Animal models of preeclampsia: an examination of usefulness and limitations based on the metabolic domino theory

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Animal models of preeclampsia are widely used to elucidate the underlying pathology and investigate the clinical utility of drugs. Although a large body of data has been generated that supports their usefulness, animal models are not necessarily suitable for all research objectives. A more thorough understanding of advantages and disadvantages of each animal model will facilitate selection of appropriate models for a given study area, thereby enabling researchers to obtain more useful research results. The metabolic domino theory offers an explanation for the time-course of increase in the risk of cardiovascular events due to complex interactions among lifestyle-related diseases, which is likened to the sequential falling of dominoes. This theory provides a valuable perspective for understanding the importance of lifestyle-related diseases, as well as the process leading to the onset of cardiovascular events. Preeclampsia has adverse effects on both pregnant women and their fetuses/infants in the short term, whereas in the long term, it also increases the frequency of cardiovascular events in patients approaching middle and old age. Therefore, from a clinical perspective, preeclampsia is considered similar to lifestyle-related diseases, and its pathology might be better understood in light of the metabolic domino theory; this, in turn, will facilitate the selection of an appropriate animal model. Various models are considered applicable for research aimed at elucidating the pathology of preeclampsia, including antiangiogenic factor overexpression models, reduced uterine perfusion pressure (RUPP) models, and risk- and regulatory-factor knock-out models. However, in light of the metabolic domino theory, these models do not accurately replicate the pathology of preeclampsia, and are thus not necessarily appropriate for the investigation of drug utility.

Introduction

Preeclampsia, which affects 2–8% of pregnant women, has a substantial impact on prognosis in both mothers and their infants.\(^1\) Approximately 15% of childbirth-related maternal deaths are attributed to preeclampsia. In addition, women with a history of preeclampsia are at increased risk of developing cardiovascular events in middle or old age. Preeclampsia has major adverse effects not only on women’s lives\(^3\) but also on perinatal outcomes of fetuses/infants, as it can lead to intrauterine growth restriction and/or premature birth.\(^3\) Reportedly, the incidence of preeclampsia is increasing in the United States, due to an increase in the proportion of pregnant women with hereditary predispositions, such as hypertension, diabetes, and obesity.\(^5\) Similarly, the incidence of preeclampsia is expected to rise in Japan, as the average age of pregnant women increases with an accompanying increase in the frequency of the aforementioned hereditary factors.\(^5\) Therefore, elucidation of the etiology and pathology of preeclampsia, and the establishment of effective control and treatment methods, are of great importance from the perspectives of both mothers and infants.

In Japan, preeclampsia is defined as a combination of hypertension (systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg) and proteinuria...
(at least 300 mg in 24 hours) occurring at week 20 of pregnancy or later and normalizing by week 12 post-delivery.\textsuperscript{6} Nearly the same definition is used in the United States and Europe.\textsuperscript{7–10}

Preeclampsia had been termed a “disease of theories”, due to its obscure etiology. However, in recent years, the “two-stage theory”\textsuperscript{11} has been proposed, which provided explanations for disease pathogenesis to a considerable extent (Figure 1).\textsuperscript{12} Stage 1 of preeclampsia is thought to begin with a zygote with paternally inherited major histocompatibility complex (MHC) molecules, which are not tolerated by maternal cells. In other words, the onset of preeclampsia is linked to the inability of endometrial, myometrial, and/or maternal immunocompetent cells at the fetomaternal interface to accept trophoblast MHC. As trophoblasts are unable to sufficiently invade the decidual side, spiral artery remodeling failure occurs, resulting in ischemia-reperfusion in the intervillous space. This causes placental oxidative stress and endoplasmic reticulum stress, leading to Stage 2 of preeclampsia. The production of antiangiogenic factors such as sFlt-1 and sEng by trophoblasts is promoted, followed by their release into the maternal circulation from the intervillus space. This manifests as various clinical symptoms, including both maternal and fetal vascular endothelial damage, maternal hypertension and proteinuria, and fetal intrauterine growth restriction. The mechanism that causes failure of spiral artery remodeling is unclear, but it is likely that interactions between trophoblast HLA-C, HLA-E, and HLA-G, and uterine natural killer cells and/or dendritic cells regulate the invasion of the decidual site by trophoblasts.\textsuperscript{13–15} As such, impaired functioning of this regulatory mechanism is considered responsible.\textsuperscript{16}

The pathogenesis of Stage 2 preeclampsia has been elucidated to a considerable extent, due in large part to animal models used in research. In the future, animal models will continue to serve as a useful means to elucidate the pathology of immunogenic maladaptation in Stage 1, which is not only the key to understanding the etiology of preeclampsia, but will also enable development of therapeutic agents. However, the question remains as to whether current animal models are appropriate for elucidating the mechanism of immunogenic maladaptation, or developing therapeutic agents. Therefore, a literature search was conducted to investigate the usefulness and limitations of current animal models.

**Animal models of preeclampsia**

Animal models of preeclampsia can be classified as (i) spontaneous, (ii) surgically induced, (iii) pharmacologically induced or substance-related, and (iv) transgenic.\textsuperscript{17} The type of animal model to use depends on the objectives of the study. Reduced uterine perfusion pressure (RUPP) models, which are prepared

![Figure 1. The mechanism of development of preeclampsia.](image-url)
by surgical ligating the uterine artery or abdominal aorta, are used to investigate the roles of antiangiogenic factors and various regulatory factors in the pathogenesis of preeclampsia. On the other hand, rats that overexpress sFlt-1 are used to elucidate the mechanisms that lead to cardiovascular events when women with a history of preeclampsia reach middle or old age. Animal models of these types are considered extremely useful for investigating the involvement of antiangiogenic factors and other regulatory factors in the pathogenesis of preeclampsia. However, placentae of different species exhibit an enormous level of evolutionary diversity, and therefore, some argue that no animal models can fully reflect the characteristics of the human placenta. Moreover, in terms of the usefulness of animal models, there are concerns that the roles of various drugs in relation to preeclampsia cannot be fully understood with animal models. In sum, animal models offer many benefits, but there are also limits to their utility. Selecting an appropriate model for use in research thus requires a thorough understanding of the advantages and disadvantages of each type of animal model.

To select an appropriate animal model, the pathology of preeclampsia should be considered from the perspective of the metabolic domino theory. Although immunogenic maladaptation is considered the principal cause of preeclampsia, hereditary factors, such as hypertension and diabetes, also play a key role. In addition, preeclampsia has long-term adverse effects on women’s health, as it could trigger cardiovascular events in middle or old age. For these reasons, the pathology of preeclampsia resembles that of metabolic syndrome, which suggests that the metabolic domino theory might offer a useful perspective in considering the pathology of preeclampsia. The term “metabolic domino theory” was coined by Itoh, who likened the pathogenesis and progression of metabolic syndrome to a row of dominoes falling. This theory helps us understand the pathogenesis and progression of metabolic syndrome, and may also facilitate an understanding of preeclampsia pathogenesis/progression. Moreover, in research using animal models of preeclampsia, this theory might provide a useful context for understanding the advantages and disadvantages of different animal models according to research objectives, and facilitate the selection of appropriate models.

**Metabolic domino theory and preeclampsia**

Complex interactions among multiple lifestyle-related diseases increase the risk of cardiovascular events.
However, patients do not develop multiple lifestyle-related diseases simultaneously, but rather, the onset of each disease occurs in a sequential fashion over the course of the patient’s life. In this respect, the issues are when and in what sequence diseases that constitute risk factors develop. The metabolic domino theory has enabled researchers to tackle these issues, in view of the time-course of disease progression (Figure 2). At the top of the causal cascade, as a ‘wobbly domino’, there are one or more lifestyle factors; when this domino falls over, pushed by obesity and/or insulin resistance, it in turn pushes others down, leading to the onset of various other lifestyle-related diseases (e.g., hypertension, hyperlipidemia, and postprandial hyperglycemia). Following the onset of these diseases, arteriosclerosis (i.e., macroangiopathy) develops, leading to cerebral stroke, dementia, and/or heart disease. Diabetes also gradually develops in parallel with these conditions. With the onset of diabetes and hyperglycemia, the progression of microangiopathy occurs, and is complicated by renal failure, retinopathy, and neuropathy. In this manner, the metabolic domino theory—in addition to facilitating an understanding of the time-course of lifestyle-related disease progression—explains how the pathology progresses over time and eventually triggers cardiovascular events, while different diseases affect each other synergistically. Therefore, in addition to complex interactions among a number of risk factors, which have long been demonstrated, attention should be paid to the progression of these risk factors and the induction of a chain reaction through the course of disease progression, according to the metabolic domino theory.

Figure 3 shows a schematic representation of preeclampsia pathogenesis based on the metabolic domino theory. Preeclampsia is thought to begin with immunogenic maladaptation i.e., zygotic MHC is not tolerated by endometrial, myometrial, and/or maternal immunocompetent cells, resulting in failure of spiral artery remodeling, insufficient invasion of the decidua by trophoblasts, and the induction of ischemia in the intervillous space. This, in turn, leads to hypoxia-reperfusion, placental oxidative stress, and endoplasmic reticulum stress, which constitute Stage 1 of preeclampsia. These stresses promote the production of antiangiogenic factors (e.g., sFlt-1, sEng), angiotensin II type-1 receptor autoantibodies (AT$_1$-AA), and inflammatory cytokines, as
well as the release of syncytiotrophoblast microparticles (STMBs). In Stage 2, these various factors transfer to the maternal circulation—rather than remaining in the intervillous space—and induce damage to vascular endothelial cells in the mother and fetus, thereby resulting in maternal hypertension and proteinuria, fetal intrauterine growth restriction, and non-reassuring fetal status. From the perspective of the metabolic domino theory, immunogenic maladaptation predicates the development of preeclampsia; however, when hypoxia-reperfusion occurs, multiple events progress simultaneously and interact with each other, thereby initiating a cascade of reactions. When antiangiogenic factors are produced, the pathology becomes more complex, and the process of pathogenesis progresses, like a chain reaction that involves numerous risk factors including antiangiogenic factors, AT1-AA, inflammatory cytokines, and STMBs. In other words, multiple ‘dominoes’ start to fall from the second half of Stage 1, with the number of falling dominoes increasing with pathological progression. Therefore, as shown in Figure 3, the ideal animal model of preeclampsia is one in which development of the condition begins with immunogenic maladaptation, followed by the fall of multiple dominoes with progressing pathology. Even with RUPP models, which are widely used and considered useful for preeclampsia research,26,27 the pathology of preeclampsia is not fully replicated, in which multiple dominoes fall, even in Stage 1, despite involving the fall of multiple dominoes in Stage 2.

**Animal models of preeclampsia: advantages and limitations**

As described above, if an animal model is to be used to study preeclampsia, it must be selected based on sufficient consideration of its advantages and disadvantages in relation to study objectives. For example, if the roles of sFlt-1 in preeclampsia pathogenesis are to be investigated, it is reasonable to use RUPP models,27 or mice that overexpress sFlt-1,28 as these models can be used for unified investigation of the effects of increased sFlt-1 on the pathology of preeclampsia. However, it remains unclear as to whether these models are appropriate for investigating the usefulness of drugs as therapeutic agents for preeclampsia. The usefulness of nicotinamide for treating preeclampsia has been demonstrated with the RUPP model27 and that of pravastatin with mice that overexpress sFlt-1.29 Likewise, numerous studies have reported on the utility of various drugs for treating the symptoms of preeclampsia.27–29 These drugs were effective in the relevant animal models, which provide hope for their therapeutic use in humans. However, to date, no studies have clearly demonstrated the effectiveness of any therapeutic or prophylactic method that was theoretically expected to be useful in treating preeclampsia. For instance, low-dose aspirin treatment (LDA) has been proposed as a prophylaxis for preeclampsia. In preeclampsia, the balance between prostaglandin-I2 (PGI2) and thromboxane-A2 (TXA2) shifts in favor of TXA2,30 and therefore, in theory, LDA should prevent preeclampsia; however, no consensus has been reached with regard to this effect.31,32 Why are there differences in findings? In preeclampsia, when the balance between PGI2 and TXA2 shifts towards TXA2, an imbalance of other vascular regulatory factors develops, resulting in a pathological state in which these factors exert effects as part of a chain reaction. Therefore, even if the balance between PGI2 and TXA2 is corrected, the pathological state is unlikely to be alleviated unless the balance of other regulatory factors is regained. In some patients, the pathology is primarily due to the imbalance between PGI2 and TXA2, so LDA may prove effective. However, in other patients (i.e., in whom the pathology primarily involves the imbalance of other regulatory factors such as endothelin or nitric oxide, and/or production of AT1-AA), LDA may not be sufficiently effective. In other words, vascular regulatory factors that lead to disorders and their severity differ among patients, and such differences may give rise to differences in LDA efficacy.

In investigating the efficacy of drugs for preeclampsia, the use of an animal model that involves the induction of immunogenic maladaptation would be appropriate, as this represents the highest point of the causal pathological cascade from the perspective of the metabolic domino theory. The RUPP rat model has been shown to replicate a pathological state that is relatively high up in the cascade,33 however, placental blood flow is surgically reduced in this model, rather than by immunogenic maladaptation, and thus, this model does not replicate spiral artery remodeling failure. In other words, the RUPP rat model does not closely resemble human preeclampsia. Similarly, transgenic mice that overexpress the Storkhead box 1 gene (STOX1) reportedly represent a model of both Stage 1 and Stage 2 preeclampsia, as research on familial preeclampsia revealed mutations in STOX1.34,35 However, from the perspective of the metabolic domino theory, these mice are not considered a model of preeclampsia onset due to immunogenic maladaptation, albeit appropriate for investigating genetic effects. Rats with anti-CD28 antibodies, which suppress CD28-dependent upregulation of regulatory T-cells that play an important role in immunogenic maladaptation, have been established as an animal model of preeclampsia. Yet, expected findings in terms of therapeutic efficacy have not been obtained.36,37 Moreover, while this model also superficially replicates the clinical symptoms of
preeclampsia, findings suggest that no animal models exist in which the pathology of preeclampsia is genuinely induced. To investigate the roles of factors expected to be involved in preeclampsia pathogenesis, therefore, a reasonable choice would be to use factor-overexpressing or factor-knockout models. However, to assess the usefulness of drugs, animal models in which many risk factors change with time, and have synergistic effects on one another, should be used in order to accurately evaluate efficacy under conditions that mimic the actual pathogenic process. In this regard, animal models in which antiangiogenic factors are overexpressed, or protective factors are knocked out, cannot be considered appropriate.

**Conclusion**

The efficacy of drugs is investigated with the ultimate objective of application to humans; therefore, research consists of intervention studies that involve invasion. Appropriate animal studies are needed in order to confirm the usefulness and safety of drugs. From this point of view—and based on the metabolic domino theory—there is a need for animal models that closely replicate the pathology in the upstream part of the causal cascade. Currently, no such animal models of preeclampsia exist. Development of ideal animal models of preeclampsia would require elucidation of the immunological mechanism of pregnancy maintenance. Moreover, to investigate the usefulness of drugs for preeclampsia, development of animal models that replicate immunogenic maladaptation, which might present considerable difficulties, will be necessary.

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**Conflict of interest statement**

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**References**