

Effects of environmental pollution with aromatic hydrocarbons on endocrine and metabolic functions of the human placenta

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Abstract

Introduction. Phenol and 1-hydroxypyrene are biological markers of exposure to polycyclic aromatic hydrocarbons (PAH) that have certain negative effects on parenchymal organs such as the human placenta. The literature presents only few reports regarding the effects of elevated PAH levels on the functions of the human placenta.

Objective. The aim of the work is to assess the effects of elevated PAH levels in excreted urine on the endocrine and metabolic functions of the human placenta obtained from a normal pregnancy.

Material and methods. Tissue material from 50 afterbirths from Płock constituted a study group, whereas 50 afterbirths from Kutno constituted a control group. Immunohistochemical reactions with the peroxidase method using LSAB kits (DAKO, Denmark) were performed. The extent and intensity of reactions were analysed. The levels of phenols and 1-hydroxypyrene in the excreted urine of pregnant women (undergoing delivery) were detected using gas chromatography and colorimetry. The statistical analysis used the PQStat v.1.6.2 software; moreover, t-student and chi-square tests were used. Differences were considered to be significant at the significance level of 95% ($p < 0.05$).

Results. The levels of phenol and 1-hydroxypyrene in the excreted urine were demonstrated to be statistically significantly higher in patients living in the area of Płock. Statistically lower expression of placental glutathione transferase and lower immunohistochemical demonstration of the placental phosphatase activity were observed in placentas from Płock. It has been demonstrated that the expression of the oestrogen receptor activity and placental gonadotropin is significantly higher in placentas from areas not contaminated with aromatic hydrocarbons (Kutno).

Conclusions. The course of pregnancy in the environment with elevated levels of aromatic hydrocarbons leads to impaired placental functioning and reduced endocrine and metabolic activity of the placenta.

Key words

Human placenta, Endocrine and Metabolic functions, Aromatic hydrocarbons, Phenol, 1-hydroxypiren

INTRODUCTION

Studies presented in this publication were a part of a large research project regarding the effects of petrochemical contamination on the health status of inhabitants of Płock. Human placenta has two major roles: it is an organ responsible for transfer of metabolites and it is a place where hormones and enzymes necessary for a growing foetus are produced or metabolised. For the foetus, it functions as lungs, gastrointestinal tract, kidneys and the whole system of endocrine glands, including liver [1, 2].

Placenta's own metabolism is extremely active. Its individual components, especially their functional performance, indicate foetal well-being. With regard to earlier studies the trophoblastic epithelium seems to be the most active component of the afterbirth. The placental connective tissue is a component that so far has been poorly studied. Few papers indicate it regulates placental functioning [3, 4, 5].

Aromatic hydrocarbons, especially phenol, are highly toxic to numerous cellular structures such as parenchymal organs (liver, lungs, placenta) and haematopoietic system [6, 7, 8]. *Liebhart and Pawiński* observed damage to the trophoblastic epithelium, its increased remodelling and renewal in human placentas from the area of Płock due to exposure to petrochemical industry in women with high urine excretion of phenol [9].

The placental barrier separating the maternal circulation from the foetal circulation reduces availability of chemical substances to the foetus to a relatively low extent.

As the levels of aromatic hydrocarbons in the air in the area around refineries and petrochemical plants are significantly higher it results in dangerous chronic intoxication that has an asymptomatic course and therefore only after certain period of time it is possible to observe numerous injuries to organs and tissues [10, 11].

Significantly elevated (significantly higher) levels of phenols and 1-hydroxypyrene in the excreted urine are biological markers of exposure (intoxication) to polycyclic aromatic hydrocarbons [12, 13, 14].

The effects of contamination with aromatic hydrocarbons on parenchymal organs may be assessed by studying and comparing afterbirths from normal pregnancies in the

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environment with increased phenol levels and afterbirths from the areas without contamination with such substances [15, 16, 17].

OBJECTIVES OF THE STUDY

The aim of the work is to assess the effects of elevated levels of aromatic hydrocarbons in the excreted urine on the endocrine and metabolic functions of the human placental tissue based on the evaluation of the extent and intensity of immunohistochemical colour-producing reactions with specific antibodies [18].

MATERIALS AND METHODS

The study material was collected from patients with a negative medical history whose pregnancies were in the environment exposed to elevated levels of aromatic hydrocarbons (AH) in the air (study group – Płock) and patients from the environment without AH contamination (control group – Kutno). A precondition for enrolling afterbirth into the study was uncomplicated physiological full-term pregnancy (39–41 week). All observed pregnancies resulted in a spontaneous labour and healthy children were born (Apgar score of 10). Prior to pregnancy patients had not been suffering from any chronic diseases, were not smokers and did not abuse alcohol or drugs. Patients in the control and study group did not differ significantly with regard to the age, parity and body weight. Children born as a result of these pregnancies did not differ statistically with regard to their birth weight. During pregnancy and delivery each patient had urine phenol levels assessed three times in order to obtain information on the total exposure to hydrocarbons as their urine metabolites are simple phenols. Phenol levels were assessed using three methods: HPLC (liquid chromatography), GC (gas chromatography) and a colorimetric method with 4-aminoantipyrine. Additionally, the urine levels of 1-hydroxypyrene were determined with gas chromatography as it is a biological marker of exposure to polycyclic aromatic hydrocarbons [13, 19].

Tissue material from 50 afterbirths from Płock constituted a study group, whereas 50 afterbirths from Kutno constituted a control group. In all cases two specimens were collected from the umbilical cord, one from the extraplacental foetal membranes and two or three specimens from the placenta itself. All 550 specimens obtained were processed in order to obtain paraffin-embedded blocks. Paraffin-embedded blocks were used to obtain slides for routine histopathological evaluation and then the most representative placental slides were selected for further immunohistochemical tests. Histopathological evaluation was performed after routine staining with hematoxylin and eosin. Preliminary results did not show significant histopathological differences in both groups of placentas, namely the study group from Płock and the control group from Kutno.

Then, immunohistochemical reactions in selected slides from 50 evaluated placentas (without specimens from the umbilical cord or foetal membranes) from the area with environmental contamination (Płock) and also in slides from 50 placentas from the control group (Kutno area) were performed. Results obtained – intensity and extent

of immunohistochemical reactions – were used as a basis to conduct a comparative analysis of expression of various reaction products indicating metabolic and endocrine functions. Immunohistochemical reactions were performed in order to detect differences with regard to maturity, metabolic performance and endocrine functioning in placentas in both assessed groups.

Immunohistochemical reactions were performed with the peroxidase method using LSAB kits (HRP Rabbit/Mouse – Universal LSAB 2 Kits by DAKO, Denmark). They are based on the sequential use of biotinylated antibody cross-linkers conjugated with streptavidin, with horseradish peroxidase. This method is extremely sensitive and low levels of primary antibodies can be detected.

Immunohistochemical reactions with the following antibodies were performed:

A. Indicating the endocrine activity of the placenta:

A1. Rb a Hu Chorionic Gonadotropin (hCG), glycoprotein, placental gonadotropin, placental beta fraction, synthesised in placental syncytiotrophoblast cells;

A2. Rb a Hu Placental lactogen (hPL), polypeptide hormone, somatomammotrophin, regarding its functions it is analogous to the growth hormone, its synthesis takes place in placental trophoblast cells;

A3. Mo o A Hu Estrogen Receptor, indicating the activity of trophoblastic cells involved in the production of the hormone, its expression indicates good performance of the foetal-placental unit;

B. Indicating the metabolic activity of the placenta:

B1. Mo a Hu Glutathione-S-Transferase Pi, known as Placental Glutathione-S-Transferase Pi, indicating Pi microsomal fraction of the placental cells, mainly epithelial cells;

B2. Mo a Hu Placental Alkaline Phosphatase, immunohistochemical demonstration of the activity of specific placental phosphatase, the best known enzyme involved in metabolic processes and active growth of the placental tissue.

Reactions were assessed with two methods. Firstly, they were assessed using an arbitrary 4-point scale: 0, 1, 2, 3, where 0 = no reaction, 3 = strong, even reaction.

Additionally, immunohistochemical reactions performed were assessed with regard to their extent and intensity using the computer system for morphometric assessment, namely Cell Soft Imaging System by Olympus; and it was possible by combining a light microscope with a digital camera (Olympus BX41, Olympus C3040) with a computer. Results of immunohistochemical tests were made more objective thanks to such an assessment. Differences were considered to be statistically significant at the significance level of 95% ($p < 0.05$).

Results obtained were subject to a statistical analysis with the PQStat v.1.6.1 Software. T-student and chi-square tests of independence were used.

RESULTS

The levels of phenol and 1-hydroxypyrene in the excreted urine were statistically significantly higher in patients living in the area of Płock (table 1).

Table 1. The urine levels of excreted phenol and 1-hydroxypyrene in pregnant women

| Study groups | Phenol met. Hplc [mg/l] (X+/-SD) | Phenol met. Gc [mg/l] (X+/-SD) | Phenol met. with 4-aminoantipyrene [mg/l] (X+/-SD) | 1-hydroxypyrene [mg/l] (X+/-SD) |
|----------------------------------------|----------------------------------|--------------------------------|----------------------------------------------------|---------------------------------|
| KUTNO n=50 | 2.025+/-0.767 | 2.044+/-0,754 | 5.059+/-0,788 | 0.225+/-0.169 |
| PLOCK n=50 | 4.419+/-2,484 | 4.455+/-2.624 | 8.735+/-2,835 | 0.519+/-0.366 |
| T-student test for independent samples | t=6.5115; k=98 p<0.001 | t=6.2443; k=98 p<0.001 | t=8.8337; k=98 p<0.001 | t=5.156831; k=98 p<0.001 |

Dimensions of placentas from the Plock group were greater, on average, compared to the Kutno group, but this difference was not statistically significant ($p=0.064$).

The analysis of the endocrine functioning of the placenta based on the extent and intensity of reactions showed differences between studied populations.

The assay for placental gonadotropin (Rb a Hu Chorionic Gonadotropin HCG antibodies) that is synthesised by the syncytiotrophoblast cells showed statistically significantly higher activity of placentas in the Kutno group compared to the Plock group ($p<0.01$) (tables 2,3).

Table 2. The intensity of reaction of placental gonadotropin in syncytiotrophoblast cells

| Groups | The intensity of the reaction | | | |
|---------------------------------|--------------------------------------------------------------------|----------------|---------------|-----------------|
| | No reaction | Trace reaction | Poor reaction | Strong reaction |
| Study group (n=50) | 0 (0%) | 10 (20%) | 15 (30%) | 25 (50%) |
| Control group (n=50) | 3 (15%) | 21 (42%) | 16 (32%) | 10 (20%) |
| Chi-square test of independence | Chi-square statistics=13.364; Degrees of freedom=3; p-value=0.0039 | | | |

Table 3. The extent of moderate and high colour-producing reactions of placental gonadotropin in syncytiotrophoblast cells

| Groups | Number (n) | Average area (X+/-SD) | |
|----------------------------------------|------------|--------------------------------|--------------------------------|
| | | [μm^2] | [%] |
| Study group (Plock) | 50 | 28913.15+/-21548.27 | 20.10+/-14.98 |
| Control group (Kutno) | 50 | 20171.51+/-13502.21 | 14.02+/-9.39 |
| T-student test for independent samples | | t=2,430789; k=98 p=0,016884 | t=2,431021; k=98 p=0,016874 |

For all reactions described below, calculation was performed as in Tables 2 and 3. These tables are not located in the text, due to the transparency of the publication. Describes only the results.

The expression of oestrogen receptors (detected by Mo o A Hu Estrogen Receptor ER antibodies) that indicate the activity of trophoblast cells involved in the production of this hormone showed significantly higher activity in the placentas from the Kutno group ($p<0.01$).

An analogous assay for the activity of placental somatomammotrophin (hCS) (Rb a Hu Placental Lactogen HPL antibodies), a polypeptide hormone previously known as placental lactogen (hPL), synthesised by trophoblast

cells showed higher average values in the Plock population; however, they were not statistically significant ($p>0.05$).

Significantly higher expression of the oestrogen receptor activity and placental gonadotropin indicates that the foetal-placental unit shows better performance in placentas from the area not contaminated with aromatic hydrocarbons (Kutno).

The analysis of the metabolic activity of placentas indicated by the expression of placental glutathione transferase (Mo a Hu Glutathione-S-Transferase Pi antibodies) that indicates the Pi microsomal fraction of epithelial cells in the placental tissue showed significantly lower activity in the placentas from the Plock group ($p<0.01$).

Similarly, immunohistochemical demonstration of the activity of placental phosphatase (Mo a Hu Placental Alkaline Phosphatase PhA antibodies), an enzyme involved in metabolic processes and active growth of the placental tissue was significantly higher in the control group ($p<0.01$).

Statistically lower expression of placental glutathione transferase and lower immunohistochemical demonstration of the placental phosphatase activity indicate reduced metabolism and impaired placental functioning in the area contaminated with aromatic hydrocarbons.

DISCUSSION

Every year, the petrochemical plant in Plock emits more than 100,000 tonnes of gas substances into the atmosphere, including sulphur and nitrogen compounds, benzopyrene, hydroxypyrene and phenol. Despite the fact that in the study period and area Polish air contamination standards were not exceeded, it was unequivocally stated that the urine levels of excreted phenol (measured with three methods) and 1-hydroxypyrene (one method) in pregnant women were statistically significantly higher compared to these levels in pregnant women from the Kutno area. 1-hydroxypyrene is a biological marker of exposure to polycyclic aromatic hydrocarbons [13, 16, 19].

It means that the Plock area is contaminated with chemical substances from the industry mentioned above, and this contamination mainly includes hydrocarbons, and additionally, all tested pregnant women were significantly affected by these substances. Papers by other authors [20, 21, 22, 23] confirm high urine levels of phenol in pregnant women living in the areas contaminated by refinery and petrochemical plants. Papers quoted showed a correlation between high levels of excreted phenol with abnormal manifestation of the afterbirth. Analyses of macroscopic, microscopic and histochemical changes were performed by such scientists as *Liebhart, Pawiński, Radowski and Wierzba* in the 1970s, 1980s and 1990s. These studies demonstrated changes in the morphology and metabolism of afterbirths such as microinfarctions and excessive fibrin deposition in the intervillous space [20, 22, 23]. *Lin, Li and Mao* observed lower birth weight of newborns born by mothers living near petrochemical plants in Taiwan [24].

In this paper authors decided to use modern immunohistochemical methods to determine whether there are changes (differences) at the level of expression of products of reactions of antigens indicating individual placental functions in afterbirths from normal pregnancies.

In order to do that a patient group was selected so as the only "pathology" was elevated concentration of phenol and 1-hydroxypyrene in the excreted urine, and there were no other differences between studied groups.

The study material was collected from patients with a negative medical history whose pregnancies were in the environment exposed to elevated levels of aromatic hydrocarbons (AH) in the air (study group – Płock) and patients from the environment without AH contamination (control group – Kutno). A precondition for enrolling afterbirth into the study was uncomplicated physiological full-term pregnancy (39–41 week). All observed pregnancies resulted in a spontaneous labour and healthy children were born (Apgar score of 10). Prior to pregnancy patients had not been suffering from any chronic diseases, were not smokers and did not abuse alcohol or drugs. Patients in the control and study group did not differ significantly with regard to the age, parity and body weight. Children born as a result of these pregnancies did not differ statistically with regard to their birth weight.

It means that the biological quality of children born in both cities is similar.

The study demonstrated that the endocrine activity of the placenta evaluated based on the activity of placental gonadotropin, expression of oestrogen receptor and activity of placental lactogen are different in these studied populations. Significantly higher expression of the oestrogen receptor activity and placental gonadotropin indicates that the foetal-placental unit shows better performance in placentas from the area not contaminated with aromatic hydrocarbons (Kutno).

Zamorska showed increased production of steroid hormones (mainly oestron and beta-oestradiol) when the activity of hydroxysteroid dehydrogenases was reduced as a compensatory phenomenon allowing to stabilise pregnancy exposed to contamination [25]. On the other hand, *Radowicki* observed reduced activity of 3-beta-hydroxysteroid dehydrogenase in placentas exposed to high levels of phenol in the excreted urine [26].

Lower metabolic activity of placentas in the study group assessed by the expression of placental glutathione transferase and placental phosphatase indicates reduced metabolism and impaired placental functioning in the area contaminated with aromatic hydrocarbons.

Other researchers studying placental metabolism also observed lower activity of lactate dehydrogenase in the trophoblast of terminal villi, more frequent incidence of microinfarctions, excessive fibrin deposition in the intervillous space and more frequent growth of the extraplacental membrane epithelium in the group of afterbirths exposed to aromatic hydrocarbons [21].

Moreover, *Radowicki* observed numerous calcium deposits on the maternal surface of placentas as evidence for impaired metabolism [27].

Wierzba, on the other hand, has shown that, the foetus is protected by activation of adaptation and compensation mechanisms that are manifested as active angiogenesis and formation of new blood vessels that provide proper nutrition and oxygen supply to the foetus and significantly greater development of mesenchymal cells compared to the control group [28].

CONCLUSIONS

1. Pregnancies in Płock are statistically significantly more exposed to aromatic hydrocarbons, especially phenol and 1-hydroxypyrene, compared to those in Kutno.
2. The endocrine activity of the placental tissue obtained after delivery from women with elevated levels of phenol and 1-hydroxypyrene in the excreted urine (Płock) is statistically significantly lower compared to patients who are not exposed to aromatic hydrocarbons (Kutno).
3. The metabolic activity of the placental tissue obtained after delivery from women with elevated levels of phenol and 1-hydroxypyrene in the excreted urine (Płock) is statistically significantly lower compared to patients who are not exposed to aromatic hydrocarbons (Kutno).

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