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Maternal insulin-like growth factor 1 and 2 differentially affect the renin–angiotensin system during pregnancy in the guinea pig

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ABSTRACT

Objective: Insulin-like growth factors (IGFs) are known to interact with the renin–angiotensin system (RAS). We previously demonstrated that administration of IGF1 to guinea pigs in early to mid pregnancy promotes placental function and fetal growth in mid to late gestation. Early administration of IGF2 had sustained, but not acute, effects on these parameters and also on placental structural differentiation. Here, we aimed to determine whether the IGFs interact with the placental RAS in early to mid gestation to modulate placental development and increase fetal growth and survival, and if IGF2 binding the IGF2R is implicated in the sustained effects of IGF2 treatment.
Design: At day 20 of pregnancy, guinea pigs were infused with 1 mg/kg/day of IGF1, IGF2, ^{Leu27}IGF2 or vehicle for 18 days and sacrificed on either day 62 (late pregnancy) or during the infusion period on day 35 (early–mid pregnancy). Placental structure at day 35 was analyzed using morphometric technique and expression of RAS genes in the placenta and placental and plasma renin activity were measured at both time points.
Results: Compared with vehicle at day 35 of gestation, IGF1 infusion reduced the total midsagittal cross-sectional area of the placenta (−17%, $p = 0.02$) and the labyrinth area (−22%, $p = 0.014$) but did not alter the labyrinth volume nor labyrinth:interlobium ratios. IGF2 treatment did not affect placental structure.
IGF1 did not alter placental mRNA for any of the RAS genes quantified at day 35 (*AGTR1, ACE, AGT, TGFB1*) but increased *TGFB1* expression by more than 16-fold ($p = 0.005$) at day 62. IGF2 increased placental expression of *AGTR1* (+88%, $p = 0.03$) and decreased *AGT* (−73%, $p = 0.01$) compared with the vehicle-treated group at day 35, and both IGF2 and ^{Leu27}IGF2 increased expression of *TGFB1* at day 62 by 9-fold ($p = 0.016$) and 6-fold ($p = 0.019$) respectively.
Both IGFs increased the ratio of active:total placental renin protein (+22% $p = 0.026$ $p = 0.038$) compared to vehicle compared to vehicle at day 35 but not 62. At day 62, IGF2-treated mothers showed a marked increase in total plasma renin (+495%) and active renin (+359%) compared to vehicle but decreased the ratio of active to total renin by 41% ($p = 0.042$). ^{Leu27}IGF2-treated animals had higher levels of placental active renin (+73%, $p = 0.001$) and total renin (+71%, $p = 0.001$) compared with the vehicle control.
Conclusions: The data obtained in the current study suggest the potential for alternate roles for the induction of the RAS after IGF treatment. IGF1 and 2 treatments increase the activation of prorenin to renin in the placenta, possibly due to increased protease activity. In addition, IGF2 treatment in early pregnancy may enhance the maternal adaptation to pregnancy through stimulation of renin in the kidney. The sustained effects on placental differentiation and function after IGF2 treatment suggest therapeutic potential for exogenous administration of IGFs in improving pregnancy outcomes.

1. Introduction

Our previous work with guinea pigs has shown major and complementary roles for increased maternal circulating IGFs in placental and fetal growth. We have demonstrated that exogenous administration of IGF1 to the mother increases placental and fetal weights in guinea pigs at day 40 of gestation [1]. Persistent effects on fetal growth at day 62

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(term 67–70 days) are seen after either IGF1 or IGF2 infusion [2]. Maternal IGF2 treatment in early pregnancy, increased the midsagittal cross-sectional area, proportion and volume of the placental region devoted to exchange (labyrinth) near term [2]. These structural changes suggest an increase in placental exchange capacity that accounts for the concomitant increase in fetal weight near term following maternal IGF2 treatment [2]. Whether the exogenous administration of IGF2 in guinea pigs in early pregnancy improves placental structural differentiation in the short term (mid pregnancy) has yet to be evaluated. Interestingly, treatment with ^{Leu27}IGF2, an analogue specific for the IGF2R, induces similar placental and fetal outcomes near term to IGF2 treatment [3] Furthermore, IGF2 signaling through IGF2R affects trophoblast survival *in vitro* [4], which indicates that the effects of IGF2 on placental development might be mediated through the IGF2R.

Both IGFs and RAS regulate placental function and development and there is cross-talk between these two systems in other tissues [5–10]; therefore, it is likely that these two systems also interact to regulate placental development. The IGF2R internalizes, degrades and activates prorenin/renin [11–14], the rate-limiting enzyme of the RAS cascade. In addition, IGF1 increases fetal renal renin secretion [9], AT_1R activity in smooth muscle cells *in vitro* [15] and vasodilation by stimulating prostanoid release in human term placental explants [16]. Angiotensin II (Ang II) binding to the AT_1R increases vasoconstriction and blood pressure, up regulates TGFβ1 mRNA synthesis [17], increases placental plasminogen activator inhibitor-1 (PAI-1) activity [18] and inhibits trophoblast invasion [18,19]. Thus, treatment of the pregnant mother with IGFs has the potential to alter placental RAS activity, trophoblast invasion and uteroplacental blood flow.

We therefore propose that the IGFs interact with the placental RAS in early to mid gestation to modulate placental development and increase fetal growth and survival; and IGF2 binding the IGF2R is implicated in the sustained effects of IGF2 treatment. To determine if this occurs, the guinea pig was used as it has a hemomonochorial placenta and highly invasive endovascular and interstitial trophoblast populations [20–22]. Furthermore, the guinea pig has a similar IGF axis to that of humans with substantial circulating IGF2 postnatally [23,24]. It has similar adaptations of this axis to pregnancy and maternal IGF actions on placental development, function and fetal growth and survival have been previously shown in this species [24–28].

2. Materials and methods

2.1. Guinea pigs

This study was approved by the University of Adelaide Animal Ethics Committee and complied with the National Health and Medical Research Council's guidelines on the treatment of animals in research. Two separate cohorts of guinea pigs were utilized. Twenty-six were used to study the acute effects of IGF infusion and sacrificed at day 35 gestation, and an additional cohort of 34 guinea pigs was studied to investigate the effects of IGF treatment near term, sacrificed at day 62. Housing and management of guinea pigs, mini osmotic pump surgery and postmortem for this cohort have previously been published for the day 35 cohort [29] and the day 62 cohort [30]. Briefly, at day 20 of pregnancy, a 200 μl mini osmotic pump (Alzet 2002, California, USA) was subcutaneously inserted behind the shoulder. Mini osmotic pumps delivered approximately 1 mg/kg/day IGF1 or IGF2 (human recombinant protein, GroPep, Australia) in 0.1 M acetic acid or 0.1 M acetic acid (vehicle) for 18 days at a flow rate of 0.51 μl/h. We have previously reported that this treatment increases the circulating concentrations of IGF1 and IGF2 by 340% and 240%, respectively [2]. The second cohort of guinea pigs also included a group treated with ^{Leu27}IGF2, which is highly selective for the type II IGF receptor with low affinity for the IGFIR and Insulin receptor [31]. Postmortem was performed on day 35 (early–mid pregnancy $n = 26$) or day 62 (near term, $n = 34$) of gestation.

2.2. Placental morphology

Analysis of placental morphology was performed using our published method [3]. Briefly, one to three placentas per dam were randomly selected for histological assessment. One representative midsagittal placental section (6 μm, the first full-thickness section cut) per placenta was stained with Masson's Trichrome to distinguish the interlobium (germinative) from the labyrinth (exchange) regions. The proportion of each region was determined by dividing the midsagittal cross-sectional area of that region by the total midsagittal cross-sectional area of the placenta. To assess the effect of IGF treatment on labyrinth composition and volume, placental sections were probed with mouse monoclonal antibodies raised against human pan cytokeratin (C2562, Sigma, USA; 1/50) and mouse anti human vimentin (3B4, Dako, Denmark; 1/50) labeling trophoblast cells and fetal capillaries The labyrinthine region of each placenta was analyzed to estimate the volume densities (Vd) of trophoblasts, fetal capillaries and maternal blood spaces. This analysis employed point counting of ten non-overlapping fields with random systematic sampling ($V_d = P_a/P_T$; where Pa is the total number of points falling on that component and P_T is the total number of points applied to the section) with an isotropic L-36 Merz transparent grid, as previously described [27]. Representative volumes of each labyrinthine component were calculated, assuming that 1 g of placenta occupies 1 cm^3, by multiplying the volume density of the labyrinthine component by weight of the placental labyrinth. The surface area per gram of placental labyrinth was quantified using intercept counting, enabling an estimation of total surface area of syncytiotrophoblast (surface area for exchange) and arithmetic mean trophoblast thickness (thickness of trophoblast layer through which substrate exchange occurs) [26].

2.3. Quantitative real-time PCR

After RNA was extracted from placental tissues, as previously published [29], reverse transcription was undertaken using the Superscript III system according to the manufacturer's specifications (Invitrogen Life Technologies, Carlsbad, California, USA). Real-time PCR was performed using a Corbett 6000 Rotor Gene System (Corbett Life Sciences, Sydney) and SYBR Green I (Applied Biosystems, Foster City, CA) chemistry to detect synthesized products. For real-time PCR, 2 μl of cDNA was added to a master mix containing 5 μl SYBR Green, 0.25 μM each of forward and reverse primers and 2 μl of water. Thermocycling parameters were set according to the manufacturer's instructions. Oligonucleotide primers were designed for *Renin (REN; Fwd-ACCCAGTACTATGGTGAGATTGGC, Rev-CCAGAGGT TGGCTGAACCTG), Angiotensinogen (AGT; Fwd-AGCACGACTTCCTGACTT GGA, Rev-TCAGACGGATGGCCCG), Angiotensin converting enzyme (ACE; Fwd-ATGGAAGCATCACCAAGGAGA, Rev-GCCTGAGGCTCCACCA CTC) Type 1 angiotensin receptor* (*AGTR1; Fwd-GCCACTGTGGGCTGTC TACA, Rev-GACGCTAGCTGAGGCGATCT*), *Type 2 angiotensin receptor (AGTR2; Fwd-CCCCTCCATGTTCTGACCTTC. Rev-CAGCTATTAATGACACCC ATCCAG)* and *Transforming growth factor β1 (TGFB1; Fwd-TGTGTGCG GCAGCTCTACAT, Rev-AGTTGGCATGGTAGCCCTTG)* genes using Primer Express (Applied Biosystems, Foster City, CA) and published sequences for conserved regions in human, rat, bovine or rabbit. Standard desalted primers were constructed by Sigma Genosys (Sigma Genosys, Sydney, Australia). The placental mRNA expression levels for *REN, ACE and AGTR1* genes were determined using the relative standard curve method for quantitation, employing the *18S* rRNA gene (Fwd-AGAACGGCTACCACATCCAA, Rev-CCTGTATTGTTA TTTTTCGTCACTACCT) as the internal control to normalize each sample. Since placental *AGT* and *TGFB1* mRNA levels were less abundant their expression was determined using the delta-delta CT method ($2^{-\Delta\Delta CT}$) for quantitation, with *18S* as the housekeeper to normalize each sample.