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Contraception 82 (2010) 572–578

Contraception

Original research article

Inhibition of vascular endothelial growth factor during the postovulatory period prevents pregnancy in the marmoset☆

Hamish M. Fraser[a,*], Keith D. Morris[a], Stanley J. Wiegand[b], Helen Wilson[a]

[a]*Medical Research Council Human Reproductive Sciences Unit, The Queen's Medical Research Institute, EH16 4TJ Edinburgh, UK*

[b]*Regeneron Pharmaceuticals, Tarrytown, New York, NY 10591, USA*

Received 11 February 2010; revised 1 April 2010; accepted 16 April 2010

Abstract

Background: This study investigated the effects of inhibition of vascular endothelial growth factor (VEGF) during the first postpartum cycle in marmosets housed with a fertile male where a 90% fertility rate is normal.

Methods: On resumption of mating, females were treated with either 25 mg/kg aflibercept, a potent VEGF inhibitor, or control Fc protein (n=6 per group) at the time of ovulation. Effects on timing of pregnancy were monitored by measuring plasma progesterone, chorionic gonadotropin (CG) and uterine palpation.

Results: In five of six Fc-treated controls, the postpartum rise in progesterone was maintained and followed by a sustained rise in CG by Day 30 posttreatment indicating pregnancy. In all six aflibercept-treated animals, progesterone secretion was suppressed in the treatment cycle and a CG rise did not occur by Day 30. Pregnancy was delayed to the next cycle, significantly extending interbirth interval compared to controls. Posttreatment deliveries and infant development were normal.

Conclusion: These results show that stringent pharmacological inhibition of VEGF suppresses luteal progesterone and prevents the successful establishment of pregnancy.

Keywords: Corpus luteum; VEGF inhibitor; Progesterone; Pregnancy

1. Introduction

Angiogenesis, the formation of new blood vessels via endothelial replication, has been shown to be intense during the first few days of the life span of the corpus luteum (CL) in all species studied, including humans [1]. Angiogenesis is primarily under the regulation of vascular endothelial growth factor (VEGF) [2]. VEGF belongs to a gene family that includes VEGF-A, B, C, D and placenta-derived growth factor (PlGF), of which VEGF-A is the principal form regulating physiological angiogenesis [2]. VEGF action is mediated via two tyrosine kinase receptors, VEGFR1 and VEGFR2. Because of its central role in angiogenesis in health and disease, a large number of compounds have been developed to target VEGF or its receptors [2], which may be used as tools to define its physiological role. We have employed aflibercept (previously known as VEGF Trap), a recombinant chimeric protein comprising portions of the extracellular domains of the human VEGFR 1 and 2 expressed in sequence with the Fc portion of human immunoglobulin [3]. Aflibercept binds all isoforms of VEGF-A, as well as PlGF, with very high affinity, preventing it from binding to and activating VEGF receptors [3]. Studies in which VEGF has been inhibited during the formation of the CL in the marmoset monkey show that angiogenesis is severely suppressed and the resulting CL is largely avascular and nonfunctional [1,4]. Furthermore, inhibition of VEGF suppresses plasma progesterone secretion at all stages of the luteal phase, irrespective of the rate of angiogenesis, indicating an additional action on ovarian function, most likely inhibition of ovarian vascular permeability [4,5].

VEGF is produced by the early CL and is maintained during the establishment of early pregnancy [6–8]. These

☆ Research was supported by the core grant (U.1276.00.002.00003.01) to Medical Research Council Reproductive Sciences Unit to HMF.

* Corresponding author. Tel.: +44 0 131 242 6222; fax: +44 0 131 242 6197.

E-mail address: h.fraser@hrsu.mrc.ac.uk (H.M. Fraser).

0010-7824/$ – see front matter.
doi:10.1016/j.contraception.2010.04.020

findings suggest that compromise of CL function, secondary to inhibition of angiogenesis and/or vascular permeability, could impair fertility. We have now directly evaluated the effect of transient VEGF inhibition on the establishment of pregnancy in marmosets to test the hypothesis that this would lead to failure in the normal rise of plasma progesterone and result in prevention of pregnancy that was reversible on resumption of normal ovulatory cycles. The marmoset offers major advantages for this study as it has an exceptionally high rate of fertility; breeding female marmosets housed in stable family groups ovulate around 11 days postpartum with a 90% fertility rate [6,9]. In addition, the cellular and molecular regulation of angiogenesis within the ovary and endometrium during the normal reproductive cycle and early pregnancy has been studied extensively in this species [1,6].

2. Materials

2.1. Animals

Experiments were carried out under the Animals Act, Scientific Proceedings (1986), and were approved by the local ethical review committee. Common marmosets (*Callithrix jacchus*) were housed in stable family groups with males of proven fertility. Female marmosets selected for the study had a history of previously giving birth to live young, then reliably becoming pregnant within 2 weeks postpartum on at least the two consecutive occasions immediately prior to recruitment. Interbirth intervals were approximately 155 days. Given a gestation period of 144 days, this indicated that ovulation and a fertile mating occurred approximately 11 days after giving birth. Marmosets meeting these criteria were identified over a 2-year period. To confirm that animals were mating prior to and at the time of expected ovulation, a vaginal lavage was collected from each marmoset on Days 7, 9 and 11 postpartum and examined for motile sperm. The vaginal wash was placed on a slide and examined for the presence of sperm using dark field microscopy. Sperm numbers were scored on a + to +++ basis.

2.2. Treatment

To block VEGF, we employed aflibercept (Regeneron Pharmaceuticals, New York) [3]. A single injection of aflibercept (25 mg/kg, s.c.) effectively inhibited VEGF for approximately 10 days in the marmoset, with maximal aflibercept concentrations in the circulation of ~100 mg/L reached at 31 h, with an elimination half-life of 59 h [5]. Therefore, in the present study, marmosets were treated with aflibercept (25 mg/kg s.c.) or a control protein (recombinant human Fc), n=6 per group, at Day 12 postpartum during the immediate postovulatory period, to block the normal luteal progesterone secretion.

Blood samples were collected beginning on Day 9 postpartum using a refined restraint device [10] and continued three times per week until pregnancy was confirmed. Pregnancy was first indicated by a continued elevation of progesterone beyond the 20 days typical of the normal luteal phase, with subsequent presence of circulating chorionic gonadotropin (CG) levels consistently greater than 20 ng/mL. Pregnancy was confirmed by manual palpation at 6 and 10 weeks posttreatment. The day of delivery, total number of offspring and surviving offspring were recorded for each pregnancy. Interbirth intervals were calculated by designating the day of birth prior to treatment as Day 1.

Each female in this study was breastfeeding twins from their prior pregnancy at the time of treatment (controls: eight females, four males; treated six females, six males). All mothers were observed to continue to suckle their infants in an apparently normal fashion throughout the treatment period. Infants began taking solid food by 40 days of age and weaning was complete by around 60 days. Body weights of the infants were recorded at approximately 10, 20, 40, 60, 80, and 100 days of age. There is no difference between growth rates between male and female infants [11], so data from both sexes were combined for analysis. Offspring conceived following the treatment cycle also were carefully monitored for any abnormalities and were then weighed routinely at intervals. Body weights were compared to growth curves recorded for infants in the colony during the previous 5 years. At 2–3 years of age, when the females had become adult, blood samples were collected three times a week for at least 2 months and plasma progesterone levels assayed to determine the occurrence of ovulatory cycles.

2.3. Assays

Plasma concentrations of progesterone [4] and free aflibercept (aflibercept not already bound to endogenous VEGF) were measured as described previously [5]. Samples for aflibercept ELISA were diluted 1:10,000 where highest concentrations of aflibercept were present and assayed neat in remaining samples. Detection limit of the assay was 1 mg/L. The marmoset does not produce luteinizing hormone (LH) as they do not express the β subunit of LH but instead express the CG β subunit. Marmoset CG was measured using a heterologous RIA [12]. HCG (code no. 75/533) was supplied by the National Institute of Biological Standards and Control (Potter's Bar, Hertfordshire) and was used for radio-iodination and as standard. The monoclonal antibody to bovine LH (518 B7) was kindly gifted by Dr. J.F. Rosner. An antibody to the LH alpha chain recognizes the marmoset homologue that is common to CG. The detection limit of the CG assay was 6 ng/mL; all samples were run in a single assay.

2.4. Statistical analyses

Fisher's exact test was used to compare the number of first postpartum cycle pregnancies between treated and control groups. With a 90% fertility rate in the first postpartum cycle being expected in controls, a size calculation was carried out assuming complete inhibition