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Treatment of Endometriosis with Local Acetylsalicylic Acid Injection: Experimental Study in Rabbits

Juliana Menezes Siqueira, MD, Adriana Beatriz Barreto, MD, and Rogério Saad-Hossne, MD, PhD*

From the Department of Surgery, Botucatu Medical School, Sao Paulo State University (UNESP), Botucatu, São Paulo, Brazil (all authors).

ABSTRACT The objective of the present study was to estimate the effects of introduction of acetylsalicylic acid solution into peritoneal implants in autologous endometrium as a method for treating endometriosis. Forty adult female rabbits were subdivided into 4 groups of 10 rabbits each, and endometriosis was induced via autotransplantation of endometrial fragments into the peritoneal cavity. At 30 days after induction of endometriosis, all animals were randomly assigned to 1 of 2 protocols. In protocol 1, animals were evaluated at 24 hours after treatment; group 1 (control) received physiologic solution, and group 2 received acetylsalicylic acid. In protocol 2, animals were evaluated at 10 days after treatment, group 3 (control) and group 4 received acetylsalicylic acid. After measuring the lesion, the endometriotic focus was removed and prepared for mounting on slides for histologic analysis. Imaging software was used for analysis of the total remaining area of endometrial tissue. The affected area in acetylsalicylic acid–treated animals was smaller than that in control animals at 24 hours and 10 days after treatment; a significant difference was found between control and treated groups ($p < .001$). Statistical analysis comparing protocols 1 and 2 demonstrated no differences between controls groups or acetylsalicylic acid groups ($p = .30$), and no differences between times ($p = .75$). Acetylsalicylic acid solution led to less growth (or higher involution) of endometrial implants. Acetylsalicylic acid injected directly into endometriotic foci was effective in their destruction. This presents new perspectives for treatment of endometriosis and for clinical applications based on further clinical studies. Journal of Minimally Invasive Gynecology (2011) 18, 800–806 © 2011 AAGL. All rights reserved.

Keywords: Acetylsalicylic acid; Endometriosis; Experimental treatment

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Endometriosis is a chronic condition characterized by the presence of endometrial tissue, glandular or stromal, outside of the uterine cavity. These ectopic and endometrial tissues are commonly observed in the ovaries, peritoneum, uterosacral ligaments, the base of the rectovaginal fundus, and extrapelvic implants. Endometriosis affects 2% to 7% of young women of reproductive age [1–3]. However, estimates indicate that at least 25% of women in their thirties can develop the disease [4].

Clinically, the condition includes both asymptomatic and symptomatic forms. The primary signs and symptoms are changes in menstrual cycle, chronic recurrent pelvic pain, and fertility disorders [5]. The prevalence of endometriosis can reach 60% when a more specific population of women with pelvic pain or infertility is considered [6–8].

The criterion standard diagnostic examination for confirmation is anatomopathologic analysis of biopsy tissue obtained via invasive methods such as videolaparoscopy and laparotomy [5]. Gross examination reveals red-blue to yellow-chestnut nodules on the serous surfaces [9]. When the disease is extensive, recurrent hemorrhage and fibrous adherences can also develop.

The first and best treatment option is surgery, to remove the endometriotic foci and to obtain the highest resolution indices. However, the recurrence rate is high at 47% [7,8,10]. Recent studies have been performed with the objectives of reducing the number of invasive procedures,

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Corresponding author: Rogério Saad-Hossne, MD, PhD, Department of Surgery, Botucatu Medical School, Paulista State University (UNESP), Distrito de Rubião Junior, s/n CEP, Botucatu, 18618-000 São Paulo, Brazil.
E-mail: rshossne@gmail.com

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improving prognosis, and decreasing the number of recurrences. Studies that used systemic drugs such as gonadotropin-releasing hormone [11], danazol [11], aromatase inhibitors [12], immunomodulators such as recombinant human tumor necrosis factor [13], and nonsteroidal anti-inflammatory drugs such as celecoxib, indomethacin, sulindac, and ibuprofen [14] have demonstrated successful ectopic implant regression. In addition, a significant reduction in peritoneal lesion size has been achieved using interferon-α 2b [15] and IL-12 via the intraperitoneal topical route [16].

However, testing treatments using new medications requires experimental models before clinical tests in human beings. The choice of model must be based on the highest physiopathogenic credibility between the animal model and human beings. There is no well-established animal model for studying endometriosis. If Sampson's theory is to be believed, primates are the ideal animal models. The theory holds that endometriosis develops due to retrograde menstruation [17] and that only primates exhibit spontaneous endometriosis. However, in addition to high maintenance, the use of primates is associated with many ethical issues [18]. Also, the presence of retrograde menstruation is not the only etiologic factor involved in the origin of this disease. Endometriosis occurs in approximately 5% of women, with more than 80% having retrograde menstruation [19]. Further, endometrial tissue is ectopically observed in patients with malformed genitalia, incompatible with reverse menstrual flow. Hence, endometriosis may not exclusively depend on Sampson's theory but on a number of factors. Some of the most well-established include a significant increase in cyclooxygenase-2 expression [20], aberrant expression of aromatase [21] and its induction by elevated prostaglandin E_2 levels [22], secretion of angiogenic factors [23], and failure of immune protection [24,25].

Experimental rat and rabbit models have been used extensively since 1984 despite nonspontaneous lesion evolution in these species [26,27]. Such trials are easily reproducible using endometrial autotransplantation, a technique in which topical endometrium is removed and implanted into the peritoneal region in immunocompetent subjects. These methods have been used to investigate the mechanisms responsible for development of peritoneal lesions. They can also be used to evaluate the effects of therapeutic drugs such as immunomodulators, anti-inflammatory agents [18,28], and acetylsalicylic acid.

In the present study, we used acetylsalicylic acid (aspirin) because of its widespread use and previous experimental data (acetylsalicylic acid and its derivatives) that we have gathered on the subject [29–34]. In these studies, we have demonstrated the effects of acetylsalicylic acid on normal tissues (rabbit liver and colonic mucosa). We injected acetylsalicylic acid (2.5% and 5.0%) into the left lobe of rabbit liver, and observed no alterations in clinical evolution, weight, or biochemical levels compared with those in control animals that received saline solution [30]. We also applied 5% acetylsalicylic acid in the form of an enema to rabbit colorectal mucosa, and also observed no alterations in colonic mucosa compared with control animals who received saline solution enemas [34].

In 2 additional studies, we evaluated the cytotoxic and antitumor effects of acetylsalicylic acid *in vitro* and *in vivo*. In the first study, we examined VX-2 tumor cells incubated with acetylsalicylic acid (2.5% and 5%), and observed that tumor cell viability was reduced progressively and that after 30 minutes all tumor cells were nonviable [29]. In the second study, we evaluated the effects of 5% acetylsalicylic acid on VX-2 liver carcinoma in rabbits, and observed experimental hepatic tumor destruction without alterations in clinical evolution, weight, or biochemical levels [31].

Thus, the objective of the present study was to evaluate the effect of acetylsalicylic acid on peritoneal implants of autologous endometrium in an experimental rabbit model of endometriosis.

Materials and Methods

The experimental protocol (No. 107/05) was approved by our ethics committee. The rabbits were maintained according to guidelines of the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research, 1996) and according to the ethical principles of the Brazilian College on Animal Experimentation (COBEA).

First Surgical Procedure: Animal Housing, Anesthesia, and Induction of Endometriosis

Forty female adult virgin rabbits weighing approximately 1500 g were kept in appropriate cages under controlled conditions of light, humidity, temperature, water, and food. All surgical procedures were performed with the animals under general anesthesia via intravenous administration of 30 mg/kg 3% sodium pentobarbital.

All animals underwent asepsis and laparotomy according to the method of Silva et al [35]. An approximately 2-cm longitudinal median incision was made in the abdominal wall, followed by removal of approximately 4 cm of the right uterine horn. The uterine fragment was immersed in 0.9% physiologic solution at 4°C for 4 minutes. A longitudinal incision was made in the removed uterine horn, withdrawing a 5.0 × 5.0-mm fragment. This was followed by separating the endometrium from the other uterine layers. The endometrium was then sutured using 6.0 polyglactin 910 (Vicryl; Ethicon, Somerville, NJ) to the parietal peritoneum, close to the Alba line, in a location where most of the vessels could be seen, and the free endometrial side was returned to the abdominal cavity lumen. After implantation, the animals were observed for 30 days, the time in which graft viability and size are maximum, i.e., 100% and 2.5 cm, respectively [35]. To reduce adherence indices in all procedures, latex gloves were first washed with 0.9% physiologic solution.