Pre-eclampsia — a disease of oxidative stress resulting from the catabolism of DNA (primarily fetal) to uric acid by xanthine oxidase in the maternal liver: A hypothesis

Roger A. McMaster-Fay*

Department of Obstetrics and Gynaecology, University of Sydney, PO Box 82, 2 Nepean Street, Emu Plains, NSW 2750, Australia

Abstract   Pre-eclamptic toxaemia or toxaemia has become outdated terminology for the disease of pregnancy called pre-eclampsia (PE) but, according to this hypothesis, these may be more relevant. This hypothesis is that PE is a toxaemia or poisoning of the blood that results in multi-organ dysfunction and injury, putting at risk the lives of both the infant and the mother. Yet these dysfunctions and injuries are reversible with the cessation of the pregnancy and the disease can be reduced with vitamins (antioxidants) and aspirin.

This hypothesis is that the PE cascade starts with excessive shedding/embolisation of trophoblast from the placenta into the maternal venous circulation. This trophoblast embolisation ('deportation') is secondary either to an excessively large amount of trophoblast tissue ('hyperplacentosis') or to vascular trophoblast injury from a faulty uteroplacental circulation. The deported nuclear rich trophoblast is largely filtered out of the circulation in the lungs, and breaks down releasing fetal DNA. Accordingly, the level of fetal DNA in the maternal circulation rises. This DNA is then broken down in the maternal liver with the hepatocytes being presented with excessive amounts of purines for catabolism. In the hepatocytes of patients who subsequently develop PE, there is activation of xanthine oxidase (XO), the more toxic iso-enzyme of xanthine oxidoreductase (XOR), with the generation of superoxide anion (O_2^-) as a by-product. Excessive superoxide production overwhelms the normal antioxidant ability of the tissues to produce oxidative stress.

In the hepatocytes, the excessive superoxide causes the peroxidation of polyunsaturated lipids to form microvesicular fat deposition. Excessive superoxide also causes hepatocellular damage with leakage of enzymes, lipids, DNA and superoxide into the circulation. In the circulation, oxidative injury of the blood corpuscles occurs releasing more DNA and accelerating purine catabolism and oxidative stress.

* Tel.: +61 2 4735 4900; fax: +61 2 4735 4911.
  E-mail address: rfay@optushome.com.au

The toxins, superoxide and the other reactive oxygen species (ROS), then travel in the arterial blood to the peripheral circulation where the microvasculature, the arterioles, capillaries, endothelial cells and venules, is injured. The damaged microvasculature leaks intravascular fluid into the extravascular compartment causing an intravascular dehydration and tissue oedema. In the kidneys, protein leaks through the damaged glomerular capillaries causing proteinuria. ROS causes arteriolar vasospasm and impairs vasorelaxation, mechanisms of hypertension. Micro-haemorrhages can occur and in the brain these, in combination with hypertension and oedema, can result in seizures or eclampsia.

Introduction

Why is it that only humans get toxaemia? Is it due to the fact that we lack the enzyme urate oxidase? Humans lack that enzyme because we have a defect in the gene that transcribes it [1] and so our UA is higher in comparison to other mammals. In humans, the final two reactions of the purine catabolic pathway are catalysed by the enzyme xanthine oxidoreductase (XOR) with the conversion of hypoxanthine to xanthine and then to UA. This reaction occurs mainly in the liver.

Hypertension, proteinuria and oedema define pre-eclampsia (PE) [2] but ''their causes are largely unknown'' [3]. For some, proteinuria is not essential to the definition [4]. Redman et al. [5] found that in terms of fetal outcome in PE, uric acid (UA) was a more important feature than hypertension. Roberts et al. [6] found that UA was ''at least as important as proteinuria in identifying pregnancies with gestational hypertension with at-risk infants.'' Many others have confirmed the importance of hyperuricaemia in PE with Yassaee [7] finding that all major measures of perinatal and maternal outcome were significantly worse in patients with higher UA. Roberts et al. [6] went on to recommend that hyperuricaemia be included in the definition of PE, at least for research purposes, as we have previously done [8,9].

As hyperuricaemia is quantitatively related to the outcome of the pregnancy for both the infant and the mother, Fay [10] proposed that UA production was an important factor in the PE disease process. He suggested that hyperuricaemia is mainly a metabolic outcome of placental injury via purine catabolism and concluded that ''the rise in UA in PE, which seems to mirror the disease process so closely, would be more logically related to some relevant process, such as placental damage, rather than some ill defined alteration in renal tubular function with, as yet, no known direct relationship to the PE disease process.''

Background

Trophoblast embolisation

Attwood and Park [11] analysed the findings from the post-mortem examination of the lungs of 220 women who died in association with pregnancy. They found embolised syncytiotrophoblast (STB) in the pulmonary capillaries of 84% of women who died of PE/eclampsia whereas they found STB present in only 27% of those dying from all other causes. They quantitated the amount of trophoblast present with a 'trophoblastic index'. Of those women who had detectable STB in their pulmonary capillaries, those dying of PE/eclampsia had more than twice the amount of STB than those dying of other causes. With PE/eclampsia the amount of STB was proportional to the severity of the disease. Blood collected from the uterine veins at the time of caesarean delivery in patients with PE had significantly more STB (microvilli) than in controls [12].

Uteroplacental circulation

In patients who develop PE, there is a failure in the development of the normal uteroplacental circulation, with a failure of the spiral arteries (the maternal vessels that supply the intervillous space of the placenta) to dilate and accommodate the ever increasing blood requirements of the pregnancy. The normal process by which this dilatation of the spiral arteries occurs is termed '*physiological change*' and this is the final stage of placentation is completed by 22—24 weeks gestation [13]. The spiral arteries that fail to develop normal *physiological change* may subsequently undergo a disease process of atheromatous change with a further narrowing and sometimes frank obstruction of these vessels and this process is termed '*acute atherosis*'.

The failure to develop a normal uteroplacental circulation results in placental injury with increased shedding of STB particles into the maternal circulation. The STB particles are made up of conglomerations of STB nuclei, the largest of which are filtered out in the maternal lungs. The STB particles and broken down releasing fetal DNA into the maternal circulation.

Fetal DNA

Cell-free fetal DNA (cffDNA) can be detected in the maternal blood in the form of the *SRY* gene from the Y chromosome from male fetal DNA. cffDNA has been found to be elevated in patients with PE with a male infant, with the elevation in cffDNA being proportional to the severity of the disease [14].

Levine et al. [15] in a large series of 120 cases and 120 controls demonstrated that in the 3 weeks before delivery the cffDNA levels in the maternal serum of PE patients rose to more than twice the levels in the controls. They also demonstrated a two-stage elevation of cffDNA in the maternal serum before the onset of PE. The first significant elevation of cffDNA occurred at the end of the second

trimester and the second elevation occurred in the last few weeks prior to the development of PE (above). These two peaks in cffDNA are synchronistic with the maldevelopment of the uteroplacental circulation of patients who develop PE, i.e. the failure of *physiological change* and the development of *acute atherosis*.

Maternal DNA

Zhong et al. [14] measured both fetal and total (fetal plus maternal) cell-free DNA in PE and found an approximate 10-fold increase in both severe PE compared with controls. They also found a threefold increase in both fetal and total DNA in mild to moderate PE.

Purine catabolism

Cell-free DNA is catabolised mainly in the (maternal) liver with the purines being broken down to form UA. Xanthine oxidoreductase (XOR) is the enzyme that catalyses the last two chemical reactions of purine catabolism in humans by converting hypoxanthine to xanthine and then xanthine to UA. XOR is a 1333 amino acid sequence protein with its gene encoded on the short arm of chromosome 2. This enzyme is found mainly in the liver (and small intestine) [16].

Serum uric acid as an index of severity of PE

Redman et al. [5] found that in pregnancy-induced hypertension, perinatal mortality was significantly higher in those with hyperuricaemia. They concluded that in terms of fetal health, hyperuricaemia was more important than hypertension. Yassaee [7] (in a report from Iran) found not only increased perinatal mortality but also an increased maternal mortality in severe PE patients with hyperuricaemia than those patients without hyperuricaemia.

Roberts et al. [6] performed a retrospective study of over 400 patients with pregnancy-induced hypertension over a 6-year period in their institution. They looked at the parameters of hypertension, proteinuria and hyperuricaemia in all possible combinations. They found that uric acid was "at least as important as proteinuria in identifying pregnancies with gestational hypertension with at-risk infants". HELLP syndrome (haemolysis, elevated liver enzymes and lowered platelets) only occurred in mothers with all three: hypertension, proteinuria and hyperuricaemia. They found that "adverse outcomes are only present with concomitantly increased uric acid" and that "the absence of hyperuricemia identifies a lower risk group". They recommended, as Chesley [17] had, that hyperuricaemia be included in the definition of PE, at least for research purposes.

Aetiology of hyperuricaemia in PE; renal *vs*. purine catabolism

The dominant paradigm has been that the hyperuricaemia of PE is a renal phenomenon [5] but a review of some older data together with more recent data, would make this paradigm incomplete. Fadel et al. [18,19] studied the uric acid/urea ratio in pregnancy. They found that in normal

pregnancy in the third trimester, the mean ratio was 0.13, in pregnancy with chronic renal disease it was 0.16 but in PE/eclampsia it was significantly higher at 0.21, with mean levels higher in more severe cases. They concluded that "factors other than reduced glomerular filtration rate must be operative in the hyperuricaemia of PE" and that "increased production of UA plays a significant role in the hyperuricaemia that characterizes PE and eclamptic pregnancies. The placenta is a possible source of the increased uric acid production in these pregnancies."

Powers et al. [20] found that patients with a combination of hypertension, proteinuria and hyperuricaemia had a 35% increase in the serum uric acid over the normal patients and yet only 11% rises in the serum creatinine. They state that the altered glomerular filtration rate "accounted for part, but not all, of the rise in serum uric acid in PE." Hayashi et al. [21] found the serum uric acid in PE was increased by approximately 60%, whereas the uric acid/creatinine clearance ratio decreased by only 17.3% which would indicate a non-renal cause for the rise in serum uric acid in PE.

These studies would indicate that the hyperuricaemia in PE is more likely due to the increased production of UA than to altered renal handling of UA.

Zhong et al. [14] found that cell-free fetal DNA makes up less than 2% of the total cell-free DNA in the maternal serum in both pregnancy and PE and yet they found a dramatic 10-fold increase in both fetal and total cell-free DNA in patients with severe PE. Thus the catabolism of this dramatically increased amount cell-free DNA is more likely to be the dominant origin of the increase in UA found in PE.

Liver enzymes (liver function tests)

Increased amounts of liver enzymes are found in the blood when there is hepatocyte injury and leakage of the intracellular contents into the circulation. Remero et al. [22] measured the liver enzyme serum glutamic oxalacetic transaminase (SGOT) in pregnancy-induced hypertension (PIH) and found elevated levels in 21% of a population of 355 patients. Those patients with liver dysfunction had increased maternal and neonatal morbidity. They also found that patients with an elevated SGOT had significantly higher serum uric acid levels then those with a normal liver function. XO, a predominately liver enzyme, has also been recently been found to be elevated in the maternal plasma in PE [23]. Ch'ng et al. [24] in a large prospective study of liver dysfunction in pregnancy found that almost all of patients with PE and abnormally elevated liver function tests had hyperuricaemia. Thus there seems to be a strong association between liver dysfunction and hyperuricaemia in PE.

Histopathological studies of the liver

Histopathological studies of the liver performed Minakami et al. [25,26] are pivotal to this hypothesis (Fig. 1). They performed a unique series of studies on liver biopsy specimens from patients with PE, histologically preparing liver tissue meticulously, to specifically examine for the lipid
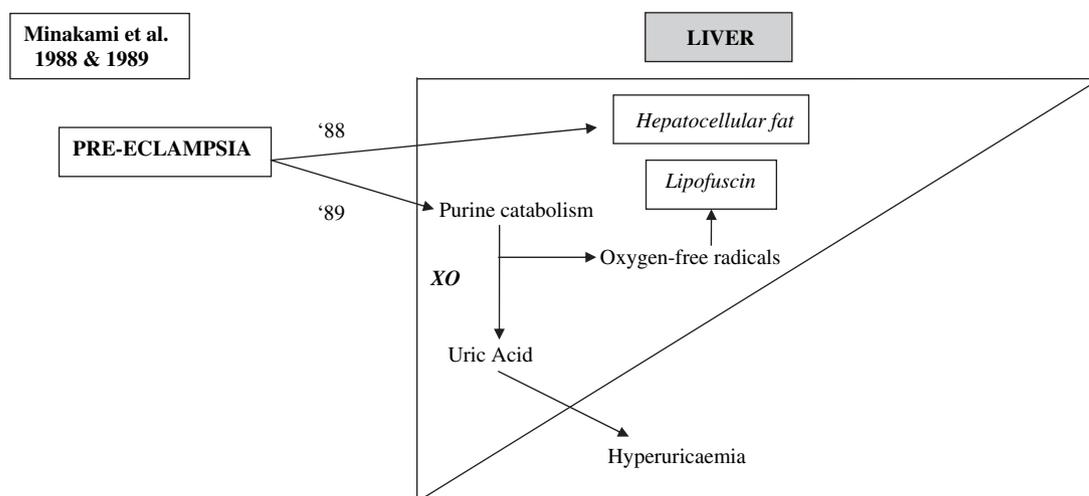
Figure 1 The Minakami models of hepatocellular fat deposition in pre-eclampsia.

content of the hepatocytes both qualitatively and quantitatively. Also their selection of patients was then and will probably remain unique. Liver biopsies were obtained from 45 patients with PE; three were post-mortem specimens from patients who died from acute fatty liver of pregnancy (AFLP); six were from live patients who also had AFLP; four were from patients with HELLP syndrome; 15 had PE with raised serum SGOT; and importantly, there were specimens from 13 with PE without any evidence of liver involvement.

Minakami et al. used special fixing and staining techniques to look for and quantify the fat in the hepatocytes and their results were remarkable. In their first study [25] they looked for microvesicular fat deposition using *oil red O staining* of *frozen sectioned* tissue. All tissues examined had appreciable amounts of small fat droplets in the hepatocytes. They quantified the size and density of these fat droplets. The amount of hepatocellular fat related to both the perinatal mortality and to the elevation of SGOT, but also (importantly to this hypothesis) to the elevation of serum UA.

In the 1989 study [26] they looked for intra-hepatocyte lipofuscin using formalin fixed tissue stained with Schmorl's ferricyanide. All the tissues examined had lipofuscin pigment present. They were then able to quantitate the proportion of the liver tissue that was occupied with the lipofuscin pigment. The amount of lipofuscin pigment granules present in the hepatocytes was again positively correlated to serum UA. They claimed that the formation of lipofuscin pigment was as a result of peroxidation of polyunsaturated lipids by oxygen-free radicals. These oxygen-free radicals as well as UA are produced by the enzyme xanthine oxidase (XO) in the liver. Similar studies of intra-hepatocyte lipid content in PE have not been performed.

Xanthine oxidase

XOR, a predominately liver enzyme, has two isoenzymes: xanthine dehydrogenase (XDH) and xanthine oxidase (XO). The dehydrogenase is the most commonly active form of

the enzyme although this can be converted to the oxidase. XO is the more toxic of these isoenzymes as it produces the superoxide anion/radical (O_2^-) as a by-product of the formation of UA. Superoxide can go on to create hydrogen peroxide (H_2O_2) and the hydroxyl radical (OH^-) all of which are referred to collectively as the reactive oxygen species (ROS) [16].

Oxidative stress

ROS becomes toxic when their production increases beyond the natural antioxidant capacity of the tissues. When the natural antioxidant capacity of the tissues becomes overwhelmed, a state of '*oxidative stress*' arises [27]. This excessive ROS causes lipid peroxidation which disrupts membrane architecture as well as effecting a lysosomal enzyme release, all of which result in tissue injury [28].

A recent review on the evidence of oxidative stress in PE [29], eight papers were found that showed a significant association between an elevation of various biomarkers of oxidative stress and PE (Table 1, [29]). Of particular interest is a recent study by Moretti et al. [30] of the chemicals in the exhaled breath of women with PE. These exhaled chemicals are representative of the chemical nature of the pulmonary arterial blood. They found significantly higher levels of the overall markers of oxidative stress in PE.

Reactive oxygen species (ROS) and antioxidants

Antioxidants are molecules that bind or 'scavenge' free radicals. In conditions of oxidative stress the levels of antioxidants fall as they scavenge the free radicals. Shaaraway et al. [31] measured the antioxidant ability of the serum in PE (mild, severe and eclampsia) and found the levels to be lower in PE than normal pregnancy, with the level inversely proportional to the severity of the disease.

Two groups have reported on both antioxidant capacities with measures of ROS in the serum of patients with PE. Karb [32] found evidence of increased superoxide generation in the serum and Harma et al. [33] found raised plasma

peroxide in patients with PE. Both studies found a reduction in the antioxidant potential capacity with PE. Harma et al. [33] combined both antioxidant capacity with ROS to develop an 'oxidative stress index' which was significantly elevated in PE.

Prophylactic treatment with antioxidants has been shown to reduce PE in high risk patients defined by a marker [34]). Subsequent antioxidant trials failed to show such a benefit but these latter trials had very broad entry criteria and thus their patients were not of high risk.

ROS and blood corpuscle injury

Excessively produced ROS in the maternal liver would leach into the hepatic venous circulation. Once in the circulation, the ROS would then react with the blood corpuscles. The WBC probably takes up or scavenges excessive ROS with Sacks et al. [35] finding a rise in the ROS content of all peripheral blood leukocytes in PE. This accumulation ROS in the WBCs would result in cell injury/apoptosis [36]. With an increased WBC turnover there would be a resultant increase in DNA in the maternal circulation. The ROS could also injure both the erythrocytes and the platelets causing haemolysis and platelet destruction and hence the clinical entity of HELLP syndrome.

Microvascular dysfunction

Del Maestro et al. [37] using an in vivo microvascular preparation of the hamster cheek pouch demonstrated the influence of the free radicals (superoxide anion and ROS) on the peripheral vasculature. The ROS was locally generated by the arterial infusion of hypoxanthine together with xanthine oxidase. Micrographically, they demonstrated arteriolar vasoconstriction, macromolecular extravasation and petechial haemorrhages in response to the ROS. These microvascular dysfunctions were then reversed by cessation of the noxious stimulus.

Endothelial cell dysfunction

The original histological description of the endothelial cell injury in PE was in the renal glomerulus. Light and electron microscopic studies describing extensive swelling and vacuolisation, with patchy collections of lipids in the endothelial cells [38]. Spargo et al. [38] coined the term 'glomerular endotheliosis' to describe this lesion. These findings, along with those of a raised serum fibronectin, a marker of endothelial injury, in PE [39] moved Roberts et al. [40] to propose that PE is an endothelial disorder. Since then there has been mounting evidence of endothelial cell dysfunction in PE with Belo et al. [41] finding a significant correlation between the rise in PAI-1, marker of endothelial dysfunction and the degree of proteinuria in PE.

Hypertension

Endothelial cells play a key role in the regulation of vascular tone and endothelial dysfunction can result in hypertension [42]. In vitro studies have shown that superoxide anion breaks down endothelium-derived vascular relaxing factor [43]. Oxidative stress contributes significantly to endothelial dysfunction in cardiovascular disease, as superoxide radicals readily inactivate nitric oxide (NO), thereby impairing vasorelaxation [16]. In the in vivo animal model of Del Maestro et al. [37] (above) infused ROS directly causes arteriolar vasoconstriction. Women who subsequently develop PET show increased vasopressor sensitivity to angiotensin II [44]. In vitro studies showed that serum from patients with PE caused increase the sensitivity of arteries to angiotensin and norepinephrine [45].

Hypothesis (Fig. 2)

The PE disease process begins with the failure of development of the normal uteroplacental circulation in the second trimester. This abnormality results in an excessive shedding of trophoblast (STB) from the placenta into the maternal venous circulation. Most of the shed STB particles are of conglomerations of STB nuclei, with the larger STB particles being filtered out of the circulation in the maternal lungs. The nuclear rich STB particles would be cleared from the circulation by the reticuloendothelial system and broken down, releasing fetal DNA, explaining the dramatically increased amount of fetal DNA found in the maternal serum in PE [14,15]. Fetal DNA is catabolised mainly in the maternal liver with the end product of purine catabolism being UA.

The final enzyme in the purine catabolic pathway in humans, XOR, has two isoenzymes: XDH and XO. XO is a potent source of the superoxide anion, which it produces as a by-product in the formation of UA. Superoxide and the other ROS are highly toxic but they are normally neutralised ('scavenged') by naturally occurring antioxidants. When these antioxidants become overwhelmed oxidative stress occurs, resulting in cellular dysfunction and damage. In the liver, where the majority of purine catabolism occurs, the hepatocytes start to accumulate peroxidised lipids, giving the appearance of microvesicular fat and lipofuscin deposition.

This hypothesis proposes a three-phase process of oxidative stress in PE (Fig. 3):

1. Fetal DNA catabolism in the hepatocyte with the production of ROS resulting in microvesicular fat accumulation. ROS is released into the hepatic venous circulation.
2. Intravascular oxidative stress including injury to the white blood corpuscles and release of maternal DNA with increased purine catabolism and accelerated production of ROS.
3. Hepatocyte injury with release of more maternal DNA and ROS as well as liver enzymes and accumulated lipids into the maternal circulation with erythrocyte and platelet injury.

Hubel et al. [46] observed such an elevation in maternal serum lipids (triglycerides and free fatty acids) in PE. They also observed a parallel rise in the maternal serum levels of the lipid peroxide metabolite malondialdehyde (MDA). According to this hypothesis, these lipids would be coming from diseased hepatocytes.
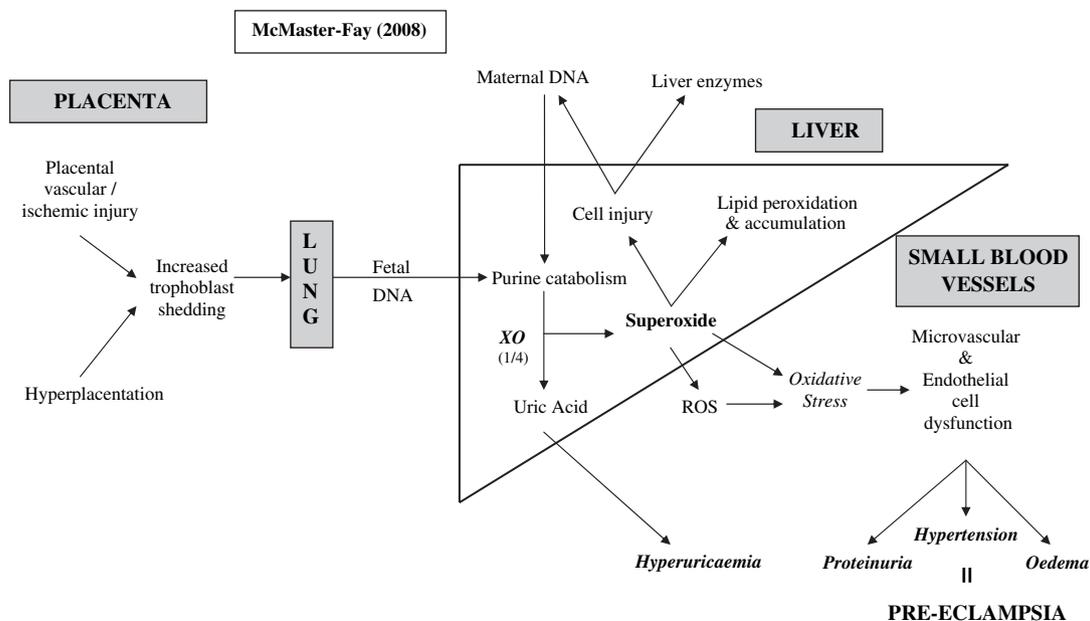
Figure 2 The hypothesis.

The superoxide and other ROS arrive at the peripheral vasculature where they cause injury, which results in the clinical syndrome of PE. This process was demonstrated in the elegant work of Del Maestro et al. [37] Vasospasm and hypertension occur as a direct effect of ROS as well as secondary to endothelial cell injury. The microvasculature leaks macromolecules, including albumen, resulting in proteinuria and oedema. Micro-haemorrhages can then occur and when these occur in the brain, seizures (eclampsia) can result.

Evolution

Fadel et al. [18] proposed that the hyperuricaemia of PE result from increased production of UA by purine catabolism with the source of the purines being placental infarctions (Fig. 3). Fay [10] proposed that, as UA levels are indicative of the severity of PE and predict perinatal mortality, the production of UA was most likely linked to an important factor in the PE process, that being placental injury. The placental injury could be infarction but also ischemia. In addition, Fay [10] proposed a 'jet hose' effect on the trophoblast, secondary to the narrowed spiral arteries and the increased blood pressure. Fay [10] further suggested that the degree hyperuricaemia is quantitatively related to degree of placental injury and hence to the outcome of the pregnancy, for both the infant and the mother. Fay [10] proposed trophoblast nuclei are the substrate for the increased purine catabolism and hence the hyperuricaemia of PE.

The group from the Magee Institute, Pittsburgh, put forward three evolving theories on the aetiology of PE, linking reduced placental perfusion to endothelial cell activation, via an intermediary, with the endothelial cell activation being the cause of the PE (Fig. 4). Roberts et al. [47] proposed a non-specific agent or agents as the
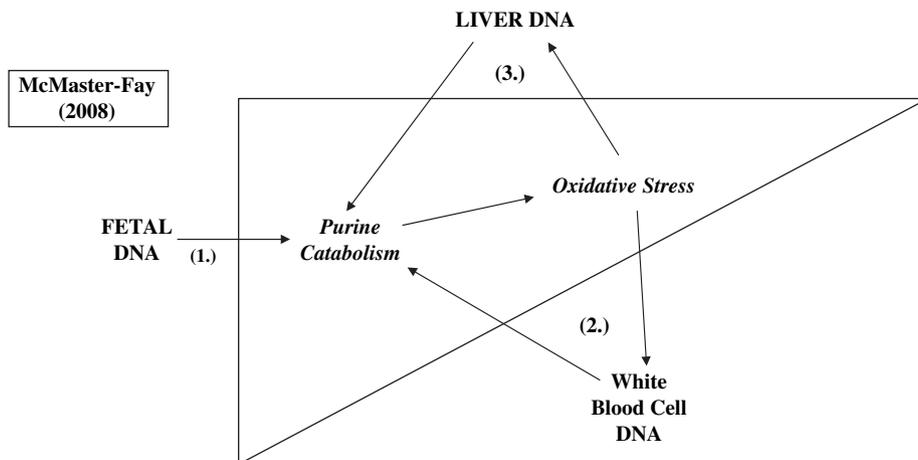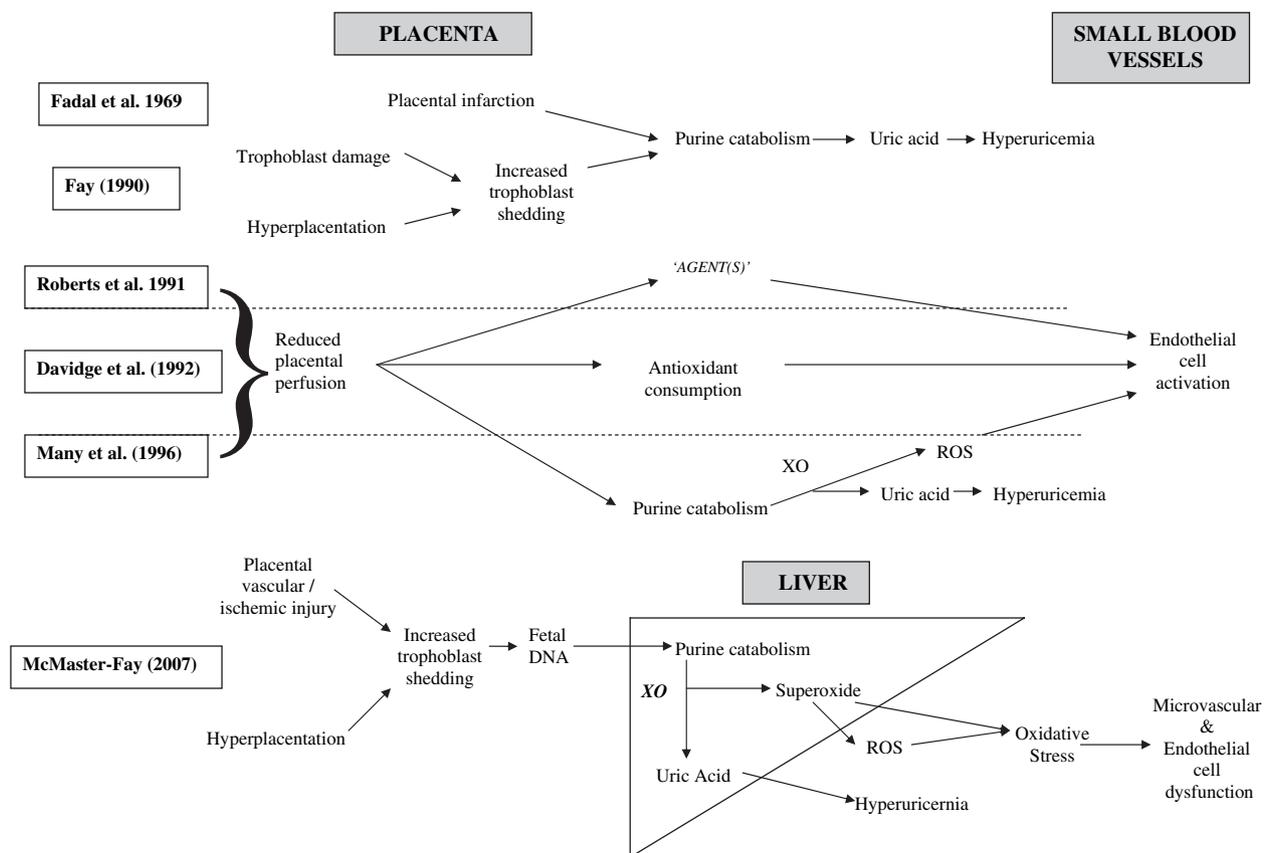

Figure 3 The three-phase process of purine catabolism in pre-eclampsia.

Figure 4   The evolution of this hypothesis of the pathogenesis of pre-eclampsia.

intermediary. Davidge et al. [48] proposed that the intermediary caused antioxidant consumption and thence endothelial cell activation. Many et al. [49] proposed that reduced placental perfusion resulted in increased trophoblast turnover and hence increased purine catabolism resulting in hyperuricaemia. The purine catabolism occurred via XO and produced ROS as a by-product and these ROS were the cause of endothelial cell activation. The Magee group did not mention the liver in any of these three papers.

Difference and importance

In a recent 15 page *Seminar* in the *Lancet* on PE, Sibai et al. [50] did not mention UA or purine catabolism. The hypothesis presented here rejects the long-held paradigm that the hyperuricaemia of PE is mainly a renal phenomenon [5]. In my hypothesis, increased production of UA through purine catabolism is a main aetiological step in the genesis of the hyperuricaemia of PE and plays a major role in the disease process of PE.

Evaluation

An allopurinol trial
This hypothesis on the pathogenesis of PE could be tested by a randomised controlled trial of prophylactic allopurinol, a methylxanthine and potent inhibitor of XO [51], in high risk pregnant patients. These drugs are not associated

with fetal anomalies and are clinically safe to use in pregnancy. Methylxanthine has been shown to inhibit PE-like signs induced in ewes by fasting [52]. Inhibition of XO by allopurinol has also been shown to improve endothelial dysfunction in patients with chronic heart failure [53], Type 2 diabetics with hypertension [54] and smokers [55].

Such a trial would be best structured in a similar fashion as the trial by Chappell et al. [34] using a mid-trimester marker of high risk status. In the style of this study [34], abnormal uterine artery Doppler blood flow studies could be used as a marker. Alternatively mid-trimester serum cffDNA could be the marker or both markers could be used together.

Computerised tomography
It is now possible to non-invasively measure the hepatocyte fat content with computerised tomography [56]. The technique has been standardised in macrosteatohepatosis [57] and it may well be useful in detecting and quantitating the microvesicular fat disease of the liver of PE [25], an important feature of the PE process in this hypothesis.

Implications and consequences

The medical and scientific revolution of the last half-century, in the Western world, has dramatically reduced the maternal and perinatal mortalities and morbidities caused by PE [58]. But the underlying pathophysiology of the disease process has still not been elucidated [3]. Thus

the improvements in outcome have come at a high price, including the training of large numbers of obstetricians, midwives and ancillary staff, along with the development large infrastructures. Unfortunately these developments have only benefited a minority of the pregnant human females on our planet with the majority (non-Westerner) still suffering the mortalities and morbidities associated with PE, which has been described by some as an ongoing 'holo-caust' [59]. Duley [60] estimates that 50,000 maternal deaths occur worldwide each year as a result of eclampsia. Most of these deaths occur in underdeveloped nations. Yas-saee [7] reported a maternal mortality rate of a stunning 17% in cases of severe PE in a university hospital in Tehran. The poorer nations of the world cannot envisage setting up the massive medical infrastructure that exits in the West to conquer the scourge of PE.

If fetal DNA screening proves to be the long waited marker for the early development of PE and allopurinol successfully interrupts the pathophysiological cascade of the disease before it manifests clinically, then these relative simple and cheap technologies, in combination with serum UA estimation, could possibly make major inroads into the injury that PE is still causing to pregnant human females throughout the world.

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