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Complement inhibition by hydroxychloroquine prevents placental and fetal brain abnormalities in antiphospholipid syndrome

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ABSTRACT

Placental ischemic disease and adverse pregnancy outcomes are frequently observed in patients with antiphospholipid syndrome (APS). Despite the administration of conventional antithrombotic treatment a significant number of women continue to experience adverse pregnancy outcomes, with uncertain prevention and management. Efforts to develop effective pharmacological strategies for refractory obstetric APS cases will be of significant clinical benefit for both mothers and fetuses. Although the antimalarial drug, hydroxychloroquine (HCQ) is increasingly used to treat pregnant women with APS, little is known about its efficacy and mechanism of action of HCQ.

Because complement activation plays a crucial and causative role in placental ischemia and abnormal fetal brain development in APS we hypothesised that HCQ prevents these pregnancy complications through inhibition of complement activation.

Using a mouse model of obstetric APS that closely resembles the clinical condition, we found that HCQ prevented fetal death and the placental metabolic changes -measured by proton magnetic resonance spectroscopy in APS-mice. Using ^{111}In labelled antiphospholipid antibodies (aPL) we identified the placenta and the fetal brain as the main organ targets in APS-mice. Using this same method, we found that HCQ does not inhibit aPL binding to tissues as was previously suggested from *in vitro* studies. While HCQ did not affect aPL binding to fetal brain it prevented fetal brain abnormal cortical development. HCQ prevented complement activation *in vivo* and *in vitro*. Complement C5a levels in serum samples from APS patients and APS-mice were lower after treatment with HCQ while the antibodies titres remained unchanged.

HCQ prevented not only placental insufficiency but also abnormal fetal brain development in APS. By inhibiting complement activation, HCQ might also be an effective antithrombotic therapy.

1. Introduction

The antiphospholipid syndrome (APS) is an autoimmune disease associated with the presence of antiphospholipid autoantibodies (aPL). APS occurs most commonly in young women of reproductive age and is characterised by adverse pregnancy outcomes frequently associated with placental pathologies [1–4].

There is growing evidence for transplacental passage of aPL, and brain abnormalities and cognitive impairment has been described in infants born to mothers affected by APS, suggesting that exposure to aPL *in utero* can affect fetal brain development and thus might induce behavioural and cognitive problems later in life [5–7]. Abnormal behaviour in the offspring of APS-mice has also been described [8,9]. Thus, the impact of maternal aPL antibodies on the offspring may extend beyond the known association with adverse pregnancy outcome.

aPL constitute a heterogeneous group of autoantibodies with

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different specificities and different target organs' principally the female reproductive system and the vascular system. β_2-glycoprotein I (β_2GPI) is the major antigen for clinically relevant antibodies in APS [4]. *In vitro* studies demonstrated that aβ_2GPI antibodies isolated from patients avidly bind to trophoblast monolayers isolated from human placentas [10,11] and to isolated mouse fetal cortical neurons [9]. Furthermore, some of these *in vitro* studies demonstrated that aPL antibodies affect trophoblast function and invasion [12,13] and mouse fetal cortical neurons cytoarchitecture [9]. Ex-vivo studies also showed robust aPL deposition in placentas in mice and humans [9,14,15]. However, no data are available on the binding of aβ2GPI antibodies and the identification of the target organs' *in vivo*.

Pregnancy complications in APS have been attributed to placental thrombosis and infarcts and management of obstetric APS is based on attenuating the procoagulant state. However, in many cases there is no evidence of decidual thrombosis or placental vasculopathy, and instead inflammatory signs are present [16–18]. Treatment with aspirin and heparin has become a conventional option for women with obstetric APS. While the use of aspirin and heparin has improved the pregnancy outcome in obstetric APS, current treatment fails in a significant number of pregnancies [19,20] raising the need to explore other treatments to improve obstetrical outcome.

Hydroxychloroquine is a medication originally used to prevent/treat malaria. This antimalarial drug has also been used to treat pregnant women with APS for many years. While recent studies suggest that HCQ might improve pregnancy outcomes in APS [21], the use of HCQ is still controversial; most of the beneficial effects of HCQ in pregnancy constitute anecdotal evidence and little is known about its mechanism of action [22–24].

Here we evaluate the effects of HCQ in the mother and developing fetus in a mouse model of obstetric APS. Knowing that complement activation plays a crucial role in the pathogenesis of adverse pregnancy and fetal outcomes in APS in mice and women [9,14,17,25–27], we hypothesised that HCQ protects pregnancy in APS by inhibiting complement activation.

2. Methods

2.1. Purification of aPL antibodies

antiβ_2GPI antibodies were isolated from 7 patients with primary APS (PAPS). Autoantibody profiles and clinical features from patients (untreated and treated with HCQ) are described in Table 1. All patients met Sidney Laboratory criteria for APS [28]. The patients were identified through the Registry of Connective tissue diseases (10/H0405/35) at St Thomas' Hospital. The NHS National Research Ethics Service approved the collection and utilization of samples for research purposes.

IgG from APS patients and from healthy non-autoimmune individuals was purified using protein G sepharose chromatography [17]. A further affinity purification step using peptides mimetic to regions of β_2GPI immobilised onto magnetic beads was performed in the IgG fractions from patients with APS in order to obtain purified aβ_2GPI antibodies. Blank et al. demonstrated that these peptides - that correspond to three epitopes located in domains I–II, III and IV of the β_2GPI molecule - bind to human anti-β_2GPI Abs and prevent fetal loss and endothelial activation in experimental APS [29]. Functionality of the isolated aβ2GPI antibodies was confirmed by ELISA. Endotoxin removal in each sample was performed using high capacity endotoxin removal spin columns (Pierce Thermoscientific). All samples showed to be endotoxin free (<0.01 ng/ml) using the LAL Chromogenic Endotoxin Quantitation Kit.

2.2. Animals

All housing and experimental procedures were performed in compliance with the UK Home Office Animals Scientific Procedures Act 1986 (Home Office project licence number 60/4305). C57BL/6 mice (2–3 months old) purchased from commercial vendors were used in all experiments. A group of females were mated with previously isolated males. The presence of a vaginal plug defined day 0 of pregnancy.

2.3. Mouse model of obstetric APS (APS-mice)

Affinity purified antibodies to β_2GPI isolated from patients and normal human IgG (NHIgG) (control antibodies) were administered

Table 1
Clinical and laboratory features of the patients used as a source of human aβ_2GPI antibodies (Pt1–Pt7) and patients that received HCQ treatment (Pt 8–Pt12). Pt indicates patient. All patients met Sidney Laboratory criteria for APS [28]. ACA = anticardiolipin, β_2 = $\beta_2$gycoprotein I (β_2GPI), PAPS = primary antiphospholipid syndrome, RM = recurrent miscarriages, DVT = deep vein thrombosis, PE = pulmonary embolism, IUGR = intrauterine growth restriction.

	Sex/age	ACA-IgG	β_2GPI-IgG	LA	Diagnostic	Clinical features	Obstetric APS
Pt 1	F/52	+	+	+	PAPS	arterial thrombosis	RM Stillbirth
Pt 2	M/62	+	+	+	PAPS	DVT, stroke	
Pt 3	F/48	+	+	+	PAPS	DVT, PE	Preeclampsia
Pt 4	F/38	+	+	+	PAPS	DVT	RM preeclampsia
Pt 5	M/54	+	+	+	PAPS	arterial and venous thrombosis	
Pt 6	F/54	+	+	+	PAPS	DVT	RM, preterm birth, stillbirth
Pt 7	F/45	+	+	+	PAPS		RM, preeclampsia
Pt 8	F/38	+	+	+	PAPS		RM Stillbirth
Pt 9	F/31	+	+	+	PAPS	DVT	RM IUGR
Pt 10	F/34	+	+	+	PAPS		Preeclampsia HELLP syndrome
Pt 11	F/32	+	+	+	PAPS		RM preeclampsia
Pt 12	F/34	+	+	+	PAPS	venous thrombosis	RM, preeclampsia