

Assessment of Superoxide Dismutase and Catalase Evolution in Different Stages of an Experimental Endometriosis

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Endometriosis is described as a gynecological disorder characterized by the presence of endometrial tissue outside the uterus; extensively explored because of its increasing incidence, with an indubitable diagnostic only after invasive surgery, with no efficient treatment, it has still many aspects to be elucidated. A growing body of facts sustain oxidative stress as a crucial factor between the numerous incriminated factors implicated in endometriosis ethiopathogeny. Reactive oxygen species (ROS) act to decline reproductive function. Our study intends to determine if an experimental model of endometriosis may be useful to assess the impact of oxidative stress on endometrial cells; we have used a murine model of 18 adult Wistar female rats. A fragment from their left uterine horn was implanted in the abdominal wall. After 4 weeks, a laparotomy was performed, 5 endometrial implants were removed, followed by biochemical tissue assay of superoxide dismutase (SOD) and catalase (CAT). At the end of the experiment, the rats were sacrificed, the implants were removed for histopathological exam and biochemical assay of antioxidant enzymes. The results revealed decreased levels of antioxidant enzymes, pointing on significant oxidative stress involvement.

Keywords : endometriosis, oxidative stress, superoxide dismutase, catalase, animal model, implant

Although endometriosis is a benign gynecologic disorder, in recent years, due to its high increased incidence, controversial ethiopathogeny and the upset generated by the invasive exploratory diagnostic, it became a major health problem during fertile life of women [1]. Defined as the presence of endometrial tissue outside the uterine cavity, endometriosis is correlated with pelvic pain and infertility and the actual therapy can only ameliorate the symptoms in endometriosis, with no other benefits [2, 3].

The management of symptoms in endometriosis seems to be difficult given the fact that we can not specify with accuracy its exact pathogenetic mechanisms. Numerous hypothesis were proposed, but none of the theories offers a complete, coherent scientifically sustained and perfectly adapted approach [4]. In this context, a noticeable factor is represented by ROS. The role of ROS in decline of reproductive function is well determined; high ROS levels are usually associated with reduced fecundity. Endometrium is a very sensitive structure and the workflow of some distinct molecular sequences generated by estrogens transform it into a very vulnerable structure. The delicate balance between oxidants/ antioxidants if compromised, distinct events will lead to local alterations. ROS have a great potential to generate endometrial oxidative injuries, both stromal and glandular components being affected [5]. Apart from complex interplay between immune, genetic, endocrine, environmental factors, based on this finding, there is increasing evidence confirming the oxidative stress involvement in endometriosis [6].

The endometrium confronted with high ROS levels and an inefficient antioxidant defense, as main features of the oxidative stress, can follow a pathway leading to endometriosis; the mechanism through which oxidative stress interfere with endometriosis development is still unclear [7, 8]. Some data relate about a self-sustaining

oxidative stress in endometrium, where cyclic bleeding will generate the destruction of red blood cells; the release of hemoglobin will allow oxygen to bind to iron and to pass to another oxidative status. The ROS will be continuously generated and will aggress the stromal and glandular endometrial components; in time, this sustained attack will generate local injuries and will create the optimal environment for endometriosis [9, 10].

One of the most important problems raised by endometriosis approach is the fact that a certain diagnosis requires an invasive technique, the laparoscopy followed by histopathologic exam to confirm the endometriotic alterations. The invasive method is not always agreed by patients, so we have to turn to a diagnosis of exclusion. For this reason, we frequently assist to a delayed diagnosis, with all its repercussions on the outcome of endometriosis [11, 12].

We think that because of many limitations of endometriosis investigation, it may be suitable for an accurate and more facile study to use experimental models with laboratory animal on purpose to obtain, to evaluate and to explain new findings in regard to endometriosis. We can observe consecutive events during endometriosis initiation and progression, focusing on the oxidative stress involvement and exploring the susceptibility of endometrium to ROS aggression and the degree of similarity with human. We can extrapolate animal experimental data to human, trying to identify the main aspects of endometriosis [13-15].

The aim of our study is to use a murine endometriosis model to explore the role of oxidative stress in its pathophysiology, to assess if endometrium is susceptible to oxidative damage and to determine the antioxidant status during endometriosis development.

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Experimental part

Materials and methods

Animal experimental protocol

18 female rats from Wistar were used for this experiment, randomly divided in two groups: control group/endometriotic implant group (a fragment from left uterine horn was sectioned and implanted in the retroperitoneal space from the same female rat). After the post surgical recuperation, all the animals were completely recovered. After 4 weeks from the beginning of the experiment, a laparotomy was performed; 4 endometrial implants were removed to observe the macroscopic aspect, to perform histologic exam and also for biochemical exam of SOD, CAT. At the end of the experiment, all the rats were euthanized, samples of normal endometrium and ectopic implants were taken for biochemical exam of SOD and CAT. The experimental procedures were realized in accordance with the mandatory principles of the Ethical Committee of the Grigore T. Popa University of Medicine and Pharmacy Iasi [16, 17].

Biochemical analysis

Antioxidant enzymes were determined. SOD was determined based on method of Sun [18]. CAT was determined based on method of Aebi [19].

Statistical analysis

All values were expressed as mean \pm SD. To determine the significance of differences between groups, one way ANOVA was used. Differences were considered statistically significant when p value $p < 0.01$.

Results and discussions

The assay of SOD activity in control and endometriotic group revealed an initial decrease after 4 weeks of the surgical intervention, followed by a constant decrease of antioxidant enzyme in endometriotic group reported to control group, at the end of the experiment (fig.1).

The other antioxidant enzyme, CAT, presented a decreased activity at 4 weeks after initial surgery, the decrease being also present at the end of the experiment (fig. 2).

Endometriosis continues to be an estrogen-dependent disorder with a complex evolution and a pathophysiology

poorly understood. The symptoms and the stages of endometriosis can be described by clinicians, but the diagnosis is difficult and for an indubitable conclusion it has to be invasive; the current treatment is not effective and can determine only a variable symptoms relief. Infertility remains a major drawback. Although endometriosis is considered as a benign disease, a large number of studies promote its possible malignant transformation and also a close relation with an increased risk of ovarian cancer [20]. All these particular features individualize endometriosis and transform it into an unsolved medical problem [21].

The theory regarding the involvement of oxidative stress in endometriosis is gaining ground. Endometrium is a dynamic structure, with cyclic sequential changes, well determined in normal state. ROS exert a very strong influence on endometrial cells and also on endothelial cells, generating different cellular dysfunctions and controlling the possible malignant transformation of normal endometrium. When activated, ROS modulate signaling pathways in a very sensitive endometrium, leading frequently to carcinogenesis [22-24]. Numerous studies highlight the connection between oxidative stress, inflammation and angiogenesis in endometriosis [25].

The experimental autotransplantation of distinct uterine segments in the abdominal wall may reproduce endometriotic areas possessing well developed estrogen and progesterone receptors which are able to react to different types of stimuli. The endometriotic implant can be used to a better approach of the mechanisms involved in the sustained process of endometrial cells proliferation and specific alterations of glandular end stromal endometrial components [26, 27].

Our study intends to reveal the importance of animal models in deciphering the contribution of oxidative stress in endometriosis, which has to be considered a multifactorial disease; some opinions sustain that the oxidative stress can be observed in endometriosis only in incipient stages [28-33]. We have monitored the induced endometriosis evolution in female rats, assessing the activity of antioxidant enzymes SOD and CAT as representative scavengers of free radicals, in different stages of the endometriotic process. It is interesting to

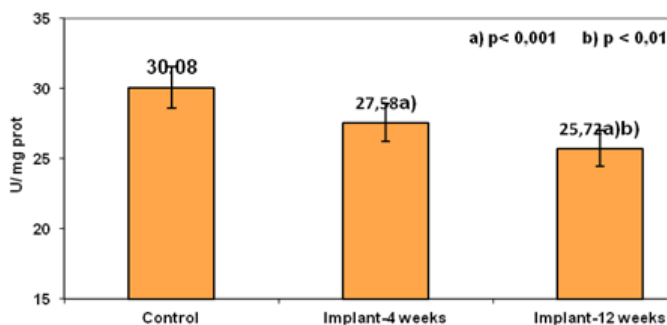


Fig. 1. SOD activity in experimental induced endometriosis

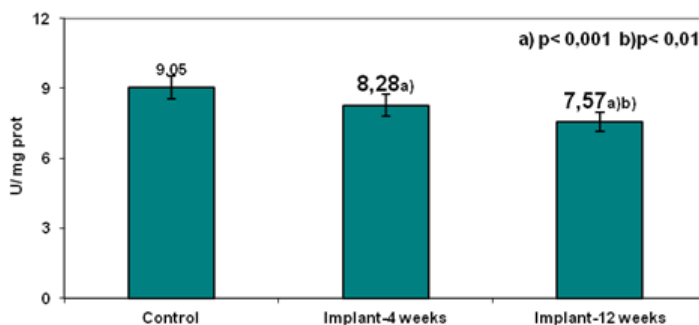


Fig. 2. CAT activity in experimental induced endometriosis

observe that SOD and catalase presented a continuous decrease. At 4 weeks after the beginning of the experiment, we examined the viability of endometrotic implant and we have the first biochimic assay of SOD and CAT. Both enzymes were decreased compared with control group, a more significant decrease was observed for SOD.

After 12 weeks when we have performed a second assay of the activity of SOD and CAT, in the context of an already induced endometriosis, antioxidant activity continued to decrease. These data support the fact that the antioxidant defense system, in our case SOD and CAT, has to comply with an oxidative aggression which is generated after the surgical implantation of the reseeded fragment of uterine horn.

During the endometriosis development, different types of processes may interfere with oxidative stress, inflammation being the most frequently invoked; the presence and the intensity of this oxidative pattern may be reflected in the amplitude of endometric injuries [34, 35].

Conclusions

The decrease of SOD and CAT in experimental induced endometriosis in female rats has to be correlated with the presence of a local oxidative stress.

The antioxidant enzymes followed a continuous decreasing trend during the experiment, so our findings sustain the presence of oxidative stress not only in the initial stages of endometriosis, but during the whole time of endometriosis.

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