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Creation of a female rabbit model for intrauterine adhesions using mechanical and infectious injury

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ABSTRACT

Background: Intrauterine adhesions (IUA) are associated with secondary amenorrhea, infertility, and recurrent pregnancy loss. An IUA animal model would contribute to research on the pathogenesis and pathologic changes of IUA and the exploration of new treatments.
Materials and methods: Eighty female rabbits were randomly divided into five groups: mechanical injury (16), infectious injury (16), dual injury (16), experimental control (16), and normal (16). Three methods were applied to establish the model: uterine curettage, uterine cavity left alone, lipopolysaccharide surgical suture in place for 48 h, and suture retention for 48 h after curettage. A sterile surgical suture was left in the uterine cavity for 48 h in the experimental control group. Histologic changes were monitored at 0, 24, 48, and 72 h and 7, 14, and 28 d after operation.
Results: The experiments revealed that endometrium injured by simple curettage or infection could be repaired. Although endometrial regeneration was severely impaired by dual injury, the ratio of the area with endometrial stromal fibrosis to total endometrial area significantly increased ($P < 0.01$), and the number of endometrial glands was significantly reduced ($P < 0.01$).
Conclusions: The method of dual injury can establish a stable rabbit IUA model.

1. Introduction

Intrauterine adhesions (IUA) refer to the partial adherence of endometrial surfaces with fibrotic tissue. Presenting symptoms are related to the degree and location of IUA and include menstrual abnormalities ranging from irregular bleeding and hypomenorrhea to amenorrhea, infertility, and recurrent pregnancy loss [1]. In 1948, Asherman [2–4] published a series of articles describing the frequency, etiology, symptoms, and roentgenologic picture of this condition, and Asherman syndrome has been used to describe the disease ever since. With the popularization of hysteroscopy and surgery, the incidence of IUA has increased and has become the second most common of female secondary infertility [5]. There are four aspects that relate to IUA: trauma [6], infection [7,8], endometrial repair disorders [9], and low estrogen levels [10]. Previous studies have focused on new treatments for preventing endometrial fibrosis and improving endometrial

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regeneration [11–13]; however, animal models to research the mechanism and curative effects of these new treatments are still scarce. It is necessary to establish an animal model of IUA to intensively research the pathogenesis and pathologic changes of IUA and to explore new treatments.

Lipopolysaccharide (LPS) is the major structural component of the cell wall of gram-negative bacteria and is also the endotoxin responsible for much of the inflammation and tissue injury associated with bacterial infections [14]. LPS-induced animal models highlight ways to explore mechanisms of multiple diseases and provide useful information on the discovery of novel biomarkers and treatment strategies [15–17]. However, reports of the effects of LPS on endometrium are rare.

The present study aimed to establish an animal model of IUA in the New Zealand white rabbits using mechanical damage, infection, and double damage as is similar to the two main factors in the pathogenesis of IUA: trauma and infection. In this study, curettage was used as a method of mechanical damage and LPS as a predisposing factor for infection. Through this study, we hope to provide an ideal animal model for the study of the exact pathogenesis of and therapy for this difficult clinical condition.

2. Materials and methods

2.1. Animals

Experimental protocols were approved by the Institutional Animal Ethics Committee. Adult female New Zealand white rabbits (Guangzhou, Guangdong, China) weighing 2500–3500 g were fed standard chow and water *ad libitum* under standardized laboratory conditions in a temperature-controlled room and light conditions (12 h light and 12 h dark) for 2 wk.

2.2. LPS surgical suture preparation

We obtained LPS from the manufacturer (derived from *Escherichia coli* 055:B5; Sigma, St. Louis, MO). A 6 mg/L solution of LPS in normal saline was stored in a 4°C refrigerator. The 10-0 medical sterile surgical sutures (10 cm) were soaked in an LPS saline solution for 24 h the day before use in the model.

2.3. Method for establishing animal models

One hundred sixty uterine horns from 80 female New Zealand white rabbits were randomly divided into five groups: normal, experimental control, mechanical injury, infectious injury, and dual injury (mechanical and infection). Twenty-four hours before the creation of the model, the rabbits were injected with human chorionic gonadotropin (50 IU) by ear vein to reach synchronization of endometrial development. All the rabbits were anesthetized with urethane (1.5 g/kg) administered intravenously through the marginal ear vein and supplemented if necessary. Throughout the surgery, particular care was taken to confirm an adequate level of anesthesia such that noxious forelimb pinching evoked no response. Two rabbits ($n = 4$ uterine horns) were killed in each group at 0, 24, 48, and 72 h and 14 and 28 d after surgery, and four rabbits were killed at 7 d. Uterine tissue samples were collected.

2.3.1. *Mechanical injury group* (n = 32 uterine horns)

The rabbits were operated on through a 0.5-cm longitudinal incision in the lowest one-third of the connection between the middle and distal uterus and then the endometrial lining of the middle and upper two-third of the uterus was scraped using a 4-mm endometrial curette. Curettage ceased when the uterine cavity stopped feeling uneven and the uterine wall became rough. The uterine tissue from two rabbits ($n = 4$ uterine horns) was collected immediately after curettage, and the abdominal incisions of the other rabbits were sutured after flushing the uterine and peritoneal cavities.

2.3.2. *Infectious injury group* (n = 32 uterine horns)

An LPS surgical suture was inserted in the uterine cavity through a uterine incision identical to that of the mechanical injury group. The tail end of the LPS surgical suture was placed through the abdominal wall, and a muscular layer was retained on the surface of the skin to facilitate removal after 48 h. Two rabbits ($n = 4$ uterine horns) were killed for the collection of uterine tissue once the LPS surgical suture was removed.

2.3.3. *Dual injury group* (n = 32 uterine horns)

After uterine curettage, performed in an identical manner to that of the mechanical injury group, an LPS surgical suture was placed in the uterine cavity (Fig. 1B). The LPS surgical suture was removed at 48 h after surgery, and two rabbits ($n = 4$ uterine horns) were killed for the collection of uterine tissue.

2.3.4. *Experimental control group* (n = 32 uterine horns)

A sterile surgical suture was left in the uterus for 48 h as an experimental control group. Similarly, two rabbits ($n = 4$ uterine horns) were killed when the surgical suture was removed.

2.3.5. *Normal group* (n = 32 uterine horns)

In the normal group, rabbit abdomens were opened to expose the bilateral uteri but were not given any treatment.

2.4. Histologic examination

The uterine tissues were fixed in 4% buffered formaldehyde, embedded in paraffin, and routinely stained with hematoxylin–eosin and Masson stains. Histologic evaluation was performed by an experienced pathologist. On each hematoxylin- and eosin-stained slice, four high-power fields were selected, the number of glands per high-power field was counted, and the mean was calculated.

On each Masson-stained slice, four high-power fields were selected, and the degree of endometrial fibrosis was calculated as the ratio between the area of endometrial stromal fibrosis and endometrial area per high-power field. The average of four fields was calculated.

2.5. Statistical analysis

Data were expressed as the mean and standard errors of the mean. Statistical analysis was performed using SPSS for