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Short communication

The effects of hydroxychloroquine on endothelial dysfunction

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ABSTRACT

Hydroxychloroquine is an anti-malarial drug which, due to its anti-inflammatory and immunomodulatory effects, is widely used for the treatment of autoimmune diseases. In a model of systemic lupus erythematosus hydroxychloroquine has been shown to exert protective endothelial effects. In this study, we aimed to investigate whether hydroxychloroquine was endothelial protective in an *in vitro* model of TNF-α and preeclamptic serum induced dysfunction. We showed that hydroxychloroquine significantly reduced the production of TNF-α and preeclamptic serum induced endothelin-1 (ET-1). Hydroxychloroquine also significantly mitigated TNF-α induced impairment of angiogenesis. These findings support the further assessment of hydroxychloroquine as an adjuvant therapy in preeclampsia.

1. Introduction

Preeclampsia is a multi-systemic disorder affecting about 5% of pregnancies [1]. It is associated with increased risks of maternal and perinatal mortality and morbidity and remains a leading cause of iatrogenic preterm birth [1,2]. While the pathophysiology of preeclampsia is yet to be fully elucidated there is growing evidence that excessive placental and systemic oxidative stress and widespread maternal endothelial dysfunction are the two main pathologies contributing to the signs and symptoms of the clinical syndrome [1,3–6].

Specifically, it is currently thought that the endothelial dysfunction is, at least in part, secondary to excessive placental release of pro-inflammatory and anti-angiogenic factors, such as tumour necrosis factor-α (TNF-α), soluble fms-like tyrosine kinase-1 (sFlt-1), soluble endoglin (sEng) and activin A into the maternal circulation [6–13]. In particular, women with established preeclampsia have significantly higher levels of TNF-α than women with a healthy pregnancy [13]. Maternal levels of TNF-α are also increased in other pregnancy complications associated with altered placental function such as fetal growth restriction and diabetes [14,15]. It has been shown that TNF-α induces endothelial dysfunction with many of the features seen in women with preeclampsia including increased endothelin-1 (ET-1) release, down-regulated endothelial nitric oxide synthase (eNOS) expression, increased NADPH oxidase activity and impaired angiogenesis [16].

Systemic lupus erythematosus (SLE), an autoimmune disease, shares many features with preeclampsia including elevated levels of TNF-α and endothelial dysfunction [17,18]. Recently, hydroxychloroquine, an antimalarial drug commonly used in the treatment of SLE, was shown to improve endothelial function in mice model of severe SLE [19]. Treatment with hydroxychloroquine is also associated with a decline in serum ET-1 levels in patients with SLE [20].

Accordingly, we aimed to determine whether hydroxychloroquine was able to mitigate the *in vitro* features of endothelial dysfunction induced by recombinant TNF-α or preeclamptic serum specifically to changes in endothelin-1 (ET-1) release and angiogenesis. To our knowledge, this is the first study to investigate the potential of hydroxychloroquine to improve TNF-α and preeclamptic serum induced endothelial dysfunction.

2. Materials and methods

Maternal sera were collected from 10 women with established preeclampsia and from five gestation-matched normotensive

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Table 1
Characteristics of pregnant women from whom serum pools were derived.

	Normotensive (n = 5)	Preeclampsia (n = 10)
Mean (±SEM) gestation at sampling (weeks)	30.5 ± 2.6	30.4 ± 3.7
Mean (±SEM) systolic blood pressure (mmHg)	107.3 ± 2.2	164.5 ± 7.5
Mean (±SEM) diastolic blood pressure (mmHg)	62.4 ± 1.6	112.8 ± 7.1
Proteinuria, g/mL 24 h	0 ± 0	1.0 ± 0.3

pregnant women, with the approval of the Monash Health Human Research Ethics Committee following written, informed consent. Sera were separated and pooled into two groups: preeclampsia and normotensive pregnancy. The patient characteristics are summarised in Table 1. Preeclampsia was defined new onset of hypertension ($\geqslant$140/90 mmHg) after 20 weeks of pregnancy with one or more of the following: renal involvement (proteinuria > 300 mg 24 h), haematological involvement (low platelets, haemolysis, DIC), liver involvement (raised transaminases), neurological involvement (seizures, headache, visual disturbance, stroke), pulmonary oedema, fetal growth restriction, or placental abruption, as per Society of Obstetric Medicine of Australia and New Zealand guidelines [21]. Exclusion criteria were pre-existing hypertension, diabetes mellitus, multiple pregnancy and treatment with magnesium sulphate.

Human umbilical vein endothelial cells (HUVECs) were isolated from term uncomplicated pregnancies (n = 8) and expanded as previously described [22]. Experiments were conducted in 96-well plates. The effect of different concentrations of hydroxychloroquine (1, 10, 100 μg/mL) (Sigma-Aldrich, Missouri, USA) on cell viability was first determined using the MTS reagent (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H* tetrazolium) (Promega, Victoria, Australia). The absorbance at 490 nm was recorded using an ELISA plate reader (SpectraMax i3, Molecular Devices, California, USA).

HUVECs were grown to confluence in 96-well plates (2×10^4 cells/well, Corning, New York, USA) and incubated with recombinant TNF-α (100 ng/mL, Life Technologies, Carlsbad, CA) or 20% preeclamptic serum in the absence or presence of hydroxychloroquine at 1 and 10 μg/mL for 24 h. The conditioned media were collected and stored at −80 °C. The levels of ET-1 in the conditioned media were measured by ELISA (R&D systems, Minneapolis, MN) according to the manufacturer's protocols.

Endothelial tube formation was performed as previously described [23], with minor modifications Briefly, pre-chilled angiogenesis μ-slides (Ibidi, Victoria, Australia) were coated with 10 μL/well growth factor reduced Matrigel (Corning, New York, USA). HUVEC cells (20,000 cells) in 50 μL complete endothelial growth media (EGM, Lonza, Victoria, Australia) were placed in the wells, treated with recombinant TNF-α (10 ng/mL, Life Technologies, Carlsbad, CA) or 5% pre-eclamptic serum in the absence or presence of hydroxychloroquine (1 and 10 μg/mL, Sigma-Aldrich, Missouri, USA) for six hours at 37 °C, 5% CO_2. The culture medium was removed from the wells, and Calcein AM fluorescent dye (Millipore, Victoria, Australia) diluted 1:500 with Hank's Balanced Salt Solution (HBSS 1:10, Gibco, Waltham, USA) was added (40 μL/well). Tubes were assessed immediately through an inverted fluorescent microscope at 4x magnification (Olympus) and quantitatively analysed (total tube lengths, branch points) using image J software (http://rsbweb.nih.gov/ij/; National Institutes of Health, Bethesda, MD).

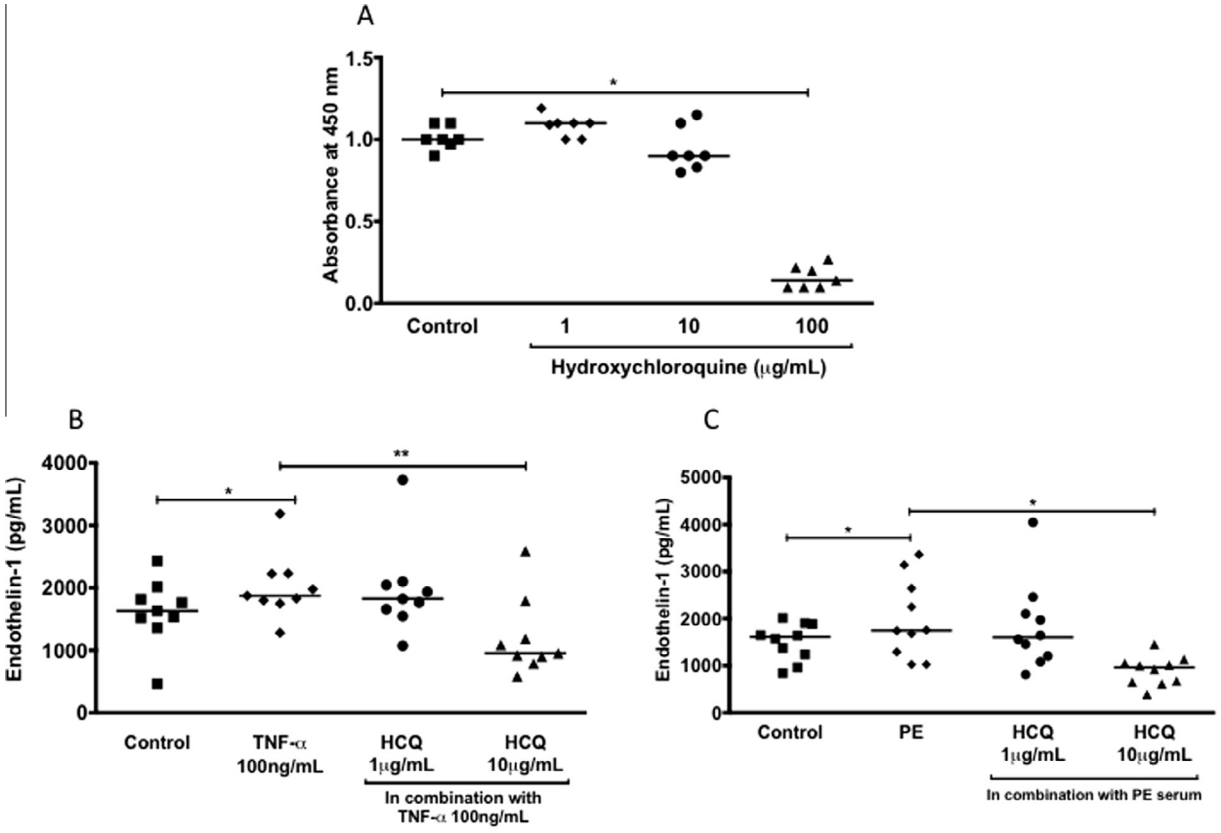

Fig. 1. (A) Hydroxychloroquine did not alter HUVEC endothelial viability at 0.1, 1 and 10 μg/mL, but reduced viability at 100 μg/mL. Data are median from seven independent biological replicates. *Denotes $p < 0.05$. (B) Recombinant TNF-α (100 ng/mL) and (C) pre-eclamptic serum (PE) increased HUVEC secretion of endothelin-1, effects mitigated hydroxychloroquine (1 and 10 μg/mL). Data are median from eight independent biological replicates and *denotes $p < 0.05$ and **$p < 0.005$.

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