Study the effect of isoproterenol to visualize cerebral blood flow by using a laser speckle imaging system in Neonatal rats


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**Abstract**

The severe stress in Neonatal rats causes hemorrhagic cerebral infarction, provoking the appearance of venous insufficiency and reduced outflow of blood from the brain. These changes in blood flow precede intracranial hemorrhage and the risk of stroke in Neonatal rats, which is an actual and acute problem of modern neonatology. We studied the contribution of the mechanisms of adrenergic vasorelaxation in impaired venous blood flow in Neonatal rats with stress-induced cerebral infarction. Neonatal rats were divided into three groups (control, pre-stroke state, and after developing a stroke). Blood flow changes were measured using laser speckle imaging systems, one of the most advanced techniques in measuring cerebral blood flow. The pharmacological stimulation of the beta-2-adrenergic receptors by the introduction of isoproterenol to the groups, the pre-stroke group revealed a higher sensitivity of animals to this effect, where the vein diameter in that group increased by 36% after injection of the isoproterenol which is 1.5 times greater than in the control. This indicates that adreno-related vasorelaxation mechanisms play a major role in disturbances in venous blood flow. Adrenergic vasorelaxation plays a major role in the critical expansion of intracranial veins and in the formation of cerebral hypotension, which gives us an indication to prevent hemorrhagic cerebral infarction in the neonate.

**Introduction**

Cerebral infarction in Neonatal is an actual and acute problem of modern neonatology (1-4). Previously, it was thought that cerebral infarction was typical only for premature babies; however, thanks to the development of optical imaging technologies for the brain, it became apparent that this pathology manifests itself with the same frequency in full-term babies as in preterm babies (2-7). Full-field imaging of blood flow can be done using laser speckle imaging. Laser speckle imaging acquired a broad interest because of the quick acceptance of blood flow research in the brain (8-10). The main problem of neonatal cerebral infarction is its interference with other symptoms, thus often not diagnosed (11-13). Treating such children is currently a lucky chance for them, not a pre-planned doctor's tactic. A detailed study of the mechanisms involved in developing brain infarction in the first days after birth is necessary to solve this problem. Cerebral circulatory disorders are one factor in developing cerebral infarction in neonatal (14-17). Despite the widespread use of optical technologies to assess changes in cerebral hemodynamics and oxygenation of brain tissue, there are no effective criteria for predicting critical disturbances in cerebral blood flow or determining the possibility of intracranial hemorrhages in the first few days after delivery. Some hypotheses postulate that the restriction in blood circulation in the neonatal brains may be the main cause of low resistance of cerebral vessels to damaging factors (18-22). Previous studies on adult hypertensive rats and neonatal with cerebral infarction established that this
pathology proceeds against the background of cerebral blood stagnation and venous insufficiency (19,23). Cerebral infarction in neonatal infants has many possible causes, including bacterial meningitis, inherited or acquired coagulopathies, trauma, and hypoxia-ischemia. However, a specific cause often cannot be identified. Neurologic symptoms in the neonatal period are often subtle and nonspecific, even in infants with large infarctions involving an entire cerebral artery distribution (24). Vessels of the brain are well-developed sympathetic innervation (25-27).

Since early ontogenesis, the sympathetic nervous system has supported the control of the myogenic tone of the cerebral arteries and veins (23,28). Adrenergic vasorelaxation plays a major role in the critical expansion of intracranial veins and the formation of cerebral hypotension (29,30). Isoproterenol is a sympathomimetic that acts almost exclusively on beta-adrenergic receptors (beta-1 and beta-2 adrenergic receptors). It is listed in the 2004 WHO Model List of Essential Medicines. It increases the heart rate for treating patients with severe bradycardia (31).

To test this hypothesis, we studied the contribution of the mechanisms of adrenergic vasorelaxation in impaired venous blood flow in Neonatal rats with stress-induced cerebral infarction using the laser speckle imaging systems technique.

Materials and methods

Ethical approve

This study was carried out at the College of Science, Mustansiriyah University, Iraq in the animal house with the ethical approve number BCSMU/0122/00019Z at January 07, 2022.

Animals

Experiments were performed on neonatal rats (n = 29), 2-3 days old after birth. We performed all procedures within the Helsinki Declaration on the humane treatment of animals during experimental work (32).

Standard conditions of rats

The rats were put in standard conditions, humidity 50%, temperature 25 ± 2 °C and 12:12 hour light/dark cycle (33).

Experimental design

Rats in total were divided into 3 groups (control, pre-stroke state, and after developing a stroke). The blood flow of mice brain was measured through a small incision in the skin in the fontanelle, while the integrity of the skull was not compromised. The sagittal sinus was chosen as a target of optical imaging because is the principal cerebral vein that collects blood from all cerebral veins and sends it via the jugular vein to the periphery (Figure 1).

Inducing a cerebral infarction

Cerebral infarction in neonatal rats was induced by exposing them to an intermittent sound with a force of 120 dB according to the following algorithm: 10 second with sound and 60 second relief (pause), for two hours, the cycle was repeated (34).

Isoproterenol administration

Cerebral blood flow was studied using an optical speckle imaging system. Isoproterenol (Sigma, 0.05 µg / kg, iv- was injected in Temporal (facial) veins) was used to activate adrenergic vasorelaxation mechanisms (35,36). The cerebral blood flow registered in the sagittal sinus was carried out within 10 minutes before Isoproterenol administration and within 30 minutes after injection in the following groups: 1) control animals (n = 10); 2) rats before cerebral infarction (4 hours after stress, n = 10); 3) rats after cerebral infarction (24 h after stress, n = 9). To exclude affection of drug administration on cerebral blood flow, the experiment included similar groups (10 rats in each group) receiving saline.

Statistical analysis

The Wilcoxon test was used to evaluate variations from baseline values. The ANOVA test was used to evaluate intergroup differences. At a p-value of 0.05, differences were deemed significant. The format of the data is mean standard error of the mean.

Results

Changes in the cerebral blood flow against a background by the development of a hemorrhagic cerebral infarction and pharmacological stimulation of beta-2 adrenergic receptors (Table 1); there were no differences in the levels of brain tissue perfusion between the groups receiving and not receiving saline. Four hours after exposure to sound stress, when intracranial hemorrhages had not yet developed, an increase in perfusion was observed by 22%, then After 24 hours, against the background of cerebral hemorrhages,
perfusion tended to increase even more than in the pre-stroke group (perfusion was 8% higher), but these changes were statistically insignificant. Compared to the control group, perfusion increased by 32% in this group (after developing a stroke group). In the next stage of the research, we investigated the effects of pharmacological stimulation of beta-2-adrenergic receptors on the perfusion of brain tissues and the state of the sagittal sinus in normal Neonatal rats and at various stages of hemorrhagic cerebral infarction. The findings demonstrated that the sagittal sinus relaxed in response to the normal administration of isoproterenol injection. Increased vein diameter by about 20%, against the background of dilatation of the main cerebral sinus, there was a decrease in perfusion by 17% (Table 1).

Table 1: Change in cerebral tissue perfusion (arbitrary units) in neonatal rats before and after administration of isoproterenol injection in both healthy rats and at different stages of cerebral stroke development

<table>
<thead>
<tr>
<th>Basal level</th>
<th>Healthy rats</th>
<th>Four hours after stress</th>
<th>24 hours after stress</th>
</tr>
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<tbody>
<tr>
<td>Before saline administration</td>
<td>277 ± 11</td>
<td>347 ± 15*</td>
<td>372 ± 11*</td>
</tr>
<tr>
<td>After saline administration</td>
<td>280 ± 9</td>
<td>342 ± 13*</td>
<td>370 ± 15*</td>
</tr>
<tr>
<td>Isoproterenol administration</td>
<td>235 ± 7</td>
<td>250 ± 12</td>
<td>337 ± 17**</td>
</tr>
</tbody>
</table>

The significant at P<0.05. * With healthy rats. • Between stressed rats.

The introduction of isoproterenol to the pre-stroke group revealed a higher sensitivity of animals to this effect, despite the stress-induced expansion of the sagittal sinus. Indeed, the vein diameter in this group after injection of the drug increased by 36%, which was 1.5 times greater than in control. Perfusion in this group under the influence of isoproterenol decreased by 27%. A progressive increase in the sagittal sinus diameter was noted against the background of the development of hemorrhagic infarction in Neonatal rats. Thus, the vessel increased 1.3 times compared with the pre-stroke group and 1.7 times compared with the control. Under conditions of excessive stretching of the vein under the load of the blood column, the vessel was insensitive to additional stimulation of vasorelaxation by isoproterenol, i.e., its diameter did not change before and after drug administration. Perfusion decreased by 9%, which was insignificant. Typical examples of changes in the speckle-imaging pattern of perfusion in the sagittal sinus area in Neonatal rats before and after administration of isoproterenol at different stages of development of cerebral stroke (Figures 2 and 3).

Figure 2: Speckle visualization of brain tissue perfusion in the area of the sagittal sinus: A. under normal conditions; B. 4 h after stress (neonatal rats pre-stroke group); C. 24 h after stress (neonatal rats with stroke).

Figure 3: The effects of isoproterenol on the perfusion of brain tissue in the sagittal sinus area: A. under normal conditions; B. 4 hours after stress (neonatal rats pre-stroke group); C. 24 h after stress (neonatal rats with stroke).

Discussion

Although stroke has long been recognized as a serious health problem causing morbidity and mortality in adults, it is also a significant contributor to acquired brain damage in neonates and throughout childhood (37,38). In our study, we choose a model of 29 neonatal rats for the experiment. From that experiment, we noted an increase in perfusion in the group after developing a stroke more than in the group pre-stroke state. Thus, stress-induced disturbances in cerebral hemodynamics developed over time, manifesting an incremental rise in blood flow to the cerebral vessels, expressed in increased perfusion. The data obtained positively correlates with previous results on neonatal and adult rats (23-25). Previous histological data showed that the pre-stroke stage is associated with the increase in the size of cerebral veins due to the accumulation of extensive blood in them in superficial areas of the brain. These changes are associated with the formation of perivascular edema, i.e. fluid pathway from the vessels. The relaxation of cerebral veins with perivascular edema is a marker of the accumulation of extensive blood in the venous system and suppression of blood outflow from the brain leading to venous insufficiency. At the same time, post-stroke time is associated with the progression of initial pathological changes in the cerebral venous system and the increasing size
of deep veins in the brain parenchyma, accompanied by accumulation of blood in the microcirculatory bed (39,40).

Clinical studies have also shown that neonatal stroke is a primary venous infarction due to a weakness of the wall of cerebral veins in neonates (41). By studying the effects of pharmacological stimulation of beta-2-adrenergic receptors on the perfusion of brain tissues and the state of the sagittal sinus, relaxation in the sagittal sinus was noted, and a reduction in perfusion in the normal administration of isoproterenol in the pre-stroke group revealed a higher sensitivity in these animals. Thus, rats at the pre-stroke stage were more sensitive to the activation of adrenergic mechanisms of vasorelaxation against the background of stress-induced expansion of cerebral veins. These results indicate that increasing the activation of beta-2-adrenergic receptors may be one of the most important mechanisms of pathophysiological relaxation for cerebral veins preceding stroke development (42). In a recent study, during cardiopulmonary resuscitation, the brain becomes ischemic, so adrenaline has been recommended may thus counteract cerebral vasoconstriction during the reperfusion period (43). A progressive increase in the diameter of the sagittal sinus was noted during the development of hemorrhagic infarction in neonatal rats. In contrast, progressive relaxation of the sagittal sinus indicates a development of venous insufficiency and a decrease in the outflow of blood from the brain, which was also shown in other studies (25,37).

Conclusion

The development of stress-induced hemorrhagic cerebral infarction in neonatal rats is accompanied by pathological relaxation of the cerebral veins, which provokes the appearance of venous insufficiency and a decrease in the outflow of blood from the brain. These critical changes in brain blood flow precede the appearance of intracranial hemorrhages, so they can be predictive criteria for the risk of developing cerebral stroke in the first days after birth. The high sensitivity of beta-2-adrenergic receptors to their pharmacological stimulation at the pre-stroke stage indicates that the adrenergic mechanisms of vasorelaxation play a major role in cerebral venous blood circulation, opening up prospects for further research studying the pharmacological correction of these processes and preventing the development of hemorrhagic cerebral infarction in neonatal.

Acknowledgments

The authors would like to thank all who pericipent in the study. Also, they would like to thank the support of the Department of Physiology at Saratov state university (Russia) and Mustansiriyah University.

Conflict of interest

The author has nothing to disclose and has no competing exist.

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دراسة تأثير الأيزوبريتينول على تدفق الدم في المخ باستخدام آلة التصوير بالبقع الليزرية في الفتران حديثي الولادة

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