Preeclampsia Foundation 2009 Vision Grant

Mid-year Report

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Project: ***Potential Therapeutic Use of Relaxin in Preeclampsia***

Our first Specific Aim is to determine whether endogenous or exogenous relaxin ameliorates symptoms of preeclampsia (e.g. proteinuria and hypertension) in the sFlt1-induced pregnant rat model of the disease. Although we ultimately propose to use viral overexpression methods to increase circulating sFlt1 levels in pregnant rats, we conducted pilot studies earlier this year using recombinant protein in nonpregnant rats. These studies were designed to assess whether we could produce hypertension and proteinuria by infusing sFlt1(aa 27-328)-Fc chimeric protein (R&D Systems, MN) by osmotic pump. Specifically, we chronically-instrumented rats with radio telemeters (Data Systems International, KS) and assessed systolic, diastolic and mean arterial pressure, pulse pressure and heart rate before, during and after sFlt1 infusion. The rationale for selecting this sFlt1 preparation over a full-length sFlt1-Fc chimera is that the truncated sFlt1 sequence does not contain the heparin-binding domains that may reduce the bioavailability of the full-length construct (S. Ananth Karumanchi, personal communication).

Recordings of baseline cardiovascular parameters were made on three alternate mornings for approximately 3-4 hours after one week recovery from surgery. Alzet model 2001 pumps containing sFlt1-Fc protein (infusion rate 30, 100 or 300ng/hr, 7 days) were then implanted in the dorsal subcutaneous space, and recordings were made on days 2, 4 and 6 of infusion (pump implantation being considered day 1). In each recording session, telemetry data was collected in 2 minute on/off cycles. Analysis was conducted on data points associated with low animal activity only (corresponding to resting/sleeping periods). In the afternoon following each recording, animals were placed into a Nalgene metabolism cage overnight, to permit timed urine collection for assessment of proteinuria. Urine was collected when rats were transferred back to their home cage the following morning. Particulate matter was removed by centrifugation and aliquots were prepared and stored at -20°C. Urinary protein was assessed at the completion of the study using the Bradford assay (BioRad, CA) and corrected for urinary volume and duration of collection. Rats were either euthanized on day 6 of sFlt1 infusion following the recording period that morning, or the pump was allowed to expire and post-infusion recordings were made for another week before euthanizing the animal. Trunk blood (3-4ml) was collected in EDTA vacutainer tubes, and plasma was dispensed into aliquots and stored at -20°C for later assessment of sFlt1 levels.

Unfortunately, despite some variation in mean arterial pressure, infusion of the sFlt1-Fc chimeric protein did not result in significant hypertension in nonpregnant female rats, nor was proteinuria observed during any period (baseline, infusion, or post-infusion), all rats producing less than 10mg urinary protein/day. Plasma sFlt1 levels were assessed using the R&D System Human VEGF-R1 Quantikine ELISA according to the manufacturer's instructions. Recovery of the VEGF-R1 standard across the range of the curve was (on average) 84.5%, although the sensitivity of the assay for the recombinant sFlt1-Fc chimeric protein was markedly attenuated (recovery ranged from 14.3 to 21.3% across the standard

curve). The diminished ability of the assay to detect sFlt1-Fc in our hands was consistent with the recovery of sFlt1-Fc in rat plasma spiked with the protein, which was only 11.3 & 16% at concentrations of 100 and 100pg/ml, respectively. Perhaps it is unsurprising then that the assay failed to detect sFlt1 in the circulation of the animals under study. Considering the lack of hypertension or proteinuria, however, these results suggest that despite the theoretically more favourable pharmacokinetic properties of the truncated sFlt1-Fc chimera, bioavailability was too low to induce hypertension and proteinuria in these nonpregnant rats. The reasons for this failure are unclear.

Nevertheless, additional progress has been made towards fulfilling the aims of the study as follows:

A murine sFlt1 construct has been obtained from the laboratory of Calvin Kuo (Stanford University), and we have engaged the services of the Viral Vector Core at the University of Florida to generate sFlt1-expressing adeno-associated viruses (AAV). We have elected to switch from adenoviruses as originally proposed to AAV because these viruses present a much lower safety hazard to laboratory personnel. In addition, we have access to novel AAV vectors with promoters that generate high expression levels of the transgene within 1 week, as required by our experimental design, as opposed to the typical time to peak expression for AAV of approximately 1-3 months. Although this will necessitate the generation of a suitable sFlt1-viral vector by us, we do not anticipate AAV generation taking any longer than the purchase of adenoviruses from QBiogene as originally proposed, as new replication-deficient adenovirus stocks would need to be prepared by the company regardless. The first installment of the PE Foundation Vision Grant funds will be put towards the generation of sFlt1-AAVs.

We have initiated collaboration with the Immunological Resource Center at the University of Illinois at Urbana-Champaign, who will generate the rat relaxin-neutralizing (MCA1) antibodies required for Specific Aim 1. The second installment of the PE Foundation Vision Grant funds will be used for the purchase of these antibodies.

Approval for the collection of subcutaneous arteries from normal and preeclamptic pregnant women (Hypothesis and Specific Aim 2) has been sought via an application to the University of Florida Institutional Review Board, and minor revisions to the protocol have been requested. We anticipate gaining approval to commence these studies by the end of this month.

Sincerely,

Jonathan T. McGuane