KATOLICKI UNIWERSYTET LUBELSKI JANA PAWŁA II KUL 1918

Endoprofem®

(JOHN PAUL II CATHOLIC UNIVERSITY)

Center for Commercialization of Science

REPORT

"Determination of the biological activity of ENDOPROFEM® in terms of steroid sulfatase enzyme inhibition"

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INTRODUCTION

Endometriosis is a chronic gynecological disease in which a tissue similar to the uterine lining, known as the endometrium, begins to grow outside the uterine cavity. This ectopic endometrial tissue may be present on the ovaries, fallopian tubes, outer surface of the uterus, and other organs in the pelvic area. Rarely, it can spread beyond the pelvic area. The disease affects approximately 10% of women of reproductive age worldwide, which is approximately 176 million women worldwide. Women who have a first-degree relative (mother, sister) with endometriosis are at higher risk, which indicates that there is a genetic predisposition to this condition. The main symptoms of endometriosis include chronic pelvic pain, painful periods, and pain during intercourse. Many women also experience heavy menstrual bleeding and fertility problems. Additionally, endometriosis can lead to fatigue, diarrhea, constipation, bloating, and nausea, especially during menstruation. The symptoms severity does not necessarily correlate with how advanced the disease is, which makes it difficult to diagnose. Being endometriosis a disorder undoubtedly related to hormonal balance, several molecular targets have been indicated that may play a significant role in its development. One of these targets is steroid sulfatase (STS, Uniprot: P08842). This enzyme catalyzes the conversion of sulfonated steroid precursors, such as dehydroepiandrosterone sulfate (DHEA-S) and estrone sulfate, into the free steroid, which interacts with hormone receptors and influences a number of biological processes, including regulation of sexual function, energy metabolism, and inflammation. This enzyme is important for hormonal wellbeing and may play a role in a variety of health disorders, including hormonal imbalances and endometriosis. The steroid sulfatase pathway provides a source of local estrogens within the endometrial lesions, thus promoting cell proliferation, inflammation, and associated symptoms of endometriosis, such as pain and infertility. By converting estrone sulfate to estrone, which can then be converted to the more active estrogen, estradiol, the STS supports the growth and maintenance of endometrial tissue. Therefore, it is crucial to search for new steroid sulfatase-inhibiting substances and preparations, which may help alleviate the symptoms of endometriosis.

References

Giudice, L. C. (2010), Clinical practice. Endometriosis, The New England Journal of Medicine, 362(25): 2389-2398.; Clemenza S. et al. (2022), Advances in targeting estrogen synthesis and receptors in patients with endometriosis, Expert Opin Investig Drugs, 31(11):1227-1238.

STUDY 1

– Determination of the effect of Endoprofem® on the activity of the steroid sulfatase (STS) enzyme isolated from the placenta

Method

In the reaction mixture of 100 µl the following were combined: STS enzyme, 3H-labeled estrone sulfate (STS substrate), unlabeled estrone sulfate (STS substrate), STS buffer and Endoprofem® at one of the following concentrations (0, 5, 10, 25, 50, 100, 250, 500, 1000, 2500 or 5000 µg/ml). The reaction was carried out for 1 hour at 37°C. The reaction was stopped by adding toluene, which extracted the reaction product – estrone. The STS activity was determined by measuring the amount of the reaction product using a radioluminometer. The IC_{50} value was determined using the GraphPad program.

Result

The inhibitory effect of Endoprofem® on STS activity was observed already from the lowest concentration (Figure 1) and the IC_{50} of Endoprofem® against STS was calculated (Figure 2), being 27.67 µg/ml.

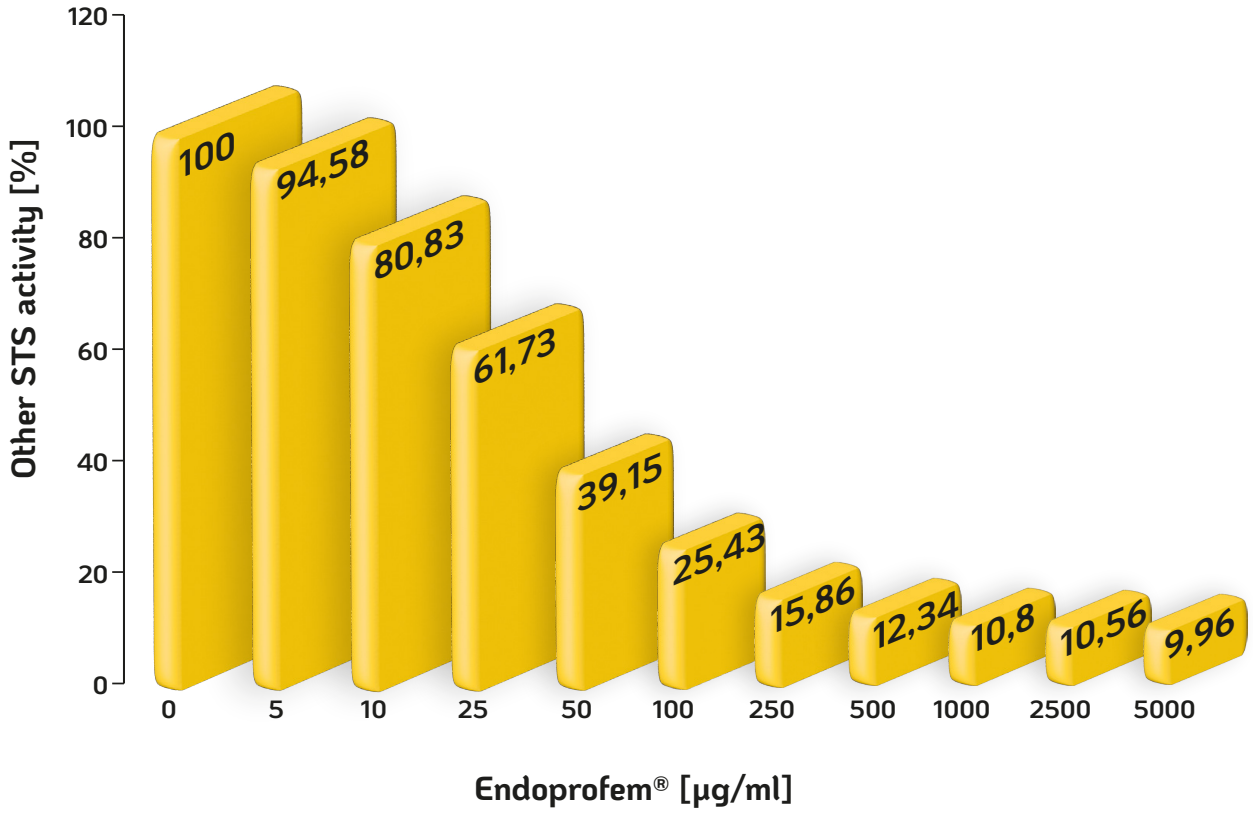

Chart 1. The effect of increasing Endoprofem® concentration on STS activity.

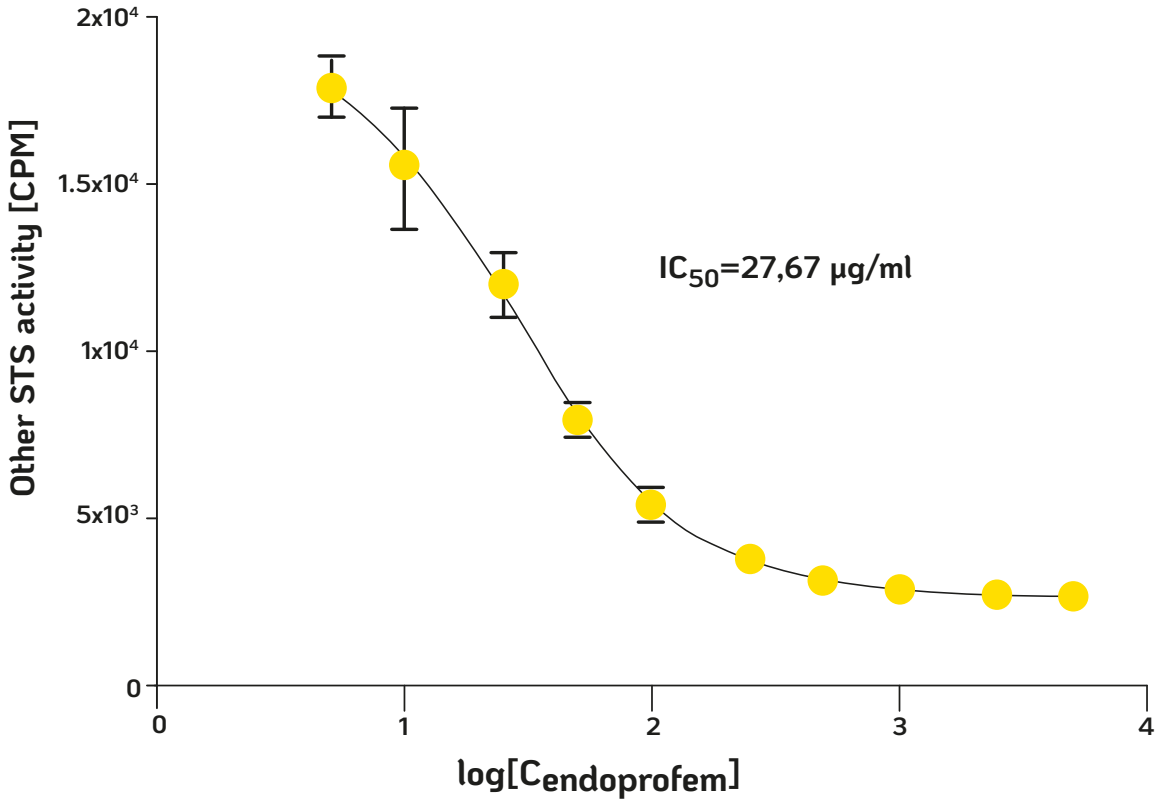

Chart 2. The effect of increasing Endoprofem® concentration on STS activity with determination of IC_{50} value.

STUDY 2

– Determination of the effect of Endoprofem® on steroid sulfatase (STS) activity in 12Z endometriosis cells

Method

In the reaction mixture of 200 µl the following were combined: 150,000 12Z cells (STS source), ^{3}H-labeled estrone sulfate (STS substrate), unlabeled estrone sulfate (STS substrate), DMEM medium and Endoprofem® at one of the following concentrations (0, 8, 16, 32, 63, 125, 250 or 500 µg/ml). It was not tested at higher concentrations due to its low solubility in the medium. The reaction was carried out for 20 hours in an incubator at 5% CO_2 at 37°C. The reaction was stopped by adding toluene, which extracted the reaction product – estrone. STS activity was determined by measuring the amount of the reaction product using a radioluminometer.

Result

A dose-dependent inhibitory effect of Endoprofem® on STS activity in 12Z endometriosis cells was observed and the IC_{50} of Endoprofem® against STS in 12Z cells was calculated (Figure 3 and 4) as **201 µg/ml.**

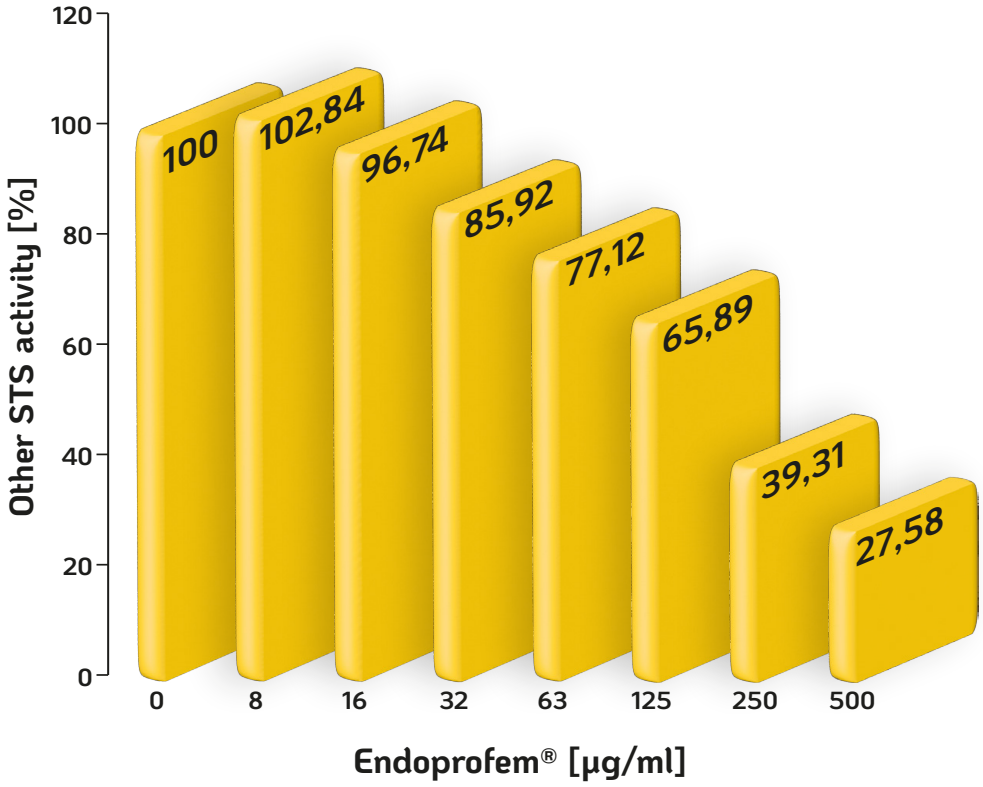

Chart 3. The effect of increasing Endoprofem® concentration in the medium on STS activity in 12Z cells.

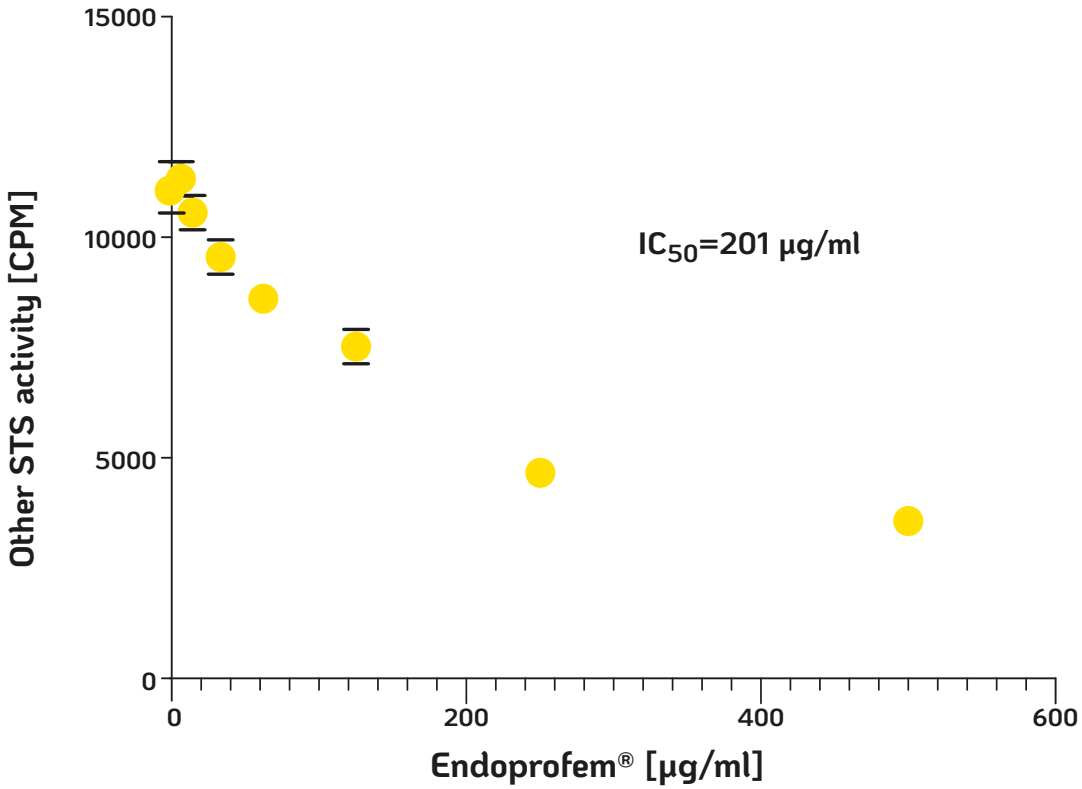

Chart 4. The effect of increasing Endoprofem® concentration on STS activity in 12Z cells with determination of IC_{50} value.

Conclusions

- Endoprofem® **inhibits** *in vitro* the activity of steroid sulfatase isolated from placenta, the IC_{50} value was 27.67 µg/ml.
- Endoprofem® **inhibits** steroid sulfatase in endometriosis 12Z cells, the IC_{50} value was 201 µg/m.

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