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Short Communication

Inhibition of embryo implantation in mice through vaginal administration of a proprotein convertase 6 inhibitor

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ARTICLE INFO

Article history:
Received 24 May 2013
Accepted 24 December 2013
Available online 13 January 2014

Keywords:
Proprotein convertase
Embryo implantation
Peptide inhibitor
Vaginal drug delivery
Contraceptive

ABSTRACT

Uterine proprotein convertase 6 (PC6) plays a critical role in embryo implantation in both mice and women. It was hypothesized that inhibiting uterine PC6 could prevent pregnancy. Vaginal administration of a PC6 inhibitor presents the ideal route for local drug delivery. A peptide-based PC6 inhibitor, C-30k-PEG Poly R that was previously shown to have properties of increased vaginal absorption and penetration was tested for its contraceptive potential in mice following vaginal administration. The study demonstrated that this approach could inhibit embryo implantation in some mice (24% completely and 47% partially inhibited).

1. Introduction

Uterine proprotein convertase 6 (PC6) is well established to play a critical role in embryo implantation in both mice and women [1,2]. When uterine PC6 production was blocked locally in mice by anti-PC6 morpholino oligonucleotides, decidualization (a critical process of implantation) was inhibited and implantation prevented [1]. The critical role of PC6 in decidualization was also proven in primary human endometrial stromal cells [2]. In addition, PCs including PC6 also play a crucial role in HIV infections [3–5]. The vagina is the entry site of sexually transmitted HIV in women, and transmission can be stopped by vaginal application of anti-HIV drugs. Therefore, we hypothesize that inhibition of PC6 in the female reproductive tract (uterus, cervix and vagina) would provide a novel approach to develop non-hormonal and female-controlled contraceptives that could also protect women from sexually transmitted HIV infections. Vaginal delivery of PC6 inhibitors to inhibit the PC6 activity in the reproductive tract presents the ideal route of administration to achieve this dual protection. In this study, we aimed to test the hypothesis that local delivery of PC6 inhibitors through the vagina can effectively inhibit embryo implantation using mice as an animal model.

Poly R (nona-D-arginine) is one of the most potent PC6 inhibitors [6]. In an effort to develop a vagina-deliverable

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1642-431X/$ – see front matter
http://dx.doi.org/10.1016/j.repbio.2013.12.008

PC6 inhibitor, we previously modified Poly R by PEGylation [covalent attachment of polyethylene glycol (PEG)], and examined the PEGylated Poly Rs for their in vitro bioactivity, stability and in vivo pharmacokinetics [7]. The study established that Poly R conjugated with a 30 kDa PEG at its C-terminus, named C-30k-PEG Poly R, is a potential PC6 inhibitor with beneficial characteristics, including enhanced vaginal absorption and penetration [7]. Here, we conducted a proof-of-concept study in mice to evaluate the contraceptive potential of C-30k-PEG Poly R in inhibiting PC6 to block embryo implantation following vaginal administration.

2. Materials and methods

C57BL/6J mice were housed and handled according to the Monash University animal ethics guidelines on the care and use of laboratory animals. All studies were approved (Animal ethics number "MMCB 2009/12") by the Animal Ethics Committee at the Monash Medical Centre, Melbourne. C-30k-PEG Poly R was produced by site-specific and covalent attachment of a 30 kDa PEG through an L-cysteine introduced to the C-terminus of Poly R (Mimotopes Pty. Ltd., Australia) [7].

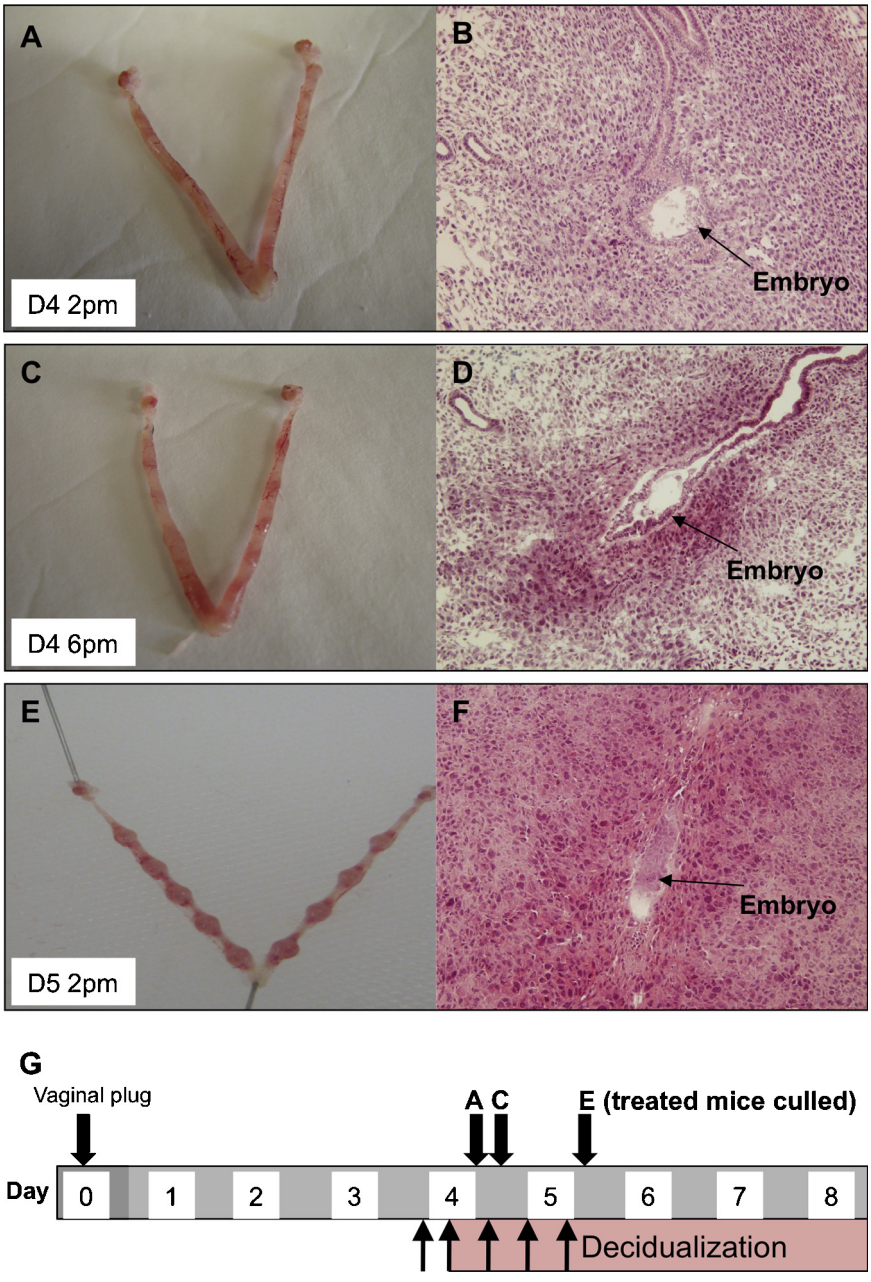

Fig. 1 – Implantation sites between Day 4 and Day 5 of pregnancy and timing of important events from Day 0 to Day 8. Appearance of mouse uterine horns with implantation sites and their cross sections stained with haematoxylin and eosin at Day 4: 2 pm (A and B); Day 4: 6 pm (C and D); and Day 5: 2 pm (E and F). (G) Timing of important events from Day 0 to Day 8. Decidualization phase is referenced from [11]. Arrows (↑) represent the time of C-30k-PEG Poly R treatment. Mice were culled at Day 5 (2 pm) for examinations.