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Mouse As A Suitable Model To Prove The Hypothesis That Defects In Embryogenesis Are Bases For The Pathogenesis Of Pre-Eclampsia

Mohammad Farhan Qureshi and Mohammad Al Bakrah
Department of Pediatrics,
Northwest Armed Forces Hospital, P.O.Box, 100, Tabuk, Kingdom of Saudi Arabia

Abstract: Pre-eclampsia is one of the most important obstetrical diseases, being a prevalent worldwide cause of mortality in women at the age of reproduction. Albuminuria is a common occurrence in pregnancy, particularly in the last trimester and may be accompanied by some edema of the ankles: these disturbances are not of serious significance unless there is also a rise in the blood pressure, when the combination of features is termed pre-eclampsia or pre-eclamptic toxemia. This occurs most commonly in primigravida and is usually mild: The changes-edema, proteinuria and hypertension-do not often increase to alarming degrees and subside usually within a few days after parturition. But in some cases the features become progressively more severe during late pregnancy and impaired renal function is reflected with a rise in the level of blood urea. In such cases, hypertensive convulsions may occur, the condition then being termed eclampsia. Death may result from uremia or hypertensive encephalopathy and considerable judgment is sometimes required to decide whether pregnancy should be allowed to continue to term.

Key words: obstetrical, mortality, pregnancy, preelampsia, primigravida

INTRODUCTION

The clinical and histological features of pre-eclampsia indicate impaired renal blood flow with narrowing of the glomerular capillary lumina. There is evidence also of a reduced uterine blood flow in pre-eclampsia, sometimes resulting in placental ischemia. The causes of glomerular changes are not known, although intravascular coagulation has been implicated by some investigators. Hypertension from any cause during pregnancy increases the risks of abortion, premature labor and retroplacental hemorrhage.

Knowledge of genetics is essential for the understanding of four groups of diseases. These are attributable to:

- Chromosome imbalance in the fertilized ovum and thus in all the individual cells;
- Inheritance of mutant genes from one or both parents;
- An interplay between environmental factors and the genetic constitution of the sufferer and
- Genetic defects (chromosomal imbalance or gene mutation) which are not inherited from the parents but arise in somatic cells during the life of the individual. Thus, whatever is the source, malformations in embryogenesis result in common human birth defects. In addition to the genetic defects, the etiology of birth defects also depends on the embryonic exposure to different intrinsic and extrinsic factors.

Different medicines used during pregnancy are a probable source of toxicity to the developing embryos. In addition, the toxic metabolites of some drugs used by men find their way to the developing embryo via semen. Experimental models have been rampantly used to determine induced malformations. Exposure of pregnant mice to trans retinoic acid, at a time when the metanephros has yet to form, causes a failure of kidney development along with caudal regression. Maternal treatment with Am580 (retinoic acid receptor alpha agonist) also induce similar patterns of kidney maldevelopment in the fetus. In metanephroi from retinoic acid-treated pregnancies, renal mesenchyme condense around the ureteric bud but then fail to differentiate into nephrons, instead undergoing involution by fulminant apoptosis to produce a renal agenesis phenotype[1]. The murine autosomal recessive juvenile cystic kidney (jck) mutation results in polycystic kidney disease.

Some medicines used for their anticonvulsant activity are known to induce embryogenic changes by elevated levels of oxidative metabolites[2]. Oxidative

Corresponding Author: Mohammad Farhan Qureshi, Department of Pediatrics, Northwest Armed Forces Hospital, Tabuk, Kingdomg Saudi Arabia

metabolites are critical in mediating phenytoin teratogenesis. Experiments on teratogenesis in animal models revealed that the oxidative metabolites of phenytoin cause congenital malformations in inbred mouse strains[3]. Oocyte mitochondrial dysfunction has been proposed as a cause of high levels of developmental retardation and arrest that occur in human preimplantation embryos generated using assisted reproductive technology[4].

Clinical studies on congenital malformations and experimental studies on teratogenesis in animal models have useful applications in preventive, dignostic and therapeutic approaches. In a study on congenital malformations, Buehler *et al.*,[2] attempted to determine whether infants who are at risk for congenital malformations could be identified prenatally by the measurement of epoxide hydrolase activity. Before fetuses at risk could be identified, they measured epoxide hydrolase activity in a randomly selected sample of amniocytes from 100 pregnant women. According to a thin-layer chromatographic assay, the randomly selected sample population had an apparently trimodal distribution, suggestive of an enzyme regulated by a single gene with two allelic forms. Fetuses homozygous for the recessive allele would have low epoxide hydrolase activity and would therefore be at risk if exposed to anticonvulsant drugs during gestation. In a prospective study of 19 pregnancies monitored by amniocentesis, an adverse outcome was predicted for four fetuses on the basis of low enzyme activity (less than 30% of the standard). In all four cases, the mother was receiving phenytoin monotherapy and after birth the infants had clinical findings compatible with the fetal hydantoin syndrome. The 15 fetuses with enzyme activity above 30% of the standard were not considered to be at risk and all 15 neonates lacked any characteristic features of the fetal hydantoin syndrome. These preliminary results suggest that this enzymatic biomarker may prove useful in determining which infants are at increased risk for congenital malformations induced by anticonvulsant drugs.

MATERIALS AND METHODS

In a study to diagnose lipid storage diseases in embryos at preimplantation stage, Epstein *et al.*,[5] adopted two parallel approaches. Firstly, activities of several sphingolipid hydrolases were determined in extracts of murine embryos and also human oocytes and polyspermic embryos. Sensitive fluorescent or fluorogenic p rocedures provided indications that Tay-Sachs, Gaucher and Krabbe diseases might be diagnosed in one human blastomere, while for Niemann-Pick disease two might be required. Secondly, pyrene lipids were administered into murine embryos and their fluorescence was quantified by computerized imaging microscopy. As a model of Gaucher disease, the fluorescent substrate pyrene glucosylceramide was administered into murine embryos in the presence or absence of an inhibitor of the enzyme beta-glucosidase. Because of decreased degradation of the substrate in enzyme-inhibited cells, the fluorescence per blastomere was considerably greater relative to those which received no inhibitor. The results indicated that lipid storage diseases might be diagnosed in single human blastomeres at the preimplantation stage, obviating the need for pre-natal diagnosis and abortion of affected fetuses.

Transgenic mice model and obstetrical pathologies: The mammalian embryo and fetus are unable to develop without a well-established, functional placenta. This transitory yet indispensable structure attaches the conceptus to the uterus and establishes the vascular connections necessary for nutrient and gaseous exchange between maternal and fetal compartments. Genetic targeting strategy allows the generation of mice lacking a specific gene. Such approaches reveal:

- The high incidence of mutant embryonic or fetal death in utero and
- The extraembryonic (placental) causes of these deaths.

Due to the similarities presented between mouse and human placenta, Sapin *et al.*[6] proposed to use the potential of mouse targeting experiments as a model in order to understand human obstetrical pathologies. The authors reviewed genes that have been demonstrated to be required in mice for implantation, choriovitelline and chorioallantoic placentation. Using examples (integrins, homeoboxs, hepatocyte growth factor or epidermal growth factor receptor...) they demonstrated the reality and efficiency of such an approach. Other candidate genes (receptor of leukemia inhibitory factor, Wnt2 or retinoic acid receptor alpha...) in order to understand, prevent and treat human obstetrical pathologies[6].

Mouse models in cardiovascular development: Several mouse models lacking coagulation factors result in impaired thrombin generation and display a phenotype of disturbed cardiovascular development. Similar phenotypes are observed in mouse models of impaired thrombin binding to its cellular receptor, protease-activated receptor-1, or of disrupted signaling via G

proteins. Most interestingly, the available data provide evidence that thrombin signaling in vascular development cannot be explained by a model based only on the classic extrinsic and intrinsic coagulation pathways. Because angiogenesis in adults follows the same signaling patterns as angiogenesis in embryos, it is important to learn about these pathways, hoping that they may serve as therapeutic targets in cardiovascular disease[7].

RESULTS AND DISCUSSION

Transient bradycardia in a mouse model: Amniotic sac puncture carried out on day 13 mouse embryos induces a high incidence of craniofacial and limb abnormalities that resemble the anomalies seen in the oromandibulofacial limb hypogenesis syndrome occasionally encountered following chorionic villus sampling carried out during early human pregnancy It has been hypothesized that this syndrome probably has a vascular basis, possibly due to hypotension and hypoperfusion of tissues secondary to placental trauma, though no detailed aetiology has so far been described, Chang and Kaufman[8] have determined embryonic heart rates in control embryos, in embryos at intervals following anaesthesia and following amniotic sac puncture. An increased duration of bradycardia is seen following this procedure which is not observed in anaesthetic-only controls and in embryos in the contralateral (non-operated) uterine horns.

Relevance of chromosome 11 in mouse models: Now that the mouse and human genome sequences are complete, biologists need systematic approaches to determine the function of each gene. A powerful way to discover gene function is to determine the consequence of mutations in living organisms. Large-scale production of mouse mutations with the point mutagen N-ethyl-N-Nitrosourea (ENU) is a key strategy for analysing the human genome because mouse mutants will reveal functions unique to mammals and many may model human diseases[9]. Genetic targeting strategy allows the generation of mice lacking a specific gene. Such approaches reveal: (i) the high incidence of mutant embryonic or fetal death in utero and (ii) the extraembryonic (placental) causes of these deaths. Due to the similarities presented between mouse and human placenta, Sapin *et al.*[6] proposed to use the potential of mouse targeting experiments as a model in order to understand human obstetrical pathologies.

Application of chromosome engineering to functional genomices: Functional modeling of human genes and diseases requires suitable mammalian model organisms. For its genetic malleability, the mouse is likely to continue to play a major role in defining basic genetic traits and complex pathological disorders. Recently, gene targeting techniques have been extended towards developing new engineering strategies for generating extensive lesions and rearrangements in mouse chromosomes. While these advances create new opportunities to address similar aberrations observed in human diseases, they also open new ways of scaling-up mutagenesis projects that try to catalogue and annotate cellular functions of mammalian genes[10].

***In vitro* fertilization and embryo transfer to produce offspring for homozygous mutant gene:** The induction of ovulation by hormone treatment, preparation of fertilized eggs by in vitro fertilization and recovery of offspring by embryo transfer were studied in five strains of mutant mice: C57BL/6-dy/dy progressive muscular dystrophy model, C57BL/6-ob/ob obesity model, BALB/c-rl/rl, BALB/c- shi/shi and C57BL/6-mld/mld motor ataxia models. The homozygotes of these mutant mice are all affected with the disease about 2 weeks after birth, followed by reproductive disturbances. Ovulation could be induced by injection with PMSG-hCG in the females. Sperm was obtained from the cauda epididymis of males and used for in vitro fertilization. The success rate of the in vitro fertilization was as low as 71.6% in C57BL/6-dy/dy mice, but was over 85% in the other strains. When 2-cell embryos obtained by in vitro fertilization were transferred to the oviducts of pseudopregnant recipients, offspring were obtained from 39.2-57.7% of the transferred embryos. These offspring developed the expected diseases about 2 weeks after birth and it was confirmed that the disease characters were reliably reproduced.

CONCLUSION

By Concluding all these results demonstrate that the experimental system of *in vitro* fertilization and embryo transfer enables production of the offspring homozygous for a mutant gene and use of them for experiments before the onset of the disease has been impossible[11].

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