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Cerebral blood flow in experimental neurotrauma

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Traumatic brain injury (TBI) posesses the largest threat facing the morbidity and mortality of the human pediatric and young adolescent population [22, 23, 29-31], and is one of the major causes of disability and long in capability in all ages. TBI to children younger than 4 years of age has been shown to cause significantly more severe cognitive and motor deficits than in older children [18]. The biologic factors that contribute to this vulnerability are not understood yet. There is also experimental evidence and relevant studies suggesting that inadequate antioxidant reserves may be a determinant, or the inflammatory response may be different in the developing brain as compared to the adult [13, 16, 20, 21]. Despite of many successful clinical and laboratory investigations on TBI, many questions and problems remain open and demand further investigation and understanding. New data, available to improve efficacy of treatment and life quality after TBI would benefit each patient with TBI.

The comprehensive understanding of pathophysiological processes developing in the injured brain always demands proper understanding of the cerebral blood flow (CBF) alterations as one of the most important and playing a significant role both in the disease development and its outcome. In many cases of clinical and animal research it has been proven the essential necessity of monitoring of blood flow measurements and there was found an interesting data concerning the flow disturbances following the injury.

Given the pathogenicity of neurotrauma, it is surprising that only few studies have examined the role of CBF disturbances in setting of TBI. The interest to CBF mostly was inspired by the clinical *post mortem* research in early 1980 s., that proves the dependence of TBI mortality to primary or secondary ischemia during autopsy [1]. Clinical investigations proved the key role of CBF in primary due to hypoperfusion) and secondary (mostly due to vasospasm) ischemia in TBI. Currently, the clinical head trauma guidelines are mainly focused on the maintenance of adequate cerebral perfusion pressure. Regularly, such an approach fails in the subacute phase, especially after severe diffuse brain damage. Possibly, treatment failure is the result of impaired autoregulation [7, 19].

It is well established that CBF decreases significantly following TBI in clinical patients [25, 32, 38] and in experimental animal models [10, 11, 25, 33]. Moderate to severe TBI lead to severe reduction of CBF causing secondary ischemia to already injured brain tissue with altered energetic metabolism and mitochondrial demands [32]. The animal studies show the correlation between trauma severity and reduction of CBF. The pathological pathways of CBF reductions are impairment of cerebral autoregulation and cerebral perfusion pressure, release of endogenous vasoconstrictors, responsible also for inflammatory response, serotonin, endothelin, alteration in brain tissue metabolism and ions transport, etc [4, 9, 35].

The application of investigation methods based on Doppler effect allows to perform continuous monitoring of cerebral blood flow and due to non-invasiveness and simplicity of application they become one of the first choice investigation methods in clinical and animal researches, as transcranial Doppler, laser Doppler, etc. The latter is on the leading position in investigations of regional cerebral blood flow.

The main idea of papers that came out in 1990s was to measure the regional blood flow by laser Doppler and compare it with the existing methods, as the well described radiolabeled microspheres [14, 36, 42], or hydrogene clearance [12] in context of TBI research.

Laboratory experiments demonstrated that TBI leads to significant reduction in cortical CBF of mature and immature experimental animals. Reduced CBF has been shown in many models of TBI. Moderate fluid percussion injury caused up to 50% reduction in CBF within 15-30 min after injury [42]. Controlled cortical impact reduced cortical CBF by about 35 to 50% [8, 10]. The most recent data of different investigators refer to continuous monitoring of CBF in animals with TBI model, during few minutes up to hours, and due to technical difficulties there is no data on subsequent next days and weeks [40], whenever clinical data show significant importance of CBF monitoring and repeated investigation during at least two-week period after TBI onset.

This paper to our knowledge is to be the first reported study of the continuous monitoring of the CBF during a 35-day-period of continuous monitoring in a large group of mature and immature murine after experimental neurotrauma.

Materials and Methods

All surgical procedures were done in accordance to and by allowance of Animal Care Protocols/Committee of the University of California in San Francisco, Ca, USA. Postnatal day 21 (n=50) and adult (n=50) C57BLK/6 mice were anesthetized with 1.25% and 2.5% 2,2,2 tribromoethanol, diluted in isotonic saline at 0.02 ml/g body weight respectively. Body temperature was maintained with a circulating water heating pad throughout the surgery and recovery. All animals fully recovered within 3 hours after surgery. Each animal was placed in a stereotaxic frame (David Koph, Tujunga, CA) for surgery. After a midline skin incision, the soft tissues were reflected and blood flow measurements were taken. The probe was placed approximately 0.5 mm above the exposed cranium. While taking the measurements, the position of the probe was fixed until the blood flow reached a constant value. Then a circular craniotomy, 5.0 mm in diameter, was made with a micro drill between bregma and lamda with a medial edge of the craniotomy 0.5 mm lateral to the midline (Fig. 1).

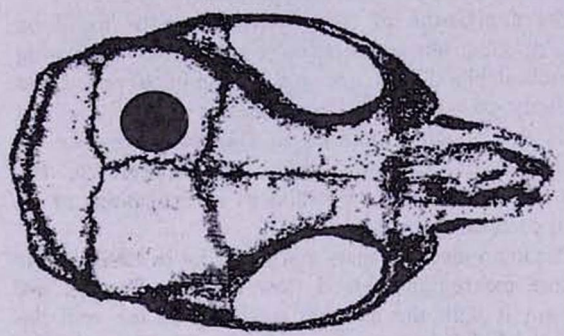

Fig. 1. The mouse skull with the pointed site of the craniotomy and further placement of laser Doppler probe

Following the craniotomy, a second blood flow measurement was made from the surface of the dura mater. When taking measurements from the dura mater, care was taken to avoid grossly visible blood vessels. In the injured group. the animal was then positioned in a stereotaxic frame of the injury device and was subjected to a controlled cortical impact injury [17], using a convex impactor tip with 3.0 mm in diameter and oriented perpendicular to the surface of the brain. The injury was generated using the following parameters: 4 m/s velocity, 1.0 mm depth of penetration and the sustained depression of 150 ms. Following cortical impact, the probe was returned to the same position and the measurement of blood flow was taken from the surface of the intact dura. Sham-operated animals underwent the same surgical procedures with the exception of cortical impact. In both groups, the scalp was then closed with sutures. Each animal was given 1.0 ml of isotonic saline subcutaneously after the operation to prevent dehydration. In order to keep all variables constant, prior to the final measurement of CBF from the same region above the site of injury, all animals were administered a surgical dosage of anesthesia and placed on a warming pad. Subsequently, the animals were deeply anesthetized. Control sham-operated animals were euthanized at 24 hours post surgery (n=10) and all other animals were euthanized at either 24 hours or 3, 7, 14 days and 5 weeks post surgery (n=10). Animals were perfused through the heart with 50 ml of 4% para formaldehyde in 0,1M phosphate buffered saline, pH 7,4 .

We used a laser Doppler device (LASERFLO BPM2, Vasamedics, US) to measure cerebral blood flow. The laser Doppler flowmeter (LDF) measures relative perfusion according to the principle of the "Doppler Effect." A transmitter/receiver probe emits a monochromatic laser light that is reflected by moving red blood cells [27, 39, 41], as well as stationary cells [27]. The moving cells according Doppler effect are causing shift in frequency. Both power and frequency of the reflected light are proportional to the blood volume and blood velocity, and the analyzer in the device computes the information of photon scatterings into electrical signals. Blood flow perfusion is calculated as a product of blood volume and velocity and is expressed as an absolute value [27]. All the obtained data were analyzed with Prism applications and evaluated by t-*test* and *ANOVA*.

Results and their Discussion

The first comparison of the blood flow measured from the intact skull and cortex proves the identity of the flow as it is shown on Figure 2. This is an important finding for the further evaluation of the cortical flow via cranial "window".

The measurements show a sufficient drop of flow right after the injury in both groups of animals. CBF falls down about 31% in adult and 33% in immature animals ($P<0.05$, the difference between two groups is not significant).

The further investigation was performed on the next day, and a tendency to restoration of the CBF was observed when the flow in adults reduced about 15% of the pre-injury level, and increased about 15 % in babies ($P<0.05$).

On the the 3d day blood flow continues to show a tendency to restoration and the differences are less prominent in adults (decreased about 5% of the pre-injury level) and in babies (increased about 8%). There was not any statistically significant difference between those values again ($P<0,05$, the difference is not significant).

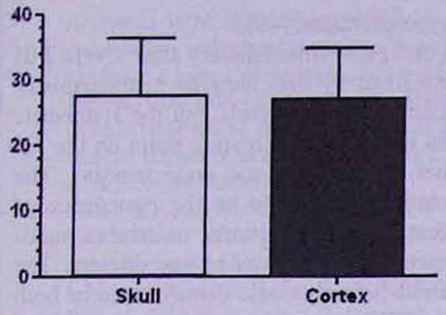

Fig. 2. Comparison of the flow measured from the skull and cortex does not reveal significant difference of LDF. y-axis is the flow measured in mlLD/min/100g tissue, (P<0.05)

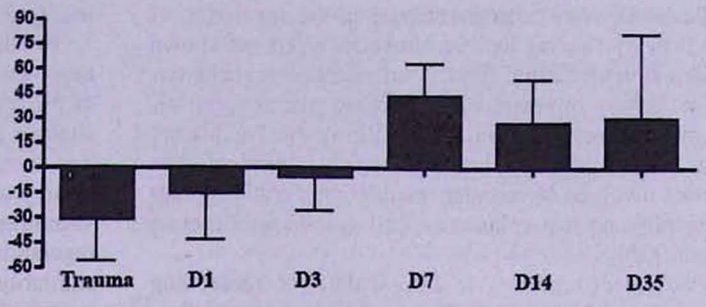

Fig. 3. The differences of LDF measurements of CBF in adult mice in different time points, based on a percentage change from baseline values (intact cortex) after experimental procedures

The most significant changes of CBF were recorded on the 7th day, when in both groups the flow reached the highest values at all investigation time points, 43 in adults and 56% in babies compared to the pre-injury level of the flow. On the 14th and 35th days we observed restoration of the flow in both groups (Fig. 3 and 4).

The mean values of the flow are presented by these numbers: the flow in the intact cortex is about 25 *mlLD/min/100gTissue* ±8 (in adults) and ±5 (in babies) (P<0.05, the difference is not significant).

The fact of decreasing of the blood flow immediately after injury still demands further understanding. Recently it was shown the connection between the CBF decrease and NO level reduction after moderate TBI, increase in the cytokines expression [2]. Moderate, parasagittal experimental TBI caused a significant decrease in absolute blood flow as measured by LDF when compared with the respective baseline control group. Changes were observed within minutes of TBI and were not significantly different at 30 and 60 min [6]. Trauma impairs the ability of the brain to regulate CBF, the cerebrovascular response after TBI may be inadequate [5]. Cerebral hypoperfusion and impaired autoregulation also have been described in cats subjected to fluid percussion injury TBI [14]. In rats subjected to experimental TBI, CBF decreased [36, 42] and

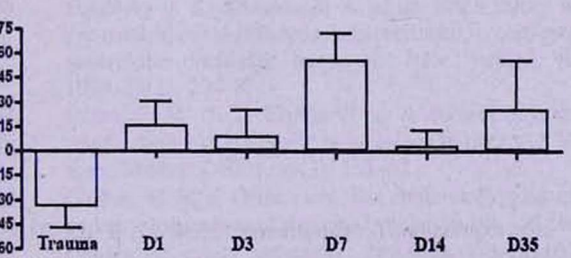

Fig. 4. The differences of LDF measurements of CBF in immature mice in different time points, based on a percentage change from baseline values (intact cortex) after experimental procedures

cerebral vessels ex vivo showed impaired vascular reactivity [34]. After weight-drop closed head injury in rats, studies using laser Doppler flowmetry suggested abnormalities of pressure autoregulation both in response to increased blood pressure [37] and hemorrhagic hypotension [19].

One of the probable ways of its understanding is cerebral circulation, dramatically reduced regional and affected general CBF. Blood flow to the brain is fundamentally controlled by changes in diameter of resistance blood vessels. In normal conditions the cerebral circulation has the ability to maintain a stable CBF over a wide range of cerebral perfusion pressures (CPP), this phenomenon is designated as cerebral autoregulation and mainly represents the capacity of the brain's resistance vessels to dilate in response to a decrease in CPP or to constrict in response to an increase in CPP. The caliber changes of the autoregulatory vessels are mediated by myogenic, metabolic, or neurogenic mechanisms [37].

The smaller arterioles dilate proportionally more than larger arterioles at a mean arterial blood pressure below physiological levels [24, 28]. However, the larger arterioles tend to be more responsive than the smaller ones at normal and increased levels of arterial pressure.

Cerebral microcirculation depends on the very delicate interaction of vasoconstrictors and vasodilators, and TBI is roughly interacting with that system, leading to release of cerebral vasoconstrictors [15] and decrease in the production, or affects the activity of vasodilators [3].

Another finding associated with CBF reduction at early time points after TBI is the increased cytokine expression and production, though the role of their activity changes is not clear yet [2]. Destruction of cortical regions could effectively produce differentation in subcortical and cortical target regions resulting in a reduced energy requirement and metabolic rates. If flow and metabolism are tightly coupled after cortical injury, reduced flow in some of these regions would follow the reduced metabolic rate [8].

The blood vessel can participate in the regulation of blood flow by altering its own structure, a process known as vascular remodeling. This is an adaptation characterized by changes in vessel wall thickness, matrix composition, and wall organization, which allows the vasculature to cope with physiological or pathological conditions. The processes involved in vascular remodeling include cellular hypertrophy and hyperplasia, as well as enhanced protein synthesis [26].

Some recent reports give similar data for succeeding next two days after controlled cortical impact injury. Performed measurements of pericontusional blood flow allow to state that cortical hypoperfusion found within the early phase following trauma is reversible and precedes a long-lasting phase of hyperperfusion [40].

So, the reduction of CBF immediately after severe TBI onset could be explained by the changing histochemistry of the brain and caliber of the vessels, but the symmetric changes in CBF in immature and mature brain on the 7th day demand further investigation and understanding. The most probable reasons of it could be the prevalence of vasodilatation effect due to impairment of cerebral autoregulation and expressed production of vasodilators. The similarity of cerebral hemodynamic disturbances in both groups proves that CBF cannot be considered as one of the reasons for increased vulnerability of the immature to neurotrauma.

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Ուղեղի արյան շրջանառությունը փորձարարական նեյրոտրավմայի ժամանակ

Վ.Մ. Մանվելյան

Գլխուղեղի արյան շրջանառության խանգարումները նեյրոտրավմայի ժամանակ մեծ դեր են խաղում հիվանդության զարգացման և հետևանքների մեջ: Տվյալ փորձարարական աշխատանքում հետազոտվում են արյան շրջանառության խանգարումները 35 օրվա ժամկետում՝ կիրառելով լազերային դոպլերի ապարատը: Պարզվել է, որ արյան հոսքը վնասվածքից անմիջապես հետո նվազում է, հետագա օրերի ընթացքում վերականգնվում, իսկ 7-րդ օրը հասնում է բարձրագույն ցուցանիշների:

Мозговое кровообращение при экспериментальной нейротравме

О.М. Манвелян

Нарушения мозгового кровообращения при черепно-мозговой травме являются одними из самых важных показателей тяжести и исхода заболевания. Несмотря на критическую роль церебральной гемодинамики, последняя изучена недостаточно и требует детального рассмотрения. В качестве экспериментальной модели выбрано контролируемое корковое повреждение у грызунов, причем измерения мозговой гемодинамики производились с применением аппарата лазерного допплера в течение 35-дневного срока.

Анализ полученных данных свидетельствует о резком падении кровотока непосредственно после травмы, постепенном восстановлении в последующие дни и характерном пике на 7-й посттравматический день. В последующие дни сохраняется тенденция к восстановлению уровня дотравматического кровотока.

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