Age-related features in systemic inflammatory response in male Wistar rats with different hypoxia tolerance

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Introduction
Organism’s hypoxia tolerance depends on many factors, including age. High newborn organism’s tolerance and high level of oxidative stress throughout aging were demonstrated by many studies. However, there is still lack of investigations, reflecting the intensity of systemic inflammatory response in different age organisms in correlation to hypoxia tolerance.

Aims: to determine the relationship between age-related tolerance to hypoxia, HIF-1 and PHD2 (prolyl hydroxylase domain protein) expression levels and the intensity of systemic inflammatory response in newborn, prepubertal and adult Wistar rats.

Methods
Systemic inflammatory response was modeled by intraperitoneal injection 15 mg/kg lipopolysaccharide (LPS) E.coli O26:B6 (Sigma). In newborn and prepubertal rats, 15 mg/kg LPS induced progressing degenerative changes and necrosis. This dose is sublethal for adult Wistar rats. The control rats were intraperitoneally injected with physiological saline. Animals were euthanized by an overdose (15 mg/kg) of anesthetic Zoletil after 24 hours of LPS injection. The histological sections of the liver were manufactured and stained by hematoxylin and eosin. The severity of pathological changes in the liver was evaluated morphometrically - the area of cells pathology and necrotic changes was estimated to the total area of the histological section. The serum collected was frozen at -70°C and was kept no longer than two months. ELISA was employed to assay proinflammatory markers neopterin (IBL) and C-reactive protein (eBioscience).

Results
Age-related differences of HIF-1α and PHD2 expression levels in the liver
Newborn rats are characterized by high mRNA HIF-1α expression level in the liver (Fig. 2), accompanied by low content of HIF-1 protein and high level of PHD2 (Fig. 3).

Prepubertal animals are the least hypoxia tolerant and their HIF-1α mRNA expression level was higher than in adult animals. The PHD2 activity in prepubertal animals was significantly reduced in comparison to newborn rats, and the HIF-1c protein level was not changed.

Fig. 2. The expression level of mRNA HIF-1α in the liver of newborn, prepubertal and adult Wistar rats. Me; 25%-75%, p – statistically significant differences, Kruskal–Wallis method.

Fig. 3. (a) – Relative protein levels of HIF-1α and PHD2 in the liver of newborn, prepubertal and adult Wistar rats, normalized on GAPDH level, representative Western Blot images are shown. (b, c) – The expression level of HIF-1α (b) and PHD2 (c) proteins in the liver of newborn, prepubertal and adult Wistar rats. Me; 25%-75%, p – statistically significant differences, Kruskal–Wallis method.

Table 1. Effect of LPS on hepatic necrosis area and serum endotoxin, neopterin and C-reactive protein content in newborn, prepubertal and adult Wistar rats in 24 h after injection, Me (25-75%). p – statistically significant differences, Kruskal–Wallis method.

<table>
<thead>
<tr>
<th>Age period</th>
<th>Group</th>
<th>Necrosis area, 10³ mkm²</th>
<th>Endotoxin, μ/l</th>
<th>Neopterin, nM/l</th>
<th>C-reactive protein, mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>Control</td>
<td>1.9</td>
<td>(0.5-3)</td>
<td>9.12</td>
<td>(7.10-10.37)</td>
</tr>
<tr>
<td></td>
<td>LPS (1.4-4.3)</td>
<td>57.0</td>
<td>(47.3-90.7)</td>
<td>7.33</td>
<td>(6.02-8.71)</td>
</tr>
<tr>
<td>Prepubertal</td>
<td>Control</td>
<td>2.6</td>
<td>(1.3-3.6)</td>
<td>2.74</td>
<td>(2.26-3.91)</td>
</tr>
<tr>
<td></td>
<td>LPS (7.9-99.5)</td>
<td>430</td>
<td>(417.8-451.3)</td>
<td>4.62</td>
<td>(3.98-5.76)</td>
</tr>
<tr>
<td>Adult</td>
<td>Control</td>
<td>0.7</td>
<td>(0.0-1.8)</td>
<td>0.84</td>
<td>(0.17-1.14)</td>
</tr>
<tr>
<td></td>
<td>LPS (1.5-6.2)</td>
<td>22.1</td>
<td>(16.3-100.0)</td>
<td>1.53</td>
<td>(1.93-1.94)</td>
</tr>
</tbody>
</table>

Conclusion
Prepubertal animals were the least hypoxia tolerant and their HIF-1c mRNA expression level was higher than in adult rats. The lowest tolerance of the prepubertal males to hypoxia correlated with the greatest manifestations of hepatic inflammation and elevated endotoxin, neopterin, and C-reactive protein levels in LPS-induced systemic inflammatory response. The obtained data should be taken into account during the development of therapeutic strategy for prepubertal children with infectious and inflammatory diseases.

References

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