Phase I Pilot Clinical Trial of Antenatal Maternally Administered Melatonin to Decrease the Level of Oxidative Stress in Human Pregnancies Affected by Preeclampsia (PAMPR Trial): Study Protocol

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PHASE I PILOT CLINICAL TRIAL OF ANTENATAL MATERNALLY ADMINISTERED MELATONIN TO DECREASE THE LEVEL OF OXIDATIVE STRESS IN HUMAN PREGNANCIES AFFECTED BY PREECLAMPSIA (PAMPR): STUDY PROTOCOL

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Running title: A pilot study of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by preeclampsia: PAMPR trial study protocol.

Key words: Preeclampsia, melatonin, clinical trial, oxidative stress.

Word Count: 1,999.

Abstract

Introduction: Preeclampsia is a common pregnancy condition affecting between 3-7% of women. Unfortunately, the exact pathophysiology of the disease is unknown and as such there are no effective treatments that exist notwithstanding prompt delivery of the fetus and culprit placenta. As many cases of preeclampsia occur in preterm pregnancies, it remains a significant cause of both maternal and perinatal morbidity and mortality. Recently, both *in vitro* and animal studies have highlighted the potential role of antioxidants in mitigating the effects of the disease. Melatonin is a naturally occurring antioxidant hormone and provides an excellent safety profile combined with ease of oral administration. We present the protocol for a Phase I pilot clinical trial investigating the efficacy and side effects of maternal treatment with oral melatonin in pregnancies affected by preterm preeclampsia.

Methods and analysis: We propose undertaking a single-arm open label clinical trial recruiting 20 women with preterm preeclampsia (24^{+0}-35^{+6} weeks). We will take baseline measurements of maternal and fetal well-being, levels of oxidative stress, ultrasound Doppler studies and other biomarkers of preeclampsia. Women will then be given oral melatonin (10 mg) three times daily until delivery. The primary outcome will be time interval between diagnosis and delivery compared to historical controls. Secondary outcomes will compare the baseline measurements previously mentioned with twice-weekly measurements during treatment, and then 6 weeks postpartum.

Ethics and dissemination: Ethical approval has been obtained from Monash Health Human Research Ethics Committee B (HREC 13076B). Data will be presented at international conferences and published in peer-reviewed journals.

Trial registration number: ACTRN12613000476730 (ANZCTR).

Article Summary

Article focus:

- The aim of the trial is to establish whether melatonin will afford a clinical or biochemical benefit in women with early-onset preeclampsia.
- To test the hypothesis, we will pose the following research aims in pregnancies affected by preterm preeclampsia:
  - To determine the effect of daily maternal oral treatment with melatonin on clinical outcomes.
  - To determine the effect of daily maternal oral treatment with melatonin on the oxidative stress response in the maternal, placental and fetal circulation.
  - To determine the effect of daily maternal oral treatment with melatonin on the clinical and biochemical measures of vascular function in the mother and fetus.

Key messages:

- Preeclampsia is a life-threatening condition for both mother and baby with increased levels of oxidative stress. Unfortunately, despite ongoing advancements in perinatal care, preeclampsia remains an incurable disease.
- Extensive animal studies demonstrate that the use of melatonin as an antioxidant in high-risk pregnancies is very promising and does not harm the developing fetus.
- This study aims to be the first human trial to assess the potential clinical and biochemical effects of melatonin in pregnancies complicated by preterm preeclampsia.

Strengths and limitations of this study:

- The strengths of this trial are that it is an appropriately designed pilot study with realistically set numbers to achieve easily measurable outcomes. Significant preparatory work has been conducted into the preliminary *in-vitro* and animal studies to guide the trial design. The trial is the first of its type world-wide and if successful, will be able to direct future randomised controlled trials.
- The limitation of this trial is that due to the nature of such a pilot study in pregnant women, the relatively small numbers of participants must act as their own pre-treatment controls. It is predicted that this limitation will be overcome in subsequent trials that will be largely informed by the outcomes of this study.

Introduction

Preeclampsia is a multi-organ syndrome of pregnancy that manifests after 20 weeks gestation with new-onset hypertension alongside maternal end-organ dysfunction and/or intrauterine fetal growth restriction. It affects between 3-7% of all pregnancies and is associated with substantial maternal and perinatal morbidity and mortality, with a significant proportion of fetal complications due to prematurity. To date, the exact pathophysiology of preeclampsia is unknown, but early placental dysfunction plays a central role in all leading hypotheses [1 2]. This placental dysfunction is thought to result in a local and systemic cascade of increasing oxidative stress in the mother, leading to endothelial dysfunction and subsequent end-organ consequences.

Placental hypoxia and reperfusion, as a consequence of abnormal placentation, result in oxidative stress leading to apoptotic and necrotic disruption of the syncytial structure. This disruption then results in the release of various factors and compounds from the intervillous space into the maternal circulation that stimulate the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF alpha), interleukin (IL)-6 and anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) [2]. The resultant effect involves potentially widespread increased oxidative stress with anti-angiogenic compromise to the maternal vasculature.

Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous lipid-soluble antioxidant hormone produced primarily by the pineal gland in humans, providing circadian and seasonal timing cues. In addition, melatonin is also a powerful antioxidant, acting both as a direct scavenger of oxygen free radicals, especially the highly damaging hydroxyl radical, and indirectly via up-regulation of antioxidant enzymes including GSHPx, GSH-reductase, superoxide dismutase and catalase [3 4].

Melatonin has several characteristics that make it an appealing treatment for use in pregnancy. Melatonin freely crosses the placenta [5] and blood-brain barrier [6], and has an excellent safety profile with no known adverse effects [7 8]. Reduced levels of melatonin are found in pregnant women with preeclampsia [9]. Placentae express receptors for melatonin [10] and thus melatonin may protect against oxidative stress generated by the dysfunctional organ, thereby inhibiting the release of vasoactive factors responsible for the clinical syndrome of preeclampsia.

Our group has previously shown that in an animal model of fetal growth restriction (FGR), melatonin administration reduced fetal hypoxia, improved neurodevelopment and decreased brain injury and oxidative stress in newborn lambs. In another published experiment we administered melatonin shortly before and during a short period of severe fetal asphyxia induced by umbilical cord occlusion at late-gestation. Melatonin prevented the formation of free radicals (hydroxyl radical) within the fetal brains and decreased lipid peroxidation and brain cell death [5]. The protective effects of melatonin on ischemia-reperfusion-induced oxidative damage to mitochondria in the rat placenta have also been published [11]. Melatonin treatment improves placental function (fetal:placental weight ratio), improves birth weight and induces antioxidant enzymes in a rat model of maternal undernourishment, known to promote oxidative stress [12].

Melatonin has been studied in several clinical trials in humans at varying gestations and for different purposes. Melatonin has been assessed in assisted reproductive technology where the quality of oocytes is important for the success of in-vitro fertilization (IVF). Melatonin is an important compound found in the follicular fluid that has been shown to be important for oocyte maturation and quality, and has been suggested to improve pregnancy outcomes with IVF [13-15]. To date, no babies born from melatonin-treated pregnancies have been shown to have any increase in abnormalities [16]. Melatonin has also been shown to up-regulate antioxidant enzymes in human pregnancies leading to the conclusion that melatonin might provide an indirect protection against injury caused by reactive oxygen species as seen in preeclampsia, FGR and fetal hypoxia [17].

Melatonin does not have any acute pharmacological effects in the nervous or vascular systems, apart from its benign but active effect on sleep mechanisms [18]. Mice that have received extremely high doses of up to 800 mg/kg melatonin did not have increased mortality and as such the median lethal dose could not be established. In humans, a Phase II clinical trial conducted in the Netherlands administered 75 mg melatonin nightly to 1400 women over 4-years, with no serious side effects reported [19]. The maternal no-adverse-effect-level (NOAEL) for melatonin has been found to be 100 mg/kg/day with the fetal NOAEL established at ≥200 mg/kg/day when administered to the mother. The maternal lowest observed adverse effect level (LOAEL) toxicity was 200 mg/kg/day [20].

Rationale

Preeclampsia is a life-threatening condition for both mother and baby affecting up to 7% of pregnancies. Despite ongoing advancements in perinatal care, preeclampsia remains an incurable disease. Extensive animal studies demonstrate that the use of melatonin as an antioxidant in high-risk pregnancies is very promising. Melatonin readily crosses the placenta and does not harm the developing fetus, not even when administered in extremely high doses. This study aims to be the first human trial to assess the potential clinical and biochemical effects of melatonin in pregnancies complicated by preterm preeclampsia.

Aims

The aim of the trial is to establish whether melatonin will afford a clinical or biochemical benefit in women with early-onset preeclampsia. To test the hypothesis, we will pose the following research aims:

1. To determine the effect of daily maternal oral treatment with melatonin on the clinical outcomes of pregnancies affected by preterm preeclampsia.
2. To determine the effect of daily maternal oral treatment with melatonin on the oxidative stress response in the maternal, placental and fetal circulation in pregnancies affected by preterm preeclampsia.
3. To determine the effect of daily maternal oral treatment with melatonin on the clinical and biochemical measures of vascular function in the mother and fetus in pregnancies affected by preterm preeclampsia.

Methods and analysis

Study design

Phase I single-arm open label clinical trial.

Subjects

We plan to recruit 20 women with preterm preeclampsia.

Study setting

We will recruit patients from the obstetric departments of Monash Health and Jessie McPherson Private Hospitals co-located in Melbourne, Australia.

Sample size

This study is a proof-of-principle Phase I trial and as such a power calculation has not been performed to determine sample size. The purpose of this trial is to establish appropriate outcome measures that can be used to calculate power for a future Phase II randomized-controlled trial for the same intervention.

Inclusion criteria

1. Be at least 18 years of age.
2. Be between 24^{+0} weeks' and 35^{+6} weeks' gestation.
3. Have a singleton pregnancy.
4. Have a diagnosis of Preeclampsia (SOMANZ criteria).
5. Be considered capable of safely continuing the pregnancy for 48 hours or more, as determined by the attending clinician.
6. Obstetrician and neonatologist believe the fetus is likely to be viable.
7. No major anomalies evident on the mid-trimester morphology scan.
8. Be capable of understanding the information provided, with use of an interpreter if required.
9. Give written informed consent.

Exclusion criteria

1. Eclampsia.
2. Current use of melatonin.
3. Contraindications to melatonin use including:
    a. Hypersensitivity to melatonin or any of its derivatives.

4. Imminent transfer to a non-trial centre due to unavailability of neonatal beds.
5. Significant uncertainty regarding gestational age.
6. Women to be treated as an outpatient.
7. Use of any of the following medications:
    a. Fluvoxamine.
    b. 5- or 8-Methoxypsoralen.
    c. Cimetidine.
    d. Quinolones and other CYP1A2 inhibitors.
    e. Carbamazepine, rifampicin and other CYP1A2 inducers.
    f. Zaleplon, zolpidem, zopiclone and other non-benzodiazepine hypnotics.

Participant enrolment

The PAMPR trial will be introduced to potential trial participants identified from various settings within the trial institutions. The clinical research team will give the potential participant an information sheet about the trial and answer any questions she or her relatives may have. Maternal and fetal assessments combined with the results of blood tests will determine whether immediate delivery is essential for the survival and wellbeing of mother and baby. If the attending clinician considers delivery within 48 hours as probable, the mother is not eligible for inclusion to the PAMPR trial. Absolute criteria for immediate delivery are not specified in this protocol and remain the responsibility of the attending clinician.

Where delivery within 48 hours is considered unlikely, the women can be approached for consent to the PAMPR trial. Ideally there should be a period of 24 hours for the women to consider whether she wishes to take part in the trial. However, it is considered clinically important to initiate treatment as soon as possible after the diagnosis of preeclampsia, therefore consent should be sought at the earliest opportunity, provided the investigator is reassured that the woman has fully understood the requirements of the trial.

Following admission to the hospital the trial medication will be written on the patient drug chart and administered through usual prescribing practices.

Intervention

Eligible women will receive melatonin (10 mg) tablets three times daily from recruitment until delivery.

Primary outcome

Interval in days from diagnosis with preeclampsia until delivery.

Secondary outcomes

Table 1. Maternal biomarkers of oxidative stress.

Maternal biomarkers of oxidative stress Samples collected at recruitment and then up to every three days until delivery		
Malondialdehyde	8-iosprostane	Total antioxidant capacity
Superoxide dismutase	Melatonin	Haeme oxygenase

Table 2. Maternal biomarkers for preeclampsia.

Maternal biomarkers of preeclampsia Samples collected at recruitment and then up to every three days until delivery		
Soluble fms-like tyrosine kinase-1	Vascular endothelial growth factor	Highly sensitive C-reactive protein
Soluble endoglin	Placental growth factor	Activin
von Willebrand Factor	Neutrophil Elastase	Platelet function tests

Table 3. Markers of preeclampsia severity.

Markers of preeclampsia severity Samples collected at recruitment and then up to every three days until delivery		
Maternal blood pressure	Level of proteinuria	Haemoglobin
Platelet count	Liver-transaminases	Renal function
Composite of preeclamptic symptoms: oedema, headache, visual disturbance, epigastric or left upper-quadrant pain		

Table 4. Markers of maternal morbidity.

Markers of maternal morbidity Samples collected at recruitment and then up to every three days until delivery		
Serum creatinine equal to or >120 micmol/L	Proteinuria equal to or >5 g/24h	Hypertension equal to or >170/110 mmHg (despite Rx)
Signs of left ventricular failure	Eclampsia	Platelets <$50x10^9$/L
Disseminated intravascular coagulation	Cerebrovascular event	Liver transaminase equal to or >500 IU/L

Table 5. Ultrasound and Doppler measurements.

Ultrasound and Doppler measurements At recruitment then every three days (biometry every 2 weeks) until delivery		
Maternal brachial artery	Maternal uterine artery	Fetal Morphology
Biometry	Amniotic fluid indices	Placental location/anomalities
Fetal characteristics: heart rate, tone, breathing, movements		
Doppler velocimetry: umbilical artery, middle cerebral artery & ductus venosus		

Table 6. Pregnancy end-points.

Pregnancy end-points Various timings		
Gestation at birth	Mode of birth	Placental histology/weight
Abnormal cardiotocogram	Labour analgesia/anaesthesia	Duration of labour stages
Labour induction/augmentation	Duration of membrane rupture to birth	Group B streptococcus infection
Cord lactates: artery & vein	Presence of meconium liquor	Intrapartum lactates
Use of antihypertensives	Use of magnesium sulfate	Use of corticosteroids

Table 7. Neonatal outcomes.

Neonatal ouctomes At birth		
Sex	Neonatal Apgar scores	Weight at birth
Length	Head circumference	Cord melatonin levels
Composite neonatal outcome: admission to NICU, duration of admission, need & duration of respiratory support, intraventricular haemorrhage, necrotising enterocolitis, abnormal neurology, mortality prior to discharge		

Table 8. Any serious adverse event.

Any serious adverse event At any time during trial

Proposed analyses

The length of gestation post recruitment will be analysed using a t-test.

The biomarkers will be analysed using a repeated measures analysis, including the baseline value as a covariate. For the primary analysis the treatment effect will be considered constant over time, secondary analyses will examine the possibility of a trend over time. Plots of mean score over time will be shown for clarification. Initially, the treatment effect will be assumed to be constant over time, but if time by treatment interaction is shown to be important by including this parameter in the model (the conventional level of p=0.05 will be used here) then further investigation into effects at differing time points will be made by analysing the least-square means as above. Plots of mean score over time will be shown for clarification.

All other continuous measures (clinical measures, etc) will be considered in the same manner as above (adjusting by baseline value if available). Dichotomous outcomes (mortality, etc) will be presented as risk ratios, with a corresponding chi-squared test performed.

Apart from baseline value, no adjustments for covariates will be made in the first instance in any of the investigations. Treatment estimates will only be adjusted when subgroups are explored. Interaction between treatment and subgroup variables will be examined in a similar fashion as above by including the relevant parameters in the model. This will be done in turn for each subgroup variable and adjusted estimates presented.

All tests are 2-sided and results will be presented as a point estimate along with 95% confidence intervals.

Adverse events

Participants will be inpatients for the duration of the trial. As such, a senior obstetric clinician who will be in ongoing communication with the research team will see them daily. The principal investigator of the PAMPR trial will be contactable by telephone at all times in the case of an adverse event. Any serious adverse events that occur after joining the trial will be reported in detail in the participant's medical notes, followed up until resolution of the event and reported to the Monash Health Health Human Research Ethics Committee, Therapeutics Committee and Therapeutic Goods Administration of Australia's Office of Scientific Evaluation immediately or within 24-72 hours.

Ethics and dissemination

Ethical approval has been obtained from the Monash Health Human Research Ethics Committee B (HREC 13076B). Data will be presented at international conferences and published in peer-reviewed journals. We will make the information obtained from the study available to the public through national bodies and charities.

Discussion

If effective, we believe that treatment with melatonin could become standard of care for women with pregnancies complicated by preterm preeclampsia. The potential benefits to both mother and baby would significantly reduce what is a terrible burden of disease internationally. Treatment with this medication also has potential use in other pregnancy disorders such as FGR and hypoxia.

We do not however, anticipate that this will be the final trial to determine whether further exploration of this area is worthwhile. We hope that the study will generate sufficient evidence that melatonin may be effective; to support a funding application for a larger randomized controlled trial. Such a trial could be designed to test the hypothesis that melatonin treatment in preterm preeclampsia afforded superior and more cost-effective outcomes prioritised by consumer groups and clinicians than existing managements.

References:

1. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension 2005;46(6):1243-9 doi: 10.1161/01.HYP.0000188408.49896.c5[published Online First: Epub Date]|.

2. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;376(9741):631-44 doi: 10.1016/S0140-6736(10)60279-6[published Online First: Epub Date]|.

3. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovascular research 2003;58(1):10-9

4. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. Journal of biomedical science 2000;7(6):444-58 doi: 25480[published Online First: Epub Date]|.

5. Miller SL, Yan EB, Castillo-Melendez M, Jenkin G, Walker DW. Melatonin provides neuroprotection in the late-gestation fetal sheep brain in response to umbilical cord occlusion. Developmental neuroscience 2005;27(2-4):200-10 doi: 10.1159/000085993[published Online First: Epub Date]|.

6. Welin AK, Svedin P, Lapatto R, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatric research 2007;61(2):153-8 doi: 10.1203/01.pdr.0000252546.20451.1a[published Online First: Epub Date]|.

7. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. Journal of pineal research 2009;46(2):128-39 doi: 10.1111/j.1600-079X.2008.00649.x[published Online First: Epub Date]|.

8. Fulia F, Gitto E, Cuzzocrea S, et al. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. Journal of pineal research 2001;31(4):343-9

9. Lanoix D, Guerin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. Journal of pineal research 2012;53(4):417-25 doi: 10.1111/j.1600-079X.2012.01012.x[published Online First: Epub Date]|.

10. Lanoix D, Beghdadi H, Lafond J, Vaillancourt C. Human placental trophoblasts synthesize melatonin and express its receptors. Journal of pineal research 2008;45(1):50-60 doi: 10.1111/j.1600-079X.2008.00555.x[published Online First: Epub Date]|.

11. Okatani Y, Wakatsuki A, Shinohara K, Taniguchi K, Fukaya T. Melatonin protects against oxidative mitochondrial damage induced in rat placenta by ischemia and reperfusion. Journal of pineal research 2001;31(2):173-8

12. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. Journal of pineal research 2009;46(4):357-64 doi: 10.1111/j.1600-079X.2009.00671.x[published Online First: Epub Date]|.

13. Tamura H, Nakamura Y, Terron MP, et al. Melatonin and pregnancy in the human. Reprod Toxicol 2008;25(3):291-303 doi: 10.1016/j.reprotox.2008.03.005[published Online First: Epub Date]|.

14. Rizzo P, Raffone E, Benedetto V. Effect of the treatment with myo-inositol plus folic acid plus melatonin in comparison with a treatment with myo-inositol plus folic acid on oocyte quality and pregnancy outcome in IVF cycles. A prospective, clinical trial. European review for medical and pharmacological sciences 2010;14(6):555-61

15. Unfer V, Raffone E, Rizzo P, Buffo S. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2011;27(11):857-61 doi: 10.3109/09513590.2011.564687[published Online First: Epub Date]|.

16. Unfer V. Personal communication via email. In: Mockler J, ed., 2012.

17. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y. Maternal-fetal transfer of melatonin in pregnant women near term. Journal of pineal research 1998;25(3):129-34

18. Barchas J, DaCosta F, Spector S. Acute pharmacology of melatonin. Nature 1967;214(5091):919-20

19. Silman RE. Melatonin: a contraceptive for the nineties. European journal of obstetrics, gynecology, and reproductive biology 1993;49(1-2):3-9

20. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicological sciences : an official journal of the Society of Toxicology 1999;50(2):271-9

Authors' contributions

SRH: research, contribution of original material, editing and approval of final manuscript; RL, EEG, EMW: editing and approval of final manuscript.

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Competing interests statement

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Strengths and limitations of this study:

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Placental hypoxia and reperfusion, as a consequence of abnormal placentation, result in oxidative stress leading to apoptotic and necrotic disruption of the syncytial structure [5]. This disruption then results in the release of various factors and compounds from the intervillous space into the maternal circulation that stimulate the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF alpha), interleukin (IL)-6 and anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) [4]. The resultant effect involves potentially widespread increased oxidative stress with anti-angiogenic compromise to the maternal vasculature.

Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous lipid-soluble antioxidant hormone produced primarily by the pineal gland in humans, providing circadian and seasonal timing cues. In addition, melatonin is also a powerful antioxidant, acting both as a direct scavenger of oxygen free radicals, especially the highly damaging hydroxyl radical, and indirectly via up-regulation of antioxidant enzymes including GSHPx, GSH-reductase, superoxide dismutase and catalase [6 7].

Melatonin has several characteristics that make it an appealing treatment for use in pregnancy. Melatonin freely crosses the placenta [8] and blood-brain barrier [9], and has an excellent safety profile with no known adverse effects [10 11]. Reduced levels of melatonin are found in pregnant women with preeclampsia [12]. Placentae express receptors for melatonin [13] and thus melatonin may protect against oxidative stress generated by the dysfunctional organ, thereby inhibiting the release of vasoactive factors responsible for the clinical syndrome of preeclampsia.

Our group has previously shown that in an animal model of fetal growth restriction (FGR), melatonin administration reduced fetal hypoxia, improved neurodevelopment and decreased brain injury and oxidative stress in newborn lambs [14]. In another published experiment we administered melatonin shortly before and during a short period of severe fetal asphyxia induced by umbilical cord occlusion at late-gestation. Melatonin prevented the formation of free radicals (hydroxyl radical) within the fetal brains and decreased lipid peroxidation and brain cell death [8]. The protective effects of melatonin on ischemia-reperfusion-induced oxidative damage to mitochondria in the rat placenta have also been published [15]. Melatonin treatment improves placental function (fetal:placental weight ratio), improves birth weight and induces antioxidant enzymes in a rat model of maternal undernourishment, known to promote oxidative stress [16].

Melatonin has been studied in several clinical trials in humans at varying gestations and for different purposes. Melatonin has been assessed in assisted reproductive technology where the quality of oocytes is important for the success of in-vitro fertilization (IVF). Melatonin is an important compound found in the follicular fluid that has been shown to be important for oocyte maturation and quality, and has been suggested to improve pregnancy outcomes with IVF [17-19]. To date, no babies born from melatonin-treated pregnancies have been shown to have any increase in abnormalities [20]. Melatonin has also been shown to up-regulate antioxidant enzymes in human pregnancies leading to the conclusion that melatonin might provide an indirect protection against injury caused by reactive oxygen species as seen in preeclampsia, FGR and fetal hypoxia [21].

Melatonin does not have any acute pharmacological effects in the nervous or vascular systems, apart from its benign but active effect on sleep mechanisms [22]. Mice that have received extremely high doses of up to 800 mg/kg melatonin did not have increased mortality and as such the median lethal dose could not be established [22]. In humans, a Phase II clinical trial conducted in the Netherlands administered 75 mg melatonin nightly to 1400 women over 4-years, with no serious side effects reported [23]. The maternal no-adverse-effect-level (NOAEL) for melatonin has been found to be 100 mg/kg/day with the fetal NOAEL established at ≥200 mg/kg/day when administered to the mother. The maternal lowest observed adverse effect level (LOAEL) toxicity was 200 mg/kg/day [24].

Rationale

Preeclampsia is a life-threatening condition for both mother and baby affecting up to 7% of pregnancies [2]. Despite ongoing advancements in perinatal care, preeclampsia remains an incurable disease. Extensive animal studies demonstrate that the use of melatonin as an antioxidant in high-risk pregnancies is very promising. Melatonin readily crosses the placenta and does not harm the developing fetus, not even when administered in extremely high doses [22]. This study aims to be the first human trial to assess the potential clinical and biochemical effects of melatonin in pregnancies complicated by preterm preeclampsia.

Aims

The aim of the trial is to establish whether melatonin will afford a clinical or biochemical benefit in women with early-onset preeclampsia. To test the hypothesis, we will pose the following research aims:

1. To determine the effect of daily maternal oral treatment with melatonin on the clinical outcomes of pregnancies affected by preterm preeclampsia.
2. To determine the effect of daily maternal oral treatment with melatonin on the oxidative stress response in the maternal, placental and fetal circulation in pregnancies affected by preterm preeclampsia.
3. To determine the effect of daily maternal oral treatment with melatonin on the clinical and biochemical measures of vascular function in the mother and fetus in pregnancies affected by preterm preeclampsia.

Methods and analysis

Study design

Phase I single-arm open label clinical trial.

Subjects

We plan to recruit 20 women with preterm preeclampsia. We will also perform a retrospective review of cases over the previous 24 months to use as historical controls for the primary outcome measure.

Study setting

We will recruit patients from the obstetric departments of Monash Health and Jessie McPherson Private Hospitals co-located in Melbourne, Australia over a 12-month period.

Sample size

This study is a proof-of-principle Phase I trial and as such a power calculation has not been performed to determine sample size. The purpose of this trial is to establish appropriate outcome measures that can be used to calculate power for a future Phase II randomized-controlled trial for the same intervention.

Inclusion criteria

1. Be at least 18 years of age.
2. Be between 24^{+0} weeks' and 35^{+6} weeks' gestation.
3. Have a singleton pregnancy.
4. Have a diagnosis of Preeclampsia (SOMANZ criteria [1]).
5. Be considered capable of safely continuing the pregnancy for 48 hours or more, as determined by the attending clinician.
6. Obstetrician and neonatologist believe the fetus is likely to be viable.
7. No major anomalies evident on the mid-trimester morphology scan.
8. Be capable of understanding the information provided, with use of an interpreter if required.
9. Give written informed consent.

Exclusion criteria

1. Eclampsia.
2. Current use of melatonin.

3. Contraindications to melatonin use including:
    a. Hypersensitivity to melatonin or any of its derivatives.
4. Imminent transfer to a non-trial centre due to unavailability of neonatal beds.
5. Significant uncertainty regarding gestational age.
6. Women to be treated as an outpatient.
7. Use of any of the following medications:
    a. Fluvoxamine.
    b. 5- or 8-Methoxypsoralen.
    c. Cimetidine.
    d. Quinolones and other CYP1A2 inhibitors.
    e. Carbamazepine, rifampicin and other CYP1A2 inducers.
    f. Zaleplon, zolpidem, zopiclone and other non-benzodiazepine hypnotics.

Participant enrolment

The PAMPR trial will be introduced to potential trial participants identified from routine antenatal clinics, pregnancy assessment units and labour ward admissions within the trial institutions as identified by the Principal Investigator. Maternal and fetal assessments by the treating clinician combined with the results of blood tests will determine whether immediate delivery is essential for the survival and wellbeing of mother and baby. If the treating clinician considers delivery within 48 hours as probable, the mother is not eligible for inclusion to the PAMPR trial. Absolute criteria for immediate delivery are not specified in this protocol and remain the responsibility of the attending clinician.

Where delivery within 48 hours is considered unlikely, the women can be approached for consent to the PAMPR trial. The Principal Investigator will provide written information about the trial and answer any questions she or her relatives may have. The patient's written informed consent to participate in the trial must be obtained before recruitment and after a full explanation has been given of the treatment options and the manner of treatment administration. Ideally there should be a period of 24 hours for the women to consider whether she wishes to take part in the trial. However, it is considered clinically important to initiate treatment as soon as possible after the diagnosis of preeclampsia, therefore consent should be sought at the earliest opportunity, provided the investigator is reassured that the woman has fully understood the requirements of the trial.

Following admission to the hospital the trial medication will be written on the patient drug chart and administered through usual prescribing practices.

The historical controls for the primary outcome measure (interval from diagnosis to delivery) will be obtained through de-identified retrospective review of medical records from Monash Health over the previous 24-month period. These historical controls will be women diagnosed with preterm preeclampsia who meet the inclusion and exclusion criteria who underwent expectant management.

Intervention

Eligible women will receive melatonin (10 mg) tablets three times daily from recruitment until delivery.

Primary outcome

Interval in days from participant diagnosis with preeclampsia until delivery compared to historical controls.

Secondary outcomes

Table 1. Maternal biomarkers of oxidative stress.

Maternal biomarkers of oxidative stress Samples collected at recruitment and then up to every three days until delivery		
Malondialdehyde	8-iosprostane	Total antioxidant capacity
Superoxide dismutase	Melatonin	Haeme oxygenase

Table 2. Maternal biomarkers for preeclampsia.

Maternal biomarkers of preeclampsia Samples collected at recruitment and then up to every three days until delivery		
Soluble fms-like tyrosine kinase-1	Vascular endothelial growth factor	Highly sensitive C-reactive protein
Soluble endoglin	Placental growth factor	Activin
von Willebrand Factor	Neutrophil Elastase	Platelet function tests

Table 3. Markers of preeclampsia severity.

Markers of preeclampsia severity Samples collected at recruitment and then up to every three days until delivery		
Maternal blood pressure	Level of proteinuria	Haemoglobin
Platelet count	Liver-transaminases	Renal function
Composite of preeclamptic symptoms: oedema, headache, visual disturbance, epigastric or left upper-quadrant pain		

Table 4. Markers of maternal morbidity.

Markers of maternal morbidity Samples collected at recruitment and then up to every three days until delivery		
Serum creatinine equal to or >120 micmol/L	Proteinuria equal to or >5 g/24h	Hypertension equal to or >170/110 mmHg (despite Rx)
Signs of left ventricular failure	Eclampsia	Platelets <50x10^9/L
Disseminated intravascular coagulation	Cerebrovascular event	Liver transaminase equal to or >500 IU/L

Table 5. Ultrasound and Doppler measurements.

Ultrasound and Doppler measurements At recruitment then every three days (biometry every 2 weeks) until delivery		
Maternal brachial artery	Maternal uterine artery	Fetal Morphology
Biometry	Amniotic fluid indices	Placental location/anomalities
Fetal characteristics: heart rate, tone, breathing, movements		
Doppler velocimetry: umbilical artery, middle cerebral artery & ductus venosus		

Table 6. Pregnancy end-points.

Pregnancy end-points Various timings		
Gestation at birth	Mode of birth	Placental histology/weight
Abnormal cardiotocogram	Labour analgesia/anaesthesia	Duration of labour stages
Labour induction/augmentation	Duration of membrane rupture to birth	Group B streptococcus infection
Cord lactates: artery & vein	Presence of meconium liquor	Intrapartum lactates
Use of antihypertensives	Use of magnesium sulfate	Use of corticosteroids

Neonatal ouctomes

At birth		
Sex	Neonatal Apgar scores	Weight at birth
Length	Head circumference	Cord melatonin levels
Composite neonatal outcome: admission to NICU, duration of admission, need & duration of respiratory support, intraventricular haemorrhage, necrotising enterocolitis, abnormal neurology, mortality prior to discharge		

Table 7. Neonatal outcomes.

Table 8. Any serious adverse event.

Any serious adverse event
At any time during trial

Proposed analyses

The length of gestation post recruitment will be analysed using a t-test.

The biomarkers will be analysed using a repeated measures analysis, including the baseline value as a covariate. For the primary analysis the treatment effect will be considered constant over time, secondary analyses will examine the possibility of a trend over time. Plots of mean score over time will be shown for clarification. Initially, the treatment effect will be assumed to be constant over time, but if time by treatment interaction is shown to be important by including this parameter in the model (the conventional level of p=0.05 will be used here) then further investigation into effects at differing time points will be made by analysing the least-square means as above. Plots of mean score over time will be shown for clarification.

All other continuous measures (clinical measures, etc) will be considered in the same manner as above (adjusting by baseline value if available). Dichotomous outcomes (mortality, etc) will be presented as risk ratios, with a corresponding chi-squared test performed.

Apart from baseline value, no adjustments for covariates will be made in the first instance in any of the investigations. Treatment estimates will only be adjusted when subgroups are explored. Interaction between treatment and subgroup variables will be examined in a similar fashion as above by including the relevant parameters in the model. This will be done in turn for each subgroup variable and adjusted estimates presented.

All tests are 2-sided and results will be presented as a point estimate along with 95% confidence intervals.

Adverse events

Participants will be inpatients for the duration of the trial. As such, a senior obstetric clinician who will be in ongoing communication with the research team will see them daily. The principal investigator of the PAMPR trial will be contactable by telephone at all times in the case of an adverse event. Any serious adverse events that occur after joining the trial will be reported in detail in the participant's medical notes, followed up until resolution of the event and reported to the Monash Health Health Human Research Ethics Committee, Therapeutics Committee and Therapeutic Goods Administration of Australia's Office of Scientific Evaluation immediately or within 24-72 hours.

Trial discontinuation or modification

Participants will be withdrawn from the trial at participant request or if they or their fetus suffer an unexpected serious adverse event as noted above. Worsening preeclampsia is within the natural history of the disease and as such will not be part of discontinuation criteria.

There will be no allowance for modification of the trial intervention.

Ethics and dissemination

Ethical approval has been obtained from the Monash Health Human Research Ethics Committee B (HREC 13076B). Data will be presented at international conferences and published in peer-reviewed journals. We will make the information obtained from the study available to the public through national bodies and charities.

Discussion

If effective, we believe that treatment with melatonin could become standard of care for women with pregnancies complicated by preterm preeclampsia. The potential benefits to both mother and baby would significantly reduce what is a terrible burden of disease internationally. Treatment with this medication also has potential use in other pregnancy disorders such as FGR and hypoxia.

We do not however, anticipate that this will be the final trial to determine whether further exploration of this area is worthwhile. We hope that the study will generate sufficient evidence that melatonin may be effective; to support a funding application for a larger randomized controlled trial. Such a trial could be designed to test the hypothesis that melatonin treatment in preterm preeclampsia afforded superior and more cost-effective outcomes prioritised by consumer groups and clinicians than existing managements.

References:

1. Lowe SA, Brown MA, Dekker GA, et al. Guidelines for the management of hypertensive disorders of pregnancy 2008. The Australian & New Zealand journal of obstetrics & gynaecology 2009;**49**(3):242-6 doi: 10.1111/j.1479-828X.2009.01003.x[published Online First: Epub Date]|.

2. Payne B, Magee LA, von Dadelszen P. Assessment, surveillance and prognosis in pre-eclampsia. Best practice & research. Clinical obstetrics & gynaecology 2011;**25**(4):449-62 doi: 10.1016/j.bpobgyn.2011.02.003[published Online First: Epub Date]|.

3. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension 2005;**46**(6):1243-9 doi: 10.1161/01.HYP.0000188408.49896.c5[published Online First: Epub Date]|.

4. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;**376**(9741):631-44 doi: 10.1016/S0140-6736(10)60279-6[published Online First: Epub Date]|.

5. Burton GJ, Jauniaux E. Oxidative stress. Best practice & research. Clinical obstetrics & gynaecology 2011;**25**(3):287-99 doi: 10.1016/j.bpobgyn.2010.10.016[published Online First: Epub Date]|.

6. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovascular research 2003;**58**(1):10-9

7. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. Journal of biomedical science 2000;**7**(6):444-58 doi: 25480[published Online First: Epub Date]|.

8. Miller SL, Yan EB, Castillo-Melendez M, Jenkin G, Walker DW. Melatonin provides neuroprotection in the late-gestation fetal sheep brain in response to umbilical cord occlusion. Developmental neuroscience 2005;**27**(2-4):200-10 doi: 10.1159/000085993[published Online First: Epub Date]|.

9. Welin AK, Svedin P, Lapatto R, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatric research 2007;**61**(2):153-8 doi: 10.1203/01.pdr.0000252546.20451.1a[published Online First: Epub Date]|.

10. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. Journal of pineal research 2009;**46**(2):128-39 doi: 10.1111/j.1600-079X.2008.00649.x[published Online First: Epub Date]|.

11. Fulia F, Gitto E, Cuzzocrea S, et al. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. Journal of pineal research 2001;**31**(4):343-9

12. Lanoix D, Guerin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. Journal of pineal research 2012;**53**(4):417-25 doi: 10.1111/j.1600-079X.2012.01012.x[published Online First: Epub Date]|.

13. Lanoix D, Beghdadi H, Lafond J, Vaillancourt C. Human placental trophoblasts synthesize melatonin and express its receptors. Journal of pineal research 2008;**45**(1):50-60 doi: 10.1111/j.1600-079X.2008.00555.x[published Online First: Epub Date]|.

14. Miller SL, Wallace EM, Walker DW. Antioxidant therapies: a potential role in perinatal medicine. Neuroendocrinology 2012;**96**(1):13-23 doi: 10.1159/000336378[published Online First: Epub Date]|.

15. Okatani Y, Wakatsuki A, Shinohara K, Taniguchi K, Fukaya T. Melatonin protects against oxidative mitochondrial damage induced in rat placenta by ischemia and reperfusion. Journal of pineal research 2001;**31**(2):173-8

16. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. Journal of pineal research 2009;**46**(4):357-64 doi: 10.1111/j.1600-079X.2009.00671.x[published Online First: Epub Date]|.

17. Tamura H, Nakamura Y, Terron MP, et al. Melatonin and pregnancy in the human. Reprod Toxicol 2008;**25**(3):291-303 doi: 10.1016/j.reprotox.2008.03.005[published Online First: Epub Date]|.

18. Rizzo P, Raffone E, Benedetto V. Effect of the treatment with myo-inositol plus folic acid plus melatonin in comparison with a treatment with myo-inositol plus folic acid on oocyte quality and pregnancy outcome in IVF cycles. A prospective, clinical trial. European review for medical and pharmacological sciences 2010;**14**(6):555-61

19. Unfer V, Raffone E, Rizzo P, Buffo S. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2011;**27**(11):857-61 doi: 10.3109/09513590.2011.564687[published Online First: Epub Date]|.

20. Unfer V. Personal communication via email. In: Mockler J, ed., 2012.

21. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y. Maternal-fetal transfer of melatonin in pregnant women near term. Journal of pineal research 1998;**25**(3):129-34

22. Barchas J, DaCosta F, Spector S. Acute pharmacology of melatonin. Nature 1967;**214**(5091):919-20

23. Silman RE. Melatonin: a contraceptive for the nineties. European journal of obstetrics, gynecology, and reproductive biology 1993;**49**(1-2):3-9

24. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicological sciences : an official journal of the Society of Toxicology 1999;**50**(2):271-9

Authors' contributions

SRH: research, contribution of original material, editing and approval of final manuscript; RL, EEG, EMW: editing and approval of final manuscript.

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Competing interests statement

SRH is funded by an Australian Postgraduate Award and Monash Health Early Researcher Fellowship. RL and EMW are funded by an NHMRC (Australia) Project Grant (# 1029148) and by the Victorian Government's Operational Infrastructure Support Program. EEG is funded by an NHMRC Senior Research Fellowship and NHMRC and NHF Australia project grant funding.

PHASE I PILOT CLINICAL TRIAL OF ANTENATAL MATERNALLY ADMINISTERED MELATONIN TO DECREASE THE LEVEL OF OXIDATIVE STRESS IN HUMAN PREGNANCIES AFFECTED BY PREECLAMPSIA (PAMPR): STUDY PROTOCOL

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Running title: A pilot study of antenatal maternally administered melatonin to decrease the level of oxidative stress in human pregnancies affected by preeclampsia: PAMPR trial study protocol.

Key words: Preeclampsia, melatonin, clinical trial, oxidative stress.

Word Count: 1,999.

Abstract

Introduction: Preeclampsia is a common pregnancy condition affecting between 3-7% of women. Unfortunately, the exact pathophysiology of the disease is unknown and as such there are no effective treatments that exist notwithstanding prompt delivery of the fetus and culprit placenta. As many cases of preeclampsia occur in preterm pregnancies, it remains a significant cause of both maternal and perinatal morbidity and mortality. Recently, both *in vitro* and animal studies have highlighted the potential role of antioxidants in mitigating the effects of the disease. Melatonin is a naturally occurring antioxidant hormone and provides an excellent safety profile combined with ease of oral administration. We present the protocol for a Phase I pilot clinical trial investigating the efficacy and side effects of maternal treatment with oral melatonin in pregnancies affected by preterm preeclampsia.

Methods and analysis: We propose undertaking a single-arm open label clinical trial recruiting 20 women with preterm preeclampsia (24^{+0}-35^{+6} weeks). We will take baseline measurements of maternal and fetal well-being, levels of oxidative stress, ultrasound Doppler studies and other biomarkers of preeclampsia. Women will then be given oral melatonin (10 mg) three times daily until delivery. The primary outcome will be time interval between diagnosis and delivery compared to historical controls. Secondary outcomes will compare the baseline measurements previously mentioned with twice-weekly measurements during treatment, and then 6 weeks postpartum.

Ethics and dissemination: Ethical approval has been obtained from Monash Health Human Research Ethics Committee B (HREC 13076B). Data will be presented at international conferences and published in peer-reviewed journals.

Trial registration number: ACTRN12613000476730 (ANZCTR).

Article Summary

Article focus:

- The aim of the trial is to establish whether melatonin will afford a clinical or biochemical benefit in women with early-onset preeclampsia.
- To test the hypothesis, we will pose the following research aims in pregnancies affected by preterm preeclampsia:
    - To determine the effect of daily maternal oral treatment with melatonin on clinical outcomes.
    - To determine the effect of daily maternal oral treatment with melatonin on the oxidative stress response in the maternal, placental and fetal circulation.
    - To determine the effect of daily maternal oral treatment with melatonin on the clinical and biochemical measures of vascular function in the mother and fetus.

Key messages:

- Preeclampsia is a life-threatening condition for both mother and baby with increased levels of oxidative stress. Unfortunately, despite ongoing advancements in perinatal care, preeclampsia remains an incurable disease.
- Extensive animal studies demonstrate that the use of melatonin as an antioxidant in high-risk pregnancies is very promising and does not harm the developing fetus.
- This study aims to be the first human trial to assess the potential clinical and biochemical effects of melatonin in pregnancies complicated by preterm preeclampsia.

Strengths and limitations of this study:

- The strengths of this trial are that it is an appropriately designed pilot study with realistically set numbers to achieve easily measurable outcomes. Significant preparatory work has been conducted into the preliminary *in-vitro* and animal studies to guide the trial design. The trial is the first of its type world-wide and if successful, will be able to direct future randomised controlled trials.
- The limitation of this trial is that due to the nature of such a pilot study in pregnant women, the relatively small numbers of participants must act as their own pre-treatment controls. It is predicted that this limitation will be overcome in subsequent trials that will be largely informed by the outcomes of this study.

Introduction

Preeclampsia is a multi-organ syndrome of pregnancy that manifests after 20 weeks gestation with new-onset hypertension alongside maternal end-organ dysfunction and/or intrauterine fetal growth restriction [1]. It affects between 3-7% of all pregnancies and is associated with substantial maternal and perinatal morbidity and mortality, with a significant proportion of fetal complications due to prematurity [2]. To date, the exact pathophysiology of preeclampsia is unknown, but early placental dysfunction plays a central role in all leading hypotheses [3 4]. This placental dysfunction is thought to result in a local and systemic cascade of increasing oxidative stress in the mother, leading to endothelial dysfunction and subsequent end-organ consequences.

Placental hypoxia and reperfusion, as a consequence of abnormal placentation, result in oxidative stress leading to apoptotic and necrotic disruption of the syncytial structure [5]. This disruption then results in the release of various factors and compounds from the intervillous space into the maternal circulation that stimulate the production of pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF alpha), interleukin (IL)-6 and anti-angiogenic factors such as soluble fms-like tyrosine kinase 1 (sFlt1) and soluble endoglin (sEng) [4]. The resultant effect involves potentially widespread increased oxidative stress with anti-angiogenic compromise to the maternal vasculature.

Melatonin (5-methoxy-N-acetyltryptamine) is an endogenous lipid-soluble antioxidant hormone produced primarily by the pineal gland in humans, providing circadian and seasonal timing cues. In addition, melatonin is also a powerful antioxidant, acting both as a direct scavenger of oxygen free radicals, especially the highly damaging hydroxyl radical, and indirectly via up-regulation of antioxidant enzymes including GSHPx, GSH-reductase, superoxide dismutase and catalase [6 7].

Melatonin has several characteristics that make it an appealing treatment for use in pregnancy. Melatonin freely crosses the placenta [8] and blood-brain barrier [9], and has an excellent safety profile with no known adverse effects [10 11]. Reduced levels of melatonin are found in pregnant women with preeclampsia [12]. Placentae express receptors for melatonin [13] and thus melatonin may protect against oxidative stress generated by the dysfunctional organ, thereby inhibiting the release of vasoactive factors responsible for the clinical syndrome of preeclampsia.

Our group has previously shown that in an animal model of fetal growth restriction (FGR), melatonin administration reduced fetal hypoxia, improved neurodevelopment and decreased brain injury and oxidative stress in newborn lambs [14]. In another published experiment we administered melatonin shortly before and during a short period of severe fetal asphyxia induced by umbilical cord occlusion at late-gestation. Melatonin prevented the formation of free radicals (hydroxyl radical) within the fetal brains and decreased lipid peroxidation and brain cell death [8]. The protective effects of melatonin on ischemia-reperfusion-induced oxidative damage to mitochondria in the rat placenta have also been published [15]. Melatonin treatment improves placental function (fetal:placental weight ratio), improves birth weight and induces antioxidant enzymes in a rat model of maternal undernourishment, known to promote oxidative stress [16].

Melatonin has been studied in several clinical trials in humans at varying gestations and for different purposes. Melatonin has been assessed in assisted reproductive technology where the quality of oocytes is important for the success of in-vitro fertilization (IVF). Melatonin is an important compound found in the follicular fluid that has been shown to be important for oocyte maturation and quality, and has been suggested to improve pregnancy outcomes with IVF [17-19]. To date, no babies born from melatonin-treated pregnancies have been shown to have any increase in abnormalities [20]. Melatonin has also been shown to up-regulate antioxidant enzymes in human pregnancies leading to the conclusion that melatonin might provide an indirect protection against injury caused by reactive oxygen species as seen in preeclampsia, FGR and fetal hypoxia [21].

Melatonin does not have any acute pharmacological effects in the nervous or vascular systems, apart from its benign but active effect on sleep mechanisms [22]. Mice that have received extremely high doses of up to 800 mg/kg melatonin did not have increased mortality and as such the median lethal dose could not be established [22]. In humans, a Phase II clinical trial conducted in the Netherlands administered 75 mg melatonin nightly to 1400 women over 4-years, with no serious side effects reported [23]. The maternal no-adverse-effect-level (NOAEL) for melatonin has been found to be 100 mg/kg/day with the fetal NOAEL established at ≥200 mg/kg/day when administered to the mother. The maternal lowest observed adverse effect level (LOAEL) toxicity was 200 mg/kg/day [24].

Rationale

Preeclampsia is a life-threatening condition for both mother and baby affecting up to 7% of pregnancies [2]. Despite ongoing advancements in perinatal care, preeclampsia remains an incurable disease. Extensive animal studies demonstrate that the use of melatonin as an antioxidant in high-risk pregnancies is very promising. Melatonin readily crosses the placenta and does not harm the developing fetus, not even when administered in extremely high doses [22]. This study aims to be the first human trial to assess the potential clinical and biochemical effects of melatonin in pregnancies complicated by preterm preeclampsia.

Aims

The aim of the trial is to establish whether melatonin will afford a clinical or biochemical benefit in women with early-onset preeclampsia. To test the hypothesis, we will pose the following research aims:

1. To determine the effect of daily maternal oral treatment with melatonin on the clinical outcomes of pregnancies affected by preterm preeclampsia.
2. To determine the effect of daily maternal oral treatment with melatonin on the oxidative stress response in the maternal, placental and fetal circulation in pregnancies affected by preterm preeclampsia.
3. To determine the effect of daily maternal oral treatment with melatonin on the clinical and biochemical measures of vascular function in the mother and fetus in pregnancies affected by preterm preeclampsia.

Methods and analysis

Study design

Phase I single-arm open label clinical trial.

Subjects

We plan to recruit 20 women with preterm preeclampsia. We will also perform a retrospective review of cases over the previous 24 months to use as historical controls for the primary outcome measure.

Study setting

We will recruit patients from the obstetric departments of Monash Health and Jessie McPherson Private Hospitals co-located in Melbourne, Australia over a 12-month period.

Sample size

This study is a proof-of-principle Phase I trial and as such a power calculation has not been performed to determine sample size. The purpose of this trial is to establish appropriate outcome measures that can be used to calculate power for a future Phase II randomized-controlled trial for the same intervention.

Inclusion criteria

1. Be at least 18 years of age.
2. Be between 24^{+0} weeks' and 35^{+6} weeks' gestation.
3. Have a singleton pregnancy.
4. Have a diagnosis of Preeclampsia (SOMANZ criteria [1]).
5. Be considered capable of safely continuing the pregnancy for 48 hours or more, as determined by the attending clinician.
6. Obstetrician and neonatologist believe the fetus is likely to be viable.
7. No major anomalies evident on the mid-trimester morphology scan.
8. Be capable of understanding the information provided, with use of an interpreter if required.
9. Give written informed consent.

Exclusion criteria

1. Eclampsia.
2. Current use of melatonin.

3. Contraindications to melatonin use including:
    a. Hypersensitivity to melatonin or any of its derivatives.
4. Imminent transfer to a non-trial centre due to unavailability of neonatal beds.
5. Significant uncertainty regarding gestational age.
6. Women to be treated as an outpatient.
7. Use of any of the following medications:
    a. Fluvoxamine.
    b. 5- or 8-Methoxypsoralen.
    c. Cimetidine.
    d. Quinolones and other CYP1A2 inhibitors.
    e. Carbamazepine, rifampicin and other CYP1A2 inducers.
    f. Zaleplon, zolpidem, zopiclone and other non-benzodiazepine hypnotics.

Participant enrolment

The PAMPR trial will be introduced to potential trial participants identified from routine antenatal clinics, pregnancy assessment units and labour ward admissions within the trial institutions ~~various settings within the trial institutions. The~~ as identified by the Principal Investigator. ~~clinical research team will give the potential participant an information sheet about the trial and answer any questions she or her relatives may have.~~ Maternal and fetal assessments by the treating clinician combined with the results of blood tests will determine whether immediate delivery is essential for the survival and wellbeing of mother and baby. If the treating~~attending~~ clinician considers delivery within 48 hours as probable, the mother is not eligible for inclusion to the PAMPR trial. Absolute criteria for immediate delivery are not specified in this protocol and remain the responsibility of the attending clinician.

Where delivery within 48 hours is considered unlikely, the women can be approached for consent to the PAMPR trial. The Principal Investigator will provide written information about the trial and answer any questions she or her relatives may have. The patient's written informed consent to participate in the trial must be obtained before recruitment and after a full explanation has been given of the treatment options and the manner of treatment administration. Ideally there should be a period of 24 hours for the women to consider whether she wishes to take part in the trial. However, it is considered clinically important to initiate treatment as soon as possible after the diagnosis of preeclampsia, therefore consent should be sought at the earliest opportunity, provided the investigator is reassured that the woman has fully understood the requirements of the trial.

Following admission to the hospital the trial medication will be written on the patient drug chart and administered through usual prescribing practices.

The historical controls for the primary outcome measure (interval from diagnosis to delivery) will be obtained through de-identified retrospective review of medical records from Monash Health over the previous 24-month period. These historical controls will be women diagnosed with preterm preeclampsia who meet the inclusion and exclusion criteria who underwent expectant management.

Intervention

Eligible women will receive melatonin (10 mg) tablets three times daily from recruitment until delivery.

Primary outcome

Interval in days from participant diagnosis with preeclampsia until delivery compared to historical controls.

Secondary outcomes

Table 1. Maternal biomarkers of oxidative stress.

Maternal biomarkers of oxidative stress Samples collected at recruitment and then up to every three days until delivery		
Malondialdehyde	8-iosprostane	Total antioxidant capacity
Superoxide dismutase	Melatonin	Haeme oxygenase

Table 2. Maternal biomarkers for preeclampsia.

Maternal biomarkers of preeclampsia Samples collected at recruitment and then up to every three days until delivery		
Soluble fms-like tyrosine kinase-1	Vascular endothelial growth factor	Highly sensitive C-reactive protein
Soluble endoglin	Placental growth factor	Activin

von Willebrand Factor	Neutrophil Elastase	Platelet function tests

Table 3. Markers of preeclampsia severity.

Markers of preeclampsia severity Samples collected at recruitment and then up to every three days until delivery		
Maternal blood pressure	Level of proteinuria	Haemoglobin
Platelet count	Liver-transaminases	Renal function
Composite of preeclamptic symptoms: oedema, headache, visual disturbance, epigastric or left upper-quadrant pain		

Table 4. Markers of maternal morbidity.

Markers of maternal morbidity Samples collected at recruitment and then up to every three days until delivery		
Serum creatinine equal to or >120 micmol/L	Proteinuria equal to or >5 g/24h	Hypertension equal to or >170/110 mmHg (despite Rx)
Signs of left ventricular failure	Eclampsia	Platelets <$50x10^9$/L
Disseminated intravascular coagulation	Cerebrovascular event	Liver transaminase equal to or >500 IU/L

Table 5. Ultrasound and Doppler measurements.

Ultrasound and Doppler measurements At recruitment then every three days (biometry every 2 weeks) until delivery		
Maternal brachial artery	Maternal uterine artery	Fetal Morphology
Biometry	Amniotic fluid indices	Placental location/anomalities
Fetal characteristics: heart rate, tone, breathing, movements		
Doppler velocimetry: umbilical artery, middle cerebral artery & ductus venosus		

Table 6. Pregnancy end-points.

Pregnancy end-points Various timings		
Gestation at birth	Mode of birth	Placental histology/weight
Abnormal cardiotocogram	Labour analgesia/anaesthesia	Duration of labour stages
Labour induction/augmentation	Duration of membrane rupture to birth	Group B streptococcus infection
Cord lactates: artery & vein	Presence of meconium liquor	Intrapartum lactates
Use of antihypertensives	Use of magnesium sulfate	Use of corticosteroids

Table 7. Neonatal outcomes.

Neonatal ouctomes At birth		
Sex	Neonatal Apgar scores	Weight at birth
Length	Head circumference	Cord melatonin levels
Composite neonatal outcome: admission to NICU, duration of admission, need & duration of respiratory support, intraventricular haemorrhage, necrotising enterocolitis, abnormal neurology, mortality prior to discharge		

Table 8. Any serious adverse event.

Any serious adverse event At any time during trial

Proposed analyses

The length of gestation post recruitment will be analysed using a t-test.

The biomarkers will be analysed using a repeated measures analysis, including the baseline value as a covariate. For the primary analysis the treatment effect will be considered constant over time, secondary analyses will examine the possibility of a trend over time. Plots of mean score over time will be shown for clarification. Initially, the treatment effect will be assumed to be constant over time, but if time by treatment interaction is shown to be important by including this parameter in the model (the conventional level of p=0.05 will be used here) then further investigation into effects at differing time points will be made by analysing the least-square means as above. Plots of mean score over time will be shown for clarification.

All other continuous measures (clinical measures, etc) will be considered in the same manner as above (adjusting by baseline value if available). Dichotomous outcomes (mortality, etc) will be presented as risk ratios, with a corresponding chi-squared test performed.

Apart from baseline value, no adjustments for covariates will be made in the first instance in any of the investigations. Treatment estimates will only be adjusted when subgroups are explored.

Interaction between treatment and subgroup variables will be examined in a similar fashion as above by including the relevant parameters in the model. This will be done in turn for each subgroup variable and adjusted estimates presented.

All tests are 2-sided and results will be presented as a point estimate along with 95% confidence intervals.

Adverse events

Participants will be inpatients for the duration of the trial. As such, a senior obstetric clinician who will be in ongoing communication with the research team will see them daily. The principal investigator of the PAMPR trial will be contactable by telephone at all times in the case of an adverse event. Any serious adverse events that occur after joining the trial will be reported in detail in the participant's medical notes, followed up until resolution of the event and reported to the Monash Health Health Human Research Ethics Committee, Therapeutics Committee and Therapeutic Goods Administration of Australia's Office of Scientific Evaluation immediately or within 24-72 hours.

Trial discontinuation or modification

Participants will be withdrawn from the trial at participant request or if they or their fetus suffer an unexpected serious adverse event as noted above. Worsening preeclampsia is within the natural history of the disease and as such will not be part of discontinuation criteria.

There will be no allowance for modification of the trial intervention.

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Ethics and dissemination

Ethical approval has been obtained from the Monash Health Human Research Ethics Committee B (HREC 13076B). Data will be presented at international conferences and published in peer-reviewed journals. We will make the information obtained from the study available to the public through national bodies and charities.

Discussion

If effective, we believe that treatment with melatonin could become standard of care for women with pregnancies complicated by preterm preeclampsia. The potential benefits to both mother and baby would significantly reduce what is a terrible burden of disease internationally. Treatment with this medication also has potential use in other pregnancy disorders such as FGR and hypoxia.

We do not however, anticipate that this will be the final trial to determine whether further exploration of this area is worthwhile. We hope that the study will generate sufficient evidence that melatonin may be effective; to support a funding application for a larger randomized controlled trial. Such a trial could be designed to test the hypothesis that melatonin treatment in preterm preeclampsia afforded superior and more cost-effective outcomes prioritised by consumer groups and clinicians than existing managements.

References:

1. Lowe SA, Brown MA, Dekker GA, et al. Guidelines for the management of hypertensive disorders of pregnancy 2008. The Australian & New Zealand journal of obstetrics & gynaecology 2009;**49**(3):242-6 doi: 10.1111/j.1479-828X.2009.01003.x[published Online First: Epub Date]|.

2. Payne B, Magee LA, von Dadelszen P. Assessment, surveillance and prognosis in pre-eclampsia. Best practice & research. Clinical obstetrics & gynaecology 2011;**25**(4):449-62 doi: 10.1016/j.bpobgyn.2011.02.003[published Online First: Epub Date]|.

3. Roberts JM, Gammill HS. Preeclampsia: recent insights. Hypertension 2005;**46**(6):1243-9 doi: 10.1161/01.HYP.0000188408.49896.c5[published Online First: Epub Date]|.

4. Steegers EA, von Dadelszen P, Duvekot JJ, Pijnenborg R. Pre-eclampsia. Lancet 2010;**376**(9741):631-44 doi: 10.1016/S0140-6736(10)60279-6[published Online First: Epub Date]|.

5. Burton GJ, Jauniaux E. Oxidative stress. Best practice & research. Clinical obstetrics & gynaecology 2011;**25**(3):287-99 doi: 10.1016/j.bpobgyn.2010.10.016[published Online First: Epub Date]|.

6. Reiter RJ, Tan DX. Melatonin: a novel protective agent against oxidative injury of the ischemic/reperfused heart. Cardiovascular research 2003;**58**(1):10-9

7. Reiter RJ, Tan DX, Osuna C, Gitto E. Actions of melatonin in the reduction of oxidative stress. A review. Journal of biomedical science 2000;**7**(6):444-58 doi: 25480[published Online First: Epub Date]|.

8. Miller SL, Yan EB, Castillo-Melendez M, Jenkin G, Walker DW. Melatonin provides neuroprotection in the late-gestation fetal sheep brain in response to umbilical cord occlusion. Developmental neuroscience 2005;**27**(2-4):200-10 doi: 10.1159/000085993[published Online First: Epub Date]|.

9. Welin AK, Svedin P, Lapatto R, et al. Melatonin reduces inflammation and cell death in white matter in the mid-gestation fetal sheep following umbilical cord occlusion. Pediatric research 2007;**61**(2):153-8 doi: 10.1203/01.pdr.0000252546.20451.1a[published Online First: Epub Date]|.

10. Gitto E, Pellegrino S, Gitto P, Barberi I, Reiter RJ. Oxidative stress of the newborn in the pre- and postnatal period and the clinical utility of melatonin. Journal of pineal research 2009;**46**(2):128-39 doi: 10.1111/j.1600-079X.2008.00649.x[published Online First: Epub Date]|.

11. Fulia F, Gitto E, Cuzzocrea S, et al. Increased levels of malondialdehyde and nitrite/nitrate in the blood of asphyxiated newborns: reduction by melatonin. Journal of pineal research 2001;**31**(4):343-9

12. Lanoix D, Guerin P, Vaillancourt C. Placental melatonin production and melatonin receptor expression are altered in preeclampsia: new insights into the role of this hormone in pregnancy. Journal of pineal research 2012;**53**(4):417-25 doi: 10.1111/j.1600-079X.2012.01012.x[published Online First: Epub Date]|.

13. Lanoix D, Beghdadi H, Lafond J, Vaillancourt C. Human placental trophoblasts synthesize melatonin and express its receptors. Journal of pineal research 2008;**45**(1):50-60 doi: 10.1111/j.1600-079X.2008.00555.x[published Online First: Epub Date]|.

14. Miller SL, Wallace EM, Walker DW. Antioxidant therapies: a potential role in perinatal medicine. Neuroendocrinology 2012;**96**(1):13-23 doi: 10.1159/000336378[published Online First: Epub Date]|.

15. Okatani Y, Wakatsuki A, Shinohara K, Taniguchi K, Fukaya T. Melatonin protects against oxidative mitochondrial damage induced in rat placenta by ischemia and reperfusion. Journal of pineal research 2001;**31**(2):173-8

16. Richter HG, Hansell JA, Raut S, Giussani DA. Melatonin improves placental efficiency and birth weight and increases the placental expression of antioxidant enzymes in undernourished pregnancy. Journal of pineal research 2009;**46**(4):357-64 doi: 10.1111/j.1600-079X.2009.00671.x[published Online First: Epub Date]|.

17. Tamura H, Nakamura Y, Terron MP, et al. Melatonin and pregnancy in the human. Reprod Toxicol 2008;**25**(3):291-303 doi: 10.1016/j.reprotox.2008.03.005[published Online First: Epub Date]|.

18. Rizzo P, Raffone E, Benedetto V. Effect of the treatment with myo-inositol plus folic acid plus melatonin in comparison with a treatment with myo-inositol plus folic acid on oocyte quality and pregnancy outcome in IVF cycles. A prospective, clinical trial. European review for medical and pharmacological sciences 2010;**14**(6):555-61

19. Unfer V, Raffone E, Rizzo P, Buffo S. Effect of a supplementation with myo-inositol plus melatonin on oocyte quality in women who failed to conceive in previous in vitro fertilization cycles for poor oocyte quality: a prospective, longitudinal, cohort study. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology 2011;**27**(11):857-61 doi: 10.3109/09513590.2011.564687[published Online First: Epub Date]|.

20. Unfer V. Personal communication via email. In: Mockler J, ed., 2012.

21. Okatani Y, Okamoto K, Hayashi K, Wakatsuki A, Tamura S, Sagara Y. Maternal-fetal transfer of melatonin in pregnant women near term. Journal of pineal research 1998;**25**(3):129-34

22. Barchas J, DaCosta F, Spector S. Acute pharmacology of melatonin. Nature 1967;**214**(5091):919-20

23. Silman RE. Melatonin: a contraceptive for the nineties. European journal of obstetrics, gynecology, and reproductive biology 1993;**49**(1-2):3-9

24. Jahnke G, Marr M, Myers C, Wilson R, Travlos G, Price C. Maternal and developmental toxicity evaluation of melatonin administered orally to pregnant Sprague-Dawley rats. Toxicological sciences : an official journal of the Society of Toxicology 1999;**50**(2):271-9

Authors' contributions

SRH: research, contribution of original material, editing and approval of final manuscript; RL, EEG, EMW: editing and approval of final manuscript.

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Competing interests statement

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