Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health 23 (2021) 205–210

Contents lists available at ScienceDirect

Pregnancy Hypertension: An International Journal of Women's Cardiovascular Health

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

Resveratrol and grape juice: Effects on redox status and nitric oxide production of endothelial cells in *in vitro* preeclampsia model

Mayara Caldeira-Dias [a], Sarah Viana-Mattioli [a], Jackeline de Souza Rangel Machado [b], Mattias Carlström [c], Ricardo de Carvalho Cavalli [b], Valéria Cristina Sandrim [a,*]

[a] Department of Pharmacology, Institute of Biosciences of Botucatu, Sao Paulo State University (UNESP), Distrito Rubiao Junior, Botucatu, São Paulo 18680-000, Brazil
[b] Department of Gynecology and Obstetrics, Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (USP), Ribeirao Preto, São Paulo 14049-900, Brazil
[c] Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm 17177, Sweden

ARTICLE INFO

Keywords:
Endothelial cells
Grape juice
Plasma
Preeclampsia
Resveratrol

ABSTRACT

Preeclampsia (PE) is a hypertensive disorder of pregnancy and it is one of the main causes of maternal and fetal morbidity and mortality worldwide. It is known that oxidative stress plays a role in its pathophysiology, therefore we investigated the effects of trans-resveratrol, a potent antioxidant, on the Nrf2/ARE pathway, nitric oxide (NO) production, and reactive oxygen species (ROS) levels in an *in vitro* model of PE. Plasma from PE patients increased ARE activity in endothelial cells compared with plasma from healthy pregnant (HP), and the addition of resveratrol was able to potentiate this increase only in PE. Resveratrol also decreased ROS levels in the cells incubated with plasma from PE. Based on these results, we performed a pilot clinical study to compare the effects of serum from PE women before and 1 h after ingestion of polyphenol-rich whole red grapefruit juice incubated on endothelial cells, since grapefruit contains large amounts of resveratrol. Serum from PE patients, obtained one hour after juice intake, decreased antioxidants markers in cells compared with the serum before juice intake, besides, it increased NO production. In conclusion, resveratrol and polyphenol-rich red grape juice have potentially beneficial effects on endothelial cells incubated with PE plasma/serum, which could aid in the management of PE.

1. Introduction

Preeclampsia (PE) affects 3–8% of pregnancies and is one of the main causes of maternal and fetal morbidity and mortality worldwide [1,2]. This multisystemic syndrome is characterized by hypertension after 20 weeks of gestation accompanied by proteinuria or other systemic alterations, such as thrombocytopenia, renal insufficiency, impaired liver function, pulmonary edema, or cerebral/visual symptoms [3]. There is no treatment, apart from the early delivery of the placenta [2]. The pathophysiology of PE is complex and is still not fully elucidated, however it is generally agreed that oxidative stress plays a role, contributing to the endothelial dysfunction and clinical manifestation of this syndrome [4]. Therefore, antioxidants, such as resveratrol, have been extensively studied to verify if they could reduce the risk and aid in the management of [5–7]. Studies showed that resveratrol can improve trophoblast and endothelial dysfunction in human cells [8–10] and it is a safe and effective adjuvant of oral nifedipine to attenuate hypertensive symptoms among PE patients [7], suggesting its potential to assist the treatment of PE.

Resveratrol is a polyphenolic compound present in a variety of fruits, mostly in red grapes. It is an antioxidant that directly scavenges free radicals and also modulates several targets, such as activation of endothelial nitric oxide synthase (eNOS), increasing nitric oxide (NO) production, and the nuclear factor-erythroid-derived 2-related factor-2 (Nrf2) [11]. Nrf2 binds to the antioxidant response element (ARE) promoter sequence, upregulating the expression of antioxidant proteins,

Abbreviations: ARE, antioxidant response element; DAF-2, diaminofluorescein; DBP, diastolic blood pressure; DCFH-DA, dichlorodihydrofluorescein diacetate; eNOS, endothelial nitric oxide synthase; GA, gestational age; GPX, glutathione peroxidase; GSH, glutathione; GSR, glutathione reductase; HO-1, heme oxygenase-1; HP, healthy pregnant; HUVEC, human umbilical vein endothelial cells; L-NAME, N^{w}-nitro-L-arginine methyl ester; miRNA, microRNA; MTT, 3-(4,5-dimethylthiazol2-yl)−2,5-diphenyltetrazolium; NA, not applicable; NO, nitric oxide; Nrf2, nuclear factor-erythroid-derived 2-related factor-2; PE, preeclampsia; ROS, reactive oxygen species; SBP, systolic blood pressure.

* Corresponding author.
E-mail address: valeria.sandrim@unesp.br (V.C. Sandrim).

https://doi.org/10.1016/j.preghy.2021.01.001
Received 1 June 2020; Received in revised form 11 January 2021; Accepted 16 January 2021
Available online 21 January 2021

including heme oxygenase-1 (HO-1) and glutathione reductase (GSR), and hence, countering oxidative stress and balancing the redox state in cells [12]. Interestingly, studies have shown that consumption of dealcoholized red wine and polyphenol-rich juices, such as grape juice, decreased blood pressure levels in humans [13,14]; however, to our knowledge, no study has focused on PE.

Progressively studies have shown the role of microRNAs (miRNAs) in the regulation of cellular redox, modulating the expression of genes associated with the Nrf2 pathway [15]. MiRNAs are small endogenous non-coding RNAs (18–24 nucleotides) that post-transcriptionally regulate gene expression resulting in the repression of protein expression [16]. Changes in the expression profile of miRNAs have been found in placental tissue and plasma/serum from PE women, suggesting a role of miRNAs in PE [17,18]. Besides, several studies have shown that resveratrol modulates miRNAs in human diseases [19], however apparently there is no study regarding PE.

Incubation of endothelial cells with plasma/serum from PE women is a well-established *in vitro* model that mimics one of the two-stages of PE, allowing the comprehension of molecular mechanisms regarding endothelial dysfunction in this syndrome [20–22].

In light of these findings, this study aimed to verify whether resveratrol could activate the Nrf2 pathway, induce the expression of antioxidant molecules and NO production, and reduce ROS in an *in vitro* model of PE. Also, we examined whether PE serum collected after grape juice intake incubated in endothelial cells could modulate those factors.

2. Materials and methods

More details for Materials and Methods in the online Supplementary Material.

2.1. Samples of in vitro studies using trans-resveratrol

For *in vitro* studies, eight PE women (PE) and four healthy pregnant (HP) were recruited from the ambulatory clinic at Hospital das Clinicas Faculdade de Medicina de Ribeirao Preto da Universidade de São Paulo (HCFMRP-USP). This sample size is considered sufficient for the *in vitro* model used, as observed in previous studies [20,21,23]. The study was approved by the Institutional Review Board at Ribeirao Preto Medical School, Brazil (reference 4682/2006, approved date June 20, 2006), following the principles of the Declaration of Helsinki, and all subjects gave written informed consent. Diagnosis criteria of PE were defined by the American College of Obstetricians and Gynecologists (ACOG) [3]. Exclusion criteria included twin pregnancy, chronic hypertension, hemostatic abnormalities, diabetes mellitus, fetal abnormalities, cancer, and cardiovascular, autoimmune, renal, and hepatic diseases. Maternal venous blood samples were collected in tubes containing heparin, rapidly centrifuged (1000*g* for 10 min) at room temperature and plasma samples were stored at −70 °C.

2.2. Pilot clinical study using red grape juice

For this pilot study, four PE patients recruited at HCFMRP-USP were consented to drink 200 mL of a commercial organic whole red grape juice (Carraro, RS, Brazil). The study was approved by the Institutional Review Board at Ribeirao Preto Medical School, Brazil (process number 2.602.100/2018, approved date April 16, 2018). PE was diagnosed according to ACOG guidelines [3]. Exclusion criteria included smokers, chronic alcohol consumption, pre-gestational obesity (BMI $>$ 30), gestational diabetes and diabetes mellitus, dyslipidemia, cardiovascular and inflammatory diseases, and cancer. Before grape juice intake, women were in a fast period of approximately 12 h. Before grape juice intake (0 h) and after 1 h (1 h), venous blood samples were collected in tubes with clot activator gel, which were rapidly centrifuged (1000*g* for 10 min) at room temperature. Serum was collected after 1 h of grape juice intake based on a previous study showing that this period was the peak absorption of polyphenols [24]. Serum samples were stored at −70 °C for *in vitro* assays.

2.3. Cell culture, plasma/serum incubation, and trans-resveratrol intervention

Human umbilical vein endothelial cells (HUVEC) (CRL 2873, American Type Culture Collection (ATCC)) were cultured until reaching 80–90% of confluence. HUVEC were incubated in supplemented DMEM with 10% (v/v) plasma from PE or HP and 1 μM of trans-resveratrol or the vehicle DMSO in the incubator for 24 h. For trans-resveratrol intervention in the *in vitro* studies, the concentration of 1 μM was chosen based on previous studies using trans-resveratrol *in vitro*, including one of our group [23,25,26]. HUVEC were also incubated with 10% (v/v) serum from PE at 0 h (before grape juice intake) and 1 h (after grape juice intake) for 24 h. Cells were used until passage 8.

2.4. Cell viability by MTT assay

Cell viability was performed using the 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium (MTT) assay as described previously [27]. Viability was compared to control (untreated cells with vehicle DMSO, 100% viability). Each sample was performed in triplicate.

2.5. Messenger RNA expression by qPCR

Total RNA from HUVEC incubated with plasma from PE and HP in the presence or absence of trans-resveratrol was isolated according to the manufacturer's protocol. Isolated RNA was consistently found to be pure. RNAs were transcribed into cDNA according to the manufacturer's protocol. qPCR reaction was performed using SYBR Green. The analyzed genes were: *NFE2L2*, *HMOX1*, and *HPRT1*. According to a previous study [21], *HPRT1* was selected as an endogenous control because it was the gene most stable in our samples. Relative quantification was calculated using the comparative 2(−Delta C(T)) method [28]. PCR reactions were performed in duplicate for each sample.

2.6. miRNA selection and expression by qPCR

miRNAs from the let-7 family (a, b, and c) were selected as they target the mRNA of the *NFE2L2* gene, based on bioinformatics analysis using two databases (Microrna.org [29] and miRWalk 2.0 [30]). Those miRNAs are expressed in HUVEC [31]. From the same isolated RNA used for messenger RNA expression, reverse transcription (RT) was performed according to the manufacturer's protocol. qPCR reaction was performed using SYBR Green. Relative quantification was calculated using the comparative 2(−Delta Delta C(T)) [28] and normalization was performed to U6 snRNA. PCR reactions were performed in duplicate for each sample.

2.7. Measurement of HO-1 concentrations

HO-1 quantification in cell supernatants was accessed using enzyme-linked immunosorbent assay kit Human Total HO-1/HMOX1 ELISA (R&D Systems, MN, USA), according to the manufacturer's protocol. Optical density was determined at 450 nm in the spectrophotometer. A standard curve was generated by incubation of HO-1 solutions (156.25–10,000 pg/mL). Each sample was performed in duplicate.

2.8. Measurement of intracellular NO

Quantification of intracellular NO was accessed by measuring the fluorescence of 4,5-Diaminofluorescein (DAF-2) diacetate. The fluorescence signal was measured (excitation 495 nm, emission 535 nm) and expressed as arbitrary units. Each sample was performed in duplicate.

2.9. Glutathione quantification

Total glutathione (GSH) quantification was performed in cell lysates using the Glutathione Colorimetric Detection Kit according to the manufacturer's protocol. Optical density was determined at 405 nm in the spectrophotometer. A standard curve was generated by incubation of oxidized glutathione standard solutions (0.78–25 μM). Each sample was performed in duplicate.

2.10. Measurement of intracellular ROS

Quantification of intracellular ROS was accessed by measuring the fluorescence of 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA). Fluorescence was determined using wavelengths of 502 nm of excitation and 523 nm of emission in a multifunctional plate reader. Tert-Butyl hydroperoxide solution at 250 μM for 2 h was used as a positive control. Each sample was performed in duplicate.

2.11. Antioxidant response element (ARE) activation by luciferase reporter assay

ARE activation was accessed using ARE Reporter Kit (BPS Bioscience, CA, USA). HUVEC were seeded into a white 96-well plate at a concentration of 1×10^4 cells per well in a final media volume of 100 μL. On the next day, cells were transfected using Lipofectamine® 2000 (Thermo Scientific) with ARE reporter or negative control reporter for 12 h. After transfection, cells were treated with plasmas in the presence or absence of 1 μM trans-resveratrol or serum from the groups for 24 h. For detecting the luciferase expression after the treatment, the Dual-Glo® Luciferase assay system (Promega, WI, USA) was used and luminescence was measured in a multifunctional plate reader (Synergy 4, BioTek®). The positive control was considered the addition of trans-resveratrol at 1 μM for 24 h (fold = 1).

2.12. Statistical analysis

Parametric *t*-tests were performed for comparisons between HP and PE groups, and comparison between 0 h and 1 h (after grape juice intake) groups. Paired *t*-test was performed for comparison within HP and PE groups between the presence and the absence of trans-resveratrol. GraphPad Prism 5.0 (GraphPad Software, CA, USA) was used for these analyses. Gene and miRNA expression data were analyzed using the GeneGlobe Data Analysis Center (Qiagen®) online platform. P-value <0.05 (two-tailed) was considered significant.

3. Results

The study design and workflow are shown in Fig. S1. Clinical characteristics of women whose plasma samples were collected and used to perform the study with resveratrol are shown in Table 1. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were significantly increased in the PE group compared with the HP group. The gestational age (GA) at delivery was significantly lower in PE compared with HP.

We did not observe a significant difference in cell viability between HP and PE and the addition of resveratrol did not change the viability of all cultures (all $p > 0.05$, Fig. S2).

We observed no significant difference in both gene expressions (*NFE2L2* and *HMOX1*, Fig. S3A and B, respectively) and miRNAs (let-7a, b, and c, Fig. S3C, D, and E, respectively) expressions in HUVEC incubated with plasma from HP and PE, and resveratrol did not alter those expressions in either the groups. To validate the effects of resveratrol alone, resveratrol without plasma was incubated in the cells and we observed increased 1.5 and 7.9-fold the expression of *NFE2L2* and *HMOX1*, respectively, compared with only cells (data not shown).

HO-1 levels in cell supernatant (Fig. 1A) were not significantly different between the groups and resveratrol did not alter the concentrations. However, when we analyzed intracellular NO levels (Fig. 1B), cells incubated with plasma from the PE group had lower levels compared with HP, and the addition of resveratrol did not alter these levels. We also measured total GSH levels in cell lysates (Fig. 1C). There was no difference between PE and HP, however, the addition of resveratrol increased GSH levels only in the HP group. ROS levels were also examined (Fig. 1D) and, although there was no difference between PE and HP groups, resveratrol was able to decrease ROS levels in PE by ~30% (±7, S.E.M.). For ROS levels, positive control was 19.78 ± 2.29 of fluorescence intensity $\times 10^3$. Finally, ARE activity (Fig. 1E) was absent in HP however, in PE it was significantly increased and resveratrol potentiated this increase (~78% ± 9).

Table 1
Clinical characteristics of pregnant enrolled in the study.

Parameters	HP (n = 4)	PE (n = 8)
GA at sampling (weeks)	36 ± 1	35 ± 2
Maternal age (years)	24 ± 3	26 ± 3
BMI (kg/m^2)	28 ± 3	30 ± 3
SBP at sampling (mmHg)	110 ± 7	133 ± 4*
DBP at sampling (mmHg)	73 ± 5	86 ± 3*
GA at delivery (weeks)	40 ± 1	35 ± 2*
Newborn weight (g)	2945 ± 135	2513 ± 419
Antihypertensive drugs at blood collection[1]		
α-Methyldopa (%)	NA	75
Nifedipine (%)	NA	12.5
Hydralazine (%)	NA	12.5

Values are the means ± S.D. or percentage. GA, gestational age; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; NA, not applicable. *$P < 0.05$ vs controls.
[1] All PE patients were under antihypertensive drug treatment.

Besides the *in vitro* study of plasma incubation from PE and HP patients and resveratrol, we performed a pilot clinical study to compare the effects of serum incubation in endothelial cells from PE women before and after 1 h of whole red grapefruit juice ingestion. Serum from the 1 h group significantly decreased ~17% ± 4 HO-1 concentration (Fig. 2A), ~50% ± 2 GSH levels (Fig. 2C) and ~69% ± 7 ARE activity (Fig. 2E) compared with 0 h (basal). However, it increased intracellular NO levels (Fig. 2B) and did not alter ROS (Fig. 2D). For ROS levels, positive control was 20.38 ± 2.29 of fluorescence intensity $\times 10^3$.

4. Discussion

This study was the first to report that plasma from PE patients (PE) increased ARE activity in endothelial cells compared with plasma from healthy pregnant (HP), and the addition of resveratrol was able to potentiate this increase. Most interestingly, serum from PE patients collected after one hour of grape juice intake decreased ARE activity and HO-1 production in endothelial cells compared with serum from PE before grape juice intake, however, it increased NO production. These findings show that after one hour of acute grape juice intake, serum from PE was able to affect endothelial cells, acting on more mechanisms compared with cotreatment of plasma from PE and resveratrol. Collectively, our results suggest that the effects of resveratrol and other components present in grape juice and its metabolites may be beneficial for endothelial cells, and therefore, grape juice intake could aid in the management of PE.

Resveratrol is a polyphenolic compound that has multiple properties and can modulate several cell-signaling molecules. Studies have shown that resveratrol improves the therapeutic outcome of patients suffering from diabetes mellitus, cancer, Alzheimer's disease, kidney diseases, inflammatory diseases, cardiovascular diseases, and complicated pregnancies [32,33]. Regarding PE, there are few studies about the effects of resveratrol, however, the results are promising [7–10,23]. Our group has recently demonstrated that resveratrol improves endothelial cell markers impaired by plasma incubation from patients before the

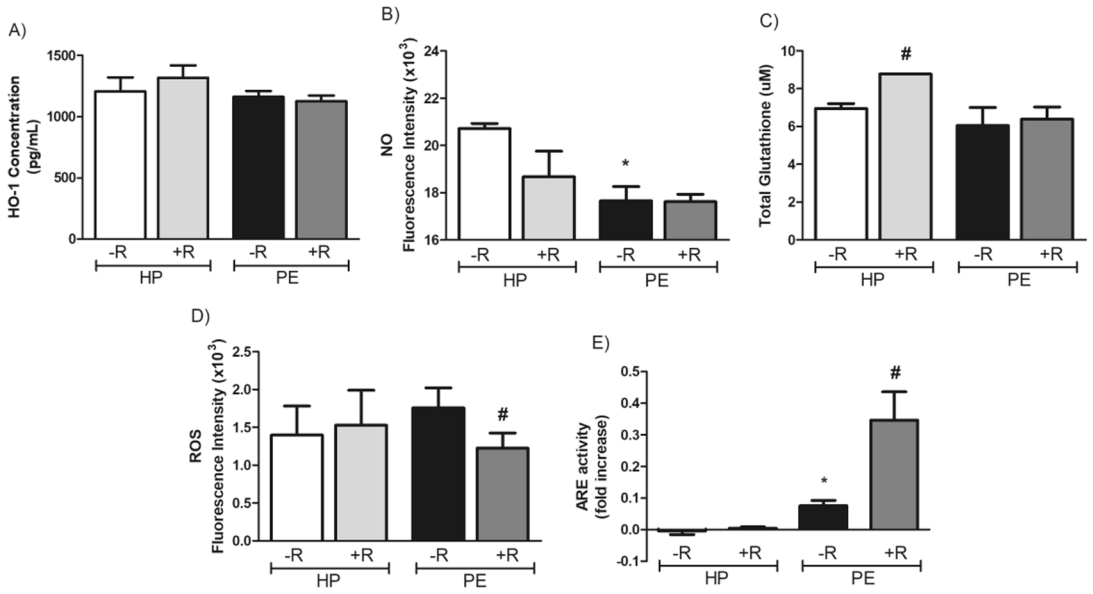

Fig. 1. HO-1 concentrations (A), NO (B), total glutathione (C) and ROS (D) levels and ARE activity (E) in HUVEC. Cells were incubated with 10% (v/v) plasma samples from preeclamptic patients (PE, n = 8) and healthy pregnant (HP, n = 4) in the absence (-R) or presence of trans-resveratrol (+R) at 1 μM for 24 h. For ROS levels, mean and standard error for positive control was 19.78 ± 2.29 of fluorescence intensity $\times 10^3$. Plasmas from PE decreased NO levels and increased ARE activity compared with HP. The addition of resveratrol decreased ROS levels and increased ARE activity only in PE group. Resveratrol was able to increase total glutathione levels only in the HP group. DMSO was used as a vehicle for trans-resveratrol. Values are means ± S.E.M. Comparisons between HP and PE groups were by the *t*-test and paired *t*-test when comparing each group with respective trans-resveratrol treatment. *$p < 0.05$ vs. HP –R, $^{\#}p < 0.05$ vs. respective group –R.

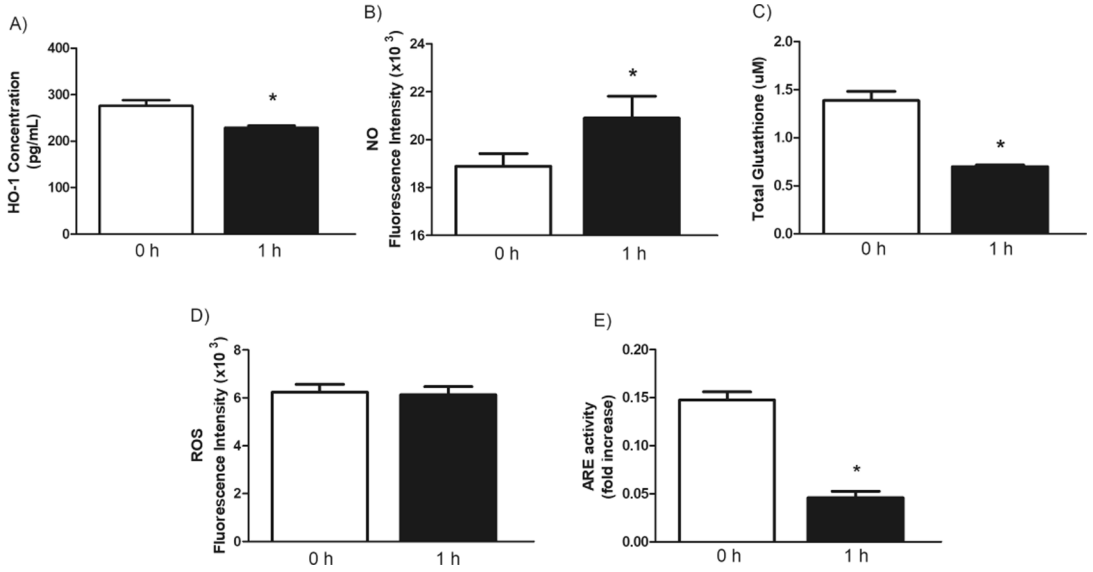

Fig. 2. HO-1 concentrations (A), NO (B), total glutathione (C) and ROS (D) levels and ARE activity (E) in HUVEC. Cells were incubated with 10% (v/v) serum from preeclamptic (PE) patients before (0 h) and after (1 h) 200 mL of whole red grape juice ingestion for 24 h. For ROS levels, mean and standard error for positive control was 20.38 ± 2.29 of fluorescence intensity $\times 10^3$. Serum from PE patients after 1 h of grape juice ingestion decreased HO-1 concentration, total glutathione levels, and ARE activity and increased NO levels compared with before. Values are means ± S.E.M. Comparisons between groups were by *t*-test. *$p < 0.05$ vs. 0 h.

development of PE symptoms, suggesting its potential in the prevention of this syndrome [23]. In this study, we observed that resveratrol also improves endothelial cell markers impaired by incubation of plasma from women with established symptoms of PE, strengthen its potential in PE management.

It is well established that deficient NO bioavailability is involved in PE pathophysiology and it contributes to the development of the symptoms of this syndrome [34]. Moreover, it was showed that resveratrol can increase NO bioavailability in endothelial cells in situations of oxidative stress [35]. We found that NO production was decreased in HUVEC incubated with plasma from PE compared with HP, however, resveratrol cotreatment with plasma from PE was not able to increase NO levels. Probably the concentration of resveratrol used was not sufficient to boost NO production. Another mechanism of action of resveratrol is the scavenging of ROS [36]. We observed that resveratrol decreased ROS production in HUVEC incubated with plasma from PE. Although it was demonstrated that oxidative stress was elevated in endothelial cells incubated with plasma from PE patients [22], we did not observe this increase in ROS production in the HUVEC incubated with plasma from PE compared to HP. We speculate that this discrepancy might be because we used an immortalized healthy cell line of HUVEC for this study, as the maternal endothelium has been

demonstrated to be impaired in PE [37]. Moreover, the incubation period and/or the quantity of plasma from PE added to these cells maybe was not enough to evidence a difference. Although incubation of endothelial cells with plasma/serum from PE women in HUVEC is a well-established *in vitro* model of PE to study endothelial dysfunction [20–22,38], our findings are limited to HUVEC cells, which are feto-placental endothelial cells. We recognize that the use of adult micro-vascular endothelial cells would strengthen our findings.

Since resveratrol also activates Nrf2 [36], which binds to ARE sequence upregulating the expression of antioxidant proteins such as HO-1, we investigated the expression of NFE2L2 and HMOX-1 genes and miRNAs from the let-7 family in our groups. miRNAs are small non-coding RNAs that post-transcriptionally downregulate gene expression [16] and miRNAs from the let-7 family target NFE2L2 [39]. We did not find a significant difference in NFE2L2, HMOX-1, and miRNAs expression between HUVEC incubated with plasma from PE and HP, and resveratrol did not affect these expressions. Regarding protein expression of HO-1, we also did not observe any significant difference, however, ARE activity was increased by plasma from PE compared with HP and resveratrol was able to potentiate this increase. Under oxidative stress conditions, the cellular antioxidant systems are activated to bring back the cell to redox equilibrium, which explains the increased ARE activity in cells incubated with plasma from PE. Resveratrol was not able to significantly increase ARE activity in HP as it was in PE, probably because oxidative stress is not as expressive as in PE, and oxidative stress is a stimulus that leads to the Nrf2 activation, which may be enhanced by the addition of resveratrol in PE. Also, we evaluated GSH levels in the HUVEC incubated with plasma from PE and HP, but there was no significant difference between the groups and resveratrol only increased GSH levels in the HP group. Indeed, resveratrol can increase GSH levels via Nrf2/Are pathway activation, which upregulated enzymes involved in GSH biosynthesis [36]. However, it was demonstrated that the glutathione system is impaired in PE through decreased glutathione peroxidase (GPX) activity associated with the synthesis of vasoconstrictive eicosanoids such as F2-isoprostanes and thromboxane, which are upregulated in PE [40]. This might explain why we did not find increased GSH levels in the PE group.

In light of the interesting results obtained with co-treatment of resveratrol and PE plasma in the endothelial cells, we decided to perform a pilot study with PE patients and acute grape juice intake to evaluate the effects of the incubation of serum collected from these patients after one hour of grape juice intake in the endothelial cells, since grapefruit contains large amounts of resveratrol [41]. This one hour period was chosen based on a previous study showing that one hour was the peak absorption of polyphenols [24]. Serum from the 1 h group increased NO production, decreased HO-1, GSH, and ARE activity, and did not change ROS concentration compared with the serum before grape juice intake incubated in the HUVEC. Since grape juices are rich in several antioxidants [41] and they might be present in the serum collected, the antioxidant systems of the cells are probably not being recruited, explaining the decreased content/activity we found in the antioxidant markers evaluated. Regarding NO production, it was showed previously that grape juice can induce eNOS expression leading to an increase in NO formation in endothelial cells [42] and causes endothelium-dependent relaxation in the coronary artery [43]. Most interestingly, it was demonstrated that consumption of grape-wine extract decreased ambulatory blood pressure in mildly hypertensive subjects [44]. Taking together, our results and these studies evidence the beneficial effects of grape juice in the vascular system, which suggest its potential to assist the management of PE.

Based on our results, it is possible to suggest that resveratrol and grape juice act on endothelial cells in different manners leading to beneficial effects in PE. Although resveratrol is a potent antioxidant and there is a lot of evidence of its potential to assist different diseases [32], grape juice is a rich source of other antioxidants besides resveratrol [41] that can act synergistically enhancing the effects of resveratrol and activating other mechanisms [45]. Also, grape juice is more accessible to patients and it is simple to include in the diet. However, it is necessary to be careful with its intake due to the high sugar content [46].

In conclusion, we found that resveratrol cotreated with plasma from PE patients and serum from women with PE collected after acute grape juice intake ameliorate endothelial cell markers and antioxidant defense. However, our results are still preliminary and there are limitations in the study. Nevertheless, our study provides more evidence for the use of resveratrol treatment and introduce the potential of grape juice supplementation in the management of PE.

Funding

This work was supported by the National Council for Scientific and Technological Development (CNPq-Brazil) [Grant Number #2014-5/305587], São Paulo Research Foundation (FAPESP-Brazil) [Grant Number #2015/20461-8] and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

All authors thank Naiara Cinegaglia for assistance in cell culture experiments.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.preghy.2021.01.001.

References

[1] J.A. Hutcheon, S. Lisonkova, K.S. Joseph, Epidemiology of pre-eclampsia and the other hypertensive disorders of pregnancy, Best Pract. Res. Clin. Obstet. Gynaecol. 25 (2011) 391–403.
[2] G.A. Dekker, Management of preeclampsia, Pregnancy Hypertens. 4 (2014) 246–247.
[3] ACOG, American College of Obstetricians and Gynecologists, Task force on hypertension in pregnancy, Hypertens. Pregnancy 122 (2013) 1122–1131.
[4] R.D. Williamson, C. McCarthy, F.P. McCarthy, L.C. Kenny, Oxidative stress in pre-eclampsia; have we been looking in the wrong place? Pregnancy Hypertens. 8 (2017) 1–5.
[5] A. Rumbold, E. Ota, H. Hori, C. Miyazaki, C.A. Crowther, Vitamin E supplementation in pregnancy, Cochrane Database Syst. Rev. 9 (2015) CD004069.
[6] A. Rumbold, E. Ota, C. Nagata, S. Shahrook, C.A. Crowther, Vitamin C supplementation in pregnancy, Cochrane Database Syst. Rev. 9 (2015) CD004072.
[7] J. Ding, Y. Kang, Y. Fan, Q. Chen, Efficacy of resveratrol to supplement oral nifedipine treatment in pregnancy-induced preeclampsia, Endocr. Connect. 6 (2017) 595–600.
[8] S. Gurusinghe, A.G. Cox, R. Rahman, S.T. Chan, R. Muljadi, H. Singh, B. Leaw, J. C. Mockler, S.A. Marshall, P. Murthi, R. Lim, E.M. Wallace, Resveratrol mitigates trophoblast and endothelial dysfunction partly via activation of nuclear factor erythroid 2-related factor-2, Placenta 60 (2017) 74–85.
[9] N.J. Hannan, F.C. Brownfoot, P. Cannon, M. Deo, S. Beard, T.V. Nguyen, K. R. Palmer, S. Tong, T.J. Kaitu'u-Lino, Resveratrol inhibits release of soluble fms-like tyrosine kinase (sFlt-1) and soluble endoglin and improves vascular dysfunction – implications as a preeclampsia treatment, Sci. Rep. 7 (2017) 1819.
[10] B. Al-Ani, Resveratrol inhibits proteinase-activated receptor-2-induced release of soluble vascular endothelial growth factor receptor-1 from human endothelial cells, EXCLI J. 12 (2013) 598–604.
[11] D. Bonnefont-Rousselot, Resveratrol and cardiovascular diseases, Nutrients 8 (2016) 250.
[12] N. Kweider, B. Huppertz, M. Kadyrov, W. Rath, T. Pufe, C.J. Wruck, A possible protective role of Nrf2 in preeclampsia, Ann. Anat. Anat. Anzeiger. 196 (2014) 268–277.
[13] G. Chiva-Blanch, M. Urpi-Sarda, E. Ros, S. Arranz, P. Valderas-Martínez, R. Casas, E. Sacanella, R. Llorach, R.M. Lamuela-Raventos, C. Andres-Lacueva, R. Estruch, Clinical/translational research dealcoholized red wine decreases systolic and diastolic blood pressure and increases plasma nitric oxide short communication, Circ. Res. 111 (2012) 1065–1068.

[14] T.E. Tjelle, L. Holtung, S.K. Bøhn, K. Aaby, M. Thoresen, S.Å. Wiik, I. Paur, A. S. Karlsen, K. Retterstøl, P.O. Iversen, R. Blomhoff, Polyphenol-rich juices reduce blood pressure measures in a randomised controlled trial in high normal and hypertensive volunteers, Br. J. Nutr. 114 (2015) 1054–1063.
[15] X. Cheng, C.-H. Ku, R.C.M. Siow, Regulation of the Nrf2 antioxidant pathway by microRNAs: new players in micromanaging redox homeostasis, Free Radic. Biol. Med. 64 (2013) 4–11.
[16] D.P. Bartel, MicroRNAs: target recognition and regulatory functions, Cell 136 (2009) 215–233.
[17] V.C. Sandrim, N. Eleuterio, E. Pilan, J.E. Tanus-Santos, K. Fernandes, R. Cavalli, Plasma levels of increased miR-195-5p correlates with the sFLT-1 levels in preeclampsia, Hypertens. Pregnancy 35 (2016) 150–158.
[18] V.C. Sandrim, M.R. Luizon, A.C. Palei, J.E. Tanus-Santos, R.C. Cavalli, Circulating microRNA expression profiles in pre-eclampsia: evidence of increased miR-885-5p levels, BJOG 123 (2016) 2120–2128.
[19] A. Amiri, M.M. Tehran, Z. Asemi, A. Shafiee, S. Hajighadimi, S. Moradizarmehri, H. R. Mirzaei, H. Mirzaei, Role of resveratrol in modulating microRNAs in human diseases: from cancer to inflammatory disorder, Curr. Med. Chem. 26 (2019).
[20] M. Caldeira-Dias, M.R. Luizon, E. Deffune, J.E. Tanus-Santos, P.P. Freire, R. F. Carvalho, H. Bettiol, V.C. Cardoso, M. Antonio Barbieri, R.C. Cavalli, V. C. Sandrim, Preeclamptic plasma stimulates the expression of miRNAs, leading to a decrease in endothelin-1 production in endothelial cells, Pregnancy Hypertens. 12 (2018) 75–81.
[21] V.C. Sandrim, M.C. Dias, A.L. de C. Bovolato, J.E. Tanus-Santos, E. Deffune, R. C. Cavalli, Plasma from pre-eclamptic patients induces the expression of the anti-angiogenic miR-195-5p in endothelial cells, J. Cell Mol. Med. 20 (2016) 1198–1200.
[22] S. Sankaralingam, H. Xu, S.T. Davidge, Arginase contributes to endothelial cell oxidative stress in response to plasma from women with preeclampsia, Cardiovasc. Res. 85 (2010) 194–203.
[23] M. Caldeira-Dias, M.F. Montenegro, H. Bettiol, M.A. Barbieri, V.C. Cardoso, R. C. Cavalli, V.C. Sandrim, Resveratrol improves endothelial cell markers impaired by plasma incubation from women who subsequently develop preeclampsia, Hypertens. Res. 42 (2019) 1166–1174.
[24] M.M. Machado, G.F.F. Dos Santos Montagner, A. Boligon, M.L. Athayde, M.I.U. M. Da Rocha, J.P.B. Lera, C. Belló, I.B.M. Da Cruz, Determination of polyphenol contents and antioxidant capacity of no-alcoholic red grape products (vitis labrusca) from conventional and organic crops, Quim. Nova 34 (2011) 798–803.
[25] Y. Takizawa, Y. Kosuge, H. Awaji, E. Tamura, A. Takai, T. Yanai, R. Yamamoto, K. Kokame, T. Miyata, R. Nakata, H. Inoue, Up-regulation of endothelial nitric oxide synthase (eNOS), silent mating type information regulation 2 homologue 1 (SIRT1) and autophagy-related genes by repeated treatments with resveratrol in human umbilical vein endothelial cells, Br. J. Nutr. 110 (2013) 2150–2155.
[26] M. Boncler, M. Różalski, U. Krajewska, A. Podsędek, C. Watala, Comparison of PrestoBlue and MTT assays of cellular viability in the assessment of anti-proliferative effects of plant extracts on human endothelial cells, J. Pharmacol. Toxicol. Methods 69 (2014) 9–16.
[27] T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, J. Immunol. Methods 65 (1983) 55–63.
[28] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[29] D. Betel, M. Wilson, A. Gabow, D.S. Marks, C. Sander, The microRNA.org resource: targets and expression, Nucleic Acids Res. 36 (2008) D149–D153.
[30] H. Dweep, N. Gretz, miRWalk2.0: a comprehensive atlas of microRNA-target interactions, Nat. Methods 12 (2015) 697.
[31] M.N. McCall, O.A. Kent, J. Yu, K. Fox-Talbot, A.L. Zaiman, M.K. Halushka, MicroRNA profiling of diverse endothelial cell types, BMC Med. Genomics 4 (2011) 78.
[32] A.P. Singh, R. Singh, S.S. Verma, V. Rai, C.H. Kaschula, P. Maiti, S.C. Gupta, Health benefits of resveratrol: evidence from clinical studies, Med. Res. Rev. (2019).
[33] J.R.T. Darby, M.H.B. Mohd Dollah, T.R.H. Regnault, M.T. Williams, J.L. Morrison, Systematic review: impact of resveratrol exposure during pregnancy on maternal and fetal outcomes in animal models of human pregnancy complications—are we ready for the clinic? Pharmacol. Res. 144 (2019) 264–278.
[34] V.C. Sandrim, A.C.T. Palei, I.F. Metzger, V.A. Gomes, R.C. Cavalli, J.E. Tanus-Santos, Nitric oxide formation is inversely related to serum levels of antiangiogenic factors soluble fms-like tyrosine kinase-1 and soluble endogline in preeclampsia, Hypertension 52 (2008) 402–407.
[35] N. Xia, U. Förstermann, H. Li, Resveratrol and endothelial nitric oxide, Molecules 19 (2014) 16102–16121.
[36] V.L. Truong, M. Jun, W.S. Jeong, Role of resveratrol in regulation of cellular defense systems against oxidative stress, BioFactors 44 (2018) 36–49.
[37] J. Roberts, Endothelial dysfunction in preeclampsia, Semin. Reprod. Med. 16 (1998) 5–15.
[38] D.J. Medina-Leyte, M. Domínguez-Pérez, I. Mercado, M.T. Villarreal-Molina, L. Jacobo-Albavera, Use of human umbilical vein endothelial cells (HUVEC) as a model to study cardiovascular disease: a review, Appl. Sci. 10 (2020) 938.
[39] Z. Ungvari, Z. Tucsek, D. Sosnowska, P. Toth, T. Gautam, A. Podlutsky, A. Csiszar, G. Losonczy, M.N. Valcarcel-Ares, W.E. Sonntag, A. Csiszar, Aging-induced dysregulation of dicer1-dependent microRNA expression impairs angiogenic capacity of rat cerebromicrovascular endothelial cells, J. Gerontol. A: Biol. Sci. Med. Sci. 68 (2013) 877–891.
[40] K. Haram, J.H. Mortensen, O. Myking, E.F. Magann, J.C. Morrison, The role of oxidative stress, adhesion molecules and antioxidants in preeclampsia, Curr. Hypertens. Rev. 15 (2019) 105–112.
[41] F. Cosme, T. Pinto, A. Vilela, Phenolic compounds and antioxidant activity in grape juices: a chemical and sensory view, Beverages 4 (2018) 22.
[42] M. Alhosin, E. Anselm, S. Rashid, J.H. Kim, S.V. Frota Madeira, C. Bronner, V. B. Schini-Kerth, Redox-sensitive up-regulation of eNOS by purple grape juice in endothelial cells: role of PI3-kinase/Akt, p38 MAPK, JNK, FoxO1 and FoxO3a, PLoS One 8 (2013).
[43] E. Anselm, M. Chataigneau, M. Ndiaye, T. Chataigneau, V.B. Schinikerth, Grape juice causes endothelium-dependent relaxation via a redox-sensitive Src- and Akt-dependent activation of eNOS, Cardiovasc. Res. 73 (2007) 404–413.
[44] R. Draijer, Y. de Graaf, M. Slettenaar, E. de Groot, C.I. Wright, Consumption of a polyphenol-rich grape-wine extract lowers ambulatory blood pressure in mildly hypertensive subjects, Nutrients 7 (2015) 3138–3153.
[45] K.L. Liao, M.C. Yin, Individual and combined antioxidant effects of seven phenolic agents in human erythrocyte membrane ghosts and phosphatidylcholine liposome systems: importance of the partition coefficient, J. Agric. Food Chem. 48 (2000) 2266–2270.
[46] S. Kuntz, S. Rudloff, H. Asseburg, C. Borsch, B. Fröhling, F. Unger, S. Dold, B. Spengler, A. Römpp, C. Kunz, Uptake and bioavailability of anthocyanins and phenolic acids from grape/blueberry juice and smoothie in vitro and in vivo, Br. J. Nutr. 113 (2015) 1044–1055.