Application of Radiotelemetric Recording to Studies of Mouse Models of Gestational Pathology (Review/Opinion)

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Summary

Mouse pregnancy provides valuable understanding of human pregnancy physiology and pathologies. Pre-eclampsia (acute onset hypertension with kidney failure and immune complications) is the most common (3-7%) human pregnancy complication, and pre-eclampsia risk is greatly elevated in diabetic women. To address gestational blood pressure control, PA-C10 radiotransmitters (DSI) were implanted into female mice that were mated post-recovery and then recorded continuously for 48 hours postpartum. A pattern of normal blood pressure fluctuation was defined (random bred CD1, inbred C57BL/6J, BALB/cJ, normoglycemic NOD and immune deficient BALB/c-Rag2$^{-/-}$ and BALB/c-Rag2$^{-/-}$/Il2rg$^{-/-}$) that aligned with changes in placental development. Patterns of NOD.scid and hyperglycemic NOD pregnancies differed, identifying NK cells and blood glucose values, respectively, as factors in gestational hemodynamics. Four to six pregnancies were sufficient to monitor the entire gestational time course due to radiotelemetric precision and data concordance between animals. This approach greatly reduced animal usage over typical methods for detailed gestational time course studies.

Keywords: blood pressure regulation, cardiac adaptation, histopathology, hypertension, mouse pregnancy

1 Introduction

Mammalian pregnancy induces major vascular changes in the uterus. In species with hemochorial placentation (humans, many non-human primates, mice, rats, and others), the endometrial stroma changes at blastocyst implantation into a transient tissue called decidua (Kennedy et al., 2007). The major decidual arteries are called spiral arteries. These derivatives of the uterine arteries are the primary transporters of nutrient-rich maternal blood to implantation sites for use by the developing placenta and fetus. Although numbers of spiral arteries (or arterioles) vary between species (from 70-100 in humans compared with 3-6 in mice), a normal physiological change, called vascular remodeling or transformation, occurs in the majority of these vessels by mid gestation (Croy et al., 2006; Pijnenborg et al., 2006). Remodeling removes or disables the contractile vascular smooth muscle, permitting great expansion of the vessel lumen. The changes increase the blood holding and delivery capacity of the spiral arteries and ensure that they cannot contract to interrupt flow to the implantation site and that blood is delivered at a much reduced pressure to developing structures. After delivery, spiral arteries regain their arterial phenotype and function. Although most of the "great human obstetrical syndromes" have been linked with incomplete or lack of spiral arterial modification (Romero et al., 2010), the mechanisms regulating this process are not fully understood. Better understood is the process of spiral arterial remodeling in mice due to

i. the availability of full gestational time course specimens for histological and molecular study,
ii. mice with gene deletions that block the remodeling process, and
iii. adoptive transfer of bone marrow-derived cells, which corrects the block and permits effective remodeling (Croy et al., 2006).

The initiation of spiral arterial remodeling has been attributed through studies in mice to a population of lymphocytes that appears in implantation sites early in post-implantation endometrial decidualization (Guimond et al., 1998). These lymphocytes, called uterine natural killer (uNK) cells, are strongly associated with vessels in the decidua basalis, and they promote remodeling through their local production of the inflammatory cytokine interferon gamma (Ashkar et al., 2000). The decidual uNK cell population declines rapidly in the 2nd half of pregnancy, once spiral arteries have remodeled and circulation has commenced into the newly completed placenta. Cultures of early human decidual tissue from elective pregnancy terminations provided data supportive of similar vascular modification roles for human uNK cells (Hazan et al., 2010), although a larger, later role for placental (i.e., fetally derived) trophoblast cells occurs in humans than in mice.

The common (3-7%) emergency condition of human pregnancy, known as pre-eclampsia, is strongly associated with incomplete spiral arterial remodeling (Redman and Sargent, 2005). The routine monitoring of blood pressure during prenatal visits to caregivers screens for this syndrome. New onset of hypertension at any time between mid-pregnancy (week 20) and postpartum, accompanied by urinary secretion of protein (i.e., kidney failure), is diagnostic. The only treatment for pre-

eclampsia is immediate delivery of the fetus and placenta (the disease is attributed to the latter, but the fetus cannot survive without a placenta). If delivery is near mid-pregnancy, the fetus will not survive; if at 24-32 weeks gestation, a long stay in the neonatal intensive care unit is needed, and infant disabilities are common. If delivery occurs after 32 weeks gestation, there is a good prognosis for immediate infant health. Exposure to stressors associated with the syndrome during fetal life are thought to be carried for life, however, and to promote risk for cardiovascular (stroke, heart attack, ischemia, etc.) and metabolic (diabetes, obesity, etc.) diseases (McDonald et al., 2008). How direct or indirect the linkage is between failure of spiral arterial modification and hypertension in pre-eclampsia is not known and requires *in vivo* study. There are limits, however, to *in vivo* studies in pregnant women. At the time of the emergency delivery of the conceptus, a decidual/endometrial biopsy can be taken for histological study. This tissue reflects the culmination of the disease processes. Biobanks at large centers are collecting tissue at the time of first trimester elective terminations, then waiting for pregnancy outcomes before dividing their samples into ~95% unaffected versus ~5% with the syndrome (Founds et al., 2009). This approach addresses the implantation site genes expressed early in patients progressing to disease, but it requires a proportionally large investment of time and research funds in the study of normal subjects. The early time point is expected to be when conditions preventing complete spiral arterial modification are established. Pre-existing diseases in women, such as obesity, and type 1 or type 2 diabetes elevate risk for pre-eclampsia 4- to 12-fold by mechanisms that are incompletely understood (Norman and Reynolds, 2011). Additionally, women conceiving through assisted reproductive methods have about a 6-fold greater risk for becoming hypertensive in later gestation (Klatsky et al., 2010). Because a number of mouse strains carry pregnancies to term without spiral arterial modification (Croy et al., 2006), and term pregnancies can be supported in diabetes-prone mice at the stages of pancreatic insulitis and early hyperglycemia (Burke et al., 2011b), we introduced the technique of chronic, continuous radiotelemetry in pregnant mice (Butz and Davisson, 2001; Woods et al., 2011) to our rodent modeling studies of pre-eclampsia. Our experiences to date are summarized below.

2 Animals and methods

Animals and Surgical Radiotransmitter Implantation. Female mice (random bred CD1; C57BL/6J (B6) and BALB/cJ, (BALB/c)) housed under modified barrier conditions, and BALB/c *Rag2*$^{-/-}$, (*Rag2*$^{-/-}$; T and B cell deficient); BALB/c *Rag2*$^{-/-}$/*Il2rg*$^{-/-}$ (*Rag2*$^{-/-}$/*Il2rg*$^{-/-}$; NK, T, and B cell-deficient, i.e., alymphoid), NOD/ShiLtJ(NOD) and NOD.CB17-*Prkdc*^{scid}/J (NOD.*scid*) housed under rigorous barrier conditions) were anesthetized and received surgical implantation of PA-C10 transmitters (Data Sciences International; DSI, St. Paul, MN) with catheter entry via the left common carotid artery, tip placement in the aortic arch, and subcutaneous battery placement in the lateral flank, as previously described (Burke et al., 2010; Butz and Davisson, 2001). After 10 days recovery in individual cages, baseline continuous recordings were made of mean, systolic, and diastolic arterial pressures (MAP, SAP, and DAP, respectively), pulse pressures, and maternal heart rates. Except for the NOD strain, syngeneic males were then added to the cages for a non-recorded interval until mating was indicated via copulation plug detection. On this morning, called gestation day (gd) 0, the male was removed and the transmitter was reactivated for continuous recording. For NOD mice, twice weekly tail vein blood samples were measured for glucose (One Touch Glucometer and strips (LifeScan, Burnaby, BC)) until hyperglycemia (>15 mmol/l) was detected in two consecutive samples. Then the hyperglycemic female and an age-matched normoglycemic (blood glucose <9 mmol/l) female were paired with normoglycemic NOD males and followed as above. A set of 6-10 telemetry-monitored gestations was our experimental goal. Thirty seconds of data were isolated for analysis each 4 min for each animal every day. All days "began" at 7 a.m.. Analyses of these data are reported elsewhere (Burke et al., 2010).

Some non-instrumented B6 females were mated by B6 or B6-Tg(UBC-GFP)30Scha/J (B6-GFP; under human ubiquitin C promoter control) males for histological studies. At least 3 pregnancies/gd are needed for each mating combination to meet minimum publication standards for identifying range of variability. After euthanasia, uteri from B6 mated females were fixed, paraffin-embedded, cut at 5 µm and dually stained with Periodic Acid Schiff's reagent, and the lectin *Dolichos biflorus* agglutinin (DBA), as previously described for subsetting of mouse uNK cells (Zhang et al., 2009). Implantation sites from B6-GFP-mated females were halved sagittally and co-stained as live tissue using PE-CD31 to identify vascular endothelium and FITC-CD45 to identify leukocytes, as previously reported (Gerber et al., 2005). All procedures were approved by Queen's University Animal Care Committee.

3 Results

3.1 2- and 3-dimensional histology of cellular interactions in implantation sites

Study of serial sections has been the classical method for understanding the relationships between cells in constantly changing mouse implantation sites. Figure 1 illustrates a 2-dimensional, paraffin-embedded section of a gd6 B6 implantation site. The refinement of whole mount immunohistochemistry provides a 3-dimensional image of the relationships between invading trophoblasts, changing blood vessels, and the early decidual influx of leukocytes. Extension of this approach to standard confocal or 2-photon confocal microscopy is possible. A study using these approaches that included virgin and postpartum uterine specimens would require euthanasia of at least 60 female mice. Each mouse would provide an independent rather than continuous measurement, and functional information such as hypertension would be deduced through the observational and interpretive skills of the microscopist.

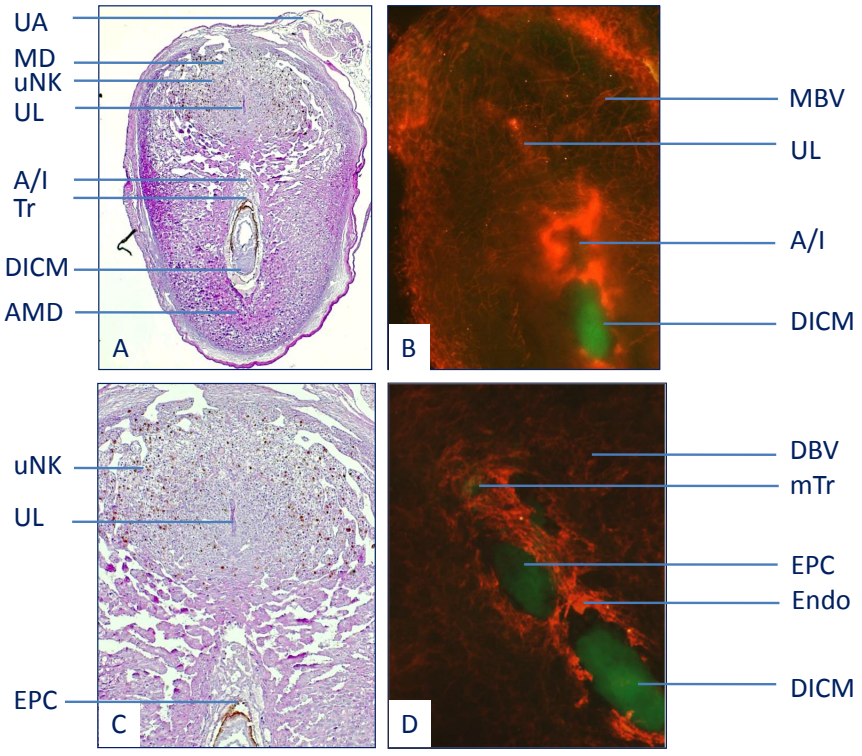

Fig. 1 Photomicrographs of mid-saggital sections of gd6.5 B6 implantation sites with the uterine suspensory ligament (also called mesometrium or broad ligament) placed towards the top

A and C show the same 2-dimensional paraffin-embedded section cut at 5 µm. The mating was made with a B6 male. These sections were dually stained with Periodic Acid Schiff's reagent and DBA lectin (visible brown stain) to reveal two subsets of uNK cells that are localized to the region where maternal angiogenesis is induced after blastocyst implantation. DBA lectin also stains the yolk sac of the conceptus. A, photographed at 25x shows the entire implantation site while C, photographed at 50x shows the invading ectoplacental cone, the tissue primordium of the placenta. Panels B and D illustrate 3-dimensional whole mount staining of a gd6.5 B6 implantation site. The mating was made with a B6 male homozygous for GFP. Thus, conceptus-derived cells are green under epifluorescence illumination. Red represents the vasculature reacted with PE-CD31. These viable, halved implantation sites also were co-stained with FITC-CD45; however, leukocytes are difficult to visualize in micrographs taken at magnifications below 100x. The leukocyte location was restricted and at the top of the images co-localized with the uNK cells shown in A and C.

Abbreviates: A/I – area of maternal angiogenesis and invasion by trophoblast of the conceptus; AMD – anti-mesometrial decidua; DBV – decidual blood vessel; DICM – primitive streak tissues derived from the inner cell mass; Endo – superbright CD31+ endothelial cells that are present from gd5.5 to 8.5 only; EPC – ectoplacental cone, the primordium of the placenta; MBV – blood vessels in the mesometrial decidua basalis; MD – mesometrial decidua; mTR – migrating invasive trophoblasts; Tr – trophoblast; UA – uterine artery in mesentery; UL – residual uterine lumen; uNK – decidual uNK cells in their mesometrial position in the decidua basalis.

3.2 Radiotelemetric probing of mouse pregnancies

We found a shared pattern of fluctuation in MAP across pregnancy in most but not all the mouse strains we studied. A drop and rebound in MAP occur between gd5-14. These are times equivalent to those in human gestation, where a drop in MAP normally occurs followed by a rebound to pre-pregnancy values (Burke et al., 2010). This is also the time during which spiral arterial modification should occur and thus may be a key time in which pathogenic processes that lead to pregnancy complications are initiated.

Most interesting was that the pattern of gestational changes in MAP coincides directly with key developmental changes in placental formation and function, a finding revealed only through the use of telemetry. Early gestational MAP is stable (gd0.5-4.5). Over this interval, the conceptus is restrained within the *zona pellucida*. Additionally, the conceptus is in the oviduct

(uterine tube) rather than the uterus until gd3.5. After arriving in the uterus, the conceptus hatches from the *zona pellucida*, then attaches to endometrium on the 4th day of pregnancy, triggering decidualization, which closes the uterine lumen and supports development of the placenta. Whole mount staining studies reveal that implantation site angiogenesis begins at gd5.0, the day the decline in MAP begins. MAP reaches its nadir at gd9-10, then increases. At gd9-10, the placenta becomes mature and its circulation opens. MAP rises from gd10. The initial rise to gd14 is more rapid than the later rise. Gd10-14 appears to reflect rapid growth of both placenta and fetus, while the later, slower rise occurs when placental growth becomes limited while fetal weight gain remains rapid. 72 hours postpartum MAP is not different from the animal's preconception baseline (Burke et al., 2010; Burke et al., 2011a; Burke et al., 2011b). This normal pattern of gestational fluctuation in MAP does not require spiral arterial modification (Burke et al., 2010). However, unbalanced NK cell to T cell ratios, as seen in NOD.scid mice, prevent the drop in gestational MAP (Burke et al., 2011a), while hyperglycemia in pregnant NOD mice prevents the rebound to preconception MAP values. In the hyperglycemic NOD mice, MAP continues to fall to parturition and is accompanied by cardiac decompensation (Burke et al., 2011b). This conclusion also was drawn from simultaneously collected heart rate data.

3.3 Limitations experienced in radiotelemetric studies of mouse pregnancies

A number of difficulties accompany the application of radiotelemetric methods to mouse pregnancy. Complications occur that are also seen in pregnancy studies of non-instrumented mice, including failure to copulate and failure to establish a pregnancy after copulation. Backup experiments for the use of these instrumented females should be planned within initial protocols. Complications that are more frequent or are limited to instrumented pregnant females include skin necrosis over the battery pack in late gestation as the gravid uterus enlarges, late fetal death, and dystocia. By deepening the pocket for the battery pack and positioning it more towards the dorsal rather than ventral flank, pressure necrosis can be eliminated (unpublished). While late fetal loss is uncommon (<5% of the instrumented females), its occurrence provided the exciting insight that this process is accompanied by hypertension and that the gain in MAP was prodromal and predicted the subsequent pregnancy failure by ~4 days (Burke et al., 2010). This finding calls into question some of the rodent models used for understanding the pathogenesis of pre-eclampsia, since pre-term deaths of some litter members are common in these models. Other complications are shared with telemetry studies of non-pregnant mice. These include differences in the size of adult mice. For example, we find BALB/c males and females about 5 grams lighter than other strains we have used, which makes their surgical preparation far more difficult. Signs of post-surgical central nervous system compromise occur that may have the reported anatomical basis of an incomplete circle of Willis or may have other causes. For example, we have rarely (2/20 mice) had successful recovery from implant surgery in B6 or 129 background mice (male or female) null for the angiogenic molecule placenta growth factor ($Pgf^{-/-}$) due to development of CNS signs in the first 48 hours after surgery. The basis for this outcome remains under investigation. Equipment failure also confounds telemetry studies. This includes incomplete or unreliable (highly variable) data collection due to battery failure or clotting at the catheter tip. In our experience, a practiced surgeon will have ~20% complications while a novice surgeon or surgeon with extended breaks between experiments may have 50-60% complications. These outcomes mean that exceptionally frequent, frank, and open communication must take place between investigators, their trainees and staff, the university veterinarian and animal care staff, and the University Animal Care Committee when telemetry is undertaken under non-conventional circumstances (in our case pregnancy and diabetes). Given the enormous amount of data generated by continuous recording and the data consistency between animals, we found that data from 4 replicate animals was sufficient to find meaningful statistical differences. When very small differences are sought, however, 6-10 animals are needed to provide robust assessments.

4 Discussion

Radiotelemetry provides the current "gold standard" for cardiovascular research studies in non-pregnant mice and rats (Kurtz et al., 2005). Telemetry's application to rodent models that address questions concerning human pregnancy is emerging but is not without difficulties. New onset hypertension is the most frequent complication of human pregnancy, and it has sequelae that elevate subsequent risk for cardiovascular disease in the mother and offspring. This drives the use of animal models to provide understanding of the mechanisms for the onset of pregnancy hypertension. It is widely held that the processes that result in hypertension during pregnancy begin early in pregnancy, well before a woman's clinical diagnosis. This complicates the conduct of human studies, since first trimester samples that do become available at the time of elective pregnancy termination are acquired before onset of clinical signs. Since approximately 5% of healthy pregnant women will become hypertensive, an investigator must plan to study 10-20 women to find one subject. Further, the *ex vivo* products of human pregnancy termination do not lend themselves to evaluation of a physiologically complex trait such as blood pressure regulation. Ethical concerns for the conceptus and wellbeing of ongoing pregnancies, plus the medical emergency status that is associated with new onset hypertension, prevent basic investigations of the pathophysiology of this problem in pregnant women. They also prevent the exploration of potential approaches for alleviation of the progress of this pathology. The transgenerational health complications and the emergency care needed for pregnant women with new onset hypertension require continued investigation of this problem in live animals. Thus, telemetry does not lead to replacement of animals in research, but it does lead to significant reduction in numbers

of animals used and to a gain in the number of parameters that can be defined from the use of each animal, i.e., refinement of animal usage. It is acknowledged that the use of telemetry in pregnant mice is a chronic intervention and that an individual animal may experience more duress than a pregnant animal being euthanized for histological tissue collection or a pregnant animal being anesthetized for examination of the cardiovascular system and fetus by ultrasound (Zhang et al., 2011).

Reduction in the use of animals comes from two sources. First, the studies are continuous. Thus, one mouse is studied before conception to define its personal baseline and every day thereafter until birth of its litter. This reduces the number of mice from 20 to 1 to provide coverage of the full gestational time course. Second, the consistency and accuracy of telemetry mean that replicate animal numbers do not need to be large. For most histological studies, 3 implantation sites from each of 3 different mothers is the minimum desired for publication. For telemetry with healthy normal mice, we have achieved statistically meaningful data from as few as 4 mice (Burke et al., 2010). More typically, 5-6 pregnant females are required (Burke et al., 2011a). These numbers have been sufficient to measure small but physiologically important changes that we were unable to detect using serial tail cuff recordings on a set of 20 pregnant mice (unpublished).

Telemetry also promotes research refinement. The PA-C10 radiotransmitters record 6 parameters simultaneously. More recent instrument systems combine this with body temperature, electrocardiograms, and other measures. Simultaneous, serial measurements in a single animal give integrative data. For example, after mid-pregnancy, hyperglycemic NOD mice had declining blood pressure. This was readily explained by the simultaneously collected heart rate data, which also declined, suggesting that progressive heart failure was accounting for the animal's inability to sustain normal blood pressure (Burke et al., 2011b). We found that ultrasound of implantation sites is highly complementary to radiotelemetry and it has successfully extended our telemetric data on maternal heart function and on placental blood flows (Zhang et al., 2011).

Telemetry also has helped us to successfully refine our experimental designs and to answer an important and basic question that could never be addressed in humans: does the mother or conceptus control the dynamic changes in blood pressure over the course of gestation? Through induction of uterine decidualization without a conceptus in radiotransmitter-implanted mice, we found that the conceptus alone controls maternal blood pressure. This is an important outcome for the understanding of human pregnancy-induced hypertension. Thus, by use of different strains of mice and protocols for pseudopregnancy, with and without decidualization, we are able to integrate information between disciplines such as physiology, development, immunology, and vascular biology over the full, dynamic, gestational time-course.

When considering whether to apply radiotelemetry to the investigation of pregnancy, a number of negative aspects also must be weighed. Equipment cost is higher than for other approaches, and transmitter refurbishing costs are an ongoing expense. We consider it false economy to "save the battery" by recording partial days because loss of data during the "off interval" prevents analysis of circadian rhythm effects, as well as the monitoring of husbandry variations or other irregularities. We employ only 24-hour continuous recording. An "in house" skilled surgeon is required if strains of mice that are not commercially available are being used, and this individual needs to have sufficient opportunity to keep in practice to achieve high levels of success. Dedicated care is needed post operatively and throughout the experimental gestation. This includes greatly increased vigilance, treatment support such as fluids and analgesia, and constant communication between animal care and scientific staff. Finally, massive amounts of data are generated. Analyses of these data require a large amount of time and must be seen as an enjoyable activity by the analyst. In our opinion, radiotelemetry has provided a defined pattern of hemodynamic performance over mouse pregnancy that now may serve as a platform to advance analysis of potential therapeutic manipulations not possible in pregnant women. This has been accomplished in studies that have reduced the numbers of animals needed to study the mouse gestational time course and refined the research questions being addressed. Radiotelemetry has improved the quality of data generated and facilitated its integration across disciplines.

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