Original Article

DOI: 10.6966/EDMJ.202412_11(4).0002

The Improvement of Endothelial Function by Guizhi Fuling Wan via Its Effects on IL-1β-Treated First Trimester Decidual Cells and Subsequent Interaction with Macrophages

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Objectives: Healthy pregnancies require healthy endothelial cells and proper angiogenesis. Endothelial dysfunction leads to abnormal implantation and adverse outcomes. Currently, no effective intervention is available for the treatment of this condition. Guizhi Fuling Wan (GFW) is a traditional Chinese medicine that has shown promise in reducing angiogenesis. We hypothesized that it would promote endothelial cell growth, reduce dysfunction, and promote blood vessel formation. Specifically, GFW promoted endothelial cell proliferation and ameliorated endothelial dysfunction, particularly in pro-inflammatory first-trimester decidual cell (FTDC)-affected macrophages.

Methods: Cell Counting Kit-8 (CCK8) assay was used to examine the effects of GFW on the proliferation of endothelial cells co-cultured with macrophages treated by conditioned media derived from IL-1β-treated FTDCs. The expression of various markers of angiogenesis and endothelial dysfunction in endothelial cells in response to macrophages affected by IL-1β-treated FTDCs in the presence or absence of GFW was assessed. Students' t-tests were used to compare the differences.

Results: GFW reduced the proliferation of human umbilical artery endothelial cells (HUAECs) in a dose-dependent manner. Macrophages enhanced endothelial proliferation in GFW-treated FTDCs. GFW modulated the effects of IL-1β-treated FTDCs on macrophages by indirectly regulating the expression of various factors by HUAECs. The expression of endothelial nitric oxide synthase (eNOS) and endothelin-1 in HUAECs was influenced by macrophages affected by IL-1β-treated FTDCs in the presence of GFW.

Conclusions: GFW might introduce both positive and negative effects on angiogenesis. It maintains a balanced homeostasis in the maternal-fetal interface via the interactions of FTDCs, macrophages, and endothelial cells in response to pro-inflammatory stimuli.

Key words: Fetal growth restriction, Spontaneous abortion, Guizhi Fuling Wan, Endothelial dysfunction, Proliferation

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Received: November 1, 2023 Accepted: January 22, 2024

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Introduction

Implantation failure accounts for 75% of pregnancy loss.[1] The maternal-fetal interface during implantation and placentation exhibits a pro-inflammatory immune profile.[2] Immune maladaptation with excessive inflammation at the maternal-fetal interface is pivotal in impaired implantation and subsequent poor placentation.[3] Thus, maintaining immune balance at the maternal-fetal interface is crucial for safeguarding tolerance to semi-allogeneic embryos and defense against pathogens. Appropriate angiogenesis and endothelial function on both sides of the maternal-fetal interface are mandatory for successful implantation, placentation, and subsequent gestation. Endothelial cells play an essential physiological role in angiogenesis and subsequent maintenance of utero-placental circulation.[4] After the blastocyst implants, development and expansion of the placental vasculature are required for adequate nutrient transport and gas-waste exchange. Blastocyst-derived extravillous trophoblasts (EVTs) traverse the decidua and the inner third of the myometrium to interact with resident decidual cells, decidual natural killer cells, and macrophages (Mϕs).

Invading EVTs transform uterine spiral arteries into high-capacitance vessels accompanied by the expression of angiogenic factors and microvascular angiogenesis.[5] In early pregnancy, decidual Mϕs and decidual cells are positioned to affect angiogenesis,[6,7] a highly regulated process. In addition to decidual vascular remodeling, sufficient proliferation of endothelial cells and proper endothelial function are essential for embryo acceptance and pregnancy maintenance. The resulting increased uterine blood flow to the intervillous space is crucial for subsequent fetal-placental development.[8,9] Adverse pregnancy outcomes such as preeclampsia, spontaneous abortion (SA), and intrauterine growth restriction (IUGR) can also result from impaired decidual vascular remodeling, which leads to endothelial dysfunction at the maternal-fetal interface.[10,11] The dysfunctional and activated endothelial cells at the maternal-fetal interface produce an array of inflammatory cytokines and vasoconstrictors, such as endothelin-1 (ET-1) and tumor necrosis factor (TNF), and enter the systemic circulation to exert detrimental effects on endothelial cells in other organ systems.[12]

Guizhi Fuling Wan (GFW) initially appeared in Chung-Ching Chang's "Synopsis of the Golden Chamber approximately 1,800 years ago. GFW is used to treat qi/blood stagnation, which is believed to activate blood circulation and alleviate blood stasis.[13] Clinically, GFW is recommended for treating threatened abortion and abdominal masses such as uterine leiomyoma.[14,15] GFW administration to pregnant women is accompanied by minimal maternal side effects. Our previous results showed that GFW improved fetal placental development in SA/IUGR-prone mice and inhibited decidual inflammation. Conditioned media derived from IL-1β- or TNF-α-treated first trimester decidual cells enhances the angiogenesis-inhibiting activity of Mϕs.[15] However, treatment of first-trimester decidual cells (FTDCs) with GFW reverses this effect, suggesting that GFW may improve placentation by promoting decidual angiogenesis in the pro-inflammatory microenvironment. We tested the hypothesis that GFW promotes endothelial cell proliferation and ameliorates endothelial dysfunction under the influence of pro-inflammatory FTDC-affected Mϕs. Direct and indirect effects of GFW on endothelial cell proliferation were also evaluated. In addition, the regulation of endothelial dysfunction of endothelial cells under the influence of Mϕs affected by IL-1β-treated FTDCs in the presence or absence of GFW was investigated.

Patients and Methods

Experimental approach

Evaluating the direct and indirect effects of GFW on the proliferation of human umbilical artery endothelial cells (HUAECs), cells were treated with various concentrations of GFW (0, 1.25, 2.5, 5.0, 7.5 μg/mL). Then, the regulation of endothelial dysfunction of HUAECs under the influence of macrophages affected by IL-1β-treated FTDCs in the presence or absence of GFW was examined.

Cell culture

FTDCs were be cultured with DMEM/F12 (D2906, Sigma-Alderich), 10% charcoal dextran stripped calf serum, 1X ITS Plus Premix (354352, Corning), 1X Pen-Strep-Amp B antibiotic (03-033-1B, BI), 2 mM L-glutamine. HUAECs were cultured in EBM-2 medium supplemented with 15% fetal calf serum (CC-3202, LONZA). HUAECs (6×10^3 cells/200 μL/well) were seeded into a 48-well plate and incubated for 18 hours. Then, various concentrations (0, 0.25, 2.5, 5.0, 7.5 μg/mL) of GFW were added into the culture. After 48 hours, the medium was replaced, followed by an evaluation of the proliferation of HUAECs by Cell Counting Kit-8 (CCK-8) assay.

Decidualization and preparation of cytokine-neutralized conditioned medium

FTDCs were seeded at a density of 1.0×10^4 cells/mL and cultured for 18 hours, followed by treatment with complete DMEM/F12 media containing 10^{-8} M estradiol (E_2) + 10^{-7} M medroxyprogesterone acetate (MPA) for 7 days. Then, the media was changed every 3.5 days. Subsequently, the cells were pre-treated with or without 1.25 μg/mL of GFW for 24 hours. Defined medium contained DMEM/F12 (D2906, Sigma-Alderich), 1X ITS Plus Premix (354352, Corning), 1X Pen-Strep-Amp B antibiotic (03-033-1B, BI), 2 mM L-glutamine, 5 μM $FeSO_4$, 50 μM $ZnSO_4$, 1 nM $CuSO_4$, 20 nM Na_2SeO_3, 50 μg/mL ascorbic acid, 50 ng/mL EGF, 10^{-8} M E_2, and 10^{-7} M MPA. After washing with 1X PBS x 2, the cells were cultured in a defined medium ± 1.25 μg/mL of GFW with or without 10 ng/mL of IL-1β for 24 hours. Then, the conditioned media was collected and centrifuged at 500 x *g* for 5 minutes with the supernatant recovered. After adding 2.2 μL of 0.5 mg/mL anti-IL-1β (α-IL-1β) antibody, the conditioned media (1 mL) was rotated for 2 hours at room temperature (25℃). After the rotation, 15 μL of 50% Sepharose G was added, followed by rotation for 1 hour at RT. Ultimately, cytokine-antibody complexes were removed by G-sepharose to generate a cytokine-neutralized conditioned medium (CNCM).

Development of macrophages from THP-1 cells

THP-1 cells, a human monocytic leukemic cell line, were cultured at a density of 2.4×10^5 cells/mL in 100 ng of phorbol 12-myristate 13-acetate (PMA)-treated RPMI-1640 media supplemented with 10% FBS and 1% penicillin/streptomycin solution (Biological Industries, Israel). THP-1 cells were cultured in 0.2 ml/transwell inserts at a density of 4.8×10^4 cells/mL with 0.4 μm pore size fitting 24-well plates for 72 hours at 37℃ in a 5% CO_2, humidified incubator. Then, macrophages were cultured with PMA-free complete RPMI-1640 for 24 hours. The cells were washed with 1X PBS x2 before adding 0.2 mL of CNCM and cultured for another 48 hours.

Co-culture of THP-1-derived macrophages and HUAECs

HUAECs with a 4.0×10^4 cells/mL density, 0.6 mL/well, were cultured in a 24-well plate for 24 hours. After washing the culture medium, the HUAECs were further cultured with an endothelial cell culture medium (CC-3202, LONZA). Similarly, the culture medium for CNCM-induced THP-1-derived macrophages was replaced by an

endothelial cell culture medium (CC-3202, LONZA) after washing and being placed in the HUAECs wells. Both cells were co-cultured at 37℃ in a 5% CO_2, humidified incubator for 24 hours.

Cell Counting Kit-8 (CCK-8) assay

CCK-8 was a metabolic activity assay to test cell proliferation and a water-soluble tetrazolium salt. WST-8 (2-[2-methoxy-4-nitrophenyl]-3-[4-nitrophenyl]-5-[2,4-disulfophenyl]-2H-tetrazolium, monosodium salt) produced a water-soluble formazan dye. Lactate dehydrogenase reduced tetrazolium salt to formazan, a colored substance. The absorbance value was read with a microplate reader to quantify the proliferation of cells. The amount of the formazan dye generated by dehydrogenases in cells is directly proportional to the number of living cells. 100 μL of cell suspension (5,000 cells/well) was dispensed in a 96-well plate and preincubated the plate for 24 hours in a humidified incubator at 37℃ in a 5% CO_2, humidified incubator. Then, HUAECs were added to the plate and incubated with CCK-8 solution (v/v: 10:1, CK04 – 05, Dojindo) for 2 hours, followed by measuring the absorbance at 450 nm using a microplate reader.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA from HUAECs was extracted using a Gene-spin Total RNA purification kit (PT-RNA-MS-50, PROTECH). Reverse transcription reactions were done by adding 0.5 μg of Oligo(dT)18 to 1 μg of total RNA and incubated at 65℃ for 5 minutes before the addition of 13 μL of reverse transcription reaction solution (4μL 5X reaction buffer, 0.5 μL of 40 U/μL RNase Inhibitor, 0.5 μL of 200 U/μL MMLV reverse transcriptase, 2 μL of 10 mM dNTP) using an MMLV reverse transcription kit. The primer sets for ET-1, endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), placental growth factor (PlGF), and AngII are listed in Table 1. The samples were incubated at 42℃ for 60 minutes in a PCR reactor. The enzyme activity was then dissipated at 70℃ for 10 minutes. Subsequently, 5 ng of cDNA, 1 μL of 1 μM forward and reverse primers, and 10 μL of PowerUp SYBR master mix (A25741, Thermo Fisher) were mixed. The reaction was performed using a StepOnePlus Real-Time PCR System with Stage 1: 50℃ for 2 minutes; Stage 2: 95℃ for 10 minutes; Stage 3: 95℃ for 15 seconds, then 60℃ for 1 minute; Stage 4: 95℃ for 15 seconds, 60℃ for 15 seconds, and 95℃ for 15

Table 1. Primer sets.

Gene	Forward (5' – 3')	Reversed (5' – 3')
AngII	ATTCAGCGACGTGAGGATGGCA	GCACATAGCGTTGCTGATTAGTC
eNOS	GAAGGCGACAATCCTGTATGGC	TGTTCGAGGGACACCACGTCAT
ET-1	CTACTTCTGCCACCTGGACATC	TCACGGTCTGTTGCCTTTGTGG
PlGF	GGCGATGAGAATCTGCACTGTG	ATTCGCAGCGAACGTGCTGAGA
VEGFA	TTGCCTTGCTGCTCTACCTCCA	GATGGCAGTAGCTGCGCTGATA
VEGFR2	GGAACCTCACTATCCGCAGAGT	CCAAGTTCGTCTTTTCCTGGGC
FLT-1	CCTGCAAGATTCAGGCACCTATG	GTTTCGCAGGAGGTATGGTGCT
LIF	AGATCAGGAGCCAACTGGCACA	GCCACATAGCTTGTCCAGGTTG
GAPDH	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

AngII: Angiopoietin 2; eNOS: endothelial nitric oxide synthase; ET-1: Endothelin-1; PlGF: Placental growth factor; VEGFA: Vascular endothelial growth factor A; VEGFR2: Vascular endothelial growth factor receptor-2; FLT-1: fms related receptor tyrosine kinase-1; LIF: Leukemia inhibitory factor; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

seconds. Melting curves were used to verify the specificity of the amplified products and the absence of primer-dimer formation. The $2^{-\Delta\Delta Ct}$ method determined the relative gene expression. The quantitation of each analyte was determined and normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

Enzyme-linked immunosorbent assay (ELISA)

Culture media of HUAECs were tested for secreted endothelin-1 (ET-1) (DET100, R&D). After adding 150 µL of Assay Diluent RD1-105 to the well, 75 µL of HUAEC CM was added and incubated for 1 hour at RT on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. After the wash × 4, 200 µL of ET-1 was conjugated to each well and incubated for 3 hours at RT on the shaker. After wash. 200 µL of Substrate Solution was added to each well and incubated for 30 minutes at RT. Finally, 50 µL of Stop Solution was pipetted to each well. The optical density was determined using a microplate reader set to 450 nm. HUAEC cell lysates were tested by eNOS (NBP2-80134, NOVUS). The standard working solution was incubated with HUAEC cell lysate for 90 minutes at 37℃. Then, 100 µL of Biotinylated Detection antibody working solution was added and incubated for 1 hour at 37℃ followed by the addition of 100 µL of horseradish peroxidase (HRP) Conjugate working solution after washing and incubated for 30 minutes at 37℃. Then, 90 µL of Substrate Reagent was added and incubated for 15 minutes at 37℃. Finally, 50 µL of Stop Solution was pipetted, and the optical density (OD value) was determined by a microplate reader set to 450 nm.

Statistics

One-way analysis of variance (ANOVA) for multiple comparisons was conducted to analyze dose-dependent inhibition of HUAEC proliferation after GFW treatment. Student's t-test was used to compare the difference in various tests between HUAECs influenced by IL-1β-treated FTDCs treated with or without GFW and control groups. All statistical differences were analyzed using SigmaPlot software 11.0 (Systat Software, San Jose, CA). $p < 0.05$ was considered statistically significant.

Results

GFW reduced HUAEC proliferation in a dose-dependent manner

The proliferation of HUAECs was decreased by 6.05 ± 1.81% after being treated with 1.25 µg/mL of GFW compared to the control (Fig. 1). Moreover, 2.50, 5.00, and 7.50 µg/mL of GFW also decreased the proliferation of HUAECs by 8.86 ± 1.92, 10.04 ± 1.81, and 11.68 ± 2.01%, respectively (Fig. 1). Dose-dependent inhibition of HUAEC proliferation was also confirmed by one-way ANOVA ($p < 0.05$).

Enhancement of endothelial proliferation by Mϕs under influence of GFW-treated FTDCs

No significant change in HUAEC proliferation was observed after co-culturing with

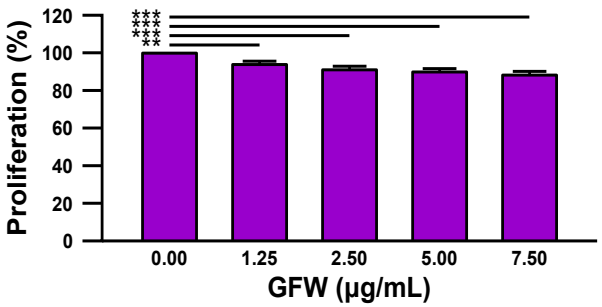

*Fig. 1 Dose-dependent inhibition of HUAEC proliferation by GFW. Proliferation of HUAECs after dose-dependent GFW treatment was measured using CCK-8 assay. Results indicate mean ± SEM. One-way ANOVA; **p < 0.01 and ***p < 0.001.*

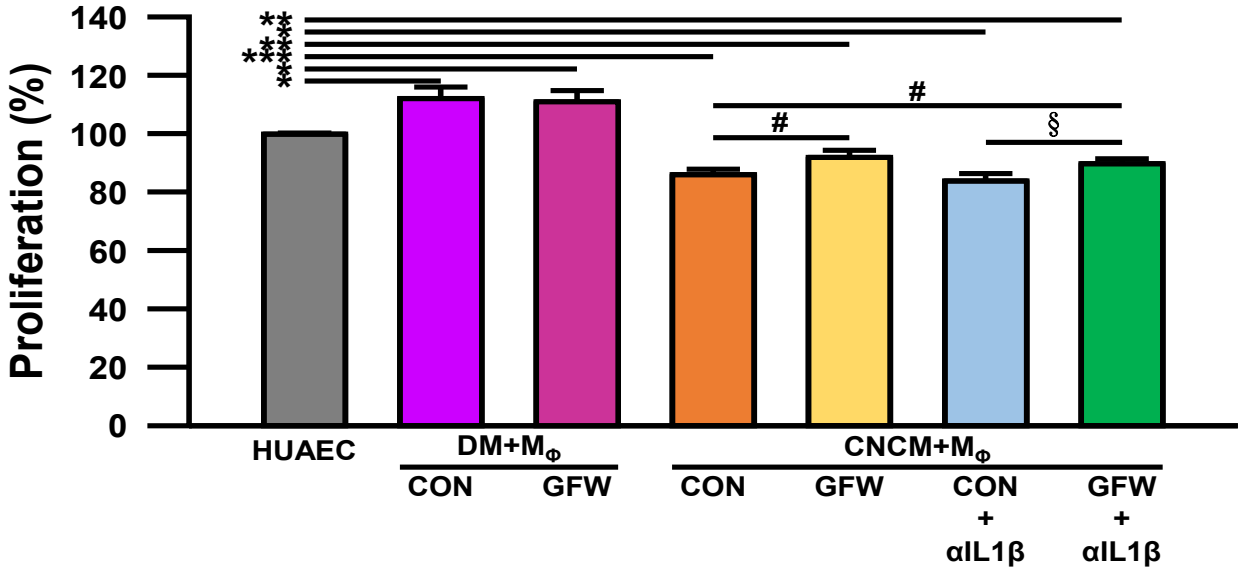

*Fig. 2 Enhancement of HUAEC proliferation by FTDC-affected macrophages in presence of GFW. CCK-8 assay was used to measure proliferation of HUAECs by IL-1β-treated FTDC-affected macrophages. Results indicate mean ± SEM. *Comparison with HUAEC; *p < 0.05, **p < 0.01, and ***p < 0.001. #Comparison to CON in CNCN + Mϕ; #p < 0.05. §Comparison to CON with αIL-1β in CNCN + Mϕ; §p < 0.05. DM + Mϕ: defined medium-treated macrophage; CNCM + Mϕ: cytokine-neutralized conditioned medium-treated macrophage; αIL1β: anti-IL-1β.*

Mϕs treated with GFW (Fig. 2). The CCK-8 assay showed that proliferation enhancement of HUAECs influenced by Mϕs were affected by IL-1β-treated FTDCs (Fig. 2). In the control group, cell proliferation was increased by 5.9% after GFW treatment. In the IL-1β-affected FTDCs group, the proliferation of HUAECs was increased by 5.92% after GFW treatment (Fig. 2).

GFW modulated effects of IL-1β-treated FTDCs on Mϕs by indirectly regulating expression of various factors in HUAECs

The morphology of HUVECs showed no differences among the treatment groups (Fig. 3A). qRT-PCR showed that the expression levels of AngII (Fig. 3B) and LIF (Fig. 3C) in HUAECs were correspondingly decreased by 0.91 ± 0.05 and 0.65 ± 0.22-fold after being influenced by IL-1β-treated FTDC-affected Mϕs. In contrast, the expression levels of AngII and LIF were reversed upon GFW treatment by 1.10 ± 0.05 and 1.53 ± 0.24-fold increases, respectively. No significant changes were found in the expression levels of eNOS (Fig. 3D), PIGF (Fig. 3E), FLT-1 (Fig. 3F), VEGF-A (Fig. 3G), and VEGFR2 (Fig. 3H) in HUAECs after indirect co-culture with IL-1β-treated FTDCs-affected Mϕs. However, the expression levels of eNOS, PIGF, FLT-1, VEGF-A, and VEGFR2 increased by 1.17 ± 0.01, 1.15 ± 0.08, 1.11 ± 0.06, 1.27 ± 0.10, and 1.10 ± 0.04-fold, respectively, after being influenced by Mϕs affected by IL-1β-treated FTDCs treated with GFW. In contrast, IL-1β-treated FTDC-affected Mϕs enhanced the expression of ET-1 (Fig. 3I) in HUAECs by 1.09 ± 0.03-fold. After adding GFW to IL-1β-treated FTDCs, the expression levels were decreased by 0.92 ± 0.04-fold.

Expression levels of eNOS and ET-1 in HUAECs were influenced by Mϕs affected by IL-1β-treated FTDCs in presence of GFW

ELISA revealed no significant changes in eNOS expression levels in HUAECs after

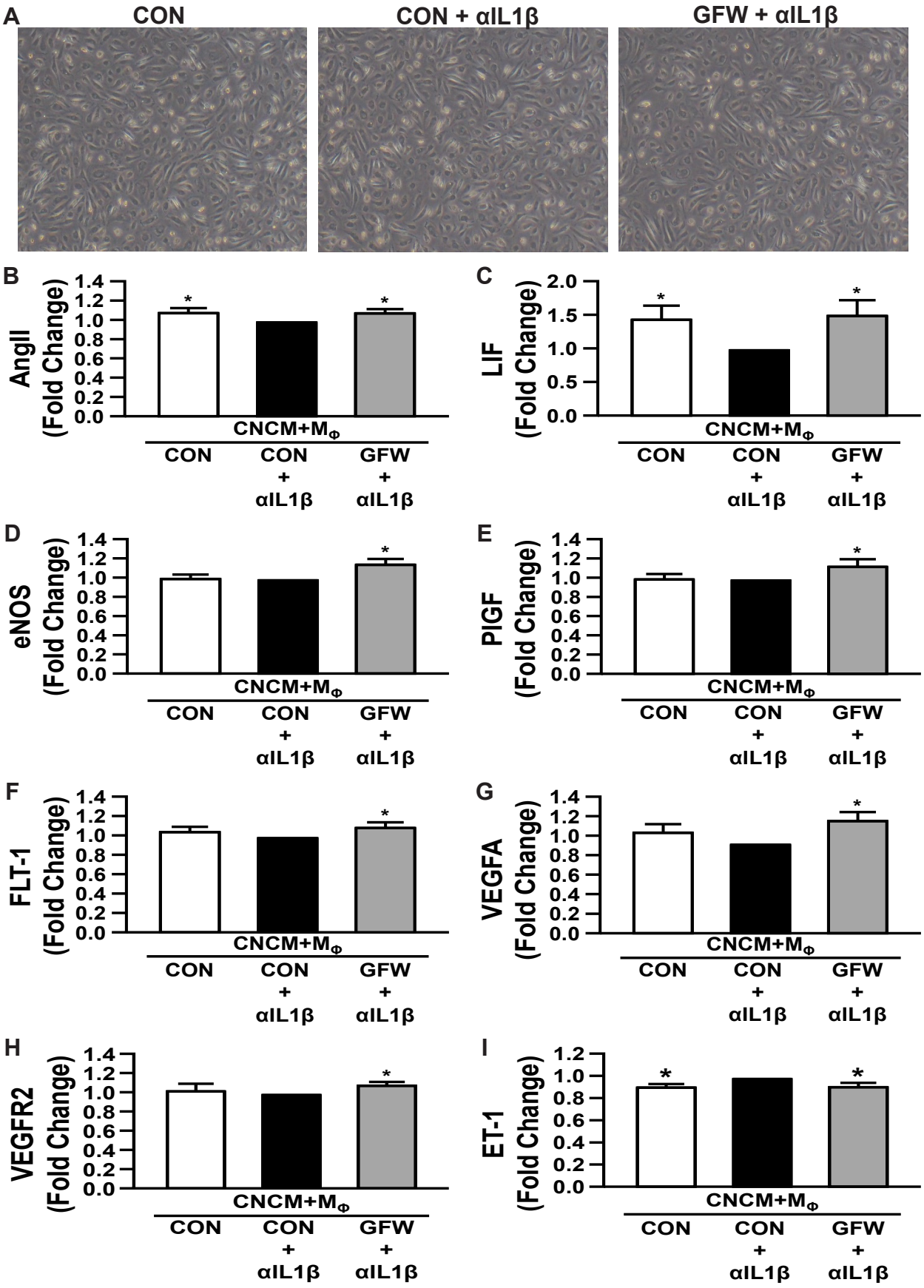

*Fig. 3 Regulation of various factors in HUAECs co-cultured with macrophages affected by IL-1β-treated FTDCs in presence of GFW.(A) The morphology of HUVECs were showed in three treatment groups. mRNA expression levels of (B) AngII, (C) LIF, (D) eNOS, (E) PIGF, (F) FLT-1, (G) VEGFA, (H) VEGFR2, and (I) ET-1 were measured using qRT-PCR. Results indicate mean ± SEM. *Comparison to CON with αIL-1β; *p < 0.05. CNCM + Mϕ: cytokine-neutralized conditioned medium-treated macrophage; αIL1β: anti-IL-1β. Magnification: 40x.*

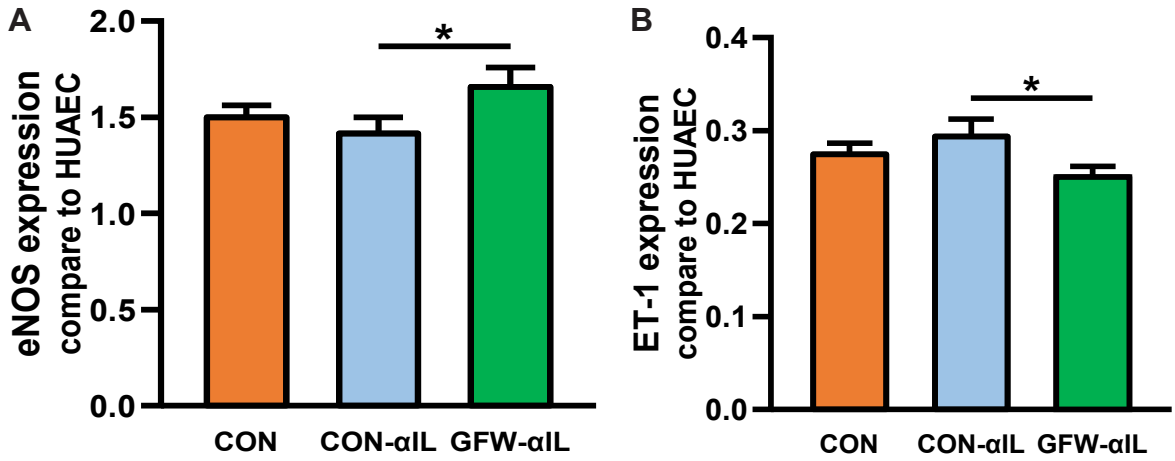

*Fig. 4 GFW indirectly regulated expression levels of (A) eNOS and (B) ET-1 in HUAECs via macrophages affected by IL-1β-treated FTDCs. ELISA was used to measure protein expression levels. Results indicate mean ± SEM. *Comparison to CON with αIL-1β; *p < 0.05. CNCM + Mϕ: cytokine-neutralized conditioned medium-treated macrophage; αIL1β: anti-IL-1β.*

indirect co-culture with IL-1β-treated FTDC-affected Mϕs (Fig. 4A). However, the expression levels of eNOS in HUAECs were increased by 1.17 ± 0.01-fold after being influenced by Mϕs affected by IL-1β-treated FTDCs in the presence of GFW. There were no significant changes in ET-1 expression levels in HUAECs after indirect co-culture with IL-1β-treated FTDC-affected Mϕs, whereas the addition of GFW to IL-1β-treated FTDCs decreased these levels by 0.88 ± 0.03-fold.

Discussion

The current study aimed to investigate the effects of GFW on pro-inflammatory conditions that potentially predispose women to adverse pregnancy outcomes, such as pre-eclampsia and miscarriage. Normal development of the embryo is dependent on successful implantation and placentation.[16] The cellular and molecular mechanisms responsible for adverse pregnancy outcomes, such as SA and IUGR, remain unclear, and no effective therapeutic or preventative intervention is currently available. Furthermore, the therapeutic mechanisms underlying the effects of GFW are not fully understood. A previous study indicated that GFW may augment decidual angiogenesis, which is beneficial for placentation.[15] GFW is a traditional Chinese herbal formula that consists of Cinnamomum cassia BLUME (Guizhi), Poria cocos WOLF (Fuling), Prunus persica BATSCH (Taoren), Paeonia lactiflora PALL (Chishao), and Paeonia suffruticosa ANDREWS (Mudanpi). Guizhi and Fuling are the most crucial components of GFW in treating inflammatory diseases such as endometriosis, as they are the emperor drugs of this formula, whereas Taoren is the minister drug. Shaoyao and Moutanpi are assistant drugs. However, further studies are necessary to determine the main components that contributed to the present study. We further examined the direct effects of GFW on endothelial cell proliferation and whether GFW-treated IL-1β-stimulated FTDCs indirectly affected endothelial cell proliferation regulated by Mϕs. Direct treatment of HUAECs with GFW inhibits their proliferation in a dose-dependent manner. Appropriate angiogenesis and endothelial function at the maternal-fetal interface are critical. A current study showed that GFW enhances HUAEC proliferation by Mϕs under the influence of IL-1β-treated FTDCs. This will open new avenues for establishing therapeutic strat-

egies to combat pregnancy complications by improving endothelial cell proliferation.

In the present study, GFW reversed the effects of IL-1β-treated FTDCs on Mϕs in regulating the gene expression of AngII, LIF, eNOS, PIGF, FLT-1, VEGF-A, and VEGFR2 in HUAECs. Hence, an increased expression of these factors was observed after the addition of GFW. In contrast, GFW inhibited the capacity of IL-1β-treated FTDCs in the enhancement of Mϕs by promoting ET-1 expression in HUAECs. According to the results, IL-1β treatment mimicked a pro-inflammatory environment; therefore, the effects of GFW were examined. Furthermore, the comparison between the control group and IL-1β + GFW verified whether GFW treatment reversed the effect of IL-1β on control levels.

The increased eNOS and decreased ET-1 expression levels in HUAECs by Mϕs affected by GFW-treated IL-1β-stimulated FTDCs suggest that GFW treatment indirectly acts through Mϕs to promote and inhibit eNOS and ET-1 production, respectively, in HUAECs. Therefore, perfusion and endothelial dysfunction at the maternal-fetal interface may be improved. Furthermore, ongoing studies have evaluated angiogenesis by examining the migration and tube formation of endothelial cells under the influence of Mϕs affected by pro-inflammatory stimulus-treated FTDCs in the presence or absence of GFW. HUAECs were obtained from umbilical arteries instead of human endometrial endothelial cells (HEECs) derived from the endometrium and were used in the current study because of their availability. Future experiments should confirm these findings using HEECs.

Kooy et al. reported increased endothelial cell proliferation in the endometrium of patients with menorrhagia,[17] suggesting the potentially detrimental effects of excessive endothelial cell proliferation on endometrial stability. In contrast, Tworry et al. revealed that increased endometrial VEGF expression may improve endometrial receptivity by promoting vascular growth and maintenance.[18] However, the impact of decreased endothelial cell proliferation in the endometrium is not fully understood. The present study showed that endothelial cell proliferation was inhibited by the direct treatment with GFW. Paradoxically, through paracrine effects, GFW boosts endothelial cell proliferation by Mϕs affected by IL-1β-treated FTDCs. The concept of Yin and Yang is pivotal to the philosophy and practices of Traditional Chinese Medicine. Thus, treatment always imposes effects on the dual energies of Yin and Yang, resulting in homeostasis. Therefore, the results of this study suggest that GFW may have both positive and negative effects on angiogenesis. It maintains homeostasis at the maternal-fetal interface via the interactions of FTDCs, Mϕs, and endothelial cells in response to pro-inflammatory stimuli.

Author Contributions

Study Design, Yu-Chieh Fang, Ya-Chun Yu and S. Joseph Huang; Data Collection, Yu-Chieh Fang and Ya-Chun Yu; Statistical Analysis, Ya-Chun Yu; Data Interpretation, Yu-Chieh Fang and S. Joseph Huang; Manuscript Preparation, Yu-Chieh Fang and S. Joseph Huang; Literature Search, Yu-Chieh Fang and Ya-Chun Yu; Funding Acquisition, S. Joseph Huang.

Funding

This research was funded by E-Da Hospital Research grants, EDAHC109002 (YCF), EDAHI110004 (SJH) and EDAHP112008 (SJH).

Institutional Review Board Statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of the E-Da

Hospital (protocol code EMRP65107N and 2019/06/06 of approval).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

Not applicable.

Acknowledgments

The authors thank Brion Research Institute of Taiwan for providing Guizhi Fuling Wan.

Conflicts of Interest

The authors have no conflicts of interest relevant to this article.

References

1. Norwitz ER, Schust DJ, Fisher SJ: Implantation and the survival of early pregnancy. N Engl J Med 2001;345:1400-8. doi: 10.1056/NEJMra000763.
2. Weiss G, Goldsmith LT, Taylor RN, et al: Inflammation in reproductive disorders. Reprod Sci 2009;16:216-29. doi: 10.1177/1933719108330087.
3. Yeh CC, Chao KC, Huang SJ: Innate immunity, decidual cells, and preeclampsia. Reprod Sci 2013;20:339-53. doi: 10.1177/1933719112450330.
4. Sen S, Rao R, Chaudhuri G: Endothelial cell function in utero-placental circulation physiology and pathophysiology. Curr Vasc Pharmacol 2013;11:730-6. doi: 10.2174/1570161111311050010.
5. Klauber N, Rohan RM, Flynn E, et al: Critical components of the female reproductive pathway are suppressed by the angiogenesis inhibitor AGM-1470. Nat Med 1997;3:443-6. doi: 10.1038/nm0497-443.
6. Croy BA, Chen Z, Hofmann AP, et al: Imaging of vascular development in early mouse decidua and its association with leukocytes and trophoblasts. Biol Reprod 2012;87:125. doi: 10.1095/biolreprod.112.102830.
7. Khaliq A, Li XF, Shams M, et al: Localisation of placenta growth factor (PIGF) in human term placenta. Growth Factors 1996;13:243-50,color plates I-II,pre.bk cov. doi: 10.3109/08977199609003225.
8. Kaufmann P, Black S, Huppertz B: Endovascular trophoblast invasion: implications for the pathogenesis of intrauterine growth retardation and preeclampsia. Biol Reprod 2003;69:1-7. doi: 10.1095/biolreprod.102.014977.
9. Moffett-King A: Natural killer cells and pregnancy. Nat Rev Immunol 2002;2:656-63. doi: 10.1038/nri886.
10. Bakrania BA, Spradley FT, Drummond HA, et al: Preeclampsia: Linking Placental Ischemia with Maternal Endothelial and Vascular Dysfunction. Compr Physiol 2020;11:1315-49. doi: 10.1002/cphy.c200008.
11. Roberts JM: Endothelial dysfunction in preeclampsia. Semin Reprod Endocrinol 1998;16:5-15. doi: 10.1055/s-2007-1016248.
12. Sen A, Most P, Peppel K: Induction of microRNA-138 by pro-inflammatory cytokines causes endothelial cell dysfunction. FEBS Lett 2014;588:906-14. doi: 10.1016/j.febslet.2014.01.033.
13. Tsai CC, Kao CT, Hsu CT, et al: Evaluation of four prescriptions of traditional Chinese medicine: syh-mo-yiin, guizhi-fuling-wan, shieh-qing-wan and syh-nih-sann on experimental acute liver damage in rats. J Ethnopharmacol 1997;55:213-22. doi: 10.1016/s0378-8741(96)01503-6.
14. Chen NN, Han M, Yang H, et al: Chinese herbal medicine Guizhi Fuling Formula for treatment of uterine fibroids: a systematic review of randomised clinical trials. BMC Complement Altern Med 2014;14:2. doi: 10.1186/1472-6882-14-2.
15. Piao L, Chen CP, Yeh CC, et al: Chinese herbal medicine for miscarriage affects decidual micro-environment and fetal growth. Placenta 2015;36:559-66. doi: 10.1016/j.placenta.2015.02.006.
16. Wulff C, Weigand M, Kreienberg R, et al: Angiogenesis during primate placentation in health and disease. Reproduction 2003;126:569-77. doi: 10.1530/rep.0.1260569.
17. Kooy J, Taylor NH, Healy DL, et al: Endothelial cell proliferation in the endometrium of women with menorrhagia and in women following endometrial ablation. Hum Reprod 1996;11:1067-72. doi: 10.1093/oxfordjournals.humrep.a019298.
18. Torry DS, Holt VJ, Keenan JA, et al: Vascular endothelial growth factor expression in cycling human endometrium. Fertil Steril 1996;66:72-80. doi.org/10.1016/S0015-0282(16)58390-3.