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THE INCREASED KINETICS OF H_2O_2-INDUCED CHEMILUMINESCENCE IN THE PATIENTS WITH LONG-TERM CONSEQUENCES AFTER CEREBRAL CONTUSION

Збільшена H_2O_2-індукована кінетика хемілюмінесценції у хворих з віддаленими наслідками після контузії головного мозку

Abstract

Purpose of the study. *The aim was to study in the patients with long-term consequences after cerebral contusion the intensity of spontaneous and H_2O_2-induced chemiluminescence in order to evaluate the early fast-flowing reactions caused by oxidative stress and associated with the formation of primary radicals such as free radical oxidations.*

Materials and Methods. *Forty-two patients with long-term consequences after cerebral contusion were investigated (39,04 ± 12,84 years mean age; mean onset years 32,56 ± 6,4) where both spontaneous and H_2O_2-induced chemiluminescences were measured directly by HPLC-chemiluminescence assay.*

Results. *The study have showed that sera of the investigated patients with long-term consequences after cerebral contusion have the increased H_2O_2-induced chemiluminescence associated with the high amplitude of «fast» burst and the tendency to increase of spontaneous chemiluminescence ($p = 0,039$ and $p = 0,58$, accordingly). Thus, the patients with long-term consequences after cerebral contusion showed the abnormal high kinetics of H_2O_2-induced chemiluminescence ($p < 0,05$). The statistically significant increase serum H_2O_2-induced chemiluminescence intensity detected in examined patients (3085,6 ± 114,2 vs 669,1 ± 214,83 controls) have showed the development of certain oxidative stress processes in this category of patients associated with the increasing of primary free*

Резюме

Мета роботи. *Вивчення у хворих з віддаленими наслідками закритої черепно-мозкової травми інтенсивності спонтанної та H_2O_2-індукованої хемолюмінісценції для оцінки їх вкладу щодо розвитку ранніх й швидких реакцій оксидативного стресу, які асоційовані з формуванням вільно-радикальних оксидантів.*

Матеріали та методи. *Сорок два хворих з віддаленими наслідками після контузії головного мозку у анамнезі (середній вік 39,04 ± 12,84; середній вік початку захворювання 32,56 ± 6,4 років) були обстежені, де спонтанна та H_2O_2-індукована хемолюмінісценції були вимірювані хемолюмінісцентним методом згідно стандартного протоколу.*

Результати. *У даному дослідженні було показано, що сироватка крові хворих з віддаленими наслідками після перенесеної контузії головного мозку мала збільшені дані щодо H_2O_2-індукованої хемолюмінісценції, яка була асоційована з збільшенням амплітуди спалаху та з тенденцією до збільшення спонтанної хемолюмінісценції ($p = 0,039$ і $p = 0,58$, відповідно). Таким чином, хворі з віддаленими наслідками після перенесеної контузії показали дуже високу кінетику H_2O_2-індукованої хемолюмінісценції ($p < 0,05$). Статистичне збільшення у сироватці крові інтенсивності H_2O_2-індукованої хемолюмінісценції (3085,6 ± 114,2 проти контролю 669,1 ± 214,83) показало розвиток певного оксидантного стресу у даній категорії пацієн-*

radical reactions and their activity were getting increased with the progression of the disease duration ($p < 0,05$).

Conclusions. *The study provides the novel data revealing the increased kinetics of H_2O_2-induced chemiluminescence in the patients with long-term consequences after cerebral contusion accompanied by the tendency to increase of spontaneous chemiluminescence that may play the certain pathogenetic role.*

Keywords: *H_2O_2-induced chemiluminescence, long-term consequences after cerebral contusion, oxidative stress.*

тів, що було пов'язане з збільшенням первинних вільних радикалів, активність котрих зростала із збільшенням тривалості посттравматичного періоду ($p < 0,05$).

Висновки. *Дослідження наводить новітні дані щодо збільшення H_2O_2-індукованої хемолюмінісценції у хворих з віддаленими наслідками після перенесеної контузії головного мозку, яка була асоційована з тенденцією до збільшення спонтанної хемолюмінісценції, що може відігравати значну патогенетичну роль.*

Ключові слова: *H_2O_2-індукована хемолюмінісценція, віддалені наслідки після перенесеної контузії головного мозку, оксідативний стрес.*

INTRODUCTION

Cerebral contusion is a form of traumatic brain injury, pathomorphologically associated with multiple microhemorrhages, small-blood vessel damages and edema in brain tissue, which occurs approximately in 20–30% of severe head injuries [1]. In the United States, the incidence of closed head injury is estimated to 200 cases per 100,000 individuals per year and in Ukraine, it is estimated around 95 to 150 cases per 100,000 individuals [2, 3]. There are two typical contusion injuries: coup brain injury, which occurs when brain is injured on the area where hit occurred and acontre-coup injury which appears when the brain area of the opposite impacted side became injured [1, 2].

From the position of bioenergy, the adequate energetic cell metabolism is also provided by the normal function of mitochondrial and neuron membranes [4]. Numerous studies predominantly from foreign researchers have found that the primary initiating element of defeated neuron membrane is strengthening of the polypeptide following with excessive accumulation of peroxides and other products of free radical oxidations (FROs) which are very toxic to any cell membranes [5–9]. Many human studies have demonstrated that post-contusion period was consisted with progressive peroxidation of mitochondrial and cellular lipids [10]. The highly-reactive free radicals are the most aggressive products of FROs because they have not only the membrane-damaging effect, but also have effect of lipids catalysts, providing autocatalysis.

Cerebral contusion results in rapid reactive oxygen species production and oxidative damage to neuronal components leading to neuronal dysfunction or cell death [4]. One of the important species of this process during any brain injuries induced by oxidative damage is the reactive nitrogen species, which are produced by the impaired brain mitochondria [6, 11, 12].

According to a number of authors, when polymorphonuclear leukocytes and brain tissue interact, cells produce chemiluminescence [13–15]. Luminol-dependent light emission from polymorphonuclear leukocytes is linked to H_2O_2 system and light emission from cell-free spontaneous or H_2O_2-induced chemiluminescence was found to be inhibited by human serum albumin which is a large molecular protein that cannot access to intracellular leukocytes sites, so, this could be used to determine the importance of extra- and intracellular events during induced chemiluminescence. There are previous experimental data regarding cell cultures, where different authors have found that proteins and albumins inhibited more than 90% of extracellularly produced and induced chemiluminescence and such results indicated that chemiluminescence of intracellular origin is limited by the generation of oxidative metabolites of FROs [16, 17].

It was shown that non-control intensification of lipids peroxidation passes in the conditions of imbalance between processes of lipid peroxidation and antyoxidative system activity [18]. Thus, the imbalance between radical-free oxidations and antyoxidative system leads to the development of oxidative stress in organism with the accumulation of excessive amount of free radical which, in its turn, again leads to changes both in the structure and peculiarities of the extra- and internal lipid membranes. This and other effects play the important role in the damage of the nervous system with the following formation of neurological deficit in posttraumatic period and a better understanding of which was a goal of this scientific research.

PURPOSE OF THE STUDY

The aim of this work was to study in the patients with long-term consequences after cerebral contusion the intensity of spontaneous and H_2O_2-induced chemiluminescence to evaluate the early fast-flowing reactions caused by oxidative stress and associated with the formation of primary and the most reactive and aggressive radicals such as FROs.

MATERIALS AND METHODS

Forty-two patients with long-term consequences after cerebral contusion with the mean age of 39,04 years and range aged between 27 and 56 years old were investigated. Patients' characteristic is presented in the table 1. Patients were enrolled in this study at the Institute of Neurology, Psychiatry and Narcology. Thirty healthy controls (mean age ± SD, 35,6 ± 9,21 years) without neurological diseases were included as a group of comparison. Demographic data from each clinical case were retrieved from the patients' history. The definition of cerebral contusion was consistent with the World Health Organization's International Statistical Classification of Diseases and Related Health Problems [3].

For the primary analysis, to assess processes associated with the formation of primary free radicals, chemoluminescent method was used. The serum intensity of super-weak luminescence was studied by using HLMC1-01 chemoluminometer where both spontaneous and induced chemiluminescences were determined by the number of flash units per second. Ferrous iron and hydrogen peroxide were used as the inducers. To measure the kinetics of spontaneous chemiluminescence 0,3 ml serum was put into a 10 × 10 × 45 quartz cuvette with 1 ml of buffer solution (1 liter buffer solution contains 121,14 g of three hundred and 7,8 g of KCl with pH 7,5) and the background of super-weak luminescence was recorded as 6 measurements for 10 s. After that, two types of chemiluminescence were studied: induced by ferrous ions with the addition of 0,05 ml Fe^{2+} to serum and chemiluminescence, induced by hydrogen peroxide with the addition of 0,05 ml 3% H_2O_2 solution to serum. Registration of the induced luminescence was carried out for a minute (6 measurements of 10 s.), followed by the recalculation of the number of flashes per 1 s. In this study, we used the method principles described by [15]: where the emission depletion, caused by an antiradical compound and added during the chemiluminescence decay, was proportional to the number of reactive species trapped.

All samples were performed in a duplicate manner with a coefficient of variation of < 10% ($p \leq 0,05$); protocols were approved by Health Research Ethics Committee; written informed consent was obtained from each patient. Data were analyzed using Excel 2016 and mean values ± standard deviations were used for data with normal distribution, to compare variables, Student's t-test was performed. The linear regression was conducted to approach the relationship between one dependent and more explanatory variable, if the aspects were not comparable, count and percentage were pointed out.

RESULTS AND DISCUSSION

The standard neurological examination has revealed the presence of focal neurological signs, indicating on mesenencephalic and brainstem structures lesions. Among the objective focal neurological symptoms, the most frequent were face asymmetry – 29 patients (69,04%), tendon reflexes increasing – 25 patients (59,52%), coordinating sphere disturbances – 23 patients (54,76%), ataxia – 18 patients (42,85%), horizontal nystagmus – 16 patients (38,09%), pathological foot reflexes – 14 patients (33,3%), rotator nystagmus – 9 patients (21,43%).

The relationship between H_2O_2-induced chemiluminescence neuron injury and the generation of endogenous lipid hydroperoxides have been investigated. Both spontaneous and H_2O_2-induced chemiluminescence endogenously were measured directly by HPLC-chemiluminescence assay. According to literature data, the formation of the most aggressive free radicals reflects the intensity and kinetics of H_2O_2–induced chemiluminescence [4, 6, 7, 11]. It is known that the luminescence level of biological systems depends on the amount of free radicals in the system, and the flash (burst) during the induction occurs at the moment of their recombination, therefore, if the amount of radicals are getting increased, then the probability of recombination have become greater and therefore, the level of luminescence is getting higher [5].

The conducted investigation have revealed that sera of the patients with long-term consequences after cerebral contusion had the increased data of H_2O_2-induced chemiluminescence associated with the high amplitude of «fast» burst and the tendency to increase of spontaneous chemiluminescence that overall, were greater in 4,6 and 0,57 points accordingly ($p = 0,039$ and $p = 0,58$) compare to control data (table 2). Thus, the patients with long-term consequences after cerebral contusion showed the abnormal high kinetics of H_2O_2-induced chemiluminescence ($p < 0,05$) indicating the oxidative mitochondrial damage.

As we can see from the table 2, in the patients with long-term consequences after contusion, the intensity of the induced serum glow was significantly higher than in the control group ($p = 0,0011$), this was evidenced as an increase in the amplitude of the «fast» outbreak in all examined patients.

To evaluate the clinical prognostic value of spontaneous and H_2O_2-induced chemiluminescence data we further segregate among the different disease duration groups. When comparing the kinetics of the intensity of H_2O_2-induced chemiluminescence in serum in the patients with different duration of the post-traumatic period, it was found that the patients with the disease duration no longer than 5 years (n = 16; 38,09%)

was characterized by 16,1% increasing in the amplitude of the «fast» bursts and a tendency to increase of the coefficient of attenuation ($p > 0,05$). In the patients with the disease duration between 5 and 15 years (n = 7; 16,66%), the growth and amplitude of the «fast» burst and coefficient of attenuation were increased till 22,7% ($p > 0,05$). In the patients with the disease duration more than 15 years (n = 19; 45,24%), the increasing of both in the amplitude of the «fast» burst and coefficient of attenuation was revealed compared to control data ($p = 0,001$), which reflect the overvoltage of both oxidant and antioxidant units.

Thereby, a significant increase of serum H_2O_2-induced chemiluminescence intensity was detected in the examined patients with long-term consequences after cerebral contusion during this study and it shows the development of certain oxidative stress pathology in this category of patients that was associated with the increasing of primary free radical reactions, the activity of which increased with the progression of the disease ($p < 0,05$). And our results were consistent with a number of some well-established observations [9, 18].

Table 1

Demographic features of all investigated patients with long-term consequences after cerebral contusion

Data	Patients (n = 42)
Male, n (%)	33 (78,57 %)
Female, n (%)	9 (21,43 %)
Mean patient age ± SD	39,04 ± 12,84
Mean onset ± SD	32,56 ± 6,4
Mean serum albumin level, g/L (ranges)	39,84 ± 6,3 (33–48)
Mean serum albumin level, g/L (ranges) in controls	46,5±2,4 (39–49)

Table 2

Spontaneous and H_2O_2-induced chemiluminescence, some FROs data in the patients with long-term consequences after cerebral contusion

Data	Patient group (n=42)	Control group (n=30)
Activity of superoxyddismutase, conventional units/mg Hb	1,2 ± 0,54	0,49 ± 0,12
Spontaneous chemiluminescence, f/s	38,41 ± 18,1	21,8 ± 6,2
Fe^{2+}-induced chemiluminescence, f/s	345,99 ± 43,7	316,6 ± 31,5
H_2O_2-induced chemiluminescence, f/s	3085,6 ± 114,2**	669,1 ± 214,83
Amplitude of fast burst (imp)	51568,41 ± 935,06*	25066,18 ± 851,84
Coefficient of attenuation (conventional units)	1,9 ± 0,37	1,5 ± 0,03

Примітка: ** $p < 0,05$ – compare to control group;*
*** $p < 0,01$ – compare to control group*

The mean spontaneous and H_2O_2-induced chemiluminescence were 38,4 ± 18,8 and 3085,6 ± 114,2 in all patients with the mean disease duration of 12,8 ± 8,45 years, accordingly, thereby, we have observed the tendency to higher spontaneous and statistically significant increased H_2O_2-induced chemiluminescence in the patients after contusion as compared to controls. Because the patients and controls were not gender matched, H_2O_2-induced chemiluminescence and the coefficient of attenuation were compared between men and women (in controls and patients). The data were not found to be comparable between male and female patients with long-term consequences after cerebral contusion ($p = 0,442$) and controls. In the same samples, these data were not correlated with the age, age of onset and albumin serum levels ($p = 0,82$ for age, $p = 0,96$ for debut age).

The revealed oxidative-antioxidative dysbalance in our patients was presented as high period of amplitude of fast burst and it was associated, in our opinion, with the deficiency of antioxidative capacity. The imbalance of lipid peroxidation plays a certain role in maintain and regulation of homeostatic processes. There is a growing evidence that lipid peroxidation occurs after mild traumatic brain injury and correlates with the long-term consequences severity ($p < 0,05$). Identification of the products of lipid peroxidation and its enzymatic or non-enzymatic nature is the essential target for the pathogenetic-based therapy in these patients.

The limitation of the present study was the lack of objective measures of cognitive functions and

neuroimaging abnormalities, such as extent of atrophy and contrast enhancing pattern to know blood-brain barrier breakage. The patients with long-term consequences after brain contusion might be necessary to provide them with the pathogenetic therapy that can improve metabolic and oxidative state and such interventions can reduce impairment during the secondary brain injury and health costs.

CONCLUSION

The study provides the novel data revealing the increased kinetics of H_2O_2-induced chemiluminescence in the patients with long-term consequences after cerebral contusion accompanied by the tendency to increase of spontaneous chemiluminescence that may play the certain pathogenetic role.

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