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Icon immunoconjugate treatment results in regression of red lesions in a non-human primate (*Papio anubis*) model of endometriosis

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ABSTRACT

Endometriosis is a common condition in reproductive-aged women characterized by ectopic endometrial lesions of varied appearance, including red, white, blue, black or powder burn coloration, which contribute to chronic pain and infertility. The immunoconjugate molecule (Icon) targets Tissue Factor, a transmembrane receptor for Factor VII/VIIa that is aberrantly expressed in the endothelium supporting ectopic endometrial tissue. Icon has been shown to cause regression of endometriosis in a murine model of disease but prior to this study had not been tested in non-human primates. This study evaluated Icon as a novel treatment for endometriosis in non-human primates (*Papio anubis*) using an adenoviral vector (*Ad*Icon) delivery system. Female baboons (n = 15) underwent surgical induction of endometriosis. After laparoscopic confirmation of endometriosis lesions 6-weeks post-surgery, the treatment group (n = 7) received weekly intraperitoneal injections of viral particles carrying the sequence for Icon, resulting in expression of the protein, while the control group (n = 8) received no treatment. Icon preferentially reduced the number and volume of red vascularized lesions. Icon may present a novel treatment for endometriosis by degrading red vascularized lesions, likely by targeting tissue factor aberrantly expressed in the lesion vasculature.

1. Introduction

Endometriosis is an estrogen-dependent disease characterized by ectopic endometrial lesions; it affects approximately 10% of reproductive-aged women and is found in 20–50% of infertile women [1,2]. These lesions differ in their degree of vascularization, which results in varied coloration. The most highly vascularized lesions are red, while less vascularized lesions may have a white, blue, black or powder burn appearance. Moreover, adhesions can also result from the disease itself or as a result of surgical interventions [3]. Most often, endometriosis is found on the pelvic peritoneum and ovaries. However, endometriosis has also been described in locations distant from the pelvis [4–8]. These lesions remote from the peritoneal cavity have been proposed to arise from stem cells originating in the bone marrow as well as from intravasation of endometrial cells into the circulation [9–11]. Aberrant genome-wide methylation in the eutopic endometrium has been associated with endometriosis, indicating that endometriosis may have an epigenetic component [12].

Hypervascularization within and around endometriotic lesions has been well-described at time of laparoscopic surgery and confirmed histologically [13]. Clinically, the pelvic fluid of women with endometriosis has greater angiogenic activity than the pelvic fluid from women without the disease [14,15]. Prior studies demonstrate that a transmembrane receptor for Factor VII/VIIa, key regulator of the extrinsic coagulation cascade, and modulator of angiogenesis, Tissue Factor (TF), is over-expressed in ectopic endometrium of women with endometriosis [16]. Endothelial TF is furthermore aberrantly expressed in the vessels of ectopic endometrial tissue [16]. Endometriosis is also associated with inflammatory cytokine and chemokine production, including interleukin-1β, which increases TF gene expression in a variety

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of cell types [16–21].

Endometriosis may dramatically impact the quality of life of those who suffer from it by causing dyspareunia, dysmenorrhea, pelvic pain, menorrhagia, menometrorrhagia, dysuria, and infertility [1,2,22–24]. Moreover, severe endometriosis can be both physically and psychologically debilitating; it is estimated that the annual per patient expense of endometriosis exceeds $10,000 due to both direct health care costs and loss of productivity [24]. Current treatments for endometriosis, however, are limited in their efficacy as return of the disease is common [25].

Novel treatment modalities aimed at preventing angiogenesis thus may be required to suppress new endometriosis lesions and the return of disease following medical or surgical management [26–28]. The immunoconjugate molecule (Icon) can degrade existing neovasculature by activating a Natural Killer (NK) cell cytolytic response against TF-bearing endothelial cells [29,30]. Icon comprises an immunoglobulin Fc domain and two low-coagulation-inducing mutated factor VII domains that bind with high affinity to TF [31,32]. In contrast with antiangiogenic treatments, which only target developing blood vessels, Icon can additionally degrade extant neovasculature. Icon's effectiveness in destroying lesions in an athymic murine model of endometriosis did not result in apparent toxicity, teratogenic effects, or reduced fertility, making it a promising candidate for clinical use [33]. Further studies in a non-human primate model phylogenetically closer to humans are required to validate these results.

Baboons (*Papio anubis)* have been used as models for human reproduction as their menstrual cycles closely approximate human menstrual cycles, with an average length of 24–38 days [34,35]. The occurrence of spontaneous endometriosis in baboons has moreover been well characterized [36–43]. These non-human primate lesions are morphologically identical to those found in humans, and they are additionally found at similar locations, primarily in the peritoneal cavity. Endometriosis has also been induced in baboons by supracervical ligation and intraperitoneal injection of endometrial tissue [44–49]. Baboon models of endometriosis have been recognized as the most appropriate non-human primate animal models of this disease [38]. In this study, we examine whether Icon can be used to treat endometriosis in a non-human primate baboon model with the ultimate goal of extending this treatment to humans.

2. Materials and methods

2.1. Subjects and ethics

We conducted all experiments involving baboons in accordance with NIH Guidelines for the Use and Care of Animals in Research and the ARRIVE guidelines. The Animal Care and Use Committee (IACUC) of the Institute of Primate Research, Nairobi, Kenya and the IACU committees of Yale University and Michigan State University approved the study, respectively.

2.2. Construction and production of adenoviral vector encoding human icon

Construction of the expression plasmid DNA for the human Icon (GenBank Accession Number AF272774) has been previously described [30]. We subcloned cDNA of human fVII(K341A)-human IgG1Fc (human Icon) into the shuttle vector pShuttle-CMV using the AdEasy system (Agilent, USA) [50]. We constructed adenoviral vector encoding human Icon, amplified the product on HEK293 cells (Human Embryonic Kidney 293 cells, Sigma Aldrich), and purified it as previously described [29,30,51,52].

2.3. Adenoviral icon gene therapy in a baboon model of endometriosis

The study was performed on 15 adult female baboons captured from the wild and maintained in captivity at the Institute of Primate Research, Nairobi, Kenya. All had normal menstrual cycles, ranging from 24 to 38 days, and did not have endometriosis or other known inflammatory conditions as determined by laparoscopy prior to the onset of the study. We induced endometriosis in all 15 baboons by intrapelvic seeding of menstrual endometrium, as previously described [45]. The animals were randomized to the treatment and control groups. We performed laparoscopy six weeks after endometriosis induction, in order to allow for at least one menstrual cycle in each baboon. We performed laparoscopies within the follicular phase, on days ranging from 0 to 9, of the baboon menstrual cycle. A single surgeon counted, measured, and characterized lesions and adhesions visually based on color in the control group and treatment group in a blinded fashion. We measured superficial lesions with calibrated probes in three-dimensions (length, width, and depth). If lesions were not superficial, we calculated volume as length × width^2. We began treatment at the time of the second laparoscopy, six weeks after endometriosis induction. The treatment group (n = 7) received weekly 20 mL intraperitoneal (I.P.) injections of 3.3×10^{10} viral particles encoding Icon in a sterile saline buffer. The control group (n = 8) had laparoscopies at the same time as the treatment group. After six weeks of treatment, we performed a third laparoscopy and again the single surgeon counted, measured, and characterized lesions and adhesions visually based on color in the control group and treatment group in a blinded fashion. All laparoscopies were filmed and conducted at the Institute of Primate Research (IPR), a World Health Organization collaborating non-human primate research facility in Nairobi, Kenya (Supplementary Images 1 and 2) [38].

2.4. Statistical analysis

We executed all statistical tests on GraphPad Prism Software for Mac (GP Inc., La Jolla, CA). The dataset used in this analysis were the changes in number and volume of lesions for each animal, before and after treatment. We used one-tailed two-sample *t* tests, with a = 0.05, to determine if Icon treatment resulted in a significant reduction in endometriosis lesion number or volume compared to the control group. We selected this test based on our hypothesis that Icon treatment would reduce lesion number and size, which was observed in prior studies [33]. We considered the lesion number to be the primary endpoint, as it was an absolute measure of change, with reduced sources of potential measurement errors, and it suggested the potential of Icon treatment to promote full disease remission. We additionally analyzed the change in lesion volume, as it represented a more nuanced measure of response to treatment and suggested the potential of Icon treatment to promote all degrees of disease remission. We did not exclude any animals from the analysis. Results are presented as p-values.

3. Results

We compared the change in number and volume of lesions (red, blue, white) and adhesions (filmy, dense) from pre-treatment laparoscopy to post-treatment laparoscopy between the control and Icon treatment groups. Although there was slight heterogeneity in the initial number of surviving lesions within this small sample size, this was not statistically significant and each animal was compared to itself in order to control for any variation in initial disease establishment.

The Icon treated animals exhibited a significant reduction in the number of red lesions between laparoscopy 2 and 3 compared to the control group animals (Icon: −2.14 ± SEM 0.99, (−48.4%) vs. control: +1.38 ± SEM 1.03, (+84.6%); p = .02, Fig. 1A). We found no significant change in number of blue lesions, white lesions, dense adhesions, and filmy adhesions between the Icon and control groups (Fig. 1B–E).

The Icon treated animals also exhibited a significant reduction in the volume of red lesions and filmy adhesions between laparoscopy 2 and 3 compared to the control group animals (Icon red lesion volume: