European Journal of Pharmaceutical Sciences 93 (2016) 132–140

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Effects of cisplatin and letrozole on surgically induced endometriosis and comparison of the two medications in a rat model

Zhanfei Li [a], Huibing Liu [b], Zhengxing He [a], Guorui Zhang [a], Jinghe Lang [a,*]

[a] Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Peking City 100005, China
[b] Department of Ultrasonography, Third affiliated hospital, Zhengzhou University, Zhengzhou City, Henan Province 450052, China

ARTICLE INFO

Article history:
Received 28 May 2016
Received in revised form 27 July 2016
Accepted 28 July 2016
Available online 29 July 2016

Chemical compounds studied in this article:
Cis-Diaminedichloroplatinum (PubChem CID: 2767)
Letrozole (PubChem CID: 3902)

Keywords:
Endometriosis
Cisplatin
Letrozole
Rat model
Medication

ABSTRACT

This study was to investigate the effects of cisplatin (CDDP) and letrozole on surgically induced endometriosis and comparison of the two drugs in a rat model. Endometriosis was surgically induced by autologous transplantation of endometrial pieces. Thirty model rats were divided into three groups, randomly. Group 1 (n = 10) served as control and received no medication. Group 2 (n = 10) received 0.2 mg/kg/day of oral letrozole. Group 3 (n = 10) received 35 mg/m^2 CDDP via peritoneal perfusion every four days. All the rats were treated for 24 days. The growth and histologic score of the implants were evaluated. The proliferation- and angiogenesis-associated proteins were assessed using immunohistochemistry and western blotting. The serum sex hormones were assayed using ELISA. After the medication, the growth and histologic score of the implants were significantly lower in the 2 and 3 groups than in the control group. The protein expressions of vascular endothelial growth factor (VEGF), aromatase P450 (P450arom), transforming growth factor-beta (TGF-β), and matrix metalloproteinase (MMP)-2, were significantly lower in groups 2 and 3 than in the control group. Further, the P450arom level was lower in the letrozole group than in the CDDP group. The TGF-β and MMP-2 levels were lower in the CDDP group than in the letrozole group. Serum T level was significantly higher in the letrozole group, and serum E_2 level was lower in the letrozole group. In conclusion, cisplatin and letrozole caused similar regression of the implants in the endometriosis model rats. But their effects on the proliferation- and angiogenesis-associated protein expressions and the serum sex hormone levels were different. Cisplatin and letrozole might cause the effects in the endometriotic foci through different mechanism.

1. Introduction

Endometriosis is defined as the development of the functional endometrial tissue outside of the uterine cavity, inducing pelvic cysts, tissue adhesion, infertility, and severe pelvic pain (Kennedy et al., 2005). It occurs primarily in reproductive age women and is rarely seen after menopause (Oner et al., 2010). Endometriosis is widely accepted as an estrogen-dependent condition. Aromatase P450 (P450arom) is the key enzyme in the estrogen production, catalyzing the conversion of androstenedione and testosterone to estrone and estradiol (Bulun et al., 2005). And aromatase's activity has been detected in the endometriotic tissue and in the eutopic endometrium of endometriosis patients (Meresman et al., 2005).

Letrozole is a third-generation aromatase inhibitor, suppressing local and systematical estrogen production (Oner et al., 2010). Its effects on endometriosis has been studied clinically and in animal experiments (Bilotas et al., 2010; Nawathe et al., 2008). Studies reported that letrozole caused obvious atrophy of the endometriotic tissues and decreased the area of the implants in the endometriosis model rats, suggesting its potential therapeutic value (Ceyhan et al., 2011; Oner et al., 2010; Yildirim et al., 2010).

In recent years, the association between endometriosis and cancer is a matter of growing concern (Wang et al., 2013). With the development of the endometriosis research, the features of endometriosis as a precursor lesion of ovarian cancer have been published in histological, biochemical, and epidemiological studies (Siufi Neto et al., 2014). Evidences have shown that endometriosis and ovarian cancer are associated at the molecular level (Meng et al., 2011). Genetic investigation even has reported gene mutations in the endometriotic lesions directly related to neoplasms (Siufi Neto et al., 2014).

Endometrioid adenocarcinoma and clear cell adenocarcinoma are the two well described pathological types resulting from the malignant transformation of endometriosis (Zhao et al., 2011). Cisplatin (*Cis*-Diaminedichloroplatinum, CDDP) is the preferred drug in the treatment of the mentioned cancers. CDDP's anti-neoplastic activity comes from its binding to DNA in target cells to induce DNA cross-links (Wang et al., 2011). Also, research found that, through a p53-associated pathway,

* Corresponding author at: Department of Obstetrics and Gynecology, Peking Union Medical College Hospital, Shuaifuyuan No.1, Dongcheng District, 100005 Peking City, China.
E-mail addresses: langjhpumch@163.com, langjh@hotmail.com (J. Lang).

http://dx.doi.org/10.1016/j.ejps.2016.07.018
0928-0987/

CDDP induces downregulation of parkin-like cytoplasmic protein (PARC) and apoptosis in the target cells, this is also believed to be involved in the anti-neoplastic mechanism (Woo et al., 2011). Based on these results, CDDP's effects on endometriosis need to be studied.

To date, no study was reported to demonstrate the effects of CDDP in an endometriosis rat model. Therefore, initially, the present study was to investigate CDDP's effects and to compare CDDP with letrozole on the regression and the protein expression of the endometriotic tissue in a rat model.

2. Materials and methods

2.1. Animals

Thirty female Sprague-Dawley rats at 8 weeks old, weighing 200–230 g, were provided by the Institute of Laboratory Animal Science, Chinese Academy of Medical Science. The Institutional Animal Care and Use Committee of the Institute of Laboratory Animal Science approved the study, and the research conformed to the institutional guidelines of Peking Union Medical College. All the rats were caged in pairs in a specific pathogen-free (SPF) facility, with a 12-h light/dark cycle and ad libitum access to food and water.

2.2. Experimental procedures and tissue collection

All animals were anesthetized through intraperitoneal administration of 3% pentobarbital sodium (Sigma, USA) at a dose of 35 mg/kg. All the rats underwent three consecutive surgical procedures.

2.2.1. Procedure 1

Vaginal smears were taken to observe the rats' estrous cycles, using Papanicolaou staining (Simsek et al., 2012). Endometriosis was induced surgically when the rat was in estrus as described by Korbel et al. with slight modifications (Korbel et al., 2010). Using aseptic technique, a ventral midline incision about 5 cm long was made to expose the reproductive organs. The left uterine horn was ligated and excised, the endometrial layer was separated and trimmed into a 5×5 mm^2 piece. Next, the trimmed endometrial piece was sutured to the inner side of the abdominal wall. Finally, the abdominal wall was closed.

2.2.2. Procedure 2

After the first endometriosis-inducing operation, all the rats were observed for 30 days without any medication. By the end of the period, all the 30 rats survived and then underwent a second exploratory laparotomy to observe the growth of the endometriotic implants. The surface sizes of the implants were recorded (length in mm × width in mm). The endometriotic tissues were photographed, then followed by the closure of the peritoneal cavity.

2.2.3. Procedure 3

All the animals were allowed to rest for three days after the second procedure. Then, the 30 rats were allocated randomly into three groups of 10 rats each. The mean sizes of the implants were similar between the three groups before the medication (Table 1). Next, the rats in group 1 ($n = 10$) served as control and received no medication. The rats in group 2 ($n = 10$) were given 0.2 mg/kg/day of oral letrozole. Letrozole tablets (2.5 mg, Femara, Novartis, Switzerland) were crushed and suspended in normal saline. The rats in group 3 ($n = 10$) were given 35 mg/m^2 CDDP via intraperitoneal injection every 4 days. The skin surface areas of the rats were calculated using the Meeh-Rubner equation $A = K \times (W^{2/3}/10{,}000)$, where A is the skin surface area (m^2), the K value is 9.1 for rats, and W is the body weight (g) (Spiers and Candas, 1984). CDDP powder (Sigma, Saint Louis, USA) was dissolved in normal saline. The medication period lasted for 24 days, which is equal to six estrous cycles of the rats. Four days after the final medication, the rats underwent the third laparotomy. The rats were anesthetized using 3% pentobarbital sodium (50 mg/kg). After the abdominal cavity was opened, the endometriotic implants were measured and photographed as previously described. Next, the blood samples were obtained from the abdominal aorta, followed by centrifugation (2000 r.p.m) to obtain serum samples, the serum samples were stored at −20 °C. Finally, the implants were excised and trimmed, a portion of each implant was fixed in formalin and then embedded in paraffin for histopathological examination and immunohistochemistry, the rest portion of each implant was first fixed in liquid nitrogen and then stored at −80 °C for further test.

Table 1
Treatment results and comparisons of the study groups.

Measures	Control	Letrozole	CDDP	*P*-value
Number of rats	10	10	10	
Mean surface area of implants (mm^2)				
Before medication	39.1 ± 6.85	37.5 ± 5.82	39.5 ± 3.24	>0.05
After medication	51.1 ± 6.89	18.3 ± 2.36	20.3 ± 3.19	<0.05
Mean histopathologic score of implants	2.6 ± 0.52	1.6 ± 0.51	1.5 ± 0.53	<0.05

Values are given as mean ± SD. CDDP: cisplatin.

2.3. Enzyme-linked immunosorbent assay

The serum levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), prolactin (PRL), testosterone (T), estradiol (E_2) and progesterone (P) were assayed using ELISA kits (R&D Systems, USA), following the manufacturer's instructions. Double replicates of each serum sample were detected in each assay. The lower detection limits were <1.0mIU/ml (LH), <0.1mIU/ml (FSH), <0.5 ng/ml (PRL), <0.1 ng/ml (T), <1.5 pg/ml (E_2), and <0.15 ng/ml (P), respectively.

2.4. Histopathological examination and immunohistochemistry

The implant specimens were sliced at 4-μm thickness. Hematoxylin and eosin (H-E) staining was used to evaluate the growth of the endometriotic tissues. In the immunohistochemistry (IHC) procedure, the slides were first deparaffinized and rehydrated, followed by incubation in a 3% H_2O_2 solution to deactivate endogenous peroxidase. After using normal goat serum to block nonspecific antigens, the slides were incubated with primary antibodies overnight at 4 °C. The primary antibodies included rabbit anti-rat VEGF, P450arom, TGF-β and MMP-2 polyclonal antibodies (1:100; Abcam, Cambridge, UK). Next, the slides were incubated with the secondary antibodies, followed by counterstaining with hematoxylin (ABC kit; Abcam, Cambridge, UK). For immunochemistry analysis, the pathologist evaluating the slides was blinded to all the groups. The slides were scored as described previously (Ceyhan et al., 2008). The percentage of positive stained cells was scored from 0 to 3 as follows: 0: <5%, 1: 5%–25%, 2: 26%–50%, and 3: >50%. The staining intensity of the cells was scored as follows: 0: negative, 1: weakly positive, 2: moderately positive, and 3: strongly positive. The IHC score for each rat was calculated by multiplying the two scores (staining intensity and percentage of positive stained cells).

2.5. Western blotting analyses

The implant samples were homogenized and lysed in a cold buffer solution. Tissue lysates were centrifuged at 13,000 rpm for 20 min at 4 °C. Protein expressions were tested using the BCA Protein Assay Kit. Protein lysate samples were separated on a 12% SDS-PAGE gel and were then transferred onto a nitrocellulose membrane. The membranes were incubated with primary antibodies overnight at 4 °C. The primary antibodies included rabbit anti-VEGF polyclonal antibody, anti-P450arom polyclonal antibody, anti-TGF-β polyclonal antibody and