можуть ослабити стінку, а також істотний градієнт тиску крові в поперечних перерізах в місцях перегину судини. Градієнт тиску може впливати на складання судини – перехід кута звивистості від тупого до гострого – еволюції звивистості. Величина градієнту тиску також істотно збільшується із зменшенням кута перегину. Отже, проведене дослідження свідчить про можливу еволюції звивистості.

Summary

BIOMECHANICAL MODELING OF DIFFERENT TYPES OF PATHOLOGICAL KINKING OF INTERNAL CAROTIDS

Kuzyk Yu.I., Ivanov D.V., Dol A.V.

Key words: kinking of carotids, the finite element method, biomechanics of pathological tortuosity.

Pathological tortuosity of internal carotid artery (PT ICA) is one of common causes of stroke. Etiology, pathogenesis and prediction of PT ICA remain little known. We studied effective and circumferential stress, distribution of hemodynamic forces in different types of PT ICA, as well as mathematical forecasting of the behavior of different types of tortuosity. We used the finite element method ANSYS. Reducing the angle of inflection, and its moving to more acute leads to the fact that kinking becomes hemodynamically significant, and the blood flow volume can be reduced by 40% or more. There are areas of high concentration of circumferential stresses that can weaken the wall, as well as a significant blood pressure gradient in the cross sections of the vessel in areas of inflection. The pressure gradient can affect the folding of the vessel - the transition from the obtuse angle to acute one – so called tortuosity evolution. The magnitude of the pressure gradient also substantially increases with decreasing angle of bend. Thus, this study suggests the possible tortuosity evolution.

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Makarenko O.M.

EFFECT OF "CEREBRAL" ON HUMAN BLOOD CELLS

Hrihoriy Skovoroda State Pedagogical University of Pereyaslav-Khmelnitskiy

The effect of trophinotropin "Cerebral", endogenous therapeutic factor, on the properties and shape of human blood cells was studied. Whole blood and plasma were incubated with "Cerebral". Incubation with "Cerebral" considerably increased concentration the "Cerebral" increased the concentration of white and red blood cells, but didn't have significant difference in vesiculability of cells compared with the control sample. Cerebral preserved the discocytic shape of erythrocytes comparable to the control.

Introduction

The group of endogenous therapeutic factors, which are actively secreted by cells of the organism being in the state of restoration after pathological process modeling or under recovery/remission have got the name of trophinotropin (TT) or neurotrophinotropic growth factors (NGF) and recently they are widely involved in pharmacological researches.

Cerebral is the agent, which separated from nerve cells of the cerebral cortex of pigs, experienced bihemispheric autohemorrhagic stroke. It contains the complex of low-molecular pharmacologically active peptides (less than 1200 Da) [1].

The study of the properties of biological membranes is necessary in assessment of the effect of various added substances on cells, tissues, and organisms.

Extracellular vesicles (EVs) are membrane-enclosed cell fragments which are shed by cells of all types into extracellular solution [2,3]. EVs which are obtained from tissues and body fluids could be used as a basis of new materials and technologies in medicine. But the mechanisms of EV's formation, properties, and effects aren't studied well.

Erythrocytes are a convenient system for study of mechanisms and interactions relevant for cell membrane, because they don't have cytoskeleton (aside from the membrane skeleton) so that these mechanisms and interactions affect the cell shape, which can be directly observed by optical microscope.

The aim of this work is to sudy in vitro effects of a "Cerebral" on blood cell membrane nanovesiculation and erythrocyte shape change.

Material and methods

Cerebral and control sample were dissolved in 2 ml of bidistilled water. Blood sampling was performed according to the Helsinki declaration considering research on humans, the Oviedo convention on human rights and biomedicine. Blood was collected from five volunteers with no record of disease (female, mean age 21 years). Volunteers fasted at least 12 hours before blood sampling. For isolation of EVs and for blood cell count, blood was withdrawn from the medial cubital vein with 21 gauge needle (MULTI Sample Needle, Nipro Corporation, Tokyo, Japan) using 2.7 mL vacutubes which contained 270 μL of sodium citrate at a concentration 0.109 mol/L (Becton Dickinson, New Jersey, USA). We took 2 epruvettes of blood from

each subject (one for the test substance and one for the control). Variation in the acquired volumes of blood did not exceed 15%. During the processing, the tubes and blood samples were kept in thermoblocks at 37 °C. For analysis of the effect of substances on the blood cell membranes, a drop of blood was inserted into an Eppendorf tube previously filled with 1 mL of bidistilled water or citrated phosphate-buffered saline (PBS). The sample was further diluted to obtain a required density of blood cells for observation. The required density rendered a monolayer of relatively closely packed cells in an observation frame under the microscope [4].

Within each sampling session, processing of blood started within 20 minutes from the sampling of the first sample. 2 ml of blood blood was centrifuged at 1550 g and 37°C for 20 minutes in a Centric 200/R centrifuge (Domel d.o.o., Železniki, Slovenia). Upper 250 μL of plasma was slowly removed and placed in a 1.8 mL Eppendorf microtube. The samples were then centrifuged at 17570 g for 5 minutes in a Centric 200/R centrifuge (Domel d.o.o., Železniki, Slovenia), so that EVs were gathered on the bottom of the microtube. The upper 210 μL of plasma was discarded and the remaining 40 μL of pellet was vortexed at 1200 g and resuspended in 210 μL of PBS (pH = 7.4). The samples were centrifuged again at 17570 g for 5 minutes. 210 μL of the supernatant was discarded and 40 μL of pellet resuspended in 60 μL of citrated phosphate-buffered saline (pH = 7.4) [4].

Concentration of EVs in isolates was determined by MACSQuant Analyzer (Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany) flow cytometer with 405 nm, 488 nm and 640 nm air cooled lasers. The MACSQuantifyTM (Miltenyi Biotec GmbH, Bergisch-Gladbach, Germany) software version 2.4. was used for data acquisition and analysis of the results. 25 L of sample was measured. The presence of residual cells and EVs was determined by forward and side scatter parameters. Typically, the region of events corresponding to EVs (marked by the red rectangle) extends over wide intervals of FSC/SSC that reflect the detected particle sizes and surface properties. The region contained more than 90 % of events in all density plots [4].

To observe the effect of “Cerebral” on erythrocytes, a drop of blood was dissolved in 1 ml of bidistilled water or phosphate buffer saline. 60 μl of dissolved blood was placed into the observation chamber (1.5 x 1 cm^2) formed on the glass by the silicon grease. 20 μl of test substance (or water for control) was added into the chamber and closed by the cover glass. The sample was observed immediately under the Leitz Aristoplan (Leitz, Wetzlar, Germany) optical microscope equipped by the Watec (Model: 902DM3S), Watec Inc., New York, USA, camera and Pinnacle Studio HD, Version 15.0.0.7593 framegraber and software, Avid Technology Inc., USA. Care was taken not to leave voids in the grease boundary in order to prevent evaporation of liquid from the observation chamber. For quantitative analysis of the effect of the substance Cerebral and its control on erythrocytes we randomly imaged at least 50 frames over the sample. We avoided regions close to the silicon grease. For other substances we imaged 20 frames over the sample.

For each sample we created an ensemble of pictures from which we omitted pictures of invalid quality. We counted the number of discocytes, echinocytes and stomatocytes in each picture. It was assumed that each picture was representative for the sample and that the number of cells of each type fluctuated around the corresponding average value. The average values of the percent of discocytes, echinocytes and stomatocytes were calculated for each sample. Altogether, more than cells were included in the analysis of each observer [4].

450 microliters of fresh blood was incubated with 50 microliters of the “Cerebral” (or water for control) for 2 hours. Then, blood cells were counted by a flow cytometer that is used for standard blood test (at the commercial laboratory Adrialab).

To determine the difference between the populations or samples, the two tailed paired t-test was used. The statistical significance of the t-test was given by the probability p. For calculation, we used Microsoft Excel software (Microsoft® Office Excel® 2007 SP3.

Results and Discussion

Results involving blood of 5 healthy subjects show that the concentration of white blood cells (WBC) was somewhat increased in sample incubated with Cerebral compared to control sample in all subjects, the concentration of red blood cells (RBC) was slightly increased in the test sample in 4 out of 5 subjects and the concentration of platelets (PLT) was slightly decreased compared to control sample in 4 out of 5 subjects. There was no trend in the mean volume of platelet (MVP). The differences between the test and the control samples were within the range of variation of the respective parameters in population of healthy subjects (Fig.1).

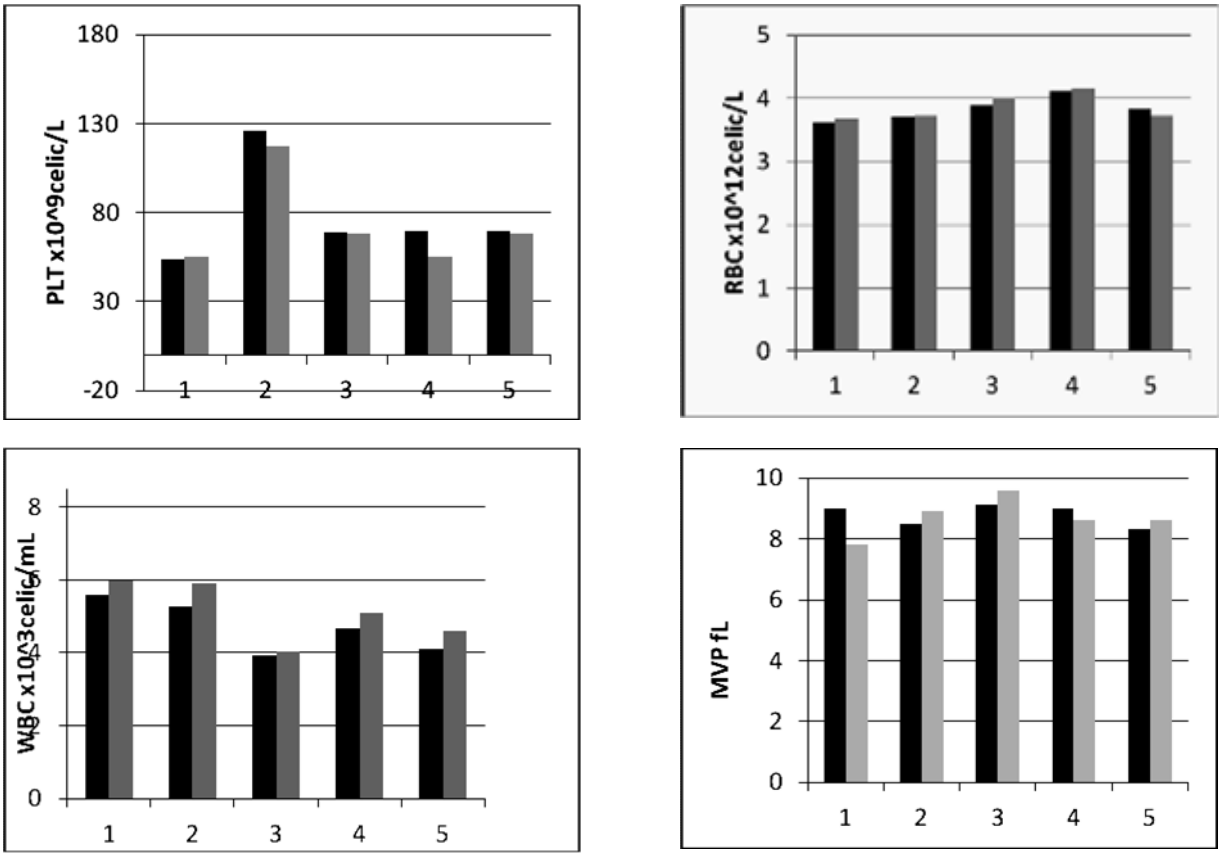


Fig.1. *Effect of substance Cerebral on blood cell count. Left (darker) columns represent the control (blood samples incubated with water) and right (lighter) columns represent the test samples. Numbers 1-5 pertain to 5 blood donors.*

In isolate from the sample incubated with Cerebral, the concentration of extracellular vesicles was 667/μl and in control sample of the same blood was incubated with the respective volume of bidistilled water the concentration of extracellular vesicles was 534/μl. The region corresponding to extracellular vesicles contained 81% of all events in the test sample and 72% in the control sample. Although the concentration of extracellular vesicles was somewhat higher in the sample incubated with "Cerebral", the difference with respect to the control sample was relatively small comparing to the variance of the concentration of extracellular vesicles in control samples. The concentrations of extracellular vesicles were in the interval from 527/μl to

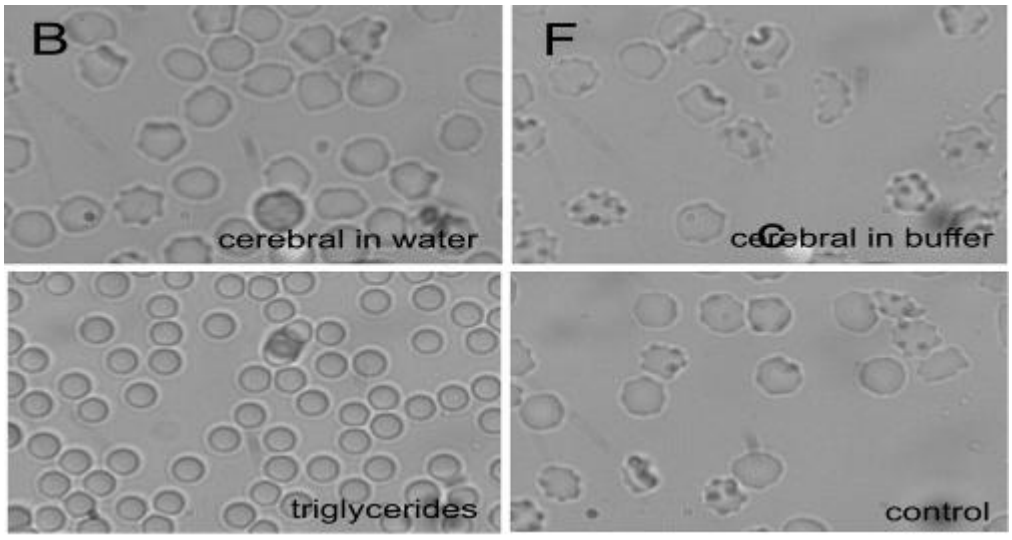


Fig.2. Effect of the "Cerebral" on the erythrocyte shape after 1 hour of incubation. Sample with triglycerides is for the comparing.

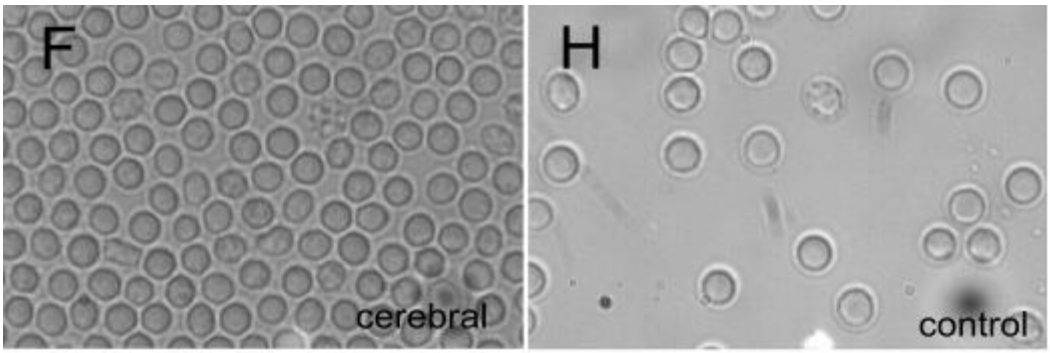
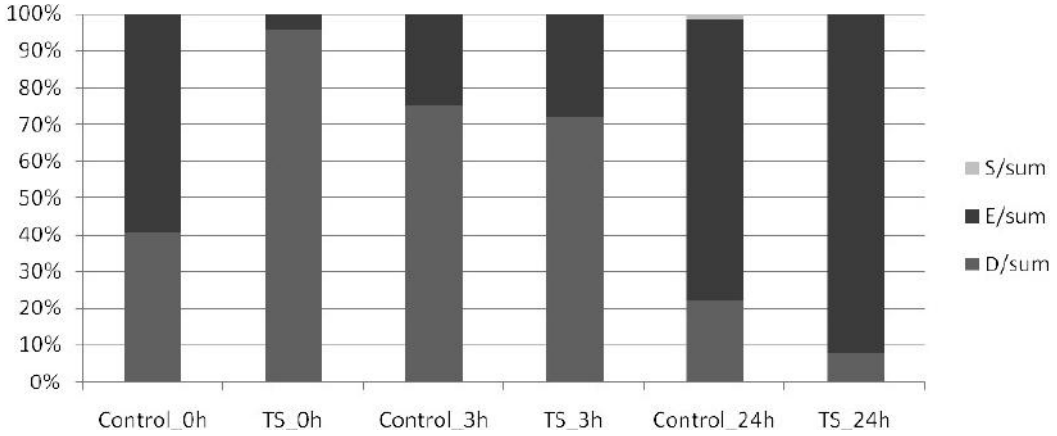


Fig.3. Effect of the "Cerebral" on the erythrocyte shape after 1 week of incubation.

We analyzed the effect of the "Cerebral" on the erythrocyte shape. We compared the number of cells and the percent of discocytes ("D"), stomatocytes ("S") and echinocytes ("E") with the control (cell solution and bidistilled water) immediately after the samples were placed into the observation chamber, after one hour, after three hours and after 24 hours.

Initially, echinocytes prevailed in the control sample over the discocytes while in the test sample, most cells were discocytic. After 3 hours, the test and the control sample were similar with majority of discocytes. After 24 hours, discocytes were still present in both samples, but the majority of cells were echinocytic. We observed a small amount of stomatocytes in the control sample after 24 hours.



Conclusions

We conclude that the "Cerebral" increased the concentration of white and red blood cells, but didn't have significant difference in vesiculability of cells compared with the control sample.

Cerebral preserved the discocytic shape of erythrocytes comparable to the control. All differences between samples except for the difference between the test and the control sample after 3 hours were statistically significant, due to a huge number of cells analyzed (more than 13000 altogether).

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Реферат

ВЛИЯНИЕ «ЦЕРЕБРАЛА» НА КЛЕТКИ КРОВИ ЧЕЛОВЕКА

Макаренко А.Н.

Ключевые слова: Церебрал, красные кровяные клетки, белые кровяные клетки, тромбоциты, везикулярность.

Изучено влияние трофинотропина "Церебрала", эндогенного лечебного фактора, на свойства и формы клеток крови человека. Цельная кровь и плазма инкубировалась с "Церебралом". Инкубация с "Церебралом" значительно увеличивала концентрацию белых и красных клеток крови, но не оказывала статистически достоверного влияния на везикулярность клеток по сравнению с контрольным образцом. «Церебрал» сохранял дискотическую форму эритроцитов в сравнении с контролем.

Реферат

ВПЛИВ «ЦЕРЕБРАЛУ» НА КЛІТИНИ КРОВІ ЛЮДИНИ
Макаренко О.М.
Ключові слова: Церебрал, червоні кров'яні клітини, білі кров'яні клітини, тромбоцити, везикулярність.

Вивчено вплив трофінотропіну "Церебрал", ендогенного лікувального фактору, на властивості і форми клітин крові людини. Цілісну кров і плазму інкубували з "Церебралом". Інкубація з " Церебралом" значно збільшувала концентрацію білих і червоних клітин крові, але не мала статистично достовірного впливу на везикулярність клітин в порівнянні з контрольним зразком. «Церебрал» зберігав дискотичну форму еритроцитів в порівнянні з контролем.

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Макаренко А.Н., Ковтун А.Н., Петров Ф.И.

ИЗМЕНЕНИЯ В ГЛИАЛЬНОЙ СИСТЕМЕ III И V СЛОЯ ЦЕРЕБРОКОРТЕКСА БЕЛЫХ КРЫС ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ВОСПРОИЗВЕДЕНИИ ОСТРОЙ ЦЕРЕБРОВАСКУЛЯРНОЙ ПАТОЛОГИИ

Киевский Национальный Университет им. Тараса Шевченко, г. Киев
ГВУЗ «Переяслав - Хмельницкий государственный педагогический университет имени Григория Сковороды», г. Переяслав-Хмельницкий
Институт продовольственных ресурсов НААН Украины, г. Киев

Работа посвящена изучению динамики глио-глиальных взаимоотношений, происходящих в III и V слоях сенсомоторной зоны цереброкортекса в условиях моделирования острого нарушения мозгового кровообращения (ОНМК). Изучены различия структурного и количественного реагирования глиальных клеток мозга и их взаимозависимости в условиях воспроизведения экспериментальной цереброваскулярной патологии, через 7 дней и 7 месяцев после этого. Взаимосвязь между клеточными элементами определяли с помощью авторских методик исследования клеточных образований мозга – глиальной формулы (ГФ) и глиального индекса количественного (ГИК). Продемонстрированы особенности и динамика количественно-качественных нарушений глиального гомеостаза мозга в условиях ОНМК.

Ключевые слова: глиальная система, цереброкортекс, глио-глиальные взаимоотношения, острая цереброваскулярная патология.

Введение

Недостаточность мозгового кровообращения – одна из самых распространенных патологий современности, требующая внедрения более эффективных форм терапии пациентов на ранних стадиях лечения [11]. Лечение пациентов с ОНМК, несмотря на существенные достижения медицинской науки, остается актуальной проблемой. Недостаточность мозгового кровообращения сопровождается тяжелой нейрональной патологией, в то время как роль глиальных элементов мозга в этом процессе изучена недостаточно. Нормальное функционирования ЦНС и выживание нейронов во многом зависит от сохранения сложной гаммы взаимосвязей между нейронами и глиоцитами. В нейронауке сформировалось устойчивое представление о нервной ткани именно как о нейроглиальной системе, в рамках которой постулируется возможность осуществления нейрональных функций только с участием глиальной составляющей [10]. Однако современная терапия цереброваскулярных патологий базируется на нейропротекторном подходе, в связи с чем, нейротропная парадигма рассматривает проблему защиты ЦНС только в плоскости нейронопротекции, не учитывая глиопротекторный аспект проблемы. Данная парадигма направлена главным образом на защиту нейронов ЦНС, в то время как реакцию глиоцитов описывают общим термином (глиоз) без детализации и конкретизации особенностей реакции различных типов глиальных клеток на действие патологических факторов ОНМК. В то же время, при развитии нейродегенеративных процессов в головном мозге при инсульте возникает системная реакция всех клеточных элементов нервной ткани. Замена доминирующего на современном этапе развития теоретического нейропротекторного подхода на системноклеточный анализ требует разработать новую парадигму лечения заболеваний ЦНС, учитывая функциональное состояние различных типов клеточных образований головного мозга и помогает объективно понять процессы, протекающие в нервной ткани (и клеточных образованиях мозга) в условиях патологии [4,11].

Цель исследования

Изучение динамики состояния глиальной системы и глио-глиальних взаимоотношений в III и V слоях сенсомоторной зоны цереброкортекса больших полушарий головного мозга крыс в условиях экспериментального воспроизведения острого нарушения мозгового кровообращения, через 7 дней и 7 месяцев после моделирования.

Объект и методы исследования

Работа была выполнена на 20 белых крысах-самцах линии Вистар, средний вес животных