Invited Paper

Cerebral venous circulatory disturbance as an informative prognostic marker for neonatal hemorrhagic stroke

Oxana Semyachkina-Glushkovskaya[a], Alexey Pavlov[a,b], Nikita Navolokin[c], Vladislav Lychagov[a], Arkady Abdurashitov[a], Ekaterina Zinchenko[a], Artemiy Gekaluk[a], Dan Zhu[d], Rui Shi[d], Qingming Luo[d] and Valery Tuchin[a,e]

[a]Saratov State University, Astrakhanskaya Str. 83, Saratov 410012, Russia;
[b]Saratov State Technical University, Politechnicheskaya Str. 77, Saratov 410054, Russia;
[c]Saratov State Medical University, Bolshaya Kazachia Str. 112, Saratov 410012, Russia;
[d]Huazhong University of Science and Technology, Wuhan 430074, China;
[e]Tomsk State University, Lenina Ave. 36, Tomsk 634050, Russia

ABSTRACT

Neonatal hemorrhagic stroke (NHS) is a major problem of future generation's health due to the high rate of death and cognitive disability of newborns after NHS. The incidence of NHS in neonates cannot be predicted by standard diagnostic methods. Therefore, the identification of prognostic markers of NHS is crucial. There is evidence that stress-related alterations of cerebral blood flow (CBF) may contribute to NHS. Here, we assessed the stroke-associated CBF abnormalities for high prognosis of NHS using a new model of NHS induced by sound stress in the pre- and post-stroke state. With this aim, we used interdisciplinary methods such as a histological assay of brain tissues, laser speckle contrast imaging and Doppler coherent tomography to monitor cerebral circulation. Our results suggest that the venous stasis with such symptoms as progressive relaxation of cerebral veins, decrease the velocity of blood flow in them are prognostic markers for a risk of NHS and are an informative platform for a future study of corrections of cerebral venous circulatory disturbance related to NHS.

Keywords: neonatal stroke, stress, cerebral veins

1. INTRODUCTION

Neonatal stroke, defined as a cerebrovascular event occurring during 28 days after birthday, is one of top ten causes of death in neonates.[1] Pathogenesis of stroke in newborns is multifactorial but there is strong evidence that abnormalities of the cerebral blood flow (CBF) make a considerable contribution to the neonatal stroke. Therefore, management of the CBF is of paramount importance in neonatal and pediatric medicine.[2,3] However, the exact role of pathological changes in the CBF is an issue to debate since the results presented in the literature are very controversial.[4,5] It remains unclear whether the CBF abnormalities can be used as prognostic criteria for a stroke in neonates or the critical alterations in the CBF may occur due to the presence of the brain hemorrhage itself.

In this experimental study on newborn rats, we aimed to determine an informative prognostic marker for a risk of NHS. With this aim, we focused on the main potential mechanism responsible for NHS – the pathological changes in cerebral hemodynamics including morphology of brain vessels as well as the pattern and complexity of the CBF on the different levels of circulatory network (arterial, venous and microcirculatory bed). A new model of NHS induced by severe sound stress was designed to investigate changes in cerebral hemodynamics in the pre-stroke and post-stroke time. We selected this model because sound-brain damages develop with the latent changes and the intracranial hemorrhages occur 24 h after stress-off in cortical and subcortical brain tissues that is close to localization as well as origin time of NHS in term neonates.[6,7] We hypothesized that this model can be a good tool for the study of pre-stroke changes in the cerebral circulation. We applied histological and coherent-domain optical methods to the study of abnormalities of cerebrovascular dynamics. The fluctuations

Further author information: (Send correspondence to O. Semyachkina-Glushkovskaya)
E-mail: glushkovskaya@mail.ru, Telephone: +7 8452 51 92 20, Fax: +7 8452 27 85 29

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in CBF were analyzed by a measure of multiscality reflecting a complexity of CBF. Since the NHS is primary venous,[8] we assume that the cerebral venous circulatory disturbance can be more sensitive to stress and more informative than microcirculatory bed to extract the hidden information for prognosis of NHS.

2. METHODS

Experiments were carried out in newborn mongrel rats 12 days old. The animals were divided into three groups: 1) intact, unstressed newborn rats (n=17, the control group); 2) stressed rats 4 h after stress-off (n=33, the pre-stroke group, rats with initial pathological changes in brain tissues and cerebral circulation); stressed rats 24 h after stress-off (n=37, the post-stroke group, rats with intracranial hemorrhages). The sound stress (120 dB, 370 Hz) was used to induce hemorrhagic stroke in newborn rats.

To assess the cerebral circulation disturbances associated with the initial and late stages of stroke, we performed the study of changes in cerebral blood flow (CBF) in newborn rats in the normal state (the control group), in the pre- and post-stroke groups using *laser speckle contrast imaging* (LSCI) and *Doppler optical coherence tomography* (DOCT). The measurement of cerebral circulation was performed through the anterior fontanel in anesthetized rats (isoflurane – an inhalational anesthetic) with fixed head and scalp incision (the *dura mater* was left intact). Anesthetic depth was assessed by periodically monitoring the rear foot reflex.

To characterize changes in complexity of cerebral circulation associated with the initial and late stages of stroke, a multifractal analysis was performed using the wavelet-transform modulus maxima method in the pre- and post-stroke groups with comparison to the control group.

The results were reported as mean ± standard error of the mean (SEM). Differences from the initial level in the same group were evaluated by the Wilcoxon test. Intergroup differences were evaluated using the Mann-Whitney test and ANOVA-2 (post hoc analysis with the Duncan's rank test). Significance levels were set at $p < 0.05$ for all analyses.

3. RESULTS

In the first step of our work, we analyzed the changes in cerebral vessels and tissues preceding and accompanying NHS using the histological method as a "gold standard" for the study of pathological alterations in the brain. With this aim, the brains were carefully removed in newborn rats in the control (intact rats without stress), the pre-stroke (4 h after stress) and the post-stroke (24 h after stress) groups and underwent histopathological analysis. Figure 1 shows the results of the morphological evaluation.

In the pre-stroke group, a congestion of excessive blood in the cerebral veins of the pia mater was observed with an increased size of the veins (Fig. 1B). Vessels of the microcirculatory bed, including small arteries, did not demonstrate clear changes at this time. Venous abnormalities were associated with the development of perivascular edema in the cortex and subcortical tissues (Fig. 1C). No brain hemorrhage was found in neonatal rats 4 h after stress.

Sound-induced intracranial hemorrhages in newborn rats developed with the latent period and occurred in the cortical and subcortical areas of the brain 24 h after stress-off. The hemorrhage was developed in 91% of animals (34 of 37). Three newborn rats without stroke were not considered in the further analysis. The mortality rate was 26% (9 of 34). Figure 1D demonstrates a typical example of multifocal hemorrhages (average size 0.005 mm^2 – 0.037 mm^2) in the cortical and subcortical tissues in newborn rats.

Notice, the clinical data suggest that hemorrhagic stroke in newborns is primary the small diffuse venous infarction in the cortex that usually occurs during with the latent period during the first 3 postnatal days.[6–10] In stress-induced model, the small venous hemorrhages in newborn rats develop in the cortex and the subcortical area with the latent time, which is 24 h. Thus, the stress-induced cortical hemorrhages in newborn animals are close to localization, type and original time of NHS.

Hemorrhages were associated with the progression of venous disturbance that we observed in the pre-stroke group. Indeed, in newborn rats venous congestion with perivascular edema developed in all regions of brain including deep veins (Fig. 1E). To contrast the pre-stroke group in newborn rats with intracranial hemorrhages,

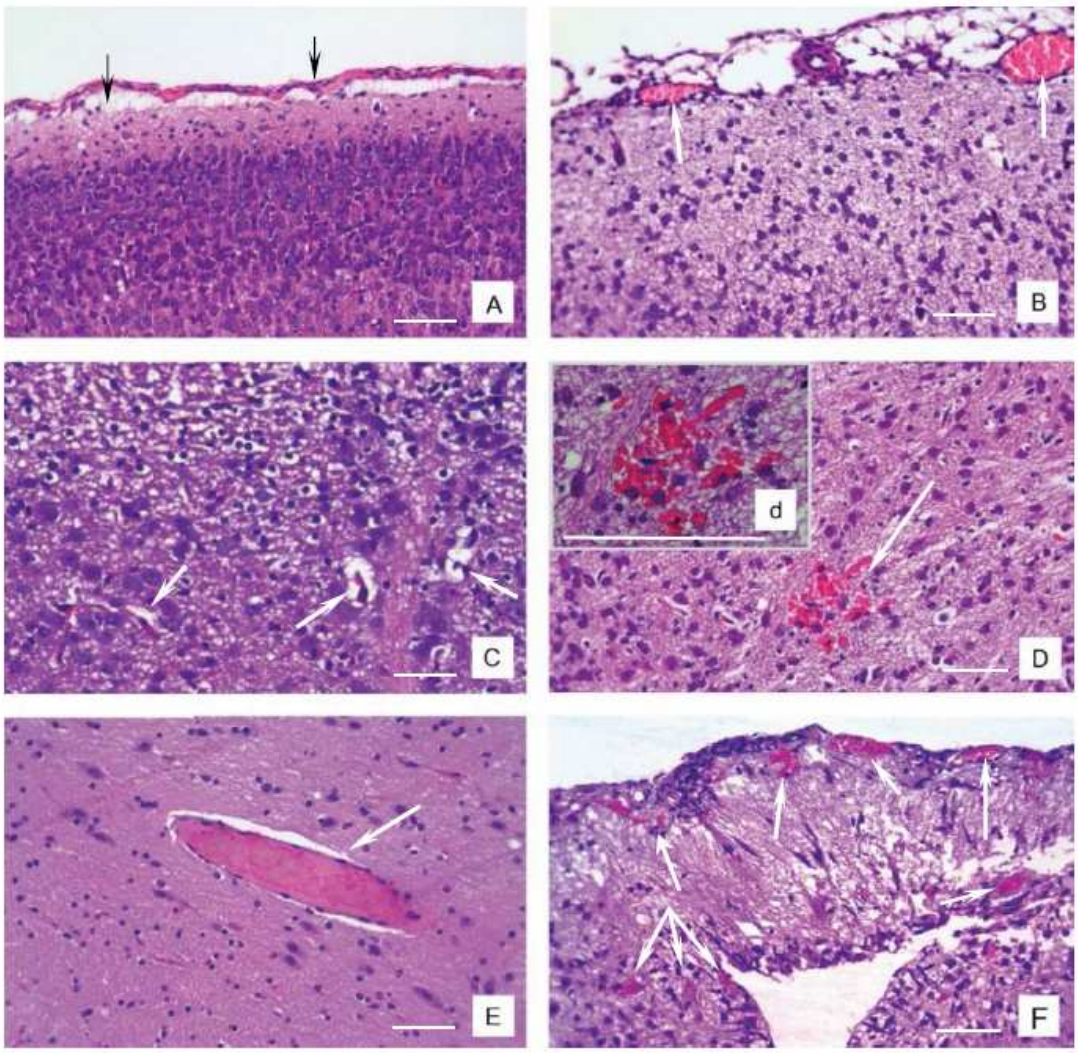

Figure 1. The typical examples of cerebral vascular and parenchyma alterations in the control, pre-and post-stroke groups. (A) – the control group including normal newborn rats without stress, arrows show the venous vessels of the pia mater. (B and C) – the pre-stroke group including newborn rats 4 h after stress without hemorrhage but with initial pathological changes in the brain: (B) – congestion of excessive blood in the cerebral veins of the pia matter (white arrows) but not in small arteries (in the middle between veins); (C) perivascular edema (arrowed) in the cortex and subcortical tissues. (D,d,E,F) – the post-stroke group including newborn rats 24 h after stress with intracranial hemorrhage and further pathological changes in the brain: (D) – low and (d) – high magnification image (774.0X) of subcortical hemorrhage (arrowed); (E) congestion of deep vein (arrowed) with perivascular edema in the cerebral parenchyma; (F) accumulation of blood in the microcirculatory vessels (arrowed) in the cortical and subcortical tissues. Hematoxylin & Eosin staining. Bars represent 10 μm (246.4X).

the blood-overloading of cerebral veins were accompanied by accumulation of blood in microcirculatory bed (Fig. 1F).

Thus, the relaxation of cerebral veins with accumulation of blood in venous network is an important component of development of intracranial hemorrhages in newborn rats in different stages from the initial pathological changes in cerebral vasculature until the incidence of hemorrhages. The particularities of cerebral veins are that they have no muscles and valves in their thin walls, therefore, they have low resistance to critical stretching occurring during blood accumulation in them. The immature brain vessels of newborns have limitation in vasorelaxation capabilities (increase of vessel size).[11] The stress-induced vasorelaxation of cerebral veins causes an

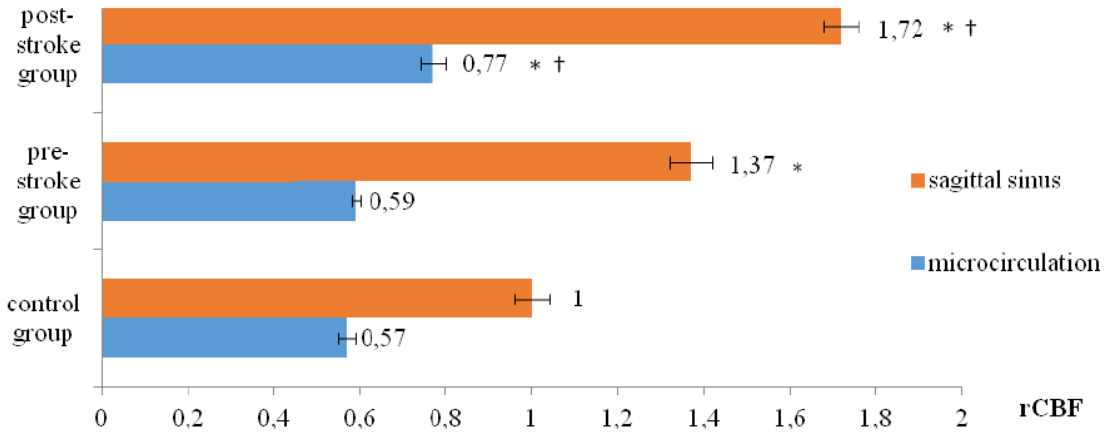

Figure 2. Relative cerebral blood flow (rCBF) assessed by LSCI in the sagittal sinus and in the small cerebral vessels (microcirculation) in newborn rats from the control, pre- and post-stroke groups: $*$ – p <0.05 vs. control group; † – between the pre- and post-stroke groups.

increase of cerebral venous pressure. A high pressure in its turn can induce easily the rupture of thin walls of immature cerebral veins of newborn rats.[12]

Since our histological data showed the stress-induced changes in cerebral veins and microvessels, in the second step of our study, we investigated the changes in cerebral circulation in the level of venous and microcirculatory network in the pre- and post-stroke groups in anesthetized newborn rats with fixed head and scalp incision (the dura mater was left intact) using laser speckle contrast imaging (LSCI) system and Doppler optical coherence tomography (DOCT). For this purpose, two regions of our interest were selected: 1) the sagittal sinus that is one of major sinuses collecting blood from the small veins of the brain and directs it into the peripheral circulation; 2) small, optically unresolvable vessels of microcirculatory bed surrounding the sagittal sinus.

Figure 2 shows changes in rCBF at different stages of the stroke in newborn rats. The latent period of stroke was characterized by increased rCBF in the sagittal sinus but not in small cerebral vessels surrounding this major cerebral vein. The indicated pathological changes in the cerebral circulation progressed to the next day after the stress-off when the stroke occurred. The post-stroke group demonstrated higher rCBF in the sagittal sinus than the pre-stroke group. Unlike the pre-stroke group, rats with the stroke showed increased rCBF in both, the sagittal sinus and microvessels. Thus, the stress-induced increase in cerebral perfusion observed earlier and more pronounced in the sagittal sinus compared with vessels of microcirculatory bed. Note, that hyperperfusion is one of the stroke-associated brain injuries.

In the first series of experiments, our histological results showed a relaxation of cerebral veins with accumulation of blood in them that preceded and accompanied NHS. Here, using LSCI, we found a progressive increase in rCBF in the sagittal sinus at the same time of the experiment. To answer the question, how the velocity of blood flow changes in the dilated cerebral veins with increased rCBF in newborn rats in different stages of stroke, we performed a measure of the velocity of blood flow and the diameter in the sagittal sinus and in the pre- and post-stroke groups using DOCT.

DOCT imaging showed that the development of stroke was accompanied by the increase in diameter of the sagittal sinus with the decrease in the velocity of blood flow, which were more pronounced in newborn rats with brain hemorrhages compared with animals from the pre-stroke group (Table 1).

At the first blush, there is an obvious conflict between LSCI and DOCT results. This contradiction can be resolved by detailed analysis of LSCI signal's nature. Briers *et al.* clearly stated that LSCI signal cannot be simply related to blood perfusion in the same way as DOCT,[13] since speckle contrast is mainly affected by both the speed of moving red blood cells (RBC), which are responsible for intensity fluctuations of the speckle field, and its concentration. The further investigation of LSCI signal nature by Kazmi *et al.* confirmed that speckle contrast reflects neither the flux nor RBC speed, but instead the product of velocity and a constant, which is

Table 1. Diameter and velocity of blood flow in the sagittal sinus in the control, pre and post-stroke groups ($*$ – p <0.05 vs. control group, † – between the pre- and post-stroke groups).

Parameters	Control group	Pre-stroke group	Post-stroke group
Diameter of the sagittal sinus, mm			
main trunk	0.33±0.01	0.60±0.03*	0.98±0.03*†
left branch	0.18±0.02	0.25±0.02*	0.39±0.03*†
right branch	0.16±0.04	0.26±0.03*	0.35±0.01*†
Blood flow velocity, mm/sec	7.00±0.08	3.03±0.02*	2.17±0.01*†

proportional to the vessel diameter.[14] Kosar *et al.* have determined dependence of LSCI signal and optical properties of tissues.[15] According to these two statements, now we can overcome confusion between LSCI and DOCT signals. It was confirmed by DOCT measurements of the hemodynamic pattern of the sagittal sinus that dilated with the progressive increase size of diameter in the pre- and post- stroke groups, respectively. As a result, we have much more RBC in the sagittal sinus (the initial stage of NHS) and in both this vessel and surrounding him mircovessels (the incidence of NHS) compare to the normal conditions. And even if the velocity of these RBC is reduced, what was measured by DOCT, the increase of RBC concentration contributes significantly to the LSCI signal and, therefore, we registered an increased rCBF value. Roughly estimation of RBC speed behavior can be done by dividing rCBF index and the corresponding vessel diameter D. In pre-stroke group this rCBF/D index is equal to 0.65, which is smaller compare to control individuals (we set rCBF/D index to unity for normal ones). Post-stroke group also shows decrease in rCBF/D index up to 0.36. According to these calculation "corrected" LSCI data is consistent with DOCT measurements.

Thus, LSCI and DOCT results demonstrate clearly that the cerebral circulatory disturbances associated with the pre- and post-stroke are accompanied by more pronounced changes in the sagittal sinus than in microcirculatory vessels. Indeed, the pre-stroke period is characterized by an increase in rCBF in dilated sagittal sinus with a decrease in the velocity of blood flow suggesting about venous stasis and accumulation of blood in cerebral veins, while the microcirculatory network demonstrates a silent response in this time of the experiment. In the post-stroke stage, rCBF increased in both venous and microcirculation network but these changes were significant in the sagittal sinus but weakly expressed in the microvessels.

4. CONCLUSION

The aim of this study was to analyze the stroke-associated brain injuries and abnormalities in the cerebrovascular dynamics for prognosis of a risk for NHS using a new sound stress-induced model of hemorrhagic stroke in newborn rats, histopathological assay and optical coherent-domain methods. Our results suggest that the cerebral veins compared with the cerebral arteries and microcirculatory vessels are more sensitive to harmful effects of stress on the brain and demonstrate significant pathological changes at the latent stage of the stroke. The persistent and progressive relaxation of the cerebral venous vessels with a suppression of the venous blood flow is an important informative platform for prognosis of a risk for NHS and further detailed investigations. Our experimental data is confirmed by clinical facts suggesting that the mechanism of the neonatal stroke is primary the venous infarction[8,9] due to a weakness in the wall of cerebral veins in neonates.[12] This conclusion is also confirmed by the similar results from other groups who showed that relaxation of cerebral veins as an important mechanism responsible for critical changes in the CBF associated with the adult stroke.[16,17]

ACKNOWLEDGMENTS

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