PG2, a botanically derived drug extracted from *Astragalus membranaceus*, promotes proliferation and immunosuppression of umbilical cord-derived mesenchymal stem cells

Yu-Hua Chao<sup>a,b,c</sup>, Kang-Hsi Wu<sup>d,e</sup>, Chiao-Wen Lin<sup>f,g</sup>, Shun-Fa Yang<sup>c,h</sup>, Wan-Ru Chao<sup>b,c,i</sup>, Ching-Tien Peng<sup>d,j</sup>, Han-Ping Wu<sup>k,l</sup>,<sup>⁎</sup>

<sup>a</sup> Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan
<sup>b</sup> School of Medicine, Chung Shan Medical University, Taichung, Taiwan
<sup>c</sup> Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
<sup>d</sup> Division of Pediatric Hematology-Oncology, Children’s Hospital, China Medical University, Taichung, Taiwan
<sup>e</sup> School of Post-baccalaureate Chinese Medicine, China Medical University, Taichung, Taiwan
<sup>f</sup> Institute of Oral Sciences, Chung Shan Medical University, Taichung, Taiwan
<sup>g</sup> Department of Dentistry, Chung Shan Medical University Hospital, Taichung, Taiwan
<sup>h</sup> Department of Pathology, Chung Shan Medical University Hospital, Taichung, Taiwan
<sup>i</sup> Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan
<sup>j</sup> Department of Biotechnology and Bioinformatics, Asia University, Taichung, Taiwan
<sup>k</sup> Division of Pediatric General Medicine, Department of Pediatrics, Chang Gung Memorial Hospital, Taoyuan, Taiwan
<sup>l</sup> College of Medicine, Chang Gung University, Taoyuan, Taiwan

**ARTICLE INFO**

**Keywords:**
Astragalus polysaccharides
Mesenchymal stem cells
PG2
Proliferation
Immunosuppression

**ABSTRACT**

*Ethnopharmacological relevance:* *Astragalus membranaceus* is used to manage the deficiency of vital energy in traditional Chinese medicine and confirmed to have many biological functions. Mesenchymal stem cells (MSCs) possess immunosuppressive effects, and are widely used for regenerative medicine and immune disorders.

*Aims of study:* This study investigated the effects of *Astragalus* polysaccharides (APS) on umbilical cord-derived MSCs (UCMSCs), including morphology, surface marker expression, proliferation, differentiation, and in-vitro and in-vivo immunosuppressive capacities.

*Materials and methods:* MSCs isolated from umbilical cords were used. PG2 injection, a botanically derived drug containing a mixture of APS, was added into the culture medium to prepare PG2-treated UCMSCs. The morphology, surface marker expression, proliferation, and differentiation of UCMSCs were determined. The in-vitro immunosuppressive effects of UCMSCs were examined by peripheral blood mononuclear cell (PBMC) proliferation assay. The in-vivo effects were evaluated by circulatory inflammation-associated cytokine levels in mice with septic peritonitis induced by cecal ligation and puncture (CLP) operation.

*Results:* Compared with control UCMSCs, UCMSCs had higher population doublings when exposed to PG2-containing medium (*P* = 0.003). The reduction rates of PBMC proliferation after phytohemagglutinin stimulation increased significantly when UCMSCs were treated with PG2 (*P* = 0.004). The serum levels of inflammation-associated cytokines, including TNF-α, IL-6, MCP-1, IFN-γ, and IL-1β, were significantly lower at 6 h after CLP in the mice receiving PG2-treated UCMSCs.

*Conclusions:* Our results demonstrated that PG2 can enhance UCMSC proliferation and their in-vitro and in-vivo immunosuppressive effects. Consequently, UCMSCs can be obtained in earlier passages to avoid senescence, and sufficient cells can be acquired faster for clinical use. With stronger immunosuppressive effects, UCMSCs may treat immune disorders more effectively. Further studies are warranted.

<http://dx.doi.org/10.1016/j.jep.2017.06.018>

Received 15 September 2016; Received in revised form 25 April 2017; Accepted 14 June 2017
Available online 23 June 2017
0178-8741/ © 2017 Elsevier B.V. All rights reserved.
1. Introduction

*Astragalus membranaceus* (Fisch.) Bunge, also called Huangqi, is one of the most popular health-promoting herbs in traditional Chinese medicine (http://www.theplantlist.org/tpl1.1/record/ild-32156). It is historically used to manage the deficiency of qi (vital energy) (McKenna et al., 2002). *Astragalus* polysaccharides (APS), the major bioactive components of *Astragalus membranaceus*, were confirmed to have a variety of important biological functions, including immunomodulation, antioxidant and anti-neoplastic properties, anti-inflammation, hepa-protection, hematopoietic promotion, neuro-protection, etc. (Jin et al., 2014).

Mesenchymal stem cells (MSCs), which were first isolated from bone marrow by Friedenstein et al. (Friedenstein et al., 1966), are considered a promising platform for cell-based therapy. However, harvesting MSCs from bone marrow involves an invasive and painful procedure. Umbilical cords are rich in MSCs which can be easily isolated and cultured (Kim et al., 2004; Wang et al., 2004; Secco et al., 2008). Our previous studies demonstrated that umbilical cord-derived MSCs (UCMSCs) could proliferate faster and better than bone marrow-derived MSCs (BMMSCs) (Wu et al., 2011; Chan et al., 2012), suggesting that umbilical cords may be considered a good source of MSCs for clinical use. Over the last decade, clinical application of in vitro expanded MSCs has been evolving rapidly for a variety of diseases (Chao et al., 2012; Wu et al., 2013; Trounson and McDonald, 2015). Given their immunosuppressive properties, MSCs are being investigated in the management of diseases associated with aberrant immune reactions (Nauta and Fibbe, 2007). In our previous studies, we found that UCMSCs had greater immunosuppressive effects than BMMSCs and could be used to treat patients with severe acute graft-versus-host diseases effectively and safely (Wu et al., 2011; Chan et al., 2012).

In vitro passaging leads to a gradual decrease in the proliferative potential of MSCs. During culture, the occurrence of MSC senescence, such as the decrease of immunosuppressive capacity, also has a great impact on the outcomes in their clinical utility (Galipeau, 2013). *Astragalus membranaceus* is a traditional herb commonly-used to reinforce vital energy, but whether the proliferative and immunosuppressive capacities of MSCs can be enhanced by *Astragalus membranaceus* has not been reported. Here, we aimed to investigate the influence of PG2 (a mixture of APS extracted from the roots of *Astragalus membranaceus*) on the characteristics of MSCs, including morphology, surface marker expression, differentiation, proliferation, and in vitro and in vivo immunosuppressive capacities.

2. Materials and methods

2.1. Chemicals and reagents

PG2 injection was purchased from PhytoHealth Corporation (Taipei, Taiwan) and reconstituted with normal saline before use. Culture medium (Dulbecco’s modified Eagle medium; DMEM), fetal bovine serum (FBS), and antibiotic-antimycotic were from Gibco (Gaithersburg, MD). Except insulin which was from Novo Nordisk A/S (Bagsvaerd, Denmark), all chemicals and reagents for osteogenic and adipogenic induction were obtained from Sigma (St Louis, MO). Reagents for immunophenotypic analysis and cytometric bead array immunoassay were purchased from BD Biosciences (San Jose, CA). Reagents used in peripheral blood mononuclear cell (PBMC) proliferation assay, including RPMI-1640 and phytohemagglutinin (PHA), were from Sigma-Aldrich (St Louis, MO). And cell proliferation reagent WST-1 was from Roche Diagnostics GmbH (Mannheim, Germany).

2.2. Isolation of MSCs from umbilical cords

The study was approved by the institutional review board of Chung Shan Medical University Hospital (CSMUH No: CS2-15091). With written informed consents from parents, umbilical cords were obtained from full-term infants immediately after birth. MSCs were isolated and cultured as per our previous studies (Chao et al., 2014; Wu et al., 2016). Shortly after, cord blood vessels were carefully removed to retain Wharton’s jelly. Wharton’s jelly was digested in 1 mg/ml collagenase and then placed in high-glucose DMEM supplemented with 10% FBS and antibiotic-antimycotic. Cells were incubated at 37 °C with 5% CO2 in a humidified atmosphere. After 48 h, the medium with suspension of non-adhered cells was discarded, and fresh medium was added. Thereafter, the medium was changed twice a week. When reaching 80–90% confluence, cultured cells were detached with trypsin-EDTA (Gibco, Carlsbad, CA) and replated for subculture. Cultured MSCs of passage 5 were used for further studies.

2.3. PG2, a preparation of APS

PG2 injection (purchased from PhytoHealth Corporation, Taiwan), a botanically derived drug, contains a mixture of APS extracted, isolated, and purified from the roots of *Astragalus membranaceus* (Chen et al., 2012; Kuo et al., 2015). The detailed information about PG2 injection can be found in the supplementary documents, and also at http://www.phytohealth.com.tw/en/product/W/product_detail. With high and consistent quality, the preparation of the raw materials, intermediates, and final products were in compliance with GMP requirements. The molecular weights of PG2 ranged between 20,000 and 60,000 Da, and the dominant polysaccharides were α,1,4-linked glucans with varying degrees of branching at the 6 positions of the backbone residues. Other polysaccharides and glycoproteins in PG2 were arabinogalactanCs, rhamnogalacturonans, and arabinogalactan proteins (Kuo et al., 2015). Each vial of 500 mg of PG2 was prepared in sterile powder, and reconstituted with 10 ml of normal saline by shaking thoroughly until completely dissolved.

2.4. Preparation of PG2-treated UCMSCs and assessment of proliferative capacity

To prepare PG2-treated UCMSCs, PG2 was added into the culture medium to a final PG2 concentration of 200 mg/l. Control UCMSCs were grown in the regular medium without PG2 (i.e. DMEM supplemented with 10% FBS and antibiotic-antimycotic). Cultured UCMSCs of 1 × 10⁶ cells were plated in 10-cm dishes and incubated at 37 °C for 72 h. Cells were then detached with trypsin-EDTA and collected. The population doubling was calculated using the following equation: population doubling = log₂ (the number of cells at harvest/the number of seeded cells).

2.5. Immunophenotypic analysis

MSCs were detached from culture dishes, and then washed and resuspended in phosphate-buffered saline (PBS; Gibco, Gaithersburg, MD). After fixing and blocking, cells were immunolabeled with fluorescein isothiocyanate or phycoerythrin-conjugated mouse anti-human antibodies specific to CD34, CD45, CD14, CD29, CD44, CD73, CD90, CD105, HLA-A, HLA-B, HLA-C or HLA-DR following the manufacturer's instruction. Nonspecific mouse IgG served as the isotype control. Data were analyzed by flow cytometry (FACSCalibur; BD Biosciences, San Jose, CA) with CellQuest software.

2.6. Assessment of osteogenic and adipogenic potential

To assess the differentiation potential, MSCs were plated in 60-mm dishes. Differentiation of MSCs was induced and evaluated as our previous studies (Chao et al., 2010, 2014; Wu et al., 2016). To induce osteogenesis, MSCs were grown in DMEM with 10% FBS, 10 mM ⍺-glycerophosphate, 0.1 μM dexamethasone, and 0.2 mM ascorbic acid. On day 14, osteogenic differentiation was demonstrated by alkaline
phosphatase (ALP) activity. For further quantification, 2 ml of 0.05 N NaOH in ethanol was added to each dish, and the extraction was measured by spectrophotometry (Ultrospec 1100 pro; Amersham Biosciences) at 550 nm.

To promote adipogenesis, MSCs were incubated in DMEM with 10% FBS, 1 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, 0.1 mM indomethacin, and 10 μg/ml insulin. On day 14, adipogenic differentiation was demonstrated by intracellular accumulation of lipid droplets stainable with oil red O. The dye content was eluted by ethanol and quantified spectrophotometrically.

2.7. PBMC proliferation assay

For use as responder cells, PBMCs were cultured in RPMI-1640 as 200 μl at 5 × 10^5 cells/ml per well in 96-well plates (Chan et al., 2012). 100 μl of 5 μg/ml PHA was added to each well as a stimulator. Suppressive effects of MSCs (PG2 treated or not) on PBMC proliferation were assessed. The ratio of PBMC:MSC was 10:1. Following co-culture for 3 days, 10 μl of cell proliferation reagent WST-1 was added to each well and absorbance was measured with a microplate reader (Molecular Devices Corporation, Sunnyvale, CA) at 550 nm. To further evaluate the influence of PG2 on PBMC proliferation, a series of PG2 concentrations in the medium was used, including 50, 100, and 200 mg/l. After incubation for 3 days, WST test was performed as previously described.

2.8. Evaluation of inflammation-associated cytokine levels in mice with septic peritonitis induced by cecal ligation and puncture (CLP) operation

CLP operation was performed to induce septic peritonitis in mice (Hubbard et al., 2005). Six-week-old male C57BL/6 mice were purchased from BioLASCO Taiwan Co. Before surgery, the mice were anesthetized by intramuscular injections of 75 mg/kg ketamine and 5 mg/kg xylazine. After laparotomy, the distal one half of the cecum was ligated with a 4-0 silk tie. A single through-and-through perforation was made in the ligated segment with a 21-gauge needle, and a 1-mm column of fecal material was extruded through the puncture site. The cecum was then replaced into the abdomen and the abdominal incision was closed. Immediately after operation, one million MSCs (PG2 treated or not) in 0.5 ml PBS were administered via intraperitoneal injection. Mice in the sham group underwent the same operative procedure but without ligation and needle perforation of the cecum; they received PBS in a volume of 0.5 ml with no cells after operation.

All mice were sacrificed at 6 h after operation, and blood was obtained by cardiac puncture immediately after death. To determine circulating cytokine levels, serum was separated by centrifugation at 10,000g for 10 min at 4 °C. The concentrations of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, monocyte chemotactic protein (MCP)-1, interferon-γ (IFN-γ), and IL-1β were measured by cytometric bead array immunoassay (BD CBA Mouse Soluble Protein Flex Set System; BD Biosciences, San Jose, CA), according to the manufacturer’s instruction. Shortly after, a mixture of capture bead reagent (50 μl) was added to a serum sample (50 μl) and incubated for 1 h at room temperature. 50 μl of the mixed PE detection reagent was then added and further incubated for 1 h. After washing, the samples were analyzed using flow cytometry (FACSCanto; BD Biosciences, San Jose, CA) with FCAP Array™ software.

2.9. Statistical analysis

Data analysis was performed using SPSS 17.0 for Windows. Students paired t-test and t-test were used to compare the characteristics of UCMSCs with and without PG2 treatment. The influence of PG2 on PBMC proliferation was tested by one-way ANOVA, and Games-Howell test for post hoc analysis. A value of P < 0.05 was considered to be statistically significant.

3. Results

3.1. Identification of UCMSCs

First, the basic properties of MSCs, including in-vitro morphology, surface marker expression, and differentiation potential (Dominici et al., 2006), were used as indicators to identify MSCs isolated from umbilical cords. In-vitro, UCMSCs adhered to culture plates and showed a spindle-shaped fibroblastic morphology. They expressed CD29, CD44, CD73, CD90, CD105, HLA-A, HLA-B, and HLA-C, and negative for CD34, CD45, CD14, and HLA-DR. Under induction conditions, UCMSCs were capable of achieving osteogenic and adipogenic differentiation. These findings were in accordance with the criteria of the International Society for Cellular Therapy (Dominici et al., 2006).

3.2. No alteration in morphology, surface marker expression, and differentiation potential after PG2 treatment

The influence of PG2 on the basic properties of UCMSCs, including morphology, surface marker expression, and differentiation potential, were evaluated. Both UCMSCs with and without PG2 treatment shared a similar spindle-shaped fibroblastic morphology in-vitro (Fig. 1) and a consistent immunophenotypic profile which was positive for CD29, CD44, CD73, CD90, CD105, HLA-A, HLA-B, and HLA-C, and negative for CD34, CD45, CD14, and HLA-DR.
Fig. 2. Differentiation potential. Under specific induction conditions, both UCMSCs with and without PG2 treatment could differentiate into osteocytes (ALP activity, ×100) and adipocytes (Oil red O stain, ×100). Further quantification by spectrophotometry revealed that PG2 treatment did not alter the capacities of UCMSCs for osteogenesis ($P = 0.865$) and adipogenesis ($P = 0.798$). $n = 10$. All procedures were performed in triplicate. Students paired $t$-test was used for statistical analysis.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>UCMSCs</th>
<th>PG2-treated UCMSCs</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proliferative potential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Population doublings</td>
<td>$1.648 \pm 0.414$</td>
<td>$1.976 \pm 0.603$</td>
<td>$0.003^*$</td>
</tr>
<tr>
<td><strong>Differentiation potential</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteogenesis (alkaline phosphatase activity)</td>
<td>$0.240 \pm 0.131$</td>
<td>$0.238 \pm 0.138$</td>
<td>$0.865$</td>
</tr>
<tr>
<td>Adipogenesis (oil red O stain)</td>
<td>$0.796 \pm 0.258$</td>
<td>$0.784 \pm 0.186$</td>
<td>$0.798$</td>
</tr>
<tr>
<td><strong>Suppressive effects of UCMSCs on PBMC proliferation after PHA stimulation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Increase of PBMC proliferation</td>
<td>$0.067 \pm 0.017$</td>
<td>$0.035 \pm 0.018$</td>
<td>$0.002^*$</td>
</tr>
<tr>
<td>Reduction rate of PBMC proliferation</td>
<td>$0.454 \pm 0.112$</td>
<td>$0.716 \pm 0.159$</td>
<td>$0.004^*$</td>
</tr>
</tbody>
</table>

PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; UCMSCs, umbilical cord-derived mesenchymal stem cells.

Data are presented as mean ± standard deviation.

$^*$ $P < 0.05$. 
CD45, CD14, and HLA-DR. Both were able to achieve osteogenic and adipogenic differentiation under specific induction conditions, as demonstrated by ALP activity and Oil red O stain, respectively (Fig. 2). As shown in Table 1 and Fig. 2, further quantification by spectrophotometry revealed that PG2 treatment did not alter the capacities of UCMSCs for osteogenesis and adipogenesis ($P = 0.865$ and 0.798, respectively).

3.3. Enhancement of UCMSC proliferation after PG2 treatment

The impact of PG2 on proliferative potential of UCMSCs was assessed. When exposed to PG2-containing medium, UCMSCs had significantly higher population doublings compared with control UCMSCs ($P = 0.003$), as shown in Table 1 and Fig. 3. An augmentation of greater than 10% could be found in eight of the ten, and greater than 30% in three. Our results indicated that PG2 had positive effects on the proliferation of UCMSCs.

3.4. Augmentation of suppression on PBMC proliferation from UCMSCs after PG2 treatment

We evaluated the suppressive effects of UCMSCs on PBMC proliferation, and compared the difference between UCMSCs with and without PG2 treatment (Table 1 and Fig. 4A). Consistent with our previous report (Chan et al., 2012), the increase of PBMC proliferation after PHA stimulation was significantly inhibited when PBMCs co-cultured with UCMSCs ($P < 0.001$). When UCMSCs were treated with PG2 before co-culture, the inhibition effects were amplified significantly ($P = 0.002$). As shown in Fig. 4B, the reduction rates of PBMC proliferation after PHA stimulation increased significantly when UCMSCs were treated with PG2 ($P = 0.004$). Our results indicated that the suppressive effects of UCMSCs on PBMC proliferation could be augmented by PG2 treatment.

The impact of PG2 on PBMC proliferation was further assessed. PBMC proliferation increased after PHA stimulation, whether PG2 was added into the medium or not (Fig. 5A). Compared with the regular medium (i.e. no PG2 within the medium), adding PG2 into the medium of 50, 100, or 200 mg/l did not significantly alter the increase of PBMC proliferation ($P = 0.975$). Our results implied that PG2 alone did not exhibit suppressive effects on PBMC proliferation (Fig. 5B).

3.5. Augmentation of immunosuppression on mice with septic peritonitis from UCMSCs after PG2 treatment

Fig. 6 illustrates the circulatory inflammation-associated cytokine levels in the mice with CLP-induced septic peritonitis at 6 h after operation. Compared with the mice in the control group (i.e. mice without any intervention) and sham group, CLP could induce inflammatory reactions in the mice, as shown in an increase in the serum levels of TNF-$\alpha$, IL-6, MCP-1, IFN-$\gamma$, and IL-1$\beta$ at 6 h after operation. These inflammation-associated cytokine levels were significantly lower in the mice receiving PG2-treated UCMSCs than in those receiving control UCMSCs after CLP, indicating the augmentation of immunosuppressive capacity in UCMSCs by PG2 treatment.

4. Discussion

Clinical application of in-vitro expanded MSCs is evolving rapidly for a variety of diseases (Chao et al., 2012; Wu et al., 2013; Trounson and McDonald, 2015). In clinical practice, it is an important concern to obtain sufficient cells with better functions in a shorter period of time. In the present study, we found that PG2 could enhance the proliferative and in-vitro and in-vivo immunosuppressive capacities of UCMSCs without alterations in their morphology, surface marker expression, and differentiation potential. To our knowledge, no other plant product has been reported to have influenced the characteristic features of MSC’s proliferative and immunosuppressive capacities. This is the first report about the effects of APS on human MSCs, and our study suggests the useful role of PG2 in the preparation of MSCs for clinical use.

In traditional medicine, Astragalus membranaceus was historically used to manage the deficiency of vital energy (McKenna et al., 2002). It is not only widely used as a Chinese medicinal herb in many Asia countries, but also popularly used in Western countries as functional food and a dietary supplement. Despite its complicated constituents, modern phytochemistry has proven that APS is the main bioactive ingredient of Astragalus membranaceus (Jin et al., 2014). PG2, a purified extract of Astragalus membranaceus, is mostly composed of APS with consistent and high quality (Kuo et al., 2015). A double-blind, randomized controlled study demonstrated that PG2 could treat cancer-related fatigue effectively in patients with advanced cancer (Chen et al., 2012). In-vitro passaging and culturing could result in the occurrence of MSC senescence, such as decreases in the proliferative and immunosuppressive capacities, and these characteristic changes may influence the outcomes in their clinical use (Galipeau, 2013). In the present study, we found that adding PG2 into the culture medium could increase the proliferative and in-vitro and in-vivo immunosuppressive capacities of UCMSCs, just like reinforcing vital energy into the MSCs.

To get sufficient cells for clinical use, in-vitro expansion of MSCs is essential. However, passing during culture causes MSCs to gradually lose their progenitor properties (Banfi et al., 2000; Dominici et al., 2006). Therefore, it is important to acquire enough MSCs of earlier passages in clinical applications. The present study demonstrated that PG2-treated UCMSCs had higher population doublings. Therefore, the time needed to acquire enough cells could be shorter, and younger cells
of earlier passages could be obtained. Our study suggested that adding PG2 into the culture medium can be useful in the preparation of MSCs for clinical use.

MSCs interact with a variety of immune cells, and have been investigated in the management of clinical diseases associated with aberrant immune responses (Nauta and Fibbe, 2007). We found that UCMSCs are able to effectively treat patients with severe graft versus host disease, which is typical of immune-mediated host tissue damage (Wu et al., 2011). Our previous studies also demonstrated the beneficial effects of MSCs on septic animals by exerting their immunosuppressive functions (Chao et al., 2014; Wu et al., 2016). Moreover, we found that UCMSCs had greater immunosuppressive effects than BMMSCs (Wu et al., 2011; Chan et al., 2012). We speculated that using MSCs with more potent immunosuppressive capacities could increase the efficacy of disease control. In the present study, there was no adverse effect of PG2 treatment on PBMC proliferation which was comparable to the findings of Zhuge et al. (2012). The increase of PBMC proliferation was inhibited when PBMCs co-cultured with UCMSCs, and the inhibition effects were amplified when UCMSCs were treated with PG2. We speculated that PG2 could increase the immunosuppressive capacities of UCMSCs which may contribute to the increase of the suppressive effects on PBMC proliferation. Moreover, the circulating levels of inflammation-associated cytokines were significantly lower in the mice receiving PG2-treated UCMSCs than in those receiving control UCMSCs after CLP-induced septic peritonitis. Our studies demonstrated that PG2 treatment increased the immunosuppressive capacities of UCMSCs, both in-vitro and in-vivo.

5. Conclusions

Our data provide the first evidence that PG2 can enhance the proliferative and immunosuppressive capacities of UCMSCs. As a commonly-used traditional medicine that is highly safe, Astragalus membranaceus can be useful in modern cell-based therapy. Further studies are warranted.

Author contributions

Yu-Hua Chao designed the study and wrote the manuscript. Kang-Hsi Wu, Chiao-Wen Lin, and Shun-Fa Yang carried out the experi-
control UCMSCs after CLP, indicating the augmentation of immunosuppressive capacity in UCMSCs after PG2 treatment. Data are presented as mean ± SEM. Students t-test was used for statistical analysis. n = 6–7 mice/group.

Fig. 6. Immunosuppressive effects of UCMSCs on the mice with CLP-induced septic peritonitis. Serum levels of inflammation-associated cytokines, including TNF-α, IL-6, MCP-1, IFN-γ, and IL-1β, were measured by cytometric bead array immunoassay at 6 h after surgery. Compared with the mice in the control group (i.e. mice without any intervention) and sham group (i.e. mice receiving sham operation), the serum levels of TNF-α, IL-6, MCP-1, IFN-γ, and IL-1β were significantly lower in the mice receiving PG2-treated UCMSCs than in those receiving control UCMSCs after CLP, indicating the augmentation of immunosuppressive capacity in UCMSCs after PG2 treatment. Data are presented as mean ± SEM. Students t-test was used for statistical analysis. n = 6–7 mice/group.

Conflict of interests

The authors declare that they have no conflict of interests.

Acknowledgments

This study was supported by the Chung Shan Medical University Hospital (CSH-2016-C-013); the China Medical University Hospital (DMR-105-039); the Ministry of Science and Technology (MOST 105-2314-B-040-017); the Research Laboratory of Pediatrics, Children’s Hospital, China Medical University; the Taiwan Ministry of Health and Welfare Clinical Trial Center (MOHW106-TDU-B-212-113004).

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jep.2017.06.018.

References


