Evidence of prokineticin dysregulation in fallopian tube from women with ectopic pregnancy

Julie L. V. Shaw, Ph.D.,[a] *Fiona C. Denison, M.B., Ch.B., M.D.,*[a] *Jemma Evans, Ph.D.,*[a] *Kimberley Durno, B.Sc.,*[a] *Alistair R. Williams, M.B., Ch.B.,*[a,b] *Gary Entrican, Ph.D.,*[c] *Hilary O. D. Critchley, M.B., Ch.B., M.D.,*[a] *Henry N. Jabbour, Ph.D.,*[a] *and Andrew W. Horne, M.B., Ch.B., Ph.D.*[a]

[a] Centre for Reproductive Biology, Queen's Medical Research Institute, University of Edinburgh; [b] Department of Pathology, Royal Infirmary of Edinburgh; and [c] Moredun Research Institute, Pentlands Science Park, Bush Loan, Edinburgh and The Roslin Institute, Royal (Dick) School of Veterinary Studies, University of Edinburgh, Roslin Biocentre, Midlothian, Scotland, United Kingdom

Objective: To demonstrate expression and regulation of prokineticins (PROKs) and their receptors (PROKRs) in fallopian tube (FT) from women who are not pregnant and women with ectopic pregnancy (EP).
Design: Tissue analysis.
Setting: Large United Kingdom teaching hospital.
Patient(s): Women undergoing hysterectomy for benign gynecological conditions (n = 15) and surgery for EP (n = 16).
Intervention(s): Quantitative reverse transcriptase–polymerase chain reaction (RT-PCR) and immunohistochemistry were used to determine FT PROK/PROKR messenger RNA (mRNA) expression and protein localization, respectively. The PROK/PROKR levels were measured in tubal explant cultures stimulated with estrogen (E) and progestogen.
Main Outcome Measure(s): Differential expression of PROK and PROKR.
Result(s): The FT PROK2 and PROKR1 mRNA levels were up-regulated during the P-dominant midluteal phase of the menstrual cycle. Increased PROKR1 expression was observed in tubal explant cultures treated with medroxyprogesterone acetate (MPA). The PROK and PROKR proteins were localized to the epithelium and smooth muscle layers of the FT. The PROKR1 and PROKR2 mRNA levels were lower in FT from women with EP compared with nonpregnant FT from the midluteal phase.
Conclusion(s): These data suggest a potential role for PROKs in FT function. The PROKs are known to affect smooth muscle contraction in the gut. Dysregulated PROK expression in FT could affect FT smooth muscle contractility and embryo–tubal transport, providing a potential cause for EP. (Fertil Steril® 2010;94:1601–8. ©2010 by American Society for Reproductive Medicine.)

Key Words: Fallopian tube, ectopic pregnancy, menstrual cycle, prokineticin, smooth muscle contractility

Ectopic pregnancy (EP) remains a considerable cause of morbidity and occasional mortality (1). One in 80 pregnancies is ectopic and more than 98% implant in the fallopian tube (1, 2). The exact mechanism leading to tubal implantation is unknown. However, given that the human embryo appears to have the ability to implant on any given epithelial surface (3), it is likely that an EP is the result of embryo retention in the fallopian tube due to fallopian tube dysfunction. Transport of the preimplantation embryo through the fallopian tube is accomplished, in part, by smooth muscle contraction (4, 5).

The prokineticins, PROK1 and PROK2, have angiogenic actions but are primarily known for their function as regulators of specific and potent contractions of smooth muscle (6). They are the cognate ligands for two closely homologous G protein-coupled receptors, PROK receptor (PROKR) 1 and PROKR2, through which either PROK can signal (6). The PROKs were initially reported to be expressed in the gastrointestinal tract, where they were shown directly to stimulate contraction of the ileum longitudinal muscle of guinea pigs (7). However, the opposing effect of relaxation through a nitric oxide-mediated mechanism has also been reported recently in the proximal colon in mice (8). In addition, it has been reported that PROK2 has no effect on forestomach or colon contraction (9), suggesting that the intracellular milieu in different tissues results in differential coupling and different phenotypic effects.

The PROKs and their receptors are expressed in the ovary, uterus, and in various tissues of pregnancy (10, 11). PROK1 is expressed in the uterine epithelium, as well as the smooth muscle cells of the myometrium, with maximum expression at the time of implantation in the P-dominant midsecretory phase (11). PROK1 is expressed in much greater levels than PROK2. The PROK2 and the PROK receptors are also expressed in the various cellular compartments of the uterus, but they do not show a temporal variation across the menstrual cycle (11), suggesting a regulatory role for PROK1 and a more permissive role for PROK2 in uterine function. Furthermore, it has been hypothesized that PROK1 facilitates successful

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Reprint requests: Andrew W. Horne, M.B., Ch.B., Ph.D., Simpson Centre for Reproductive Health, Room S7129, Royal Infirmary of Edinburgh, 51 Little France Crescent, Edinburgh EH16 4SA, Scotland, United Kingdom (FAX: 131-242-2686; E-mail: andrew.horne@ed.ac.uk).

TABLE 1

Clinical information for fallopian tube samples across the menstrual cycle.

Sample	Cycle phase	Serum estrogen (pmol/L)	Serum P (nmol/L)	Reason for surgery	Uterine pathology
1	Follicular	1,022.87	0.81	HMB	Adenomyosis
2	Follicular	940.44	3.82	HMB, PP	Adenomyosis
3	Follicular	829.42	4.19	HMB, PP	Adenomyosis, fibroid
4	Follicular	770.63	5.16	HMB	No abnormality
5	Follicular	116.3	2.88	HMB, PP	No abnormality
6	Follicular	55.0	2.2	HMB, PP	Adenomyosis, fibroids
7	Midluteal	549.91	88	Dysmen	No abnormality
8	Midluteal	242	53.1	HMB, dysmen	No abnormality
9	Midluteal	201	24.55	HMB, PP	Fibroid
10	Midluteal	399.8	80.45	HMB, dysmen	Adenomyosis, fibroids, polyp
11	Midluteal	1,633.0	54.38	HMB, dysmen	No abnormality
12	Midluteal	424	76.9	PP	No abnormality
13	Midluteal	266	37.1	HMB	No abnormality
14	Midluteal	331	83.5	HMB	No abnormality
15	Midluteal	242.9	38.1	HMB	No abnormality

Note: HMB = heavy menstrual bleeding; Dysmen = dysmenorrhea; PP = pelvic pain.

Shaw. Prokineticin expression in human fallopian tube. Fertil Steril 2010.

intrauterine embryo implantation through induction of leukemia inhibitory factor expression (12), which has been shown to promote adhesion of trophoblast cells to extracellular matrix proteins, in vitro.

It is therefore plausible that the PROKs and their receptors could regulate smooth muscle contraction in the human fallopian tube facilitating controlled and timely embryo transport into the uterine cavity, and that attenuated expression could lead to embryo retention and EP. However, PROK/PROKR expression has, to our knowledge, never been demonstrated in human fallopian tube. In the present study, we report expression and tissue localization of the PROKs and PROKRs in human fallopian tube, their regulation by P, and show that PROKR expression is dysregulated in EP.

MATERIALS AND METHODS

Collection of Human Tissues

Ethical approval for this study was obtained from the Lothian Research Ethics Committee (04/S1103/20) and informed written consent was obtained from all patients. Fallopian tube tissues from the ampullary region of the fallopian tube were collected at the time of hysterectomy (n = 15) or during surgical management of tubal pregnancy (n = 16). Women were between 18 and 45 years of age (mean age for hysterectomy patients was 40 ± 4 years and mean age for patients with tubal EP was 31 ± 5 years). Samples were prepared by either [1] short-term storage in phosphate-buffered saline (PBS) before explant culture; [2] immersion in RNAlater (Ambion, Austin TX) at 4°C overnight and then flash frozen at -80°C for RNA extraction; or [3] fixation in 4% neutral-buffered formalin overnight at 4°C followed by storage in 70% ethanol, and embedding in paraffin wax for immunohistochemical staining. The menstrual cycle phase of each patient at the time of hysterectomy was determined using the following parameters: [1] last menstrual period confirmed by patient, [2] histologic examination and staging of an endometrial biopsy taken with the fallopian tube by a gynecological pathologist, and [3] measurement of E_2 and P levels in serum of patients. The clinical data corresponding to each sample are listed in Tables 1 and 2. None of the gynecological conditions listed have been reported to date to affect PROK/PROKR expression in the endometrium, therefore it was assumed that they would have no effect on expression in the fallopian tube. Fallopian tubes removed during surgical management for EP were all from the ampullary region of the fallopian tube. Gestational age, hCG, and P levels of each EP are listed in Tables 1 and 2.

Hormonal Treatment of Fallopian Tube Explants

Fallopian tube tissue culture was performed, in triplicate, using tissue from three different patients. Two of the fallopian tubes were from the follicular phase of the menstrual cycle and one was from the late luteal phase. Each tissue was cut into small pieces (2–3 mm), which were placed in each well of a 12-well dish and cultured in Rosewell Park Memorial Institute (RPMI)

TABLE 2

Clinical information for fallopian tube samples from ectopic pregnancies.

Sample no.	Gestation length (d)	Serum hCG (IU/L)	Serum P (nmol/L)
16	58	15,956	67.43
17	50	487	39.21
18	59	2,056	24.58
19	53	1,854	63.07
20	52	2,425	61.68
21	56	225	20.36
22	64	1,383	24.35
23	78	1,204	134.70
24	56	203	19.77
25	49	502	30.54
26	64	5,981	158.13
27	44	508	7.06
28	44	12,161	10.64
29	46	10,285	31.70
30	52	1,082	23.92

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