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Article Title:

Warfarin ineffective as symptomatic therapy for erythropoietic protoporphyria

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Warfarin ineffective as symptomatic therapy for erythropoietic protoporphyria

Autosomal recessive erythropoietic protoporphyria (EPP) is an inherited cutaneous porphyria, with an incidence of approximately 1:200,000 people.[1] It arises most commonly due to a mutation in the *FECH* gene, causing reduced activity of the ferrochelatase enzyme that catalyses the insertion of iron into protoporphyrin IX (PPIX) to form haem.[1] As a result, PPIX accumulates within the erythrocytes, liver and skin. Retention within the skin causes acute, profoundly debilitating photosensitivity when exposed to sunlight.[1] Conventional treatments have been largely ineffective, with sun avoidance the main management, resulting in restriction of daily activities and poor quality of life.[1] Alfamelanotide (human α-melanocyte–stimulating hormone) implants have been approved in some European countries as a possible treatment option for EPP with minor side effects, but currently this is not approved for use in Australia.[2] Management also seeks to prevent the progression of possible liver disease to liver failure.

We originally reported a case study in a patient who had complete resolution of EPP symptoms, with administration of therapeutic warfarin following aortic valve replacement.[3] His symptoms recurred after ceasing warfarin prior to an elective surgical procedure, and resolved upon recommencement. Given this outcome, we sought to evaluate the efficacy and safety of warfarin therapy in providing symptomatic relief to individuals with EPP.

Australians with EPP (*FECH* gene mutation) were recruited from the multi-disciplinary porphyria service at The Royal Melbourne Hospital. Participants were assessed for warfarin sensitivity by screening for; CYP2C9 gene polymorphisms (known to affect plasma levels of warfarin and it's metabolites) and VKORC1 gene polymorphisms (known to influence the anticoagulant effect). A single-blinded, crossover study was applied. Participants were administered daily doses of warfarin, with change to the fixed dose every 3-months for a total of 12 months. Participants were initially commenced on 1mg of warfarin, then 0mg, then 0.5mg, with a 1-week washout period in between change of dose. A further open-label period, aiming for therapeutic international normalised ratio (INR 1.5-2.5) was also undertaken. Participants were seen monthly and assessed for sun tolerability, pain and completed a daily sun diary, dermatology quality of life questionnaire (DLQI) and a validated EPP symptomatic scoring questionnaire, adapted from Biolcati *et al*.[4] Monthly laboratory measures included coagulation analysis and red blood cell (RBC) porphyrin and total plasma porphyrin levels.

Five participants were recruited (Table 1); with three intermediate metabolisers of warfarin. Primary endpoints were analysed based on week 12 results (1mg warfarin dosing) and results of open-label therapeutic warfarin dosing. Two participants (normal warfarin metabolisers) entered into the open-label period with a maximum INR of 1.7 and 2.2. A comparison was undertaken between outcome measures at baseline (0mg of warfarin) and maximum dose of warfarin administered for each participant (Table 2). Mean compliance of daily medication administration for all participants was 94.6 ± 3.2% (SD). There was no associated improvement in EPP symptoms from analysis of quality of life questionnaires. Biochemical RBC and total plasma porphyrin levels showed no improvement in either low dose or therapeutic doses of warfarin.

We were limited in the capacity of sunlight exposure throughout phases of this study, where seasonal changes (winter) resulted in decreased visible light wavelengths and sunlight contact with skin, which is important in the pathogenesis of the skin related EPP symptoms. We attempted to minimise this effect, by instigating the open-label extension period during the intense sunlight of the Australian summer months. Limitations also were noted in

relation to poor compliance of the daily skin diaries, with mean daily compliance of 21 ± 4.8% (SD). The intermediate warfarin metaboliser participants elected not to enrol into the extension study, due to a perceived lack of efficacy during the blinded period. It remains unknown whether these participants may have benefited from therapeutic doses, as the patient we described in the initial case report was also an intermediate metaboliser of warfarin. In the extension study, we were able to obtain safe INR values of 1.9, which may have been lower than the therapeutic dose that allowed resolution of symptoms in the original case report, which aimed for a target INR of 2.5.[3]

Whilst our original case study suggested an antioxidant and anti-inflammatory effect of warfarin in mitigating the symptoms of EPP, this pilot study was not able to replicate this. Given the paucity of effective treatments, further investigation into warfarin and other antioxidant agents should still be considered, especially given the outcome of the initial case study.

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The study was approved by The Melbourne Health Human Research Ethics Committee (HREC/15/MH/231) and conforms to the recognised standards according to the Declaration of Helsinki.

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Tables:

Table 1: Erythropoietic protoporphyria patient characteristics

Characteristic	**P01**	**P02**	**P03**	**P04**	**P06***
Sex	Male	Female	Female	Female	Male
Age (years)	41.8	51.3	39.3	24.2	47.6
Body Mass Index (kg/m²)	27.1	23.9	33.8	25.1	30.2
Years since diagnosis	38	47	26	14	42
Photosensitivity?	Y	Y	Y	Y	Y
Intermediate metaboliser to Warfarin?	Y°	N	N	N	Y°
Period on study (weeks)	10	72	51	65	25

*Participant P05 withdrew before commencement of study; Y = Yes; N = No;

° Intermediate metaboliser to both CYP2C9 and VKORC1 gene polymorphisms

Table 2: Individual results of erythropoietic protoporphyria patients comparing outcomes measures of placebo to maximum dosing periods

Characteristic	P01	P02	P03	P04	P06*
Baseline period (0mg warfarin)					
Season	Summer	Summer	Summer	Summer	Autumn
INR	1.0	1.0	0.9	0.9	1.0
DLQI Score	9	4	3	9	17
EPP QoL Score	26	25	12	27	23
RBC Porphyrin (µmol/L)	69.9	43	41.6	12.8	79
Total Plasma Porphyrin (mg/L)	891	634	698	324	1062
Maximum warfarin dose					
Season	Winter	Summer	Autumn	Summer	Autumn
Warfarin dose (mg)	1.0	8.0	1.0	6.0	1.0
INR	1.3	1.7	1.1	2.2	1.1
DLQI Score	6	1	5	2	8
EPP QoL Score	29	29	14	18	22
RBC Porphyrin (µmol/L)	75.6	37.5	64.0	12.9	82
Total Plasma Porphyrin (mg/L)	702	262	680	230	522

*P05 withdrew before study commencement; INR = International Normalised Ratio;

DLQI = Dermatology Life Quality Index; EPP QoL = Erythropoietic Protoporphyria Quality of Life;

RBC = Red Blood Cell

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