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Endometriosis prophylaxis and treatment with the newly developed xenogenic immunomodulator RESAN in an animal model

Krzysztof Szymanowski a,*, Karolina Chmaj-Wierzchowska a, Andrey Yantczenko b, Joanna Niepsuj-Biniaș a, Ewa Florek c, Tomasz Opala a, Marek Murawski d

a Department of Mother’s and Child’s Health, K. Marcinkowski University of Medical Sciences, Poznan, Poland
b The Scientific Research Enterprise RESAN, Vitebsk, Belarus
c Department of Toxicology, Laboratory of Environmental Studies, K. Marcinkowski University of Medical Sciences, Poznan, Poland
d I Department of Gynecology and Obstetrics, Medical University of Wroclaw, Poland

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ABSTRACT

Objective: The objective was to assess the effectiveness of the newly developed immunomodulator RESAN in the prophylaxis and treatment of endometriosis induced in rats.

Study design: The study was performed on 58 Wistar rats. Twelve weeks before endometriosis induction, the RESAN vaccine was administered to 24 rats (100 mg i.m. and 100 mg s.c.). Endometriosis induction was performed in 48 rats, which were divided into two groups: group I, the prophylaxis group, consisting of 24 previously vaccinated rats; and group II, the treatment group, comprising the other 24 rats, which had not been vaccinated. The graft (4 mm × 4 mm) of endometrium was attached to the parietal peritoneum. A sham operation was performed in 10 rats (group III). After 3 months, a second laparotomy was performed in all animals, and endometriotic foci were excised when present. RESAN was administered to the group II animals. After an additional 3 months, a third laparotomy was performed in all animals of the three groups.

Results: Positive, histologically confirmed endometriosis was found in 4.3% of the animals in group I and in 69.6% of group II rats (p < 0.0001). Macroscopic assessment revealed endometriosis in 21.7% and 91.3% of animals in groups I and II, respectively (p < 0.0001). At final laparotomy, 3 months after excision of the previously suspected foci, no signs of endometriosis were found according to both macroscopic assessment and histological examination. During the second laparotomy intraperitoneal adhesions were present in 13.0% of the animals in group I and in 61.0% of those in group II. No adhesions were present in group III. At the final laparotomy, the adhesions were present in only three of the animals in group II (p < 0.0009).

Conclusions: RESAN seems to be effective in both the prophylaxis and treatment of endometriosis, as well as in the prophylaxis of adhesions. Histological confirmation of endometriosis should be mandatory.

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1. Introduction

Endometriosis is defined as the presence of functioning endometrial glands and stroma outside the uterus, predominantly within the peritoneal cavity [1]. In most cases, the spread of extrauterine endometrial tissue appears to result from retrograde menstruation and capillary or lymphatic dissemination. Endometrial cells implanted ectopically respond to cyclical changes in estrogen and progesterone with proliferation and secretion. Their presence in extrauterine areas can initiate immune and inflammatory responses that lead to pain and peritoneal adhesions, and may interfere with fertility. Immunologic factors may affect a woman's susceptibility to the implantation of exfoliated endometrial cells. Immune alterations include increased number and activation of peritoneal macrophages, decreased T cell reactivity and natural killer cell cytotoxicity, increased circulating antibodies, and changes in the cytokine network. There is substantial evidence that immunologic factors play a role in the pathogenesis of endometriosis and endometriosis-associated infertility, due to adhesion formation. Decreased natural killer cell cytotoxicity leads to an increased likelihood of the implantation of endometriotic...
tissue. In addition, macrophages and a complex network of locally produced cytokines modulate the growth and inflammatory behavior of ectopic endometrial implants. The prevalence of endometriosis is 7–17% among reproductive age women [2–4]. However, these numbers reach 20–50% in patients suffering from infertility, and 40–60% in those with dysmenorrhea [5,6]. There is no truly effective method of endometriosis treatment, a circumstance caused by an insufficient understanding of its pathogenesis.

The adhesion of endometrium refluxed through the fallopian tubes is one of the first and necessary stages of the implantation theory, which leads to the question, why do these misplaced endometrial fragments only survive in some women? An immunological/inflammatory etiology has been conjectured, as demonstrated by increased concentrations of activated macrophages, cytokines, and T and B cells [7]. What happens in immunological surveillance in the very early stages of endometriosis in women remains unclear. Clinical trials in vivo, involving early endometriotic foci development, cannot be performed for obvious ethical reasons. Therefore, our knowledge of endometriosis development arises mainly from experimental works and animal studies. In oncology, bacillus Calmette-Guérin (BCG) and granulocyte–macrophage colony-stimulating factor (GM-CSF), among others, are used as boosters for the immune system. In clinical settings, both have been administered with tumor cells, to make the immune system more responsive to the antigens presented [8,9]. Some of these experiences may be useful for endometriosis therapy. In animal studies, systemic prophylaxis with BCG caused an inhibitory effect on endometrial transplantation [10,11]. Balasch et al. [12] presented data from a prospective randomized controlled trial on the effect of pentoxifylline on endometriosis patients [13].

The aim of the present study was to assess the effectiveness of the anticancer vaccine RESAN in the prophylaxis and treatment of endometriosis induced in rats.

2. Materials and methods

The study was conducted after approval by the Regional Animal Research Committee. The experiment was performed in accordance with both the Ministry and High Education Report of 1959, and the UNESCO Declaration of Animal Rights from the 1978 (Paris) guidelines.

Sexually mature female Wistar white rats bred at Jeleniogorskie Zakłady Farmaceutyczne ‘Polfa’ (Jelenia Gora, Poland) were housed and cared for by the Department of Toxicology, Karol Marcinkowski University of Medical Sciences in Poznan, as previously described [14–18]. The experiment was performed on 58 rats, which were kept in an environmentally controlled area and maintained with water and chow ad libitum. Twelve weeks before the endometriosis induction, the RESAN vaccine was given to 24 rats (group I, prophylaxis). The RESAN vaccine is a complex of molecules extracted from xenogenic tissues, i.e., G. domesticus. It contains glycoproteins (fraction α2B), peptides and carbohydrate fragments of more than 40 different common tumor antigens. The glycoproteins imitate 6–50 fragments (a length of 7–30 amino acids) of each antigen [19]. One milliliter of the suspension (with 200 mg of the antigens) was divided into two parts, one of which was given intramuscularly and the second subcutaneously into the upper part of the buttock, according to the manufacturer’s instructions. Endometriosis induction was performed in 48 rats (24 had been vaccinated previously with RESAN, group I, and 24 had not, group II, the treatment group) as previously described, with minor modifications [15,16]. Shortly afterward, a mid-ventral laparotomy was performed aseptically under pentobarbital anesthesia. A 3-cm segment of the right uterine horn was ligated and excised. After immersing the excised horn in the sterile culture medium, the tissue was divided into parts for further experiments. In one part the endometrium was detached from the muscular layer, forming a graft measuring 4 mm × 4 mm, which was then attached to the parietal peritoneum on the right side of the abdominal wall. Sutures of 6–0 nylon were used to attach the graft. Microsurgical techniques were applied in the steps described above. The abdominal wall was closed with a 1-0 vicryl running suture. In 10 animals, a sham operation was performed (group III, controls). After opening the abdominal wall, section and ligation of the right uterine horn were performed. Next, a 6–0 nylon suture was attached to the same place in the abdominal wall as in the study animals. All the other procedures were performed in exactly the same manner as in groups I and II.

Three months later, a second laparotomy was performed in all animals. We searched for endometriotic foci and adhesions. After visualization, description and photographic documentation were completed, then, excision of the endometriotic foci was performed in groups I and II. Finally, the left uterine horn was excised for parallel investigation. During the second laparotomy, RESAN was given to the animals in group II. All suspected endometriotic foci were embedded in 4% formaldehyde for histology. After 3 months, a third laparotomy was performed in all animals. Once again, we searched for endometriotic foci and the suspected lesions were excised for histology. All the tissue fragments acquired during the second and third laparotomy were fixed, cut on microtome, and stained with hematoxylin and eosin. All tissue sections were assessed for endometriosis by the same person (K.S.) in a blinded manner. A second assessment was performed by the consulting histologist with 100% accuracy. For statistical analysis, Fisher’s test was used to compare groups I and II. A p value < 0.05 was considered significant.

3. Results

Two animals failed to complete the entire program. One rat died because of postoperative hemorrhage 2 days after the second operation (group I), and the second expired because of contamination caused by self-opening of the abdominal wall a day after the first operation (group II).

Positive, histologically confirmed endometriosis induction was found in 4.3% of the animals in group I and in 69.6% in group II (p < 0.001). Macroscopic assessment revealed endometriosis in 21.7% and 91.3% of the animals in groups I and II, respectively (p < 0.0001; Table 1). At the third laparotomy (after previous excision), no signs of endometriosis were found on both macroscopic assessment and histological examination (Figs. 1–3).

During the second laparotomy, intraperitoneal adhesions were found in 13.0% of the animals in group I and in 61.0% in group II (Table 2). No adhesions were found in group III. At the third laparotomy, the adhesions were present only in three animals in group II.

4. Discussion

Clearly, the immune system is involved in endometriosis. What is not clear, however, is whether and to what extent this
involvement is a primary response leading to the initiation, promotion, and progression of the disease or whether it is a secondary response to the ectopic endometrial growth in an attempt to restore homeostasis [20]. Because immunomodulation could be the key to finding more effective treatment for endometriosis, numerous studies have shown changes in the immune response in women with endometriosis; despite this, however, no effective remedy has been found [12]. For obvious reasons, many results come from animal studies. The rat model has been used by our group for endometriosis studies since 1988 [15]. The anatomic similarity of human and rat endometrium seems clear, although it is recognized that the type and duration of the cycles differ and the spontaneous luteal phase is absent in rats. This anatomic similarity is probably one of the factors that have enabled a high percentage of successful, surgical endometriosis inductions [10,11,15,16]. In our earlier experiments, the percentage of successful endometriosis induction reached 75–80%. This figure is in agreement with the results of present study. Because we have observed previously that there is no difference in endometriosis frequency according to the phase of the estrus cycle at the time of endometriosis induction, we did not obtain vaginal smears prior to the operations. We are conscious that the rat model is not “true” endometriosis and that there may be some differences between transplanted endometrium and endometrioma; however, this model seems to be the best, due to its simplicity and low costs.

After vaccination with RESAN, we obtained a reduction in endometriosis induction from 69.6% to 4.3%. If we take into account macroscopic assessment of endometriosis, the results would be very similar (91.3 vs. 21.7). Even in the best clinics, where the teams have to face endometriosis on a daily basis, there are some differences between the histological and macroscopic assessments of endometriosis, but these differences decrease with increasing experience. In some cases, however, histologists have real problems in arriving at the proper diagnosis. Sometimes specimen size is limited, and endometrial glands are not always present. The growing role of laparoscopy in the 1990s introduced new trends in which the diagnosis of peritoneal endometriosis could be made only with visual inspection. This fact leads us to ask, which data in our experiments are correct? Although we think the question is valid, the differences between groups were immense, according to both macroscopic and microscopic assessment. Vaccine producers have suggested that the antigens in their vaccines and in human endometrium and fibroids might be similar. This could explain, to some extent, the specific inhibition of endometriosis growth after implantation into the peritoneum. The question that remains, though, is why did we find such a big difference in adhesion formation between groups I and II? This is probably due to the time between vaccination and implantation allowing some self-defense mechanisms to be triggered, which caused quick rejection of the explants, thus avoiding adhesion formation. Sham operations were performed in 10 animals with the goal of observing the influence of laparotomy and nylon sutures attached to peritoneum on the formation of adhesions and/or lesions similar to endometriosis. It is reasonable to ask why we did not find any adhesions in this group. There are three possible explanations. First, in the sham operation group there were no previous vaccinations; thus, the results should be comparable to

<table>
<thead>
<tr>
<th>Assessment</th>
<th>Group I (n=23)</th>
<th>Group II (n=23)</th>
<th>Group III (n=10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macroscopic</td>
<td>3 13.0</td>
<td>14 61.0</td>
<td>0 0</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
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Table 2
Formation of the adhesions after endometriosis induction.
those for group II. Second, we did not leave endometrial fragments in the peritoneal cavity, as they represent a stimulus to adhesion formation. Finally, the operations in the sham group were shorter, because after uterine horn incision, no other time-consuming procedures were performed. Which element could be considered the most helpful in explaining the results obtained?

In our model, we decided to extend the time between vaccinations and the further steps to 3 months. We wanted to make the results more valuable. Ideally, vaccines should trigger long-term changes in immunity. Postponing the consecutive steps of the experiment, compared with other studies, enabled us to work on so-called established endometriosis.

We have found many differences between eutopic endometrium in women with and without endometriosis [21]. However, actually, we do not know which specific antigens differentiate endometrioma from eutopic endometrium. Thus, manufacturing a specific, anti-endometriotic vaccine seems to be impossible. However, we hope that RESAN triggered some nonspecific mechanisms of immunological surveillance, including natural killer cells and macrophages. We realize that immunotherapy concerned with the generation of a specific antibody response would have immense value in endometriosis treatment. Such a conclusion parallels those of cancer studies [22,23].

In summary, our results are similar to those obtained by Gul et al. [11] and Itil et al. [10], although our groups are slightly larger. Moreover, histological assessment seems to be better than photobiomicroscopy and sham operations allowed us to make the deduction process a little more meticulous.

The vaccine used by us is registered in Belarus and Mexico; however, one should consider whether it should be more properly characterized as an immunomodulator. There is a very close analogy to DETOX (detoxified Freud’s adjuvant), which is widely accepted in the literature as an immunological adjuvant. DETOX exerts both humoral and cellular immunity [24,25].

Certainly, we are conscious of the very serious limitations in transferring our laboratory results to humans, but we assume that step-by-step our understanding of endometriosis is improving. Presently, our group is working on an analysis of the immunological changes that take place after endometriosis induction and excision, as well as those due to RESAN administration.

References