Research Article

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The Small Intestine in the Acute Period of Spinal Injury: Early Metabolic Disorders According to Fluorescence-Lifetime Imaging FLIM

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RELEVANCE A special place in the development of enteral insufficiency is given to dysproteinemia, which is one of the leading causes of the development of decubital ulcers in patients with spinal cord injury. Early enteral nutrition partially solved this problem, but the incidence of bedsores still remains high and reaches 68%. The risk of metabolic disorders in the acute period of spinal injury is largely determined by non-occlusive intestinal ischemia against the background of spinal shock, neurohumoral dysregulation; intra-intestinal and intra-abdominal hypertension; change in intestinal microflora. Pathological changes in the intestinal wall occur during the first 20 days after injury and further exacerbate chronic maldigestion, malabsorption, intestinal dyskinesia in patients with traumatic spinal cord disease. New knowledge about the features of early enteral nutrition in patients in the acute period of traumatic spinal cord disease will reduce the risk of decubitus ulcerative defects.

AIM OF THE STUDY To study the dynamics of metabolic processes in the tissues of the small intestine in the acute period of spinal injury.

MATERIAL AND METHODS Wistar rats (n=22). Spinal injury was simulated by acute complete transection of the spinal cord at the level of Th5–Th6 vertebrae. The assessment of metabolic changes in the cells of the serous membrane of the intestine was performed immediately, 3 and 24 hours after injury. The metabolism was assessed in vivo using fluorescence time-resolved macroimaging technology FLIM by autofluorescence in the spectral channel of the metabolic cofactor nicotinamide adenine dinucleotide (phosphate).

RESULTS The acute period of spinal cord injury is accompanied by a change in the endogenous autofluorescence of the serous membrane of the small intestine: a statistically significant decrease in the mean fluorescence lifetime (τm), the lifetime of the long component (τ2), and the relative contribution of the long component (a2) in 24 h after injury was recorded. The changes observed using FLIM confirm the catabolic type of metabolism in the tissues of the small intestine after spinal cord injury.

CONCLUSION For the first time in the experiment in vivo it has been shown that the acute period of spinal injury is accompanied by a violation of metabolic processes in the tissues of the small intestine. This fact requires a more balanced approach in calculating the calorie content of nutrients used for early enteral nutrition in patients with spinal cord injury.

Keywords: spinal injury, autofluorescence, FLIM, metabolism, catabolism, bedsores, decubital ulcers, sarcopenia, energy metabolism
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NAD(P)H – nicotinamide adenine dinucleotide (phosphate)
TSCI – traumatic spinal cord injury
FAD – oxidized flavin adenine dinucleotide
FLIM – fluorescence lifetime imaging

INTRODUCTION

Enteral insufficiency is one of the most significant and least studied components of traumatic spinal cord disease (TSCI). It is known that enteric insufficiency in patients with spinal cord injury is characterized by impaired absorption, secretory, motor, evacuation, immune, and barrier functions of the digestive tract [1–3]. At the same time, enteral insufficiency becomes the main cause of dysproteinemia, imbalance of minerals and electrolytes [4-6]. Metabolic disorders potentiate muscle cachexia and increase the risk of developing decubital ulcers [7–9]. That is why the study of development mechanisms, prevention and treatment of enteral insufficiency in patients with TSCI is a task of paramount clinical importance [10–12].

Recommendations "For the treatment of acute complicated and uncomplicated spinal trauma in adults" and other authoritative sources suggest early enteral nutrition with a high content of protein products as a preventive and therapeutic measure [13, 14], since the key pathogenetic component of enteral failure associated with TSCI is dysproteinemia [15–18]. However, it is also known that the digestion, absorption and assimilation of protein products is an energy-consuming process, in which food thermogenesis reaches a level of 30–32% [19–23]. Perhaps a significant cause of dysproteinemia in patients after spinal cord injury is metabolic imbalance caused by a deficiency in energy metabolism. However, an objective assessment of metabolic disorders in the intestinal wall after spinal cord injury is a difficult task: for a long time there was no appropriate research equipment to record functional metabolic changes.

The possibility of monitoring metabolic processes in vivo appeared with the development of the technology for noninvasive analysis of metabolic cofactors in living cells, namely, with the introduction of time-resolved fluorescence imaging (fluorescence lifetime imaging, FLIM) into research practice [24–27]. The technology has demonstrated high efficiency in the study of metabolic processes in various tissues. FLIM is able to detect changes in the balance of glycolytic and oxidative metabolism of cells based on the detection of autofluorescence of dehydrogenase cofactors — reduced nicotinamide adenine dinucleotide (phosphate) (NAD(P)H) and oxidized flavin adenine dinucleotide (FAD). Of these, the NAD(P)H fluorescence decay rates have a simpler and more unambiguous interpretation — the free form of NAD(P)H, which has a short fluorescence lifetime (~0.45 ns), is associated with glycolysis, and the bound form with a longer fluorescence lifetime (~2–3 ns) is associated with mitochondrial

respiration. In addition to metabolic cofactors, collagen and elastin proteins, which change their structure during catalytic imbalance of metabolism, can contribute to tissue autofluorescence. The advantage of FLIM macroimaging over two-photon time-resolved microscopy is the ability to quickly examine sufficiently large tissue areas, which is of interest for further use of this method in the clinic [1–7, 28]. Potentially complex objective diagnostics of these metabolites and proteins can be the best way to assess the intensity of catabolism in the tissues of the intestinal wall.

Thus, to test the hypothesis about the trigger mechanism of enteral insufficiency in the acute period of TSCI, it is advisable to use the FLIM diagnostic technology. Despite the high clinical significance of the topic, similar studies have not been previously conducted in Russia or worldwide.

Purpose of the study: to study the dynamics of metabolic processes in the tissues of the small intestine in the acute period of spinal injury in an animal experiment.

MATERIAL AND METHODS

Wistar rats (males, weighing from 230 to 285 g, n = 22). The keeping of animals in a certified vivarium of the Federal State Budgetary Educational Institution of Higher Education "PIMU" of the Ministry of Health of Russia and research work were carried out in accordance with international rules ˝Guide for the Care and Use of Laboratory Animals˝, and met the requirements of the European Convention for the Protection of Vertebrate Animals Used for Experiments or Other Uses for scientific purposes" dated March 18, 1986. The study was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education PRMU of the Ministry of Health of Russia, protocol No. 17 dated 10/11/2019. Surgical interventions during the experiment were performed under general anesthesia with a mixture of solutions of 3.5% tiletamine hydrochloride, zolazepam and 2% xylazine hydrochloride (in a volume proportional to body weight), which were administered intraperitoneally.

Of the 22 animals, in 20 cases spinal injury was modeled, after which metabolism of the small intestinal wall was studied; 2 animals were included in the control group; intestinal samples were taken from them for histological examination without modeling a spinal injury (Fig. 1).

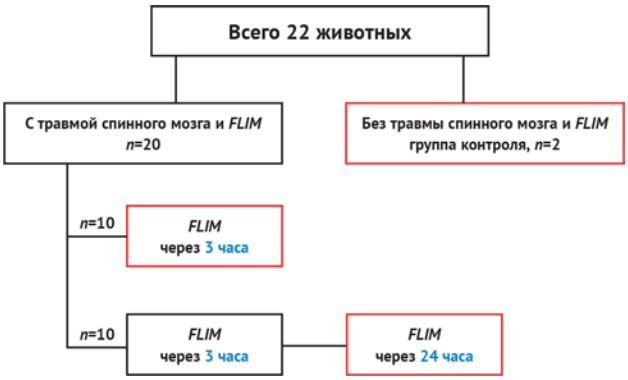

Fig. 1. Scheme of the experiment. Red frames — the stage of sampling the small intestine for histologic examination

Note: FLIM - fluorescence-lifetime imaging

At the first stage of the experiment, all 20 animals underwent a median laparotomy 2 cm long under general anesthesia. For the study, a 5 cm long section of the small intestine, located 18–20 cm distal to the ligament of Treitz, was selected and brought into the wound. Registration of autofluorescence of intestinal tissues was carried out from the side of the serous membrane of the small intestine. Metabolic parameters were recorded before spinal injury and at different time intervals (3 and 24 hours) after the simulated injury.

The modeling of spinal injury consisted of complete transection of the spinal cord at the level of Th5 – Th6 vertebrae after laminectomy [29, 30] (Fig. 2A). The wall of the small intestine was scanned from the side of the serous membrane: immediately after the injury and 3 hours after the injury in 10 laboratory animals, in which, after this period of time, a section of the small intestine was taken for histological examination. In other 10 animals, an additional metabolic study of the intestinal wall was performed 24 hours after injury, followed by sampling of histological material.

Changes in metabolic processes were recorded in vivo in the spectral channel of the nicotinamide adenine dinucleotide cofactor NAD(P)H. We used an original two-channel confocal FLIM/PLIM macroscanner (Becker&Hickl, Germany) with single-photon fluorescence excitation using picosecond lasers (Fig. 2c) [25, 30]. The macroscanner makes it possible to obtain fluorescent time-resolved images from the field of view up to 16 × 16 mm in size with a spatial resolution of up to 15 μm [27]. Registration of the fluorescence lifetime was carried out according to the principle of time-correlated counting of single photons TCSPC. Fluorescence was excited with a picosecond laser at a wavelength of 375 nm and detected in the range of 435–485 nm. The excitation radiation power was 12 mW, the photon collection time was 60 s. The number of photons per pixel was at least 5,000. The experiments were carried out in a darkened room with detectors isolated from external illumination. For scanning, the object was placed under the lens of the macroscanner (Fig. 2C), positioned, and manual focusing was carried out, taking into account the desired area of interest.

FLIM data analysis was performed using the SPCImage 9.87 program (Becker&Hickl, Germany). Fluorescence decay curves were approximated by a biexponential model (χ^2 0.8–1.2). In the course of the work, the following parameters of endogenous fluorescence decay of the intestinal tissue were analyzed: the weighted average lifetime (τ_m), the fluorescence lifetimes of the short and long components (τ_1 and τ_2) and their relative contributions (a_1 and a_2, $a_1 + a_2 = 100\%$) [23, 25]. The short component corresponds to the free form of NAD(P)H, its long component corresponds to protein-bound NAD(P)H. For calculations, 2–3 areas of interest were identified in each image, excluding areas with artifacts and the presence of blood (Fig. 3).

As a result, endogenous fluorescence decay parameters were calculated for 50 areas of the small intestine from the side of the serous membrane (18 before spinal injury, 16 — 3 hours after injury, and 16 — 24 hours after injury). Accordingly, FLIM data were distributed into three groups: normal, 3 and 24 hours after injury.

After fluorescent imaging, the studied sections of the intestine of experimental animals were taken for pathomorphological examination in 10 animals 3 hours after injury and in other 10 animals after 24 hours. Samples were fixed for 24 hours in 10% buffered formalin, then they were subjected to standard wiring and paraffin embedding procedures. Transverse histological sections 5 μm thick were made from the middle part of the sample and stained with hematoxylin and eosin. Histological preparations were interpreted by an independent pathologist.

For statistical data processing, the IBM SPSS Statistics program was used . 20. Assessment of the statistical significance of differences when comparing groups by quantitative characteristics was carried out using the Wilcoxon test for non-parametric samples. Data are presented as Me [Q1; Q2], where Me is the median, Q1 is the lower quartile, Q2 is the upper quartile, n is the volume of the analyzed subgroup, p is the statistical significance of the differences. The critical significance level was taken equal to 5% ($p \leqslant 0.05$).

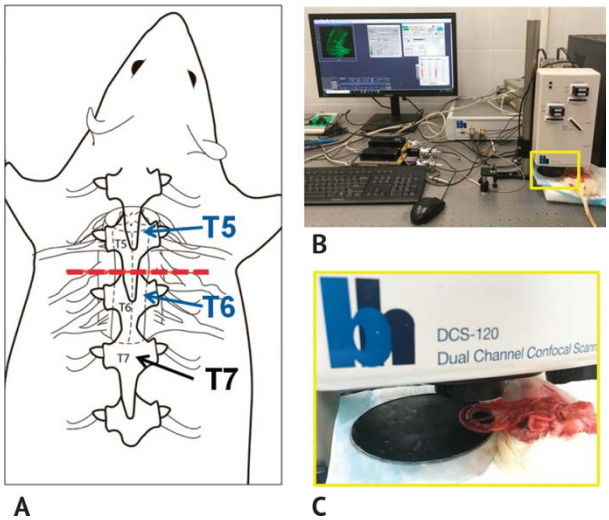

Fig. 2. A — scheme of spinal injury; B — FLIM-macroscanner and registration of endogenous fluorescence from the surface of the rat small intestine in vivo; C — enlarged fragment of image B

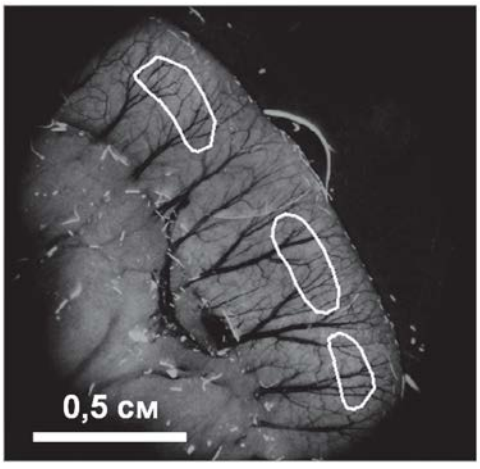

Fig. 3. An example of the selection of regions of interest on FLIM images of the rat small intestine from the side of the serosa to calculate the decay parameters of endogenous fluorescence

RESULTS

In the course of the study by the FLIM method, it was found that the initial value of the weighted average life time (τ_m) in the serous membrane is 1.50 [1.24; 2.08] ns. 3 hours after the injury, this indicator did not change statistically and amounted to 1.46 [1.02; 1.86] ns (p = 0.461), and only after 24 hours after spinal cord injury did the parameter τ_m statistically significantly (p = 0.0001) decrease to 1.22 [1.11; 1.35] ns. The values of short τ_1 and long τ_2 lifetimes in the control before injury were ~0.5 ns and ~5.8 ns. At the same time, the short survival time remained unchanged during the entire observation period after injury, which corresponds to the concept of the stability of the free form of NAD(P)H in the cellular microenvironment. The long survival time shortened to 3.8 ns (p = 0.0001) 24 hours after injury. The values of the percentage contribution of the short component (a_1) were equal to 76.7% [72.3; 81.3] at baseline, 78.2% [74.6; 81.3] after 3 hours (p = 0.272) and 78.9% [75.3; 79.7] after 24 hours (p = 0.019). As for the percentage contribution of the long component (a_2), the initial values of this indicator varied at around 23.1 [18.6; 27.6]%, after 3 hours no statistical decrease was recorded - 21.9 [18.6; 25.3]%, a statistically significant decrease in this indicator to 21.3 [20.0; 22.2% was observed after 24 hours (p = 0.0001) (table)

Table

Autofluorescence lifetime parameters calculated for the serosa of the small intestine

Parameter	Norm (before injury)	After 3 hours after injury		After 24 hours after injury	
	Me [Q1; Q2]	Me [Q1; Q2]	p *	Me [Q1; Q2]	p *
τ_m (ns)	1.49 [1.20; 2.09]	1.45 [1.01; 1.86]	0.461	1.19 [0.98; 1.32]	0.0001
τ_1 (ns)	0.52 [0.46; 0.58]	0.54 [0.40; 0.62]	0.959	0.53 [0.48; 0.60]	0.441
τ_2 (ns)	5.8 [4.0; 6.3]	5.7 [3.2; 6.6]	0.573	3.8 [3.0; 4.5]	0.0001
a_1 (%)	76.7 [72.3; 81.3]	78.2 [74.6; 81.3]	0.272	78.9 [75.3; 79.7]	0.019
a_2 (%)	23.1 [18.6; 27.6]	21.9 [18.6; 25.3]	0.268	21.3 [20.0; 22.2]	0.0001

N about te: * - Wilcoxon test, comparison of this group with the "norm" group

Figure 4 shows typical macro-photos (A–C), macro- FLIM images of the small intestine of rats for the τμ παραμετερ (D–F) and a comparison diagram for the τμ parameter in the studied groups (G).

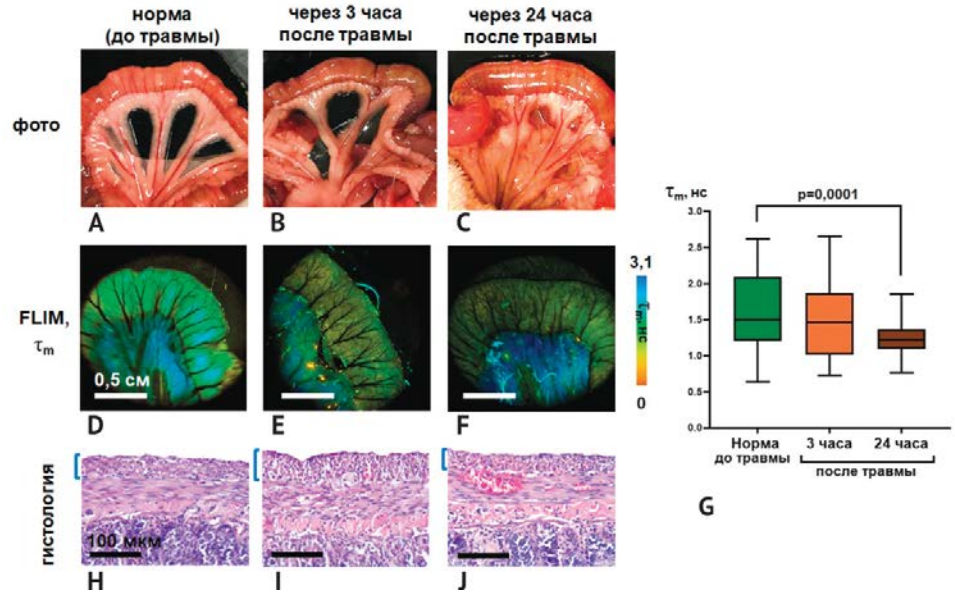

Fig. 4. Macrophoto, macro-FLIM and histology of the intestine of rats from the serosa in different periods after spinal injury. A–C: macro photo; D–F: FLIM-images, parameter τ_m (D–F); G: comparison diagram of the parameter τ_m in the studied groups; H–J: histological images, stained with hematoxylin and eosin. The blue bracket indicates the thickness of the serous layer. A, D, H: normal group; B, E, I — injury group after 3 hours; C, F, J: injury group after 24 hours

The visually normal intestine had a pink color (Fig. 4A) and actively peristalted. After the spinal injury, no obvious pathological processes were observed, however, by the end of 3 hours, the peristalsis of the intestinal wall visually decreased, the serous membrane became somewhat dull relative to the norm (Fig. 4B). 24 hours after the spinal injury, the macroscopic picture was consistent with the signs of dynamic intestinal obstruction: the intestinal loops were swollen, there were no peristaltic movements, the intestinal wall was somewhat edematous, the serous membrane was dull with areas of significant hyperemia (Fig. 4C).

According to the morphological study, 3 hours after the spinal injury, no changes in the serous membrane were observed (Fig. 4I) and histologically, the tissues corresponded to the normal state (Fig. 4H). The mild inflammation was observed in the serous membrane in 25% of cases after 24 hours, severe diffuse inflammation up to the formation of small foci of necrosis was observed in 42% of cases. Neutrophilic granulocytes predominated among inflammatory cells in all cases. Also, in 58% of cases, acute dyscirculatory disorders of microcirculation were recorded (42% moderate and 16% severe), in the remaining 42% of microcirculation disorders were not detected. These changes in 92% of the samples were accompanied by mild focal edema (Fig. 4J).

THE DISCUSSION OF THE RESULTS

The data obtained indicate that the acute period of spinal cord injury is accompanied by disruption of metabolic processes in the tissues of the intestinal wall. It is well known that many pathological changes in the small intestine, primarily acute disorders of absorption and digestion, begin from its mucous membrane. Based on these fundamentally sound ideas, the "ideal experiment" would be a study that allows non-invasive assessment of metabolic changes in the mucous membrane and enterocytes. This study would probably make it possible to obtain even more pronounced dynamics of the studied parameters. However, at the current level of technology development, atraumatic FLIM study of the mucous membrane in vivo is impossible due to the lack of endoscopic sensors of the required size. To access the mucosa, it would be necessary to perform an enterotomy, which would inevitably lead to acute disturbances in blood circulation and the structure of the intestinal wall, and to distortion of metabolic parameters. For this reason, in real experimental conditions in vivo, the study of the intestinal wall from the side of the serous membrane is least susceptible to unwanted data distortions. In addition, the dynamics of changes recorded in the serous layer can be most useful for creating clinical diagnostic FLIM technology of the intestine in conditions of abdominal surgery; the serosa of the small intestine is more accessible for diagnosis than the mucous membrane.

It is worth noting that the values of the long component of autofluorescence lifetime τ_2 recorded in the small intestine are atypical for NAD(P)H, which may indicate the contribution of other fluorophores with long lifetimes, for example, collagen (fluorescence lifetime ~3.6 ns) [27] or fatty acids (fluorescence lifetime >7 ns). It is known that during a catabolic metabolic imbalance, the structure of collagen and elastin is one of the first to be destabilized; in this case, dysproteinemia and sarcopenia are accompanied by a decrease in the concentration of NAD+ in tissues and cells [21, 22].

A significant decrease in the value of τ_2 24 hours after a spinal injury indicates, first of all, changes in the fluorophore composition of the tissue, associated, for example, with a violation of the structure of collagen or reduced absorption of lipids. Changes in the relative contributions of the short (a_1) and long (a_2) components do not exclude disturbances in the balance of free and bound forms of NAD(P)H caused by modifications of energy metabolism. The contribution of the short component (a_1) significantly increases relative to the norm, which usually indicates a shift in metabolism towards glycolysis and confirms the catabolic orientation of metabolism in the tissues of the small intestine. Perhaps this condition is associated with the disturbed perfusion of the small intestine tissue, on the one hand, and its sympathetic innervation, on the other. This fact deserves attention, since the currently known provisions on early enteral nutrition with a high protein content are at odds with the principles of nutritional thermogenesis, where additional, and often useful energy is spent on its absorption and transformation. Therefore, the magnitude of nutritional thermogenesis should be regulated by more careful control of the chemical composition of absorbed nutrients by patients with spinal cord injury and can serve as an additional characteristic of food and prepared meals along with energy value. Maintaining the correct energy balance is crucial in plastic metabolism, which allows avoiding the development of sarcopenia, maintaining the proper muscle frame in the victim, and reducing the risk of decubital ulcers [31].

Thus, the study demonstrates the important role of controlling the chemical composition of nutrients, maintaining a balance between the required percentage of protein intake in this category of patients and the energy expended on their digestion.

CONCLUSION

Thus, for the first time, using the modern optical macro-FLIM method, an in vivo study of the dynamics of metabolic changes in the tissues of the small intestine during spinal injury was carried out in an experiment on animals. It has been shown that traumatic disease of the spinal cord in the acute period is accompanied by disturbed metabolic processes in the serous membrane of the intestinal wall 24 hours after the complete intersection of the spinal structures at the level of Th5– Th6 vertebrae. The severity of pathological processes progresses over time, namely, in the studied periods up to 24 hours. The data obtained indicate catabolic metabolic processes in the tissues of the small intestine after spinal cord injury with increased anaerobic glycolysis processes. Timely and correct selection of nutritional preparations used to stop enteral insufficiency, as one of the predictors of the formation of bedsores in traumatic spinal cord disease, will reduce the risks of their formation and improve the quality of life of victims.

The pathophysiological phenomena obtained in this study may be one of the important mechanisms influencing the occurrence of surgical complications in patients with traumatic spinal cord disease. The established patterns can serve as the basis for active intra-intestinal therapy aimed at maintaining metabolic processes in the intestinal wall with a subsequent reduction in energy costs for protein digestion while maintaining its required concentration in the body of the victim and reducing the percentage of formation of decubitus ulcers in the acute and long-term period of the course of traumatic spinal cord disease.

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