human THP-1 cells and rat bone marrow-derived macrophages by helicobacter pylori lipopolysaccharide, Infect. Immun. 63 (1995) 1183–1187.
[40] M. Daigneault, J.A. Preston, H.M. Marriott, M.K. Whyte, D.H. Dockrell, The identification of markers of macrophage differentiation in PMA-stimulated THP-1 cells and monocyte-derived macrophages, PLoS One 5 (2010) e8668.
[41] C. Li, Y. Cheng, C. Hsieh, T. Tsai, Pharmacokinetics of schizandrin and its pharmaceutical products assessed using a validated LC–MS/MS method, Molecules 23 (2018) 173.
[42] Z. Wang, L. You, Y. Cheng, K. Hu, Z. Wang, Y. Cheng, J. Yang, Y. Yang, G. Wang, Investigation of pharmacokinetics, tissue distribution and excretion of schisandrin B in rats by HPLC–MS/MS, Biomed. Chromatogr. 32 (2018) e4069.
[43] H.J. Forman, H. Zhang, A. Rinna, Glutathione: overview of its protective roles, measurement, and biosynthesis, Mol. Asp. Med. 30 (2009) 1–12.
[44] K. Ko, B.Y. Lam, Schisandrin B protects against tert-butylhydroperoxide induced cerebral toxicity by enhancing glutathione antioxidant status in mouse brain, Mol. Cell. Biochem. 238 (2002) 181–186.
[45] L. Li, Q. Pan, W. Han, Z. Liu, L. Li, X. Hu, Schisandrin B prevents doxorubicin-induced cardiotoxicity via enhancing glutathione redox cycling, Clin. Cancer Res. 13 (2007) 6753–6760.
[46] N. Urban, D. Tsitsipatis, F. Hausig, K. Kreuzer, K. Erler, V. Stein, M. Ristow, H. Steinbrenner, L. Klotz, Non-linear impact of glutathione depletion on *C. elegans* life span and stress resistance, Redox Biol. 11 (2017) 502–515.
[47] M. Song, J.A. Kellum, H. Kaldas, M.P. Fink, Evidence that glutathione depletion is a mechanism responsible for the anti-inflammatory effects of ethyl pyruvate in cultured lipopolysaccharide-stimulated RAW 264.7 cells, J. Pharmacol. Exp. Ther. 308 (2004) 307–316.
[48] J. Kefer, A. Rahman, K.N. Anwar, A.B. Malik, Decreased oxidant buffering impairs NF-kappaB activation and ICAM-1 transcription in endothelial cells, Shock 15 (2001) 11–15.
[49] J.J. Jones, J. Fan, A.B. Nathens, A. Kapus, M. Shekhman, J.C. Marshall, J. Parodo, O.D. Rotstein, Redox manipulation using the thiol-oxidizing agent diethyl maleate prevents hepatocellular necrosis and apoptosis in a rodent endotoxemia model, Hepatology 30 (1999) 714–724.
[50] T. Nguyen, P. Nioi, C.B. Pickett, The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress, J. Biol. Chem. 284 (2009) 13291–13295.
[51] N. Chen, M. Ko, Schisandrin B-induced glutathione antioxidant response and cardioprotection are mediated by reactive oxidant species production in rat hearts, Biol. Pharm. Bull. 33 (2010) 825–829.
[52] P.Y. Lam, C.W. Yan, P.Y. Chiu, H.Y. Leung, K.M. Ko, Schisandrin B protects against solar irradiation-induced oxidative stress in rat skin tissue, Fitoterapia 82 (2011) 393–400.
[53] P.K. Leong, P.Y. Chiu, H.Y. Leung, K.M. Ko, Cytochrome P450-catalysed reactive oxygen species production mediates the (-) schisandrin B-induced glutathione and heat shock responses in AML12 hepatocytes, Cell Biol. Int. 36 (2012) 321–326.
[54] E. Hakala, L. Hanski, H. Uvell, T. Yrjönen, H. Vuorela, M. Elofsson, P.M. Vuorela, Dibenzocyclooctadiene lignans from *Schisandra spp.* selectively inhibit the growth of the intracellular bacteria *Chlamydia pneumoniae* and *Chlamydia trachomatis*, J. Antibiot. 68 (2015) 609.
[55] S. Lin, M. Fujii, D. Hou, Molecular mechanism of apoptosis induced by schizandrae-derived lignans in human leukemia HL-60 cells, Food Chem. Toxicol. 46 (2008) 590–597.
[56] S. Jeong, S. Kim, T. Kwon, K. Yu, S. Kim, Schizandrin prevents damage of murine mesangial cells via blocking NADPH oxidase-induced ROS signaling in high glucose, Food Chem. Toxicol. 50 (2012) 1045–1053.
[57] K. Zeng, T. Zhang, H. Fu, G. Liu, X. Wang, Schisandrin B exerts anti-neuroinflammatory activity by inhibiting the toll-like receptor 4-dependent MyD88/IKK/NF-κB signaling pathway in lipopolysaccharide-induced microglia, Eur. J. Pharmacol. 692 (2012) 29–37.
[58] S. Zhu, Y. Wang, M. Chen, J. Jin, Y. Qiu, M. Huang, Z. Huang, Protective effect of schisandrin B against cyclosporine A-induced nephrotoxicity *in vitro* and *in vivo*, Am. J. Chin. Med. 40 (2012) 551–566.
[59] S.Y. Park, S.J. Park, T.G. Park, S. Rajasekar, S. Lee, Y. Choi, Schizandrin C exerts anti-neuroinflammatory effects by upregulating phase II detoxifying/antioxidant enzymes in microglia, Int. Immunopharmacol. 17 (2013) 415–426.
[60] W. Hou, W. Gao, D. Wang, Q. Liu, S. Zheng, Y. Wang, The protecting effect of deoxyschisandrin and schisandrin B on HaCaT cells against UVB-induced damage, PLoS One 10 (2015) e0127177.
[61] Q. Lai, Z. Luo, C. Wu, S. Lai, H. Wei, T. Li, Q. Wang, Y. Yu, Attenuation of cyclosporine A induced nephrotoxicity by schisandrin B through suppression of oxidative stress, apoptosis and autophagy, Int. Immunopharmacol. 52 (2017) 15–23.
[62] M. Dalod, R. Chelbi, B. Malissen, T. Lawrence, Dendritic cell maturation: functional specialization through signaling specificity and transcriptional programming, EMBO J. 33 (2014) 1104–1116.
[63] L. van de Laar, P.J. Coffer, A.M. Woltman, Regulation of dendritic cell development by GM-CSF: molecular control and implications for immune homeostasis and therapy, Blood **119** (2012) 3383–3393.
[64] J. Banchereau, R.M. Steinman, Dendritic cells and the control of immunity, Nature 392 (1998) 245–252.
[65] A. Vecchiarelli, D. Pietrella, P. Lupo, F. Bistoni, D.C. McFadden, A. Casadevall, The polysaccharide capsule of *Cryptococcus neoformans* interferes with human dendritic cell maturation and activation, J. Leukoc. Biol. 74 (2003) 370–378.
[66] M.C. Kuppner, A. Scharner, V. Milani, C. Von Hesler, K.E. Tschop, O. Heinz, R.D. Issels, Ifosfamide impairs the allostimulatory capacity of human dendritic cells by intracellular glutathione depletion, Blood 102 (2003) 3668–3674.
[67] A. Agrawal, P. Kaushal, S. Agrawal, S. Gollapudi, S. Gupta, Thimerosal induces TH2 responses via influencing cytokine secretion by human dendritic cells, J. Leukoc. Biol. 81 (2007) 474–482.
[68] W. Chanput, J.J. Mes, H.J. Wichers, THP-1 cell line: an in vitro cell model for immune modulation approach, Int. Immunopharmacol. 23 (2014) 37–45.
[69] D. Flego, M. Bianco, A. Quattrini, F. Mancini, M. Carollo, I. Schiavoni, A. Ciervo, C.M. Ausiello, G. Fedele, *Chlamydia pneumoniae* modulates human monocyte-derived dendritic cells functions driving the induction of a type 1/type 17 inflammatory response, Microb. Infect. 15 (2013) 105–114.
[70] M.G. Netea, C.H. Selzman, B.J. Kullberg, J.M. Galama, A. Weinberg, A.F. Stalenhoef, Van der Meer, W.M. Jos, C.A. Dinarello, Acellular components of *Chlamydia pneumoniae* stimulate cytokine production in human blood mononuclear cells, Eur. J. Immunol. 30 (2000) 541–549.
[71] S. Prebeck, C. Kirschning, S. Durr, C. da Costa, B. Donath, K. Brand, V. Redecke, H. Wagner, T. Miethke, Predominant role of toll-like receptor 2 versus 4 in *Chlamydia pneumoniae*-induced activation of dendritic cells, J. Immunol. 167 (2001) 3316–3323.
[72] F. Cao, A. Castrillo, P. Tontonoz, F. Re, G.I. Byrne, *Chlamydia pneumoniae*-induced macrophage foam cell formation is mediated by toll-like receptor 2, Infect. Immun. 75 (2007) 753–759.
[73] L. Hanski, P. Vuorela, Lead discovery strategies for identification of *Chlamydia pneumoniae* inhibitors, Microorganisms 4 (2016) 43.
[74] M. Krull, J. Kramp, T. Petrov, A.C. Klucken, A.C. Hocke, C. Walter, B. Schmeck, J. Seybold, M. Maass, S. Ludwig, J.G. Kuipers, N. Suttorp, S. Hippenstiel, Differences in cell activation by *Chlamydophila pneumoniae* and *Chlamydia trachomatis* infection in human endothelial cells, Infect. Immun. 72 (2004) 6615–6621.
[75] N. Käding, I. Kaufhold, C. Müller, M. Szaszak, K. Shima, T. Weinmaier, R. Lomas, A. Conesa, P. Schmitt-Kopplin, T. Rattei, Growth of *Chlamydia pneumoniae* is enhanced in cells with impaired mitochondrial function, Front. Cell. Infect. Microbiol. 7 (2017) 499.
[76] A. Mouithys-Mickalad, G. Deby-Dupont, J. Dogne, X. de Leval, S. Kohnen, R. Navet, F. Sluse, M. Hoebeke, B. Pirotte, M. Lamy, Effects of COX-2 inhibitors on ROS produced by *Chlamydia pneumoniae*-primed human promonocytic cells (THP-1), Biochem. Biophys. Res. Commun. 325 (2004) 1122–1130.
[77] R. Sessa, M. Di Pietro, G. Schiavoni, A. Macone, B. Maras, M. Fontana, C. Zagaglia, M. Nicoletti, M. Del Piano, S. Morrone, *Chlamydia pneumoniae* induces T cell apoptosis through glutathione redox imbalance and secretion of TNF-α, Int. J. Immunopathol. Pharmacol. 22 (2009) 659–668.
[78] J. Shin, M. Ebersold, M. Pypaert, L. Delamarre, A. Hartley, I. Mellman, Surface expression of MHC class II in dendritic cells is controlled by regulated ubiquitination, Nature 444 (2006) 115.
[79] E. Walseng, K. Furuta, R.S. Goldszmid, K.A. Weih, A. Sher, P.A. Roche, Dendritic cell activation prevents MHC class II ubiquitination and promotes MHC class II survival regardless of the activation stimulus, J. Biol. Chem. 285 (2010) 41749–41754.