Environmental Research 132 (2014) 328–333

Contents lists available at ScienceDirect

Environmental Research

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

Metal status in human endometrium: Relation to cigarette smoking and histological lesions

Piotr Rzymski [a,*], Paweł Rzymski [b], Katarzyna Tomczyk [b], Przemysław Niedzielski [c], Karol Jakubowski [c], Barbara Poniedziałek [a], Tomasz Opala [b]

[a] Department of Biology and Environmental Protection, Poznan University of Medical Sciences, Rokietnicka 8, 60-806 Poznań, Poland
[b] Department of Mother's and Child's Health, Gynecologic and Obstetrical University Hospital, Poznan University of Medical Sciences, Poznań, Poland
[c] Department of Analytical Chemistry, Faculty of Chemistry, Adam Mickiewicz University, Umultowska 89, 61-614 Poznań, Poland

ARTICLE INFO

Article history:
Received 15 January 2014
Received in revised form
7 April 2014
Accepted 9 April 2014

Keywords:
Human endometrium
Metal content
Metalloestrogens
Cigarette smoking

ABSTRACT

Human endometrium is a thick, blood vessel-rich, glandular tissue which undergoes cyclic changes and is potentially sensitive to the various endogenous and exogenous compounds supplied via the hematogenous route. As recently indicated, several metals including Cd, Pb, Cr and Ni represent an emerging class of potential metalloestrogens and can be implicated in alterations of the female reproductive system including endometriosis and cancer. In the present study, we investigated the content of five metals: Cd, Cr, Ni, Pb and Zn in 25 samples of human endometrium collected from Polish females undergoing diagnostic or therapeutic curettage of the uterine cavity. The overall mean metal concentration (analyzed using microwave induced plasma atomic emission spectrometry MIP-OES) decreased in the following order: Cr > Pb > Zn > Ni > Cd. For the first time it was demonstrated that cigarette smoking significantly increases the endometrial content of Cd and Pb. Concentration of these metals was also positively correlated with years of smoking and the number of smoked cigarettes. Tissue samples with recognized histologic lesions (simple hyperplasia, polyposis and atrophy) were characterized by a 2-fold higher Cd level. No relation between the age of the women and metal content was found. Our study shows that human endometrium can be a potential target of metal accumulation within the human body. Quantitative analyses of endometrial metal content could serve as an additional indicator of potential impairments of the menstrual cycle and fertility.

1. Introduction

Industrialization and urbanization increase the risk of daily exposure to a variety of chemical contaminants. One of the most serious health threats associated with environmental pollution is recognized in various toxic metals. Some of these elements can accumulate in biota (including human) and usually possess long half-lives (Järup, 2003; Rzymski et al., 2014; Liu et al., 2014). In humans, exposures to metals occur mainly through inhalation of polluted air, consumption of contaminated food and cigarette smoking. This is then followed by metal transport with blood in general circulation, partial secretion with urine and feces, and deposition within the human body in e.g. kidneys, liver, lungs as well as bones (Sumino et al., 1975; Brune et al., 1980; Minoia et al., 1990; D'Haese et al., 1999; Barregard et al., 2010; Miculescu et al., 2011). As shown in numerous studies, these elements can adversely alter the human female reproductive system. Several studies associated metals exposure with increased risk of infertility (Chang et al., 2006; Kim et al., 2013), pregnancy loss (Ajayi et al., 2012), endometrial (Akesson et al., 2008) and uterine cancers as well as endometriosis (Jackson et al., 2008).

The endometrium, the innermost glandular tissue of the uterine cavity, is a thick, glandular layer undergoing cyclic changes and periodic discharge. It is composed of two layers, a functional layer (shed during menstruation) and a basal layer that is attached to the myometrium. Due to a large number of blood vessels, it can be sensitive to different endogenous (e.g. hormones) and exogenous compounds supplied through the hematogenous route. At the same time the endometrium plays a significant role in preparation for implantation and serves as a location for development of the placenta (Ralph and Wynn, 1989). Prevailing disorders of the female reproductive system include endometrial cancer and endometriosis (the presence of endometriotic tissue outside the uterus) which underlines the potential susceptibility of this tissue to different environmental factors (Siegel et al., 2012; Nassif et al., 2013). Although toxic metals have received a great deal of attention in both toxicological and epidemiological studies and been implicated in a possible role in the development of endometriosis

* Corresponding author.
E-mail address: rzymskipiotr@ump.edu.pl (P. Rzymski).

http://dx.doi.org/10.1016/j.envres.2014.04.025
0013-9351/

and uterine myomas (e.g. through up-regulation of estrogen receptor-α) as well as alterations of reproductive function (Cunzhi et al., 2003; Silva et al., 2012a; Pollack et al., 2013), only little is known as to their status in endometrial tissue and the factors which can potentially influence it.

The present study was undertaken in order to study the content of one of the most abundant metals in the environment, Cd, Cr, Ni, Pb, and Zn in human endometrium. From these, Cd, Cr, Ni and Pb were found to potentially give rise to estrogen agonist responses (Stoica et al., 2000; Martin et al., 2003) whereas altered status of Zn has been associated with infertility and fetal loss (Graham et al., 1994; Dickerson et al., 2011). We also aimed to investigate the relations between endometrial metal content and histological image, thickness of the endometrium, female age and history of cigarette smoking. We believe this is the first although preliminary report to indicate that the endometrium can be one of the target tissues of systematic metal deposition, suggesting that it can be highly sensitive to the exposures of different metals resulting from a harmful lifestyle.

2. Material and methods

2.1. Study group

Samples of endometrium were obtained from 25 white Caucasian females undergoing diagnostic or therapeutic curettage of the uterine cavity under general anesthesia in the Gynecologic and Obstetrical University Hospital in Poznań, Poland. The causes of intervention were abnormal excessive bleeding, bleeding after menopause or hypertrophy of the endometrium estimated in ultrasound examination (Aloka SSD3500, Japan). All procedures were performed as a routine medical treatment. Endometrial samples were obtained using a surgical stainless steel curettage instrument. During this procedure time of contact of a curettage instrument and sampled tissue did not exceed 1 s. To maintain diagnostic quality, samples were divided using plastic instrument into $0.2 \times 0.2 \times 0.2$ mm pieces for metal content analyses (one sample per female due to limited tissue material) and the rest of the material was sent for routine pathological examination. The tissue samples utilized for metal analyses were first profusely flushed (to remove blood) and then immersed in sterile distilled water (Polpharma, Poland), and placed in cryogenic tubes (Nunc™, ThermoScientific, United States) and stored at -40 °C prior to determination. None of the studied individuals were exposed to occupational sources of metals. Based on a short questionnaire, the smoking habits of the investigated females were specified. The study was approved by the local bioethical committee of the Poznan University of Medical Sciences, Poznan, Poland and every patient undersigned the written consent.

2.2. Metal content analyses

Tissue samples were handled using plastic instruments with special care taken to avoid any contamination. Thawed tissues were dried in an oven at 40 °C, flushed twice with MilliQ water (Millipore, USA) to ensure the removal of blood remnants dried to a constant weight and then weighed in an electronic microscale (0.1 mg accuracy). Complete digestion was performed with suprapur 14 mol/L HNO_3 (Sigma-Aldrich, Germany) in sealed plastic tubes using an oven (80 °C). The concentration of metals (Cd, Cr, Ni, Pb and Zn) in the investigated samples was determined by a microwave induced nitrogen plasma atomic emission spectrometer (MP-AES by Varian, Australia) equipped with a nitrogen generator. The following wavelengths were applied: Cd – 228.802 nm, Cr – 425.433 nm, Ni – 232.0 nm, Pb – 405.781 nm and Zn – 213.857 nm. Each determination was performed in triplicate, values were averaged. The calibration was performed using multi-element standard analytical solutions (Merck, Germany). The limit of detection was 1.00 μg/kg for each analyzed metal. Prior to the analysis, the detection method was validated with reference material ERM-CE278K (mussel tissue). The recovery rate exceeded 90% for all determined elements, at low relative standard deviation (RSD) values (below 5%). Moreover, a control without any tissue but containing HNO_3 was performed in order to exclude the interference of any procedure step on metal content determination – all analyzed elements were below limits of detection. The final concentrations were given as μg metal per kg of dry tissue.

2.3. Statistical calculations

The results were analyzed using STATISTICA 10.0 software (StatSoft, U.S.A.). Gaussian distribution was tested with Shapiro-Wilk's test, and because most of the data did not meet this assumption, non-parametric methods were employed. To evaluate differences between two independent groups the Mann–Whitney *U* test was used. Relations between two datasets were determined with Spearman's rank correlation coefficient. $P < 0.05$ was considered as statistically significant.

3. Results

Characteristics of the study group are presented in Table 1. Cd, Cr, Ni, Zn were detected in all investigated endometrial tissues whereas Pb was found in only 22 samples (88%). The overall mean metal concentration decreased in the following order: Cr > Pb > Zn > Ni > Cd (Table 2). Statistically relevant and positive correlations between levels of the investigated metals were found with the strongest observed between Zn and Ni (Table 3).

There was no significant relationship between the age of the women and the content of any studied metal (Table 4). Moreover, female ≤ 40 years old did not differ in endometrial metal contents with female > 40 years old (Table 5).

A significantly greater endometrial content of Cd (difference in medians 4.4 μg/kg,) and Pb (difference in medians 15.5 μg/kg) was found in females with a smoking history (both current and former) than in those women who had never smoked. Moreover, the women who had formerly smoked were found to demonstrate significantly higher Cd (difference in medians 4.2 μg/kg) and Pb (difference in medians 18.4 μg/kg) content than those who had never smoked. There was, however, no statistical difference ($p > 0.05$) in metal content between current smokers and not current smokers (Fig. 1). Furthermore, significant positive correlations were found between both, time (years) of cigarette smoking and number of cigarettes smoked in the past per day, and the endometrial content of Cd and Pb. No significant associations with smoking habits were found for other metals (Table 4).

Endometrial tissue with recognized histological lesions was characterized by significantly higher Cd content ($p < 0.001$), increased 3-fold as compared to normal endometrium. However, no significant

Table 1
Characteristics of the study group.

Age (years) (mean ± SD[a])	45.0 ± 13.6
Endometrial thickness (mm) (mean ± SD[a])	10.5 ± 3.9
Histological image	
No lesions (*n*)	14
Lesions (*n*)	11
Endometrial simple hyperplasia (*n*)	4
Endometrial polyposis (*n*)	5
Atrophic endometrium (*n*)	2
Smoking history	
Non-smokers	12
Current smokes	3
Former smokers:	10
Years of smoking (mean ± SD[a])	12.4 ± 12.5
Smoking frequency range (cigarettes per day) (mean ± SD[a])	9.6 ± 7.4

[a] SD – standard deviation.

Table 2
Level of metals in human endometrial tissue (μg/kg dry tissue; *n*=25).

Metal	Median	Interquartile range
Cr	20.0	10.1–35.9
Cd	3.5	2.3–7.1
Ni	5.0	1.3–11.1
Pb	18.5	7.0–27.0
Zn	7.3	2.4–35.9