Functional and morphological changes in the mother-placenta-fetus system during chronic hypoxia (experimental study)

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ABSTRACT

The aim of work was to study the characteristics of uteroplacental blood flow in pregnant rats, tissue oxygenation and the morphological features of the main internal organs (heart, liver, kidneys) of newborns during chronic intrauterine hypoxia. The rats (8 animals) were divided into 2 experimental groups. The first (hypoxia) group was subjected to hypoxia throughout pregnancy (21 days). The second (control) group was not exposed to any effect throughout the pregnancy. On the 20th day of gestation in both groups, a Doppler ultrasound study of uterine-fetal blood flow and measurement of the placenta was performed. Newborn rat pups of the first day of life were subjected to oxygenation degree measurement. After delivery, the number of rats in the litter was counted and their body weight was measured. After the measurements the laboratory animals were withdrawn from the experiments and morphological study of their internal organs was performed. The studies have shown a clear relationship between the characteristics of uterine blood flow and the presence or absence of hypoxia. In chronic hypoxia, there were low rates of arterial blood flow, an increase in the peripheral resistance of uterine vessels. All this indicates the defective formation of the placenta and impaired blood flow with the development of placental insufficiency. It was also found that hypoxia in the antenatal period adversely affects the number and somatometric indicators of newborn rats in the offspring. Histological examination of the tissues of the heart, liver and kidney of newborn rat pups showed signs of impaired blood circulation, dystrophic and necrobiotic changes in the parenchyma.

Keywords: chronic hypoxia, newborn morphology, placental insufficiency, ultrasound, oxygen saturation

1. INTRODUCTION

Fetal hypoxia is a frequent complication of pregnancy and childbirth. The increase in the number of children born with hypoxia is observed due to the growth of extragenital and obstetric pathology in pregnant women, on the background of which placental insufficiency develops and, as a result, intrauterine growth retardation. Transferred prenatal hypoxia and impaired blood circulation are the main background state of development in a child in subsequent neurological and somatic pathology.1,2 Thus, placental insufficiency (PI) is the most important problem of modern obstetrics inasmuch as in children with a history of chronic intrauterine hypoxia (CIH), physical developmental delay, morphological and functional damage of various organs and systems are observed.1,3

In clinical practice, the methods of intravitational fetal blood circulation are frequently implemented using Doppler sonophoresis. Doppler ultrasound provides a non-invasive method for the study of fetal hemodynamics. A study of the uterine and umbilical arteries provides information on perfusion of the uteroplacental and fetoplacental circulation.4 Previously, a study on the effect of chronic hypoxia on the reproductive system of newborns was performed.5 As a result of the research, it was found that hypoxia in the antenatal period adversely affects the number and somatometric parameters of newborn rats in the offspring. Histological examination of testicular tissue showed a significant decrease in the number of tubules in the visual field, a decrease in the diameter and area of the tubules with a simultaneous increase in the stroma area, a decrease in proliferative potential and an increase in apoptosis of gonocytes, Leydig and Sertoli cells in rats of the studied group.5
The aim of this study was to evaluate the characteristics of uteroplacental blood flow in pregnant rats, tissue oxygenation and morphological features of the main internal organs (heart, liver, kidneys) of newborns during chronic intrauterine hypoxia.

2. MATERIALS AND METHODS

2.1 Animals

The experiments were performed with 8 healthy white outbred adult rats-females weighing 180-260 g, obtained from the vivarium of the Saratov State Medical University n.a. V.I. Razumovsky. Animals received a standard diet once a day, with free access to water. Laboratory rats were divided into 2 groups. The first (experimental) group (5 animals) was subjected to hypoxia throughout pregnancy (21 days). The timing of pregnancy began with the discovery of spermatozoa in the vaginal smear of a rat. The modeling of hypoxia was carried out in accordance with the method of N.N. Karkishchenko (2010). The second (control) group (3 animals) was not exposed to any effect throughout pregnancy.

All procedures with animals were done in accordance with the guidelines for experimental (preclinical) study of new pharmacological substances (R.Yu. Khabriev, 2005), the “Guide for laboratory animals and alternative models in biomedical technologies”, the Geneva Convention on "International principles of biomedical research using animals" (1985), and the Helsinki Declaration (2000) on the humane treatment of animals.

2.2 Ultrasound procedure

On the 20th day of gestation in both groups, a Doppler ultrasound study of the uterine-fetal blood flow and measurement of the placenta was performed. The uterus transabdominal examination was performed using real-time Voluson E8 ultrasound system (GE Healthcare, US) with a high-frequency 10 MHz linear transducer. During the studies, the peak systolic velocity of the arterial blood flow (PSV), final diastolic velocity (EDV), resistance index (IR), systolic-diastolic ratio (SD), and placenta thickness were calculated.

2.3 Oxygenation measurement

Newborn rat pups of the first day of life were subjected to oxygen saturation measurement. The experimental studies were performed using a USB4000-Vis-NIR multichannel optical spectrometer with a QR400-7-Vis/NIR fiber-optic probe (Ocean Optics, USA) in the spectral range of 400–1000 nm at room temperature. The fiber-optic probe included six illumination fibers around one receiving fiber. For provide of uniform illumination of detection area the probe was placed above skin surface on the distance 2 mm. Five spectra were measured for each animal. A halogen lamp (HL-2000, Ocean Optics, USA) was used as a light source. The spectrometer was calibrated using a WS-1-SL (Labsphere, USA) reflectance standard with a smooth surface.

To exclude the influence of baseline scattering and changes thereof on the spectra, all obtained were corrected for baseline in the following fashion:

\[ D_{\text{corr}}(\lambda) = D(\lambda) - (a + m\lambda) \]

where \( D(\lambda) = -\log R(\lambda) \) is the apparent optical density measured in course of the experiments, \( R(\lambda) \) is reflectance, \( \lambda \) is wavelength, \( D_{\text{corr}}(\lambda) \) is the apparent optical density corrected for the underlying scattering, \( m \) is the slope of the least squares fitted line to the data between 640 and 820 nm, and \( a \) is the intercept of this regression.

For estimation of the oxygen saturation, four different algorithms presented in details in the Refs. [8-12, 14, 15] was used. Here \( [\text{HbO}_2] \) and \( [\text{Hb}] \) are the concentrations of oxy- and deoxyhemoglobin, respectively.

Method 1 [10]:

\[ \text{SatO}_2 = \alpha \left( \frac{D_{270} - D_{557} - D_{537} - D_{445}}{13} \right) \frac{1}{H + \beta} \]

where \( \alpha = 31 \), \( \beta = 1 \), and \( H = \frac{D_{445} - D_{530}}{16} - \frac{D_{530} - D_{445}}{25} \) is the hemoglobin index. Subscripts show corresponding wavelengths.
Method 2 [8, 11]: 
\[
\text{SatO}_2 = \frac{[\text{HbO}_2]}{[\text{HbO}_2]+[\text{Hb}]}; \quad [\text{HbO}_2] = \frac{D_{574}^{553} - D_{553}^{553} \times K_1}{K_2}; \quad [\text{Hb}] = \frac{D_{553}^{553} \times \varepsilon_{\text{oxy}}^{553}}{\varepsilon_{\text{deoxy}}^{553}}; \quad K_1 = \frac{\varepsilon_{\text{deoxy}}^{574}}{\varepsilon_{\text{deoxy}}^{553}}. 
\]

Superscripts show corresponding wavelengths. \( \varepsilon_{\text{oxy}}^{\lambda} \) and \( \varepsilon_{\text{deoxy}}^{\lambda} \) are the molar extinction coefficients for oxy- and deoxyhemoglobin, respectively, at wavelength \( \lambda \).

Method 3 [12]: 
\[
\text{SatO}_2 = \frac{\mu_{\text{Hb}}(\lambda_1) - \mu_{\text{Hb}}(\lambda_2)}{\mu_{\text{Hb}}(\lambda_1) + \mu_{\text{HbO}_2}(\lambda_1)} \times R(\lambda), \quad \text{where } R(\lambda) \text{ is the measured reflectance at the chosen wavelength}; \quad \lambda_1 = 560 \text{ nm}, \ \lambda_2 = 545 \text{ nm}. \quad \mu_{\text{Hb}} \text{ and } \mu_{\text{HbO}_2} \text{ are the absorption coefficients of deoxygenated and oxygen-saturated blood, respectively [13].}
\]

Method 4 [8, 14, 15]: 
\[
\text{SatO}_2 = \frac{(D_{577} - D_{586}) - 9}{17} \times \frac{(D_{569} - D_{586})}{1.49(D_{569} - D_{586})} \times 100\%. \quad \text{Subscripts show corresponding wavelengths.}
\]

2.4 Morphological study

After delivery, the number of rats in the litter was counted and their body weight was measured. After withdrawn of newborn animals from experiments, an autopsy study was performed and attention was paid to the general appearance of the organs, their shape, appearance on the incision, and blood filling. For morphological studies, samples of the internal organs were fixed in buffered 10% formalin.

After standard histological passage in alcohols with increasing concentration, the biomaterial was embedded in paraffin. Serial sections of 4-5 \( \mu \)m thick were prepared on a rotary microtome and stained with hematoxylin and eosin.

In the statistical analysis of the results, the computer software packages IBM SPSS Statistics 24.0 and Microsoft Office Excel 2007 were used.

It was found that the distribution of the studied parameters in the sample sets is different from the normal one, therefore, for comparative analysis, non-parametric statistics was used with the calculation of the median, interquartile range, confidence levels of differences between groups according to the Mann-Whitney test. Differences were considered statistically significant at \( p < 0.05 \).

3. RESULTS AND DISCUSSION

3.1 Ultrasound procedure

When examining the placenta in the experimental group, heterogeneity of its structure with echo-negative sites was observed, its thickness was 0.28 cm. It was also noted that in the experimental group 1 empty fetal egg was found. In the control group, the placenta was homogenous, dense, the thickness was 0.41 cm.

When measuring the blood flow, an increase in peripheral resistance indices (IR and S/D) in the experimental group (Me RI - 0.575; Me S/D - 2.36, Table 1) almost 2 folds compared with the control group were registered. This indicates a decrease in blood flow and an increase in peripheral resistance of uterine vessels.

Another feature of uterine perfusion in the experimental group was a decrease in the absolute values of the arterial blood flow velocity (PCV and EDV) compared with the control group (Table 1). Such changes in the parameters of uteroplacental circulation indicate a decrease in uteroplacental perfusion in pregnant rats.
Figure 1. Indicators of blood flow in the uterine artery. A-control group; B-hypoxia.

Figure 2. Fetal eggs in the uterus. A - 3 gestational eggs in sight in the control group; B - empty gestational egg in the experimental group.

Figure 3. Thickness of the placenta. A - control group; B - experienced group.
According to the results of the non-parametric test Mann-Whitney, significant differences were found in the experimental and control groups in all parameters (Table 1).

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Experimental group (median, percentile)</th>
<th>Control group (median, percentile)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCV</td>
<td>[12.43; 11.185; 13.185] *</td>
<td>[18.36; 18.15; 19.74] *</td>
</tr>
<tr>
<td>EDV</td>
<td>[5.22; 4.79; 5.61] *</td>
<td>[11.5; 11.16; 12.165] *</td>
</tr>
<tr>
<td>RI</td>
<td>[0.575; 0.555; 0.6] *</td>
<td>[0.38; 0.38; 0.39] *</td>
</tr>
<tr>
<td>S/D</td>
<td>[2.36; 2.24; 2.5] *</td>
<td>[1.62; 1.61; 1.64] *</td>
</tr>
<tr>
<td>D, thickness of the placenta, cm</td>
<td>0.28</td>
<td>0.41</td>
</tr>
</tbody>
</table>

Note: the differences are significant: * - p <0.05

3.2 Oxygenation measurement

Results of the oxygenation measurements are presented in Table 2. Since correction of the experimentally measured reflectance spectra (see, Subsection 2.3) changes the shape of the spectra we have performed calculation of oxygenation degree both with and without the reflectance spectra correction. As it can be seen from Table 2, Method 1 is not sensitive to the correction. For Methods 2 and 3 the correction increases oxygenation degree, and, on the contrary, for Method 4 it decreases oxygenation degree. Moreover, the absolute value of the degree of oxygenation calculated using different methods varies greatly in magnitude, from 27.5±2.7% (Method 3) to 61.23±18.1% (Method 4) in the control group and from 26.72±8.27% to 59.92±31.3% (Method 4) in the group with hypoxia.

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Method 1 with spectra correction</th>
<th>Method 1 without spectra correction</th>
<th>Method 2 with spectra correction</th>
<th>Method 2 without spectra correction</th>
<th>Method 3 with spectra correction</th>
<th>Method 3 without spectra correction</th>
<th>Method 4 with spectra correction</th>
<th>Method 4 without spectra correction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>41.38±7.09</td>
<td>41.51±7.18</td>
<td>52.71±4.46</td>
<td>47.38±0.42</td>
<td>31.32±1.59</td>
<td>27.94±1.72</td>
<td>47.48±4.67</td>
<td>72.73±5.05</td>
</tr>
<tr>
<td>Rat2</td>
<td>24±6.63</td>
<td>23.9±6.69</td>
<td>38.39±3.06</td>
<td>45.64±0.51</td>
<td>28.73±2.81</td>
<td>25.02±2.46</td>
<td>29.22±5.78</td>
<td>38.99±8.16</td>
</tr>
<tr>
<td>Rat3</td>
<td>39.83±2.68</td>
<td>39.94±2.72</td>
<td>52.98±3.33</td>
<td>47.61±28</td>
<td>33.25±2.19</td>
<td>29.56±1.82</td>
<td>56.37±7.58</td>
<td>74.66±3.44</td>
</tr>
<tr>
<td>Mean value</td>
<td>35.06±9.76</td>
<td>35.12±9.87</td>
<td>48.03±7.83</td>
<td>46.88±0.99</td>
<td>31.1±2.83</td>
<td>27.5±2.7</td>
<td>44.36±13</td>
<td>61.23±18.1</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>24.77±15.75</td>
<td>24.83±15.88</td>
<td>42.36±18.37</td>
<td>44.05±4.85</td>
<td>22.21±7.33</td>
<td>19.91±5.5</td>
<td>39.97±36.61</td>
<td>23.64±30.28</td>
</tr>
<tr>
<td>Rat2</td>
<td>40.87±6.11</td>
<td>40.97±6.16</td>
<td>54.38±13.8</td>
<td>47.78±1.43</td>
<td>42.27±11</td>
<td>37.33±10.5</td>
<td>65.72±30.03</td>
<td>61.27±8.36</td>
</tr>
<tr>
<td>Rat3</td>
<td>32.01±6.96</td>
<td>32.7±6.05</td>
<td>53.1±7.46</td>
<td>47.37±54</td>
<td>30.02±3.89</td>
<td>25.96±3.25</td>
<td>50.1±11.1</td>
<td>75.16±23.45</td>
</tr>
<tr>
<td>Mean value</td>
<td>37.03±7.69</td>
<td>37.11±7.79</td>
<td>56.38±3.87</td>
<td>47.85±51</td>
<td>30.75±2.24</td>
<td>26.72±2.16</td>
<td>65.22±16.49</td>
<td>83.62±17.79</td>
</tr>
<tr>
<td>Rat5</td>
<td>27.46±14.9</td>
<td>27.41±15.11</td>
<td>47.59±6.69</td>
<td>46.75±76</td>
<td>27.88±6.62</td>
<td>23.7±6.52</td>
<td>41.98±27.07</td>
<td>60.3±39.87</td>
</tr>
<tr>
<td>Mean value</td>
<td>32.43±11.8</td>
<td>32.46±11.9</td>
<td>50.76±11.6</td>
<td>46.76±2.55</td>
<td>30.63±9.19</td>
<td>26.72±8.7</td>
<td>49.87±22.9</td>
<td>59.92±31.3</td>
</tr>
</tbody>
</table>

At the same time, comparing the degree of oxygenation, measured by different methods for the control group and group with hypoxia, clearly shows that there are no statistically significant differences in the oxygenation degree.

3.3 Morphological study

The number of rats in the litter in the experimental and control groups differed slightly. So, in the experimental group, the average number of rats was 17, in the control - 21. There was a significant decrease in body weight in the offspring (Me - 5.8) of the experimental group compared to the control (Me - 6.4).

In a macroscopic examination of the internal organs of rats of the first and second groups, the picture was not significantly different. At the same time, during the histological study, the following changes were identified:

**Liver.** When viewing microscopy in the liver tissue of animals of the control group, the beam structure was preserved, histoarchitecture was not disturbed, focal lymphohistiocytic infiltration was noted, and moderate central venous plethora was observed. In the liver tissue of rats in the experimental group, multiple focal small and large hemorrhages were noted, the expansion of the Disse spaces, and in some places the cytoarchitecture of the liver was disturbed, with marked dystrophic changes in hepatocytes (Fig. 4 A, B).

**Heart.** Weak, predominantly perivascular edema was observed in the heart tissue of rats in the control group, and granular dystrophy was observed in cardiomyocytes. In the heart tissue of animals in the experimental group, there was a
pronounced vascular plethora, small foci of hemorrhages in the parenchyma, and interstitial edema phenomena. Cardiomyocytes were in a state of hypertrophy (Fig. 5 A, B).

**Kidneys.** In the tissue of the kidneys of rats in the control group, there was a degeneration of the epithelium of the tubules. In the experimental group, stromal edema, dystrophy, focal necrosis and desquamation of the tubular epithelium were observed (Fig. 6 A, B).

Figure 4. Liver tissue of newborn rats. A - control group; B - experimental group. Staining with hematoxilin and eosin. ×246.4.

Figure 5. Heart tissue of newborn rats. A - control group; B - experimental group. Staining with hematoxilin and eosin. ×774.
4. CONCLUSION

The experiments showed a clear relationship between the features of changes in uterine blood flow and experimental hypoxia. In chronic hypoxia, there were low rates of arterial blood flow, an increase in the peripheral resistance of uterine vessels. All this indicates the defective formation of the placenta and impaired blood flow with the development of placental insufficiency.

It has been established that hypoxia in the antenatal period negatively affects the number and somatometric indicators of newborn rats in the offspring. Histological examination of the tissues of the heart, liver and kidney of newborn rat pups shows signs of impaired blood circulation, dystrophic and necrotic changes in the parenchyma.

ACKNOWLEDGEMENTS

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