Effect of 5-aminolevulinic acid (ALA) doses and oestrogen/progesterone on protoporphyrin IX (PpIX) accumulation in human endometrial epithelial cells

Butowska W[1], Warchoł W[2], Nowak-Markwitz E[3], Wołuń-Cholewa M[1]

[1]Department of Radiobiology and Cell Biology, [2]Department of Biophysic,
[3]Clinic of Gynaecological Oncology and Department of Perinatology and Gynaecology
University of Medical Sciences, Poznan, Poland

Abstract

Endometriosis represents one of the most frequent causes of restricted fecundity. Despite the progress in medicine, appropriate diagnosis and treatment pose significant problems. The aim of this study was to evaluate ALA-induced PpIX fluorescence of normal endometrial epithelial cells for the diagnosis of endometriosis. PpIX-fluorescence was measured after stimulation with estradiol-17 beta (E2) or with estradiol-17 beta (E2) and progesterone (P) and after incubation with ALA under a confocal microscope. The epithelial cells showed a significantly higher fluorescence of PpIX in the course of 24 and 48h incubation with hormones, than the cells without stimulation. After 72h, a significant decrease in cellular PpIX concentration was noted. The results suggested that E2 and P were required to convert ALA to PpIX in epithelial cells and increased PpIX concentration in a time-dependent fashion.

Key words: endometriosis, *in vitro*, protoporphyrin IX, 17ß-estradiol, progesterone.

Introduction

Endometriosis is widely encountered in women during the period of sexual maturity. It is appraised to affect 3-10 % women in the generative age but, among infertile women and in women with pain in the pelvis, the incidence is supposed to reach 20-90% [1]. In clinical practice, the only reliable way to diagnose endometriosis is to visualize its typical alterations in the course of laparoscopy or laparotomy and to confirm the changes by histopathology [2]. In recent years, a photodynamic technique has been introduced to clinical practice, both for diagnostic and therapeutic purposes. It uses fluorescent drugs that concentrate preferentially in tumours and other hyperproliferative tissues. At present, among substances, applied in the photodynamic approach, a particular attention is focused on 5-aminolevulinic acid (ALA), which is a physiological requirement for heme production in cells. One of the compounds, which arises during heme biosynthesis, is protoporphyrin IX (PpIX), used in photodynamic therapy (PDT) as a photosensitizer. Till now, only few studies have been performed on PpIX accumulation in presence of ALA in endometrial cells [3, 4]. The studies have shown that, when exposed to ALA, the cells are more effective in the accumulation of PpIX. In animal models, endometrial grafts to peritoneum have been shown to accumulate PpIX in such a way that they could be detected, using PDD and could be subjected to photodynamic therapy [5, 6]. Nevertheless, in none of the above studies have female sex hormone effects on the obtained results been considered. This may explain the divergences in the extent of PpIX accumulation in myometrium and endometrium. Still, the results have demonstrated the capacity of the endometrium to accumulate more PpIX, as compared to other tissues, and allow to suggest that the phenomenon can be used for diagnosis and treatment of endometriosis, when the hormonal condition of female body is taken into account. Considering the above data, recognition of PpIX accumulation in isolated cells of endometrial uterine epithelium, as affected by estrogen and progesterone, may be of practical, not just theoretical, significance for the definition of requirements of photodynamic therapy.

ADDRESS FOR CORRESPONDENCE:
Maria Wołuń-Cholewa
Department of Radiobiology and Cell Biology
University of Medical Sciences of Poznań
Święcickiego 6, 60-781 Poznań, Poland
e-mail: doskon@amp.edu.pl

Figure 1. Epithelial cells, isolated from normal endometrium (A) and preincubated with E2 for 48h and, then, incubated with 2 mmol/l ALA for 2 h (B).

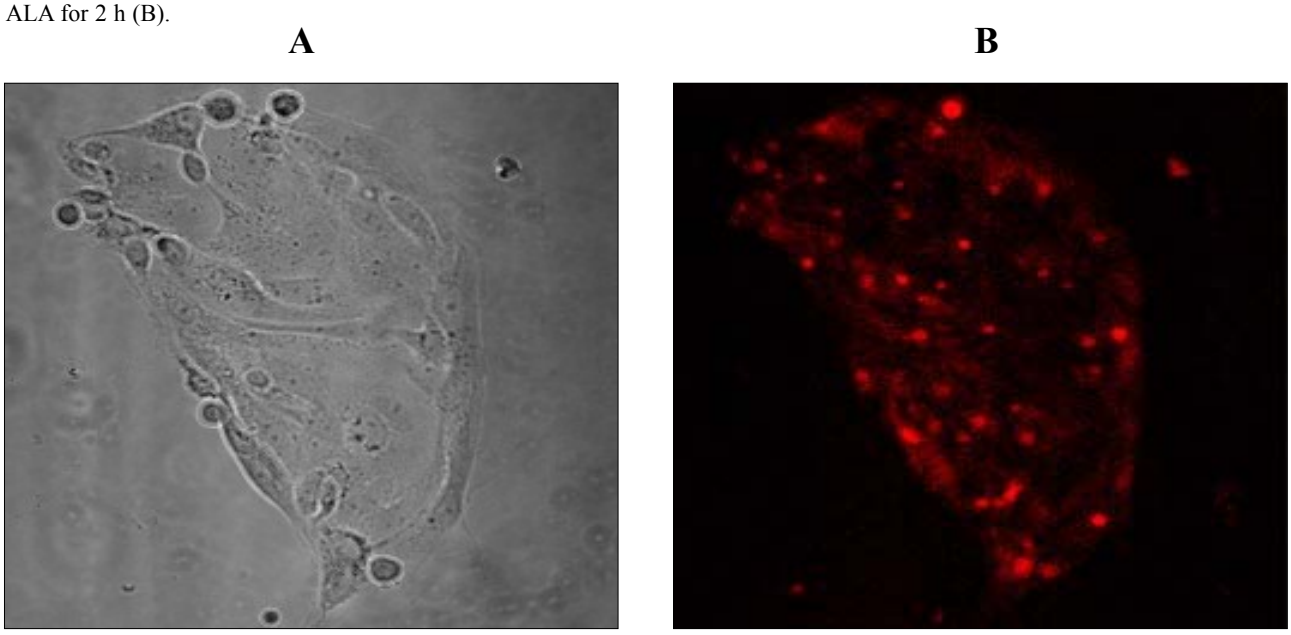


Figure 2. Alterations in the PpIX content in epithelial cells during incubation with E2 and ALA.

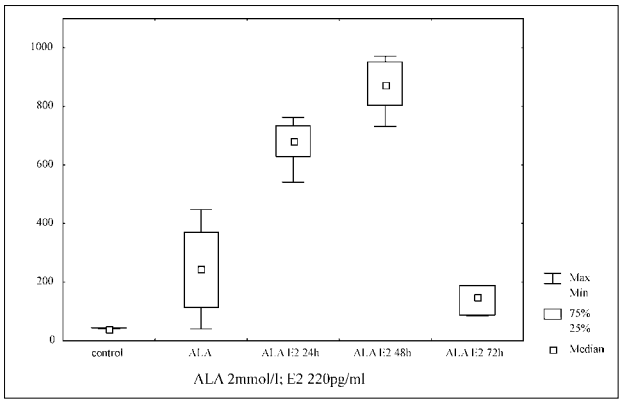
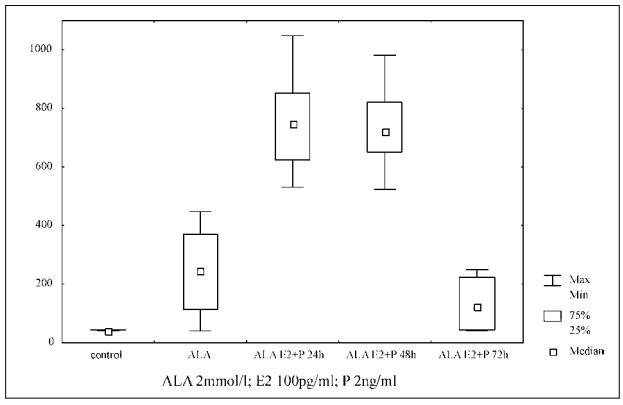


Figure 3. Alterations in the PpIX content in epithelial cells during incubation with E2/P and ALA.



Methods

The studies were performed on human primary endometrial epithelial cells, originating from normal uterine cavity. The cells were isolated and cultured as described by Ryan et al. [7]. The cultures of epithelium cells were conducted in presence of estradiol-17 beta (E2) and progesterone (P) in concentrations typical for the follicular stage (E2 alone, 220 pg/ml) or the luteal stage (E2 100 pg/ml and P 2 ng/ml) for a period of 24, 48 or 72h (the hormone doses were selected to correspond to their blood levels during normal menstrual cycles in women) [8]. The effect of 5-aminolevulinic acid (ALA) concentration on the accumulation of protoporphyrin IX (PpIX) in cells was defined in the cells, following their incubation with 2.0 mmol/l ALA for a period of 2h (ALA concentration and the duration of incubation were selected on the basis of published data and our preliminary results) [4, 9]. Following that time, PpIX fluorescence in the cells was evaluated, using a confocal microscope (LSM 510, Zeiss). The estimations took advantage of PpIX-exciting laser beam of 458 nm wavelength, while the emitted light was analysed, using a 585 nm filter. PpIX content was evaluated, using the CytFlu 1.2 software and expressed as a product of an average intensity of fluorescence and percentage surface bearing area over the level of background (%AIF). In each experiment, cells of the control group, incubated in the same way, but in the culture medium devoid of ALA, were evaluated. Statistical evaluation of the obtained results involved the nonparametric U-Mann Whitney's test. P value <0.05 was considered to represent threshold of significance.

Results and Conclusion

Results of PpIX-specific fluorescence estimation in a confocal microscope, following the incubation with ALA and E2, are presented in Fig. 1. Protoporphyrin IX content, following 2h incubation with 2 mmol/l ALA without steroid treatment, was 244 %AIF. Following the 24h/E2 treatment of the epithelial cells, the intensity of the PpIX-specific fluorescence significantly increased ($p<0.001$) and, then, after subsequent 48h, again slightly increased to end up in a significant

decrease ($p<0.001$) after 72h (Fig. 2). The maximal peak of PpIX fluorescence (873 %AIF.) was noted on the 48th hour of the experiment (Fig. 2). PpIX-specific fluorescence in cells of the control group did not significantly change in the course of the entire experiment and never exceeded the value of 41 %AIF. A separate cycle of experiments was devoted to alterations in the PpIX content in epithelial cells, which were preincubated with E2 and P for the 24, 48 and 72h, then, transferred to the medium with ALA. The obtained results are illustrated in Fig. 3. An augmented and similar content of PpIX was observed after 24h and 48h. A significant decrease ($p<0.001$) in the cellular PpIX content was noted after 72h from the steroid treatment. The results demonstrated that incubation in a steroid-containing media (E2, P) and ALA induced an accumulation of PpIX in epithelial cells. Moreover, the application of E2 for 48h resulted in a significant, three-fold increase in PpIX-related fluorescence. An evident decrease in fluorescence intensity was noted, following the 72h incubation with oestrogen. The observed alterations should be interpreted as a result of an equilibrium between the synthesis of PpIX in the epithelial cells and the removal of PpIX, due to the binding of the compound to iron, its transformation to heme or its efflux out of the cells [10]. In the case of E2 and P treatment, the maximum value of PpIX-fluorescence should, for certain time (between 24 and 48h), exhibit a plateau. Probably, the synthesis of PpIX will balance off its elimination. The decrease in PpIX content after 72h in the studied cells might have reflected either an efflux of the compound or an increased activity of ferrochelatase, which catalyses the binding of PpIX with iron. The data on PpIX accumulation in endometrial cells, as related to the presence of 17ß-estradiol or progesterone in the incubation medium, may provide indications as to the menstrual cycle phase(s) in which PDD and/or PDT for endometriosis treatment should be performed. It is concluded that, for the diagnosis and treatment of endometriosis, the hormonal condition of female body must be taken into account.

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